DISSERTATION

EXPLORATION OF BISON INDUSTRY PRACTICES AND MITOCHONDRIAL METABOLISM

Submitted by

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ABSTRACT

EXPLORATION OF BISON INDUSTRY PRACTICES AND MITOCHONDRIAL METABOLISM

The objectives of this study were to benchmark the United States bison meat industry and to compare the mitochondrial metabolism of beef and bison. The first project evaluated bison industry stakeholder perceptions on management, animal welfare, and meat quality with in-person and online surveys. The stakeholder surveys identified animal handling, bison behavior, employee training, facility design, and transportation duration as the most critical factors that could impact animal welfare in the bison production system. The stakeholders understood that animal welfare is a crucial component of bison production and directly affects meat quality. The second project analyzed multiple live animal factors to benchmark their influence on specific meat quality attributes. Live animal production parameters of bison (n = 2,284; Bulls n = 1,101; Cows n = 199; Heifers n = 984) such as distance traveled, season, number of head bumps in the chute, sex class, and live weight were associated ($P < 0.05$) with differences in fat thickness, ribeye area, blood splash presence, and instrumental color of bison meat. The third experiment compared the mitochondrial metabolism of bison and beef. The left side masseter muscle of crossbred Angus steers (n = 12) and bison (n = 12) were collected within 60 minutes postmortem. The oxygen consumption rate of permeabilized muscle fibers at specific respiration states was evaluated utilizing the Oroboros O_2K high-resolution respirometry system. The abundance of electron transport chain protein in bison and beef muscle was measured using gel electrophoresis. All mitochondrial data were analyzed as mixed models with species as the fixed effect, and day confounded with sex class as the random effect, using JMP Pro 16. No differences were found in oxygen consumption flux (JO_2) between bison and beef under baseline, Leak respiration (LEAK; $P = 0.8813$), rotenone respiration (ROT; $P = 0.1071$), and carbonyl cyanide m-chlorophenyl hydrazone respiration (CCCP; $P = 0.7502$) respiration states. Bison permeabilized muscle fibers had a higher ($P = 0.0016$) JO_2 during max OXPHOS (+D) and produced more hydrogen peroxide ($P = 0.0234$) during this respiration state compared to beef. Respiration control rate (RCR) did not differ ($P = 0.2928$) between beef and bison permeabilized muscle samples. Bison muscle samples contained lower relative abundance of the electron transport chain complexes II ($P = 0.0057$) and III proteins ($P = 0.0020$) than beef. Additionally, bison and beef had similar concentrations of citrate synthase in the masseter muscle ($P = 0.4650$). Results from these experiments can be used as an industry reference to monitor improvements in bison animal welfare and meat quality. Additionally, information regarding mitochondrial metabolism can serve as the foundation for future research to further investigate differences in efficiency between bison and beef.

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DEDICATION

I dedicate this dissertation to my late godfather Hernando Velasco.

Querido padrino, tu ausencia no ha sido fácil de procesar. Pero como nos pediste en tus últimas palabras, intento recordar con más frecuencia los momentos felices que vivimos. Tú y yo sabemos que en ámbito académico nunca fui un estudiante excepcional, pero aun así siempre me apoyaste y me instruiste. Recuerdo claramente el último día que te vi, te platiqué mi situación y me quejé del estrés que da hacer un Doctorado. Me escuchaste pacientemente y dijiste "síguele echando muchas ganas, que tienes muchas bocas que callar." Después cambiamos el tema y empezaste con tus bromas. Por unos minutos todo era risas y felicidad. Voy a seguir tu ejemplo y siempre dejar a las personas mejor de como estaban. Te dedico esta disertación que representa mi esfuerzo de tres años y lo importante que eres para mí.

Atentamente,

David Velazco

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CHAPTER 1: LITERATURE REVIEW

AMERICAN BISON

Bison Industry

The American bison meat industry represents a niche sector within the agricultural market that has been experiencing continuous growth (USDA, 2024). As the industry grows, it becomes crucial to quantify live animal and carcass characteristics that can directly influence carcass value, such as dressing percentage, fat thickness, ribeye area, and meat color. These parameters are not only important to determine market prices but also serve as indicators of meat quality and can be related to animal welfare. Moreover, live animal production parameters, including distance traveled to the plant and season, have been associated with variations in meat quality, indicating the importance of preslaughter management. Accurate quantification and understanding of management factors, meat quality, and animal welfare characteristics can lay the groundwork for future research to increase meat quality and enhance bison welfare.

American bison (*Bison, bison, bison*) are often referred to as buffalo; however, bison and buffalo are distinct species as bison belongs to the genus Bison and buffalo belongs to the genus Bubalus (Arthun and Holechek, 1982). The American bison is distinguished from other bovids by having a large, heavy skull, a hump along the frontal section of the dorsal process, and smaller appearing hindquarters (compared to the forequarters). Also, American bison are the largest terrestrial mammal that inhabits the Western Hemisphere (Plumb et al., 2014). In the 1880s, the American bison (or Plains Bison) population was affected by the hunting practices of Euro-Americans (Kolipinski et al., 2014). Once roaming in high numbers in the grasslands of North America, the bison population diminished close to extinction due to overhunting and habitat loss during the 19th century (Tokarska et al., 2011). Their remarkable recovery from near extinction has led to their recognition as one of the most emblematic species representing the United States. Currently, bison are used for meat consumption in the United States. Paradoxically, their consumption helps increase the number of bison in North America (Tielkes and Altmann, 2021) as most of the bison in the U.S. are used for commercial purposes (CENSUS, 2022).

Market Value and Size

According to the USDA National Agricultural Statistic Service's 2022 Census of Agriculture, the United States had approximately 192,477 bison raised for livestock across 47 states (CENSUS, 2022). The top bison-producing states are South Dakota, Nebraska, and Montana. The number of farms increased from 1,775 in 2017 to 1,986 in 2022 (CENSUS, 2022). In recent years, there has been a consistent upward trend in number of bison slaughtered over the past decade (USDA, 2024). According to the National Monthly Bison Report by the United States Department of Agriculture (USDA, 2024), 68,939 bison were slaughtered in the United States from January to December 15th, 2023, compared to 65,927 in 2022, and 59,614 in 2021 (USDA, 2024). Therefore, the trend is for the bison industry to continue expanding. In the United States, there are nine federally inspected bison slaughter facilities spread across seven states within the United States (APHIS, 2024).

In the United States, the primary export market for bison is Mexico. However, the volume of this export is relatively modest compared to other meat species. The National Monthly Bison Report (USDA, 2023a) reported that 675,432 (lbs) of boneless fresh chilled bison meat was exported to Mexico from January to December 15th, 2023. An increase in bison exports could be anticipated if markets in other countries start recognizing bison as an alternative meat option. This projection is predicated on assuming the industry maintains its current expansion trajectory. The potential for increased exports underscores the demand for bison meat and the opportunities that this presents for the industry.

Bison Industry Associations

Due to the historical significance and the symbolic representation of bison, numerous associations have been established to promote and expand the bison meat industry. The National Bison Association (NBA) is a non-profit entity that plays a pivotal role in developing the bison industry by fostering a collective environment for senior and new stakeholders. Currently, the NBA has a robust membership of over 1,100 individuals spanning all states within the United States, in addition to 10 members located internationally (www.bisoncentral.com). In 2018, the United States farm bill authorized the USDA's National Institute of Agriculture to identify centers of excellence (USDA-NIFA. SEC 1673), and as a result of this initiative the South Dakota State University Center of Excellence for Bison Studies was established. The research from this Institute focuses on enhancing bison health and increasing the overall economic value of tribal and private bison herds.

Bison Behavior and Morphology

Regardless of the increase in demand, bison are not usually considered "livestock" (Duysen et al., 2017) and are typically recognized as a non-domestic or exotic species (Firmage-O'Brien, 2008; FSIS, 2019). In addition to the differences in terminology, bison are also considered flightier and more fearful than beef cattle (Grandin, 2000), which can represent a challenge for workers handling this species. According to the study by Duysen et al. (2017), worker injuries occurred at three of the ten bison slaughter facilities included in the experiment from 2014 to 2015. Although bison are ruminants raised for meat, like beef cattle, their distinct behavioral patterns can pose significant risks to personnel involved in their care and management. In May 2024, the Meat Institute released the updated version of the Meat Industry and Animal Welfare Guidelines and Audit. This is the first version that contains information of transportation, animal handling, stunning, and welfare practices specifically designed for bison. This tool also describes how bison behaves differently than other cattle species. Specifically, bison are either completely still or running full speed. Bison move better in groups and are likely to enter in a pen running and then turn around abruptly imposing worker hazards (MIAHG, 2024). The MIAHG suggests that bison handlers intervene minimally in the moving process and that in some instances the electric prod must be used sparingly (MIAHG, 2024). These behavioral differences emphasize the importance of implementing appropriate safety measures and protocols to mitigate potential hazards in the workplace. Besides differences in behavior, bison and beef cattle have distinct anatomical differences. Bison have a sizeable dorsal process with more fat and muscle on the cranial side of their carcass, while the muscling on the caudal end is comparatively less (Koch et al., 1995). These anatomical characteristics make bison capable of performing agile actions such as jumping cattle guards (Lott, 1974) and impose great risk in inadequate facilities. Therefore, processing facilities should have specialized infrastructures that account for the anatomical and behavioral characteristics of bison.

