THESIS

PRE-SLAUGHTER FACTORS AFFECTING MOBILITY, BLOOD PARAMETERS, BRUISING, AND MUSCLE PH OF FINISHED BEEF CATTLE IN THE UNITED STATES

Submitted by

Paxton Sullivan

Department of Animal Sciences

In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Fall 2023

Master's Committee:

Advisor: Lily Edwards-Callaway

Ann Hess
Mahesh Narayanan Nair

ABSTRACT

PRE-SLAUGHTER FACTORS AFFECTING MOBILITY, BLOOD PARAMETERS, BRUISING, AND MUSCLE PH OF FINISHED BEEF CATTLE IN THE UNITED STATES

Decades of work have focused on reducing fear, stress, and discomfort in cattle moving through the pre-slaughter phase by improving and promoting low-stress animal handling, transportation, and management processes. Even still, there is limited information about the effects of pre-slaughter factors on animal welfare and meat quality outcomes in finished cattle in the United States. The objective of this study was to track individual animals through the slaughter process to identify pre-slaughter factors associated with key welfare and quality outcomes. A total of 454 cattle from one slaughter facility were included in the study. Pre-slaughter factors assessed included: distance traveled, lairage density, lairage duration, season, and truck waiting time. Animal-related characteristics, i.e., body weight, breed, and sex, were also recorded. One trained observer scored mobility of all study cattle using the North American Meat Institute's 1-4 scale (i.e., normal to extremely reluctant to move). Postmortem, exsanguination blood was collected on animals and analyzed for cortisol, creatine kinase, and lactate. Carcass bruising was scored using a modified version of the National Beef Quality Audit's bruise scoring methodology (i.e., no bruise, one bruise that was ≤ the size of a deck of cards, one bruise that was > than the size of a deck of cards, and multiple bruises). Ultimate muscle pH was measured 32 to 36 hours postmortem. Multi-predictor models were selected for each outcome variable using Aikake Information Criterion (AIC). Continuous outcome variables were analyzed using linear mixed-effects models and categorical outcome variables with mixed-effects logistic regression models.

Increased truck waiting time was associated with increased cortisol ($P = 0.04$) and lactate ($P = 0.02$) concentrations. Similarly, an increase in lairage duration was associated with an increase in creatine kinase ($P = 0.05$) and the odds of cattle being bruised ($P = 0.03$). Less space allowance per animal in lairage was associated with increased odds of cattle having impaired mobility ($P = 0.01$). There was a seasonal effect for many of the measured outcomes; the summer season was associated with greater lactate concentrations ($P < 0.0001$), increased odds of impaired mobility ($P < 0.0001$), and increased odds of carcass bruising ($P = 0.003$). The findings of this study indicate that many of the pre-slaughter factors assessed influence key welfare and meat quality outcomes of finished beef cattle, warranting future research and consideration.

ACKNOWLEDGEMENTS

To my advisor and mentor – Dr. Lily Edwards-Callaway, from the very bottom of my heart, thank you. Thank you for your tireless commitment to excellence, which permeates everything you do and, in turn, everything your colleagues and students do, for fostering my love for science and for teaching me how to be a better scientist each day, for your genuine and unwavering support of all that I do, and for allowing me the opportunities and new experiences to continue to grow, develop, and learn at every juncture of my journey here. Also, I am thankful for all the small things that really were not so small, like the early morning and late-night emails, text messages, and phone calls, and all the open-door office visits when I had a question or just needed your guidance; these things never went unnoticed. I am certain that I am a better researcher, teacher, student – and above all, a better person for working and learning alongside you.

To my committee members – Drs. Ann Hess and Mahesh Nair, I would like to thank you both for your precise and technical contributions to this thesis, as your expertise and guidance have made this work more robust. I have felt and appreciated your support every step of the way.

To faculty members in the Department of Animal Sciences – Drs. Jason Ahola, Catie Cramer, Terry Engle, Noa Román-Muñiz, and John Wagner. Each of you has played a key role in my success here, supporting and championing my journey in graduate school in so many capacities. You serve as mentors, leaders, teachers, advocates, and role models to me. And as I reflect on my time at CSU, you all make up some of my favorite parts of it. Above all, thank you for your trust and belief in me; your sincere words of encouragement (whether in the hallways, in your offices, classrooms, etc.) always meant more to me than you could know.

To my many colleagues and friends in the Department, particularly the members of the Livestock and Behavior lab group (Melissa, Corley, Lily K., Lauren, David, Claire) – thank you for your friendship and your support of me. We have come a long way together, learning about ourselves and science along the way. I am so very thankful for every moment we have shared – all the early morning and late-night data collections, coffee sessions in the office, and post-work dinners that reminded me how lucky I was to have you all as colleagues and, most of all, friends.

To my family – Mom, Dad, and James. When you first dropped me off for my first day as an undergraduate at CSU, I don't think anyone anticipated that I would still be here six years and almost two degrees later. Yet, throughout my journey here, you have been everything I needed you to be and more. You are my biggest cheerleaders and most fervent supporters. I especially appreciate that you always check in on me, even when I am not very good at checking in on you sometimes. You never tire of telling me how proud you are of me and that you love me every chance you get. These small but meaningful actions encourage me to preserve even when the days are hard. My most cherished accomplishments are the titles you gave me – daughter and sister. I love you; I love you; I love you!

DEDICATION

To every student that I have ever had the privilege of teaching, but most importantly, learning from. The future of agriculture looks bright because you are a part of it – keep going!

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CHAPTER 1: INVESTIGATING THE IMPACT OF PRE-SLAUGHTER MANAGEMENT FACTORS ON MEAT QUALITY OUTCOMES IN CATTLE RAISED FOR BEEF: A SCOPING REVIEW[1]

1.0 Introduction

Animal well-being in the beef production chain is particularly relevant as producers, consumers, and retailers of animal-derived proteins increasingly regard food animal welfare as a chief concern (Clark et al., 2016; Wigham et al., 2018; Edwards-Callaway and Calvo-Lorenzo, 2020); this concept is reflected in many programmatic animal welfare documents and guidelines set forth by food companies (Nestle, 2014; JBS, 2019; Cargill, 2022) as well as global (OIE, 2016; GRSB, 2022) and national entities (Brazil, Macitelli et al., 2018; NAMI, 2021; Australian Animal Welfare Standards and Guidelines, 2022). Although a beef animal's welfare is impacted throughout its entire lifetime in the production chain, its welfare is particularly important during the pre-slaughter management period – this includes the time between transport from the ranch or feedlot of origin to the abattoir through the stunning or slaughter process. During the pre-slaughter period, cattle are exposed to a wide range of novel stimuli (e.g., mixing with other animals, interaction with animal handlers, new environments); consequently, cattle may become stressed or fatigued, potentially resulting in compromised welfare and subsequent adverse meat quality outcomes (Wigham et al., 2018).

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[1]Sullivan P, Davis M, Bretón J, and Edwards-Callaway L (2022). Investigating the impact of pre-slaughter management factors on meat quality outcomes in cattle raised for beef: A scoping review. *Front. Anim. Sci.* 3:1065002. doi: 10.3389/fanim.2022.1065002.

A large body of work on the effect of different pre-slaughter management factors on various meat quality outcomes exists (Ferguson and Warner, 2008; Schwartzkopf-Genswein et al., 2012; Losada-Espinosa et al., 2018); still, challenges persist for examining the impacts of pre-slaughter stressors on meat quality. These challenges are not only due to the highly variable transport, handling, and lairage practices worldwide, but also because there are many intrinsic and extrinsic factors that influence meat quality outcomes. Pre-slaughter factors reported to impact meat quality outcomes include weather (Scanga et al., 1998), transport duration (Jones and Tong, 1989; Gallo et al., 2003), animal handling practices (Warriss, 1990; Frimpong et al., 2014), and lairage duration (Loredo-Osti et al., 2019; del Campo Gigena et al., 2021; Steel et al., 2021); furthermore, how these factors interact with each other to influence product quality is complex, and thus, the nature of these relationships have not been fully elucidated.

Carcass bruising and dark cutting beef, otherwise known as dark, firm, and dry (DFD) beef, are two quality defects of particular note due to their industry prevalence and implications for economic loss; in the United States, the 2016 National Beef Quality Audit (NBQA) reported 38.9% of fed steers and heifers, 42.9% of bulls, and 64.1% of cows were bruised (Eastwood et al., 2017). In Mexico, Miranda-de la Lama and others (2012) reported a 92% bruise prevalence among a population of over 8,000 beef carcasses; additionally, South American studies have reported similarly high numbers (60%, Huertas et al., 2015; 89.1%, da Silva Frasão et al., 2014), highlighting that carcass bruising is not an issue unique to North America. Yet, industry reports have estimated that carcass bruising costs the U.S. beef industry $35 million each year (Lee et al., 2017) – this significant monetary loss warrants further investigation into how and when bruising occurs along the supply chain.

Additionally, findings from 2016 NBQA identified that 1.9% of carcasses exhibited dark cutting (Boykin et al., 2017) – a costly quality defect that can result in decreased consumer eating satisfaction (Wulf et al., 2002; Węglarz, 2011; Grayson et al., 2016; Loudon et al., 2019) and a shortened shelf-life (Newton and Gill, 1981). Congruent with the findings of the 2016 NBQA, Steel et al. (2021) reported a dark cutting frequency of 2.8% in Australian beef carcasses; in contrast, Loredo-Osti et al. (2019) and Pérez-Linares et al. (2015) in Mexico and Arik and Karaca (2017) in Turkey reported substantially higher numbers (13.45%, 39%, and 24.78%, respectively). The variation in the frequency of dark cutters across the scientific literature suggests that the dark cutting condition is multifaceted, and various animal characteristics, production systems, and management factors may impact an animal's physiological reactions to stress, postmortem metabolism, and subsequently, meat quality. Furthermore, the variation in dark cutter frequency may also be explained by thresholds for classifying dark cutting across the literature, which also vary considerably (pH of 5.8 to 6.2, Jeremiah et al., 1991; 5.9 or greater, Ferguson et al., 2001; 6.0 or greater, Apple et al., 2006). Even still, the characteristic dark color associated with dark cutting beef is unfavorable to consumers and continues to have significant economic implications (Ponnampalam et al., 2017), accounting for a nearly 170-million-dollar loss to the United States beef industry annually (Underwood et al., 2007).

Although the pre-slaughter period is a necessary step in the food production chain and there has been a focus on minimizing animal fear and distress during this time by improving animal handling and management practices (Grandin, 2019; Edwards- Callaway and Calvo-Lorenzo, 2020; Grandin, 2020), cattle are subject to many inherent stressors during transport from farm to slaughter that have consequences for meat quality. To the authors' knowledge, a systematic review of the literature charting the impact of different pre-slaughter management practices on meat quality outcomes of beef cattle has not been published. This scoping review was conducted to investigate, synthesize, and report on the size and scope of the research evaluating management's impact during the pre-slaughter period on product quality. The research question was "How do pre-slaughter factors affect meat quality outcomes in cattle raised for beef?" For the global beef industry to continue to progress and evolve, understanding how cattle management during this important juncture in the food supply chain affects meat quality and economic outcomes is critical. Doing so will help inform industry stakeholders of best practices that will improve meat quality, enhance profitability, and ultimately, promote the sustainability of beef production globally.

This scoping review had two primary objectives: (1) to catalog pre-slaughter management factors that impact meat quality outcomes, and (2) to identify indicators used to evaluate the impact of pre-slaughter management factors on meat quality outcomes. The secondary objective was to gain an understanding of the relationship between the pre-slaughter phase and end-product quality.

2.0 Methods

Following the methodologies for performing scoping reviews first described by Arksey and O'Malley (2005) and further refined by Levac et al. (2010), as well as the reporting guidelines from the PRISMA checklist and flow diagram (Page et al., 2021), this scoping review was conducted to investigate, synthesize, and report on the size and scope of the research evaluating the impact of management factors during the pre- slaughter period on meat quality outcomes of beef cattle.

2.1 Eligibility criteria

All peer-reviewed and primary studies written in English were eligible for initial inclusion in this scoping review; no year exclusion was applied other than the default year ranges set forth by each database. The population of interest was cattle raised for beef as their primary purpose in the food supply chain during the pre-slaughter period, such as heifers and steers finished in feedlots. Cattle that became beef at some point in their lifetime, such as culled dairy cattle, were outside the scope of this particular review and therefore excluded, as meat quality is usually not a driving factor in these production systems. However, due to the highly variable nature of different beef- fattening systems globally, studies were included in the analysis when they evaluated dairy breeds and it was clear that the animals were raised for beef as their primary purpose, for example, surplus male dairy calves raised for bull beef in Spain and the United Kingdom (Rutherford et al., 2021).

For the purposes of this scoping review, the pre-slaughter period was defined as the 96 hours prior to loadout from the farm or ranch of origin through stunning or slaughter at the processing plant, which included transport and lairage, among a variety of other pre-slaughter practices and factors. Pre-slaughter factors of interest included slaughter practices, abattoir factors, feed or water management, environmental factors, handling practices, lairage practices, and transportation. Although not pre-slaughter factors, animal characteristics (e.g., breed type, sex class, animal, source, etc.), were quantified in this review due to the high proportion of studies not only reporting these population characteristics, but also acknowledging that they have some effect on cattle in the pre- slaughter period.

Articles were deemed eligible for inclusion if they met the population parameters outlined above (i.e., cattle raised for beef in the pre-slaughter period) and reported at least one meat quality outcome or carcass characteristic, or both; a key feature of a majority of the articles included in the final search was the reporting of meat quality outcomes in addition to other carcass characteristics, such as carcass weight, quality or yield grades, dressing percentage, fat thickness, and loin muscle (LM) area. The authors recognize that most of the aforementioned carcass traits will not be impacted by pre-slaughter factors, instead they are heavily influenced by animal characteristics and on-farm management practices; still, many papers reported these outcomes, so they were also quantified in this review. Meat quality outcomes of interest for this review included a wide range of outcomes. Although not an exhaustive list, the most common meat quality outcomes assessed were pH, bruising, and color. To be included in the final analysis, articles had to meet three specific criteria: (1) the population of interest (beef cattle), (2) the appropriate context (pre-slaughter period), and (3) the outcomes of interest (meat quality traits).

2.2 Search process

Three databases were used to search for all relevant articles, which included CAB Abstracts, PubMed, and Web of Science Core Collection. Filters were used in each database to further refine the search results, which included filters for peer- reviewed, English studies. The search string refinement was an iterative process that included discussion among all of the co-authors about the population, pre-slaughter factors, and outcomes of interest; this process guided the development of a comprehensive search that would capture all articles eligible for inclusion in the analysis. The final search string was developed with the guidance of a librarian knowledgeable in conducting scoping reviews. Details about the search strings for each database can be found in **Table 1**.

2.3 Selection process

Citations from all three databases were downloaded to Zotero (Zotero, Fairfax, VA), an open-source citation management software, for further appraisal by another reviewer – duplicate citations were also screened for and removed at this time. This initial appraisal involved screening titles for the three inclusion criteria. Concomitantly, articles at this stage were removed based on exclusion criteria in titles; exclusion keywords included dairy, veal, poultry, broiler, swine, pig, sheep, lamb, goat, fruit, or review.

