DISSERTATION

# BENCHMARKING AND ANALYSIS OF CURRENT PRE-SLAUGHTER MANAGEMENT FACTORS AND THEIR INFLUENCE ON WELFARE AND MEAT QUALITY OUTCOMES IN FED BEEF CATTLE


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In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

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Fall 2023

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ABSTRACT

BENCHMARKING AND ANALYSIS OF CURRENT PRE-SLAUGHTER MANAGEMENT FACTORS AND THEIR INFLUENCE ON WELFARE AND MEAT QUALITY OUTCOMES IN FED BEEF CATTLE

Several factors related to pre-slaughter management of fed beef cattle and their impacts on welfare and meat quality have been identified and discussed thoroughly in previous literature. However, a full catalog of these factors and indicators used to evaluate their impacts on cattle welfare is missing. Additionally, benchmarked data for these factors and welfare and meat quality outcomes, and an analysis of their relationships on a large scale is underrepresented in current literature. The objectives of the first chapter of this dissertation were to catalog pre-slaughter management factors, identify indicators used to evaluate their impacts, and ultimately gain a further understanding of the relationships between pre-slaughter management factors and cattle welfare. This review included an in-depth analysis of 69 studies from across the globe that identified factors related to transportation and handling using behavioral and physiological indicators to measure welfare that were the most researched throughout the studies. The discussion of this review also identified pre-slaughter factors that require benchmarking and/or more research on their potential impacts on cattle welfare. Thus, the objectives of the second chapter in this dissertation was to benchmark pre-slaughter management factors at a collection of commercial fed cattle processing facilities. This data collection took place at five commercial processing facilities in the West, Midwest, and Southwest regions of the United States from March 2021 to July 2022. Data were collected on a total of $n$ = 637 slaughter lots representing $n$

= 87,220 head of cattle. Transportation factors such as distance travelled and the time cattle waited on the truck to unload after arriving at the facility, space allowance in lairage for cattle, lairage duration and cattle mobility was recorded. Environmental factors were later recorded from an online weather service, and cattle characteristics and several meat quality factors including bruising, quality grading, carcass weight and dark cutting were obtained from plant records. Descriptive statistics were calculated for both the lot and individual animal level depending on the variable. Cattle travelled on average, 155.8 ± 209.6 km (Mean ± SD) to the processing facility from the feedlot, waited 30.3 ± 39.7 minutes to unload at the plant and were held in lairage for 200.7 ± 195.0 minutes. The mean lairage density was 3.1 ± 2.0 $m^2$/animal, and a majority of cattle (91.8%, *n* = 77,645) were scored as having normal mobility. Carcass bruising prevalence was 69.7% (*n* = 57,099), and of those that were bruised, 65.2% (*n* = 39,856) had multiple bruises. Having this baseline benchmarking data outlines not only areas that need further improvement, but also areas in this sector that the industry has already improved upon. This benchmarking data also identified the need for additional analysis on the relationships between these factors and outcomes. Therefore, the objective in the final chapter of this dissertation was to assess the effects of these factors on select welfare and meat quality outcomes in fed beef cattle. Using the same data set and methods as in the second chapter, any slaughter lots with no response variables or < 75% of predictor variables present were excluded. A total of n = 619 slaughter lots representing 84,508 head of cattle were used for further analysis. Descriptive statistics for this subset of data and linear and logistic regression models were performed to assess relationships. Statistical significance was determined at $P < 0.05$. Predictor variables of interest included plant, breed, sex class, operation shift at the plant, distance travelled, truck waiting time to unload, lairage duration and space allowance, THI, and wind

speed. Outcome variables of interest included mobility, bruising, dark cutting, quality grades, and hot carcass weights. All outcomes of interest were associated with several pre-slaughter factors of interest, particularly plant and cattle breed. Increased odds of impaired mobility were associated with increased distance travelled (1.001, 1.000 – 1.001; OR, CI) and truck waiting time (1.003, 1.001 – 1.004; OR, CI). Increased odds of carcass bruising were associated with decreases in distance travelled (0.997, 0.996 – 0.998; OR, CI), but increases in space allowance in lairage (1.035; 1.017 – 1.053; OR, CI). Cattle that experienced increases in lairage duration were associated with decreased hot carcass weights ($P < 0.0367$) and increased odds of cark cutting (1.034, 1.001 – 1.068; OR, CI). Additionally, cattle that were slaughtered during the first shift of operation at the plant were associated with decreased odds of being bruised (0.806, 0.772 – 0.842; OR, CI), being classified as a dark cutter (0.416, 0.336 - 0.514; OR, CI), and having a poorer quality grade (0.777, 0.657 - 0.920; OR, CI). Results from these studies identify areas where further and more detailed research is needed to fill knowledge gaps and fully understand these relationships. This research also has the potential to aid in informed decision-making regarding cattle management during the pre-slaughter period and further educate the industry on sustainable management practices.

# ACKNOWLEDGEMENTS

The main project of this dissertation was supported by the Agriculture & Food Research Initiative Competitive Grant no. 2019-67015-29578 from the USDA National Institute of Food and Agriculture; thank you for your funding and contributions to research in this space. Thank you to the processing companies, plant employees, and the over 30 other students who joined on data collection trips and assisted with data management. This project would truly not have been possible without you all. A special thank you to the over 80,000 cattle that were included in this study, your experiences will help improve the welfare of future cattle. Thank you to the other students, faculty, and staff for their contribution to the review process, guidance, patience, and support throughout writing the scoping review.

Thank you to my advisor, Dr. Edwards-Callaway, for your support and dedication to my education for the past few years; you are truly an inspiring mentor and you have taught me so much. Thank you to my committee members (Drs. Mahesh Nair, Ann Hess, and Daniel Mooney) as well. This project and future projects stemming from this data would not be possible without your expertise and support.

## DEDICATION

This dissertation is dedicated to researchers, ranchers, employees, government agencies, trade associations, organizations, industry stakeholders, and additional members of the livestock industry who are passionate and dedicated to the betterment of animal welfare throughout the supply chain. Thank you for all that you have done and continue to do for this industry and its animals; your efforts have not gone unnoticed.

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# CHAPTER 1: INVESTIGATING THE IMPACT OF PRE-SLAUGHTER MANAGEMENT FACTORS ON INDICATORS OF FED BEEF CATTLE WELFARE – A SCOPING REVIEW[1]


**Summary**

The impact of pre-slaughter management practices on fed beef cattle welfare is a multifaceted and well researched subject matter. Factors such as transportation, handling, lairage time and several animal characteristics can directly impact the cattle's behavior, mobility, blood lactate and cortisol levels, likelihood of injury and ultimately overall cattle welfare. Therefore, the objectives of this scoping review were to (1) catalog pre-slaughter management factors that impact fed beef cattle welfare at the time of slaughter, (2) identify indicators used to evaluate the impact of pre-slaughter management on fed beef cattle welfare at slaughter, and (3) gain further understanding of the relationship between pre-slaughter management factors and fed beef cattle welfare outcome indicators at slaughter. A total of 69 studies were included in final analysis for this review, including studies from six geographic regions around the globe. Studies involving alternative slaughter methods (e.g., religious stunning or mobile slaughter) were not included in the formal analysis of this review, but still merited an in-depth discussion within this paper. After reviewing the studies, a total of 37 pre-slaughter factors and 69 indicators of welfare were measured throughout. Pre-slaughter management factors were then categorized by: animal characteristics; environmental characteristics; handling; lairage; transportation; and water/feed. Outcome indicators of welfare were categorized into: behaviors; health, injury and disease;


[1] Davis, M., P. Sullivan, J. Bretón, L. Dean, and L. Edwards-Callaway. 2022. Investigating the impact of pre-slaughter management factors on indicators of fed beef cattle welfare – a scoping review. Frontiers in Animal Science. 3. Available from: https://www.frontiersin.org/articles/10.3389/fanim.2022.1073849

physiological; and stunning and insensibility. Pre-slaughter factors relating to transportation and handling, and welfare outcomes measured by behaviors and physiology were of the most researched throughout the studies. The results of this review offer a catalogue of commonly researched factors and indicators of welfare measured during the pre-slaughter phase, as well as the relationships between them. This review also offers further substantial evidence that a multitude of events in the pre-slaughter phase affect fed beef cattle welfare and a collection of highly applicable welfare indicators to expedite further research on the effects of pre-slaughter factors and the application of improved practices.

## 1. Introduction

Animal welfare is a highly researched (Broom, 2011; Gallo and Huertas, 2016; Edwards-Callaway and Calvo-Lorenzo, 2020; Nalon et al., 2021) and multifaceted topic which addresses an animal's individual state in regards to its ability to cope with its environment (Broom, 1986). Interestingly, there is no universally accepted definition of animal welfare or definitive guidance on what specifically constitutes 'good' or 'bad' welfare for each species (Wigham et al., 2018). For example, the American Veterinary Medical Association refers to animal welfare as the state of the animal, the treatment it receives, and that protecting an animal's welfare means providing for its physical and mental needs (AVMA, 2022). Whereas, the World Organization for Animal Health describes animal welfare as the physical and mental state of an animal in relation to the conditions in which it lives and dies (OIE, 2022). While these definitions share a similar foundation, they vary slightly in their notions of what is causing the impacts on animal welfare itself. Animals that experience negative or poor animal welfare will attempt to cope and counteract any adverse effects from their environment (Broom, 1986, 1988). Lack of success in their attempt to counteract poor conditions may lead to detrimental effects both mentally and physically (e.g., stress, behavior or sickness), and these effects can be observed and measured. Along with the ethical and moral aspects of ensuring acceptable welfare standards for animals, improving an animal's welfare has the potential to positively influence economic outcomes (Gibson and Jackson, 2017).

Globally, efforts have been made across every stage of cattle production to develop standards, protocols, and regulations, both voluntary and involuntary, to help ensure rearing conditions promote positive animal welfare states (NFACC, 2013; AHA, 2016; Executive Council, 2018; NAMI, 2021; NCBA, 2022). Even when these protocols are followed, there will undoubtedly be scenarios in the animal's life where a multitude of stressors may lead to compromised welfare conditions and induce distress, fear, injury or pain. One of these scenarios is the pre-slaughter phase of cattle production. Loading of meat animals for transportation to slaughter initiates the beginning of a potentially stressful process as animals are moved from a familiar environment to the slaughterhouse, where the animals are exposed to potential stressors, such as handling and mixing with other animals (Terlouw et al., 2008). Although the cattle industry has made great efforts to reduce stress during pre-slaughter processes (da Costa et al., 2012; Grandin, 2012; Edwards-Callaway and Calvo-Lorenzo, 2020), there are some unavoidable events that can impact welfare even if relatively brief. Several studies have previously explored the impacts of various stressors in the pre-slaughter phase on animal welfare outcomes (Hogan et al., 2007; Terlouw et al., 2008; Weeks, 2008; Mendonça et al., 2016; Njisane and Muchenje, 2017a; Castro de Jest et al., 2021), and attention has been given to reducing the negative impacts associated with pre-slaughter management (Grandin, 2014; Frisk et al., 2018; Grandin and Cockram, 2020).

The pre-slaughter phase of the cattle production is comprised of several components including loading, transportation to the slaughter plant, unloading, lairage, handling and slaughter. Several other factors are also important components of this phase such as individual animal characteristics, transportation conditions, stocking densities, adverse weather conditions and other novel environmental factors. Collectively, this wide array of factors makes it difficult to determine which ones specifically are contributing to an animal's welfare state (Bourguet et al., 2010). Different approaches to measuring animal welfare at slaughter plants have been employed including the use of: animal-based scoring systems, qualitative behavior assessments, weighted scoring systems, morphometric indicators, and biological and hematological measures (Grandin, 2010; Velarde and Dalmau, 2012; Losada-Espinosa et al., 2018; Wigham et al., 2018). Often studies focusing on cattle welfare during the pre-slaughter phase include the evaluation of a key predictor on welfare outcomes; for example, research exists studying the effects of: transportation stress on physiological responses (Chacon et al., 2005), animal handling on injuries (Jarvis et al., 1996b; Valadez-Noriega et al., 2018), physiological responses and behavior (Ahsan et al., 2014; Hagenmaier et al., 2017; Hultgren et al., 2020) and lairage duration on behavior and blood constituents (Liotta et al., 2007; Özdemir et al., 2022). Common physiological responses to stress include elevated levels of blood cortisol and catecholamines (e.g., epinephrine) as well as changes in heart rate and body temperature (Burdick et al., 2010; Abubakar et al., 2021). Changes in behaviors vary widely as there are several behavioral aspects involved in indicators of welfare of cattle at slaughter that can be either positive (e.g., lying or ruminating) or negative (e.g., aggressive interactions with other cattle or vocalizing) (Tarrant et al., 1988; Cockram, 1990; Grandin, 1998b).

As animal welfare continues to grow as a critical component of slaughter in the beef industry, a significant number of studies have emerged covering the impacts of pre-slaughter management factors and their effects on indicators of fed beef cattle welfare (Losada-Espinosa et al., 2018, 2021; Edwards-Callaway and Calvo-Lorenzo, 2020; de Marchi et al., 2022). However, a formal review and compilation of these studies does not exist. A comprehensive evaluation of the existing literature is necessary to provide an overview of animal welfare indicators measured during the pre-slaughter phase and pre-slaughter management factors that may impact these indicators.

The research question for this scoping review was "What are measurable management factors and subsequent indicators of welfare in fed beef cattle at slaughter, and what are the relationships between them?" The research question for this scoping review was addressed through three main objectives. The first objective was to catalog pre-slaughter management factors that impact fed beef cattle welfare at the time of slaughter. The second, was to identify indicators used to evaluate the impact of pre-slaughter management on fed beef cattle welfare at slaughter. Lastly, the third objective was to gain further understanding of the relationship between pre-slaughter management factors and fed beef cattle welfare outcome indicators at slaughter.

## 2. Materials and Methods

This scoping review was conducted using the methodological framework outlined by Arksey and O'Malley (2005) and advanced by Levac et al. (2010), and utilized the reporting guidelines from the PRISMA checklist and flow diagram (Page et al., 2021).

### *2.1. Eligibility Criteria*

#### 2.1.1. Population, Factors & Outcomes

The population of interest for this scoping review was beef cattle (i.e., fed beef cattle), and the studies of interest were included if they investigated pre-slaughter management factors and indicators of animal welfare. While bulls and cows are not considered as fed beef cattle in the United States, several of the studies from other geographic regions reported mainly processing bulls and cows and therefore these animal types were included in the population when they were considered primarily beef animals. Pre-slaughter management factors were defined as any procedures adopted by workers, slaughter facilities or companies during loading, transportation, unloading, lairage and the actual slaughter process. Other factors present during the pre-slaughter period that were considered relevant included animal characteristics (e.g., breed), environmental characteristics (e.g., temperature and humidity), handling, water and/or feed access, and additives fed one month prior to slaughter were also included in analysis. All types of slaughter facilities, stunning methods, and pre-slaughter management strategies were included to ensure there was a comprehensive list to catalog pre-slaughter management factors that may impact fed beef cattle welfare at the time of slaughter. Including all slaughter facility types, pre-slaughter management strategies and slaughter methods in the discussion also provides a comprehensive catalog of strategies and methods to identify potential areas of the pre-slaughter process that may warrant further research attention. Welfare outcomes were broadly defined as any measure related to one of the three conceptions of animal welfare as outlined by Fraser et al. (1997): biological functioning, affective states, and natural living. For example, biological functioning included cattle health and condition and physiological parameters; affective states included reactivity, head posture, emotional states, and vocalizations; natural living included social and sexual behaviors, eating, drinking and lying down. The outcomes of pre-slaughter management investigated were categorized as: behavior; health, injury and disease; physiological; and stunning and sensibility.

2.1.2. Limitations

Only papers written in English were included. Research articles covering alternative slaughter types (e.g., religious slaughter, on-farm slaughter, mobile slaughter, and slaughter without captive bolt stunning first) were included in the discussion, but not included in the final analysis. Due to the differences of slaughter methods and systems' possible impact on welfare outcomes, the decision was made to discuss these papers separately.

*2.2. Search*

2.2.1. Information Sources

A librarian knowledgeable in systematic reviews was consulted when identifying databases for the literature search. Three data bases were ultimately selected for this scoping review: PubMed, CAB Abstracts (EBSCO; including a filter for peer-reviewed articles), and Web of Science. The concepts used in the database searches were the population of interest (i.e., fed beef cattle), the location in the supply chain (i.e., pre-slaughter), pre-slaughter management factors, and welfare outcomes. Other livestock species (e.g., dairy) not of interest were excluded in the searches. The final searches were performed on August 2$^{nd}$, 2022.

*2.3. Search Strategy*

Initial search strategies were tested on PubMed against a few core references to verify that the core studies were present in the search results. After consultation with the librarian and other research members, a final search string was developed. The same search string was used for all three databases, and peer-reviewed was selected as a filter when available, which was only for CAB abstracts. The final search string and information on the databases searched is available in Table 1.1.

*2.4. Selection of Sources*

All sources from the three databases were collated, uploaded to Zotero, an open-source citation management software (Zotero, Fairfax, VA), and software tools were used to remove duplicates. One reviewer performed the first level of screening of titles to remove any sources that were not relevant to the concepts. For the second level of screening, abstracts and full-text articles were reviewed by two independent reviewers to further exclude sources according to the inclusion criteria. If there was disagreement between the reviewers, each paper was then reviewed together and discussed until an agreement was made. Only primary studies, written in English that covered the inclusion criteria were included in this study. One additional article was discovered post-database searches after reviewing the bibliographies from the original database search results and was included in the final analysis.

*2.5. Data Charting and Synthesis of Results*

Two reviewers discussed and determined the variables of interest to extract from the articles. All articles were reviewed by one researcher and variables of interest were extracted and collated into Microsoft Excel (Microsoft Corporation, Redmond, WA) for each article. The variables were then summarized and organized into categories of predictor variables and outcome variables, and the number of articles investigating each individual variable was reported. Geographic region(s) of each study was also extracted and reported using the regions defined by the United States Department of Homeland Security's office of Immigration Statistics (DHS, 2022).

## 3. Results

### *3.1. Study Selection*

From the three databases searched, 5,197 records were found and downloaded, and 862 of them were removed as duplicates. A total of 4,335 records were then screened for this scoping review. As a result of title screening, 4,041 records were removed for irrelevant topics, leaving 294 records for abstract and full-text screening. When inclusion and exclusion were criteria were applied, 216 records were excluded based on the wrong population, wrong outcomes, wrong location in the beef supply chain, non-English studies, or non-primary studies (i.e., studies that reviewed existing literature). One additional article was found post-database search leading to a total of 78 articles and 1 conference abstract (Mach et al., 2008) included based on the criteria. The authors removed studies from formal analysis where alternative slaughter methods were used (10), because of the possibility of the differing slaughter methods having an impact on welfare outcomes but included them in their own section of the discussion. Therefore, a total of 69 studies were included in the formal analysis. A flow diagram outlines the study selection process in Figure 1.1.

### *3.2. Study Characteristics*

Studies were distributed across six different geographical regions with the majority of studies conducted in Europe (*n* = 30), followed by North America (*n* = 15), South America (*n* = 11), Africa (*n* = 8), Oceania (*n* = 4) and Asia (*n* = 3). Two studies took place in two regions (Grandin, 2001; Teke, 2013). Proportions of each geographic region's representation is displayed in Figure 1.2. Studies that reported when their data was collected ($n$ = 24) took place between the years of 1981 and 2020, and all studies included in the final analysis were published between 1984 and 2022. The number of animals used or observed in the studies ranged from 5 to 290,866, with the average number of animals being approximately 5,718. Eight studies did not report the number of animals included. Over 39 breeds and crosses of cattle were represented, with the most common breed being of Charolais influence, followed closely by Limousin, Angus, Friesian and Hereford. Eighteen studies did not report the breeds of cattle used or observed. Cattle ages studied ranged from 9 months to 9 years, however the majority of studies included cattle ranging from 12 to 24 months, and 39 studies did not report the ages of cattle. Studies with mixed sex (heifers, steers, bulls and/or cows) were the most common ($n$ = 25), followed by steers ($n$ = 14), bulls ($n$ = 13), heifers ($n$ = 1) and cows ($n$ = 1). Fifteen studies did not report the sex of cattle used or observed. Cattle originated from several sources; however, farms were the most common (21), followed by unspecified animal handling facilities ($n$ = 7), feedlots ($n$ = 6), pastures ($n$ = 5), markets ($n$ = 1), multiple origins ($n$ = 8) and 21 studies did not specify the origin of their cattle.

### *3.3. Measures*

Predictor and outcome variables of interest were identified by the author of the scoping review as relevant to pre-slaughter management factors (predictors) and their effects on indicators (outcomes) of welfare and were then collated into categories. A breakdown of predictor and outcome variables measured for each category is summarized in Table 1.2. In the behaviors category of Table 1.2, all behaviors were collated and summarized into larger behavioral categories; the authors recognize that some of the specific behaviors could have been classified into multiple categories but based on the context of the paper and the reason the measurement was taken as part of the specific study, behaviors were classified as presented.

3.3.1. Predictors

After reviewing the 69 studies, a total of 37 unique predictor variables were categorized into the following categories: animal characteristics, environmental characteristics, handling, lairage, transportation or water/feed. The number of studies including a predictor within each category were recorded and are displayed in Figure 1.3. Some studies explored more than one predictor, therefore, the total number of transportation predictors ($n$ = 71) reported in Figure 1.3 exceeds the total number of studies included in the final analysis ($n$ = 69). Out of these five categories, transportation was studied the most ($n$ = 71) in papers reviewed for this scoping review. Within transportation, distance and duration of transportation was measured the most, followed by the mention of general transportation. General transportation in this case meaning the study transported animals and looked at downstream effects of this factor, but no other specific characteristics of the experience were evaluated. The second most studied factor was handling ($n$ = 56). Within handling, general handling was measured the most frequently. General handling, similar to general transportation, meaning the study observed animal handling and downstream effects of this factor, but again, no other specific characteristics of the experience were evaluated. The handling category also included slaughter practices such as stun to stick time (i.e. the time from being stunned to exsanguinated) and stun quality (e.g., missed stuns or number of stuns to render the animal unconscious). The evaluation of animal characteristics was included in 43 studies and the most commonly measured animal characteristic was origin of cattle prior to being transported to the slaughter facility. Environmental characteristics ($n$ = 33) included temperature and humidity, noise levels and facility design and condition. The most measured lairage predictors ($n$ = 32) were lairage duration and density. The least measured factor across the studies was water and feed ($n$ = 6). This category included measures such as fasting duration, water consumption and alternative feeding systems or feed additives prior to transport for slaughter.

### 3.3.2. Outcomes

After reviewing the 69 studies, a total of 69 outcome variables were measured and categorized into the following categories: behavior; cattle health, injury and disease; physiological or stunning and insensibility, and the number of studies measuring each category were recorded and are displayed in Figure 1.4. Out of these categories, physiological parameters were evaluated the most ($n$ = 68) in studies reviewed for this scoping review. Within physiological parameters, blood parameters were measured substantially more than others such as vital measurements or urine parameters. Cattle behaviors were the next commonly measured indicators of welfare ($n$ = 40), with the most frequently measured being the frequency of falls, vocalizations and slips. Cattle health, injury and disease ($n$ = 12) was commonly assessed by recording injuries, mortality and mobility or lameness at the slaughter plant. The final category, stunning and insensibility indicators ($n$ = 7), were measured as post-stunning behaviors or checking for signs of insensibility and bleed out time. Frequently measured behavioral signs to check for insensibility were the lack of corneal reflex ($n$ = 5) and rhythmic breathing ($n$ = 4).

## 4. Discussion

### *4.1. Summary of Main Findings*

#### 4.1.1. Factors and Outcomes

Transportation and handling were the most highly researched pre-slaughter factor categories found in this collection of papers. The effects of transportation on beef cattle welfare are extensively researched and reviewed (Fike and Spire, 2006; Huertas et al., 2010; Schwartzkopf-Genswein et al., 2012, 2016) as transportation presents a period in the pre-slaughter phase that is considered one of the most stressful events that cattle must experience during their lifetime (Chambers and Grandin, 2001; Schuetze et al., 2017). For some cattle, depending on the production system they have been raised in, transport to the slaughter plant may be the first time they have been in a trailer. Livestock transportation regulations and policies vary greatly across areas of the world (e.g., Twenty-Eight Hour Law, 1994; CARC, 2001; European Council, 2005a) as do cattle production systems and associated infrastructure (i.e., pasture-based vs. feedlot; Gonzalez et al., 2022) which can all shape transportation logistics and thus impacts on welfare. There are many different components to transportation as well, including but not limited to, transportation duration and distance, the microclimate in the trailer, and driver experience, which have all been incorporated into the studies included in this review (e.g., Tarrant et al., 1992; González et al., 2012; Chulayo et al., 2016; Valadez-Noriega et al., 2018). Animal handling was also highly represented as a pre-slaughter management factor of interest. There have been considerable efforts in educating stakeholders about low-stress handling techniques as the benefits to reducing animal stress are clear (Grandin, 2012, 2014). Additionally, animal handling is a component of pre-slaughter management that can be more easily controlled and manipulated as compared to other factors such as the environment or facility design and thus a target area to identify improvements. Lastly, many of the industry regulations and guidelines globally highlight the need to have trained employees that understand the basic principles of low-stress animal handling (Government of Canada, 2018; OIE, 2018; GRSB, 2021) and thus this area has perhaps received more attention in relation to cattle welfare at slaughter.