Quality Audits in the Meat Industry

Because the meat industry is constantly changing, it is useful to use frequent evaluations to adapt to the current needs. To achieve this, the beef meat industry has utilized the National Beef Quality Audit (NBQA). The NBQA is a national collaborative initiative between academia, industry, and government that assesses welfare conditions and quality outcomes of beef produced in the U.S. Originally, the NBQA began in the early 1990s to help improve the beef industry by establishing benchmarks and determining where improvements in the industry could be made by beef producers to ultimately improve the quality of the beef they produce. However, in recent years the NBQA started to measure more than carcass characteristics and focuses also on cattle breeds, mobility scores, and carcass bruising (Shook et al., 2008; Eastwood et al., 2017; Harris et al., 2018). These factors not only reflect welfare conditions but also describe live animal factors that can potentially affect meat quality and production efficiency. This undertaking has been performed regularly six times since 1990 and has led to notable improvements in animal well-being, beef tenderness, and other aspects that have improved demand for U.S. beef. The NBQA has three main components. The first component focuses on interviewing market segments that purchase beef, the second consists of evaluating live beef animals and carcass characteristics at beef processing facilities, and the third aims to disseminate the information gained to cattle producers to implement changes to improve the overall beef industry. The bison industry does not have a program that evaluates the live animal factors related to well-being that could potentially affect the quality of bison meat products. Therefore, an understanding of current industry production characteristics could lay the foundation for future research and potentially lead to the implementation of a program that aims to enhance both bison welfare conditions and meat quality.

Transportation

Bison are produced both in the United States and Canada. However, a portion of the Canadian bison is imported live into the United States for slaughter and further processing (USDA, 2024), and this involves long-distance transportation from producers to processors. Distance traveled is a critical characteristic of transportation as it can directly influence the risk of injury to livestock (Nielsen et al., 2011). Bison are typically transported in a cattle truck and the transportation duration of bison to the slaughter plant is approximately 2-12 hours (Galbraith, 2011), although weather and road conditions may alter these travel times. It is important to note that bison have no access to water or feed during transportation. Transporting and handling bison is a process that carries a significant risk of causing injuries and bruising to the animals (McCorkell et al., 2013; Rioja-Lang, 2019), especially because both male and female bison have horns (Vervaecke et al., 2005) which have the potential to induce injury. Welfare during transport is of paramount importance for two reasons. First, from an ethical standpoint, it is essential to ensure the adequate welfare of animals during handling and transportation. Second, injuries and bruising during transport can lead to an increase in trim loss, directly impacting efficiency and profitability (McCorkell et al., 2013). Previously, the quantification of visual bruising has been utilized as an indicator of welfare (Sullivan et al., 2022) and trim loss (Kline et al., 2020). Therefore, bison producers could benefit from specific bruise information to enhance animal welfare and reduce trim loss. To the authors' knowledge, there is no current information regarding bison transportation and its effect on bruise prevalence. Because of the bison's behavior, the possibility of bison physically injuring each other during loading and unloading (regardless of distance traveled) should be considered.

Slaughter Methods

Before an animal can be humanely slaughtered, it is typically restrained, and adequate tools should be utilized. Bison have a more significant flight zone and sizable frontal cranium bone than cattle (CFIA, 2019; Rioja-Lang, 2019). Based on the authors' observation, traditional captive bolt tools are only utilized when bison are restrained with a specialized head restrainer. A penetrating stunner has proven to be efficient to stun bison when placed 2.5 cm above an imaginary line connecting the center of the horns (MIAHG, 2024), however, most bison are killed with a shot in the skull. According to the Canada Food Inspection Agency's guidelines for stunning techniques of mammalian food animals' section 4.1 (CFIA, 2019), bison should be restrained in a sturdy chute and shot at the intersection of an imaginary line drawn between both eyes and the base of the horns. This should be done with ammunition that does not exceed 2000ft/s for velocity nor 1000ft/lb for energy to avoid ricochet and skull perforation (CFIA, 2019), but that is strong enough to penetrate the skull. The FSIS directive 12600.1 suggests that exotic animals in the United States must be slaughtered following the CFR 9 part 352 (CFR 9, 1978). This article states that exotic animals should be slaughtered according to the CFR title 9 volume 2 part 3.13.16 which describes that an adequate caliber should be used to kill the animal while the animal is in a calm state to ensure efficiency. Animal behavior and the use of firearms to slaughter bison can produce hazardous conditions in the slaughter plants. Thus, appropriate training and adequate facility design are essential to reduce worker endangerment in bison slaughter facilities (Duysen et al., 2017). On-farm slaughter is a unique way of slaughtering cattle that can be practiced with livestock. This process can be done in multiple manners; however, the most common ways utilize a portable on-farm restraining chute. Research suggests that these procedures result in lower frequencies of violent cattle behavior, including backing, kicking, and fighting (Hultgren et al., 2020). A new alternative bison slaughter method in the United States is to shoot them from a distance while free roaming, typically with a long-range bolt action rifle equipped with a telescopic sight and a silencer to ensure accuracy and reduced noise (wildideabuffalo.com). The on-farm slaughter is employed to avoid transportation stress and injury.

Bison Meat Quality Characteristics

Bison meat is often presented as a nutritious and environmentally friendly source of protein in popular news media articles (Sims, 2016; Gazdziak, 2018; Hobbs et al., 2006). According to the Food Safety and Inspection Service (FSIS, 2019) labeling guidelines, growth hormones are not approved for exotic species, including bison. Additionally, the National Bison Association (NBA,

2018) code of ethics requires that members abide by all laws and jurisdictions that apply to bison producers, including restricting growth hormones and non-therapeutic antibiotics. Thus, bison meat products represent a red meat alternative for consumers who demand growth hormone-free products. Bison meat is frequently regarded as a nutritious source of lean protein because it typically contains lower fat and cholesterol than beef (USDA, 2022a, 2022b). A study by McDaniel et al. (2013) analyzed the health impact of bison meat consumption compared to beef consumption over seven weeks and found that bison ingestion led to decreased inflammation, reduced oxidative stress, and a lower atherosclerosis risk than beef intake. Moreover, Popoola et al. (2021) utilized an online free word association survey to understand the perception of Canadian participants (n = 145) regarding red meat from multiple species. The authors found that bison meat was most associated with natural, lean, indigenous, and wildlife words, indicating that consumers perceive bison as a low-fat meat alternative (Popoola et al., 2021). Lower fat content is a characteristic of high importance as consumers are willing to pay more for products that are low in fat and viewed as nutritious (Teixeira and Rodrigues, 2021). Although bison meat is considered a wholesome, healthy meat product, its consumption is low compared to beef (Williamson et al., 2014) possibly because of the lower slaughter numbers of bison in the United States. Therefore, improvements in production and marketing could plausibly increase the sale of meat bison products.

Sensory Profile

The sensory characteristics of meat are complex. There are three main sensory traits: tenderness, juiciness, and flavor (O’Quinn et al., 2018). If one of these traits fails to meet consumer requirements, the overall palatability of meat can be compromised (O’Quinn et al., 2018). These sensory characteristics are greatly influenced by the cooking method before consumption (McClenahan et al., 2001). Calkins and Hodgen (2007) described that flavor arises from the synchronous sensory interpretation of umami, salty, bitter, sour, and sweet tastes. Thus, flavor is a sophisticated, multifaceted attribute of meat products (O'Quinn et al., 2024).

Recent research on bison flavor has focused on the specific flavor-related precursors of bison meat products. Williamson et al. (2014) reported that flavor precursors in bison meat, including mannose, ribose, cysteine, valine, leucine, and glutamate, increased after 21 days of wet-aged storage at 4°C. Williamson et al., (2014) also showed that unsaturated free fatty acids that affect the Maillard reaction, such as linoleic acid and arachidonic acid, also increased during storage. Additionally, cooked bison samples (121°C for 15 minutes) assessed by a trained sensory panel had an increase in vinegar/sour aroma, tenderness, and juiciness, whilst decreasing connective tissue and chewiness at aging day 15, indicating an optimal wet-aging time for bison meat products (Williamson et al., 2014).

A challenge for bison meat products is that they can produce off-flavors (Juarez et al., 2019; Koch et al., 1995). This off-flavor development could be because bison has less intramuscular fat (USDA, 2022b) and a different fatty acid profile (Galbraith et al., 2016) than other species, such as beef. Thus, when bison meat is cooked, lipophilic compounds that usually remain in the fat (with enough content) can be volatilized, influencing the meat's organoleptic characteristics (Gardner and Legako, 2018; O'Quinn et al., 2024). Future research must delve deeper into understanding the flavor profile of these products.

Meat tenderness can be defined as the perception by consumers of easy mastication, during which meat structure is disorganized (Lepetit and Culioli, 1994), and it is one of the most critical quality attributes of meat products that affect consumer satisfaction (Sasaki et al., 2014; Miller et al., 2001). Łozicki et al. (2017) compared multiple quality attributes of domestic cattle (Bos taurus), zubron (Bos taurus x Bison bonasus), and European bison (Bison bonasus), and found lower shear force values for beef than European bison. In contrast, Koch et al. (1995) reported that bison had less marbling and yet lower shear force values compared to beef. These contradictory results could be because multiple factors, including collagen content, muscle fiber type, and aging time, can affect the overall tenderness of meat products (Warner et al., 1998). Additionally, Rinse and Chill® is a technology that is used in the bison meat industry that could further influence bison meat tenderness. Rinse and Chill® (Rinse and Chill, WI, USA) is a technology that involves pumping a solution containing 98.5% water and 1.5% mixture of dextrose, maltose, and sodium phosphates through the vascular system of bison before exsanguination. Kethavath et al., (2022) reported that bison longissimus lumborum steaks treated with Rinse and Chill® had Warner-Bratzler shear force values 24% lower than untreated controls. Previous studies have suggested that the perception of tenderness in beef is significantly influenced by its fat content (O'Quinn et al., 2012; Garmyn et al., 2011). As previously mentioned, grain fed-bison has less fat content than grain-fed beef (USDA, 2022b; Rule et al., 2002). Given the contrasting results regarding bison tenderness, more studies are needed to understand whether the differences in tenderness between species are due to marbling content, Rinse and Chill® application, or other factors such as postmortem enzymatic activity.