In a subsequent round of screening, two reviewers independently screened abstracts; if there was a disagreement between the two reviewers, a consensus about whether to include or exclude the article was reached through discussion. In the rare occurrence that agreement could not be met after discussion among the two parties, a third party was consulted to make the final decision as to whether or not the article met all inclusion criteria. Lastly, the full text of each article that was kept after two screening rounds was retrieved and managed in Zotero. In the third and final round of screening, each full text of this final subset of articles was screened again for inclusion criteria by an independent reviewer. Although the search process was extensive and included relevant databases, the articles represented in the final search may not represent all relevant literature; for example, if a paper did not include relevant terms in the keywords, title, or abstract it may not have been captured in the selection process.

2.4 Data extraction process

After three rounds of screening, a final subset of articles was retrieved and managed in Zotero. One reviewer independently extracted data from each article pertinent to this scoping review's aims. A data extraction form was used to keep track of information about a multitude of parameters, including details relevant to the study location, population, pre-slaughter management factors (and at what time point they occurred, i.e., pre-transport, during transport, or at the abattoir), and meat quality outcomes or carcass characteristics.

3.0 Results

3.1 Study selection

A total of 3,747 records were obtained from three databases (CAB Abstracts, 989; PubMed, 1,713; Web of Science, 1,045) in August 2022. From those 3,747 records, 3,217 non-duplicate records were screened for inclusion in this scoping review. Citations underwent three rounds of screening in which reviewers applied inclusion and exclusion criteria to each article to determine their eligibility for inclusion in the final synthesis. After title and abstract screening, 98 full-text articles were assessed for eligibility, 13 of which were excluded for various reasons, which included wrong population, pre- slaughter management factor not assessed, non-target outcome, or non-English publication. After three rounds of screening, a total of 85 articles met the inclusion criteria and were included in the final review (n=85). Summary statistics were calculated in Microsoft Excel (Microsoft Corporation, Redmond, WA) for all variables of interest. Unless otherwise indicated, the following results are reported as (n, percentage). More detailed information regarding the citation identification, screening, and inclusion processes is included in **Figure 1**.

3.2 Study characteristics

3.2.1 Article characteristics

Of the 85 articles included in the final synthesis, 36 unique journals were represented. Meat Science was the most common journal, accounting for 28.2% (n=24) of the articles. The second most common journal was Veterinary Record (6, 7.1%), followed by Animals, Journal of Animal Science, and Livestock Science, accounting for four articles each (4.7%), or a cumulative 14.1% (n=12) of the papers. Overall, publication dates ranged from 1979 – 2022, with a median publication date of 2011. Nearly half of the papers were published in the last decade (i.e., 2012 –

2022; 42, 49.4%) and thirty-four percent of articles were published within the last five years (i.e., 2017 – 2022; 29, 34.1%). The most frequent publication dates were 2019 and 2020 (8, 9.4% and 7, 8.2%, respectively).

3.2.2 Population characteristics

Forty-one percent (n=35) of studies were conducted in the European region, followed by the South American (21, 25%), Oceanic (12, 14%), and North American (10, 12%) regions. Africa and Asia represented regions with the fewest number of studies (3, 3%; 4, 5%, respectively; **Figure 2**). The number of animals in each study varied considerably – ranging from 16 to 2.7 million cattle; 263 was the median sample size per study. Seven (8.2%) of the 85 articles reported large sample sizes (i.e., 127,838 – 2,672,223) – these were epidemiological studies that spanned multiple years and therefore included a large number of animals. Consequently, the mean was influenced by these epidemiological studies, and thus, the mean sample size did not provide an accurate representation of the average sample size; 63.5% of the studies had sample populations of less than 500.

Regarding animal-related factors, roughly half of the studies (43, 50.5%) reported a single sex class. The remaining studies reported two or more sex classes (33, 38.8%) or none at all (9, 10.6%). Bulls, i.e., uncastrated male bovines of any age including bull calves for the purposes of this review, were the most frequent sex class reported (37, 43.5%) by any paper, followed by steers (34, 40.0%), heifers (21, 24.7%), and then cows (18, 21.2%). A small subset of articles categorized cattle as either female or male with no further specifications – these accounted for 8.2% (n=7) and 10.6% (n=9) of the papers, respectively. Fifty- six percent of the articles reported using a single breed (n=48), while the remaining papers reported either two or more breeds (17, 20.0%) or did not report one (20, 23.5%). Predominantly British or Continental (24, 28.2%) and Bos indicus breeds (24, 28.2%) were the most common breed types among the 85 studies, with dairy breeds (14, 16.5%) and British or Continental crosses (13, 15.3%) included in fewer studies. Approximately ten percent (9, 10.6%) of articles reported breeds native to their respective countries, e.g., native African, Chinese, Italian, and Spanish breeds, while the fewest articles reported dairy beef crosses (4, 4.7%).

3.3 Reporting characteristics

3.3.1 Pre-slaughter management factors

A key feature of many of the studies included in this review was the reporting of multiple pre-slaughter management factors, particularly at different timepoints in the final marketing phase, for example, measuring the effects of both transport and lairage duration or handling stress at loading and unloading on "x" response variable(s). Studies were grouped by the pre-slaughter management factor they evaluated, which included eight main categories: slaughter practices (n=5), abattoir factors (n=9), feed or water management (n=9), environmental factors (n=23), animal characteristics (n=29), handling practices (n=35), lairage practices (n=36), and transportation (n=46; **Figure 3**); a total of 55 studies (64.7%) reported pre-slaughter factors in two or more of these categories. Slaughter practices included different stunning methods (e.g., electrical versus captive bolt stunning) and slaughter procedures (e.g., time between stunning and exsanguination). Abattoir factors included variables related to abattoir size and scale (Guarnido-López et al., 2022). The feed or water management category was comprised of variables relating to fasting animals prior to slaughter or providing animals with feed prior to slaughter, or both. This category also included a few studies assessing the impact of pre-slaughter administrations of a bovine appeasing substance (Cappellozza et al., 2020), glycerol (Egea et al., 2015), or other nutritional supplement on meat quality (Grumpelt et al., 2015). The next most reported pre-slaughter management category was environmental factors, which represented studies that evaluated season or weather conditions as predictors (Brown et al., 1990; Kreikemeier et al., 1998; Nanni Costa et al., 2003) or the effects of stressful conditions (e.g., noises and disturbances in the environment; Wythes et al., 1988a; Peña et al., 2014; Pighin et al., 2015; Reiche et al., 2019) on meat quality outcomes. Animal characteristics was a broad category that included animal-related factors (breed type, sex class, and horn status; Wythes et al., 1979b; Tyler et al., 1982; Fabiansson et al., 1984; Kawecki et al., 2020), as well as information relative to the animals' source, which included farm or ranch of origin (Mounier et al., 2006), marketing method (e.g., direct to abattoir versus transfer through multiple stakeholders before slaughter; Ferguson et al., 2007; Vimiso and Muchenje, 2013; Loudon et al., 2019), and production type (e.g., grass versus grain finished; del Campo Gigena et al., 2010; López-Pedrouso et al., 2020). Handling practices included factors such as prod use, handling time, or handling stress (Marıáet al, 2004; Chacon et al., 2005; Nanni Costa et al., 2005; Nanni Costa et al., 2006). Mixing animals, whether in transport or lairage, was considered a handling practice for the purposes of this review; a total of 16 papers studied the effects of mixing during the pre-slaughter period (Bartošet al., 1988; Lahucky et al., 1998; Lahucky et al., 1999). The lairage practices category included lairage duration (n=34) and pen density (n=4; Mach et al., 2008; Hoffman and Lühl, 2012; Romero et al., 2017; Loredo-Osti et al., 2019); just one study in this category assessed the effect of water showering in lairage during cold weather (n=1; Zhao et al., 2022). A notable gap in this body of work is the lack of research focused on heat mitigation during lairage. The transportation category included the most studies and included factors related to trailer motion (Kehler et al., 2022), loading density, transport distance, transport duration (Villarroel et al., 2003a; Villarroel et al., 2003b; Polkinghorne et al., 2018), transport method (e.g., truck, rail, boat, walking, etc.), and vehicle type (Silva et al., 2016;

Mendonça et al., 2018; Mendonçaet al., 2019; Ferreira et al., 2020). The majority of the papers in this category (37 of the 46 papers; **Figure 3**) evaluated transport distance or duration, or both.

3.3.2 Meat quality outcomes

Meat quality is a multifaceted term that encompasses both objective and subjective measurements; Becker (2002) categorizes meat quality outcomes into two broad categories: quality attributes and quality characteristics. Quality attributes are features of the meat that impact consumer satisfaction, such as flavor, tenderness, and juiciness, while quality characteristics are features that can be objectively measured, such as water holding capacity, quality grade, and instrumental color, (Becker, 2002). This particular scoping review includes a breadth of quality attributes and characteristics, some of which have been demonstrated to be influenced by factors in the pre-slaughter period (e.g., dark, firm, and dry beef), while others are influenced very little by pre-slaughter stress (e.g., quality and yield grade), instead animal characteristics and feeding management plays a greater role in these outcomes. Therefore, although this review quantified many aspects of meat quality in the literature, this review's main objective was to focus on the meat quality outcomes most impacted by the pre-slaughter period.

Studies were grouped by the meat quality outcomes they evaluated, which included eight major categories: sensory traits, cooking loss, water-holding capacity (WHC), tenderness, carcass traits, color, bruising, and pH (**Figure 4**); the majority of the studies (51, 60.0%) reported two or more of these categories. The most frequently assessed meat quality outcome in any of the studies was pH (n=59; measured at approximately 24 hours post-mortem by the vast majority of the studies), followed by bruising (n=35), and color (n=30). The carcass trait category, reported in 21 studies, included a variety of carcass characteristics, such as hot carcass weight (HCW), dressing percentage, carcass fat (i.e., carcass fat score, fat thickness, and rib fat), LM area, quality grade, and yield grade. Instrumental tenderness was also evaluated in 21 studies, followed by WHC (n=13), cooking loss (n=12), and sensory traits (i.e., consumer and trained sensory panels; n=9; **Figure 4**). **Figure 5** shows the breakdown of meat quality outcomes which were assessed using predictors in each phase of pre- slaughter management – the three phases were: pre-transport (i.e., up to 96 hours prior to loading), during transport (i.e., total time in transport, including periods of rest), and at the abattoir (i.e., from unloading at the abattoir through stunning). Muscle pH was most commonly assessed with predictors at the abattoir (n=37), followed by the transport (n=31) and pre-transport (n=11) phases. Conversely, the effects of transportation were most commonly evaluated on bruising (n=23) with the fewest number of studies assessing pre-transport factors on the incidence of carcass bruising (n=8). A consistent trend across all of the categories was that there were relatively few studies evaluating the impact of pre-transport factors on meat quality. The remaining six categories (color, carcass traits, tenderness, WHC, cooking loss, and sensory traits) regularly reported predictors in the "at the abattoir" phase more than any of the other two phases.

Due to the variable methods for measuring carcass bruising and inconsistent reporting of results, only a subset of studies that reported bruising prevalence by a percentage of the population is depicted in **Table 2**. Additionally, some studies, such as Miranda-de la Lama et al. (2012) and Eastwood et al. (2017), simply benchmarked bruising prevalence in a given population and did not assess the effect of a specific pre-slaughter parameter on bruising – these studies were excluded from **Table 2**.

In this subset of papers (n=21), bruise prevalence ranged from 8.6 percent to 100 percent of the populations of interest with a mean prevalence of 61.3 percent. Overall, bruise prevalence was high across all of the studies and varied by region, breed type, and sex class (**Table 2**).

Moreover, the large variation in bruising prevalence across studies may reflect differences in methodologies for measuring carcass bruising, which differed across studies.

4.0 Discussion

4.1 Main findings

The primary objectives of the current study were to catalog pre-slaughter management factors that impact meat quality outcomes and to identify indicators used to evaluate the impact of pre-slaughter management factors on meat quality outcomes. The secondary objective was to gain an understanding of the relationship between the pre-slaughter phase and end- product quality. To the authors' knowledge, this is the first global and comprehensive review of the scientific literature on the impacts of pre-slaughter management practices on meat quality outcomes for beef cattle. A total of 85 peer-reviewed journal articles were identified through a systematic search for primary studies evaluating the impact of pre-slaughter management factors on meat quality outcomes and carcass characteristics.

4.1.1 Global implications

Overall, studies assessed many different pre-slaughter management factors that encompassed all facets of this terminal step in the production chain – ranging from hours or days pre-transport (mixing groups of cattle up to 96 hours pre- transport, Wythes et al., 1979a; administering glycerol 24 hours prior to slaughter, Egea et al., 2015; fasting cattle for 48 hours prior to transport, Dodt et al., 1979) up to the time of slaughter (pre-slaughter restraint procedures, Mpamhanga and Wotton, 2015; stunning methods, Önençand Kaya, 2004; Barrasso et al., 2022). Overall, the range of the entire pre-slaughter period varied greatly across studies, ranging from just a few hours to multiple days in length. The highly variable nature of pre- slaughter factors reported in the literature is reflective of the diversity in beef production systems globally, which include variable animal characteristics, environmental conditions, and consumer demands (Gonzalez et al., 2022). Due to these vast differences in beef production systems, studies in different geographic regions are designed to address system-specific challenges which may not be prioritized in or applicable to other areas in which cattle management differs. European and South American countries were significantly represented in this review; cumulatively, these regions comprised nearly two-thirds of the studies. This is indicative of the established beef production systems in Europe and South America – Brazil is ranked second and the European Union is ranked third in global beef production (Gonzalez et al., 2022). Additionally, South America exports the most beef globally (OECD-FAO, 2022); their responsibility to meet the expectations of high animal welfare and meat quality standards of their global trade partners is a potential reason for the extensive literature in this area. Moreover, European consumers increasingly value animal welfare; in 2016, more than half of European citizens surveyed expressed a strong concern for animal welfare (European- Commission, 2016). Historically, this increased concern and awareness of well-being of food animals has dictated demand for welfare-friendly products and influenced on-farm management practices (Veissier et al., 2008; Miranda-de la Lama et al., 2017; Alonso et al., 2020); therefore, the body of work from Europe was expected given their long-standing and robust animal welfare standards and guidelines. Contrarily, Asian and African countries were under-represented, accounting for just 8% of the studies. This under- representation may be due to the lack of substantial exports, critical harvesting capacity, and consumer demand for animal welfare. China was the world's largest beef importer in 2021 (Gonzalez et al., 2022), which potentially impacts the focus on exploring impacts on meat quality within Chinese production systems. Additionally, the harvesting capacity in many African countries is currently underdeveloped, which could contribute to the relatively lower numbers of papers found in these regions. However, the authors anticipate that as these countries' beef production systems continue to grow and evolve to meet increasing consumer demands concerning supply and animal welfare, so too will the body of work on how aspects of humane animal handling and care impact meat quality.