#### 4.1.2. Factor and Outcome Relationships

To assess the relationships between pre-slaughter management factors and outcome indicators of welfare for fed beef cattle, the following discussion will be arranged by the categories of outcomes and a discussion on each one's relationship with pre-slaughter management factors. Main factors and outcomes will be highlighted throughout this discussion. Relationships between variables with less research or no significant impact will be limited in discussion.

#### 4.1.3. Behavior

Allowing an animal to perform natural highly-motivated behaviors has been a foundational component of many animal welfare frameworks (e.g., the Five Freedoms, FAWC, 1993; Fraser's Three Circles, Fraser et al., 1997; the Five Domains Mellor et al., 2020). Additionally, current frameworks for welfare assessment encourage both the promotion of positive experiences and the reduction of negative experiences (Green and Mellor, 2011). Even though the pre-slaughter phase represents a relatively short period within the animal's lifetime, it is still important to provide cattle with the opportunity to express positive natural behaviors such as lying, exploring, and ruminating. Upon arrival at a slaughterhouse, the state of the animal will depend upon the nature and duration of their experience from their place of origin, and the new physical and social environment is likely to affect the ability of the cattle to rest and relax

(Cockram, 1990). In several studies, the frequency of both positive (e.g., ruminating, eating or drinking) and negative (e.g., aggression or vocalizing) behavioral states were measured (Cockram, 1990; Jarvis et al., 1996b; Njisane and Muchenje, 2014, 2017b; del Campo Gigena et al., 2021). Lying time can be an important welfare indicator in cattle (Haley et al., 2000; Tucker et al., 2021). In a slaughter plant, lying behavior would most commonly occur during lairage as cattle experience a period of inactivity (i.e., no handling) during holding. Studies have shown that lying behavior tended to increase with lairage duration, where 26% of focal animals were lying down by 3 hours in lairage (Jarvis et al., 1996b), however, this is highly influenced by time of day, activity within the pen and origin of cattle (Cockram, 1990, 1991; Jarvis et al., 1996a). Cockram (1990) reported that the mean number of head alert reactions to external stimuli in cattle originating from a market decreased with time in lairage and occurred the least in steers over heifers with a space allowance of greater than $5m^2$ per animals, a sign that cattle were acclimating to the new environment. One study comparing cattle that did not experience transportation or lairage with ones that were transported and held for durations up to 24 hours, found reduced aggression frequency (0.00 compared to 0.57 at 24 hours) (e.g., butting) with conspecifics in the group that went straight to slaughter (Özdemir et al., 2022); this is a logical finding as there were reduced opportunities for animal-to-animal interactions in the direct slaughtered group. Cattle are also motivated to spend time eating and drinking (Schütz et al., 2018); the availability of food and water for cattle during this pre-slaughter phase varies across production systems. The origin of the cattle (i.e., market vs. farm) has been shown to influence drinking time; cattle from markets drank for significantly longer and more often at the plant than cattle coming from farms (Jarvis et al., 1996b). This may be indicative of an extended period of water deprivation for cattle originating from markets. Related, cattle being transported from farms over 80 miles away from the abattoir drank significantly more than cattle transported for shorter distances (Jarvis et al., 1996a) from the extended water deprivation period. Cattle ruminate while resting, and time spent ruminating is a direct indicator of animal welfare status in ruminants (del Campo et al., 2021). del Campo et al. (2021) reported that as lairage time increased (from 3 to 15 hours), the frequency of rumination decreased from approximately 40% to 15% of time; one study reported that rumination decreased by as much as 34% when cattle were held for over 12 hours (Mach et al., 2008), a finding that was likely partially related to gut fill. In summary, many factors can influence the expression of behaviors during the pre-slaughter phase, however, past research does show that cattle still have the ability to express highly motivated behaviors at different points in the process.

Movement behaviors, such as slipping, falling, jumping, and loss of balance were frequently measured throughout multiple studies. Studies have demonstrated that these types of behaviors are impacted by both animal handling techniques (Cockram and Corley, 1991; Hemsworth et al., 2011; Huertas et al., 2018; Cevallos-Almeida et al., 2021) and facility condition or type (Grandin, 1996; Disanto et al., 2014; Romero et al., 2017; Willson et al., 2021) and many of them can negatively impact animal welfare by increasing the risk of injury to the animal. Generally, these movement behaviors are observed during periods of animal handling which can occur at multiple times throughout the pre-slaughter period allowing for assessment at a variety of locations and stages. Additionally, these types of behaviors are relatively easy to observe and quantify and are thus practical methods of assessing welfare in a slaughter plant environment. Loading and unloading of cattle are activities during the pre-slaughter period in which handling intensity increases and thus several studies have evaluated the impacts on welfare outcomes from these events (Jarvis et al., 1996b; von Holleben et al., 2003; Hagenmaier et al., 2017). Loading has been shown to be more stressful for cattle than unloading, as the frequency of falls (approximately 33%) and balking (approximately 50%) was greater during loading than unloading (approximately 15% and 10%, respectively; María et al., 2004), possibly due to the cattle being moved into an unfamiliar environment. However, unloading can be more difficult when temperatures are lower, when the duration of transportation is longer, and if cattle are mixed immediately before loading (Mounier et al., 2006), demonstrating the interactive effects of multiple pre-slaughter factors. Once cattle are loaded onto a trailer there are limited opportunities to assess behavior without the use of technology (i.e., cameras in the trailer, accelerometer on the cattle) but it has been reported that during transport, cattle frequently lose their balance with sudden stops or on roads with curves and gravel portions (Tarrant et al., 1988; Wikner et al., 2003). Additionally, increases in transportation duration (60 minutes versus 180 minutes) and greater Temperature-Humidity Index (THI) classes (<60, ≥60<70 and ≥70) have been associated with increased restlessness frequency (7.0% during 60 minutes of transportation and 11.8% during 180 minute transportation duration, and 8.1% in THI class <60, 9.8% in ≥60<70 and 10.8% in ≥70) in cattle on trailers during transport (Costa et al., 2003). Within the slaughter plant cattle have the potential to slip and fall in areas where active handling occurs such as the holding pens, the single-file alley, or moving into the stun box or restrainer system (Costa et al., 2006).

Vocalization is another behavior that is measured as a welfare indicator in research studies focusing on pre-slaughter management; similar to the movement behaviors, vocalization can be measured at a variety of time points throughout the pre-slaughter period and is relatively simple to quantify. Cattle have been observed to vocalize at the plant from aversive and novel events such as prodding with electric prods, slipping at or near the stunning box, missed or inadequate stuns, sharp edges from poor facility conditions, being moved through holding pens to the single-file, or excessive pressure from restraint devices (Grandin, 1998b, 2001; Cevallos-Almeida et al., 2021). Due to these events, measuring vocalizations is a valuable indicator of animal welfare and it can be compared across animal handling facilities (Grandin, 2017). Increased vocalizing has been observed more in cattle being moved after overnight lairage (number of vocalizations ranged from 0-29 per group) compared to cattle being moved after a shorter lairage duration (ranged from 0-4 per group; Cockram and Corley, 1991), likely because cattle housed overnight had time to rest and recuperate. Again, there are many factors that impact cattle behavior during the pre-slaughter phase and vocalization is a welfare indicator that is responsive to changes in handling and the environment that cattle experience.

Many of the above descriptions of behavioral outcomes include some component of animal handling. The impact of human-animal interaction on animal welfare is well-documented (Mota-Rojas et al., 2020; Acharya et al., 2022). As noted above, animal handling during this pre-slaughter phase can have negative impacts on welfare outcomes; most of the research found focused on negative actions of the animal handlers during intense animal handling events (e.g., loading, movement at the plant) and the subsequent cattle behavioral responses (e.g., balking, falling, vocalizing; Cockram and Corley, 1991; Grandin, 2001; Hemsworth et al., 2011; Doyle et al., 2016). Probst et al. (2013) took a different approach and explored positive handling interactions prior to the commencement of the pre-slaughter period. In their study, Probst et al. (2013) discovered that gentle touching and increased interactions with handlers in the few weeks prior to slaughter decreased avoidance distances in cattle at the abattoir, suggesting that positive human handling prior to being transported for slaughter can reduce an animals' fear of humans. While gentle touching of each animal is not practical in most commercial settings, these results do raise the question of whether increased animal handler interactions of other manners (e.g., walking through the pens or moving cattle) may be a plausible solution to decreasing stress responses in cattle prior to slaughter.

#### 4.1.4. Health and Injury

There are several pre-slaughter management factors that impact the prevalence of cattle injury and health outcomes. Previous literature has identified relationships between pre-slaughter management factors specifically during transport and welfare outcomes, including weight loss, traumatic lesions and even death, for both cattle and other livestock species (Knowles et al., 1993, 1994; Villarroel et al., 2001; Gallo et al., 2003). Overall health and well-being can be impaired and the onset of severe stress can be observed in animals being transported (Minka and Ayo, 2010). A survey of transporters commercially hauling cattle for long durations reported that cattle condition was good (98.66% of journeys), fair (1.28%) and poor (0.05%) at time of loading, but the prevalence of journeys with ‘fair’ or ‘poor’ conditioned cattle increased 2.7-fold and 4.2-fold upon unloading (96.3% good, 3.49% fair and 0.21% poor) as a result of transportation stress (González et al., 2012). Another survey of livestock haulers reported that almost half of its participants (48.6%) observed up to five animals with injuries inflicted during transportation to the plant, however, this could be a low estimate as drivers have been documented to have limited ability to recognize pain and injuries in animals (Valadez-Noriega et al., 2018).

An animal’s ability to move through the pre-slaughter phase and navigate the different transport and handling systems is critical. Cattle mobility and lameness are important considerations and have been focused on significantly more in recent years within the fed cattle industry, particularly in the United States (Edwards-Callaway and Calvo-Lorenzo, 2020). Mobility is a multifactorial issue impacted by not only weight, THI, sex, and days on feed (DOF; (González et al., 2012; Edwards-Callaway et al., 2017; Martinez et al., 2021; Mijares et al., 2021), but also transportation (e.g., distance hauled) and lairage factors (e.g., lairage duration; Hagenmaier et al., 2017). One study within this review, however, reported a rather low prevalence of lameness (0.7%; Burgstaller et al., 2022) compared to other studies ranging from as low as 1.1% to 54.8% of cattle reported as clinically lame (Dudley, 2017), likely because the sample size of this study consisted of mostly young bulls and calves rather than older cattle. Older cattle are typically more prone to mobility issues due to age-related variations in their physiological status (Gavrilova and Sementovskaya, 2021). Individual animal characteristics (e.g., horned or different weight categories), truck flooring conditions, falls during loading or unloading, and trailers with inadequate conditions such as loose or protruding boards, screws or nails have also been shown to be associated with increased injury and lesions in cattle at the abattoir (Lee et al., 2017; Brito et al., 2019).

#### 4.1.5. Physiological

Studies in this review included several physiological measures ranging from vital parameters to metabolites measured in biological samples, however, blood metabolites were the most commonly measured within this category, comprising approximately 65% of studies. Hematological blood indicators are known to be the primary determinants of an animal's ability to adapt to its environment and thus are useful parameters to include in welfare assessment (Anderson et al., 1999). Specific hematological parameters such as neutrophil/lymphocyte ratios, cortisol, adrenaline and noradrenaline are commonly used to measure the stress response to pre-slaughter management in cattle (Warriss, 2010; Cucuzza et al., 2014; Mirzad et al., 2018).

Adrenal hormones in particular, such as cortisol, represent one of the most important physiological parameters to measure the stress response as various stressors can result in its release, and is used in several studies assessing the impact of pre-slaughter management on cattle welfare (Mitchell et al., 1988; Ndlovu et al., 2008; Burdick et al., 2010; Tarantola et al., 2020). Common blood parameters measured in studies included in this review included cortisol, creatine kinase (CK) and catecholamines such as epinephrine and norepinephrine but the selection of physiological parameters measured varies substantially between studies. As cattle attempt to maintain homeostasis, adaptive responses such as these physiological changes attempt to restore that balance (Carrasco-García et al., 2020).

Generally, regardless of the pre-slaughter predictors assessed, most studies have found that physiological parameters change in response to management manipulations (Rulofson et al., 1988; Tarrant et al., 1988; Chacon et al., 2005; Tadich et al., 2005; Romero et al., 2014). For example, transportation effects on blood parameters were highly researched in studies included in this review. Transportation has been shown to induce changes in the composition of blood along with other parameters such as heart rate, hormones, metabolites, enzymes and even skin dehydration (Fazio and Ferlazzo, 2003; Gregory, 2008), however, these effects will vary based on transportation duration, road conditions, and age, breed, and previous experiences of the cattle. Several studies discovered that transportation stress was substantial enough to elevate blood concentrations of antidiuretic hormone (Ballarin et al., 2006), epinephrine and norepinephrine concentrations (Mitchell et al., 1988; Rulofson et al., 1988), glucose (Jarvis et al., 1996a), lactate (Chacon et al., 2005; Gruber et al., 2010), cortisol (Villarroel et al., 2003; Romero et al., 2014; Bertoloni et al., 2016; Capra et al., 2019), LDH (Birhanu et al., 2019), and creatine phosphokinase (CPK, i.e., creatine kinase or CK; Tarrant et al., 1992; Tadich et al., 2005). Cattle can be transported in various ways across the globe and thus transport conditions vary greatly; in the US, cattle are transported using livestock trailers, whereas other geographic regions such as Oceania may export cattle for slaughter overseas via ship. Cattle transported by sea are usually subjected to longer transport times and extended periods of feed withdrawal (Phillips, 2008). Route of transportation has also shown differences in plasma CK levels as cattle transported by sea and road experienced mean plasma CK levels of 1,137.86 (IU/L)compared to cattle subjected to only road transportation and a saleyard pathway (596.79 IU/L; Loudon et al., 2019), however, both transportation pathways resulted in cattle with elevated plasma CK levels as published normal basal concentrations range from 35 to 280 IU/L (Radostits et al., 2000).

Lairage at the slaughter plant presents a particularly challenging period prior to slaughter as several factors such as lairage duration, mixing with unfamiliar cattle, stocking density and temperature microclimates differ in severity at each plant and offer their own varying degrees of stress for cattle (Edwards-Callaway and Calvo-Lorenzo, 2020). Several studies explored the impact of varying lengths of time spent in lairage on physiological outcomes (Tadich et al., 2005; Liotta et al., 2007; Giannetto et al., 2011; de Marchi et al., 2022). Allowing animals to rest in lairage for longer durations has shown a reduction in: CK and LDH activity (Chulayo and Muchenje, 2017), glucose, lactate and protein blood levels (Pighin et al., 2015) and cortisol concentrations (Liotta et al., 2007; Chulayo et al., 2016). Grosskopf et al. (1988) reported that blood parameters such as total plasma cortisol decrease in value as time in lairage increased (203 nmol/L immediate slaughter, 128 nmol/L after 3 hours of lairage and 85 nmol/L after overnight lairage), however, plasma creatine kinase was higher in cattle with a lairage period of 3 hours (513 U/L) compared to cattle slaughtered immediately upon arrival to the abattoir (372 U/L), with a decrease in values in cattle held overnight (112 U/L). Differing results between studies likely are partially due to differences in other pre-slaughter management practices, such as transportation time. The stress impacts of mixing non-familiar groups of cattle is a highly researched area (Šímová et al., 2016; Hubbard et al., 2021), particularly during the pre-slaughter phase. Warriss et al. (1984) found that when mixing unfamiliar cattle during lairage, those that exhibited the most animal-to-animal interactions compared to other animals had greater levels of CPK and free fatty acids (FFA) concentrations and decreases in plasma lactate, indicating that liver and muscle glycogen was depleted from the stress of social mixing.

The pre-slaughter process involves several instances where cattle will be moved to different locations (e.g., loading or unloading, moving from holding pens to the drive alley and through the single file) all of which present a challenge if facilities and handling techniques are suboptimal (Disanto et al., 2014). High levels of cortisol can be attributed to inadequate handling of cattle during the pre-slaughter and slaughter phase (Mitchell et al., 1988; Carrasco-García et al., 2020; Guarnido-Lopez et al., 2022). Cattle exposed to low-stress slaughter conditions (i.e., less time being transported and moved through the slaughter process at the abattoir) resulted in lower heart rates and stress hormone concentrations (Reiche et al., 2019). Cattle that fell from slippery flooring or improper handling, were lame, or were injured during transportation showed mean levels of CK and cortisol increasing by 43U/I and 8 ng/ml, respectively, compared to those without injury (von Holleben et al., 2003). When stunning animals, it has been discovered that animals needing to be stunned more than once had increased blood cortisol concentrations compared to those that only required one stun to be rendered unconscious (Chulayo et al., 2016; Njisane and Muchenje, 2017b). Studies such as the ones presented here offer further evidence on the importance of animal handling and stunning training, and how they are essential in improving efficiency and animal welfare (Ceballos et al., 2018; Večerek et al., 2021).

Potential relationships between behavior and blood parameters have also been explored (Burdick et al., 2010; Stockman et al., 2012). Temperamental cattle have been reported to have greater cortisol and epinephrine concentrations compared to calmer cattle post-transportation (Burdick et al., 2010). Stockman et al. (2012) found that cattle scored as 'nervous' or 'anxious' prior to being slaughtered had greater plasma lactate concentrations than those that were calmer; this effect was also associated with the amount of time waiting to be slaughtered (i.e., end of the queue vs. the beginning of the queue). Other animal characteristics such as breed, which also could influence temperament, has also been explored (Doornenbal et al., 1988; Prisacaru, 2014). In one study examining the relationships between Bonsmara, Nguni and Angus breeds and physiologic parameters, Bonsmara cattle were reported to have the greatest concentrations of adrenaline, noradrenaline and dopamine and Nguni had the greatest serum cortisol concentrations suggesting that both breeds had increased responses to the stress associated with the pre-slaughter period (Ndlovu et al., 2008). In a similar study, Angus cattle had lower levels of urinary creatinine compared to other breeds such as Limousin and Blond d'Aquitain, suggestive of a lesser stress response to slaughter events such as transportation and stunning (Bourguet et al., 2015). Results from these studies suggest the possible need for breed specific pre-slaughter management strategies, however, further research is warranted in this area, specifically investigating differences between breeds underrepresented in current research.

Vital parameters such as heart rate were also commonly measured as indicators of welfare after particularly stressful events during the pre-slaughter phase. Heart rate variability in cattle can be used to quantify stress from physical, emotional and pathological origins (von Borell et al., 2007), all of which can be caused by events in the pre-slaughter phase. For example, cattle recovered resting heart rates during longer journeys compared to those only transported for thirty minutes where all cattle maintained elevated heart rates (Chacon et al., 2005), suggesting that time to recuperate during transportation may be beneficial. Also, cattle that showed more resistance to handling during human exposure prior to transport for slaughter have also shown to have faster heart rates during loading (Terlouw et al., 2012).

### 4.1.6. Stunning and Insensibility

The actual act of slaughter can have a significant impact on animal welfare and thus is an important consideration when evaluating the pre-slaughter period. In non-religious slaughter systems, an animal will be stunned prior to further processing, required by law (e.g., The Humane Methods of Slaughter Act, USDA-FSIS, 1978), in order to render the animal insensible to pain. Despite the significance of ensuring effective stunning to overall welfare, few studies in this review measured post-mortem behaviors of cattle following stunning (Grandin, 1998a; Miranda-de la Lama et al., 2012; Mpamhanga and Wotton, 2015; Romero et al., 2017; Cevallos-Almeida et al., 2021). These studies were primarily assessing behaviors indicative of return to sensibility which included eye reflexes, blinking, rhythmic breathing and righting reflexes (Grandin, 1994). Agitation just prior to stunning, such as using a pre-slaughter crush restraint for the purpose of cattle identification, have resulted in a less effective stun as rhythmic breathing, rotation of the eyeball and limb movement at sticking in stunned cattle was significantly reduced when crush restraint was not used (Mpamhanga and Wotton, 2015). A notable point to consider is employee behavior related to the stunning and exsanguination process; stunning operators can become fatigued, resulting in cattle that are not efficiently stunned and the need for multiple stunning attempts to render the animal unconscious (Grandin, 1998a). The single study measuring pre-slaughter effects on bleed-out times did not find any significant effects of genotype, transportation groups or durations of lairage on bleed out times (Njisane and Muchenje, 2017b).

#### 4.1.7. Gaps in Research

Several pre-slaughter factors and indicators of fed beef cattle welfare have been identified in this review; however, some underrepresented welfare-friendly practices and useful indicators of welfare should be investigated further. While factors such as transportation, handling, animal and environmental characteristics, and lairage factors have been extensively measured in studies assessing pre-slaughter management on welfare outcomes, factors related to water and feed provision have not been thoroughly researched in this space. Fasting duration for both feed and water have been studied (Jarvis et al., 1996a, 1996b; Clariget et al., 2021), but little information is known about the implications of these factors on the welfare of cattle. Analyzing the interactions between fasting durations and water deprivation on both cattle condition and cattle responses to stressful experiences would be beneficial to understand.

Behavioral and physiological parameters were included in the majority of studies in this review, however indicators of welfare related to cattle health, injury and disease and stunning and insensibility were underrepresented. Although in the United States there has been considerable attention paid to cattle mobility at the slaughter plant (Eastwood et al., 2017; Lee, 2017), mobility and lameness were measured in less than 5% of papers in this review. Heat stress is another area of concern particularly for cattle in the finishing and pre-slaughter phases of the industry as climate change continues to impact both cattle welfare and production efficiency (Berman, 2019; Lees et al., 2019). Several recent studies have been conducted exploring the implementation of heat mitigation strategies and their benefits for cattle in the beef supply chain

(Mitlöhner et al., 2002; Rusche et al., 2021; Davis et al., 2022). Heat stress behaviors such as open-mouth breathing was only measured in one study in this review (Hagenmaier et al., 2017). Surprisingly, the impacts of heat mitigation on cattle welfare during the pre-slaughter phase was not measured in any of the studies included in this review.

*4.2. Global Differences*

Although animal welfare is relevant to beef cattle production systems globally, there is a clear difference in how welfare is studied across global geographic regions, as was identified in this scoping review. It is important to consider the vast differences in both supply chain structures and management systems (Aghwan and Regenstein, 2019; Gonzalez et al., 2022), but also perceptions about animal welfare across different areas of the world (Toma et al., 2012; Alonso et al., 2020; Abdulhaleem, 2022), specifically between developed and developing nations. Perceptions about animal welfare are impacted by cultural, socioeconomic, and religious factors and thus differences in animal care practices are likely to be observed between different countries (Karesh, 1995; Agoramoorthy and Hsu, 2012; Abdulhaleem, 2022). Developed nations often have the capacity to invest resources in more progressive animal welfare efforts while developing countries may be faced with challenges that supersede animal care concerns, such as political instability, food insecurity, and human health and well-being (Karesh, 1995). However, public concern for higher standards of animal welfare is increasing throughout the world, even in developing countries (Harper and Henson, 2001). Additionally, as this scoping review focused on pre-slaughter management specifically, trends in meat consumption and dietary rules differ globally and therefore could impact the research focus in certain geographic regions (Eliasi and Dwyer, 2002). Thus, it is important to note that the exclusion of non-English papers likely limits the cultural and geographic diversity of research studies found in this scoping review.

The World Organization for Animal Health (OIE), a globally recognized entity, has developed a framework with specific recommendations for how member countries should construct their animal care standards (OIE, 2018) for a selection of livestock species. Other globally recognized organizations such as the Global Roundtable for Sustainable Beef (GRSB) work to encourage learning and adoption of best animal health and welfare practices across sections of the globe (GRSB, 2022). The GRSB encompasses twelve national roundtables (e.g., Mexican, Brazilian, European, Southern Africa and Australian roundtables), serving their members, regions and countries with projects and initiatives for a more sustainable, efficient, and profitable beef industry that would include promoting and progressing cattle welfare. While the efforts of organizations like these are profound, there is a large gap in animal welfare conditions between developed and developing countries (e.g., countries in Europe versus countries in Africa and Asia) often posing a challenge when trying to establish universal benchmarks for animal welfare progress. In the area of slaughter welfare, the OIE Terrestrial Code includes a chapter outlining animal welfare considerations for food animals during pre-slaughter and slaughter processes (OIE, 2016; Canadian Beef, 2021; OECD, 2022); slaughter processes are defined as "any procedure that causes the death of an animal by bleeding" thus encompassing both religious and non-religious methods.