Oxidation in Meat Products

Lipid oxidation in meat products is a well-known autocatalytic process that leads to rancidity, producing off odors and flavors (Gray and Monahan, 1992). The significance of lipid oxidation in meat products cannot be overstated, as it results in degradation in quality characteristics. This degradation can lead to undesirable quality changes, which may be subject to potential rejection by consumers (Dominguez et al., 2019; 2021). Lipid oxidation is a progressive sequence of reactions involving free radicals, comprising initiation, propagation, and termination

(Min and Ahn, 2005). The initiation period is a chemical process that can occur in multiple reaction series such as Fenton chemistry and autoxidation. Fenton chemistry occurs when hydrogen peroxide reacts with iron in the ferrous state, producing a hydroxyl radical, a hydroxide, and iron in the ferric state (Tang et al., 2021). Autoxidation involves the formation of free radicals due to environmental substrates such as oxygen metals and or light (Tang et al., 2021; Fenton, 1984). During the propagation stage, free radicals interact with proteins, metals, and mono or poly-unsaturated fatty acids, creating a variety of lipid radicals that, in the presence of oxygen, continue to enhance oxidative autocatalytic reactions (Min and Ahn, 2005). Lastly, during the termination stage, free radicals stabilize by reacting with each other, producing stable compounds, and stopping the reaction (Tang et al., 2021; Min and Ahn, 2005).

The type of livestock and their dietary feeding regimens significantly impact the oxidation processes in meat products. This is primarily due to the saturation level of fatty acids and the total fat composition within the meat (Rule et al., 2002). Thus, when discussing lipid oxidation in meat, it is important to describe species and dietary practices. Certain inherent attributes of meat composition may lead to effects contrary to initial expectations. For example, although grass-fed bison contains more vitamin E (alpha-tocopherol, which is a known antioxidant) compared to beef (Łozicki et al., 2017), it experiences accelerated lipid oxidation during retail display, resulting in elevated thiobarbituric acid reactive substances (TBARS) values (Galbraith et al., 2016). This phenomenon is potentially attributable to bison’s fatty acid composition, which contains polyunsaturated fatty acids (PUFAs) in quantities more than triple those found in beef (Galbraith et al., 2016). Additionally, research by Rule et al. (2002) presented that beef and bison in a grass-fed finishing system produced higher PUFAs in their total fat composition compared to the grain-finished systems in their longissimus dorsi muscles. Notably, these differences in percentage fatty acid composition do not necessarily translate to increased overall fat content per unit of weight. This is because grain-finished bison will typically contain more fat within muscle per unit of weight, resulting in greater total mono and polyunsaturated fatty acid content compared to grass-finished bison (Janssen et al., 2021).

Meat Color

Consumers purchase fresh meat products using visual characteristics as the primary indicators of freshness and wholesomeness, making meat color one of the most essential characteristics influencing buying decisions (Mancini and Hunt, 2005; Suman et al., 2014). Meat color is primarily influenced by the condition of myoglobin present on its surface. Myoglobin, in its deoxygenated form, known as deoxymyoglobin, imparts a purple-red color (Mancini and Hunt, 2005). This state is typically observed in freshly cut meat before it has been exposed to air. Upon exposure to oxygen, myoglobin transitions into the oxymyoglobin state, which is ferrous, and this results in the meat producing a cherry-red color that is often associated with freshness. However, when meat is stored for extended periods or is not preserved in adequate conditions, it can undergo a process where water binds to the myoglobin, converting it into metmyoglobin, which is in a ferric state. This change results in the meat exhibiting a brown color, leading to a decrease in a* values and the production of discolored meat, which is typically less favored by consumers. (Mancini and Hunt, 2005; Suman and Joseph, 2013; Holman et al., 2017; Mancini et al 2018). Meat color is significant, as it has substantial implications for food waste and economic loss. A recent study by Ramanathan et al. (2022) presented that the U.S. beef industry suffers an annual financial loss of approximately $3.73 billion because of meat discoloration, which translates to a yearly wastage of 194.7 million kg. There is currently no data regarding bison meat waste because of discoloration.

Thus, future research should analyze what the implications of bison meat discoloration are regarding food waste and economic loss.

Muscles are highly organized structures designed for specific functions, and therefore contain distinct myoglobin contents depending on the fiber type. Type I muscle fibers rely on oxidative metabolism and have high myoglobin content (Picard and Gagaoua, 2020). In contrast, type II muscle fibers are more glycolytic (Lee et al., 2010) and contain less myoglobin (Picard and Gagaoua , 2020). Intermediate muscle fiber types share characteristics with types I and II (Picard and Gagaoua, 2020). Muscle fiber type is dependent on multiple factors, including muscle function and species, all of which can affect meat color. For instance, bison meat has a darker appearance than beef (Koch et al., 1995; Sood et al., 2020). This difference could be because bison meat has less content of αW (type II) muscle fiber than beef (Koch et al., 1995; Aalhus et al., 2009), and αW muscle fibers have less myoglobin content than αR (type I; Picard and Gagaoua, 2020). Therefore, the inherently greater myoglobin content in bison meat (Galbraith et al., 2016) may be responsible for the darker color. These fiber type differences not only affect the visual appearance of meat but also influence the color stability of meat products. Type I muscle fibers have been associated with rapid rates of discoloration, and type II has been associated with increased color stability (Picard and Gagaoua, 2020).

Bison meat tends to undergo rapid discoloration under aerobic packaging (Galbraith et al., 2011; Sood et al., 2020). This difference in discoloration is of interest because bison and beef myoglobin are structurally identical (Joseph et al., 2010). Galbraith et al. (2016) compared beef (grain-fed) and bison (grass-fed) longissimus steaks under aerobic packaging at retail display conditions, and bison steaks underwent oxidation at a higher rate during three days of retail display. Due to its color-liable nature, bison meat is often sold in vacuum packages (Pietrasik et al., 2006).

Previous studies have described the role of mitochondria in the color of meat (Tang et al., 2005; Mancini et al., 2018; Kiyimba et al., 2022). However, to the author's knowledge, there is no research directly comparing the mitochondrial activity of bison and beef. Investigating differences in mitochondrial metabolism could give insight into why bison meat discolors at a faster rate compared to beef.

MITOCHONDRIA

Bison and beef myoglobin share the exact amino acid sequences (Joseph et al., 2010). However, their meat quality characteristics, such as color and discoloration rate, differ. This difference in color stability could be due to mitochondrial biochemistry. Therefore, the forthcoming discussion will describe mitochondrial functionality, including oxygen consumption rates, reactive oxygen species (ROS) formation, and specific functionality of electron transport chain proteins and their association with meat color.

Mitochondrial Functionality

Mitochondria are the powerhouse of cells because they are responsible for making ATP (the energy currency of living systems) required for multiple metabolic purposes. Mitochondria contain an outer membrane that is somewhat permeable and an inner membrane that is highly impermeable, the space in between these two membranes is called the inter-membrane space. There are two types of mitochondria typically found in skeletal muscle: the subsarcolemmal and the intermyofibrillar. The subsarcolemmal mitochondria reside on the periphery of the muscle fibers and beneath the sarcolemma, whereas the intermyofibrillar mitochondria are in between myofibrils (Ramos et al., 2020). During energy metabolism, glycolysis converts one molecule of glucose into 2 molecules of pyruvate and two molecules of ATP. Pyruvate can enter the mitochondria to be converted into acetyl CoA in the presence of oxygen. Acetyl CoA is modified by undergoing the Krebs cycle to recharge reducing equivalents such as NADH and $FADH_2$. Under aerobic conditions, these electron carriers can react with proteins found in the inner membrane of the mitochondria to produce a proton gradient in the inter-membrane space and, with this energy, synthesize ATP. There are five complexes found in the inner mitochondrial membrane: complex I (NADH-ubiquinone oxidoreductase), complex II (succinate-ubiquinone oxidoreductase), complex III (Ubiquinol-cytochrome C oxidoreductase), complex IV (cytochrome C oxidase) and complex V (ATP-synthase). Under aerobic conditions, NADH reacts with complex I and is oxidized (supercharging complex I), pumping protons into the intermembrane space. Additionally, one electron is transferred to vitK2 or coenzyme Q. Similarly, $FADH_2$ interacts with complex II under aerobic conditions, and it is also oxidized, passing its electrons to vitK2 or coenzyme Q, but protons are not introduced by this complex (complex II does not cross the intermembrane space). Electrons from complexes I and II supercharge complex III, pumping additional protons into the intermembrane space against the concentration gradient. Cytochrome C transfers electrons from vitK2 and Coenzyme Q to complex IV and uses oxygen as the final electron acceptor to produce water and pump more protons into the intermembrane space. Using all the gradient force from the protons pumped into the intermembrane space, complex V spins and converts ADP and inorganic phosphate into ATP. However, mitochondrial metabolism may change depending on the conditions they are subjected to. After the animal has been exsanguinated, essential nutrients and oxygen needed for oxidative phosphorylation no longer reach the muscle cells (Matarneh et al, 2023). To maintain minimal energy levels, the muscle cell shifts its metabolism from aerobic to anaerobic leading to the formation of lactate, which is ultimately converted to lactic acid dropping the pH of meat (Paredi et al., 2012). Although the animal is dead, the muscle mitochondria are still capable of respiring oxygen and undergoing biochemical reactions (Tang et al., 2005; Mancini et al., 2018). It has been found that muscle mitochondria can still respire after 3 weeks of fresh storage, however, this is largely dependent on muscle and storage conditions (Grabez et al., 2015). This is important because the storage conditions of muscle can influence mitochondrial respiration and, thereby, meat color and shelf life.

Assessing Mitochondrial Metabolism

There are multiple ways to analyze mitochondrial metabolism. Some methods utilize an isolation method that requires differential centrifugation to separate mitochondria from other organelles (Liao et al., 2019). This isolation can be done to quantify mitochondrial content in a sample; however, isolated mitochondria can also be used to quantify respiration rates and reactive oxygen species production with an Oroboros respirometer. This Oroboros system measures oxygen depletion rates in a sealed chamber to present respiration rates during multiple steps in the electron transport chain. Additionally, this system can measure ATP and ROS production. The relative efficiency of complexes can be analyzed via the use of complex-specific inhibitors, comparing oxygen consumption before and after the addition of enzyme inhibitors (Bioblast, 2022).