4.1.2 Muscle pH

Commercial transportation of livestock to slaughter has continually been identified as a factor that has implications for animal welfare and meat quality outcomes (Tarrant, 1990; Ferguson and Warner, 2008; Schwartzkopf-Genswein et al., 2012); therefore, it is not surprising that the majority of papers included in this review evaluated the effect of transport-related factors on meat quality outcomes. In total, 25 papers assessed the impact of transport distance or duration on pH; of those 25 papers, only seven observed that as cattle traveled for longer distances or durations, muscle pH increased (1 hour versus 24 hours, Tarrant et al., 1992; 92 minutes versus 265 minutes, Marenčićet al., 2012; 75-130 km versus 180-250 km, Silva et al., 2016; less than 125 km versus 300 km, Arik and Karaca, 2017; 366 km versus 1012 km, Chulayo and Muchenje, 2017; 7-10 hours versus 12-15 hours, Romero et al., 2017; 3 hours versus 12 hours, Burns et al., 2019). The remaining subset of papers (n=18) reported no significant findings between distance travelled and muscle pH (see for example, Marıáet al., 2003 and Lacerda et al., 2021). The variation in transport times included in this review represents both the highly variable transport practices and regulations between different geographical locations (Twenty- Eight Hour Law, 1994; CARC, 2001; Council Regulation, 2005).

Under conditions of high metabolic demand, i.e., chronic pre-slaughter stress, initiation of the sympathetic nervous system drives the antemortem breakdown of muscle glycogen, disrupting the muscle's normal postmortem metabolism and thus reducing pH decline. This cascade of events results in a higher ultimate muscle pH producing a lean with a characteristic dark, purplish-red color; this combination of parameters results in what is referred to as dark cutting beef (DCB). The major challenge associated with evaluating and managing the dark cutting condition in cattle is that the cause of DCB is multifactorial, and factors contributing to its prevalence are found throughout the supply chain, beginning with on-farm management and ending with lairage at the abattoir. Not only are cattle subjected to novel humans, animals, and environments during this time, but they may also experience social disruption, feed and water deprivation, and weather extremes, among various other stressors (Ferguson and Warner, 2008; Edwards- Callaway and Calvo Lorenzo, 2020); thus, attributing the occurrence of dark cutting to a single pre-slaughter factor is difficult and may explain the variable results demonstrated across the scientific literature. Additionally, the inconsistent findings may be, in part, due to the range of breed types and sex classes evaluated in the literature, as previous research has reported that animal-related factors (i.e., breed type, sex class, age) may also influence the incidence of dark cutting (Scanga et al., 1998; Page et al., 2001). There are many other quality defects associated with DCB aside from its characteristic dark color, many of which were assessed in studies included in this review – these defects include reduced tenderness (Carrasco- Garcıáet al., 2020; Sierra et al., 2021), higher water holding capacity (Arik and Karaca, 2017), and poor palatability (Węglarz, 2011; Loudon et al., 2019). In commercial settings, lean color is most often assessed visually due to its association with high muscle pH (Page et al., 2001), however, more objective measures for classifying DCB have been identified, including instrumental color and pH measurements which are more often used in research settings. The authors suggest that a potential reason for the relatively greater number of studies that assessed pH and bruising were due to the ability for researchers to collect these measurements in a plant setting, compared to other instrumental measurements for tenderness and cooking loss, for example, which require samples to be taken from the plant for further laboratory analysis.

A consistent trend across all of the papers was using pre-slaughter factors at the abattoir, i.e., the time from unloading at the plant through stunning or slaughter, to evaluate meat quality outcomes. As an example, the effect of lairage duration on muscle pH was a concept that was extensively explored. The relatively high number of studies assessing the effect of lairage practices on meat pH is not surprising given the opportunity for cattle to be exposed to a multitude of novel stimuli during this time; in holding pens, cattle may be mixed with unfamiliar animals, deprived of feed for extended periods, exposed to variable weather conditions, and experience increased handling intensity. Lairage conditions and duration tend to vary by region; for example, fed cattle in North American plants are typically processed on their arrival day and spend relatively short periods in holding pens (personal communication, L.N. Edwards-Callaway), while Oceanic and South American countries tend to have more extended lairage periods to allow animals to rest after long transport (Ferguson and Warner, 2008). This concept was reflected in the studies presented in this scoping review, as conditions and duration of lairage varied substantially across regions, e.g., lairage duration ranged from hours (del Campo Gigena et al., 2021) to multiple days (Liotta et al., 2007). Overall, many papers assessed the effect of lairage duration on muscle pH, of which only three evaluated the impact of pen density on the quality outcome. The relatively few numbers of studies assessing pen density on pH was surprising as overcrowding cattle in lairage pens may impact their ability to access water, comfortably lie down, and move around freely – all of which could have an impact on muscle pH if cattle are overcrowded for extended periods of time and unable to rest and rehydrate. Some studies discovered a significant association between longer lairage times and high muscle pH (Wythes et al., 1988b; Strappini et al., 2010; Loredo-Osti et al., 2019; Steel et al., 2021) while others discovered the opposite (i.e., as lairage duration increased, pH decreased, Warriss et al., 1984; Bartoš et al., 1993; Kuzmanovic and Elabjer, 2000; Teke et al., 2014). A potential explanation for the studies that reported that longer lairage times lowered muscle pH is that cattle may have been able to restore their glycogen stores partially or completely before slaughter, therefore avoiding the dark cutting condition; however, research has demonstrated that the glycogen repletion rate in muscles of stressed cattle is slow (i.e., 1.5 mmoles/g/day; Tarrant, 1989). In order for cattle to replenish glycogen stores antemortem certain conditions need to be met, such as resting and refeeding, which has been shown to increase glycogen repletion to 6.3 mmoles/g/day (Tarrant, 1989) and subsequently, lower muscle pH (Shorthose et al., 1972; Wythes et al., 1980; Warriss et al., 1984). Even though there is conflicting research on the topic, existing evidence suggests that there could be an association between lairage time and dark cutting carcasses (i.e., the incidence of dark cutting increases with longer lairage). Still, more research is needed to fully understand this relationship. We postulate that since dark cutting is the result of chronic pre-slaughter stress, it may be possible that although animals become agitated and fatigued during this period, their stressors are not intense enough to drive the depletion of muscle glycogen and thus, contribute to the occurrence of dark cutting. Taken together, the existing body of work on lairage management on meat quality outcomes warrants future investigation into what is an optimal duration of rest, recognizing that this may be influenced by many animal and environmental factors; additionally, certain slaughter plants may not be able to accommodate ideal lairage times due to both purchasing and scheduling logistics, and facility limitations.

4.1.3 Carcass bruising

From the 15 papers that assessed the effect of transport distance or duration on carcass bruising, eight papers reported significant findings (i.e., longer transport increased bruising incidence; Jarvis et al., 1995; McNally and Warriss, 1996; Hoffman et al., 1998; Vimiso and Muchenje, 2013; Silva et al., 2016; Mendonça et al., 2018; Bethancourt-Garcia et al., 2019a; Brito et al., 2019). Similar to evaluating the effects of transport on muscle pH, ample challenges exist for assessing and managing bruising as there are multiple opportunities for bruising to occur along the supply chain (e.g., mixing cattle with different horn statuses, Shaw et al., 1976; Wythes et al., 1979b; high stocking densities, Tarrant et al., 1988; Brennecke et al., 2020; Ferreira et al., 2020; rough pre-slaughter handling conditions, Jarvis et al., 1995; McNally and Warriss, 1996; Mendonça et al., 2018).

Bruising is a quality issue that also has a significant welfare component; not only is bruised meat removed from the carcass at the slaughter plant and not used for human consumption, but also animals experience some level of fear, distress, or pain during an event that would cause an impactful bruise (Edwards- Callaway and Kline, 2020). The loss from bruising comes from the actual reduction in yield from bruise removal, the devaluing of cuts that may have been partially impacted by a bruise, the increased labor required to remove the bruises during processing, and the reduced efficiency associated with slower line speeds (McNally and Warriss, 1996; Edwards-Callaway and Kline, 2020). The economic impact of bruising is substantial and has been estimated to cost the beef industry in the millions or billions of dollars annually depending on the country (Huertas et al., 2015; Henderson, 2016; Lee et al., 2017), thus incentivizing producers and processors to focus on identifying management practices that could reduce bruise prevalence.

The prevalence of bruising across studies is highly variable, ranging from 8.6% to 100% (**Table 2**). Although there were several studies reporting bruise frequency of less than 25% for at least one population group (Bethancourt-Garcia et al., 2019a, b; Strappini et al., 2010; Brito et al., 2019), the majority of studies reported relatively high bruise prevalence with some reporting over 90% bruising (Jarvis et al., 1995; Jarvis et al., 1996; Huertas et al., 2018; Brennecke et al., 2020; Ferreira et al., 2020) in their study populations. Although not all bruises are the same size or severity, these bruise frequencies are substantial and cause concern both from an economic and welfare standpoint. Interestingly, the majority of studies assessing pre-slaughter management on bruising were conducted in South America, and although the impact of transportation characteristics on bruise prevalence is not consistent across studies, it is worth considering the transport conditions in South American countries. Although published statistics on average transport distance, routes, and times across countries are scarce, in South America, most beef production systems are pasture-based (Gonzalez et al., 2022), and these more remote or rural regions of cattle production could have challenges with transport infrastructure (McManus et al., 2016) that may have a downstream impact on bruising.

Bruises vary in size, shape, location, pattern, and severity which all contribute to determining what could have caused the injury (Edwards-Callaway and Kline, 2020). It is challenging to compare bruise prevalence across studies primarily due to the range of methodologies used to quantify and characterize bruising. Often studies will report the presence or absence of bruises (i.e., the frequency of bruising) in addition to the location on the carcass (Kline et al., 2020; Teiga-Teixeira et al., 2021). Many studies will use some type of carcass map in order to identify the location of the bruise (Strappini et al., 2012; Romero et al., 2013; Mendonça et al., 2019; Bethancourt-Garcia et al., 2019a; Bethancourt-Garcia et al., 2019b; Kline et al., 2020);

although these maps do vary, the general concept of dividing the carcass into clear regions remains consistent across studies. In order to estimate the economic loss from bruising it is necessary to have some evaluation of size and weight of the bruise in addition to location. The NBQA has utilized a bruise scoring system based on a visual estimation of the weight of the bruise using a 10-point scale which are collapsed into broader classifications (i.e., minimal, major, critical, and extreme; Texas A & M University, 2016). Another commonly used scoring system is the Australian Carcass Bruise Score System (Anderson and Horder, 1979) which uses an estimate of bruise diameter to calculate a surface area of the bruise which is then categorized as slight, medium, or heavy; many studies in this scoping review used this methodology for quantifying carcass bruising (Wythes et al., 1979a; Wythes et al., 1979b; Wythes et al., 1985; Tarrant et al., 1988; Wythes et al., 1989; Tarrant et al., 1992; Romero et al., 2013; Vimiso and Muchenje, 2013). With any of these described systems it is important to assess interobserver reliability as many of the systems require using visual observation to make estimates of length or weight which can be challenging. Additionally, some of the systems are highly complicated, and although manageable in research settings, they would not necessarily be beneficial in a commercial setting to track bruising internally. Because bruising can only be assessed during post-mortem processing, studying factors that may impact bruising is challenging; numerous observations must be made ante-mortem and individual animal or group (i.e., lot) information must be tracked through the slaughter process, which can require substantial data collection inputs depending on the facility. Some studies have measured bruise age by visual appraisal using the method described by Gracey et al. (1999) to determine when during the pre-slaughter process bruising could have occurred (Hoffman and Lühl, 2012; Vimiso and Muchenje, 2013; Mpakama et al., 2014). Although bruise color does change with age, visual appraisal may not be the preferable method of assessment due to low reliability and accuracy (Strappini et al., 2009).

4.1.4 Beef sensory quality

Fulfilling our second primary objective, which was to identify indicators used to evaluate the impact of pre-slaughter management factors on meat quality outcomes, the authors discussed in depth the implications of the pre-slaughter period on the most commonly reported meat quality outcomes in the literature – carcass bruising and postmortem muscle pH. The remaining meat quality categories, tenderness, water-holding capacity, cooking losses, and sensory traits, did not warrant extensive discussion in this review as relatively few studies overall assessed the effects of pre-slaughter factors on these specific outcomes. However, we would be remiss not to discuss that tenderness is one of the most important drivers of beef palatability, alongside juiciness and flavor, (O'Quinn et al., 2018), which can be impacted by an abundance of pre-harvest (e.g., breed and age of animal, production system, stress prior to harvest, etc.) and post-harvest (e.g., in-plant practices, ageing method and length, packaging system, cooking method, etc.) factors (Santos et al., 2021). In the subset of papers that measured instrumental tenderness, there was conflicting results on the influence of the pre-slaughter period on tenderness; for example, some papers observed that longer transport decreased tenderness (Warner-Bratzler shear force, Guarnido-López et al., 2022; trained sensory panel, Villarroel et al., 2003a), while more studies observed no effect of transport on tenderness at all (Marıáet al., 2003; Ferreira et al., 2006; Polkinghorne et al., 2018; Lacerda et al., 2021); there was a similar trend for the effect of the lairage period on tenderness. Due to the multifaceted influence of pre- and post-harvest factors on tenderness, quantifying the effect of the pre-slaughter period on this quality attribute is challenging – a common theme discussed in many of the papers assessing the impact of pre- slaughter stressors on tenderness included in this review (see for example, Ferguson et al., 2007; Gruber et al., 2010; Polkinghorne et al., 2018). Taken together, this body of work suggests that the pre-slaughter period has a relatively minor influence on beef tenderness (independent of differences in muscle pH) and that other pre-harvest variables, such as production type (e.g., grass- fed vs grain fed), breed or breed-type (e.g., Bos indicus vs Bos Taurus), supplements (e.g., beta-agonist-fed vs beta-agonist- free), and age of animal, likely contribute more significantly to measurable differences in tenderness.

4.1.5 Gaps in knowledge

As demonstrated by this review, a critical gap in research exists regarding the effect of heat mitigation during lairage on various outcomes; from the 85 studies included in this review, only one quantified the effects of water showering in lairage on beef meat quality (Zhao et al., 2022); it is important to note that this study was conducted in cold weather and does not necessarily contribute to the body of work on heat mitigation. Little industry information exists quantifying heat mitigation strategies and effectiveness throughout the beef supply chain. However, a recent survey of beef cattle processors characterized the use of different heat abatement strategies at slaughter plants in the United States; Davis and others (2022) reported that sprinklers or misters were most commonly used among beef processors. When asked if heat mitigation provides benefits during lairage, one survey respondent stated that the use of heat mitigation results in ”quality benefits such as reduced dark cutters,” while others stated that heat abatement results in ”less stress” and cattle that ”are more comfortable” (Davis et al., 2022); this preliminary data from the United States suggests that processing plants both appreciate and value the benefits of heat mitigation on animal welfare and quality outcomes, which is not congruent with the available literature in this area. In a comprehensive review of the impacts of shade on cattle well-being, Edwards-Callaway and others (2021) highlighted the need to quantify the effects of shade on cattle at packing plants due to the importance of heat stress to animal welfare and economic performance outcomes. While no studies to date have been performed to assess the impact of heat mitigation during lairage on meat quality outcomes, multiple studies have assessed the use of shade in feedlot settings and found that shaded cattle experienced less heat stress and better performance outcomes (e.g., higher dry-matter intake, average daily gain, and final body weight) than unshaded cattle (Mitlöhner et al., 2002; Sullivan et al., 2011; Hagenmaier et al., 2016). More strikingly, Mitlöhner et al. (2002) found that shaded feedlot cattle experienced an approximate 50% reduction in dark cutting compared to unshaded cattle. Additionally, several studies have reported that weather significantly affects the occurrence of dark cutters (Scanga et al., 1998; Marenčićet al., 2012; Steel et al., 2021), which warrants further consideration into how implementing heat abatement strategies during lairage, while even in the short-term, may impact meat quality. The authors anticipate that the focus of heat mitigation on cattle well-being and meat quality will begin to intensify as global climate change continues to evolve and have ramifications for extreme weather events, drought, and cattle death loss associated with extreme heat stress.