As outlined in the results, articles studying pre-slaughter management factors and their impacts on indicators of welfare in fed beef cattle are spread widely across the global geographic regions. The greatest percentage of studies were conducted in Europe; Europe is the third largest beef producing region globally (Canadian Beef, 2021; OECD, 2022). The European Union (EU) has numerous and advanced laws protecting the welfare of farmed animals (Simonin and Gavinelli, 2019) with provisions for both welfare on-farm, during transport, and at slaughter

(European Council, 2005b, 2009; European Union, 2008). Governmental institutions in the EU use a polling instrument, the Eurobarometer, to regularly assess consumer insights on a variety of political and social subjects, including animal welfare (European Union, 2022). In the 2016 report, 94% of EU respondents indicated it was important to protect animal welfare and more than half (59%) indicated they would pay more for products that came from "animal welfare-friendly" production systems (European Commission, 2016). European countries are often regarded as having progressive legislation for animal welfare protections (Caporale et al., 2005) with consumers that have high expectations for farmed animal welfare (Martelli, 2009; Alonso et al., 2020). In a systematic review of studies exploring public attitudes and perceptions towards farm animal welfare, nearly three-quarters of the included studies were conducted in Europe (Clark et al., 2016) emphasizing the relative importance that this area of the world may place on aspects of welfare.

Interestingly, the North American and South American regions combined only accounted for approximately one-third of the research studies yet countries within these regions are some of the largest beef producers in the world; the United States and Brazil are the top two beef producing nations globally, together accounting for over a third of the world's beef production (Canadian Beef, 2021; OECD, 2022), and coincidentally were the top two countries in North and South America in studies included in this review. Studies in large commercial slaughter facilities, like many of those found in North and South America, are challenging to coordinate and can be expensive to execute. The authors speculate that funding mechanisms for research of this nature across countries could be different in number of opportunities, sponsor interests, and grant amounts contributing to these geographic differences.

Conversely, studies originating from developing geographic regions such as Africa and Asia were underrepresented in this scoping review. This gap in animal welfare research in these regions is likely due to many factors such as economic status of specific countries and cultural or religious predispositions of how animals should be treated (Abdulhaleem, 2022). Public concern for animal welfare comes predominantly from urbanized populations and is inversely proportional to the population size engaged in agriculture, which is this case is many of these developing countries as populations are heavily engaged in agriculture (Harper and Henson, 2001). Public and consumer concern can positively drive legislation to achieve some minimum standards of welfare conditions (Désiré et al., 2002; Asebe et al., 2015; Alonso et al., 2020; Abdulhaleem, 2022), however, many developing countries within the regions of Africa and Asia do not have the same concern for animal welfare as education and awareness of the topic is limited (Abdulhaleem, 2022). Additionally, many developing countries do not have the resources to provide animal care to the standards that developed countries are able to do (Rahman et al., 2005). Sinclair et al. (2017) explored attitudes of stakeholders in Asia towards animal welfare during slaughter and transport and found that government laws, religion, and peer attitudes towards welfare were among the greatest ranked influencing factors.  These examples should not be generalized to every developing country, but this discussion may help clarify some of the differences in welfare research attention across regions.

Oceania encompasses a geographically, socially and economically diverse region, where concerns for animal welfare vary greatly (Rahman et al., 2005). Studies conducted in Oceania had low representation in this review and all studies were conducted in Australia. New Zealand and Australia have strong legislation at the government and community level that work to improve and regulate animal welfare, however, several countries (e.g., Tonga, Wallace and

Futuna) are in desperate need for development and updates in animal welfare legislation, as well as practicing veterinarians to implement animal related policies and practices (Rahman et al., 2005). Rahman et al. (2005) also explains that many populations in oceanic countries, like other developing countries, lack awareness of animal welfare issues.

Efforts are being made to narrow the gap in animal welfare legislation, awareness, and research across demographic regions of the globe by increasing the online availability of animal welfare guidelines and best practices (Bayne and Turner, 2019). The accessibility of these resources can contribute to the development of standards and practices in developing countries, however, some developing countries lack the resources to efficiently adopt practices of already developed countries, and therefore these countries will need to evolve their own standards based on their own priorities (Rahman et al., 2005).

### *4.3. Alternative Slaughter Methods*

Alternative slaughter was identified as a process that followed religious slaughter laws, used a head restraint during stunning, performed electrical stunning, or slaughtered and processed animals outside of a permanent facility. Ten papers were identified as studies categorized as alternative slaughter: religious slaughter (Bourguet et al., 2011; Ahsan et al., 2014; Bozzo et al., 2018; Alam et al., 2020; Abubakar et al., 2021; Imlan et al., 2021), electrical stunning followed by exsanguination (*n* = 1; Minka and Ayo, 2007), on-farm and mobile slaughter (*n* = 2; Hultgren et al., 2020, 2022) and conventional slaughter with a head restraint (*n* = 1; Ewbank et al., 1992).

4.3.1. Religious Slaughter

The welfare of animals during religious slaughter has been extensively discussed (Adams and Sheridan, 2008; Anil, 2012; Downing, 2015; Farouk et al., 2016). Due to the nature of religious slaughter (i.e., in most instances, no stun delivered prior to exsanguination), welfare concerns have been identified including an increase of stress for the animals (Bozzo et al., 2018), casting procedures (Ahsan et al., 2014), incorrect knife use (i.e., dull or small; Ahsan et al., 2014; Alam et al., 2020) and sensibility (Alam et al., 2020). Some of the studies in this category explored different aspects of religious slaughter impacts on cattle welfare, such as: a general focus on welfare outcomes during the religious slaughter process (i.e., not treatment comparisons; Ahsan et al., 2014; Alam et al., 2020); a comparison of welfare outcomes in cattle experiencing religious slaughter as compared to conventional slaughter (e.g., stunning with a captive bolt stunner prior to exsanguination; Bourguet et al., 2011; Bozzo et al., 2018); and a comparison of different restraint devices used during religious slaughter on welfare outcomes (Imlan et al., 2021). All of these studies included one or more of the following measurements to aid in welfare assessment: blood parameters, electroencephalography (EEG) analysis, post-exsanguination animal responses (e.g., signs of insensibility), behavioral reactions to lairage conditions, electrical prod use, pre-slaughter handling (e.g., slipping and falling), and characteristics of the neck cut that could impact welfare (e.g., cuts and stabs). The last study in this category examined the relationship between transport distance and stocking density on the trailer and cortisol response and EEG parameters of animals slaughtered using religious methods (Abubakar et al., 2021). Several of the same or similar welfare outcomes were measured as those found the formal analysis portion of this review. For example, antemortem behaviors and blood parameters were measured frequently throughout these studies and the studies included in the review.

Although a direct comparison between welfare outcomes in studies utilizing religious slaughter methods as compared to non-religious methods was not performed, it is worth noting that some of the results reported in these studies did cause concern for animal welfare. For example, religiously slaughtered animals had an increase in blood parameters such as cortisol (Bozzo et al., 2018) indicating increased stress, and observed corneal reflex (Bourguet et al., 2011) indicating sensibility post-slaughter as compared to conventionally slaughtered cattle. Some pre-slaughter handling techniques of animals during the religious slaughter process also raises welfare concern due to practices such as casting; Ahsan et al. (2014) observed animals pushed onto hard concrete, dragging by tails, bounding of all four legs for several minutes' pre-slaughter, and vocalization during casting and post-cut. Consideration should be given to how the actual slaughter method (e.g., stunning and exsanguination, exsanguination only, etc.) could impact results when exploring other pre-slaughter management practices.

4.3.2. On Farm and Mobile Slaughter

On-farm and mobile slaughter has become an area of interest, mainly due to the stress transportation induces on cattle which is avoided in on-farm scenarios, and it is anticipated that more research will be conducted in this area as this type of processing becomes more popular (Johnson et al., 2012; Friedrich et al., 2015; Hultgren et al., 2020, 2022). Hultgren et al. (2022) studied slaughter by rifle from a distance (ranging 6-12 m), focusing on blood parameters and pre and post slaughter behaviors (e.g., walking, exploring, sniffing, and vocalizing) as indicators of stress. Hultgren et al. (2020) conducted a study to assess the stress related behaviors in a mobile slaughter scenario compared to a stationary slaughter plant. The measurements recorded in this study focused on the actions of the animal handlers (e.g., touching, hitting, prodding, and tail-twisting) and subsequent animal responses (e.g., slipping, backing up, and vocalizing). The study concluded that more handling actions by stockmen, such as during the transportation process, increased stress behaviors in animals (Hultgren et al., 2020).

### *4.4. Limitations*

These studies were evaluated solely on the pre-slaughter factors that they measured and the resulting indicators of welfare, this review did not compare methodology for measuring these factors or welfare indicators. This sector of the beef industry requires continued improvement and advancement from researchers on narrowing down which pre-slaughter factors are the most impactful and which measures of animal welfare provide the most accurate depiction of the animal's current state. This review only covered the pre-slaughter phase of the beef industry, therefore past experiences, stressors, and challenges faced by animals in these studies varied and therefore may have affected their responses to stressors during the pre-slaughter phase. In addition to the vast difference in measures assessed across studies, several studies also proved difficult to compare as cattle populations varied in their characteristics such as breed, age and sex, and place of origin. Although studies included in this review were conducted in differing geographic regions, the exclusion of non-English papers likely limits the fully robust global potential of this review.

### *4.5. Conclusions*

Undoubtedly, fundamental factors such as the effects of transport conditions, reactions of the animals to novel environments and underlying commercial pressures that impose 'speed' on workers at the plant (Wigham et al., 2018), all contribute to animal welfare impacts. It is both essential and imperative for beef processors, producers and industry stakeholders to understand the direct relationships between management decisions and beef cattle welfare. This review highlights that there are several pre-slaughter management factors that contribute to animal welfare, as well as several measurable outcomes to assess fed beef cattle welfare at slaughter. A majority of management factors affecting beef cattle welfare were centered around transportation and animal handling, and frequently assessed indicators of welfare included physiological and behavioral measures. The results of this review continue to demonstrate that animal welfare is complex and identifying precise events or stages in the pre-slaughter phase that generate the most notable outcomes is difficult as the impacts are likely multifactorial and dynamic. Systematically compiling these factor and outcome measures, as well as their relationships, is essential to provide an accurate description of hazards to fed beef cattle welfare and hazard occurrence within the pre-slaughter sector of the industry. Animal welfare surveillance activities during the pre-slaughter management period may provide a framework that not only enables the timely detection of hazards and threats, but also identifies approaches that either support or drive different risk management strategies to be adopted by the public and private sectors (Losada-Espinosa et al., 2018). From this review, there is substantial evidence demonstrating that a majority of events in the pre-slaughter phase inflict multiplicative stressors on an animal that negatively impact their welfare. However, the results from this review also provide a collection of welfare indicators that can be used to facilitate further research on examining how new and existing management factors impact cattle welfare.

**Tables and Figures**

**Table 1.1.** Databases and search string for a scoping review covering pre-slaughter management factors and their impacts on indicators of fed beef cattle welfare.

| Database | Interface | Dates Included[1] |
|---|---|---|
| PubMed | NCBI | 1950 – 2022 |
| Cab Abstracts[2] | CABI | 1973 – 2022 |
| Web of Science Core Collection | Web of Science | 1945 – 2022 |
| **Search String (All Databases)** | | |
| (Fed OR Native OR Cattle OR Heifer OR Steer OR Beef OR "Beef Cattle" OR "Fed Cattle" OR "Fed Beef") AND ("Preslaughter management" OR Preslaughter OR Slaughter OR Antemortem OR Harvest OR Pre-Harvest OR Pre Harvest OR Preharvest OR Abattoir) AND (Lairage OR Transport* OR Handling OR Mitigation OR Management OR Weather OR Pens OR "Holding Pens") AND (Stress OR Welfare OR Behavior OR Mobility OR Physiologic OR Biomarker OR Blood OR Well Being OR Pain OR Injury OR Lactate OR Cortisol OR Epinephrine OR Metabolite OR Distress) NOT (Dairy OR Broiler* OR Poultry OR Swine) | | |

[1]Dates shown above were provided by each database's respective preset year range. Date exclusions were not applied during database searches.
[2]'Peer Reviewed' was applied as a filter on this database during the search.

**Table 1.2.** Preslaughter management factors and fed beef cattle welfare indicator categories with subsequent measurable variables of interest from studies included in analysis (n = 69) for a scoping review.

| | **Category** | **Measurable Variables** |
|---|---|---|
| **Predictor** | Animal Characteristics | Origin of Cattle, Temperament, Presence of Horns, Sex, Breed, Age and Health/Condition Upon Arrival to the Slaughter Plant |
| | Environmental Characteristics | Bedding/Substrate Availability, Time of Day, Temperature/Humidity, Shadows, Light, Noise, Facility Size and Facility Design/Condition |
| | Transportation | Group Size, Driver Experience, Cattle Location in Trailer, Trailer Type, Trailer Condition, Stocking Density, Travel Distance/Duration, Social Mixing, Wait Time to Unload and General Transportation |
| | Handling | Prior to Transport, Loading/Unloading Duration, General Animal Handling, Stun Quality and Stun to Stick Time |
| | Water/Feed | Alternative Feeding System/Feed Additives, Fasting Duration and Water Consumption |
| | Lairage | Group Size, Social Mixing, Lairage/Resting Duration and Lairage Density |
| **Outcome** | Cattle Health, Injury & Disease | Mortality, Mobility/Lameness, Injuries and General Health/Condition |
| | Behavior | • Movement (e.g., slip, fall, walking)<br>• Social (e.g., aggression, mounting)<br>• Fight, Flight or Freeze (e.g., reactivity, avoidance, evacuations, kick, vocalize)<br>• Stress Response (e.g., muscle tremors, open mouth breathing, eliminating)<br>• Standing (e.g., idling, immobile)<br>• Emotional States and Temperament<br>• Eating, Drinking and Ruminating<br>• Lying and Sitting<br>• Exploratory<br>• Vigilance |
| | Stunning & Insensibility | Post-Stunning Behaviors/Insensibility Checking (e.g. rhythmic breathing or corneal reflex), and Bleed Out Time |
| | Physiological | Vital and Blood Parameters, Electroencephalogram Recording and Brain, Foot, Skin, Liver, Adrenal Gland, Saliva and Urine Samples |

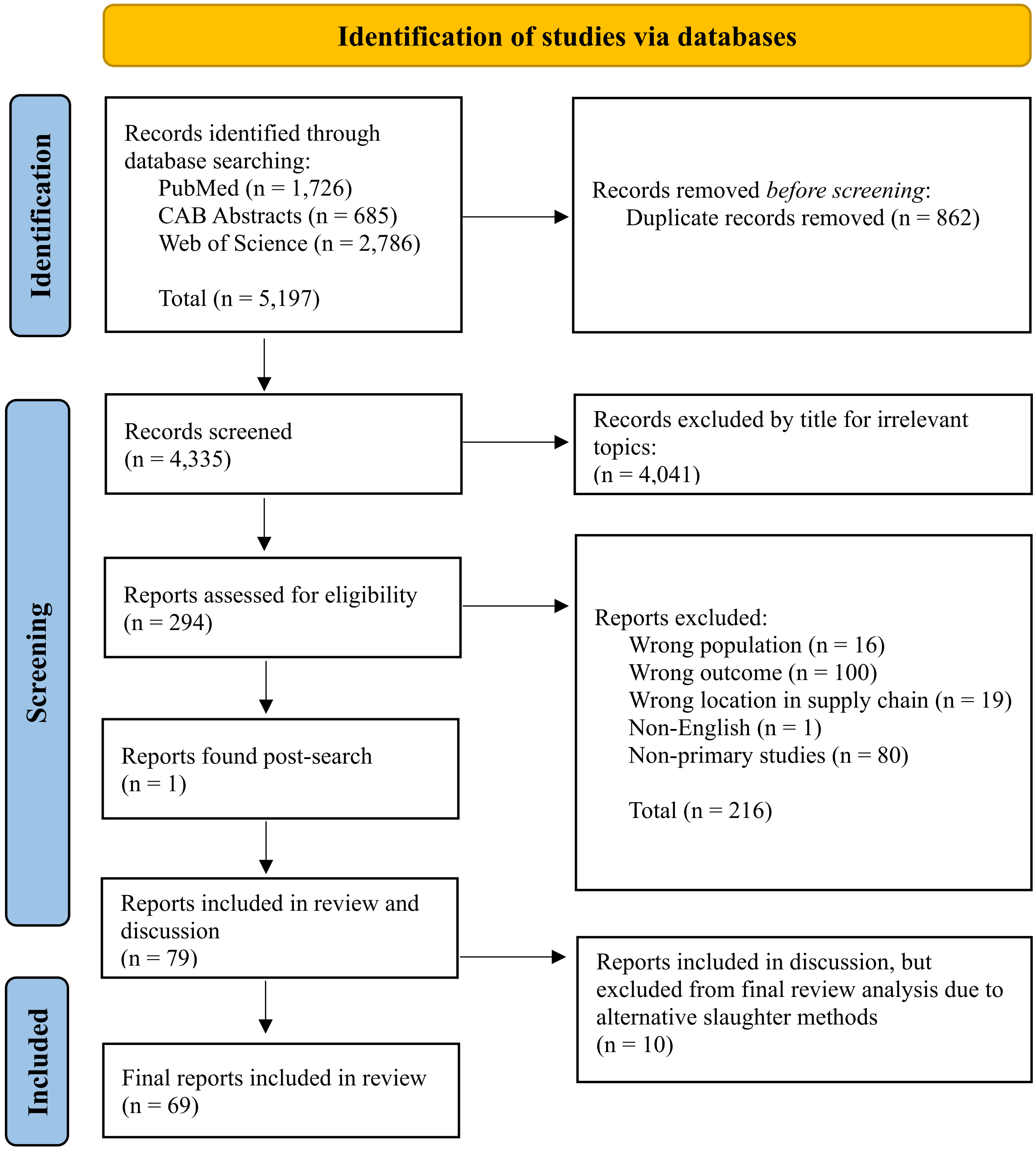


**Figure 1.1.** PRISMA flow diagram outlining the identification and inclusion of articles depicting pre-slaughter management factors and fed beef cattle welfare outcomes. Flow chart adapted from the PRISMA 2020 Statement (Page et al., 2021).

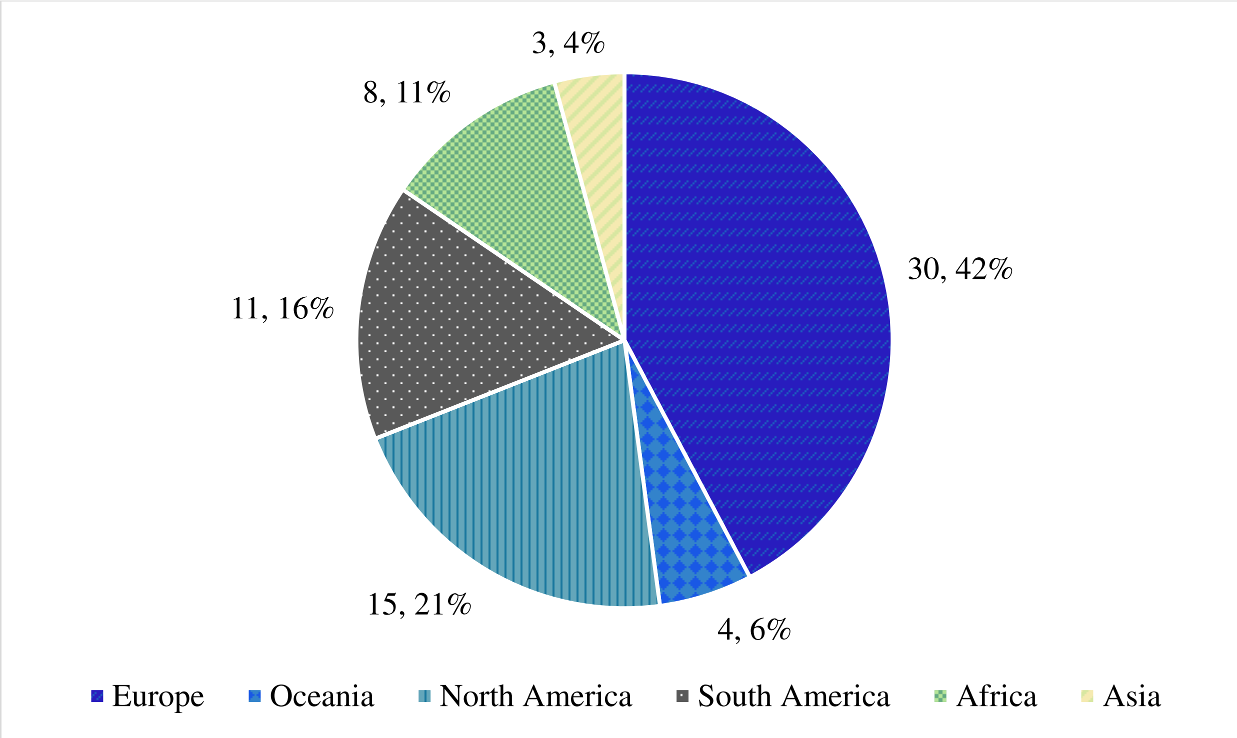


**Figure 1.2.** Number and percentage of studies included in the final scoping review analysis reported by demographic region1 (n = 692). Results are reported as (n, %).
[1]Study locations were categorized into geographic regions as defined by the United States Department of Homeland Security's Office of Immigration Statistics (DHS, 2022).
[2]Two studies took place in more than one region.

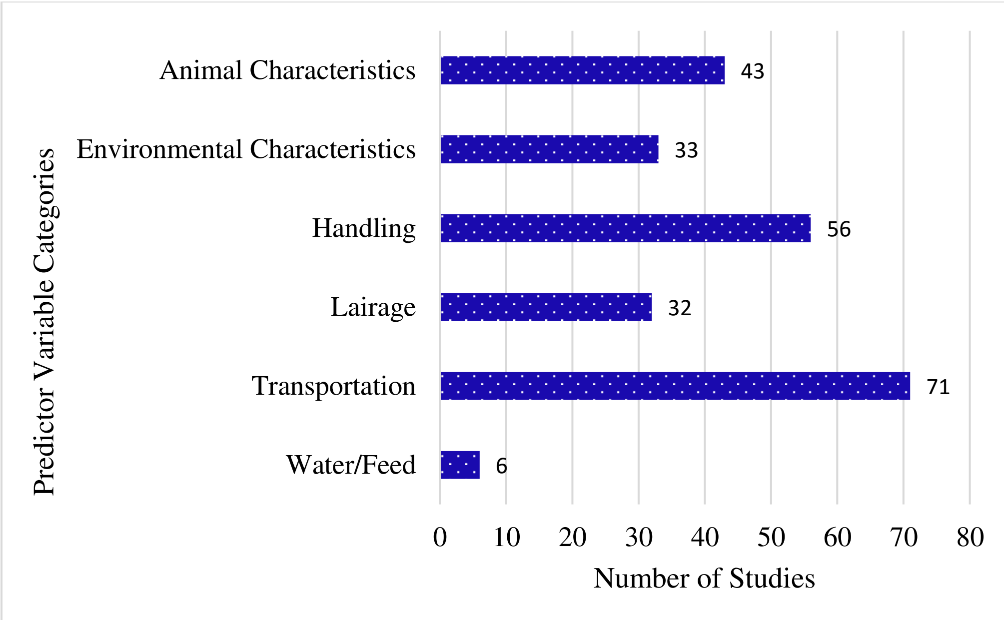


**Figure 1.3.** Number of studies per each predictor category included in the final scoping review analysis out of the total collection ($n$ = 69[1]).
[1]Several studies reported multiple factors within each category, therefore the total number of variables reported in each category represent more than total studies included in the analysis.