Research suggests that the isolation of mitochondria affects its morphology and thereby affects its functionality (Picard et al., 2011). To avoid this, muscle mitochondria respiration rates can be analyzed without extraction to obtain biologically relevant respiration readings and avoid mitochondrial extraction effects. Muscle cells can be permeabilized by immersing the muscle fibers in ice-cold BIOPS solution and with the usage of tweezers, pulling the muscle fibers apart. This is done to increase the surface area and allow for deeper oxygenation. Using permeabilized muscle fibers for mitochondrial analysis provides a more realistic quantification of mitochondrial metabolism by maintaining their morphology and function.

Oxygen Consumption Rate

Mitochondrial oxygen consumption rate can influence meat color because mitochondria can directly compete for oxygen with myoglobin (McKeith et al., 2016). As the goal of myoglobin is to temporarily store oxygen in the muscle cell, which then will be transferred via facilitated diffusion for mitochondrial oxidative phosphorylation (Kamga et al., 2021), it can be hypothesized that mitochondria have a higher affinity for oxygen compared to myoglobin (McKeith et al., 2016; Kiyimba et al., 2022). Therefore, high rates of mitochondrial respiration could result in the prevention of oxymyoglobin formation and the production of a dark meat color (Ramanathan and Mancini, 2018). Alternatively, when oxygen consumption by the mitochondria is minimal, a substantial amount of the available oxygen can attach to myoglobin (Mancini and Hunt, 2005). This attachment results in an increased production of oxymyoglobin, which imparts a distinctive cherry-red color to the meat. This phenomenon is important for consumers, as the meat's color is a primary determinant of its freshness and quality (Tomasevic et al 2021).

Beef muscles that are color labile, such as the psoas major, gluteus medius, and triceps brachii, exhibit a high rate of oxygen consumption and a low depth of oxygen penetration (Mckenna et al., 2005). On the contrary, muscles that are color stable, including the longissimus lumborum, longissimus thoracis, and tensor fasciae latae, demonstrate a low rate of oxygen consumption and an increased depth of oxygen penetration during display (Mckenna et al., 2005). Muscles with higher oxygen consumption rates tend to be more color-labile, leading to darker meat, whereas those with lower rates exhibit a more stable color. This describes the importance of oxygen consumption as a factor in meat coloration and its subsequent impact on consumer perception and marketability.

Myoglobin and Mitochondrial Redox Interactions

The two main antioxidant systems found in meat are direct NADH-dependent reductase activity and non/enzymatic metmyoglobin reduction (Bekhit and Faustman 2005). The enzyme NADH-dependent reductase catalyzes a biochemical reaction that converts metmyoglobin, a form of myoglobin with a ferric iron atom, to ferrous myoglobin, where the iron atom is in a ferrous state (Ramanathan and Mancini, 2018). This enzymatic conversion occurs in the presence of the coenzyme NADH. In this reaction, NADH acts as a reducing agent, donating electrons to facilitate the reduction of metmyoglobin to ferrous myoglobin (Arihara et al., 1995). Under normal physiological conditions in living tissue, electrons flow from Complex I (NADH ubiquinone oxidoreductase) to Complex IV (cytochrome C oxidase) in the electron transport chain. However, in postmortem conditions, given the presence of sufficient amounts of succinate (a TCA cycle intermediate) and NAD, the electron flow can reverse. This results in the generation of NADH from NAD, a process known as reverse electron transport (Belskie et al., 2015). The NADH produced can then participate in either enzymatic reduction reactions or non-enzymatic metmyoglobin reduction (Ramanathan and Mancini, 2018). Furthermore, NADH acts as a reducing agent, donating electrons to other molecules in enzymatically catalyzed reactions (Bekhit and Faustman, 2005).

In addition to the previously mentioned reducing agents, cytochrome C plays a significant role in metmyoglobin reduction (Ramanathan and Mancini, 2018). Cytochrome C is involved in the transfer of electrons between Complex III (Ubiquinol-cytochrome C oxidoreductase) and Complex IV (cytochrome C oxidase). In this process, cytochrome C acts as an electron donor, which could potentially be harnessed for the reduction of metmyoglobin. Specifically, the electrons donated by cytochrome C could reduce the ferric iron atom in metmyoglobin to a ferrous state, thereby converting metmyoglobin to deoxymyoglobin (Tang et al., 2005).

Mitochondrial Respiration States

Mitochondrial respiration states are associated with mitochondrial response to cellular energy demands in the presence of specific substrates. By studying these states, we can gain insights into the efficiency of ATP production and oxygen consumption. To the author's knowledge, there is currently no data regarding differences in respiration rates between beef and bison species. An understanding of the respiration states is crucial when analyzing mitochondrial metabolism and efficiency.

There are five respiration states which were thoroughly described by Chance and Williams (1955). The state one respiration, often referred to as basal mitochondrial respiration or LEAK ((L): Ln), involves the addition of mitochondria into a medium containing a fixed amount of oxygen present, and naturally occurring inorganic phosphate in the absence of ADP. The state one oxygen consumption is supported by unknown endogenous substrates such as TCA intermediates and naturally present ADP (Chance and Williams, 1955). The state 2 respiration (Rox), also known as substrate-limited respiration, refers to the stabilization of mitochondrial oxygen consumption after the addition of specific mitochondrial respiration substrates (Chance and Williams, 1955). The state 3 respiration state (OXPHOS: (P)) describes the maximal mitochondrial oxygen consumption in the presence of high ADP, inorganic phosphate, and TCA cycle substrates such as pyruvate, malate, glutamate, and succinate, which produce both NADH and FADH2 (Chance and Williams, 1955). State 4 respiration ((L): Lt), also known as Lt-LEAK respiration, involves mitochondrial oxygen consumption when all ADP has been phosphorylated to ATP. This oxygen consumption occurs via intrinsic naturally occurring uncoupling of the inner mitochondrial membrane that allows protons through, leading to oxygen consumption and water formation (at complex IV) without the production of ATP (Chance and Williams, 1955). Finally, state 5 occurs when all the oxygen has been consumed, leading to anoxia (Chance and Williams, 1955). With the addition of an uncoupler, it is possible to induce maximum oxygen flux through the artificial introduction of protons to the mitochondrial matrix, increasing oxygen consumption and leading to the electron-transfer (E) capacity respiration state (Gnaiger, 2009). By utilizing this information, it is possible to calculate the L/E coupling-control ratio by dividing LEAK respiration by ET capacity. This can be used to define the degree of mitochondrial uncoupling (or dyscoupled in the case of disease; Gnaiger, 2009). Additionally, the coupling efficiency (j E-L) can be calculated using the following equation jE-L = 1 – (L/E), where 0.0 represents zero coupling and 1.0 is a fully coupled system. Cytochrome C is known to activate apoptosis in skeletal muscle cells (Zou et al., 1999; Zhang et al., 2017). Therefore, outer membrane integrity is of importance when analyzing mitochondrial oxygen respiration rates. If the membrane has been damaged, it can cause the release of cytochrome C, inducing programmed cell death and reducing oxygen consumption by tissue weight. An increase in respiration after the addition of cytochrome C into the respirometer can determine if the mitochondrial membrane has been damaged (Volani et al., 2017; Fischer et al., 2022) and troubleshoot the teasing process to determine if the differences in respiration are a representation of treatment differences rather than induced by cell death.

Reactive Oxygen Species (ROS)

As discussed previously, mitochondria use oxygen as the final electron acceptor in the oxidative phosphorylation process. Due to the reactive nature of oxygen, it has the potential to generate ROS. These substances are defined as oxygen-containing species that are more reactive than oxygen itself (Freinbichler et al., 2011). During the aging process, the level of ROS tends to increase (Zou et al., 2022). Thus, differences in ROS formation postmortem could reflect differences in antioxidant capacities between muscles (Anderson and Neufer, 2006) and potentially animal species. The most common free radical produced by mammalian mitochondria is superoxide (Mailloux, 2020). This free radical is usually stabilized by superoxide dismutase (SOD). The mitochondrial variant of this enzyme is known as SOD2 (superoxide dismutase 2) and it's in charge of the dismutation of superoxide into hydrogen peroxide (H_2O_2) (Powers et al., 2022). A common mechanism that mitochondria utilizes to stabilize superoxide and other highly reactive free radicals such as H_2O_2 is tripeptide glutathione peroxidase (GPX; γ-L-glutamyl-L-cysteinyl-glycine; mGSH). This enzyme can maintain the mitochondrial oxidative balance by reducing H_2O_2 and other hydroperoxides to form water or alcohol (Mari et al., 2009; Radak et al., 1995). Studies have reported greater GPX activity and lower thiobarbituric acid (TBA) values in chickens fed a selenium-supplemented diet (DeVore et al., 1983), however, studies performed in beef cattle have not found a direct relationship between GPX activity and lipid oxidation or pigment (O'Grady et al., 2001). Thus, further investigation is needed to understand the relationship between GPX activity and meat color.

The H_2O_2 levels postmortem can provide insights into the metabolic processes that occur in live animal conditions. For instance, an accumulation of ROS postmortem may indicate a higher or lower muscle redox capacity. The study of ROS in bison muscle, particularly in comparison to beef, is an unexplored area of research. This research gap represents a significant opportunity for future studies to investigate whether there are inherent differences in oxidative stress and muscle metabolism between bison and cattle, which could have implications on meat quality and shelf life. Such comparative studies could also contribute to a better understanding of species-specific muscle metabolism.

Chapter 2 of this dissertation examines the benchmarking of the United States bison meat industry, focusing on stakeholder perceptions, production parameters, and live animal factors related to animal well-being that affect meat quality. Chapter 3 of this dissertation compares the mitochondrial metabolism of bison and beef describing respiration state-specific oxygen flux, hydrogen peroxide production and overall mitochondrial efficiency.