4.1.6 Limitations

Three electronic databases were used to search for literature pertaining to the impact of pre-slaughter management factors on meat quality outcomes. The final search string was developed with the guidance of a librarian knowledgeable in conducting scoping reviews, thereby increasing the quality and rigor of this particular review. A limitation of this study, however, is that a single reviewer screened all of the full-text articles for inclusion criteria, potentially introducing bias into the included papers. Additionally, the population of interest for this review was restricted to cattle in the food supply chain destined to become beef as their primary purpose, which limited the scope of our search. For example, in the United States, the cull cow market represents a significant component of the beef supply chain accounting for nearly 20% of the U.S. beef supply annually (USDA-NASS, 2021), yet we did not capture this important population. The welfare of cull cattle in the final marketing phase is of particular concern since they are exchanged through multiple stakeholders and travel longer distances on their journeys to specialized processing plants (USDA, 2018; Edwards-Callaway et al., 2019). Similar challenges associated with the transport of cull cattle to slaughter have been identified in Europe (Dahl- Pedersen et al., 2018) and Canada (Stojkov et al., 2020). Due to the difference in welfare challenges and meat quality priorities, this type of animal was not included in this scoping review. Future research is needed to understand the impacts of the pre-slaughter phase on this more vulnerable population. Lastly, the exclusion of non-English studies restricted the scope of this review by potentially precluding important research that has contributed to key findings in this body of work.

4.1.7 Conclusions

Following the methodologies for performing scoping reviews first described by Arksey and O'Malley (2005) and further refined by Levac et al. (2010), as well as the reporting guidelines from the PRISMA checklist and flow diagram (Page et al., 2021), this scoping review was conducted to investigate, synthesize, and report on research evaluating the impact of management factors during the pre-slaughter period on meat quality outcomes for beef cattle. Most of the research in this space has assessed the effects of transportation, lairage, and handling practices on a suite of meat quality outcomes, primarily muscle pH, bruising, and color. However, the complexity of the pre-slaughter period poses many challenges for assessing and managing meat quality issues associated with stress before slaughter. Except for bruising (which was mainly evaluated with predictors related to transport), studies evaluated the remaining meat quality categories with predictors at the abattoir (e.g., lairage duration and density, slaughterhouse handling practices, mixing groups of cattle, etc.). A common trend across all categories was that relatively few studies evaluated the impact of pre-transport factors on product quality. The substantial variation in findings across all the studies included in the review and inconsistent reporting of those results is evidence of the challenges associated with quantifying the impact of the pre-slaughter period on meat quality. Future research should consider implementing large-scale research endeavors to better account for variations in animal characteristics and management practices so that the relationship between management during the pre-slaughter period and meat quality outcomes may be more fully elucidated; charting the relevant literature's main findings and research gaps is an important step towards this goal.

Table 1. Database and search string information for a scoping review on the impact of pre-slaughter management factors on meat quality outcomes in cattle raised for beef.

Database	Interface	Dates Included[1]	Search Terms (used in all databases)
CAB Abstracts	CABI	1973-2022	All fields = (fed OR native OR cattle OR heifer OR steer OR beef OR "beef cattle" OR "fed cattle" OR "fed beef" OR "grain-fed beef" OR "grain-fed cattle") AND ("preslaughter management" OR pre slaughter OR preslaughter OR pre-slaughter OR slaughter OR antemortem OR harvest OR pre harvest OR preharvest OR pre-harvest OR abattoir) AND (transport* OR handling OR mitigation OR management OR weather OR lairage OR pens OR "holding pens") AND ("meat quality" OR quality OR "dark cut*" OR pH OR "carcass trait*" OR "carcass characteristic*" OR performance OR bruis* OR carcass*) NOT (dairy OR veal OR poultry OR broiler* OR swine OR pig* OR sheep OR lamb* OR goat* OR fruit)
PubMed	NCBI	1950-2022	
Web of Science Core Collection	Web of Science	1945-2022	

[1]A year exclusion was not applied for any of the three databases. The date ranges depicted above represent each database's respective preset year range.

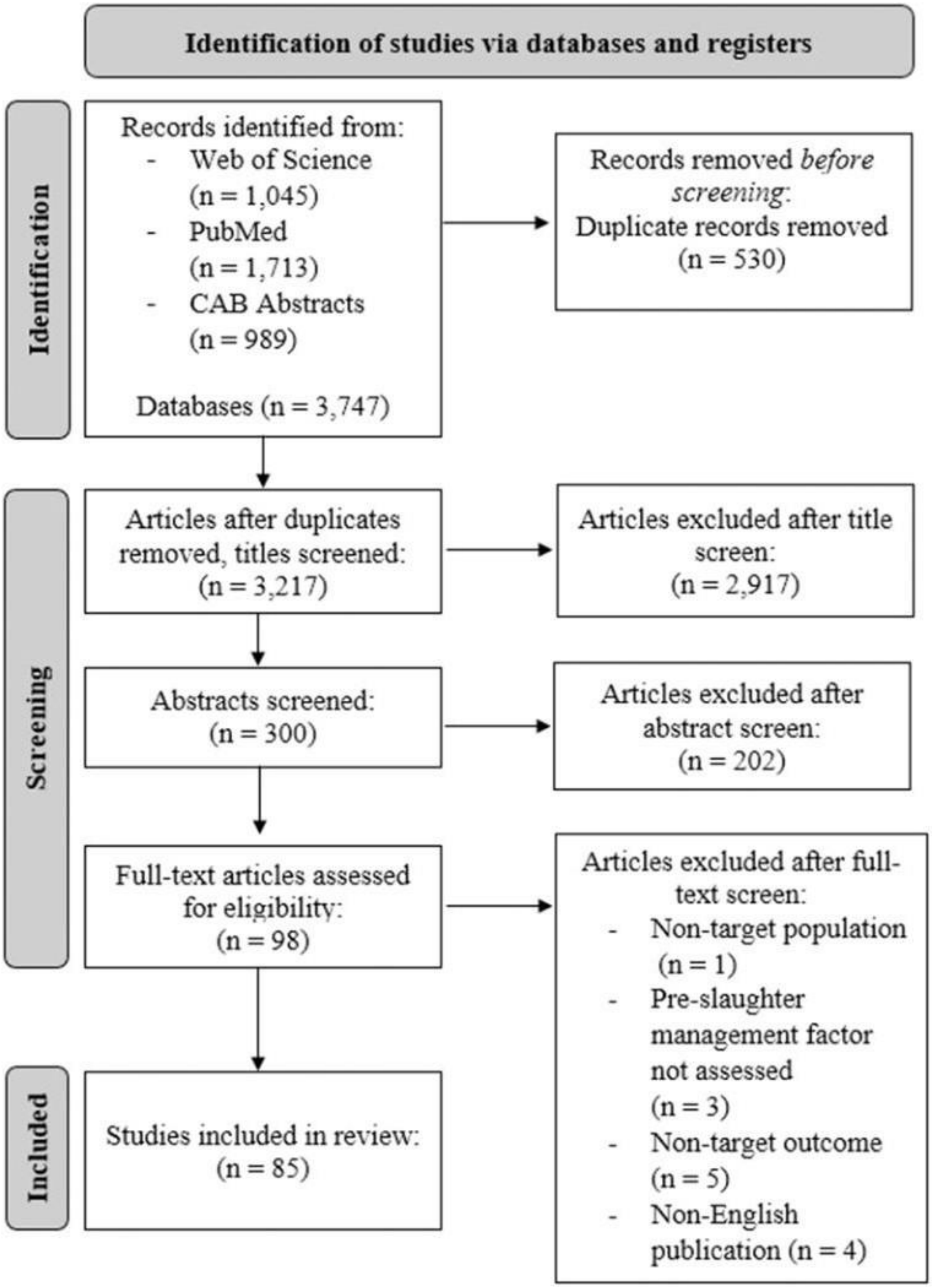
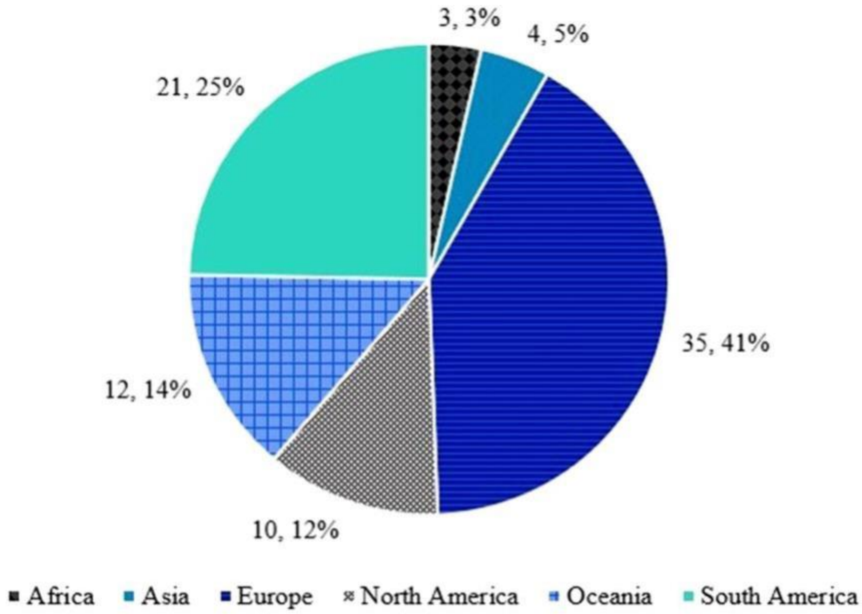


Figure 1. A PRISMA flow chart depicting the inclusion and exclusion of articles through three rounds of screening. The final number of articles included in the review is also represented.



[1]Geographic regions were adapted from regional groups determined by the U.S. Department of Homeland Security's Office of Immigration Statistics.

Figure 2. Number of studies by geographic region[1] (*n*=85). Results are reported as (*n*, percentage).

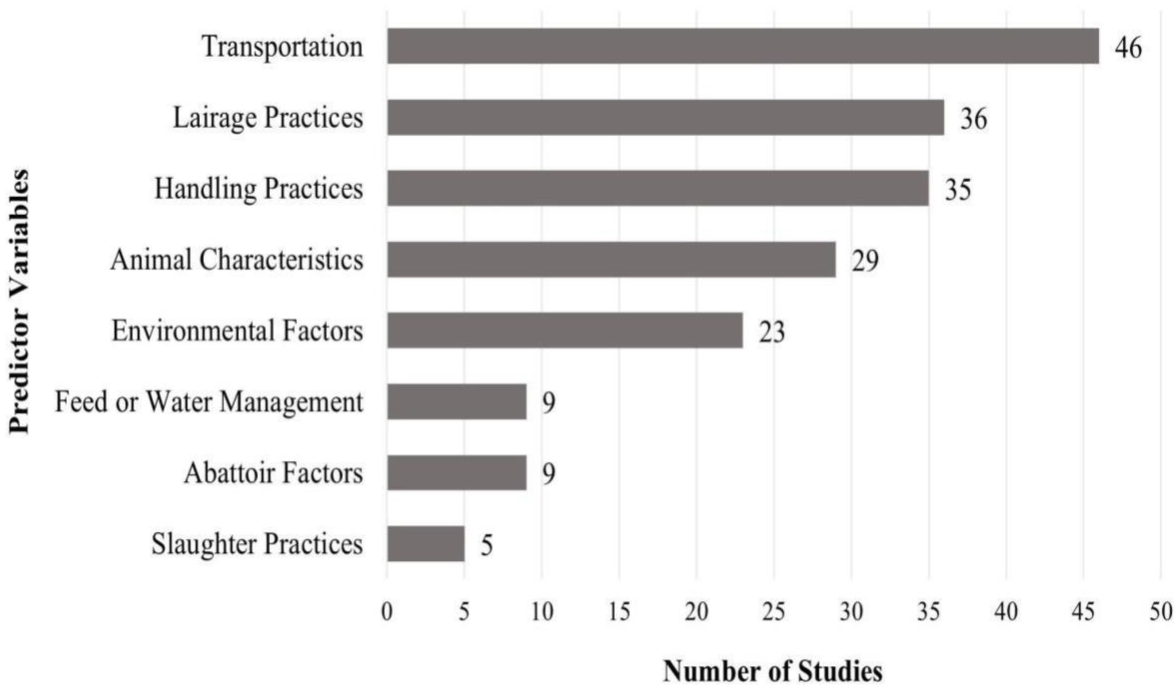


Figure 3. Number of studies that reported pre-slaughter management factors as predictors for meat quality outcomes (*n*=85). Pre-slaughter management factors were categorized into eight broad categories, including slaughter practices, abattoir factors, feed or water management, environmental factors, animal characteristics, handling practices, lairage practices, and transportation. Some studies may have researched more than one pre- slaughter factor.

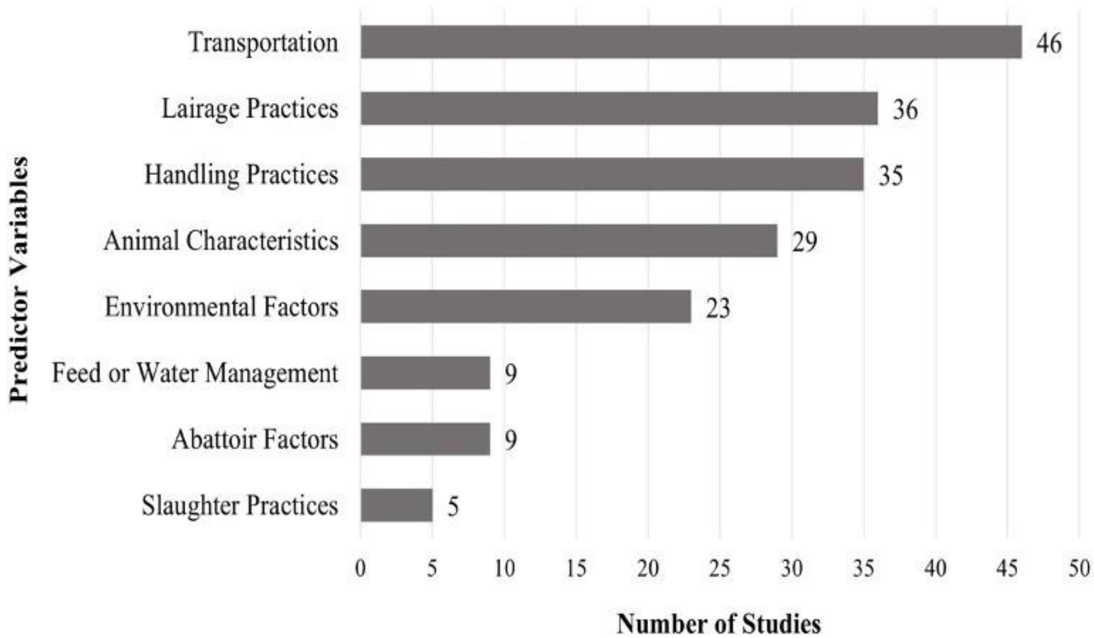


Figure 4. Number of studies that reported meat quality outcomes in response to pre-slaughter management factors (*n*=85). Meat quality outcomes were categorized into eight overarching categories, including sensory traits, cooking loss, water holding capacity, tenderness, carcass traits, color, bruising, and pH. Some studies may have measured more than one outcome.