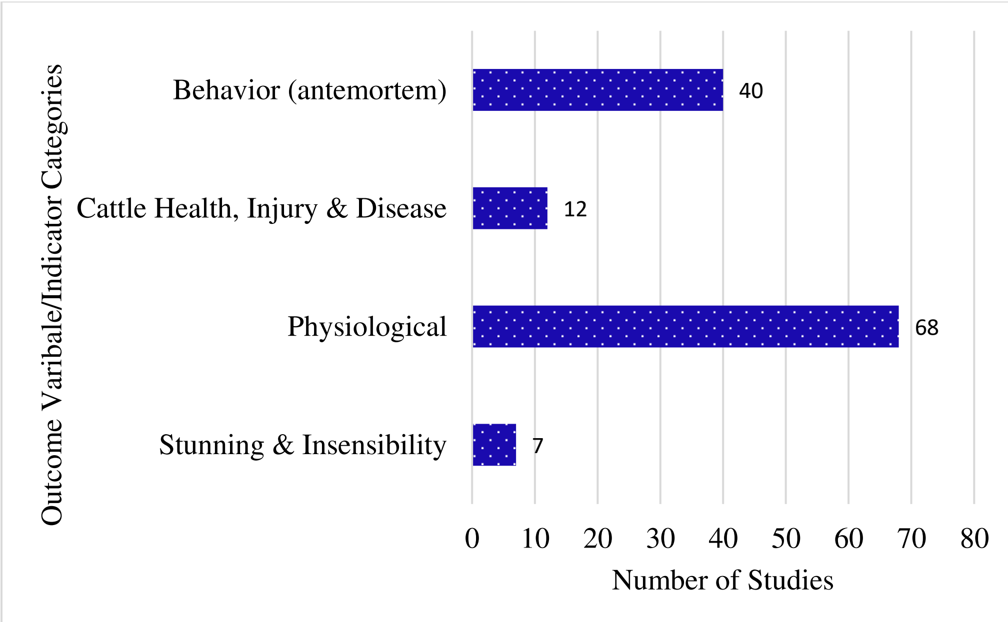


**Figure 1.4.** Number of studies per each outcome category included in the final scoping review analysis out of the total collection ($n$ = 69[1]).
[1]Several studies reported multiple outcomes within each category, therefore the total number of variables reported in each category represent more than total studies included in the analysis.

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# CHAPTER 2: BENCHMARKING CURRENT PRE-SLAUGHTER MANAGEMENT FACTORS, WELFARE INDICATORS, AND MEAT QUALITY OUTCOMES AT COMMERCIAL FED CATTLE PROCESSING FACILITIES IN THE UNITED STATES[2,3]


## Summary

Pre-slaughter management factors and their impacts on cattle welfare and meat quality are well documented in current literature. However, certain management factors related to transportation and lairage are underrepresented. Benchmarking pre-slaughter management factors that can impact welfare and meat quality outcomes will enable the industry to identify areas for improvement. The objective of the current study was to benchmark pre-slaughter management factors for a nationwide sample of commercial fed cattle processing facilities. Five processing facilities in the West, Midwest, and Southwest regions of the United States were sampled from March 2021 to July 2022. Data were collected on a total of *n*=637 slaughter lots representing *n*=87,220 head of cattle. Variables of interest included general cattle characteristics, distance travelled to the plant, truck wait times to unload, environmental conditions, lairage density, and lairage duration. Additionally, mobility was scored using a 4-point locomotion scale (1 being normal, not lame, to 4 being extremely reluctant to move). Carcasses were also observed for bruising using the following scale: no bruises, bruises smaller or larger than a deck of cards, and having multiple bruises. Descriptive statistics were performed on the data at the lot


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[2] Davis, M. K., P. A. Sullivan, A. M. Hess, M. N. Nair, D. F. Mooney, and L. N. Edwards-Callaway. 2023. Benchmarking current pre-slaughter management factors, welfare indicators, and meat quality outcomes at commercial fed cattle processing facilities in the United States. Manuscript submitted for publication.


[3] This project was supported by Agriculture & Food Research Initiative Competitive Grant no. 2019-67015-29578 from the USDA National Institute of Food and Agriculture.

and individual animal level. On average, cattle travelled 155.8 ± 209.6 km (Mean ± SD) from the feedlot to the processing facility and waited 30.3 ± 39.7 minutes to unload. Once in lairage pens, cattle were held for 200.7 ± 195.0 minutes. The mean lairage stocking density was 3.1 ± 2.0 $m^2$ per head. A majority of the cattle scored a mobility score of 1 (*n*=77,645, 91.8%), 7.8% (*n*=6,125) were scored as a 2 and the remaining less than one percent of cattle were scored as either a 3 or 4 (*n*=265). Carcasses with bruises less than or equal to the size of a deck of cards (*n*=22,672, 27.1%) were less frequent than bruises measuring greater than the size of a deck of cards (*n*=34,427, 42.6%). Of carcasses that were bruised, 65.2% (*n*=39,856) had multiple bruises of varying size. This baseline data on pre-slaughter management factors identifies opportunities for improvement in wait times, lairage densities, and factors that cause bruising. Future studies should explore the relationships between these factors and their impacts on welfare and meat quality, report the economic value of these outcomes, and explore industry acceptability and adoptability of optimal welfare factors.

## 1. Introduction

As a complex and critical time in the beef supply chain, the pre-slaughter phase (beginning when animals are loaded for transport at the feedlot and ending with stunning and slaughter) represents a period in which cattle may encounter multiple stressors. In the United States, federal regulations (9 CFR Part 313; United States Electronic Code of Federal Regulations, 2023) require that livestock be handled with “minimum excitement and discomfort”, and this is applicable to all preslaughter processes. Most processing facilities in the United States also adhere to the recommended animal handling guidelines developed by the North American Meat Institute (NAMI, 2021) to promote and monitor animal welfare within their facilities. These guidelines focus on general livestock handling, transportation factors, humane handling and stunning at the plant, as well as providing thresholds of acceptability for several pre-slaughter management factors (e.g., water provision in lairage, or truck waiting time to unload animals at the plant). Many processing companies will set internal standards and thresholds that are stricter than those outlined in the NAMI guidelines to ensure they are meeting and maintaining animal welfare related goals (e.g., JBS USA, 2021). Additionally, consumer pressure for high standards of animal welfare at slaughter facilities (Edwards-Callaway and Calvo-Lorenzo, 2020) is undoubtably a major factor in the global push for additional focus on animal welfare standards and requirements during the pre-slaughter period (Terlouw et al., 2008; Choe, 2018).

As cattle are exposed to the multiple stressors during this pre-slaughter phase (e.g., transportation, commingling, novel environments, interactions with unfamiliar humans) they exhibit physiological and behavioral changes as they attempt to cope with the new environment. The stress induced by these challenges can impact both cattle welfare and meat quality. There are many indicators that can be used to assess cattle welfare during the pre-slaughter period (Davis et al., 2022b). Davis et al. (2022b) reported that physiological parameters, primarily evaluation of metabolites indicative of stress in exsanguination blood or urine, and behavior, most often including the frequency of falls and vocalizations, were the most common welfare parameters evaluated in the reviewed body of literature. Although some of the behavioral parameters cited are currently included in the monitoring of animal handling at plants, some of the physiological parameters are not as commonly integrated into monitoring systems, perhaps due to the complexity, expense, and logistics related to their measurement. Although cattle mobility has garnered increased focus as an important and relevant welfare metric for fed cattle arriving at slaughter facilities (Losada-Espinosa et al., 2018; Edwards-Callaway and Calvo-Lorenzo, 2020; Mijares et al., 2021), mobility as a welfare indicator was not as prevalent in the research included in the Davis et al. (2022b) scoping review. However, cattle mobility has been included in other national benchmarking efforts (e.g., Elanco's Cattle Mobility Assessment Program), and recently added to the National Beef Quality Audit (NBQA; Eastwood et al., 2017) underlining its importance. Additionally, the prevalence of carcass bruising and dark cutting (DC) carcasses across studies is highly variable, yet well researched (Sullivan et al., 2022), and offer insight as significant indicators of welfare. These welfare indicators, among others, serve as useful tools to assess and monitor animal welfare in real-time.

Although great progress has been made in the United States cattle industry to ensure that stress is minimized and positive animal welfare states are promoted during this terminal point in cattle production cycle (NAMI, 2021; NCBA, 2022), it is important to monitor how preslaughter management factors impact cattle welfare and meat quality. A growing body of research on pre-slaughter stress not only in the United States, but globally, articulates the strong relationship between pre-slaughter management factors and animal welfare and meat quality outcomes (Adzitey, 2011; Edwards-Callaway and Calvo-Lorenzo, 2020; Davis et al., 2022b; Sullivan et al., 2022). Factors related to transportation, animal handling, and lairage are consistently among the most commonly researched predictors for welfare outcomes of beef cattle at slaughter (Miranda-de la Lama et al., 2014; Davis et al., 2022b). However, two recent review papers investigating the impacts of pre-slaughter management factors on beef cattle welfare and meat quality concluded that truck waiting time at the plant to unload cattle, lairage duration, and density as influencing factors are underrepresented in current literature despite their relevance to the pre-slaughter period (Davis et al., 2022b; Sullivan et al., 2022). Additionally, some of these factors (e.g., truck waiting time) are included in recommended handling guidelines used by auditing organizations (e.g., Food Safety Net Services; 2023), and processing facilities, emphasizing the need to understand how they may impact welfare and meat quality.

There is little aggregate data at a national scale benchmarking some of these critical pre-slaughter management factors, such as time to unload and lairage density and duration. There have been some national efforts to quantify a selection of preslaughter management factors and cattle welfare and meat quality outcomes. The National Cattlemen's Beef Association (NCBA) NBQA assesses current transportation, mobility and quality characteristics of U.S. cattle on a large scale (Boykin et al., 2017; Eastwood et al., 2017), yet is not animal welfare focused and thus does not offer an in-depth assessment of additional welfare indicators. The NBQAs evaluate the beef industry efforts every five years to improve beef quality, and since the beginning of the quality audits in 1991, significant progress in beef quality has been made and continued progress will hopefully be evident in future nationwide benchmarking efforts. By benchmarking animal welfare throughout the pre-slaughter phase, the industry has the same opportunity to identify needs and make improvements. Understanding how cattle are managed during the pre-slaughter period can provide valuable information to help stakeholders identify potential areas for improvement in addition to demonstrating the progress that has been made in managing cattle during this critical time. Thus, the objective of this study was to benchmark fed cattle pre-slaughter management factors, welfare indicators, and meat quality outcomes at commercial processing facilities in the United States.

## 2. Materials and Methods

*2.1 Ethical Statement*

All animal data was collected via observation or obtained from plant records, and therefore the Colorado State University Animal Care and Use Committee granted this research exempt from IACUC oversight (IACUC Exemption #2019-080-ANSCI).

*2.2 Characteristics of Processing Facilities*

Five United States Department of Agriculture (USDA) inspected commercial processing facilities in the West, Midwest, and Southwest regions of the United States were sampled from March 2021 to July 2022. Data were strategically collected at each facility multiple times throughout the collection process to capture variability in environmental conditions throughout the seasons. Four plants operated two 8-hour production shifts per day averaging approximately 4,000 to 5,000 cattle slaughtered per day with chain speeds ranging from approximately 250 to 390 cattle per hour. One plant operated only one shift per day slaughtering approximately 1,200 cattle per day at a line speed of approximately 150 cattle per hour. In total, data were collected on $n$ = 637 slaughter lots (i.e., groups of cattle originating from the feedlot that will remain a group throughout the process and given a slaughter lot identification number) representing $n$ = 87,220 head of cattle. For plants with two shifts, data were collected from both shifts to capture additional variability (shift 1: $n$ = 248 lots, 48.1%; shift 2: $n$ = 268 lots, 51.9%). Slaughter lots of cattle were tracked, using their individual lot identification number, from arrival at the facility through the slaughter process and both antemortem and postmortem observational measurements were collected.

### *2.3 Cattle Population*

Sex class (steer, heifer, or mixed sex lot), hide color (no black [i.e., all red, white, gray, etc.], all black, > 50% black, ≤ 50% black, or Holstein patterned), and general breed type (predominately *Bos taurus*, *Bos indicus,* or Holstein) were recorded at the lot level. Cattle were considered to have *Bos indicus* influence if more than 25% of the cattle within the lot possessed two or more of the typical breed characteristics (e.g., large droopy ears, excess skin on their dewlap and/or prepuce, or a large hump on their withers). The population of cattle within the lots in this study were predominately > 50% black-hided steers ($n$ = 391 lots, 65.9% and $n$ = 337 lots, 56.4%, respectively) of *Bos taurus* influence ($n$ = 520 lots, 88.7%). The number of animals per lot was recorded during observation and the average live weight for each lot was obtained from plant records when available. The average live weight in this study was 623.6 ± 60.4 kg (Mean ± SD).

### *2.4 Transportation*

The slaughter lots of cattle in this study originated from feedlots where they were loaded onto cattle trucks and transported to their respective processing plant. Feedlots of origin were recorded for each lot and Google Maps (Google LLC, Mountain View, CA, USA) was used to calculate an approximate transport distance (Distance Travelled) between each feedlot and the plant. Multiple routes were often suggested in Google Maps, however the first route recommended, typically representing the shortest distance and/or travel time, was selected. Upon arrival at the plant, each truck's arrival time was recorded and later subtracted from the time the cattle were unloaded (i.e., the time at which the first animal stepped off the truck), to calculate the amount of time cattle waited on the trucks at the plant to unload (truck waiting time). Truck waiting time for each truck within the lot was then averaged to calculate truck waiting time at the lot level. Cattle at each plant were handled and managed by plant employees according to each plant's standard operating procedures beginning at unloading from the trucks.

*2.5 Lairage*

After unloading, cattle were moved to lairage pens. While in the lairage pens at each plant, cattle were provided ad libitum access to water and during the warmer months, sprinklers were turned on at either 26.7 or 32.2°C depending on the plant. One plant had permanent shade cloths above all pens with sprinklers underneath. Most plants had stamped concrete flooring in the handling areas and lairage pens, however, one plant had rubber matted floors in all handling areas and lairage pens except for the unloading docks and scale. Cattle receiving times varied by plant, therefore slaughter lots of cattle were occasionally held overnight in lairage before slaughter the next day.

Prior to being slaughtered, all cattle were inspected by a USDA antemortem livestock inspector. After antemortem inspection, cattle were moved through the drive alleys, into the single-file and up to the center track restrainer where they were rendered insensible using pneumatic captive-bolt stunners and exsanguinated before further processing. One day a week, one plant used a head restrainer for Halal slaughter on lots of Holstein and native cattle. Immediately following the neck cut made by a certified Imam, a captive bolt hand-held stunner was used to render the animal insensible.

2.5.1 Mobility and Heat Stress Scoring

Multiple trained mobility scorers were used throughout the study period. Training videos were used to ensure that scorers received a Kappa coefficient for inter-observer reliability ≥ 0.80 (i.e. strong to almost perfect agreement; McHugh, 2012) compared to a gold standard scorer to qualify as a mobility scorer. Cattle mobility was scored at one time point throughout the pre-slaughter process, typically when in the drive alley as cattle were moved to slaughter after resting time in lairage or when moving between lairage pens during antemortem inspection. Mobility scorers observed cattle from catwalks above the pens or drive alleys when available. A four-point mobility scoring scale (1 being normal with no apparent lameness to 4 being extremely reluctant to move; Table 2.1; NAMI, 2015) created by NAMI was used to score cattle mobility. The number of animals scoring in each category was tallied and then the frequency of each mobility category was calculated per lot. The mobility scorer also recorded hide color and breed type at the lot level, and assessed cattle in lairage for signs of open mouth breathing (OMB) as a measurement of heat stress using an adapted definition developed by Mader et al. (2006; increased respiration and heavy open mouth panting with saliva usually present, and may be accompanied by a protruding tongue and the neck extended forward). For each lot, the total number of cattle exhibiting signs of OMB was recorded. The number of cattle reported as dead or a downer (i.e., arrived dead, died during lairage, or could no longer stand or walk on its own and therefore was euthanized) was also recorded for each lot.

### 2.5.2 Lairage Duration and Density

The total time that cattle spent in pens (Lairage Duration) for each lot was calculated by subtracting the time at which the middle truck of the lot unloaded from the time at which the mobility scorer started mobility scoring that lot of cattle. Lairage Density reported as $m^2$/animal for each lot was calculated by using the total square meters of each pen and dividing by the number of cattle in that pen.

*2.6 Environmental Conditions*

Temperature (°C), humidity (%), precipitation (cm) and wind speed (km/h) were recorded at three time periods during each lot's pre-slaughter process (i.e., the average time of all truck arrival times at the facility, the average time of all unloading times, and the average time of all mobility scoring) using an online commercial weather service (Weather Underground, San Francisco, CA, USA). Environmental conditions were then averaged over the three time points for each lot. Temperature and humidity were then used to calculate a Temperature Humidity Index (THI) score for each lot using the equation $THI = 0.8*T + RH*(T-14.4) + 46.4$ where T is ambient or dry-bulb temperature (°C) and RH is relative humidity expressed as a proportion (LiveCorp and Meat and Livestock Australia, 2023).

*2.7 Bruising and Meat Quality*

2.7.1 Bruise Scoring

Multiple trained bruise scorers were used throughout the study period. In-plant training videos and in-person viewing experiences were used for bruise scoring training to ensure that scorers received a Kappa coefficient for inter-observer reliability of $\geq 0.80$ compared to a gold standard scorer to qualify as a bruise scorer. Bruise scoring occurred on the slaughter line after the hide was removed and prior to carcass splitting at each plant. The bruise scoring system used in this study was adapted from the NBQA bruise scoring system (a ten-point scale visually estimating bruise size, weight, location, and severity). The NBQA's severity categories range from minimal to extreme where the largest bruise size in the minimal category is deemed equivalent to the size of a deck of cards, therefore a deck of cards was used as the threshold in the simplified scoring system in the present study. Three mutually exclusive categories were used to assess carcass bruising in this study; individual carcasses were scored and recorded as either having no bruises, one bruise that was ≤ to the size of a deck of cards, > than the size of a deck of cards, and an additional category recording if there were multiple bruises was also used (the size of the largest bruise was also noted). Within each bruise category, the number of carcasses with a bruise of the specified size was tallied and then the frequency of each category was calculated per lot. This system was used to simplify the assessment of bruises in real-time at large plants with fast chain speeds for hours at a time.

2.7.2 Carcass Characteristics

After carcasses were chilled at each plant for approximately 24 hours postmortem, all standard operating procedures for processing were performed by plant employees. After USDA employees assigned a quality grade (QG) and yield grade (YG) to each carcass, USDA grades, hot carcass weights (HCW), dressing percentages (DP), and number of DC for each lot were obtained from each plant's records. One plant reported dark cutting, quality, and yield grades for each carcass side, therefore in some cases the number of carcasses reported does not equal a whole number; this is evident in Table 2.4. In the event that one carcass side was condemned, then the other carcass side is still represented in the data.

*2.8 Statistical Analysis*

All statistical analyses were performed in SAS 9.4 (SAS institute, Cary, NC). Slaughter lot ($n$ = 637) was used as the observational unit. For all variables of interest, descriptive statistics were summarized at the lot level. Continuous variables were summarized by their minimum, mean, maximum and standard deviation and categorical variables by relative frequency. The relative frequencies for the categorical variables of mobility, downer or dead cattle, OMB, bruising, DC, QG and YG were calculated as a proportion using individual animal data ($n$ = 87,220). Percent within optimal range for the variables truck wait time, lairage density and THI values were also calculated in SAS 9.4 (SAS institute, Cary, NC) using industry guidelines. For truck waiting time, 60 min was used as the maximum optimal threshold as the NAMI Recommended Animal Handling Guidelines and Audit Guide states that the plant will receive full points during the audit if unloading of cattle is started within 60 min of the truck's arrival at the plant (NAMI, 2021). For lairage density, a minimum density of 2.04 $m^2$ was used as the optimal threshold as the average live weight across all lots was 623.6 kg, which was closest to the recommended density (2.04 $m^2$) for a group of animals with an average live weight of 635 kg as also stated in the NAMI Recommended Animal Handling Guidelines and Audit Guide (NAMI, 2021). A THI value below 72 is considered normal with no risk of heat stress in the Australian Veterinary Handbook for Cattle, Sheep and Goats (LiveCorp and Meat and Livestock Australia, 2023), and therefore 72 was used as the maximum threshold for optimal values for THI.

## 3. Results

Within each company and plant, differences in pre-slaughter management, facility design, and data management resulted in inconsistent sample sizes across each variable as noted in the tables throughout. It is also important to consider that the results presented in this study are averages or frequencies from the entirety of the project and span all four seasons, therefore do not represent season specific challenges that may have occurred (e.g., environmental conditions).

### *3.1 Cattle Population*

Detailed cattle population characteristics are presented in Table 2.2. A total of $n$ = 637 slaughter lots representing $n$ = 87,220 head of cattle were sampled in this study. Sex class, breed type and hide color were recorded for 598, 586 and 594 lots, respectively. Over half (56.4%, $n$ =

337) of the lots were steers and 31.9% (*n* = 191) were heifers and the remaining 11.7% (*n* = 70) were lots of mixed sex. A majority of lots (*n* = 520, 88.7%) consisted of cattle with predominantly *Bos taurus* influence and 11.3% (66) of lots consisted of either Holsteins (*n* = 21, 3.6%) or cattle that were ≥ 25% *Bos indicus* influence (*n* = 45, 7.7%). Slaughter lots with no black-hided cattle were infrequent (*n* = 20, 3.4%), whereas lots of cattle with greater than 50% black or all black cattle made up 65.9% (*n* = 391 lots) of the sample population. A little less than one third of the lots consisted of cattle with ≤ 50% black hides (*n* = 162, 27.3%). The number of cattle per slaughter lot averaged 136.9 ± 95.1 (Mean ± SD) with a range of 1 to 929 cattle, and average live weight was 623.6 ± 60.4 kg ranging from 361.5 to 766.4 kg.

### *3.2 Pre-slaughter Management Factors*

#### 3.2.1 Transportation and Lairage

Descriptive statistics on transportation and lairage factors are presented in Table 2.3. Cattle in the present study originated from 148 different feedlots throughout the West, Midwest, and Southwest regions of the United States and travelled on average 155.8 ± 209.6 km (Mean ± SD) to the plant, ranging from 2.7 to 1,332.5 km. Once trucks arrived at each processing facility, trucks waited on average 30.3 ± 39.7 minutes ranging from 0 to 574.2 minutes before unloading their cattle. A total of *n* = 521 (85.8%) slaughter lots of cattle were unloaded within the optimal time limit of 60 min.

In lairage, slaughter lots spent on average 200.7 ± 195.0 minutes waiting before slaughter, and the durations ranged from 4 to 1,071.5 minutes. Lairage density, or sometimes referred to as stocking density, was 3.0 ± 2.0 $m^2$/animal with a range of 0.6 to 31.7 $m^2$/animal, and *n* = 509 (83.6%) slaughter lots of cattle experienced lairage densities within the threshold minimum limit of 2.04 $m^2$/animal.

3.2.2 Environmental Characteristics

THI, precipitation and windspeed descriptive statistics are reported in Table 2.3. Temperature and relative humidity were used to calculate a THI score for each lot which averaged 60.4 ± 13.6 and ranged from 18.9 to 81.5 over all lots in the current study. A total of *n* = 449 (72.2%) of cattle slaughter lots experienced weather conditions with THI values below the optimal maximum threshold of 72. The occurrence of precipitation during data collection dates was low (0.004 ± 0.03 cm) throughout the study, however, wind speed varied from 0 to 56.3 km/h and averaged 18.1 ± 10.1 km/h.

*3.3 Pre-slaughter Management Outcomes*

3.3.1Mobility and Open-Mouth Breathing

All pre-slaughter management outcomes reported as a frequency are presented in Table 2.4. Cattle with a mobility score of 1, or no mobility impairment, was the most common amongst the cattle within the study population (*n* = 77,645, 91.8%), whereas cattle scoring a 2, or exhibiting minor stiffness or a slight limp, were less frequent (*n* = 6,125, 7.8%). Cattle with a mobility score of a 3 or 4 (i.e., extreme stiffness or reluctancy to move) equated to less than 0.4% (*n* = 265) of the study population. Dead or downer cattle were rarely observed in the current study (*n* = 17, 0.03%), as well as signs of OMB (*n* = 41, 0.06%).

3.3.2 Bruising and Carcass Characteristics

Carcasses exhibiting bruising, regardless of size, occurred more frequently (*n* = 57,099, 69.7% of the time) than carcasses with no apparent bruising (*n* = 24,858, 30.3%; Table 2.4). Of carcasses that were bruised, approximately two-thirds (*n* = 39,856, 65.2%) exhibited multiple bruises. Data on the number of DC carcasses within each lot was acquired for 374 of the lots in this study due to differences in company records. Out of those lots, DC occurred in 1.6% (*n* = 873) of carcasses. More than three-quarters of carcasses in the present study received a QG of Choice or higher (*n* = 66,226, 79.2%), 19.0% (*n* = 16,104) and 0.5% (*n* = 475) of carcasses received a QG of Select or Standard, respectively, and the remaining less than 2% (*n* = 1,162.5) of carcasses were placed in the "Other" category (i.e., carcasses were graded as Cutter, Canter, No Roll, etc.). The yield grade of individual carcasses varied from 1 to 5, with a majority of the carcasses grading as either a 2, 3 or 4 (*n* = 78,014, 89.6%), and 6.4% (*n* = 5,169) and 4.0% (*n* = 2,879) grading as a 1 or 5, respectively. Hot carcass weights ranged from 301.6 to 675.7 kg and averaged 396.3 ± 38.5 kg, and DP ranged from 57.9 to 67.7% and averaged 63.6 ± 1.5% (Table 2.5).