CHAPTER 2: BENCHMARKING THE UNITED STATES BISON MEAT INDUSTRY: STAKEHOLDER PERCEPTIONS, PRODUCTION PARAMETERS AND LIVE ANIMAL FACTORS AFFECTING MEAT QUALITY

Summary

This project evaluated bison industry stakeholder perceptions on management, animal welfare and meat quality with in-person and online surveys. Additionally, multiple live animal factors were measured to benchmark their influence on specific meat quality attributes. From the stakeholder surveys, animal handling, bison behavior, employee training, facility design, and transportation duration were identified as the most critical factors that could impact animal welfare in the bison production system. Moreover, the stakeholders understood that animal welfare is a critical component for bison production and that it directly affects meat quality. Live animal production parameters such as distance traveled, season, number of head bumps in the chute, sex class and live weight were associated with differences in fat thickness, ribeye area, blood splash presence and instrumental color of bison meat. The results from this study can be used as a baseline for industry improvements and future research.

INTRODUCTION

The United States bison industry is growing both in size and popularity, with bison slaughter numbers increasing steadily over the past several years (USDA, 2022a; 2023a; 2024a). According to the United States Department of Agriculture (USDA) National Monthly Bison Report (USDA, 2023a), a total of 68,939 bison were harvested in the United States from January to December 15th, 2023. In comparison, the number of bison slaughtered during the corresponding period in the years 2022 and 2021 were 65,927 and 59,614 respectively (USDA, 2024a). There are nine federally inspected bison slaughter facilities, which are distributed across seven states within the country (USDA, 2023b). Media articles indicate that consumers perceive bison meat products as a healthy sustainable protein (Sims, 2016; Gazdziak, 2018; Madhivi, 2020) compared to other livestock and poultry meat. Additionally, bison meat is often considered a healthy lean protein as it typically contains less fat and cholesterol compared to beef (USDA, 2022b). A recent study with human subjects examining the effect of consuming bison meat compared to beef by analyzing blood lipids, oxidative stress markers, and endothelial function for seven weeks indicated that the consumption of bison results in reduced inflammation, lower oxidative stress and lower atherogenic risk compared to beef consumption (McDaniel et al., 2013). The lower cholesterol and fat content inherent in bison meat may be the primary factors contributing to its recognition and value among consumers. Therefore, the nutritional composition of bison meat is a healthy alternative in the market, attracting health-conscious consumers.

There are some distinct behavioral differences between bison and domestic cattle (Rioja-Lang et al., 2019). Therefore, the facility design, transportation logistics, and animal handling of bison tend to be modified from beef cattle to accommodate differences in bison behavior. During the initial stages of bison production, the animals spend most of their time grazing in pastures, after which bison are usually transported directly to the slaughter plant or to a feedlot for finishing (Tielkes and Altman, 2021). Transporting bison can result in injuries during the process, and this can affect trim loss (McCorkell et al., 2013). Studies have shown that blood cortisol levels (an indicator for stress) were greater in bison that had been transported versus bison that were slaughtered on-farm (Galbraith, 2011). Additionally, bison have horns that can cause bruising during transportation and handling, leading to trim loss during fabrication (McCorkell et al., 2013).

Although both cattle and bison are ruminants, their meat quality attributes differ. Bison meat is darker than beef due to differences in the muscle fiber types and greater myoglobin concentration in bison (Galbraith et al., 2014). Moreover, bison meat color is unstable compared to beef and browns relatively quickly during retail display in oxygen permeable packaging (Sood et al., 2020). Unlike beef, bison longissimus lumborum muscles contain minimal marbling (Ding et al., 2016) and are darker (Galbraith et al., 2014), which impedes the direct application of the USDA grading system that exists for beef to bison. Another factor that could potentially affect bison meat grading is diet (Marchello and Driskell, 2001). Previous studies have indicated that corn-based diets could potentially generate bison with higher marbling compared to grass fed (Janssen et al., 2021). Moreover, there is currently limited research on bison producer perspectives regarding quality grading application to their products.

The beef industry has been able to continuously improve quality using the National Cattlemen's Beef Association (NCBA) National Beef Quality Audit (NBQA) data which has been conducted since the early 1990's. The NBQA benchmarks meat quality and welfare attributes of cattle arriving at slaughter plants approximately every five years and serves as a baseline for the beef industry in the United States. The beef industry has made significant improvements in animal welfare and meat quality attributes utilizing the NBQA information (e.g., reduction of injection site lesions, awareness of carcass bruising). However, similar benchmarking does not exist for the bison industry and to the authors knowledge there is no data on stakeholder perceptions related to bison meat production and carcass quality outcomes at slaughter that impact carcass value. As the bison industry continues to grow, benchmarking will be instrumental in establishing guideposts, identifying needs to drive quality enhancements, and to ultimately drive improvement and profitability throughout the supply chain. Therefore, the objectives of this study were 1) to understand the perceptions of individuals that work in the bison industry regarding animal welfare, management, and meat quality, 2) to benchmark live animal and carcass characteristics of bison, and 3) to determine specific antemortem factors that impact quality characteristics of bison meat.

MATERIALS AND METHODS

Survey Population and Recruitment

The project was approved by Colorado State University's (CSU's) Institutional Review Board prior to study initiation (IRB Approval #2871). The target population for this survey consisted of cow-calf producers, finishers, processors, distributors, and other individuals that work in the bison industry. The survey was distributed during the National Bison Association (NBA) Winter Conference in Denver, CO, on January 20th, 2022. Researchers hosted a booth at the conference and asked attendees (total conference attendees n = 245) if they were interested in filling out a survey. Attendees could take the survey on an iPad (7th Generation, Apple Inc., Cupertino, CA) or on a personal device. Additionally, all conference attendees received a flyer with conference registration materials which included a quick response (QR) code to take the survey. Respondents were not offered any incentive for participation. Participation was voluntary, and respondents could skip questions or stop the survey at any time. The only required question was the initial question confirming consent to participate. One week after the conference ended, an email was shared with approximately 1,300 members of the National Bison Association inviting them to participate in the survey.

Survey Format

The survey was created in Qualtrics (Qualtrics, WA. USA) and developed by an interdisciplinary team with proficiency in animal welfare and meat science. The survey was tested before distribution to ensure functionality and clarity. The survey had a total of 22 questions including Likert, open ended, and multiple answer questions (Supplementary Material). The survey included questions about the current bison industry growth, animal welfare, marketing, and bison meat quality. Demographic questions included age, gender, race, time working in the industry, size of operation, location of operation and sector of the industry they worked. The survey was designed to take less than 30 minutes to complete. Many questions had a text option to write a personalized answer if none of the options given represented the respondent´s point of view.

Live Animal Data Collection

This study was observational, therefore an IACUC waiver was granted (IACUC #: 2672). This study was conducted from November 2021 to December 2022. A CSU research team visited 3 bison processing plants in the Western U. S. These plants harvested approximately 50 to 180 bison per day.

Antemortem Information

The slaughter date, lot number, lot head count, and producer were collected by a CSU student at the live animal holding pens. The estimated distance traveled to the slaughter facility was calculated using the shortest distance produced by GoogleMaps (Google, CA, USA) using the producer or feeder location to the slaughter facility (distance in Km). Season was recorded based on the following date cutoffs: Spring - March 1st to May 31st; Summer - June 1st to August 31st; Fall - September 1st to November 30th; Winter - December 1st to February 29th in leap year. Producer information and sex class was provided by the processing facilities. Sex classes were bulls, cows, and heifers.

A trained graduate student with past experience evaluating animal handling and movement and scored mobility, electric prod use and vocalization when animals moved from the holding pens to the holding chute. Mobility was tallied for number of dead/downers, normal moving animals (moved without any problems), and abnormal (limping or refusing to move). The electric prod use was tallied for the number of animals prodded (not for number of times prodded) and the number of animals that grunted (yes/no) was also recorded at this time.

Animal live weights were collected either in the restraining chute (built-in scale) or immediately after stunning the animal (before exsanguination). At the restraining chute, grunt score (yes/no) and number of head bumps in the chute were recorded. Visual mud coverage was scored either before the bison entered the restraining chute or after the bison was stunned and hung (this was plant-dependent). The number of head bumps in the chute was defined as when the animal intentionally and forcefully hit the inner surface of the chute. If the plant did not have restraining chute where bison could head bump, head bumps were recorded in the single file prior to the final restrainer. Visual mud coverage was assessed with an ordinal scale from 1 to 3 (1- less than 1/3 mud coverage, 2- between 1 and 2/3 mud coverage, 3- more than 2/3 mud coverage). A specific carcass ID was assigned at the processing facility (added post-harvest) and was used to trace each individual carcass through the slaughter facility. Hot carcass weights were collected after the bison were fully processed (skinned, eviscerated, dismembered, and trimmed). Cold carcass weights were taken before the carcasses were cut into primals after chilling for approximately 18 to 23 h.

Bruising Score

A carcass map, adapted from (Strappini et al., 2012) was used to determine bruise location of each individual carcass (Figure 1). The specific modification was the addition of two sections representing the front legs of the animal (R8 and L8). Bruising was marked on the carcass map where the bruise was observed. Additionally, the length of the longest part of the bruise was used to estimate bruise size using a 0 to 4 visual scoring system: 0 = no bruise; 1 = greatest length between 0.1 and 7.62 cm, 2 = between 7.63 and 15.24 cm; 3 = between 15.25 and 30.48 cm and

4 = greater than 40.48 cm. Multiple bruises within a section were recorded, however, only the highest bruise for each specific section was considered in this study.

Meat Quality Measurements

The following measurements were taken approximately 12 hours postmortem in the carcass coolers. The carcasses were ribbed between the 12th and the 13th rib and allowed to bloom in the coolers for one hour. The right side of the carcass was used to get the following measurements: instrumental color, ribeye area, backfat thickness and blood splash presence. If the right side of the carcass was too damaged to analyze or if the ribbing was not done properly, measurements were taken on left side of the hanging carcass. Instrumental color was collected using a HunterLab MiniScan XE Plus spectrophotometer (illuminant A and 10° standard observer; HunterLab, VA, USA) presenting values according to the CIELAB color space ($L^*a^*b^*$). An average of 3 readings was used, and these measurements were taken across the longissimus thoracic area avoiding large intramuscular fat. The colorimeter was calibrated before the start of every session and its aperture was cleaned between all carcasses to avoid the accumulation of fluids or fat. The ribeye area was calculated using a transparent grid (loin eye area grid) with squares of specific known area. Trained researchers placed the grids over the ribeye and counted the squares to calculate the ribeye area (cm^2) of the longissimus thoracic muscle. Fat thickness was measured between the 12th and the 13th rib. This was done two-thirds of the length of the ribeye from the medial plane to the lateral plane and the exterior fat measurement was taken from the sagittal plane towards the medial plane using a stainless-steel ruler. The presence of blood splash was recorded (yes or no); blood splash was present if the longissimus thoracic muscle had presence of ecchymosis.