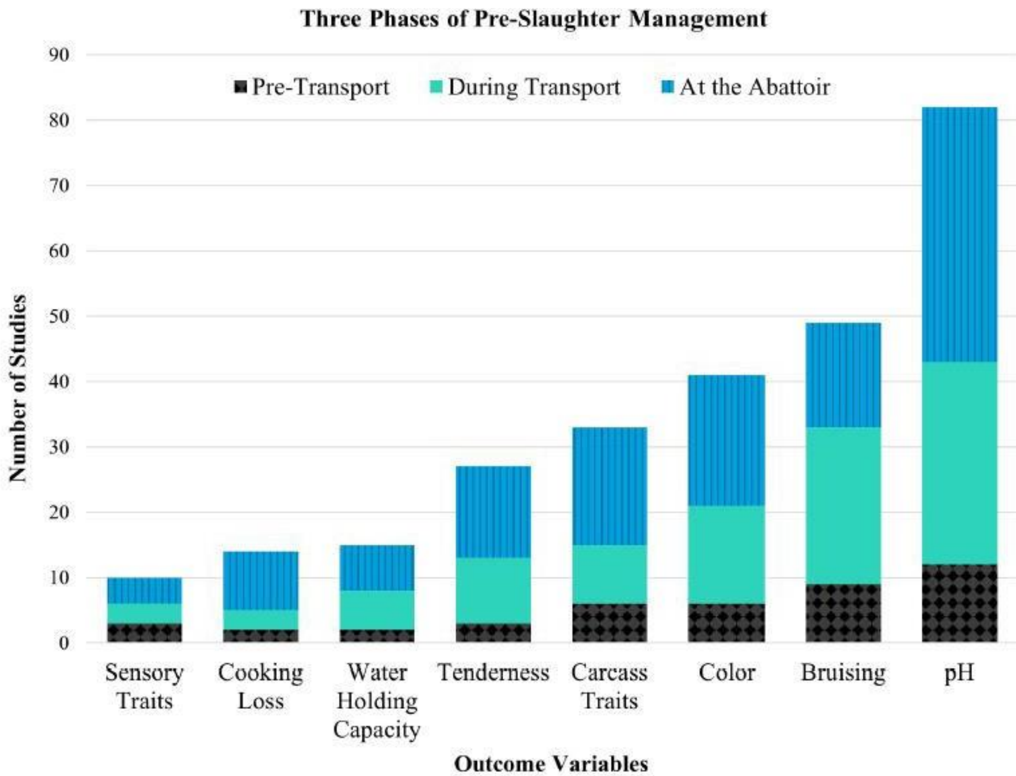


Figure 5. Number of studies that reported meat quality outcomes by using factors in each phase of pre-slaughter management (*n*=85). Some studies may have researched more than one pre-slaughter factor and measured more than one outcome.

Table 2. Bruise prevalence by region, breed type, and sex class (*n*= 21).

Reference	Region	Breed Type	Sex Class(es)[1]	Bruise Prevalence, (%)[2]	n, total[3]
Bethancourt-Garcia et al., 2019a[4]	South America	Bos indicus	Cows, heifers, steers	17.2, 38.6	154,100
Bethancourt-Garcia et al., 2019b[4]	South America	Bos indicus	Cows, heifers, steers	20.9, 79.1	154,100
Brennecke et al., 2020	South America	Bos indicus	Heifers, steers	87, 100	270
Brito et al., 2019	South America	Bos indicus	Heifers, steers	18.4, 70.6	414
Carrasco-García et al., 2020	North America	Bos indicus	Steers	81	448
da Silva Frasão et al., 2014	South America	—	Cows	89.1	320
del Campo Gigena et al., 2021	South America	British or continental, Bos indicus	Steers	48.3	60
Ferreira et al., 2020	South America	Bos indicus	Heifers, steers	96.1, 100	701
Hoffman et al., 1998	North America	—	Cows	48.3	3,955
Huertas et al., 2018	South America	British or continental	—	90.5	8,132
Jarvis et al., 1995	Europe	British continental-cross	Bulls, heifers, steers	97	3,296
Jarvis et al., 1996	Europe	British continental-cross, Dairy	Bulls, cows, heifers, steers	99	220
Kline et al., 2020	North America	British or continental, Dairy	Bulls, cows, steers	28.1, 42.6	9,544
Liotta et al., 2007	Europe	British or continental	Bulls	35.9	28
McNally and Warriss, 1996	Europe	—	Bulls, cows, heifers, steers	59	16,600
Mendonça et al., 2018	South America	Bos indicus	Cows, steers	44, 64	4,438
Nanni Costa et al., 2005	Europe	Dairy	Bulls	72.4	105
Nanni Costa et al., 2006	Europe	British or continental	Bulls	66.9	142
Romero et al., 2013	South America	Bos indicus	Bulls, cows, heifers, steers	37.5	1,179
Strappini et al., 2010	South America	—	Cows, heifers, steers	8.6, 20.8	127,838
Vimiso and Muchenje, 2013	Africa	—	—	41.1, 63.1	315

[1]Most studies did not report individual bruising frequencies for individual sex classes; the classes indicated above simply demonstrate all of the possible sex classes that were evaluated by a study, which were often grouped into a single population.

[2]Values separated by a comma represent studies that reported a bruising prevalence for two populations, such as for different sex classes or slaughterhouses.

[3]For papers that reported bruise prevalence for two populations, this number represents the total number of carcasses assessed for bruising in a study, irrespective of population breakdown.

[4]Bethancourt-Garcia et al., 2019a and b appear to be representing the same population, but this was not definitely stated in either reference.

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CHAPTER 2: PRE-SLAUGHTER FACTORS AFFECTING MOBILITY, BLOOD PARAMETERS, BRUISING, AND MUSCLE PH OF FINISHED BEEF CATTLE IN THE UNITED STATES

1.0 Introduction

Heightened consumer awareness and interest in how food animals are raised has increased the demand for welfare-friendly labeling of food products (Kehlbacher et al., 2012; Alonso et al., 2020) and influenced how food animals are cared for in many capacities (Broom, 2010; Clark et al., 2016). Against this changing landscape, in part driven by consumer perceptions, corporations have continued advancing their commitments to animal welfare and solidifying it as a core business tenet and a pillar of corporate social responsibility (Maloni and Brown, 2006). For example, McDonald's, one of the largest beef purchasers in the world, has aligned their practices (e.g., responsible sourcing of animals and antibiotic use) with the Global Roundtable for Sustainable Beef's (GRSB) Core Principles, which incorporate animal health and welfare (McDonald's, 2022; GRSB, 2023), further highlighting the industry's commitment to meeting new animal welfare standards and evolving consumer expectations. Nevertheless, animal welfare has long been established as a critical component of beef cattle slaughter (Grandin, 2006; Grandin, 2012), evidenced by regulatory requirements (HMSA, 1978; OIE, 2016), widely accepted industry guidelines (Leary et al., 2016; NAMI, 2021), and company-specific animal welfare programming governing the humane slaughter of livestock (Nestle, 2014; JBS, 2019; Cargill, 2023a).

Undoubtedly, though, cattle still experience some level of stress as they are marketed through the final stages of the beef supply chain, which involves many inherent stressors (Chulayo et al., 2012; Edwards-Callaway and Calvo-Lorenzo, 2020); cattle are loaded onto a truck from their feedlot of origin, transported to the processing plant, and rested in lairage pens before being slaughtered. Numerous pre-slaughter factors have been documented to impact cattle welfare (reviewed by Davis et al., 2022a) and beef quality outcomes (reviewed by Sullivan et al., 2022). However, some pre-slaughter factors are more highly researched than others – these include transportation (Tarrant, 1990; Knowles, 1999; Schwartzkopf-Genswein et al., 2012), animal handling (Warriss, 1990; Grandin, 2014; Gallo et al., 2022), and lairage practices (Gallo et al., 2003; Liotta et al., 2007; Teke et al., 2014). Other pre-slaughter factors, such as the time trucks wait to unload upon arrival to the plant, the amount of space given to cattle in lairage pens, and the use of heat mitigation (i.e., shade) at the plant are under-represented in the current literature (Sullivan et al., 2022) and warrant further exploration in order to understand their impacts on welfare and quality outcomes.

Although a large body of work has focused on minimizing and preventing the incidence of adverse welfare events during pre-slaughter procedures, inevitably, stressors are an inherent component of the pre-slaughter period. In response to stressful stimuli, cattle may elicit a range of physiological reactions that are measurable and serve as helpful tools for assessing animal welfare at slaughter (Grandin, 2014). Creatine kinase (CK), an indicator of muscle damage and fatigue (Mpakama et al., 2014), is an enzyme that helps skeletal muscle maintain homeostasis in locations where adenosine triphosphate (ATP) activity is high, i.e., the muscle is under high metabolic demand (Diene and Storey, 2009). Physically demanding events, such as handling (high stocking density, Tarrant et al., 1992) and long transport (Tadich et al., 2005), can alter the muscle membrane's permeability, stimulating the release of CK from the interstitial space and thereby increasing concentrations of CK in the circulatory system. A similar phenomenon occurs when the tissue is damaged or injured – physical muscle damage, such as a bruise, is associated with higher

CK levels in the blood (Wickham et al., 2012). Lactate, an indicator of muscle fatigue, physical effort, and stress, is routinely measured at slaughter and becomes elevated when catecholamine concentrations (e.g., epinephrine and norepinephrine) are high, initiating the antemortem breakdown of muscle glycogen and thus the formation of lactate (Thomson et al., 2015). When an animal is chronically stressed prior to slaughter, glycogen in the muscle is catabolized and, if to a significant enough extent, will inhibit the muscle's normal postmortem pH decline, resulting in beef that is high in pH (typically greater than 6.0) with a characteristic dark, purplish-red color, otherwise known as dark cutting beef (DCB; Ponnampalam et al., 2017). Studies have found that muscle pH increases as cattle spend longer durations in transport (Tarrant et al., 1992; Silva et al., 2016) and lairage (Strappini et al., 2010; Loredo-Osti et al., 2019), although it should be noted that research has also shown that transport and lairage duration did not increase muscle pH or result in DCB (transport, Lacerda et al., 2022; lairage, Teke et al., 2014). It is a challenge to pinpoint one specific factor contributing to DCB as many pre-slaughter, environmental, and animal-related factors influence an animal's physiological reactions to stress (i.e., the extent of glycogenolysis) and thus the prevalence of DCB.

In a recent review, Losado-Espinosa and others (2018) compiled and rated the validity and feasibility of measuring cattle welfare indicators (CWI) at the time of slaughter; CK and lactate were rated as highly valid CWIs, while cortisol was rated as a CWI with low validity since its concentration is time-dependent and can be influenced by many factors that are not always indicative of cattle having experienced an adverse event (e.g., courtship and mating, Broom, 1988). Even then, cortisol is used frequently to assess the physiological responses of cattle to handling and transport practices during the pre-slaughter phase (Chulayo et al., 2016; Abubakar et al., 2021). In addition, Losado-Espinosa et al. (2018) reported that pH measurements were rated as having both high validity and feasibility as a welfare indicator in production settings, meaning that it was not only a highly researched measurement but also a measurement that is relatively easy to collect. Not unexpectedly, CK, lactate, and cortisol were rated as lowly feasible to measure in plants (Losado-Espinosa et al., 2018) as collecting and maintaining the integrity of blood samples at the time of slaughter can require substantial preparation, labor, and data collection inputs, particularly in large commercial facilities. Even though physiological measurements such as those described above are typically considered lowly feasible to measure in plant settings, they are still possible to collect given the right conditions, such as having accessible labor, equipment, and resources, and are useful when determining how an animal has coped with stress in the pre-slaughter period.

Along those lines, other less expensive and non-invasive indicators, i.e., mobility and carcass bruising, have been utilized to observe the impact of pre-slaughter factors on animal welfare (NAMI, 2016; Texas A & M University, 2016). While data from the 2016 National Beef Quality Audit (NBQA) indicated that the vast majority of cattle sampled (96.8%) received a mobility score of 1 (i.e., walks easy, no apparent lameness; Eastwood et al., 2017), the frequency of cattle with impaired mobility arriving at slaughter plants (i.e., score of 2 or greater), albeit a small number ranging from 3.8 to 25.5% in fed cattle populations (Mijares et al., 2021), is still significant and warrants attention. Numerous risk factors, such as high temperatures (Gonzalez et al., 2012), transport and lairage duration (Edwards-Callaway et al., 2017, Hagenmaier et al., 2017, respectively), and heavier body weights (Thomson et al., 2015), are associated with the prevalence of impaired mobility in finished cattle at slaughter, which underscores the challenges associated with assessing and managing the multifactorial outcome. Still, mobility remains an important welfare indicator and is used in industry benchmarking studies, such as the NBQA, and in internal and third-party audits (NAMI, 2021) to assess and manage cattle welfare at plants. Additionally, carcass bruising is a quality defect with significant implications for both animal welfare and economics; not only is there some level of pain and distress associated with a bruise (i.e., hemorrhage of tissue from ruptured blood vessels beneath the skin' surface, Strappinni et al., 2010), but also there is lost value associated with having to remove bruised tissue from the carcass (Edwards-Callaway and Kline, 2020). Factors such as mixing cattle with horns (Shaw et al., 1976), high stocking density (Ferreira et al., 2020), and rough handling (Mendonca et al., 2018) have been identified as factors impacting the prevalence of bruising in beef cattle. However, assessment methods and frequency of bruising vary considerably across animal populations, regions, and production systems (Miranda-de la Lama et al., 2012, Strappinni et al., 2012; Lee et al., 2017), making it difficult to compare results across studies and to determine when and where bruising is occurring along the supply chain. Nevertheless, according to industry benchmarks, bruising impacts approximately 40% of fed steers and heifers (Eastwood et al., 2017), costing the US beef industry millions of dollars in loss annually (Lee et al., 2017).

While there is a plethora of information from global studies about animal welfare and meat quality and their relationship to pre-slaughter stress, there is actually a dearth of information about these relationships among finished beef cattle in North American production systems (Sullivan et al., 2022). Further, it is challenging to draw conclusions from studies conducted in varying parts of the world and with diverse cattle populations, as the final marketing phase varies considerably (e.g., transport type and conditions, lairage duration, slaughter practices) across the globe. Not only is very little information known about the impact of specific pre-slaughter factors on welfare indicators in cattle (and to what extent) but also, most of the available information is measured or observed in groups of animals (i.e., slaughter lots) and not on individual animals. Therefore, the objective of the present study was to track individual animals throughout the slaughter process to identify pre-slaughter factors associated with key welfare and quality outcomes in finished beef cattle.