## 4. Discussion

Benchmarking this breadth of pre-slaughter management factors, welfare indicators, and meat quality outcomes at this scale, in real-time, has not previously been done or reported for fed cattle in the United States. Although the number of cattle in this study represents a small subset of the number of all cattle commercially processed in the United States each year (0.24% of the approximately 34 million cattle slaughtered in 2022; USDA-NASS, 2023a), the data set still remains representative of the characteristics of fed cattle slaughter in the United States. For example, several variables in the present study reflect current trends in the industry (e.g., breed types and sex class proportions, cattle mobility, and carcass characteristics) and have been reported on a larger scale formerly (i.e., the NBQA; Boykin et al., 2017; Eastwood et al., 2017) with similar cattle population characteristics. Many of the variables in this study, however, (e.g., truck waiting time to unload cattle, lairage duration, and density) have never been benchmarked at this scale previously, or there is limited knowledge of their importance and relationship to both animal welfare and meat quality. Additionally, Texas, Nebraska, Kansas, Oklahoma and California currently represent the top five states for cattle inventory (USDA-NASS, 2023b), all of which are located in the same regions where the plants and feedlots of cattle origin were located in this study.

*4.1 Pre-slaughter Management Factors*

In terms of cattle characteristics, steers continue to make up the majority of cattle slaughtered in federally inspected facilities in the United States, and the frequency reported in the present study similarly reflects that. Steers represented 56.4% (*n* = 337 lots) of cattle slaughtered in the present study, similar to the 2022 USDA slaughter statistics which comprised of 47.0% steers (USDA-NASS, 2023a). Heifers represented 31.9% (*n* = 191) of cattle in the present study, similar to the 2022 livestock slaughter summary reported number of 30.6% (USDA-NASS, 2023a). The values in the livestock report, however, are based on individual animal numbers and not slaughter lots as represented in the current study; in the current study, lots of mixed sex were also recorded and could be one reason why reported frequencies of each sex class differ. Regional and company differences may also contribute to variation in sex class percentages slaughtered.

Greater emphasis has been placed on breed type and primary hide color for several years within the industry (e.g., several certified beef programs that promote beef coming from Angus influenced or black-hided cattle; USDA-AMS, 2023). In the current study, the majority of lots (*n* = 391, 65.9%) consisted of > 50% or all black-hided cattle; in line with the 2016 NBQA data (57.8% black-hided; Eastwood et al., 2017). The percentage of Holstein lots in this study, although, was less than what was reported in the last NBQA (3.5%, *n* = 21 vs 20.4%, respectively; Eastwood et al., 2017). However, a recent similar study reported a slightly less percentage of Holstein cattle lot slaughtered (16%; Lee et al., 2017), suggesting that these differences in hide color and breed type, similar to differences in sex class, are likely due to differences between regions and plant cattle procurement. The *Bos taurus* breed type currently dominates the U.S. cattle industry; past estimates suggest that *Bos indicus* influenced cattle typically only represent approximately 8% of the total U.S. cowherd (Cundiff et al., 2012); similar to the current study findings of 7.7% ($n$ = 45 lots). *Bos indicus* cattle tend to have a shorter, smoother hair coat allowing them to withstand and survive in higher temperatures and humidity (Forbes et al., 1998), and therefore were typically only recorded in data collected from plants in the Southwest region of the United States.

Transportation is a highly researched, reviewed and regulated component of the pre-slaughter period, not only for beef cattle, but for all livestock species (FAO, 2001; Davis et al., 2022b; Hultgren et al., 2022). Transportation represents one of the most stressful events that cattle may experience in their lifetime even in favorable conditions (Kettlewell and Mitchell, 2005; Schuetze et al., 2017), and some cattle may experience transportation for the first time when transported to slaughter depending on their origin or production system. Different components of transportation such as duration, distance travelled, trailer stocking densities, and environmental conditions can be variable and have known impacts on several cattle welfare indicators, and subsequent carcass and meat quality (Tarrant et al., 1992; Gonzalez et al., 2012a; Gonzalez et al., 2012b; Chulayo et al., 2016). In this study, a wide range of transportation distances were recorded (2.7 to 1,332.5 km), consistent with transportation distances reported in the last NBQA (12.9 to 1,400.1 km; Eastwood et al., 2017). Increased distances travelled, (i.e., longer transport durations) may lead to increased risk of injury, dehydration, fatigue, and/or mortality for the cattle being transported (Minka and Ayo, 2007; Schwartzkopf-Genswein et al., 2012; Šímová et al., 2016; NAMI, 2021). While there are currently no regulations regarding transportation distances in the United States, a federal regulation exists restricting the amount of time livestock can be transported without rest, feed, and water. As its name suggests, the Twenty-Eight Hour Law mandates that cattle cannot be transported longer than 28 hours (Twenty-Eight Hour Law, 1994), however, this law is not strictly enforced and is difficult to do so. Although not directly measured in this study, based on the maximum distances travelled, cattle in this study likely were not transported for more than 28 hours. There are a number of factors that could influence where cattle are shipped for slaughter including but not limited to company procurement agreements, location of a plant in the area, cattle type, and scheduling needs. Transportation distances as reported in this study and do not necessarily accurately estimate transportation duration as distance alone does not capture delays during transport or road conditions, all of which may in some capacity have an impact on animal welfare and meat quality (Schwartzkopf-Genswein et al., 2012; Miranda-de la Lama et al., 2014).

To the authors' knowledge, truck wait times to unload cattle at slaughter facilities has not been benchmarked or researched previously despite the existence of current acceptable thresholds for truck wait time in industry audit guides; the NAMI Recommended Animal Handling Guidelines and Audit Guide (NAMI, 2021) requires 60 minutes or less wait time to unload in order to receive full points on this audit criterion. A total of $n$ = 521 (85.8%) of slaughter lots in this study were within this optimal threshold underlining an important success in this sector of the industry. A majority of processing plants have policies in place to minimize wait times at the plant by implementing a scheduling system to coincide with the slaughter schedule and holding pen capacity, nevertheless, delays and various unforeseen events can result in a backlog of trucks waiting to unload. In the current study, the average truck waiting time was approximately 30 minutes, however, the wide range of wait times, with a maximum of 574 minutes (approximately 9 and a half hours) is a concern. This extended time on the truck adds to the aforementioned risks of injury, fatigue, or potentially death from the increased standing time and/or weather-related stress, especially when cattle are without adequate airflow (Minka and Ayo, 2007; Goldhawk, 2014; Schuetze et al., 2017; NAMI, 2021). Additionally, processing companies often have protocols in place that require management personnel to instruct truck drivers to continue driving when wait times to unload at the plant are long and temperatures exceed the company's maximum heat threshold (e.g. 27 °C; personal communication L.N. Edwards-Callaway), however, many drivers do not leave, likely due to risk of missing their opportunity to unload or not wanting to incur additional fuel costs with the added distance.

Lairage duration, or the time that animals spend resting in holding pens at the processing plant prior to being slaughtered, varies from plant to plant. Generally, during this time, antemortem inspection by the Food Safety Inspection Service inspectors occurs; antemortem inspection can take place at different times (i.e., during unloading or immediately before slaughter) depending on the facility. Lairage also allows for the entirety of a slaughter lot of animals to arrive, in most cases, prior to being sent to slaughter together, and allows the animals time to rest following transportation (Warriss et al., 1992; Edwards-Callaway and Calvo-Lorenzo, 2020). Some plants receive cattle the evening prior to the next kill shift to rest in lairage overnight until the morning. Regardless of lairage duration, federal regulations in humane livestock slaughter require that cattle always have access to water, access to feed if held longer than 24 hours, and have sufficient space to lie down when held overnight (Code of Federal Regulations. Humane Slaughter of Livestock., 1979). Surprisingly, there is no industry benchmarking data on current lairage durations at processing plants. Only a few studies have researched the effects of varying lairage durations on fed cattle welfare during the pre-slaughter period at plants in the United States. There is currently contradicting research on the impact of lairage duration on cattle welfare and meat quality outcomes (Ferguson et al., 2007; Teke et al., 2014; Özdemir et al., 2022). For example, one study found that longer lairage durations (58 h vs. 31 h) significantly increased ultimate pH values (Liotta et al., 2007), whereas another study found no effect on ultimate pH for cattle held in short versus long lairage durations (3 h vs. 18 h, respectively; Ferguson et al., 2007). Lairage duration may be a difficult variable to compare across studies as perceptions of “short” versus “long” lairage durations and conditions during lairage vary from study to study that may confound the results. In the current study, a wide range of lairage durations were recorded (4 to 1,071.5 minutes), but the average duration was approximately 200 minutes, or just over three hours. This benchmarking information could be valuable for future analyses exploring how lairage duration impacts fed beef cattle welfare and subsequent carcass quality.

Stocking densities in lairage are largely determined by the NAMI space allowance guidelines as well as by available space for incoming cattle and the need to maintain groups of cattle together without mixing (NAMI, 2021). Both the plant to plant and in-plant variation in lairage stocking density is highly variable. As mentioned earlier, federal regulations in humane livestock slaughter require cattle have sufficient space to lie down when held overnight (Code of Federal Regulations. Humane Slaughter of Livestock., 1979) but is not prescriptive in the exact space needed per animal. The NAMI guidelines also have recommendations for various space allowances for cattle based on average weight, however, this parameter is not included in the audited segment of this tool (NAMI, 2021); space allowance is a secondary audit criterion and is included as essentially a note in the audit. While the average lairage density in the present study was 3.1 $m^2$/animal, (well above the minimum space allowance of 2.04 $m^2$/635 kg. animal in the NAMI guidelines; NAMI, 2021) the minimum reported density of 0.6 $m^2$/animal is over three times less than the minimum recommendation. At this space allowance, water access and room available to lie down is limited, and increased temperature and humidity in the pen microclimate, similar to during transportation, is likely to occur (Weeks, 2008; Goldhawk et al., 2014; Edwards-Callaway and Calvo-Lorenzo, 2020). While the average stocking density of lairage pens is well within regulations and guidelines, and 83.6% ($n$ = 509) of slaughter lots were above the optimal minimum threshold of 2.04 $m^2$/animal, increased attention and focus on ensuring that all stocking densities in lairage are following federal regulations and the NAMI guidelines is critical for allowing cattle the space and opportunity to access water and lie down.

Cattle are highly motivated to seek shade on hot days (Schütz et al., 2008), and not being able to find or use shade on a hot day can impact the animal's biological functioning and ability to express natural behaviors, may cause discomfort or result in a negative affective (mental) state, and ultimately may decrease productivity (Polsky and von Keyserlingk, 2017). In terms of final product quality, chronic and acutely heat stressed cattle have demonstrated decreased carcass weights, fat thickness, meat tenderness, increased muscle pH, and poor meat color (Nardone et al., 2010; Summer et al., 2019). Therefore, implementing a management strategy to decrease the heat load that cattle may endure in lairage pens during the warmer months is critical for overall cattle welfare and ultimate meat quality. The Temperature Humidity Index (THI) provides thresholds of when heat mitigation or applying different handling methods should be implemented to help alleviate heat stress in cattle (Chichester and Mader, 2012; UNL Beef, 2014). The severity of heat stress is related to the ambient temperature and the level of humidity (Gantner et al., 2011), both of which are factored into the equation for calculating a THI value

(THI = 0.8*T + RH*(T-14.4) + 46.4) where T is ambient or dry-bulb temperature (°C) and RH is relative humidity expressed as a proportion; LiveCorp and Meat and Livestock Australia, 2023). The thermal comfort zone for feedlot animals or mature cows generally ranges from -13 °C to 25 °C , and *Bos indicus* cattle can tolerate temperatures above 32 °C (Gantner et al., 2011; Chichester and Mader, 2012). Generally a THI value of < 72 is optimal for cattle to exhibit no signs of heat stress and anything above that value can result in mild to very severe heat stress (LiveCorp and Meat and Livestock Australia, 2023). In the present study, THI values averaged 60.4, and 72.19% (*n* = 449) of cattle slaughter lots experienced weather conditions below the threshold of a THI value of 72 for heat stress symptoms to occur in cattle. However, a maximum value of 81.5 was reached at one point during the duration of the study which is within the range of causing severe stress to cattle (LiveCorp and Meat and Livestock Australia, 2023). In that regard, it is likely that research in this space will evolve and progress as temperatures around the world continue to rise.

Commercial beef slaughter plants across the U.S. have adopted several widely used forms of heat mitigation for cattle in lairage pens. Sprinklers or misters and shade are the most widely used forms of heat mitigations, however, several other techniques such as the use of fans or the use of one technique in conjunction with another are common factors among slaughter facilities (Davis et al., 2022a). Wind speed and precipitation, or nature's natural heat mitigation, act similarly to fans or sprinklers that may be added to lairage pens to alleviate heat stress. Heat mitigation via sprinklers allow for soaking of cattle hair coats to completely wet the cow, while fans facilitate air movement and increase convection to decrease respiration rates and overall body temperatures (Armstrong, 1994; West, 2003; Polsky and von Keyserlingk, 2017). Therefore, additional natural environmental conditions (i.e., wind speed and precipitation) that could impact heat stress were included in this benchmarking data. Precipitation throughout the data collection was low, however, averaging 0.004 cm. Conversely, increased wind speeds were recorded more frequently with an average of 18.1 km/h and a maximum speed of 56.3 km/h at one time point, both of which surpass the recommended air circulation speeds of 3.5 to 5 mph (5.6 to 8.0 km/h) to increase convective heat transfer and enhance evaporation in resting, feeding and holding areas for cattle (McFarland, 2022). However, windspeeds and precipitation in this study represent an average across all plants throughout all seasons, and therefore some plants may have experienced little to no wind at all. Additionally, low prevalence of precipitation reported in this study furthers the need for added, maintained, and managed sprinkler systems in lairage pens to combat added heat stress or used as preventative measures.

*4.2 Pre-slaughter Management Outcomes*

Open mouth breathing or a high respiration rate, typically seen as a response to heat stress, has been researched extensively in feedlot and dairy settings (Mader et al., 2006; Hagenmaier et al., 2016; Unruh et al., 2017; Ruban et al., 2020), however, is not commonly measured in lairage during the pre-slaughter phase for beef cattle and therefore offers an opportunity to be considered in future studies. A recent scoping review by Davis et al., (2022b) identified only one study researching OMB during lairage and these authors reported no cattle exhibiting signs of OMB during lairage (Hagenmaier et al., 2017). Similarly, in the current study, only 0.06% ($n$ = 41) of the cattle exhibited OMB. OMB may not be readily observed when animals are being actively handled or when they are not settled (i.e., moving around) during lairage. The authors of this present study suggest exploring other methods to capture OMB either during a time when most animals in a holding pen are resting or using video recording, so that observers are not present.

The beef industry in recent years has pursued an increased focus on the mobility of finished cattle as it is not only a welfare concern, but can impact overall efficiency (Edwards-Callaway et al., 2017). Elanco's Cattle Mobility Assessment Program database dates back to 2016 with historical data representing over 12 million head of cattle to serve as mobility benchmarking information for the industry. Additionally, the NBQA has recently added mobility scoring to their most recently published (2016) and future quality audits (Eastwood et al., 2017) highlighting the importance the industry places on monitoring mobility. Mobility issues in finished cattle are multifactorial and could be impacted by sex, body weight, days on feed, increased THI, handling practices particularly during loading and unloading for transport, and transportation practices or conditions (Gonzalez et al., 2012a; Edwards-Callaway et al., 2017; Lee et al., 2018; Mijares et al., 2021). Just over 91% ($n$ = 77,645) of cattle in the present study showed no signs of impaired mobility, a small decrease from the previous NBQA's 96.8% (Eastwood et al., 2017). On the contrary, Mijares et al. (2021) reported considerably lower frequency of normal mobility (74.6%). However the Mijares et al. (2021) study was conducted during the COVID-19 pandemic recovery with the intention of characterizing the impacts that some of the repercussions of the pandemic had on this sector of the supply chain. The mobility scoring system used in the Elanco Cattle Mobility Assessment Program, the NBQAs, and by Mijares et al. (2021) was the same as the one used in the current study, however, the location where cattle were scored differed. For example, mobility scores in the current study were recorded as cattle were either moved between holding pens during antemortem inspection at the plant, or as they were moved into the drive alley just prior to slaughter, and therefore were not scored immediately following transportation such as in the NBQA. There was a small percentage of animals (0.002%, $n$ = 1) that had severely impaired mobility (i.e., a score of 4). Generally, an animal that scores a 4 and is immobile will be euthanized as it will not be capable of moving through the handling facility to be slaughtered. The percentage of dead and downer (or non-ambulatory) cattle was low (0.03%, $n$ = 17)), similar to previously published data from other cattle transportation studies (e.g., 0.022% reported as non-ambulatory and 0.011% dying during transportation; (Gonzalez et al., 2012a). Continuing to improve efforts with animal handling, transportation, and identification of animals unfit for transport can further progress animal welfare and comfort in this sector, decrease the overall number of dead or downer cattle, improve mobility of cattle upon arrival at the slaughter facility, and allow for efficiency at the plant.

Carcass bruising as a postmortem welfare outcome serves as an indicator of a variety of potential issues that may have occurred during the pre-slaughter period including inappropriate handling, rough transportation, or the presence of cattle with horns (Warriss et al., 1995; Huertas et al., 2010; Strappini et al., 2010; Kenny and Tarrant, 1987; Jarvis et al., 1995; Hoffman et al., 1998; Mendonça et al., 2019). Economic loss due to carcass bruising is also a major concern as an estimated $35 million is lost annually in the U.S. from trimming or cutting out bruises on the slaughter line that result in carcass downgrades (Gallo et al., 1999; Lee et al., 2017). The 2016 NBQA reported that approximately 38.9% of fed steer and heifer carcasses had at least one bruise (Eastwood et al., 2017), whereas 69.7% ($n$ = 57,099) of carcasses in the present study were scored as having at least one bruise. The bruise scoring system in the present study was based strictly on size (i.e., length of the bruise), whereas the NBQA uses a scoring system based on increasing size for the first three categories and then increasing weight for the remaining seven categories. The differences between the bruise scoring systems used could have contributed to differences in reported bruising prevalence between studies. For instance, when data collectors are required to make finer discriminations (i.e., choose between more options such as in the 10-point bruise scoring scale used in the NBQA) reliability is therefore much more difficult to obtain (McHugh, 2012). The prevalence of bruising is also highly dependent on geographic location as well, as studies in Chile, the United Kingdom, and Uruguay have reported a wide range of carcass bruising prevalence(12.3%, 97%, and 60%, respectively; Jarvis et al., 1995; Strappini et al., 2010; Huertas et al., 2015). The prevalence of carcass bruising is increasing in recent years (Eastwood et al., 2017), and further identification and management of factors causing bruising is pertinent to the reduction of this welfare and quality issue.

Beef classified as DC is characterized by a darker color and is viewed as unfavorable to consumers (Ponnampalam et al., 2017). This occurs when cattle experience high metabolic demand (e.g., from pre-slaughter stress) and depletion of muscle glycogen stores, which limits the postmortem pH decline, resulting in a higher ultimate muscle pH and a darker, purplish-red muscle color (Lister, 1988). A reduction in DC carcasses serves as an economic benefit as DC is estimated to cost the United States beef industry nearly $170 million annually (Underwood et al., 2007). The percentage of DC carcasses in the present study (1.6%, $n = 873$) is comparable to the 1.9% reported in the previous NBQA (Eastwood et al., 2017). Globally, the prevalence of DC reported across studies is variable and greater than that reported in the current study with studies from Mexico and Australia reporting DC prevalence of 2.8% (Pérez-Linares et al., 2015) and 39% (Steel et al., 2021), respectively. The impacts on DC beef are multifactorial and vary based on characteristics of the animal, production system, management practices, environmental factors, location, or the various identified thresholds for classifying dark cutting throughout literature (Sullivan et al., 2022).

Additional carcass characteristics such as YG and HCW reported in this present benchmarking study are in line with industry averages reported in the previous NBQA (Boykin et al., 2017). Additionally, an average DP of 63.6% across all lots is similar to the average DP of 63% for beef in the United States (Campbell, 2022). The majority of the carcasses in the present study were categorized as Choice (70.6%, $n$ = 59,338), highly similar to the previous NBQA's findings of 68.8% (Boykin et al., 2017). Quality grade has the potential to be affected by pre-slaughter stress as lean color is a factor considered in an overall QG, and as mentioned earlier with DC, lean muscle color can potentially be affected by pre-slaughter stress.

## 5. Conclusions

With the continued focus, research, and auditing of beef cattle during the pre-slaughter period, the industry has continued to advance and promote animal welfare focused practices. In an effort to report industry progress and identify areas needing further attention, the objective of this study was to benchmark pre-slaughter management factors, welfare indicators, and meat quality outcomes for fed beef cattle at five commercial processing facilities in the United States. In the present data, average truck waiting times to unload were below the recommended 60 minutes, and average lairage densities were well above the minimum recommendations outlined in the aforementioned NAMI guidelines (NAMI, 2021). Moreover, more than 90% of cattle had normal mobility. While it is important to highlight successes in this sector of the beef industry, it is also necessary to identify areas needing further attention. Maximum truck waiting times to unload at the plant, lairage density minimums, and the percentage of bruised carcasses, all of which are multifactorial in nature will require significantly more research and technological advancements to overcome. In summary, benchmarking pre-slaughter management factors and subsequent welfare and meat quality outcomes for fed beef cattle in the United States is crucial to determine if the industry is meeting the standards and regulations set by governing bodies, identify areas where improvements have been made, and identify specific areas that may require further research and attention.  Furthermore, this data set can be used to investigate these relationships, report the economic value of specific welfare outcomes, and ultimately survey industry acceptability and adoptability of identified optimal management factors to improve welfare and meat quality within this sector of the beef supply chain.

**Tables**

**Table 2.1.** North American Meat Institute (NAMI, 2015) mobility scoring system for finished cattle.

| Mobility Score | Definition |
|---|---|
| **1** | Normal, walks easily, no apparent lameness, no change in gait. |
| **2** | Exhibits minor stiffness, shortness of stride, slight limp, keeps up with normal cattle. |
| **3** | Exhibits obvious stiffness, difficulty taking steps, obvious limp, obvious discomfort, lags behind normal cattle. |
| **4** | Extremely reluctant to move even when encouraged, statue-like. |

**Table 2.2.** Characteristics of fed cattle slaughter lots during the present study.

| Characteristic | *n* | Frequency (%) |
|---|---|---|
| **Sex Class (*n* = 598)** | | |
| *Steers* | 337 | 56.4 |
| *Heifers* | 191 | 31.9 |
| *Mix*[1] | 70 | 11.7 |
| **Breed Type[2] (*n* = 586)** | | |
| *Bos taurus or < 25% Bos indicus Influence* | 520 | 88.7 |
| *≥ 25% Bos indicus Influence* | 45 | 7.7 |
| *Holstein* | 21 | 3.6 |
| **Hide Color (*n* = 594)** | | |
| *No Black* | 20 | 3.4 |
| *All Black* | 36 | 6.1 |
| *> 50% Black* | 355 | 59.8 |
| *≤ 50% Black* | 162 | 27.3 |
| *Holstein* | 21 | 3.5 |

[1]Slaughter lots consisting of both steers and heifers were considered a mix lot.

[2]Cattle were considered to have *Bos indicus* influence if more than 25% of the cattle within the lot possessed two or more of the typical breed characteristics (e.g., large, droopy ears, excess skin on their dewlap and/or prepuce, or a large hump on their withers).

**Table 2.3.** Potential risk factors during pre-slaughter transportation and lairage associated with animal welfare and meat quality outcomes at the slaughter lot level.

| Variable | *n* | Minimum | Mean | Maximum | SD | *n*,% Optimal[3] |
|---|---|---|---|---|---|---|
| **Transportation** | | | | | | |
| *Distance Travelled (km)* | 604 | 2.7 | 155.8 | 1,332.5 | 209.6 | – |
| *Truck Waiting Time (min)* | 607 | 0.0 | 30.3 | 574.2 | 39.7 | 521, 85.8 |
| **Lairage** | | | | | | |
| *Lairage Duration (min)* | 572 | 4.0 | 200.7 | 1,071.5 | 195.0 | – |
| *Lairage Density ($m^2$/animal)* | 609 | 0.6 | 3.1 | 31.7 | 2.0 | 509, 83.6 |
| **Environmental Characteristics** | | | | | | |
| *Temperature Humidity index (THI)*[1, 2] | 622 | 18.9 | 60.4 | 81.5 | 13.6 | 449, 72.2 |
| *Precipitation*[2] *(cm)* | 622 | 0.0 | 0.004 | 0.25 | 0.03 | – |
| *Wind Speed*[2] *(km/h)* | 622 | 0.0 | 18.1 | 56.3 | 10.1 | – |

[1]THI score was calculated using the equation: THI = 0.8*T + RH*(T-14.4) + 46.4 where T is ambient or dry-bulb temperature (°C) and RH is relative humidity expressed as a proportion (LiveCorp and Meat and Livestock Australia, 2023).
[2]Temperature and humidity used to calculate THI, precipitation and wind speed were recorded using an online commercial weather service (Weather Underground, San Francisco, CA, USA).
[3]Percent within optimal range is based on thresholds stated in the NAMI Recommended Animal Handling Guidelines and Audit Guide (NAMI, 2021) and the Australian Veterinary Handbook for Cattle, Sheep and Goats (LiveCorp and Meat and Livestock Australia, 2023). The threshold used for truck waiting time was 60 minutes or less, the threshold used for lairage density was 2.04 $m^2$ or above, and the threshold for THI value used was 72 or below.