Statistical Analysis

The survey data were exported from Qualtrics (Qualtrics, WA. USA) to Microsoft Excel (Microsoft, WA. USA). Descriptive statistical analysis was performed using JMP (Statistical Discovery, NC. USA) software. Live animal and postmortem data were exported from Microsoft Excel (Microsoft, WA. USA) to JMP (Statistical Discovery, NC. USA). Descriptive statistics, linear regression and logistic regression were performed. Linear regression was used for dressing percentage, ribeye area, fat thickness and instrumental color. Logistic regression was done for bruising and blood splash. Model selection was done by stepwise backwards elimination based on parameter significance ($P<0.05$) keeping only significant parameters in the models. Factors considered for dressing percentage were number of head bumps in the chute, ribeye area, fat thickness, distance traveled, sex class, mud score, bruise score (bruised or not), season, plant, live weight, and grunt score (yes/no). Factors considered for ribeye area were fat thickness, distance traveled, sex class, mud score, bruise score, season, plant, live weight, and grunt score. Factors considered for fat thickness were ribeye area, distance traveled, sex class, mud score, bruise score, season, plant, live weight, and grunt score. Factors considered for instrumental color were number of head bumps in the chute, ribeye area, distance traveled, fat thickness, sex class, mud score, bruise score, season, plant, live weight, and grunt score. Lot was included as a random effect in all linear regression models. Factors considered for bruising regression were number of head bumps in the chute, ribeye area, fat thickness, distance traveled, sex class, mud score, season, blood splash and live weight. Factors considered for blood splash regression were number of head bumps in the chute, ribeye area, fat thickness, distance traveled, sex class, mud score, bruise score, season, and live weight. For logistic regression models, lot was not included due to confounding with other variables. Heat maps for bruising by sex class were created in a binary manner (bruise present in a carcass section: yes or no), counting the total percent not bruised and the percent bruised (regardless of size) for a specific carcass section and values were presented in percentages. The dressing percentage was calculated by dividing the hot carcass weight by the live weight and expressing the result as a percentage.

RESULTS

Survey

Seventy individuals (out of 245 attendees) completed surveys at the NBA conference, and fifty-two individuals (out of 1300 members) completed the survey online after the conference, totaling 122 responses. Ten respondents completed less than 10% of all questions, and two individuals were not involved in the industry, so these were removed from the final analysis. A total of 110 surveys were included in the analysis.

Demographics

Respondent demographics are reported in Table 1. Seventy three percent (n = 80) of the respondents were men, and 24% (n = 27) were women. The majority of respondents (87%, n = 93) identified as White. Sixty percent (n = 66) of the respondents had been working in the bison industry for more than 10 years. The survey was answered by cow-calf producers (n = 76), finishers (n = 42), retailers (n = 29), processors (n = 13), and others (n = 28; many of the respondents were involved in more than one type of operation). In a question that allowed for multiple answers, many respondents indicated that their businesses were in the Midwest and West regions of the United States (40.9%, n = 45, and 36.6%, n = 40, respectively). Fewer respondents worked in the Southwest (10.9%, n = 12), Southeast (5.4%, n = 6), Northeast (4.5%, n = 5), and Alaska and Hawaii (1.8%, n = 2).

Industry Growth and Improvement

Seventy-three percent (n = 81) and 21% (n = 23) of the individuals strongly agreed and agreed, respectively, that the bison industry should continue to expand and grow. Individuals were asked a question that allowed for multiple answers about which attributes of the bison industry would benefit from improvements. Most selected responses (Figure 2) from a provided list of options were marketing (64%, n = 70), animal health (46%, n = 51), animal welfare (38%, n = 42) and production cost (38%, n = 42).

Animal welfare

Approximately 96 % (n = 106) of the individuals agreed or strongly agreed that animal welfare is a critical component of bison production (Table 2). Almost all respondents (99%, n = 108) indicated they believe that animal welfare impacts meat quality. Approximately 32% (n = 36) of the individuals strongly agreed and agreed 28% (n = 31) that transportation to the slaughter plant is an area of concern due to its potential impacts on animal welfare. In a question that allowed for multiple answers, individuals were asked to select the factors that affect animal welfare within the bison industry from a provided list of options. Most selected responses were facility design (80%, n = 88), animal handling (78%, n = 86), employee training (56%, n = 62), and transportation duration (56%, n = 62; Figure 3).

Meat Quality

Sixty seven percent (n = 74) of the individuals stated that the most important quality attribute of bison meat is flavor while other answers (color, health/nutrition, juiciness, quality, tenderness and other) were selected by less than 11% (n = 12) of respondents. When asked if there were quality differences between grain-fed and grass-fed bison, 41% (n = 46) and 37% (n = 41) of the individuals strongly agreed and agreed with the statement, respectively (Table 3). When asked if grass-finished bison should be marketed as a premium product, 25% (n = 28) of the individuals strongly agreed, and 25% (n = 28) agreed. In a question that allowed for multiple answer selection from a provided list of options. The most selected options were healthy (84.54%, n = 93), lean meat (73.63%, n = 81), meat quality (70%, n = 77), and unique flavor (54.54%, n = 60) were selected as attributes of bison that provide added benefit/value to consumers (Figure 4). Twenty-two percent (n = 24) of respondents strongly agreed and 32% (n = 35) agreed that a carcass grading system would benefit the US bison industry.

Benchmarking

This study was conducted from November 2021 to December 2022. A total of 72 lots were evaluated from three bison processing facilities in the US over 23 data collection days. A total of 2,284 bison (Bulls n = 1,101; Cows n = 199; Heifers n = 984) were included in the study. Nine animals arrived dead (in the truck), and four had to be euthanized outside of the processing facility due to handling difficulties. It is important to note that the incidents of animals arriving dead or needing to be euthanized outside the processing facility (holding pens) were not isolated for a single day but occurred throughout the project. Of the bison evaluated in this experiment, only 0.56% of bison scored an abnormal mobility score. Approximately 10% of the bison were electrically prodded before being restrained in the knock box or restrainer. Ninety-one percent of the bison (n = 2,022) had a hide mud coverage (Table 4) visual score of 1 (less than 33% of the hide was covered). Only 0.35% (n = 8) of bison grunted while restrained and thirty-two percent (n = 728) of bison forcefully head bumped when restrained in the holding chute. The average blood splash in this study was 4.3%. Sixty-eight percent of bison (n = 1,556) did not head bump at all, 29.6% (n = 676) head bump between 1 and 5 times, 1.7% (n = 39) head bump between 6 and 9 times and 0.5% (n = 13) head bump 10 or more times.

The average distance bison traveled from the producer to the slaughter processing facility was 823.7 ± 583.6 km (Mean ± SD; Tabe 5). The average live weight was 455.8 ± 75.1 kg (Mean ± SD). The average hot carcass weight was 276.4 ± 49.7 kg (Mean ± SD), and the average cold weight was 271.0 ± 49.1 kg. The dressing percentage (Mean ± SD) was 60.5 ± 3.3%. The average fat thickness (Mean ± SD) was 1.4 ± 1.1 cm. Average bison ribeye area was 62.6 ± 9.8 cm^2 (Mean ± SD). The average L^* value for bison longissimus thoracis was 32.9 ± 2.8 (Mean ± SD), the average a^* value was 18.4 ± 2.2, and the average b^* value was 12.7 ± 1.9 (Table 5).

Bruising

Approximately 97% percent of the bison in this study had at least one bruise. Specific percentages for each bruise size and location by sex class are presented in Table 6. Heat maps (Figure 5) demonstrate the frequency of bruising across different carcass regions by sex class. All sex classes had prominent bruise frequency on two bruise sections in the caudal side of the carcass toward the medial plane (R6 and L6). Bulls had more bruising on their dorsal process compared to the rest of their body. Cows and heifers had numerically higher frequency of bruising on the lateral frontal sides of their carcasses (4R and 4L) compared to bulls.

Linear regression

Factors associated with dressing percentage were ribeye area, fat thickness, sex class, plant, and bruise score ($P<0.05$; Table 7). Greater ribeye area and fat depth were associated with greater dressing percentage ($P<0.0001$). Additionally, bruise score was also associated with slightly higher dressing percentages ($P = 0.0431$). Cows had reduced dressing percentage compared to heifers ($P<0.0001$).

Factors associated with ($P<0.05$; Table 7) the ribeye area were sex class, plant, and live weight. Greater live weight was associated with greater ribeye area ($P<0.0001$). Bulls had greater ribeye areas compared to heifers ($P<0.0001$), and cows had lower ribeye areas compared to heifers ($P<0.0001$).

Factors associated with ($P<0.05$; Table 7) fat thickness were sex class and live weight. As live weight increased ($P<0.0001$) fat thickness increased. Bulls had decreased fat thickness compared to heifers ($P<0.0001$) whereas cows had greater fat thickness compared to heifers ($P = 0.0088$).

Factors associated with ($P<0.05$; Table 8) L^* were plant, fat thickness, ribeye area, number of head bumps in the chute, distance traveled, and sex class. The greater the fat thickness ($P<0.0001$), ribeye area ($P<0.0001$), and number of head bumps in the chute ($P = 0.001$) the higher the L^* values.

Factors associated with ($P<0.05$; Table 8) a^* were plant, number of head bumps in the chute, ribeye area, live weight, fat thickness, and sex class. The greater the number of head bumps in the chute ($P = 0.017$), ribeye area ($P<0.0001$), live weight ($P<0.0001$), and fat thickness ($P<0.0001$) the highest a^* values after one hour of bloom time. Bulls and cows had lower a^* values as compared to heifers ($P<0.0001$).