2.0 Materials and Methods

2.1 Ethical Statement

Due to the noninvasive nature of all animal measurements and observations taken as a part of this study, an exemption was filed and granted by the Colorado State University Animal Care and Use Committee (IACUC Exemption #2019-080-ANSCI).

2.2 Slaughter Facility and Cattle Population

For one week each in January and July 2022, data were collected at one federally inspected slaughter plant in the Southwestern region of the United States (U.S.) that processes fed cattle. The slaughter facility operates two 8-hour production shifts, with an average slaughter capacity of 5,000 cattle per day and a chain speed ranging from 325 to 375 cattle per hour. Data were recorded over two periods to capture variability in weather across the winter and summer seasons (represented by January and July, respectively; **Table 3**). Due to logistical and personnel capabilities and the complexity associated with tracking animals throughout the slaughter process, samples were taken only from cattle slaughtered on the first shift, which ran from approximately 0630 to 1500 hours daily. Researchers tracked individually marked animals throughout the slaughter process and collected both antemortem and postmortem measurements.

All cattle in the study were handled according to the plant's standard operating procedures, including being unloaded from a truck, moved into lairage pens by facility employees, and inspected by a United States Department of Agriculture (USDA) veterinarian before slaughter. During antemortem inspection, cattle were moved out of their pen to another pen of a comparable size. In the lairage pens, cattle were provided with ad libitum access to water and, in the summer season, were cooled with sprinklers when temperatures exceeded 26°C. All lairage pens and handling areas had stamped concrete flooring and were uncovered. After being inspected, cattle were moved through the handling area to a center-track restrainer, where they were rendered insensible with a pneumatic captive-bolt stunner (Jarvis Products Corporation, Middletown, CT, USA) and subsequently exsanguinated before further processing.

A total of 454 cattle were included in this study (Winter, n = 199; Summer, n = 255). Population characteristics are presented in **Table 4.** In brief, the individually identified cattle population included fed steers (287, 63.22%) and heifers (167, 36.78%) from local commercial feed yards that were predominately black-hided with Bos Taurus influence (286, 63.70% and 438, 96.90%, respectively). Individual live weights were not measured at the slaughter facility; consequently, live weights for individual animals were estimated by dividing the animal's hot carcass weight (HCW) by the dressing percentage (DP) for the animal's respective slaughter lot. The study population had an average live weight of 610.67 ± 71.61 kg (mean ± SD) and ranged from 371.38 to 837.07 kg.

2.3 Individual Animal Identification

Trucks arrived from approximately 0400 to 1130 hours daily for the first shift. Every truck's arrival time, defined as the time a truck arrived at the plant regardless of if they had to wait outside a fenced-in area before checking in, and unloading time, defined as the time that the first animal walked off of the truck, was recorded. The time cattle spent waiting on the truck at the plant was calculated by subtracting a truck's time of arrival from the time cattle were first unloaded from it. The first and one of the last two trucks to arrive from a slaughter lot were chosen for study inclusion to capture variation in truck waiting times. Approximately ten slaughter lots (i.e., twenty trucks) were sampled per day.

From each study truck, five to six animals were randomly selected and marked during unloading to track them throughout the entire slaughter process. One deck of cattle were unloaded at a time (approximately 20 animals) and were moved into a pen scale, where two researchers individually marked cattle with a livestock breeding patch (Estrotect Heat Detector; Rockway Inc., Spring Valley, WI, USA) applied with adhesive glue (Kamar Adhesive; Kamar Products Inc., Zionsville, IN, USA). The patch was placed along the dorsal topline of each animal, usually between the shoulders or on the rump. Multiple patch colors were used to distinguish individual animals within a slaughter lot.

Google Maps (Google LLC, Mountain View, CA, USA) was used to calculate an estimated transport distance between the feedlot of origin and the plant. When multiple routes were available, the first route suggested by Google Maps was selected; this route typically represented the route with the shortest distance.

2.4 Mobility Scoring and Open-Mouth Breathing

Cattle mobility was scored at a single time-point using the North American Meat Institute (NAMI) mobility scoring system (1-4 scale; normal to extremely reluctant to move; **Table 5**; NAMI 2016). One researcher with extensive experience assessing fed cattle mobility scored all cattle included in the study from a catwalk above the lairage pens, which typically occurred when the animals were being moved from their holding pen to the staging area just prior to slaughter. A second researcher recorded an individual animal's hide color and breed type (i.e., predominately Bos taurus vs. predominantly Bos indicus). Cattle were determined to have Bos indicus influence when they possessed two or more typical breed characteristics, including having a large hump above the shoulders, droopy ears, and excess skin on their dewlap and prepuce; only 3% of all cattle sampled met this criterion. In addition to mobility, open-mouth breathing was assessed in the lairage pens using a modified version of the panting scoring system developed by Mader et al. (2006). However, due to the absence of heat stress signs observed by the researcher, heat stress was not analyzed in the present study.

2.5 Blood Collection and Analysis

Exsanguination blood was collected from all study animals. Immediately after exsanguination, researchers collected blood in a plastic cup and subsequently transferred it into two pre-labeled tubes: a 5 mL glucose determination tube with additives of Sodium Fluoride as a stabilizer and Potassium Oxalate as an anticoagulant, and a 10 mL serum tube with silicone coating as a clot activator (BD Vacutainer; Becton, Dickson and Company, Franklin Lakes, NJ, USA).

Approximately thirty minutes after collection, whole blood was analyzed for lactate with a handheld lactate meter and test strip (Lactate Plus; Nova Biomedical Corp., Waltham, MA, USA). The remaining blood tubes were left to clot at room temperature for approximately 30 minutes before being centrifuged (Centrifuge 5810; Eppendorf North America, Framingham, MA, USA) at 2,000 x g for 15 minutes at 4°C. The resulting serum was pipetted into two 2.5 mL microcentrifuge tubes and placed on dry ice for transport; samples were stored at −80°C until further analysis by a third-party laboratory. Serum was analyzed for creatine kinase (CK) using a Cobas C 501 Analyzer (Roche Diagnostics, Indianapolis, IN, USA). Additionally, serum cortisol was assayed in duplicate using a commercial radioimmunoassay kit (ImmunoChem™ Cortisol Coated Tube RIA kit; MP Biomedicals, LLC., Santa Ana, CA, USA). Project intra- and interassay coefficients of variability (CV) for serum cortisol averaged 12.8% and 13.3%, respectively.

2.6 Meat Quality

2.6.1 Bruise Scoring

After the hide was removed but before the carcasses were split, three trained researchers (in rotation) scored carcass bruising and recorded carcass identification numbers. Researchers were trained against a bruise-scoring expert using a series of in-plant videos and real-time experience and had to earn a Kappa coefficient, an estimate of intra-observer reliability, of 0.80 or higher to qualify for bruise-scoring. The methodology employed in this study was adapted from the National Beef Quality Audit (NBQA) bruise scoring system, which uses a 10-point scale to estimate bruise size and weight visually. The scale employed in the present study used four categories based on the upper bound of the NBQA's "Minimal" category (i.e., a bruise the size of a deck of cards) in order to simplify bruise assessment in real-time at fast chain speeds. The four categories were no bruise, one bruise that was ≤ the size of a deck of cards, one bruise that was > than the size of a deck of cards, and multiple bruises (the size was noted); all categories were mutually exclusive.

2.6.2 Carcass Characteristics

Carcass processing occurred according to the plant's standard operating procedures. After carcasses were chilled at -2 to 1°C for approximately 24-hours postmortem, plant employees ribbed carcasses between the 12^{th} and 13^{th} rib before USDA employees evaluated and ranked carcasses for USDA quality and yield grades. In addition to USDA grades, HCW, dressing percentage, and dark cutter data were obtained for each carcass from plant records.

2.6.3 Muscle pH

Approximately 32 to 36 hours postmortem, muscle pH was determined by using a portable pH meter. Measurements were determined with the Hanna Portable Meat pH Meter (Hanna

Instruments, Woonsocket, RI, USA) for the first data collection, but due to in-plant environmental conditions, the Orion Star A121 Portable pH Meter (Thermo Scientific, Waltham, MA, USA) with the Orion 8104BN ROSS Combination Rugged Bulb pH Electrode (Thermo Scientific, Waltham, MA, USA) was used for the second data collection. In addition to the electrode, the Orion Stainless-Steel Automatic Temperature Probe (Thermo Scientific, Waltham, MA, USA) was used to compensate for variations in pH measurements due to changes in ambient temperature. The probe was inserted into the exposed *longissimus thoracis* muscle (referred to as the ribeye) between the 12^{th} and 13^{th} ribs on the right side of the carcass. A single measurement was taken for each carcass. Between measurements, the probe was rinsed with de-ionized water and blotted dry, and instrument calibration was conducted after every 20 samples.

2.7 Statistical Analysis

All statistical analyses were performed using R 4.2.2 (R Core Team, 2021), with individual animal serving as the observational unit. Descriptive statistics were calculated for all variables of interest; continuous variables were summarized by their minimum, mean, maximum, and standard deviation (SD), and categorical variables by their relative frequency. For analysis purposes only, some variables were collapsed into smaller categories. Due to the low frequency of mobility scores 3 and 4 observed, mobility was categorized as a binary variable (Normal Mobility, i.e., a score of one; Impaired Mobility, i.e., a score of 2 or higher). Bruise scores were similarly collapsed into a binary variable for analysis (None/Minimal, i.e., no bruise or one bruise ≤ the size of a deck of cards; Greater than Minimal, i.e., one bruise > than the size of a deck of cards or multiple bruises of any size).

2.7.1 Regression Analysis

Mixed model analysis was used to account for lot effects as there were multiple animals observed per slaughter lot. Continuous variables, including cortisol, creatine kinase (CK), lactate, and muscle pH, were analyzed using linear mixed-effects models (lme4 v1.1-31 and lmerTest v3.1-3); diagnostic plots were used to assess model fit and ANOVA assumptions. To account for non-normality, a logarithmic transformation was performed on CK data for analysis and later back transformed for reporting purposes. Mobility was analyzed using a mixed-effects binary logistic regression (Base R v4.2.2). Bruising was analyzed using a binary logistic regression (Base R v4.2.2); in this model, high model complexity generated a non-convergence issue. To solve this issue, slaughter lot was excluded as a random effect in the bruising model only.

Type III F-tests (car v3.1-1) were used to analyze all models. Additionally, pairwise comparisons for categorical variables were calculated using estimated marginal means (emmeans v1.8.4-1). Statistical significance was determined at $P \leq 0.05$ and statistical trends were declared at $0.05 < P \leq 0.10$. The following results are reported as (*n*, %) unless otherwise noted.

2.7.2 Model Selection

Akaike Information Criterion (AIC) was used to evaluate regression models with multiple predictors. The variables season, body weight, sex, breed, truck waiting time, distance travelled, lairage density, lairage duration, mobility (except for the mobility model), and bruising (except for the bruising model) were considered for all full models during the model selection process. In addition, lactate was added as a predictor variable for the mobility and pH models. For each response variable of interest, a full model was fit separately (meaning all possible and relevant predictor variables were included), an automated model selection was applied (package: MuMIn v1.47.1), and the model with the lowest AIC was selected. For a subset of response variables, a small number of two-way interactions were included in the model selection process based on their relevance to the specific research question and previous subject matter knowledge (e.g., season by lairage duration); however, none of the lowest AIC models included interactions and were therefore excluded from further analysis.

3.0 Results

3.1 Pre-Slaughter Management Factors

Descriptive statistics of preslaughter management factors are outlined in **Table 6**. In the current study, cattle were transported 68.70 ± 44.70 km (mean ± SD) and waited in the truck at the plant for an average of 54.43 ± 38.30 minutes. The shortest amount of time cattle waited to be unloaded was six minutes, with a maximum waiting time of 208.00 minutes (about 3 and a half hours). The average lairage duration was 152.07 ± 38.30 minutes and ranged from 92.00 to 253.00 minutes (about 4 hours). Lairage density, measured in m^2 of pen allowance per individual animal, averaged 3.67 ± 1.75 m^2 and ranged from 2.05 to 13.24 m^2. Postmortem, study animals recorded HCWs ranging from 237.68 to 530.70 kg with a mean of 388.43 ± 45.45 kg. In addition, the average dressing percentage in this study was 63.60 ± 0.70% and ranged from 61.80 to 65.10%.

3.2 Mobility

Of the 438 cattle assigned an individual mobility score, 67.12% (n=294) were assigned a score of 1 (i.e., normal mobility; **Table 7**). The vast majority of cattle with impaired mobility were given a score of 2 (n=140, 31.96%). Greater mobility scores (i.e., 3's and 4's) were less frequently observed, i.e., less than one percent (n=4, 0.91%) of cattle included in the study scored a 3, and none scored a 4. Results of the mobility regression analysis identified two factors significantly associated with the outcome: lairage density and season (**Table 8**). A one-unit (m^2/animal) increase of space allowance per animal in lairage was associated with decreased odds of cattle with impaired mobility (i.e., scoring a 2 or higher; OR: 0.83, CI: 0.73, 0.96). Compared to cattle slaughtered in the summer season, those slaughtered in the winter season had reduced odds of exhibiting impaired mobility (OR: 0.26, CI: 0.13, 0.51).

3.3 Blood Parameters

3.3.1 Cortisol

Serum cortisol averaged 21.70 ± 11.30 ng/mL (mean ± SD) and ranged from 0.08 to 77.40 ng/mL (**Table 9**). Overall, there was evidence of an association ($P \leq 0.05$) between body weight, bruising, and truck waiting time with cortisol concentrations (**Table 10**). Greater average cortisol concentrations were associated with heavier cattle ($P = 0.049$), cattle that waited longer in the truck at the plant ($P = 0.038$), and those with carcass bruising compared to those without carcass bruising (23.0 ± 0.89 vs. 20.3 ± 1.05 ng/mL (predicted mean ± SE); $P = 0.015$). In contrast, there was no evidence of an association between sex or lairage density on cortisol concentrations ($P = 0.130$ and $P = 0.128$, respectively).

3.3.2 Creatine Kinase

Serum CK concentrations ranged from 240.00 to 8622.00 U/L with an average value of 949.00 ± 933.00 U/L (**Table 9**). Results indicate that greater average CK concentrations tended to be associated with heifers versus steers ($P = 0.054$), the winter versus summer season ($P = 0.055$), and in cattle that were held in lairage for longer durations ($P = 0.051$; **Table 10**) but were not statistically significant ($P > 0.05$).

3.3.3 Lactate

Study cattle had a mean lactate concentration of 5.72 ± 2.46 mmol/L, ranging from 0.70 to 14.80 mmol/L (**Table 9**). Results of lactate analysis identified multiple factors associated with the blood parameter, including body weight, truck waiting time, lairage duration, season, and mobility (**Table 10**). Greater average lactate concentrations were associated with heavier cattle ($P < 0.05$) and in cattle that waited on the truck for more extended periods of time ($P = 0.023$). There was also a tendency for lactate to be elevated in cattle held in longer lairage ($P = 0.052$). Season was associated with greater lactate, as cattle that were slaughtered in the winter had significantly lower lactate levels than cattle slaughtered in the summer (3.54 ± 0.22 vs. 7.22 ± 0.17 mmol/L (predicted mean ± SE); $P < 0.0001$). Additionally, mobility score was associated with lactate, as cattle with impaired mobility had lower blood lactate than cattle with normal mobility (5.20 ± 0.18 vs. 5.56 ± 0.14 mmol/L; $P = 0.027$).