**Table 2.4.** Cattle welfare and meat quality outcome variables associated with pre-slaughter management risk factors.

| Variable | *n Lots* | *n* Animals/Carcasses | Frequency (%) |
|---|---|---|---|
| **Mobility Scores[1]** | 614 | | |
| *1* | | 77,645 | 91.8 |
| *2* | | 6,125 | 7.8 |
| *3* | | 264 | 0.3 |
| *4* | | 1 | 0.002 |
| **Downer or Dead Cattle** | 614 | 17 | 0.03 |
| **Open Mouth Breathing[2] (OMB)** | 614 | 41 | 0.06 |
| **Bruise Scores[3]** | 612 | | |
| *None* | | 24,858 | 30.3 |
| *≤ Deck of Cards* | | 22,672 | 27.1 |
| *> Deck of Cards* | | 34,427 | 42.6 |
| *Multiple[4]* | | 39.856 | 65.2 |
| **Dark Cutting[5]** | 374 | 873 | 1.6 |
| **Quality Grades[5]** | 618 | | |
| *Prime* | | 6,888 | 8.6 |
| *Choice* | | 59,338 | 70.6 |
| *Select* | | 16,104 | 19.0 |
| *Standard* | | 475 | 0.5 |
| *Other* | | 1,162.5 | 1.3 |
| **Yield Grades[5]** | 618 | | |
| *1* | | 5,169 | 6.4 |
| *2* | | 28,946 | 33.0 |
| *3* | | 37,121 | 42.3 |
| *4* | | 11,947 | 14.3 |
| *5* | | 2,879 | 4.0 |

[1]Mobility scores are defined as: 1 = normal, walks easily, no apparent lameness; 2 = exhibits minor stiffness, shortness of stride, slight limp, keeps up with normal cattle; 3 = exhibits obvious stiffness, difficulty taking steps, obvious limp, obvious discomfort, lags behind normal cattle; and 4 = extremely reluctant to move even when encouraged by a handler, statue-like (NAMI, 2015).

[2]Open mouth breathing (OMB) was assessed by recording the number of cattle in each lot exhibiting signs of OMB. A definition adapted from Mader et al. (2006; increased respiration and heavy open mouth panting with saliva usually present and may be accompanied by a protruding tongue and the neck extended forward) was used.

[3]Individual carcasses were scored and recorded as either having no bruises (none), one bruise that was ≤ to the size of a deck of cards, one bruise that was > than the size of a deck of cards,

and if it had multiple bruises where the size of the largest bruise was noted. Scores were then summarized at the lot level.

[4]Multiple is expressed as the proportion of carcasses scored as either ≤ a deck of cards or > a deck of cards that were also scored as having multiple bruises.

[5]One plant reported dark cutting, quality, and yield grades for each carcass side. In the event that one carcass side was condemned, then the other carcass side is still represented in the data.

**Table 2.5.** Cattle meat quality outcome variables associated with pre-slaughter management risk factors.

| Variable | *n* | Minimum | Mean | Maximum | SD |
|---|---|---|---|---|---|
| *Hot Carcass Weight (kg)* | 612 | 301.6 | 396.3 | 675.7 | 38.5 |
| *Dressing Percentage (%)* | 610 | 57.9 | 63.6 | 67.7 | 1.5 |

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# CHAPTER 3: THE INFLUENCE OF PRE-SLAUGHTER MANAGEMENT FACTORS ON WELFARE AND MEAT QUALITY OUTCOMES IN FED BEEF CATTLE IN THE UNITED STATES


**Summary**

One sector of the beef industry that continues to garner attention is the pre-slaughter phase. Cattle are transported from their place of origin to a slaughter facility, experiencing transportation, lairage, environmental factors, novel environments, and several other challenges. A large body of literature has focused on understanding how and why the pre-slaughter phase impacts cattle welfare and meat quality, however, research encompassing several pre-slaughter management factors and their impacts on specific welfare and meat quality outcomes is limited. The objective of this study was to assess the effects of pre-slaughter management factors on welfare and meat quality outcomes in fed beef cattle in the United States. Transportation factors, environmental characteristics, lairage factors, cattle characteristics and several meat quality variables were collected from five federally inspected commercial processing facilities in the United States. After excluding slaughter lots that included <75% complete data sets, a total of 619 slaughter lots representing 84,508 head of cattle were used for further analysis. Predictor variables of interest included processing plant, cattle breed, sex class, operation shift at the plant, distance travelled to the plant, truck waiting time to unload at the plant, lairage duration and space allowance, temperature humidity index (THI), and windspeed. Outcome variables of interest included cattle mobility, carcass bruising, dark cutting (DC), quality grades (QG), and hot carcass weights (HCW). All statistical analysis was performed using SAS 9.4. Logistic and linear regressions were used to analyze the associations between the predictor and outcomes variables of interest. Increases in distance travelled (1 km; OR, CI; 1.001, 1.000 – 1.001) and truck waiting time (1 minute; OR, CI; 1.003, 1.001 – 1.004) were associated with increased odds of mobility impairment. Decreases in distance travelled were associated with increased odds of carcass bruising (10 km; OR, CI; 0.997, 0.996 – 0.998). Cattle in lairage for longer duration were associated with decreased hot carcass weights ($P < 0.0367$) and increased odds of dark cutting (60 minutes; OR, CI; 1.034, 1.001 – 1.068). This research has the potential to aid in informed decision-making regarding cattle management in the pre-slaughter phase to work towards continued progress on challenges with animal welfare and meat quality within this sector.

## 1. Introduction

The management of cattle during the pre-slaughter period has become an industry sector facing increased scrutiny not only among consumers (Wigham et al., 2018), but among the stakeholders and members of the supply chain alike due to its cumulative effects on animal welfare and meat quality (Edwards-Callaway and Calvo-Lorenzo, 2020). The pre-slaughter period is defined as the time when fed cattle leave their place of origin (e.g., a feedlot) and are transported to a slaughter facility. During this period, cattle are subjected to variety of stressors including increased handling, novel environments, and mixing with unfamiliar cattle that undoubtably have a multiplicative impact on an animal's welfare and subsequent meat quality (Warriss, 1990; Wigham et al., 2018). The intensity and duration of the pre-slaughter process and its associated stressful events vary based on location (i.e., differences in environmental characteristics), processing facility management (i.e., differing protocols), and an individual animal's response to stress (Ferguson and Warner, 2008). It is well documented that levels of fear, stress, and discomfort that animals may experience during the pre-slaughter process can negatively affect their overall welfare state and final product quality (Ferguson and Warner, 2008; Cockram, 2017; Edwards-Callaway and Calvo-Lorenzo, 2020). Additionally, there is significant financial loss from meat quality defects such as bruising or dark cutting in beef that occur during the pre-slaughter period that decrease the yield or profit of saleable meat (Warriss, 1990; Kline et al., 2020).

Ensuring and measuring animal welfare at slaughter are necessary components of management. For example, federal regulations (Code of Federal Regulations. Humane Slaughter of Livestock., 1979; Twenty-Eight Hour Law, 1994) and industry guidelines were created to ensure proper care throughout the slaughter process. There are several ways to measure animal welfare at slaughter. Depending on the goal, it can be more complex measurements (i.e., measuring physiological indicators in exsanguination blood) or more simple measurements (i.e., observing animal handling, behavior, or signs of injury or disease) can be taken (Davis et al., 2022). One commonly measured indicator of pre-slaughter welfare is mobility scoring using the North American Meat Institute's (NAMI) scoring system (NAMI. North American Meat Institute Scoring System, 2015). Cattle mobility is an important production and welfare issue with pre-identified risk factors occurring during the pre-slaughter phase such as increased temperatures and heat stress (González et al., 2012a; Lee et al., 2018), heavier body weights and handling practices at slaughter (Edwards-Callaway et al., 2017), and transportation conditions (González et al., 2012a). Scoring mobility has also recently been added to the National Beef Quality Audits (NBQA) that occur every few years.

Meat quality issues discovered postmortem (e.g., carcass bruising and dark cutting) can provide some insight into the animal's welfare state antemortem. In the United States, the 2016 National Beef Quality Audit (NBQA) reported that 38.9% of fed steers and heifers, 42.9% of bulls, and 64.1% of cows were bruised (Eastwood et al., 2017), and an additional benchmarking study found bruise prevalence of 69.7% in fed cattle (Davis et al., 2023). With carcass bruising costing the U.S. beef industry an estimated $35 million each year (Lee et al., 2017), there is a significant need for continued research on bruise risk factors. Dark cutting is another quality issue stemming from chronic stressful events antemortem with can result in depletion of muscle glycogen, in turn leading to lead to high ultimate pH of meat (Scanga et al., 1998; Ponnampalam et al., 2017). The reason for dark cutting is multifactorial and various stressors such as feed and water deprivation, transportation, and lairage conditions may impact an individual animal's responses to stress, postmortem metabolism, and final product quality (Ponnampalam et al., 2017; Sullivan et al., 2022). Dark cutting beef is not only unfavorable to consumers due to its darker appearance (Ponnampalam et al., 2017), but it also has a shortened shelf-life (Newton and Gill, 1981), and is discounted and downgraded in terms of quality grade accounting for nearly $170 million in losses annually for the U.S. beef industry (Underwood et al., 2007). Findings from Boykin et al., (2017) in the 2016 NBQA, and the benchmarking paper by Davis et al., (2023), identified that 1.9% and 1.6%, respectively, of fed cattle carcasses exhibited signs of dark cutting. Pre-slaughter stressors such as fasting, transportation, unfamiliar environments also have significant impacts on additional carcass characteristics such as hot carcass weights and quality grades (Smith et al., 1982; Warriss, 1990), likely with similar financial losses for the industry.

Several recent review papers have indicated the need for continued research in the pre-slaughter period focusing specifically on transportation, lairage, and environmental factors due to their cumulative effects on cattle welfare and final product quality (Schwartzkopf-Genswein et al., 2012; Tucker et al., 2015; Schwartzkopf-Genswein et al., 2016; Davis et al., 2022; Sullivan et al., 2022). There is far less research on the effects of pre-slaughter period on cattle welfare and meat quality following transportation to the plant (i.e., truck waiting time to unload at the plant or animal space allowance in lairage and lairage duration) compared to during transportation. Additionally, there is a limited body of research that provides a holistic overview of animal welfare and meat quality outcomes impacted by several varying pre-slaughter management factors. Addressing these gaps in research knowledge will ultimately lead to optimized transportation and management practices and guidelines that are acceptable to both industry stakeholders and consumers alike.

As evident from benchmarking and auditing efforts and continued research that have improved quality and animal handling, this sector of the beef industry has been successful in improving both cattle welfare at slaughter and ultimate meat quality. However, there are opportunities for improvement to continue identifying best management practices during the preslaughter period. The effects of several critical pre-slaughter management factors on animal welfare, meat quality, and economic performance outcomes for fed beef cattle have not yet been thoroughly quantified in the United States. With approximately 34.3 million cattle commercially slaughtered in 2022 (USDA-NASS, 2023), small changes in the pre-slaughter process through improved management practices or animal handling has the potential to impact the welfare of millions of cattle in the future. Research identifying more specific relationships between pre-slaughter management practices and fed beef cattle welfare and meat quality outcomes is warranted. Therefore, the objective of this study was to assess the effects of pre-slaughter management factors on welfare and meat quality outcomes in fed beef cattle in the United States.

## 2. Materials and Methods

*2.1 Ethical Statement*

This research was granted an exemption (IACUC Exemption #2019-080-ANSCI) by the Colorado State University Animal Care and Use Committee as all animal data collected was observational.

*2.2 Data Collection*

This article is an extension of a previously published manuscript by Davis et al., (2023).

2.2.1 Processing Facility Characteristics

Data were collected from five federally inspected commercial processing facilities in the West, Southwest and Midwest regions of the United States from March 2021 to July 2022. Data were recorded for a total of 637 slaughter lots consisting of 87,220 total head of cattle. Sex class, general breed type, number of cattle, and average live weight for each lot was obtained from the plant while on-site. Four of the processing plants operated two 8-hour shifts per day and slaughtered approximately 4,000-5,000 cattle per day, while the fifth plant operated one shift per day and slaughtered approximately 1,200 cattle per day. Lairage pens at each plant provided ad libitum access to water for cattle, and sprinklers were used as heat mitigation by all plants during the warmer months. While most plants had stamped concrete flooring in the handling areas and holding pens, one plant added rubber mats to all handling areas and holding pens except for on the scale and unloading docks. Receiving times for cattle at the plant varied from plant to plant, and slaughter lots of cattle were occasionally held overnight. Slaughter lots of cattle were tracked throughout the pre-slaughter and slaughter process to obtain ante- and postmortem observational measurements.

2.2.2 Cattle Population and Pre-slaughter Management Factors

Sex class, breed type (*Bos taurus*, *Bos indicus,* or Holstein), number of cattle, and average live weight for each lot was obtained from the plant while on-site. Cattle were recorded as *Bos indicus* if greater than 25% of the cattle within the lot has two or more breed characteristics consistent with *Bos indicus* (e.g., a large hump on their withers, excess skin on their dewlap and/or prepuce, or large droopy ears). Other cattle not possessing the characteristics of *Bos indicus* were either categorized as *Bos taurus or* were clearly Holsteins. Live weight was either provided by the plant or calculated using the truck net weights and number of cattle in each truck. In brief from Davis et al., (2023), the cattle population was predominately black-hided *Bos taurus* steers ($n$ = 391 lots, 65.9% black-hided; $n$ = 520 lots, 88.7% *Bos taurus*; and $n$ = 337 lots, 56.4% steers, respectively). The number of cattle per lot in the previous study averaged 136.9 ± 95.1 (Mean ± SD), and live weight averaged 623.6 ± 60.4 kg.

Transport distance from to the plant for each lot was calculated using Google Maps (Google LLC, Mountain View, CA, USA). Once trucks arrived at a processing facility, each truck's individual arrival time was recorded to calculate truck waiting time before unloading cattle at the facility; these times were then averaged for each lot. Environmental conditions (Temperature; °C, humidity; %, precipitation; cm, and wind speed; km/h) were recorded at three time points for each lot gathered from an online weather service (Weather Underground, San Francisco, CA, USA) and then averaged per lot. Temperature and Humidity were then later used to calculate a Temperature Humidity Index (THI) score for each lot using the equation $THI = 0.8*T + RH*(T-14.4) + 46.4$ where T is ambient or dry-bulb temperature in °C, and relative humidity (RH) is expressed as a proportion (LiveCorp and Meat and Livestock Australia, 2023).

2.2.3 Pre-slaughter Management Outcomes

While in lairage, cattle mobility was scored by trained scorers either on a catwalk or in an alley way as cattle were moved from lairage pens to slaughter. Mobility scores were given for each individual animal using the North American Meat Institute (NAMI) cattle mobility scoring scale (1: no apparent lameness, normal, walks easily; 2: exhibits minor stiffness, shortness of stride, slight limp, keeps up with normal cattle; 3: exhibits obvious stiffness, difficulty taking steps, obvious limp, obvious discomfort, lags behind normal cattle; and 4: extremely reluctant to move, statue-like; NAMI. North American Meat Institute Scoring System, 2015). The frequency of each mobility score category was then calculated per lot. The total time that cattle slaughter lots spent in lairage (subtracting the time at which the lot was unloaded at the facility from the time the lot was moved for slaughter), and the lairage stocking densities ($m^2$/animal was calculated using the total square meters of the pen and the number of animals in each pen) were also recorded. Postmortem, individual carcasses were bruise scored by trained scorers immediately following hide removal using an adapted version of the National Beef Quality Audit (NBQA) bruise scoring system (Eastwood et al., 2017) using three mutually exclusive categories (i.e., carcasses were scored as either having no bruises, one bruise that was ≤ to the size of a deck of cards, or one bruise that was > than the size of a deck of cards), and scorers could also report if a carcass had multiple bruises and the assigned bruise category was determine by the size of the largest bruise on the carcass in this case. The frequency of each bruise category was then calculated per lot. After carcasses were chilled for 24 hours, carcass characteristics including quality grade (QG), number of dark cutting (DC) carcasses, and hot carcass weights (HCW) for each lot were later obtained from each plant's records. One plant reported carcass characteristics for each carcass side, however, if one carcass side was condemned, then the other carcass side was still represented in the data set.

### *2.3 Statistical Analysis*

All statistical analyses were performed in SAS 9.4 (SAS institute, Cary, NC). Descriptive statistics for the full data set of 637 slaughter lots can be found in (Davis et al., 2023).

#### 2.3.1 Regression Analysis and Model Selection

Prior to model selection, slaughter lots of data with no response variables or lots with < 75% of predictor variables present were excluded from further analysis resulting in a total of 619 slaughter lots (the number of slaughter lots for each variable varies based on missing data) consisting of 84,508 head of cattle for data analysis. The descriptive statistics for this subset of lots for the predictor and outcome variables of interest used for regression analyses are presented in Tables 3.1, 3.2 and 3.3. Predictor variables of interest included: plant, breed, sex class, operation shift at the plant (i.e., 1 or 2), distance travelled when transported from the feedlot to the plant, truck waiting time to unload cattle at the plant, lairage duration, space allowance in lairage, THI, and wind speed. Primary response variables of interest included mobility, bruising, DC, QG, and HCW. Bruise scoring categories were collapsed into a binary variable (Not Bruised/Bruised). Regression analyses were used to assess relationships between the predictor variables of interest (pre-slaughter management factors and animal characteristics) and the response variables of interest (mobility, bruising, DC, QG, and HCW) that were chosen for analysis based on their known relationships with pre-slaughter stress. Bruising and DC were analyzed using binary logistic regressions (Bruised/Not Bruised; Dark Cutter/Not a Dark Cutter with Bruised and Dark Cutter as the event; PROC LOGISTIC; SAS 9.4). Mobility and QG were analyzed using ordinal logistic regression (in the directions of increased mobility impairment and poorer quality grade; PROC GLIMMIX with the Laplace method; SAS 9.4). The continuous variable, hot carcass weight (HCW), was analyzed using a multiple linear mixed-effects regression model to determine relationships between the same list of predictor variables of interest (PROC MIXED; SAS 9.4) using the restricted maximum likelihood method, and diagnostic plots were used to assess model fit. A group analysis was used to account for lot in the binary logistic regressions, and slaughter lot was included as a random effect in the ordinal and linear mixed models. Statistical significance was determined at $P < 0.05$. For each primary response variable, separate full models were fitted that included all possible predictor variables on interest. Manual backwards elimination was then applied as the variable with the largest *P*-value was removed from the model during each step and a new model was fit; this process was repeated until all variables in the model had *P*-values < 0.05.

## 3. Results

### *3.1 Descriptive Statistics*

In the subset of data from the $n = 619$ slaughter lots, 56.4% of cattle were steers, 88.8% of cattle were of *Bos taurus* influence, and 57.2% of cattle were slaughtered on the first shift of operation (Table 3.1). Cattle were transported 155.4 ± 210.2 km (Mean ± SD) and held in lairage for 200.8 ± 195.0 minutes at a space allowance of 3.1 ± 2.0 $m^2$/animal (Table 3.2). Table 3.2 also reports an average THI value of 60.4 ± 13.6 and wind speeds of 18.1 ± 10.1 km/h. Hot carcass weights (HCWs) averaged 397.0 ± 38.6 kg (Table 3.2). A majority of the cattle in the study had a mobility score of 1 (91.8%), were bruised (69.8%), and were graded as Choice (70.6%; Table 3.3). Of the plants that reported dark cutting (DC) data for their carcasses, 1.7% of carcasses were classified as DC.

*3.2 Mobility*

Plant, breed, truck waiting time, distance travelled, THI, wind speed, space allowance in lairage were identified as having significant associations with mobility ($P < 0.05$; Table 3.4). In comparison to plant 5, a multiplicative decrease in the odds of an animal showing signs of increased mobility impairment was associated with plant 1 (OR, 95% CI; 0.384, 0.298 – 0.494), plant 2 (OR, 95% CI; 0.432, 0.334 – 0.559), plant 3 (OR, 95% CI; 0.839, 0.652 – 1.080), and plant 4 (OR, 95% CI; 0.802, 0.607 – 1.060). Compared to slaughter lots of Holstein cattle, cattle lots of "*Bos taurus* influence or less than 25% *Bos indicus* influence" were associated with a multiplicative increase in the odds of an animal showing signs of increased mobility impairment (OR, 95% CI; 1.082, 0.728 – 1.609), whereas cattle lots of "≥ 25% *Bos indicus* influence" were associated with a multiplicative decrease in the odds of an animal showing signs of increased mobility impairment (OR, 95% CI; 0.690, 0.419 – 1.134). A multiplicative increase in the odds of an animal showing signs of increased impaired mobility was associated with a one unit increase in truck waiting time (1 minute; OR, 95% CI; 1.003, 1.001 – 1.004), distance travelled (1 km; OR, 95% CI; 1.001, 1.000 – 1.001), and space allowance in lairage (1 $m^2$/animal; OR, 95% CI; 1.088, 1.041 – 1.1138). Conversely, a multiplicative decrease in the odds of an animal showing signs of increased mobility impairment was associated with a one unit increase in THI value (OR, 95% CI; 0.994, 0.988 – 0.999) and wind speed (1 km/h; OR, 95% CI; 0.989, 0.980 – 0.997).

*3.3 Bruising*

Plant, breed, sex class, shift, distance travelled, THI, wind speed, lairage duration and space allowance in lairage were identified as having significant associations with bruising ($P < 0.05$; Table 3.5). In comparison to plant 5, a multiplicative decrease in the odds of a carcass being bruised was associated with plant 1 (OR, 95% CI; 0.271, 0.252 – 0.292), plant 2 (OR, 95% CI; 0.489, 0.457 – 0.524), and plant 4 (OR, 95% CI; 0.990, 0.926 – 1.059), whereas plant 3 was associated with a multiplicative increase in the odds of a carcass being bruised (OR, 95% CI; 1.513, 1.404 – 1.631). Compared to slaughter lots of Holstein cattle, cattle lots of *Bos taurus* influence or < 25% *Bos indicus* influence, and cattle lots ≥ 25% *Bos indicus* influenced were associated with a multiplicative decrease in the odds of a carcass being bruised (OR, 95% CI; 0.714, 0.641 – 0.796; 0.715, 0.629 – 0.812, respectively). In comparison to slaughter lots of mixed sex, lots consisting of only steers were associated with a multiplicative increase in the odds of their carcass being bruised (OR, 95% CI; 1.150, 1.081 – 1.225), and lots consisting of only heifers were associated with a multiplicative decrease in the odds of their carcass being bruised (OR, 95% CI; 0.986, 0.923 – 1.052). Cattle that were slaughtered during the first shift of operation per day were associated with a multiplicative decrease in the odds of their carcass being bruised (OR, 95% CI; 0.806, 0.772 – 0.842) compared to cattle slaughtered during the second shift. A multiplicative increase in the odds of a carcass being bruised was associated with a one unit increase in space allowance in lairage (1 $m^2$/animal; OR, 95% CI; 1.035, 1.017 – 1.053). However, a multiplicative decrease in the odds of a carcass being bruised was associated with a one unit increase in distance travelled (10 km; OR, 95% CI; 0.997, 0.996 – 0.998), THI value (OR, 95% CI; 0.989, 0.987 – 0.990), wind speed (1 km/h; OR, 95% CI; 0.994, 0.992 – 0.996), and lairage duration (60 minutes; OR, 95% CI; 0.990, 0.983 – 0.997).