Logistic Regression

Season, sex class, live weight, and plant were associated with bruising ($P<0.05$; Table 9). The odds of being bruised increased as live weight increased, with an odds ratio (OR) of 1.003 and a confidence interval (CI) range of 1.0012 to 1.005. Additionally, the odds of being bruised were greater in cows as compared to heifers (OR: 3.561, CI: 1.604, 12.005) and reduced in bulls as compared to heifers (OR: 0.298, CI: 0.151, 0.505). The odds of being bruised increased in Fall compared to Winter (OR: 0.228, CI: 0.185, 0.434) but there was no evidence of a difference between Summer and Spring when compared to Winter ($P = 0.7691$ and $P = 0.0573$ respectively).

Season, live weight, plant, and number of head bumps in the chute were associated with blood splash in bison longissimus thoracis ($P < 0.05$; Table 9). The odds of having blood splash increased with live weight (OR: 1.001, CI: 1.000, 1.003). Odds of blood splash increased with number of head bumps in the chute (OR: 1.136, CI: 1.017, 1.252). The odds of having blood splash were higher in the Summer (OR: 3.698, CI: 2.272, 7.878) but there was no difference between Spring and Fall ($P = 0.0663$ and $P = 0.1820$ respectively).

DISCUSSION

Survey

The NBA is a non-profit national organization representing stakeholders across the bison supply chain. Most of the respondents in this study worked for more than one sector of the industry (e.g., both as cow-calf producer and finisher). This is different compared to the beef industry, where the producers are mostly segmented into cow-calf producers, finishers, packers, and retailers and tend to work separately (Buhr et al., 1997). More than half of the respondents had been working in the industry for more than 10 years, suggesting that most of the individuals have long-term involvement in the bison industry. The majority of the stakeholder businesses were located in the Midwest and West regions of the United States, indicating a concentration of the bison industry in those regions. Most individuals employed in the bison industry are identified as white men, constituting 73% (n = 80) of the workforce. The average age for stakeholders was 53 years old, with a maximum age of 85 years and a minimum of 22 years.

Most survey respondents agreed that the bison industry should continue to expand. The increase in the bison slaughter numbers over recent years indicates that the industry has been steadily growing (USDA, 2024a). Respondents indicated that the bison industry would benefit from enhancements in marketing, animal health, animal welfare, and production cost. Although details were not asked about marketing needs, an example of an area where increased marketing efforts could be beneficial is in the nutritional information about bison products. However, the majority of the individuals either strongly agreed or agreed that bison is appropriately marketed based on its unique characteristic. This suggests that even though the majority of respondents agreed that bison meat is appropriately marketed these individuals are invested in the growth and continuous improvement of the bison industry and so are likely always looking for innovative ways to promote and market their products. Bison meat is a healthy lean protein source (McDaniel et al., 2013; USDA, 2022a; 2022c), and it is important that consumers recognize the nutritional value of these products. Yang and Woods (2013) investigated the effects of knowing the nutritional value of bison ground meat on consumer willingness to pay and indicated that some consumers do not know about the benefits of consuming bison as compared to other animal proteins. Moreover, consumers that knew about the nutritional value of bison meat were willing to pay \$2.68 to \$2.81 more (from the total price) for these bison ground products. These findings are also in agreement with Qasmi et al. (2015), who also found that providing nutritional information about the product significantly increased consumer willingness to pay for ground bison. Similarly, a survey in Canada reported that consumers associated bison meat with terms such as “lean”, “game” and "expensive", but most participants had never consumed it or would only consume it on special occasions (Popoola et al., 2021).

In this study, bison stakeholders indicated that animal welfare was critical to produce bison meat and that it directly affected the quality of the product. It is well-documented that inappropriate welfare, handling, and transportation can have negative effects on meat quality in beef (Xing et al., 2019; Carrasco-Garcia, 2020; Diro et al., 2021; Sullivan et al., 2022). Additionally, bison stress could be potentially hazardous for animal handlers, however, there is limited data on bison workers injuries (Duysen et al., 2017). Bison are readily agitated, and they tend to have a sizable flight zone (Rioja-Lang et al., 2019) sometimes making them more challenging to handle, particularly when stressed. This represents a challenge for bison producers because bison generally must be transported to the slaughter plant and restrained prior to harvesting. This process requires several human-animal interactions that even if performed in a proper manner can be stressful for the animals. Moreover, a challenge with bison is that they tend to herd side by side. This can lead to animals crowding in the single-file chute (Grandin and Deesing, 2014) which is used in many handling facilities. There is currently very limited research on the effects of transportation on bison animal welfare. Survey respondents suggested that improvements in facility design, animal handling, employee training and transportation duration should be made to meet and maintain optimal animal welfare standards in the bison meat production system. Therefore, more research is needed to develop new strategies that could be applied to enhance bison animal wellbeing and overall meat quality during the marketing process.

The meat derived from bison is characterized by a dark red color and typically exhibits a low degree of intramuscular fat content (Koch et al., 1995; McDaniel et al., 2013). Approximately half of respondents agreed that grass-fed bison should be marketed as premium, however, a quarter of respondents disagreed with this statement. Fat content and type are considered important factors in developing desired meat flavors (Calkins and Hodgen, 2007). In the current survey, leanness was not the most selected response in terms of meat quality. Instead, respondents in the current study identified flavor as the most important quality attribute of bison meat products. Bison has been characterized to have a strong aroma and aftertaste, and still ranked as high as beef and greater than elk on a consumer overall liking preference test (Popoola et al., 2019). Moreover, bison meat has been associated with positive terms such as "lean" and "nutritious" (Popoola et al., 2019). Because feeding regimens affect the fatty acid profile and content of bison (Rule et al., 2002), additional research could show the potential of feeding strategies in altering the nutritional composition of bison meat, thereby yielding benefits for the industry.

The National Cattlemen's Beef Association (NCBA) has been using the National Beef Quality Audit (NBQA) data to continuously improve the beef industry's quality since the early 1990s. Over time, the focus of the NBQA has expanded from traditional attributes of beef quality to include indicators of animal welfare such as cattle mobility, live animal defects, and carcass bruising (Eastwood et al., 2017). This research initiative serves as a baseline for the U.S. beef industry, benchmarking meat quality and welfare attributes of cattle arriving at slaughter plants approximately every five years. It also records outcomes such as mobility upon arrival at the plant, cattle breeds, carcass bruising, quality grades, and yield grades (Shook et al., 2008; Eastwood et al., 2017; Harris, 2018). In addition to in-plant data collection, the NBQA actively collects stakeholder input. This process provides a deeper understanding of the current challenges and perceptions of the beef industry. It successfully identifies stakeholder perceptions of animal management and meat quality through surveys and in-person interviews (Shook et al., 2008; Boykin et al., 2017). This comprehensive approach allows for the timely identification of the industry’s needs that are constantly changing. Information obtained from the NBQA has been used to support the continuous improvement of quality and value across all sectors of the beef supply (Moore et al., 2012). In contrast, the bison industry does not have a similar mechanism to collect and understand stakeholder perceptions of successes, challenges, and needs.

The beef industry has been able to narrow the gap between expected eating experience and the actual eating experience by developing the USDA quality grading system (Liu et al., 2022). The U.S. grading system for beef segregates carcasses into different quality grades, primarily, prime, choice and select. There is currently no official grading system for bison meat in the United States. Canada has a grading system that evaluates bison carcasses based on animal maturity, muscling, muscle color and external fat color and fat depth. This system has 10 quality grades (A1–4, B1–3, and D1–3) separated into two different maturity classes: Maturity Class I = youthful, includes A1–4 and B1–3; and Maturity Class II = mature, includes D1–3 (Janssen et al., 2021). In the current study, approximately one-third of respondents neither agreed nor disagreed that a grading system would benefit the United States bison industry. However, approximately half of the respondents agreed that a carcass grading system could benefit the United States bison industry. Potential challenges associated with a bison meat grading system are that to have a grading system there needs to be more consistency in meat quality and a market for graded products. A carcass grading system could be challenging to develop because bison steaks have very little marbling compared to traditional fed cattle (Ding et al., 2016). If the differences between products are not easily detectable, it can be challenging to categorize meat based on these characteristics. If a grading system were to be developed it would likely need to include other quality attributes other than marbling.

Antemortem and Postmortem Outcomes

In this study bulls (48.2%) represented the majority of bison observed, followed by heifers (43.1%), and cows (8.71%). Although not directly quantified, the majority of bison included in this study were grain-fed. The USDA National Monthly Bison Report (USDA, 2023c) reported that the U.S. grain fed bison population had a similar distribution with the majority of bison being bulls (67.7%) followed by heifers (28.29%) and aged cows (3.9%). Average carcass weights were numerically similar between this study (276.4 kg) and the USDA National Monthly Bison Report (266 kg; USDA, 2023c). Thus, the current study population is representative of the current United States bison industry.

Animals are exposed to multiple stressors during transportation to the slaughter plant (Deters and Hansen, 2020). During this process cattle can experience a wide variety of conditions such as unfamiliar environments, comingling with unfamiliar animals, food and water deprivation, loud noises, and extreme temperatures (Edwards-Callaway and Calvo-Lorenzo, 2020). In this study the average distance bison traveled was 823 km. Multiple producers included in this study raised bison in Canada to be slaughtered in the U.S. and likely explains, in part, the long transport distance. According to the USDA National Monthly Bison Report (2023a) a total of 2,753 bison were exported just during the month of November from Canada to be slaughtered in the U.S. Additionally, because the bison industry is relatively small (USDA, 2022a; 2023a; 2024), there are less options to where producers can ship their animals and thus bison may need to travel greater distances to be processed. Nielsen et al. (2011) reported that increased distance traveled can increase the risk of injury in multiple species such as poultry, sheep, cattle, and horses and thus it is important to evaluate animal welfare upon arrival at the plant. Additionally, McCorkell et al., (2013) found increased trim loss (from bruising) on bison that were transported in comparison with bison that were slaughtered on-farm. In this study, as distance traveled increased, L^* of bison longissimus thoracis decreased. Previous studies have also demonstrated the same inverse relationship between distance traveled with L^* values in cattle (Vimiso and Muchenje 2013). Stress can change multiple physiochemical parameters in muscle leading to a high ultimate pH (Lahucky et al., 1998). In beef, such high ultimate pH leads to muscles that are darker in color with lower L^* value compared to normal pH muscles (Hughes et al., 2017).