3.4 Meat Quality Parameters

3.4.1 Bruising

Approximately one-third (161, 36.02%) of study cattle had either no carcass bruising (89, 19.91%) or a single small bruise (72, 16.11%; **Table 7**). A smaller proportion of cattle had a single large bruise (28, 6.26%), with the majority of cattle having multiple bruises of varying sizes (Small: 122, 27.29%; Large: 136, 30.43%). Multiple factors were associated with carcass bruising, including lairage duration, season, and sex (**Table 8**). Odds of carcass bruising were positively associated with lairage duration (OR: 1.01, CI: 1.0007, 1.0122). Compared with the summer season, the winter season was associated with reduced odds of cattle becoming bruised (OR: 0.48, CI: 0.30, 0.78). Similarly, the odds of having a bruise were reduced in heifers compared with steers (OR: 0.47, CI: 0.27, 0.81). In contrast, there was no evidence of an association between body weight or mobility on bruise incidence (OR: 0.997, CI: 0.994, 1.00; OR: 0.69, CI: 0.45, 1.07, respectively).

3.4.2 Muscle pH

Study carcasses had a mean postmortem muscle pH of 5.53 ± 0.12 and ranged from 5.20 to 6.56 (n = 414; **Table 9**). Less than one percent of carcasses were classified as dark cutters (pH range: 6.15 to 6.56; 3, 0.72%). Mobility was identified as a factor associated with muscle pH, as pH was lower in cattle with impaired mobility compared to cattle with normal mobility (pH of 5.54 ± 0.009 vs. 5.51 ± 0.011; P = 0.029; **Table 10**). There was also a tendency for body weight to decrease pH (P = 0.059). Lastly, there was no evidence that muscle pH was associated with season (P = 0.206).

4.0 Discussion

A multitude of work has focused on reducing fear, stress, and discomfort in cattle being moved through the final marketing phase by improving and promoting low-stress animal handling, transportation, and management processes during this time (Edwards-Callaway and Calvo-Lorenzo, 2020). Not only do consumers increasingly care about how their food is raised (FMI, 2022), but also companies have incorporated animal welfare into all segments of their operations and have established it as a core pillar of corporate social responsibility (Mendez and Peacock, 2022). For example, packing companies have implemented new standards for cattle transporters, such as requiring drivers shipping cattle to their plants to be Beef Quality Assurance Transport (BQAT) certified (Cargill, 2023b). Even more recently, conversations and efforts around enhancing cattle comfort at the plant, for example, by providing shade or rubber mats in lairage, have become more frequent (Tyson, 2019; Davis et al., 2022). Even still, there is limited information about the effects of pre-slaughter factors on animal welfare and meat quality outcomes, particularly among finished beef cattle in the U.S. A recent scoping review investigating the impact of the pre-slaughter period on beef quality outcomes reported that only 10 of the 85 studies assessed were conducted in North America, with even fewer studies (*n* = 5) conducted in the U.S. (Sullivan et al., 2022). The objective of the current study was to track individual animals throughout the slaughter process to identify pre-slaughter factors associated with key welfare and quality outcomes in finished beef cattle.

Although data collection occurred at a single beef processing facility, the sample population was representative of cattle entering the food supply chain from conventional beef-fattening systems in the U.S. Data from the 2016 National Beef Quality Audit (NBQA), a tool used to benchmark progress in the U.S. beef industry, indicated the majority of all sampled cattle were black-hided and steers (57.8%, Eastwood et al., 2017; 66.5%, Boykin et al., 2017, respectively), aligning with the characteristics of the cattle population sampled in this study which followed similar trends (approximately 60% were steers and black-hided). On average, carcasses in this study weighed 388 kg and is aligned with findings from the 2016 NBQA which reported an average HCW of 390 kg among 8,493 fed steer and heifer carcasses (Boykin et al., 2017). While the primary focus of this study was to investigate the impact of pre-slaughter factors on mobility, blood parameters, bruising, and muscle pH for individual animals, a trend that emerged from our findings was that animal-related factors, namely body weight and sex, were associated with a few of the measured outcomes. In the current study, body weight was identified as a factor associated with serum cortisol concentration and blood lactate; a one-kg increase in body weight was associated with an increase in both blood parameters. In a study conducted in finishing pigs, live weight itself had a limited effect on blood lactate, however, the effect of handling intensity on lactate was strong as lactate concentrations increased with the intensity of handling (Hamilton et al., 2004); this is a finding supported by Hagenmaier et al. (2017) in a group of finished beef cattle.

The current literature suggests that other factors, such as the quality and intensity of animal handling, has a significant impact on an animal's physiological response to stress and warrants consideration. Sex was another animal-related characteristic associated with carcass bruising; heifers had less bruising compared with steers. Other studies have identified sex as a risk factor for bruising (Mendonca et al., 2018; Sanchez et al., 2022), although the findings are highly variable and reasons for this are unclear. It is possible that behavioral differences between different sex classes may explain the variation in bruise prevalence of animals (Kline et al., 2020).

The transportation of livestock to slaughter is continually regarded as one of the most stressful events in an animal's life, having implications for both animal welfare and meat quality (Miranda-de la Lama, 2014; Schuetze et al., 2017). Exposure to variable and often times extreme environmental conditions, loud noises and vibrations, novel handlers and animals, and extended periods of feed and water deprivation are just a few aspects of livestock transport that may lead to cattle becoming stressed, fatigued, or injured on the road (Wigham et al., 2018). With these things considered, industry-accepted recommendations and guidelines (Leary et al., 2016; NAMI, 2021), regulations governing the transport of livestock (Twenty-Eight Hour Law, 1994), and good practices in animal handling and transport (e.g., BQAT training and certification), are in place to help ensure animal welfare is maintained during this critical juncture of the final marketing phase. Transportation conditions, such as distance or duration travelled, can be highly variable across different regions and production systems, even within the U.S. (Gonzalez et al., 2022). Interestingly, though, transport distance was not identified as a factor associated with any measured outcome in this study. This finding is surprising because the effects of transportation have been repeatedly explored and identified as an important pre-slaughter factor for a suite of welfare and quality outcomes (Schwartzkopf-Genswein et al., 2012), many of which were measured in this study (e.g., mobility, bruising, and muscle pH). There are several potential reasons for transport distance not emerging as an impacting factor in the current study. Cattle were transported from their feedlot of origin to the plant for relatively short distances, as the mean transport distance was 68.7 km, and no cattle were transported for distances greater than 206.0 km. For reference, similar studies in the U.S. have reported mean transport distances ranging from 172.0 km to 218.5 km (Mijares et al., 2021; Hagenmaier et al., 2017; Eastwood et al., 2017). Additionally, many other transport-related characteristics, including driver experience (Tarrant, 1990), trailer type (Silva et al., 2016), loading density (Tarrant et al., 1992; Bethancourt-Garcia et al., 2019), and trailer microclimate (Schuetze et al., 2017), have been shown to impact beef cattle welfare and meat quality. Although these factors were not assessed in this study, it is possible that they may explain the variation in our measured outcomes better than transport distance alone and should be considered in future work.

Once cattle arrive at the slaughter plant they often have to wait to be unloaded for a variety of reasons (e.g., pen capacity, plant breaks, arrivals outside of the scheduled time; personal communication, L.N. Edwards-Callaway). To the author's knowledge, the impact that the time cattle wait to unload upon arrival to the plant (i.e., truck waiting time) has on cattle welfare and meat quality has not been studied yet. Truck wait time is a component of the North American Meat Institute's (NAMI) Transportation Audit (NAMI, 2021). The audit criterion indicates that trucks should be unloaded within 60 minutes of arrival time to the plant; each additional 30 minutes of waiting time results in a one-point deduction on the audit, and trucks that wait for longer than 120 minutes, without reason, receive zero points (NAMI, 2021). It is unclear within the audit guidelines why 60 minutes is considered an acceptable amount of waiting time. On average, study cattle waited to unload for approximately 54 minutes, which is under the 60-minute threshold determined by NAMI. However, it should also be noted that there was a small subset of study cattle that waited for extended periods of time, i.e., 32 of 454 cattle waited longer than 120 minutes, of which 12 waited between three and three and a half hours to unload in the summer season (almost 2.5 times longer than the audit criteria indicates). The plants make concerted efforts to minimize truck waiting times by optimizing purchasing and scheduling logistics, but a combination of internal (e.g., facility limitations, unexpected operating delays, shift changes) and external (e.g., feedlot logistics, early or late arrivals, road conditions) factors make managing truck wait times at the plant a challenge. Still, the long wait times recorded in this study, even though infrequent, are significant and warrant attention. More research is needed to determine if the currently accepted guidelines for wait time to unload are acceptable or need revision.

In the current study, truck waiting time was identified as a factor associated with two of the three blood parameters indicative of stress (e.g., cortisol and lactate) with an increase in cortisol and lactate levels observed with increases in truck waiting time. While other studies have not directly evaluated the impacts of truck waiting time, research have assessed physiological reactions to stress in relation to being transported. Romero et al. (2014) and Capra et al. (2019) measured cortisol on the farm prior to transport and at the time of slaughter and observed significant increases in cortisol in cattle transported for more prolonged periods. In addition, Gruber and others (2010) measured behavioral and physiological reactions to stress after a 64 km transport to a commercial slaughter facility. Cattle that exhibited behavioral symptoms of stress post-transport had greater plasma lactate concentrations at slaughter, potentially indicative of an adrenergic stress response to transport itself (Gruber et al., 2010). Furthermore, even though the impact of the time cattle wait to unload at the plant has not been quantified, a truck's microclimate (e.g., heat, humidity, noxious gas concentration, and overall air quality) at rest is different than it in motion (Goldhawk et al., 2014; Scheuetze et al., 2017); in particular, ventilation and airflow are altered which could have deleterious effects on cattle. Seventeen of the 32 cattle that waited for longer than 120 minutes on the truck were slaughtered in the summer season. The average temperature during this season was 33.9°C (range: 26.7 to 42.2°C) with an average Temperature Humidity Index (THI) of 78.2 (range: 73.0 to 83.8). According to Meat and Livestock Australia, the mean THI observed in this study (78.2) is considered Mild Stress and the maximum THI (83.8) is considered Severe Stress (LiveCorp and Meat and Livestock Australia Veterinary Handbook, 2023). Although unknown, the effects of longer wait times coupled with high temperatures and heat load risk (i.e., Mild/Severe Stress) could have significant implications for animal welfare and meat quality and should be explored further. In market-weight pigs, an exploration of the effects of additive stressors (i.e., handling intensity, floor space during transport, and distance handled) on a host of metabolic responses found that blood lactate concentration increased linearly as pigs experienced more stressors throughout the pre-slaughter period (Ritter et al., 2009). Therefore, the time cattle wait to unload likely has cumulative effects on an animal's physiological reaction to stress, and it is just one of many factors that may impact an animal in the pre-slaughter phase.

Although regulatory requirements governing the humane slaughter of livestock do not explicitly stipulate how much space an animal should be given in holding pens at the slaughter facility, federal regulations do require that animals have access to water in pens, are provided feed if held for longer than 24 hours and are given adequate space to lie down if held overnight (USDA FSIS, 1987). Additionally, industry guidelines such as those developed by NAMI provide guidance on how much space cattle should be allotted in holding pens and indicate that space allocations could vary based on weather, animal size, and duration of lairage (NAMI, 2021). In brief, NAMI recommendations indicate that a 545 kg animal should be allotted 1.87 m^2 in lairage, which increases with heavier body weights (up to 2.22 m^2; NAMI, 2021); the creation and implementation of a tiered, weight-based pen density system was added to the NAMI guidelines in a 2017 revision (Kline et al., 2019). Cattle in the current study weighed 610 kg on average and were allotted an average of 3.67 square meters of lairage pen space each (which is more space than the largest allotment recommended by NAMI). Even still, pen density was associated with an important welfare indicator; cattle with less space in lairage were at greater risk for having impaired mobility, potentially indicating that the currently accepted recommendations for lairage density may need to be reevaluated as cattle demographics continue to evolve (i.e., type, body weight, frame size).

A limited number of studies have assessed the effects of pen density on welfare (Davis et al., 2022) or meat quality outcomes in beef cattle (Sullivan et al., 2022). Also, to the author's knowledge, no studies exist on the impact of lairage density on beef cattle mobility. Mobility represents a significant challenge for the beef industry as it has implications for animal welfare and production efficiency (Edwards-Callaway et al., 2017). Mobility challenges at the plant can reduce operating speeds which can have a significant economic cost (i.e., production minutes lost). Nearly a decade ago, highly-publicized reports of cattle arriving at slaughter plants with severely impaired mobility heightened industry stakeholder's interest in and concern for cattle mobility (Beef Magazine, 2013; NPR, 2013). Since then, a tremendous amount of effort has focused on understanding risk factors associated with impaired mobility to assess and manage it more effectively (Boyd et al., 2015; Hagenmaier et al., 2017; Lee et al., 2018; Mijares et al., 2021). Even still, challenges persist for assessing and managing cattle mobility because of numerous pre-slaughter, animal-level, and management-related risk factors – including but not limited to environmental conditions (Gonzalez et al., 2012), lairage duration (Hagenmaier et al., 2017), body weight (Edwards-Callaway et al., 2017), sex (Lee et al., 2018), and days on feed (DOF, Mijares et al., 2021). Although pen density has not yet been identified as a risk factor for impaired mobility, future research should explore how cattle spend their time in lairage. Since overcrowding cattle in lairage pens negatively impacts their ability to comfortably lie down or move around freely, this could worsen mobility outcomes and is area worthy of consideration.

Cattle in high-density pens are more susceptible to heat stress because their ability to dissipate a high heat load effectively is impaired (Edwards-Callaway et al., 2021); increases in stocking densities can impact a pen's microclimate by reducing overall airflow and increasing humidity (Weeks, 2008; Davis et al., 2022b). It should be noted that observers in the current study assessed cattle for signs of heat stress (i.e., open mouth breathing) during lairage using a modified version of a panting score system developed by Mader et al. (2006); but, due to no heat stress signs observed, it was not analyzed. Many challenges are associated with observing heat stress in lairage pens; numerous factors, such as the overcrowding of cattle in pens, time of observation, and severity of heat stress, all impact a researcher's ability to observe it. In recent years, particularly in light of extreme heat events and cattle death loss in feedlots (Drovers, 2022; NPR, 2022) a heightened focus has been placed on the importance of providing cattle with heat mitigation, such as sprinklers, fans, shade, or a combination thereof during lairage (Davis et al., 2022b). In the current study, sprinklers were used in lairage as a form of heat mitigation when temperatures exceeded 26°C; however, depending on where cattle were standing in the pen and the consistency of the sprinkler application, cattle may not have had equal access to heat mitigation. Future research should explore the application and effectiveness of shade provision in lairage and the impact of pen-level temperature and humidity on animal outcomes.