*3.4 Dark Cutting*

Plant, breed, shift, wind speed, lairage duration, and space allowance in lairage were identified as having significant associations with DC ($P < 0.05$; Table 3.6). The number of DC carcasses in each lot for Plants 1 and 2 were not obtained and therefore were not included in this analysis. In comparison to plant 5, a multiplicative increase in the odds of a carcass being classified as a DC was associated with plant 3 (OR, 95% CI; 2.875, 1.979 – 4.176) and 4 (OR, 95% CI; 4.564, 3.197 – 6.514). Compared to slaughter lots of Holstein cattle, cattle lots of *Bos taurus* influence or < 25% *Bos indicus* influence were associated with a multiplicative decrease in the odds of a carcass being classified as a DC (OR, 95% CI; 0.714, 0.472 – 1.080), and cattle lots ≥ 25% *Bos indicus* influenced were associated with a multiplicative increase in the odds of a carcass being classified as a DC (OR, 95% CI; 1.197, 0.748 – 1.916). Cattle slaughtered during the first shift were associated with a multiplicative decrease in the odds of their carcass being classified as a DC (OR, 95% CI; 0.416, 0.336 – 0.514) compared to cattle slaughtered on the second shift. A multiplicative increase in the odds of a carcass being classified as a DC was associated with a one unit increase in lairage duration (60 minutes; OR, 95% CI; 1.034, 1.001 – 1.068) and space allowance in lairage (1 $m^2$/animal; OR, 95% CI; 1.092, 1.049 – 1.136). Conversely, a multiplicative decrease in the odds of a carcass being classified as a DC was associated with a one unit increase in wind speed (1 km/h; OR, 95% CI; 0.981, 0.972 – 0.989).

*3.5 Quality Grades*

Plant, breed, sex class, shift truck waiting time, and THI were identified as having significant associations with QG ($P$ <0.05; Table 3.7). Compared to plant 5, a multiplicative increase in the odds of a carcass having a poorer QG was associated with plant 2 (OR, 95% CI; 4.185, 3.277 – 5.344), plant 3 (OR, 95% CI; 1.322, 0.995 – 1.757), and plant 4 (OR, 95% CI; 4.894, 3.819 – 6.273). Conversely, plant 1 was associated with a multiplicative decrease in the odds of a carcass having a poorer QG (OR, 95% CI; 0.960, 0.748 – 1.231). In comparison to slaughter lots of Holstein cattle, cattle lots of *Bos taurus* influence or < 25% *Bos indicus* influence and cattle lots ≥ 25% *Bos indicus* influenced were associated with a multiplicative decrease in the odds of a carcass having a poorer QG (OR, 95% CI; 0.530, 0.358 – 0.784; 0.583, 0.364 – 0.934, respectively). Compared to slaughter lots of mixed sex, lots consisting of only steers were associated with a multiplicative increase in the odds of their carcass having a poorer QG (OR, 95% CI; 1.301, 1.038 – 1.630), and lots consisting of only heifers were associated with a multiplicative decrease in the odds of their carcass having a poorer QG (OR, 95% CI; 0.729, 0.573 – 0.928). Cattle slaughtered during the first shift were associated with a multiplicative decrease in the odds of their carcass having a poorer QG (OR, 95% CI; 0.777, 0.657 – 0.920) compared to cattle slaughtered during the second shift. A multiplicative decrease in the odds of a carcass having a poorer QG was associated with a one unit increase in truck waiting time (1 minute; OR, 95% CI; 0.997, 0.995 – 0.999), whereas a multiplicative increase in the odds of a carcass having a poorer QG was associated with a one unit increase in THI value (OR, 95% CI; 1.012, 1.006 – 1.017).

### *3.6 Hot Carcass Weight*

Plant, breed, sex class, THI, and lairage duration were identified as having significant associations with hot carcass weight (HCW; $P < 0.05$; Table 3.8). In comparison to plant 5, HCW were lighter in plants 1 through 4 ($P$ <.0001). Hot carcass weights were heavier in slaughter lots of cattle of *Bos taurus* influence or < 25% *Bos indicus* influence (estimate = 31.2117, $P$ <.0001) and cattle lots ≥ 25% *Bos indicus* influence (estimate = 23.8301, $P = 0.0043$) compared to Holstein cattle. Slaughter lots consisting only of steers (estimate = 28.3222, $P$ <.0001) had heavier HCW, and slaughter lots consisting only of heifers (estimate = -10.1478, $P = 0.0176$) had lighter HCW compared to lots of mixed sex. Hot carcass weights also were lighter in environmental conditions with increased THI value (estimate = -0.3810, $P$ <.0001) and when cattle were held in lairage for longer durations (1 minute, estimate = -0.01711, $P = 0.0367$).

## 4. Discussion

### *4.1 Study and Plant Characteristics*

The results of this study provide a holistic view of the effects of pre-slaughter management factors on specific animal welfare and meat quality outcomes, several of which have not been researched previously in this sector of the industry. This data set combines aggregate data from multiple plants in various regions of the United States and is highly representative of current fed cattle slaughter statistics. Additionally, many of the variables in this data set reflect current trends in the industry (i.e., cattle characteristics, mobility, and carcass characteristics; Boykin et al., 2017; Eastwood et al., 2017), making it highly representative of the pre-slaughter sector of the current fed beef cattle industry. A common theme in the results from this study were differences between plants and shift for many of the outcome variables; mobility scores, the prevalence of bruising and dark cutting (DC), quality grades (QG) and hot carcass weights (HCW) differed by plant. These differences are likely due to company differences in management and protocols, but also regional differences (e.g., environmental conditions, breed type, and proximity of plant to feedlot) that may have impacted these welfare and meat quality outcomes. Additionally, the results in the current study are reflective of differences between shifts at the plant. Differences in employees and management from the first to second shift, as well as truck arrival and wait times to unload at shift change could influence the management of animals throughout the pre-slaughter phase. For example, cattle slaughtered during the first shift of operation per day compared to the second were associated with decreased odds of being bruised, decreased odds of being classified as a DC, and decreased odds of the carcass having a poorer QG, underlining the importance of consistent employee training and management across each shift.

*4.2 Mobility*

Cattle lameness is an abnormal behavior normally caused by pain from various pathological or underlying health conditions (Webster et al., 2005; Archer et al., 2010). Pain that causes lameness in cattle negatively affects the animal's welfare. Increased concern for cattle mobility occurred approximately a decade ago when cattle arriving at packing plants were reported to have elevated mobility impairment issues (Huffstutter and Polansek, 2013). In response to this issue, a tool was created by industry experts to monitor fed cattle mobility known as the NAMI Mobility Scoring System (NAMI. North American Meat Institute Scoring System, 2015). This scoring tool is still widely used today (NAMI. North American Meat Institute Scoring System, 2015; NCBA, 2023). With the heightened industry awareness around mobility as a welfare challenge in cattle, the scoring of mobility has been included in recent industry benchmarking efforts. The 2016 NBQA (Eastwood et al., 2017) and the current study report high percentages of cattle with normal mobility (i.e., a score of 1; 96.8% and 91.8%, respectively). However, there is still much to learn about the risk factors associated with the pre-slaughter period that cause or aide in the mobility impairment of cattle. Cattle are transported a wide range of distances from feedlots to processing plants and many of these distances have been reported previously benchmarking studies (12.9 to 1,400.1 km, Eastwood et al., 2017; 2.7 to 1,332.5 km in the current study). The continual consolidation of both feedlot operations and processing plants has increased the distances that animals are transported to reach these facilities (Speer et al., 2001). Additionally, economic drivers (i.e., better prices in distance markets) may increase transportation distance as well (Schwartzkopf-Genswein et al., 2012). In the present study, increased distance travelled was associated with increased odds of mobility impairment in cattle, which is similar to findings from González et al. (2012a) and Mijares et al. (2021) who reported longer durations and distances that cattle spent on the truck, the greater the likelihood of them developing mobility impairment, or becoming non-ambulatory. González et al. (2012a) additionally reported that the likelihood of animals being affected by transportation increased significantly after 20 h and sharply after 30 h or more on the truck, an indication of the onset of fatigue or complete exhaustion as energy and water resources are deficient. Animals experience additional stress over the course of the journey as a result of the physical activity required to maintain balance and remain standing (González et al., 2012a). The degree of stress, however, depends heavily on the condition of the animals at the time of loading (i.e., if an animal already has impaired mobility and is therefore unfit for transport) and the length of time animals have to cope with the condition (González et al., 2012a). It is important to note, however, that transportation distances reported in the current study do not necessarily translate directly to estimating transportation duration that may include delays or road conditions that have added impacts on animal welfare and meat quality (Schwartzkopf-Genswein et al., 2012; Miranda-de la Lama et al., 2014). Similarly, in the current study, longer truck wait times to unload cattle at the plant were also associated with increased odds of cattle experiencing increased mobility impairment. Wait times to unload cattle from trucks at the plant have not been extensively researched. Previous studies (Mounier et al., 2006; Warren et al., 2010; González et al., 2012b) have documented average wait times to unload at 15.4, 30, and 25 minutes, respectively. Mounier et al. (2006) used truck wait times to unload in an analysis of unloading score but found no evidence of an association, and the follow-up study to the benchmarking efforts by González et al. (2012b) reported that wait times to unload are one reason for extended transportation journey time (González et al., 2012a). The results in the present study align with previous literature indicating that general increases in time on the truck is associated with impaired mobility in cattle from increased standing time (González et al., 2012a). Previous research and the results from the current study suggest the need for route optimization and streamlined protocols for efficient and timely delivery of cattle to the processing plants. Additionally, specific research on the change in mobility scores throughout the pre-slaughter period (before transport, after transport, and after lairage) may provide some added insight as to what specific factors are impacting cattle mobility the most.

Space allowance for animals in holding pens is one area typically included in plant audits and is federally regulated. Federal regulations state that animals must have access to water and have sufficient space to lie down when held overnight (Code of Federal Regulations. Humane Slaughter of Livestock., 1979), but do not provide strict stocking density or space allowance guidelines while in lairage. The NAMI guidelines have recommendations for stocking densities for cattle in lairage pens based on average pen weight; however, verification of recommended stocking density is not included in the scored portion of this tool and is considered a secondary item that asks if the holding pens “appear to be overcrowded” (NAMI, 2021). Stocking densities in lairage do in fact have effects on cattle welfare outcomes as decreased space available to lie down forces cattle to stand for longer periods on hard surfaces such as concrete, that further exacerbates mobility issues (Boyd et al., 2015). Decreased space between individual cattle will also increase the microclimate within the pens (i.e., the temperature and humidity) with reduced air flow leading to heat stress in hot weather (Albright, 1990; Weeks, 2008; Edwards-Callaway and Calvo-Lorenzo, 2020), coupled with limited access to water, may lead to additional concerns such as impaired mobility (González et al., 2012a; Lee et al., 2018). In the present study, however, increased space allowance was associated with increased signs of impaired mobility in cattle, suggesting the need for further research in this space. There is limited additional research on the effects of space allowance in lairage on cattle mobility to begin with, and this should be explored further to provide additional evidence, such as increased risk of injury or increases in stress that would impact cattle welfare and meat quality. These findings can promote the need for monitored and heavily regulated stocking densities in lairage. Specific research focusing on the changes in cattle mobility throughout the pre-slaughter process, or analyzing cattle behavior during lairage (e.g., lying down or drinking) may provide insight on factors affecting cattle mobility pre-slaughter.

Increased THI values (i.e., increased temperature and humidity) have traditionally been found to negatively impact cattle mobility (González et al., 2012a; Lee et al., 2018; Mijares et al., 2021). However, the present study reports decreased odds, albeit slight, of an animal showing signs of increased mobility impairment with increased THI values. THI values in the current study never exceeded a value of 81.5, suggesting that THI may not have ever been high enough to be a leading cause of mobility impairment in the current study's cattle. Conversely, increased wind speeds were associated with decreased odds of an animal showing signs of mobility impairment, suggesting that increased air flow may be alleviating heat stress (Marchesini et al., 2018; FASS, 2020) and, therefore, may be decreasing the odds of any additional cattle from showing signs of heat exhaustion and therefore mobility impairment as well. Wind speed during the pre-slaughter period is not typically measured, but results such as decreased mobility impairment may provide some additional evidence for the necessity of additional heat abatement technologies to be used during lairage.

Information regarding the impact of sex class and breed type on mobility is limited, however, one study found that heifers had a 43.86% increase in the percentage of mobility scores $\geq 2$ compared to steers (Mijares et al., 2021). Conversely, a study by González et al. (2012a) reported that males (steers or bulls) were more likely to be lame and non-ambulatory during the pre-slaughter phase compared with females (heifers or cows). There was no evidence of an association between sex class and mobility in the current study, however. To the author's knowledge, there is currently no published research on differences in mobility scores at the plant by breed. However, breed is often confounded by sex class (i.e., at a fed cattle plant, Holsteins cattle are steers), yet additional research in this space may provide further insight on handling and managing different cattle breeds differently. In the current study, compared to Holstein cattle, *Bos taurus* cattle had increased odds of having mobility impairment, and *Bos indicus* cattle had decreased odds of having mobility impairment that may be attributed to industry trend towards heavier *Bos taurus* cattle. Recognizing cattle that are unfit for transportation, minimizing standing time, providing cattle enough time to rest and recuperate, and investigating methods for improving cattle mobility in lairage (e.g., the addition of rubber mats) should all be considered by processing facilities and transporters alike to improve cattle welfare.

### *4.3 Bruising*

Carcass bruising serves not only as an indicator of pre-slaughter cattle welfare (Romero et al., 2012; Huertas et al., 2015) but also affects final meat quality and potential economic loss resulting in an estimated $35 million lost annually in the U.S (Gallo et al., 1999; Lee et al., 2017). Several potential risk factors for bruising during the pre-slaughter period have been identified, including transportation factors, animal handling, animal characteristics, and stocking density (McNally and Warriss, 1996; Strappini et al., 2009; Hoffman and Luehl, 2012; Mendonca et al., 2018). Bruise prevalence in the industry remains high (38.9%, Eastwood et al., 2017; 69.8%, in the current study), warranting continued research on the potential causes of bruising in addition to methods to mitigate the occurrence. There are several ways to evaluate and score bruising (e.g., a ten-point size and weight-based scale used by the NBQA; Eastwood et al., 2017; a four-point scale based on bruise depth and severity; Strappini et al., 2010). The authors decided to simplify carcass bruising using a binary variable (bruised/ not bruised) for the current study to aid in interpretation (i.e., applicability) and comparison to other studies irrespective of the bruise scoring systems used in each.

In the present study, breed type was associated with the odds of a carcass being bruised; specifically, Holstein cattle were associated with an increased odds of being bruised. Similarly, previous studies by Lee et al. (2017) and Kline et al. (2020) reported a greater prevalence of bruising in Holstein cattle compared to beef breeds. Larger frame sizes in Holstein cattle compared to their beef breed counterparts (Long et al., 1979; Tatum et al., 1986) can lead to more traumatic events (i.e., injury to the topline) during transportation from both decreased space allowance and clearance when entering or exiting the trailers likely increasing the prevalence of subsequent carcass bruising (Lee et al., 2017). Another cattle characteristic, sex class, was also found to be associated with carcass bruising in the present study. Slaughter lots consisting of only steers were associated with increased odds of being bruised and slaughter lots of only heifers were associated with decreased odds of being bruised in comparison to slaughter lots of mixed sex. The impact of sex class on bruising prevalence is variable across studies. The findings in the current study are similar to previous literature by Romero et al. (2013) that reported increased bruise prevalence in male cattle compared to females. Additionally, Mpakama et al. (2014) specifically reported increased serum creatine kinase levels in males compared to females which the authors linked to stress and bruising. The findings of increased bruising in steers may be due to their larger body mass compared to heifers, or additional agnostic behaviors occurring between cattle that result in injury and/or damage to cattle that should be researched further, particularly in lairage. Conversely, other studies have reported increases in bruising in female cattle compared to steers and bulls (Yeh et al., 1978; Hoffman and Luehl, 2012), however, these female cattle were classified as cows rather than heifers, which may account for some of the differences observed.

Additional factors that were found to be associated with carcass bruising in the current study included space allowance in lairage, lairage duration, and distance travelled. More specifically, increases in space allowance per animal was found to be associated with increased odds of carcass bruising. There is limited research on the effects of space allowance in lairage on the prevalence of carcass bruising in beef cattle, however, review papers of road transport of cattle have identified that there is substantial research on the effects of stocking density (i.e., space allowance) during transport on bruise prevalence (Knowles, 1999; Strappini et al., 2009). Studies by Eldridge and Winfield (1988) and Tarrant et al. (1988, 1992) reported increased bruise prevalence in cattle transported at high stocking densities, however, contrasting the results of the current study. It may be possible that cattle with too much space are more likely to move around more or come in contact with objects that would cause bruising more frequently. Research focusing on the optimum space allowance in lairage is needed to further explore this theory. In the current study, the decreased odds of carcass bruising in cattle that experienced increased lairage durations is congruent with findings from a similar study by Strappini et al., (2010). However, other studies have found contrasting results of increased prevalence and risk of bruising in cattle that spent longer durations in lairage (McNally and Warriss, 1996; Romero et al., 2013). Lairage durations are inevitably difficult to compare across studies as “short” and “long” lairage durations and lairage conditions vary from plant to plant and, therefore, across studies. Additional studies across varying lairage conditions with consistent lairage durations may aid in a greater understanding of its effects. As mentioned previously, additional research focusing on cattle behavior during lairage may also aid in a better understanding of the relationships between lairage factors and welfare and meat quality outcomes.

Interestingly, increased distances travelled in the present study were associated with decreased odds of carcass bruising, contrasting previously literature that has reported increases in bruise prevalence with extended distances travelled (Marshall, 1977; Hoffman et al., 1998; Bethancourt-Garcia et al., 2019). However, one study by Mendonça et al., (2019) reported that short journeys (<120 km) resulted in greater rates of bruising to the hip and round, and the greatest number of front bruises occurred in the shortest (<120 km) and greatest (>240 km) distances. In the current study, transportation distances ranged from 2.7 to 1,332.5 km and averaged 155.4 km. Previous studies and the current study likely differ in additional transport conditions such as road conditions, delays during transport, or environmental conditions that could also influence bruising. Animals transported long durations are more likely to have compromised welfare from exposure to extreme temperatures, deprivation of food, water, rest, and increased risk of injury exacerbated by length of exposure compared to animals transported for shorter distances (Nielsen et al., 2011; Mendonça et al., 2019). However, it is important to note that the associated negative aspects that accompany transportation may be the consequence of many factors, not just distance travelled. Moreover, after animals have adapted to transportation conditions and have calmed down, the impacts of transportation distance may be minimized in comparison to other factors such as trailer stocking density, trailer design, road conditions or driver behavior/skill (Tarrant, 1990; Strappini et al., 2009; Hoffman and Luehl, 2012). There was no evidence of associations in the present study with bruise prevalence and truck waiting time and there is no additional research measuring this relationship. Much like lairage duration, further research across varying transport conditions and research on the effects of truck waiting time in varying conditions may aid in a greater understanding of its effects.

Stressful environmental conditions during the pre-slaughter period play a role in affecting welfare and meat quality outcomes of cattle (Chulayo et al., 2012; Schwartzkopf-Genswein et al., 2016; Birhanu, 2020). In particular, variations in carcass bruising may be accounted for by changes in weather conditions (i.e., temperature, humidity; Grandin, 1980). However, there is limited published data on the associations between environmental weather conditions and carcass bruising in cattle. In the present study, increases in THI values were associated with decreased odds of bruising, contrasting the results of increased bruise prevalence when daily minimum temperatures increased, as reported by Hoffman and Luehl (2012). However, the study by Hoffman and Luehl, (2012) also reported that maximum temperatures did not affect bruise levels. The authors speculate that increases in temperature and humidity may decrease cattle levels of movement in an effort to decrease heat load, that would result in fewer interactions with their surroundings that may inflict injury. To the authors' knowledge, there is currently no published data on the associations between wind speed and carcass bruising, yet the present study reports decreased odds of bruising with increased wind speeds. It could be possible that much like improved air flow in trailers during transportation in warmer temperatures improves animal welfare in the pre-slaughter period (Miranda-de la Lama et al., 2014; Schuetze et al., 2017), increased wind speeds throughout the entirety of the pre-slaughter period may also improve animal welfare in regards to decreased risk of injury and/or bruising as well. The need for further research measuring the relationship between air flow, wind speed, and other environmental conditions and animal welfare outcomes such as injury has been outlined before by Schwartzkopf-Genswein et al., (2016), and this research is still warranted today. Processing plants and transporters need to pay special attention to specific risk factors for bruising in cattle and ways to mitigate them.

*4.4 Dark Cutting*

In cattle that are under high metabolic demand, such as from pre-slaughter stress, activation of the sympathetic nervous system breaks down muscle glycogen, decreasing the glycogen stores, which in turn limits the pH decline postmortem and thus disrupting the muscle's normal postmortem metabolism and pH decline (Scanga et al., 1998; Ponnampalam et al., 2017; Sullivan et al., 2022). This higher ultimate muscle pH results in a darker, purplish-red colored lean with reduced shelf-life commonly referred to as dark cutting (DC) beef or dark, firm, and dry (DFD) meat (Ponnampalam et al., 2017; Terlouw et al., 2021). Several factors during the pre-slaughter phase such as transportation (Wythes et al., 1988; Marenčić et al., 2012), lairage (Wythes et al., 1988; Teke et al., 2014; Loredo-Osti et al., 2019), environmental conditions (Scanga et al., 1998; C. Steel et al., 2022), and specific animal characteristics such as breed type and sex class (Scanga et al., 1998; Page et al., 2001) have previously been reported to influence DC in beef.

In the current study, several of these same factors (i.e., breed type, environmental conditions, and lairage) were found to be associated with DC. For example, in comparison to slaughter lots of Holstein cattle, *Bos taurus* cattle were associated with decreased odds of DC, whereas *Bos indicus* cattle were associated with increased odds of DC. Congruent with these results, Cook (1998) and Viljoen (2007) reported that *Bos indicus* or Brahman-influenced cattle had significantly darker meat. Holstein cattle, much like *Bos indicus* cattle, are more heat tolerant than *Bos taurus* cattle (Santana et al., 2017; Forbes et al., 1998) making them more susceptible to colder temperatures or temperature fluctuations compared to *Bos taurus* cattle.

This susceptibility to weather changes may be an additional reason why Bos taurus cattle were associated with decreased odds of DC in the current study. However, it is important to note that there was no evidence of THI being associated with DC in the current study, but temperature and THI have been identified in previous studies (Scanga et al., 1998; Steel et al., 2022) to have an association with DC. The absence of an association between THI and DC in the current study could be due, in part, to the average THI value being 60.4, well within the range where cattle should be comfortable and exhibiting no signs of heat stress (LiveCorp and Meat and Livestock Australia, 2023). An additional environmental factor, wind speed, was identified in the current study to have a negative association with DC. In previous literature, intermediate wind speeds (10-15 km $h^{-1}$) at the abattoir have resulted in lower incidences of dark beef (Murray, 1989), and increased wind speeds within 7 days of departure from the feedlot to the processing facility was also associated with lower risk of DC beef (Steel et al., 2022). These findings are congruent with associations with DC in the current study. These results may be due, in part, to an increase in air movement aiding in evaporative cooling from the skin (Vermunt and Tranter, 2011) and serve as additional evidence for the implementation of heat mitigation methods such as fans to decrease this occurrence of DC.

Interestingly, there was no evidence of another animal characteristic, sex class, being associated with dark cutting in the present study. A greater incidence of DC beef in both steers and bulls has been outlined in previous literature across all sectors of the beef industry (Jeremiah et al., 1991; Mohan Raj et al., 1992; Page et al., 2001; Miguel et al., 2014) that may be attributed to their flighty and aggressive temperaments (Warriss et al., 1984; Ponnampalam et al., 2017). None of the transportation factors measured in this study were associated with dark cutting. Past research has reported that transportation duration and rest stops during transportation affect final meat pH. However, cattle were transported by various means in previous literature (e.g., by rail or road) and for various durations that may have greatly differed from the journeys of cattle in the present study (Wythes et al., 1988; Brown et al., 1990; Marenčić et al., 2012). In the current study, increased space allowance was also associated with increased odds of DC. Romero et al. (2017) reported contradicting findings, stating that a higher prevalence of DFD meat was associated with higher stocking densities in lairage. It is unclear as to why increased space allowances for cattle in the current study was associated with increased odds of DC, however, further research on cattle behavior while in lairage and risk factors for dark cutting, as mentioned previously, may help explain this. Lairage durations have also been shown to be related to increased incidence of DC beef in previous literature, and in the current study, longer lairage durations were associated with increased odds of DC. Other studies have reported greater incidence of DC in cattle that were held for longer lairage duration (Puolanne and Aalto, 1981; Fabiansson et al., 1984; Bartoš et al., 1993; Steel et al., 2021), and a study by Loredo-Osti et al., (2019) found reduced probability of dark cutting (7.21% to 0.02%) with a reduction from 14.9 to 3.0 hours in lairage. However, Brown et al. (1990) reported a greater incidence of DC in cattle that were slaughtered on the day of arrival (5.5%) to the slaughter plant compared to holding them in lairage overnight before slaughter (3.1%). Other authors suggest that 3 hours in lairage is an appropriate amount of time for rest for many species and that longer lairage duration may be counterproductive (Gallo et al., 2003; Diaz et al., 2014). The mean lairage duration in the current study was 200.8 minutes, or approximately three and a half hours. However, lairage durations ranged from 4.0 to 1,072.0 minutes. Lairage durations and environments also differ substantially from plant to plant, which may also play a role in the varying results. Further research pinpointing specific risk factors during the pre-slaughter period on DC can help improve animal welfare and meat quality, and ultimately reduce economic loss. Additionally, this growing body of research emphasizes the need for slaughter plants to focus on reducing extended lairage durations and focusing on cattle comfort while in lairage.

*4.5 Quality Grades*

Quality grading (QG) beef carcasses is based on two characteristics: the degree of marbling (i.e., the primary determination of quality grade), and the degree of maturity (Hale et al., 2013). Literature exploring the associations of pre-slaughter factors with QG is limited; current literature is more focused on meat quality (i.e., pH) or overall eating quality. However, QG encompasses factors that affect meat palatability (i.e., tenderness, flavor, and juiciness), and the QG is only given if color, texture, and firmness of the lean is normal (i.e. if the carcass has not already been given a discounted grade; USDA Market News Service, 2023). Thus, a deviation in normal lean color (i.e., DC beef) results in a discounted carcass grade classified as a DC (currently averaging a $36.67/cwt discount from the base price as of October 2$^{nd}$, 2023; USDA Market News Service, 2023).

There are also several pre-slaughter factors that are associated with QG, such as breed type, sex class, transportation, and environmental factors. In comparison to slaughter lots of Holstein cattle, cattle lots of *Bos taurus* and *Bos indicus* influence were associated with decreased odds of a carcass having a lower QG. These results contradict the findings of the 2016 NBQA (Boykin et al., 2017), where dairy breed carcasses on average had better quality grades ($717 \pm 1.7$; least square means ± SEM) indicative of a higher quality grade than native and *Bos indicus* breeds ($705 \pm 0.9$, and $667 \pm 4.7$, respectively). In contrast, a Bertrand et al., (1983) reported higher QG in Angus cattle compared to Hereford, Holstein, and Brown Swiss cattle breeds. A sample population encompassing more U.S. regions and slaughter plants may find results congruent with the 2016 NBQA. Another animal characteristic, sex class, was found to be associated with QG in the current study. Compared to slaughter lots of mixed sex, steer slaughter lots were associated with an increase in the odds of a carcass having a poorer QG, and heifer lots were associated with a decrease in the odds of a carcass having a poorer QG, contrary to the 2016 NBQA that reported slightly higher QGs in steers than heifers (708 ± 0.9, and 704 ± 1.5, respectively).

While not all environmental factors were found to have evidence of an association with quality grades in the present study (i.e., wind speed), an increase in the odds of a carcass having a poorer QG was associated with an increase in THI value. Increased THI values are generally indicative of warmer seasonal temperatures that may cause heat stress in cattle, resulting in decreased feed intake, animal growth, and production efficiency (Brown-Brandl, 2018). This decrease in production efficiency may lead to poorer QG and may not be strictly related to THI values during the pre-slaughter period. For example, more feedlot heifers in shaded pens graded USDA Choice than those in unshaded pens, which resulted primarily from the prevalence of DC being decreased by approximately half in carcasses from shaded heifers compared to unshaded (Mitlöhner et al., 2002). This data supports the concept that the associations between increased temperatures and THI values and increased prevalence in DC in previous studies (Scanga et al., 1998; C. Steel et al., 2022) may be contributing to the decreases in quality in the present study. One transportation factor was found to be associated with QG in the current study; a decrease in the odds of a carcass having a poorer QG was associated with an increase in truck waiting time. To the author's knowledge, truck waiting time and QG associations have not been previously researched in cattle. Additional research on this topic is warranted to fully understand this relationship and determine if these results are common. In general, truck waiting time to unload should be minimized to alleviate its negative effects on cattle welfare, especially in the warmer months.

*4.6 Hot Carcass Weight*

Hot carcass weight (HCW) is the hot (pre-chilled) weight of the carcass post-slaughter and after removal of the head, hide, intestinal tract, and additional internal organs (UGA Extension, 2017), and is used to determine yield grade and dressing percentage (i.e., the percentage of the live animal that ends up as a carcass and is calculated using the formula: [HCW ÷ live weight] x 100%). Much like QG, there are price discounts that are associated with carcass size (i.e., weighing less than 600 pounds and more than 900 pounds; USDA Market News Service, 2023). Pre-slaughter challenges expose cattle to risks of fear, dehydration, and hunger, increased physical activity, and fatigue (Carrasco-García et al., 2020), many of which have the potential to affect HCW postmortem. For example, long periods without feed and water (i.e., during transportation) may result in what is called “tissue shrink” caused by extra-cellular and intra-cellular fluid loss that will decrease the final carcass weight (Barnes et al., 2017).

Specific animal characteristics such as breed type and sex class also have the potential to affect HCW. In the present study, HCWs were heavier in cattle of *Bos taurus* influence or < 25% *Bos indicus* influence and cattle lots of ≥ 25% *Bos indicus* influence compared to Holstein cattle. These results are similar to those reported in other studies that have concluded that even at the same live weight, dairy breeds will typically have lower dressing out percentages and thus lower carcass weights, compared to beef breeds due to higher proportions of non-carcass tissues (i.e., intestines or organs) removed from the carcass (Kempster et al., 1982; Preston and Willis, 1982). Not surprisingly, slaughter lots of steers had heavier HCW, and slaughter lots of heifers had lighter HCW compared to lots of mixed sex in the current study. Previous studies have reported that steers generally weigh more than heifers in live weight and in HCW, attributed to differences in growth rates between the sexes, which is lower in heifers (Boykin et al., 2017; Augusto et al., 2019).

Impacts on HCW were also associated with environmental conditions with increased THI values. A decrease in HCWs during periods of increased THI values could be due to heat stress conditions that cattle may be enduring in the time leading up to slaughter that would cause decreased feed intake and thus limit animal growth (Brown-Brandl, 2018), however, that was not measured in the current study. There have been reported associations between decreased hot carcass weights and increased fasting durations (Clariget et al., 2021), and thus, it is not surprising that HCW in the current study were lighter when cattle were held in lairage for longer durations without feed. Cattle lose live weight rapidly within the first 24 hours without access to feed and water, and this fasting duration can have detrimental effects on carcass shrinkage and muscle quality (Jones et al., 1990). There was no evidence of additional pre-slaughter factors in the current study (e.g., operation shift, transportation factors, space allowance in lairage, and wind speed) having an association with HCW. On average, transportation distance and truck waiting times were short (155.4 km and 30.3 minutes, respectively; Table 2) and therefore, a majority of the journeys may not have resulted in enough time to make a significant difference in final carcass weights in the current study. In general, minimizing the amount of time that cattle are without food and water and focusing on heat mitigation strategies throughout the pre-slaughter period may help alleviate losses in carcass weight.

### *4.7 Conclusions*

The beef industry is continually evolving and adapting to both internal and external pressures for high quality beef, and in their efforts to do so, changes in cattle welfare and meat quality have followed suit. Substantial efforts made within the beef industry to promote and manage animal welfare and subsequent meat quality have not gone unnoticed. However, there continues to be room for improvement in this ever-changing industry. Results from the current study have identified areas where further research is needed to fill knowledge gaps and fully understand the impacts of pre-slaughter management factors on welfare and meat quality outcomes and more specific origins of factors that affect these outcomes. These results will also hopefully aid in informed decision-making regarding cattle management during the pre-slaughter phase. The outcomes of a project of this magnitude have the potential for industry stakeholders to further question and evaluate current management practices and hopefully make significant and positive changes in the pre-slaughter sector of the industry. Continued multidisciplinary research, recommended management practices put into practice, and education of industry stakeholders, plant employees and truck drivers are imperative to the development and implementation of pre-slaughter management practices that are sustainable on all accounts.

**Tables**

**Table 3.1.** Descriptive statistics of categorical fed cattle slaughter lot characteristics used for regression analyses.

| Characteristic | *n Lots* | Frequency (%) |
|---|---|---|
| **Sex Class (*n* = 594)** | | |
| *Steers* | 335 | 56.4 |
| *Heifers* | 190 | 32.0 |
| *Mix*[1] | 69 | 11.6 |
| **Breed Type[2] (*n* = 582)** | | |
| *Bos taurus or < 25% Bos indicus Influence* | 517 | 88.8 |
| *≥ 25% Bos indicus Influence* | 44 | 7.6 |
| *Holstein* | 21 | 3.6 |
| **Shift (*n* = 617)** | | |
| *1* | 353 | 57.2 |
| *2* | 264 | 42.8 |

[1]Slaughter lots with steers and heifers mixed together were considered a mix lot.
[2]Cattle were recorded as *Bos indicus* if ≥ 25% of the cattle within the lot had two or more of the breed's characteristics (e.g., a large hump on their withers, excess skin on their dewlap or prepuce, and large, droopy ears).

**Table 3.2.** Descriptive predictor statistics of numeric transportation, lairage, and environmental characteristics of the slaughter lots used in regression analyses.

| Variable | *n* | Minimum | Mean | Maximum | SD |
|---|---|---|---|---|---|
| **Transportation** | | | | | |
| *Distance Travelled (km)* | 598 | 2.7 | 155.4 | 1,332.5 | 210.2 |
| *Truck Waiting Time (min)* | 603 | 0.0 | 30.3 | 574.0 | 39.7 |
| **Lairage** | | | | | |
| *Lairage Duration (min)* | 572 | 4.0 | 200.8 | 1,072.0 | 195.0 |
| *Space Allowance ($m^2$/animal)* | 606 | 0.6 | 3.1 | 31.7 | 2.0 |
| **Environmental Characteristics** | | | | | |
| *Temperature Humidity index (THI)*[1, 2] | 619 | 18.9 | 60.4 | 81.5 | 13.6 |
| *Wind Speed*[2] *(km/h)* | 619 | 0.0 | 18.1 | 56.3 | 10.1 |

[1]THI value was calculated as: THI = 0.8*T + RH*(T-14.4) + 46.4 where T is the ambient temperature in °C and RH is the relative humidity proportion (LiveCorp and Meat and Livestock Australia, 2023).

[2]Temperature and humidity used to calculate THI, and wind speed were recorded from a commercial weather service online (Weather Underground, San Francisco, CA, USA).

**Table 3.3.** Frequencies and descriptive statistics of specific cattle welfare and meat quality outcomes used for regression analyses.

| ***Categorical Variables*** | | |
|---|---|---|
| **Variable** | ***n Lots*** | **Frequency (%)** |
| **Mobility Scores[1]** | 610 | |
| *1* | | 91.8 |
| *2* | | 7.8 |
| *3* | | 0.3 |
| *4* | | 0.002 |
| **Bruise Scores[2]** | 598 | |
| *None* | | 30.2 |
| *≤ Deck of Cards* | | 27.2 |
| *> Deck of Cards* | | 42.6 |
| *Multiple[3]* | | 65.2 |
| **Bruise Prevalence[4]** | 598 | |
| *Bruised* | | 69.8 |
| *Not Bruised* | | 30.2 |
| **Dark Cutting[5]** | 361 | 1.7 |
| **Quality Grades[5]** | 604 | |
| *Prime* | | 8.6 |
| *Choice* | | 70.6 |
| *Select* | | 19.0 |
| *Standard* | | 0.5 |
| *Other* | | 1.3 |

| ***Continuous Variable*** | | | | | |
|---|---|---|---|---|---|
| **Variable** | ***n*** | **Minimum** | **Mean** | **Maximum** | **SD** |
| **Hot Carcass Weight (kg)** | 598 | 301.6 | 397.0 | 675.7 | 38.6 |

[1]Mobility scores were defined as: 1 = normal, no apparent lameness; 2 = exhibits minor stiffness, keeps up with normal cattle; 3 = exhibits obvious stiffness, lags behind normal cattle; and 4 = extremely reluctant to move, statue-like (NAMI, 2015).

[2]Individual carcasses were scored as either having no bruises (none), one bruise that was ≤ to the size of a deck of cards, one bruise that was > than the size of a deck of cards, and if it had multiple bruises in which case the size of the largest bruise was noted. Scores were then summarized at the lot level.

[3]Multiple is expressed as the proportion of bruised carcasses that had multiple bruises.

[4]Bruising was summarized and analyzed as a binary variable. Therefore, the prevalence of those bruises versus not bruised is reported in this table.

[5]One plant reported information regarding dark cutting, quality, and yield grades as data for each carcass side. Therefore, if one carcass side was condemned, the other carcass side is still represented in the data set.

**Table 3.4.** Fed cattle **mobility**[1] ($n$ = 72,204) ordinal logistic regression analysis. Odds ratio (OR) associated with the odds of an increased mobility score (increased lameness). An OR > 1 indicates that the variable is associated with a multiplicative increase in the odds of an animal showing signs of increased lameness, whereas an OR < 1 indicates that the variable is associated with a multiplicative decrease in the odds of an animal showing signs of increased lameness.

| Variable | Estimate | SE | Odds Ratio (95% CI) | *P*-value |
|---|---|---|---|---|
| Plant | | | | |
| *1* | -0.9584 | 0.1292 | 0.384 (0.298 – 0.494) | <.0001 |
| *2* | -0.8389 | 0.1310 | 0.432 (0.334 – 0.559) | <.0001 |
| *3* | -0.1757 | 0.1287 | 0.839 (0.652 – 1.080) | 0.1721 |
| *4* | -0.2203 | 0.1421 | 0.802 (0.607 – 1.060) | 0.1209 |
| *5* | *Referent* | | | |
| Breed | | | | |
| *Bos taurus or < 25% Bos indicus influenced* | 0.07886 | 0.2023 | 1.082 (0.728 – 1.609) | 0.6967 |
| *Cattle ≥ 25% Bos indicus influenced* | -0.3717 | 0.2538 | 0.690 (0.419 – 1.134) | 0.1430 |
| *Holstein* | *Referent* | | | |
| Truck Waiting Time (minute) | 0.002597 | 0.000963 | 1.003 (1.001 – 1.004) | 0.0070 |
| Distance Travelled (km) | 0.000614 | 0.000185 | 1.001 (1.000 – 1.001) | 0.0009 |
| THI[2] | -0.00650 | 0.002923 | 0.994 (0.988 – 0.999) | 0.0262 |
| Wind Speed[3] (km/h) | -0.01156 | 0.004237 | 0.989 (0.980 – 0.997) | 0.0064 |
| Space Allowance ($m^2$/animal) | 0.08439 | 0.02279 | 1.088 (1.041 – 1.138) | 0.0002 |

[1]Mobility was originally scored as either: 1 = normal, walks easily, no apparent lameness; 2 = exhibits minor stiffness, shortness of stride, slight limp, keeps up with normal cattle; 3 = exhibits obvious stiffness, difficulty taking steps, obvious limp, obvious discomfort, lags behind normal cattle; and 4 = extremely reluctant to move even when encouraged by a handler, statue-like (NAMI, 2015).

[2]THI value was calculated using the equation: THI = 0.8*T + RH*(T-14.4) + 46.4 (LiveCorp and Meat and Livestock Australia, 2023) where T is the ambient or dry-bulb temperature in °C, and RH is the proportion of relative humidity.

[3]Wind speed was recorded from an online commercial weather service's report (Weather Underground, San Francisco, CA, USA).

**Table 3.5.** Fed cattle **bruising**[1] ($n$ = 68,607) binary logistic regression analysis. Odds ratio (OR) associated with the odds of a carcass being bruised to some degree. An OR > 1 indicates that the variable is associated with a multiplicative increase in the odds of a carcass being bruised to some degree, whereas an OR < 1 indicates that the variable is associated with a multiplicative decrease in the odds of a carcass being bruised to some degree.

| Variable | Estimate | SE | Odds Ratio (95% CI) | *P*-value |
|---|---|---|---|---|
| Intercept | 1.7381 | 0.0552 | 5.687 (5.104 – 6.336) | <.0001 |
| Plant | | | | |
| *1* | -0.9822 | 0.0205 | 0.271 (0.252 - 0.292) | <.0001 |
| *2* | -0.3921 | 0.0181 | 0.489 (0.457 - 0.524) | <.0001 |
| *3* | 0.7375 | 0.0231 | 1.513 (1.404 - 1.631) | <.0001 |
| *4* | 0.3136 | 0.0215 | 0.990 (0.926 - 1.059) | <.0001 |
| *5* | *Referent* | | | |
| Breed | | | | |
| *Bos taurus or < 25% Bos indicus influenced* | -0.1124 | 0.0221 | 0.714 (0.641 – 0.796) | <.0001 |
| *Cattle ≥ 25% Bos indicus influenced* | -0.1118 | 0.0297 | 0.715 (0.629 – 0.812) | 0.0002 |
| *Holstein* | *Referent* | | | |
| Sex Class (lots) | | | | |
| *Steer* | 0.0982 | 0.0136 | 1.150 (1.081 - 1.225) | <.0001 |
| *Heifer* | -0.0564 | 0.0147 | 0.986 (0.923 - 1.052) | 0.0001 |
| *Mix* | *Referent* | | | |
| Shift | | | | |
| *1* | -0.1079 | 0.0111 | 0.806 (0.772 – 0.842) | <.0001 |
| *2* | *Referent* | | | |
| Distance Travelled (10 km)[2] | -0.00027 | 0.000058 | 0.997 (0.996 - 0.998) | <.0001 |
| THI[3] | -0.0115 | 0.000704 | 0.989 (0.987 - 0.990) | <.0001 |
| Wind Speed[4] (km/h) | -0.00613 | 0.00112 | 0.994 (0.992 - 0.996) | <.0001 |
| Lairage Duration[5] (60 minutes)[3] | -0.00017 | 0.000056 | 0.990 (0.983 - 0.997) | 0.0027 |
| Space Allowance ($m^2$/animal) | 0.0346 | 0.00887 | 1.035 (1.017 - 1.053) | <.0001 |

[1] Bruise scoring categories were collapsed into a binary variable for simplicity in analysis (not bruised/bruised).

[2] 10 km was used as the unit for distance travelled in this model as a more desirable scale to develop an odds ratio > or < than 1.000.

[3] THI value was calculated using the equation: THI = 0.8*T + RH*(T-14.4) + 46.4 (LiveCorp and Meat and Livestock Australia, 2023) where T is the ambient or dry-bulb temperature in °C, and RH is the proportion of relative humidity.

[4] Wind speed was recorded from an online commercial weather service's report (Weather Underground, San Francisco, CA, USA).

[5] 60 minutes was used as the unit for lairage duration in this model as a more desirable scale to develop an odds ratio > or < than 1.000.

**Table 3.6.** Fed cattle **dark cutting** (*n* = 44,568) binary logistic regression analysis. Odds ratio (OR) associated with the odds of a carcass being classified as a dark cutter. An OR > 1 indicates that the variable is associated with a multiplicative increase in the odds of a carcass being classified as a dark cutter, whereas an OR < 1 indicates that the variable is associated with a multiplicative decrease in the odds of a carcass being classified as a dark cutter.

| Variable | Estimate | SE | Odds Ratio (95% CI) | *P*-value |
|---|---|---|---|---|
| Intercept | -4.1292 | 0.1398 | 0.016 (0.012 – 0.021) | <.0001 |
| Plant[1] | | | | |
| *3* | 0.1979 | 0.0905 | 2.875 (1.979 - 4.176) | 0.0286 |
| *4* | 0.6601 | 0.0841 | 4.564 (3.197 - 6.514) | <.0001 |
| *5* | *Referent* | | | |
| Breed | | | | |
| *Bos taurus or < 25% Bos indicus influenced* | -0.2843 | 0.0805 | 0.714 (0.472 – 1.080) | 0.0004 |
| *Cattle ≥ 25% Bos indicus influenced* | 0.2321 | 0.1042 | 1.197 (0.748 – 1.916) | 0.0259 |
| *Holstein* | *Referent* | | | |
| Shift | | | | |
| *1* | -0.4389 | 0.0541 | 0.416 (0.336 - 0.514) | <.0001 |
| *2* | *Referent* | | | |
| Wind Speed[2] (km/h) | -0.0197 | 0.00462 | 0.981 (0.972 - 0.989) | <.0001 |
| Lairage Duration (60 minutes)[3] | 0.000559 | 0.000274 | 1.034 (1.001 - 1.068) | 0.0415 |
| Space Allowance ($m^2$/animal) | 0.0878 | 0.0203 | 1.092 (1.049 - 1.136) | <.0001 |

[1]Plants 1 and 2 did not record the number of dark cutting carcasses and therefore were not included in the analysis.

[2]Wind speed was recorded from an online commercial weather service's report (Weather Underground, San Francisco, CA, USA).

[3]60 minutes was used as the unit for lairage duration in this model as a more desirable scale to develop an odds ratio > or < than 1.000.

**Table 3.7.** Fed cattle **quality grade** ($n$ = 75,083) ordinal logistic regression analysis. Odds ratio (OR) associated with the odds of a carcass having a poorer quality grade. An OR > 1 indicates that the variable is associated with a multiplicative increase in the odds of a carcass having a poorer quality grade, whereas an OR < 1 indicates that the variable is associated with a multiplicative decrease in the odds of a carcass having a poorer quality grade.

| Variable | Estimate | SE | Odds Ratio (95% CI) | *P*-value |
|---|---|---|---|---|
| Plant | | | | |
| *1* | -0.04101 | 0.1269 | 0.960 (0.748 - 1.231) | 0.7466 |
| *2* | 1.4314 | 0.1248 | 4.185 (3.277 - 5.344) | <.0001 |
| *3* | 0.2792 | 0.1450 | 1.322 (0.995 - 1.757) | 0.0542 |
| *4* | 1.5881 | 0.1266 | 4.894 (3.819 - 6.273) | <.0001 |
| *5* | *Referent* | | | |
| Breed | | | | |
| *Bos taurus or < 25% Bos indicus influenced* | -0.6350 | 0.1998 | 0.530 (0.358 - 0.784) | 0.0015 |
| *Cattle ≥ 25% Bos indicus influenced* | -0.5403 | 0.2406 | 0.583 (0.364 - 0.934) | 0.0247 |
| *Holstein* | *Referent* | | | |
| Sex Class (lots) | | | | |
| *Steer* | 0.2628 | 0.1152 | 1.301 (1.038 - 1.630) | 0.0225 |
| *Heifer* | -0.3164 | 0.1231 | 0.729 (0.573 - 0.928) | 0.0102 |
| *Mix* | *Referent* | | | |
| Shift | | | | |
| *1* | -0.2517 | 0.08582 | 0.777 (0.657 - 0.920) | 0.0034 |
| *2* | *Referent* | | | |
| Truck Waiting Time (minute) | -0.00313 | 0.000956 | 0.997 (0.995 - 0.999) | 0.0010 |
| THI[1] | 0.01157 | 0.002720 | 1.012 (1.006 - 1.017) | <.0001 |

[1]THI value was calculated using the equation: THI = 0.8*T + RH*(T-14.4) + 46.4 (LiveCorp and Meat and Livestock Australia, 2023) where T is the ambient or dry-bulb temperature in °C, and RH is the proportion of relative humidity.

**Table 3.8.** Multivariable linear mixed-effects regression model for associations with pre-slaughter management factors and hot carcass weight (HCW) in fed beef cattle slaughter lots ($n$ = 514).

| Variable | Estimate | SE | *P*-value |
|---|---|---|---|
| Intercept | 417.5900 | 9.1827 | <.0001 |
| Plant | | | |
| *1* | -32.4438 | 4.8187 | <.0001 |
| *2* | -56.9314 | 4.3017 | <.0001 |
| *3* | -33.1834 | 4.0148 | <.0001 |
| *4* | -48.4733 | 4.1031 | <.0001 |
| *5* | *Referent* | | |
| Breed | | | |
| *Bos taurus or < 25% Bos indicus influenced* | 31.2117 | 6.8722 | <.0001 |
| *Cattle ≥ 25% Bos indicus influenced* | 23.8301 | 8.3102 | 0.0043 |
| *Holstein* | *Referent* | | |
| Sex Class (lots) | | | |
| *Steer* | 28.3222 | 4.0095 | <.0001 |
| *Heifer* | -10.1478 | 4.2604 | 0.0176 |
| *Mix* | *Referent* | | |
| THI[1] | -0.3810 | 0.09622 | <.0001 |
| Lairage Duration (minutes) | -0.01711 | 0.008168 | 0.0367 |

[1]THI value was calculated using the equation: THI = 0.8*T + RH*(T-14.4) + 46.4 (LiveCorp and Meat and Livestock Australia, 2023) where T is the ambient or dry-bulb temperature in °C, and RH is the proportion of relative humidity.

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