The findings of this study indicate that seasonality was a significant risk factor for mobility and bruising; the summer season was associated with a higher risk for impaired mobility and the incidence of 'Greater than Minimal' carcass bruising. In a study by Mijares and others (2021), a greater THI was associated with an increase in the percentage of cattle scoring a 2 or greater on the NAMI mobility scoring system; in their study (holding all other factors constant), an increase on the THI scale from Mild Stress (i.e., $72 \leq THI < 79$) to Severe Stress (i.e., $79 \leq THI < 89$) increased the relative risk of mobility scores of 2 or greater by approximately 125%. In addition, although season has repeatedly been identified as a risk factor for carcass bruising (Huertas et al., 2015), the findings are highly variable across the available literature and contributes to the ongoing challenges associated with assessing and managing bruising in the beef supply chain. Comparing risk factors associated with bruising across studies is difficult because numerous variables, including sex class, animal type, quality of animal handling and facilities, and methodologies used to quantify and characterize bruising, may confound the prevalence of bruising (Sullivan et al., 2022). Although, animal handling was not assessed in the current study, we can postulate that mobility and bruising were potentially related. For example, if an animal was more difficult to move (e.g., it had impaired mobility), that is potentially when handling outcomes worsened. In a population of culled cattle, Strappini and others (2013) tracked the incidence of traumatic events in the pre-slaughter period and their relationships to carcass bruising; they discovered 27% of bruises resulted from negative human-animal interactions, which occurred most frequently during the loading and unloading of animals (Strappini et al., 2013). This research emphasizes the importance of proper animal handling at the plant and identifies an area in need of additional exploration in the future.

Many factors, including lairage duration, lairage density, environmental conditions, animal condition, and others, interact with each other to have multifactorial effects on bruising, underscoring the complexity associated with pinpointing factors influencing the quality defect. Not only is there an animal welfare component to bruising, but also, bruising has numerous downstream effects which are estimated to cost the U.S. beef industry millions of dollars annually (Lee et al., 2017). This loss is attributed to overall reductions in carcass yield due to trimming, the devaluing of cuts affected by bruising, and the increased labor needed to trim bruises out (McNally and Warriss, 1996). Therefore, substantial work has been done to understand the risk factors associated with bruising to reduce its prevalence (Kline et al., 2020; Sanchez et al., 2022; Zanardi et al., 2022). In the current study, the risk of carcass bruising was positively associated with lairage duration and is consistent with other work that has also found that bruising worsens with longer durations of lairage (Warriss et al., 1990; Hoffman and Luhl, 2012). As cattle spend more time in the lairage pens, they have more opportunities to become bruised through negative animal-animal, human-animal, and animal-facility interactions (Strappini et al., 2013). Strappini and others (2013) found that approximately one-third of bruising in their study was inflicted during the lairage period. Some extent of pain, suffering, and distress is associated with a forceful impact that would cause a bruise on an animal (Edwards-Callaway and Kline, 2020), a notion supported by findings of this study; greater average cortisol concentrations were associated with cattle that had carcass bruising compared to those without bruising, indicating some level of distress was experienced by the impacted animal. However, it should be noted that the observed differences in cortisol concentrations in the current study was small and may be biologically irrelevant. Still, Warriss and Brown (1985) measured cortisol in exsanguination blood and found a positive correlation between plasma cortisol concentration and carcass bruising in pigs.

A large body of work exists on the impact of pre-slaughter factors on postmortem muscle pH in beef cattle. A scoping review about the effect of pre-slaughter management practices on meat quality outcomes identified that pH was the most highly researched outcome among papers included in the review (i.e., pH was assessed in nearly 70% of the included studies; Sullivan et al., 2022). The mean pH of carcasses measured in this study was 5.53 and fell within the acceptable range of ultimate pH for beef (5.5–5.6; Sakowski et al., 2022). Interestingly, less than 1% of all study carcasses (3 of 414) exhibited some degree of dark cutting. Other large, epidemiolocal-type studies have also evaluated dark cutting in beef cattle and found similarly low incidence rates (0.3–1.6% among 724,639 cattle, Kreikemeier et al., 1998; 0.05–0.64% among 2.6 million cattle, Scanga et al., 1998; 2.8% among 142,228 cattle, Steel et al., 2021). Due to a low incidence of dark cutters in the sample population, we could not explore the impact of the pre-slaughter management factors measured in this study on dark cutting.

In an exploration of dark cutting in a population of nearly 150,000 Australian grain-fed beef carcasses, Steel and others (2021) reported that environmental conditions (e.g., increased wind speeds and rain during lairage) and lairage duration were associated with an increased risk of dark cutting, a finding supported by a large body of work. Warren and others (2010) found that cattle lairaged overnight were more likely to dark cut than cattle slaughtered on the day of their arrival, and Loredo-Osti et al., 2019 reported that a reduction in lairage time from 14.9 to 3.0 hours would decrease the probability of dark cutting from 7.21 to 0.02%. Other studies have documented numerous other factors associated with dark cutting. For example, time of the year (Kreikemeier et al., 1998; McGilchrist et al., 2014); duration of transport (Gallo et al., 2003; Chulayo and Muchenje, 2017; Burns et al., 2019), and animal related factors (Scanga et al., 1998) are just a few that has been reported previously. It is clear that identifying a single pre-slaughter factor associated with this defect is challenging, mainly because numerous and compounding intrinsic and extrinsic factors contribute to its occurrence. While dark cutting is a costly quality defect (carcasses can be discounted between 20 to 50 dollars per cwt, USDA AMS, 2023) and there is economic incentive to minimize it, it is extremely difficult to manage; factors contributing to its occurrence exist throughout the entire final marketing phase, some of which cannot be controlled (e.g., fluctuations in temperature 24 hours prior to slaughter; Scanga et al., 1998). In order to fully investigate relationships between pre-slaughter factors and dark cutting, future research may begin to explore dark cutting in targeted populations of cattle who may be at higher risk for dark cutting or during specific times of the year when dark cutting incidence is known to be higher.

Presumably, pre-slaughter factors (e.g., truck waiting time, lairage duration) differ by production shift with many large commercial beef processing plants in the U.S. operating on two 8-hour shifts. The plant may experience unexpected downtime, delays in receiving cattle, or pen capacity limitations throughout the day, which could potentially exasperate some of the factors measured in this study. A limitation of the current study is that samples were collected only from cattle slaughtered on the first shift. Although the trends would likely be similar to those found in the current study, it is possible that the magnitude of these relationships would differ if animals arriving during the second shift had been included in this study. Although animal behavior and handling outcomes were not assessed in the present study, variations in behavior and handling are likely associated with many of the studied outcomes – namely, mobility and bruising. Future research should investigate how cattle spend their time in lairage and, more specifically, if their access to water, space to lie comfortably, and room to move around freely impacts animal-based outcomes. Further, the relationship between mobility and handling outcomes and its downstream effects on bruising and muscle pH could lead to understanding how best to manage cattle with impaired mobility in the pre-slaughter phase to maximize welfare and quality outcomes.

The results of this study support a growing body of work that highlights the importance of the pre-slaughter period for animal welfare and meat quality. This project also identifies areas for continuous improvement, such as reducing the time trucks wait to unload at the plant, shortening the duration of lairage, and providing cattle with more space in lairage pens. Additionally, there was a seasonality effect to many of the measured outcomes. Although environmental conditions (e.g., temperature, humidity, wind speeds) are challenging to manage, opportunities exist for plants to modify their practices at times of the year when cattle are most susceptible to adverse welfare outcomes. Providing heat mitigation (i.e., shade) during lairage may help cattle cope with high heat loads and is just one example of the opportunities that exist to improve the welfare of finished cattle arriving to slaughter plants. However, additional exploration is needed to quantify the effects of shade on cattle at plants. Presumably, exposure to many stressors during the final marketing phase has a compounding impact on cattle welfare and meat quality, and the factors identified in this study are just a few of many important pre-slaughter factors requiring further investigation. Lastly, a significant amount of work has been done to improve the transport, handling, and management of cattle in the pre-slaughter phase, which is evident in the findings of this study. Continuing to promote and improve good practices in animal handling and transport will be key for advancing animal welfare in the beef supply chain.

Table 3. Minimum, mean, and maximum ambient temperature, humidity, and temperature humidity index (THI) by season.

Season	Temperature[1], °C			Humidity[1], %			THI[2]		
	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max
Summer	26.70	33.90	42.20	13.00	25.20	43.00	73.00	78.20	83.80
Winter	-6.11	8.33	15.60	14.00	32.20	68.00	27.60	50.20	59.00

[1]Temperature and humidity were recorded using an online commercial weather service (Weather Underground, San Francisco, CA, USA).

[2]THI was estimated using the following equation: THI = 0.8*T + RH*(T-14.4) + 46.4, where T denotes temperature in °C and RH denotes relative humidity as a proportion (LiveCorp and Meat and Livestock Australia Veterinary Handbook, 2023).

Table 4. Animal characteristics of study cattle, including sex class, breed type, hide color, and age.

Characteristic	***n***	**Frequency, %**
Sex class (*n*=454)		
Heifer	167	36.78
Steer	287	63.22
Breed type[1] (*n*=452)		
Bos taurus	438	96.90
Bos indicus	14	3.10
Hide color (*n*=449)		
Black	286	63.70
Not Black	163	36.30
Cattle age (*n*=414)		
Under 30 months	401	96.86
Over 30 months	13	3.14

[1]Cattle were classified as having Bos indicus influence when they possessed two or more typical breed characteristics, which included having a large hump above the shoulders, droopy ears, or excess skin on the dewlap and prepuce.

Table 5. Mobility scoring system for finished cattle developed by the North American Meat Institute (NAMI).

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 6. Description of pre-slaughter management factors measured as potential risk factors associated with animal welfare and meat quality outcomes. All measurements were taken on an individual animal basis.

Variable	***n***	**min**	**mean**	**max**	**SD**
Distance traveled, km	454	2.74	68.70	206.00	44.70
Truck waiting time, min	454	6.00	54.43	208.00	38.61
Lairage duration, min	446	92.00	152.07	253.00	38.30
Lairage density, m^2/animal	451	2.05	3.67	13.24	1.75

Table 7. Descriptive statistics for mobility, bruise score, yield grade, and quality grade for individual animals.

Variable	*n*	Frequency, %
Mobility score[1] (*n*=438)		
1	294	67.12
2	140	31.96
3	4	0.91
4	0	0
Bruise score[2] (n=447)		
None	89	19.91
One small	72	16.11
One large	28	6.26
Multiple small	122	27.29
Multiple large	136	30.43
Yield grade (*n*=447)		
1	37	8.28
2	255	57.05
3	139	31.10
4	15	3.36
5	1	0.22
Quality grade (*n*=446)		
Prime	11	2.47
Choice	291	65.25
Select	137	30.72
Standard	4	0.90
Cutter	3	0.67

[1]Mobility scores were classified as follows: 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, 2016).

[2]Bruise score categories were classified as follows: None = no bruise present; One small = one bruise that was less than or equal to the size of a deck of cards; One large = one bruise that was larger than the size of a deck of cards; Multiple = two or more bruises and the size of the largest bruise was noted.

Table 8. Multivariable logistic regression models for categorical variables, including mobility and bruising. Estimates for each variable and reported *P*-values are from solutions for fixed effects of the respective regression model.

Variable	Estimate	SE	*P*-value	OR (95% CI)
***Mobility* (*n*=406)**				
Intercept	1.1435	0.5897	0.0525	3.14 (0.99, 9.97)
Bruising				
None/Minimal	Referent	–	–	–
Greater than Minimal	-0.4435	0.2276	0.0513	0.64 (0.41, 1.00)
Lairage density, m^2/animal	-0.1820	0.0716	0.0110	0.83 (0.73, 0.96)
Lactate, mmol/L	-0.1223	0.0711	0.0853	0.89 (0.77, 1.02)
Season				
Summer	Referent	–	–	–
Winter	-1.3662	0.3572	0.0001	0.26 (0.13, 0.51)
Truck waiting time, min	0.0057	0.0031	0.0693	1.01 (1.00, 1.01)
***Bruising* (*n*=431)**				
Intercept	1.9956	1.3381	0.1359	7.36 (0.54, 104.00)
Bodyweight, kg	-0.0026	0.0018	0.1508	0.997 (0.994, 1.00)
Mobility				
Normal	Referent	–	–	–
Impaired	-0.3709	0.2232	0.0965	0.69 (0.45, 1.07)
Lairage duration, min	0.0063	0.0029	0.0294	1.01 (1.00, 1.01)[1]
Season				
Summer	Referent	–	–	–
Winter	-0.7251	0.2429	0.0028	0.48 (0.30, 0.78)
Sex				
Steer	Referent	–	–	–
Heifer	-0.7620	0.2854	0.0076	0.47 (0.27, 0.81)

[1]95% CI has been rounded and does not include 1.

Table 9. Description of physiological stress parameters (i.e., lactate, creatine kinase, and cortisol) and postmortem muscle pH.

Variable	*n*	min	mean	max	SD
Cortisol, ng/mL	441	0.08	21.70	77.40	11.30
Creatine kinase, U/L	450	240.00	949.00	8622.00	933.00
Lactate, mmol/L[1]	431	0.70	5.72	14.80	2.46
Muscle pH[2]	414	5.20	5.53	6.56	0.12

[1]Lactate was analyzed in whole exsanguination blood with a portable lactate meter approximately 30 minutes postmortem.

[2]Muscle pH was determined with a portable pH meter approximately 32 to 36 hours postmortem.

Table 10. Multivariable linear mixed-effects regression models for continuous variables, cortisol, creatine kinase (CK), lactate, and muscle pH. Estimates for each variable and reported *P*-values are from solutions for fixed effects of the respective regression model.

Variable	Estimate	SE	***P*-value**
Cortisol, ng/mL **(*n*=433)**			
Intercept	7.4214	6.4020	0.2472
Bodyweight, kg	0.0189	0.0096	0.0488
Bruising			
None/Minimal	Referent	–	–
Greater than Minimal	2.6904	1.0972	0.0146
Lairage density, m^2/animal	-0.5357	0.3510	0.1283
Sex			
Steer	Referent	–	–
Heifer	2.7331	1.7778	0.1304
Truck waiting time, min	0.0363	0.0173	0.0380
Creatine kinase[1], U/L **(*n*=442)**			
Intercept	666	78.7	< 0.0001
Season			
Summer	Referent	–	–
Winter	0.8810	0.0567	0.0553
Sex			
Steer	Referent	–	–
Heifer	0.8800	0.0563	0.0541
Lairage duration, min	1.0000	0.0008	0.0509
Lactate, mmol/L **(*n*=409)**			
Intercept	4.3262	1.0905	< 0.0001
Bodyweight, kg	0.0028	0.0014	0.0498
Mobility			
Normal	Referent	–	–
Impaired	-0.3611	0.1631	0.0274
Lairage duration, min	0.0052	0.0027	0.0523
Season			
Summer	Referent	–	–
Winter	-3.6800	0.2798	< 0.0001
Sex			
Steer	Referent	–	–
Heifer	0.4660	0.3045	0.1321
Truck waiting time, min	0.0063	0.0027	0.0227
Muscle pH **(*n*=401)**			
Intercept	5.6470	0.0504	< 0.0001

Bodyweight, kg	-1.666×10^{-4}	8.786×10^{-5}	0.0594
Mobility			
Normal	Referent	–	–
Impaired	-0.0269	0.0122	0.0285
Season			
Summer	Referent	–	–
Winter	-0.0200	0.0155	0.2056

[1]A logarithmic transformation was performed on CK data for analysis and back transformed for reporting purposes.

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