Animal mobility is commonly used in the cattle industry to evaluate welfare both on-farm and at the slaughter plant (Edwards-Callaway and Calvo-Lorenzo, 2020; Mijares et al., 2021; BQA FA, 2021). Only 0.5% of bison in the current study had an abnormal mobility score. During this study, four bison had to be euthanized outside the processing facility (in holding pens or single file), but not because of mobility challenges. Sometimes bison can be challenging to move and to reduce stress from more intensive handling and prevent animals from becoming more agitated and potentially riskier to handle, plant employees may elect to stun the animal prior to entering the restraint area.

In addition to mobility, several animal handling metrics were recorded during this study. The most common handling tools used for moving bison during this experiment were sorting paddles. Electric prods were not used as the primary driving tool. There is a limited amount of data regarding electric prod use in bison. In this study, approximately 10% of bison were electrically prodded before being restrained in the knock box or restrainer. The beef industry has been able to reduce electric prod use in feedlots and slaughter facilities by raising awareness around best animal handling practices and in-plant audits utilizing the guidelines and audit tool from the North American Meat Institute (NAMI, 2021). These guidelines and audit tool suggest that plants use electrical prod on 25% or less of their cattle and pigs (NAMI, 2021). Developing more handling guidelines and audit tools specific to bison could be a valuable resource for the industry.

Vocalization has been used as a reliable indicator of beef cattle distress (Watts and Stookey, 1999; Davis et al., 2022). In this study, grunting was assessed in individual bison in the handling area prior to and during stunning. Only 0.3% of bison grunted before being slaughtered. Anecdotally, plant employees have shared that bison rarely vocalize while they are being moved through the facilities. Vocalization may not be an appropriate indicator of bison stress. Overall, these handling measurements highlight the use of low-stress handling practices in all three plants included in the study.

The last handling parameter measured was head bumping in the chute or at the single file door prior to restraining. This is a behavior that is worth exploring in future studies as it happened frequently in this study with head bumping being observed in one-third of the bison. From the plants analyzed, two plants had knock boxes. So, in this design, animals could bump their heads into the front of the knock box. The other plant had a center-track restrainer; in this plant, the bison head bumped at a single file door before the restrainer. In this study the number of head bumps was associated with greater probability for blood splash. This could be because the force exerted back by the chute surface during head bumping can travel through the animal's spine, potentially bursting blood vessels. Linear regression results indicated that the number of head bumps in the chute also resulted in increased L^*, a^*, and b^* values. To the authors' knowledge this has not been reported elsewhere and further work is needed to understand the reasons for bison head bump and its impact on quality.

Mud has been recognized as a factor that could potentially impact animal welfare, behavior, and performance in cattle (Morrison et al. 1970; Grandin, 2016; Dickson et al. 2022). Currently the NBQA evaluates mud/manure based on the location on the animal and the proportion of coverage (none, small, moderate, large, and extreme; Eastwood et al., 2017). Mud coverage is of high importance because manure or mud can act as a transportation means for bacteria and could potentially be introduced in meat products generating higher risk of food born illness (Hauge et al., 2012). The majority of bison in the current study had minimal mud coverage (less than 1/3 of hide covered). Similarly, the NBQA (2016) reported that 70.3% of cattle had small mud coverage and 22.9% had moderate mud coverage (Eastwood et al., 2017). Eastwood et al., (2017) suggested that the difference in mud scores by location during their quality audit (legs 40.8%, belly 33.0%, tail region 15.5%, side 6.8% and top line 3.9%) and the previous audit by McKeith et al., (2012) (legs 36.8%, belly 23.7%, tail region 13.7%, side14.9%, and top line 11.0%) are likely due to seasonality effects. In the current study, no seasonal effects were observed in bison mud scores. Based on the findings in this study, mud coverage may not represent a welfare challenge for the bison industry because it remains low for the majority of bison regardless of season.

A bruise is defined as the rupture of vascular supply in tissues that allows for the accumulation of blood and serum typically after trauma that did not cause external laceration (Hoffman et al. 1998). Approximately 97% percent of bison in the current study had at least one bruise, and the vast majority of bruised bison had multiple bruises. Bruises along the dorsal midline were frequent for all bison. These results are similar to those of Hoffman and Luhl (2012), who found high bruise percents on the rump sections of the beef carcasses and suggested that it may be due to high truck densities. Previous studies also suggest that both pre-slaughter handling and sex class can influence bruising (Jarvis et al., 1995). In this study, greater odds of being bruised were related to sex class, plant, and season. In the current study, bison cows had increased odds of being bruised as compared with heifers. These results are in agreement with Yeh et al., (1978) who identified that beef cows had greater bruising and a greater amount of trim loss compared to bullocks. Additionally, Kline et al., (2020) reported that beef cows had greater frequency of bruising than steers. Distance traveled did not influence the probability of bruising in this study. Typically, both male and female bison have horns (Vervaecke et al., 2005). Horns could cause bruising during the handling, loading, and transporting of bison because they provide a small surface area on which low amounts of force could produce high pressures capable of bruising or piercing muscles. The presence of horns can further influence meat safety. Muscle is considered essentially sterile on the inside (Gill et al, 1978), however, if a horn penetrates, the muscle is no longer considered sterile, and it should be trimmed off from the carcass. Additionally, after a trauma, muscle sustains a higher pH that can support the growth of spoilage bacteria (Cruz-Monterrosa et al., 2017). Moreover, an increase in carcass bruising has been related to lower dressing percentages (because bruises are removed from the carcass during trimming) and inferior meat quality in cattle (Warner et al. 1998).

According to the USDA beef carcass price equivalent index value (USDA, 2024b) choice steer and heifer dressing percentages were 62.4% and 62.2%, respectively. In this study bison had a slightly lower dressing percentage averaging 60.5%. These results are contradictory to Koch et al. (1995) who reported that bison (*Bison bison*) had a greater dressing percentage than beef (*Bos taurus)* cattle (62.6% in bison vs. 60.7% in beef). Koch et al. (1995) reported their average bison live weight as 431.3kg which is numerically similar to the average live weight (455.8kg) in this study. Variations in dressing percentage can be impacted by many variables. In this study, sex class, ribeye area, plant, bruising, and fat thickness were associated with changes in dressing percentage. Unexpectedly, bruise presence was associated with greater dressing percentages but the magnitude of difference between bruised and unbruised carcasses was minimal. Further research on bruise size and trimming weights could potentially explain the effect of bruising on dressing percentage of bison. Other studies have indicated that factors such as animal age, diet, and sex class could also affect cattle dressing percentage (Coyne et al., 2019).

Chronic antemortem stress in cattle can negatively influence meat quality (Vimiso and Muchenje 2013), reducing the amount of glycogen in muscle resulting in meat with a high pH (>5.8) and a darker color (Mounier et al., 2006). Acute antemortem stress can also affect meat quality (Warner et al., 2007) and can lead to pale soft and exudative (PSE) like condition during which pH declines rapidly at high temperatures resulting in protein denaturation producing higher initial *L** and *a** values (Sammel et al, 2002; Nair et al, 2016). In this study, longer distance traveled was associated with lower *L** values. The linear regression estimate represented only a 0.0011 unit decrease in *L** values per unit (km) of distance traveled. However, as the average distance traveled in this study was high (823.7 ± 583.6 km), changes in *L** values could be indicative of a relationship between distance traveled and chronic stress, because of the cumulative effect each km has on the *L** value. Additionally, the number of head bumps in the chute was associated with greater *L** values possibly due to acute stress muscle metabolism. Therefore, multiple antemortem factors such as distance traveled, plant, number of head bumps in the chute and fat thickness can influence *L** values in bison meat.

Color is one of the most important quality attributes of meat that influences consumer purchase decisions (Suman et al., 2014). Linear regression indicated the factors associated with greater (*a** values) were plant, fat thickness, ribeye area, number of head bumps in the chute and live weight. Multiple factors such as genetic background, sex, age, diet, and breed can impact meat color (Raes et al, 2003). Additionally, the difference in the processing and chilling conditions could have contributed to the variation in *a** values between the plants. Another factor that could have an influence on meat color is the Rinse & Chill (Rinse & Chill. WI, USA) technology used in one of the plants. This technology is used to circulate a cold solution composed of approximately 98.5% water and 1.5% mixture of dextrose, maltose, and sodium phosphates through the bison's vascular system, which could affect the appearance of the meat (Hwang et al., 2022). Research suggests that Rinse & Chill results in meat with lower pH, longer sarcomere length, improved redness after blooming and lower shear force values when compared to traditional chilling methods (Kethavath et al., 2022). Bison meat has a greater concentration of polyunsaturated fatty acids compared to beef and typically undergoes rapid discoloration (Galbraith et al., 2016; Roberts et al, 2017; Hasan et al. 2021). This could be a reason why bison meat is commonly sold in vacuum packages (Pietrasik et al., 2006). Since the vacuum bags have little to no oxygen, myoglobin is in the deoxy-myoglobin state resulting in a purple color (Seideman et al., 1984). Studies have found that if the consumers are educated about beef color in a vacuum package, they are more likely to purchase vacuum packaged products compared to uneducated consumers (Lynch et al., 1986). Therefore, consumer education could be an area of opportunity for the bison meat industry.

The average ribeye area for bison in the current study was 62.6 cm^2 which is much lower than the average ribeye area (89.5 cm^2) for fed beef reported in the 2016 National Beef Quality Audit (Boykin et al., 2017). Linear regression indicated that the factors associated with greater ribeye area were live weight, sex class, and plant. Previous studies in beef have also indicated that carcass weight can be correlated with greater ribeye areas (Boykin et al. 2017). However, greater carcass weights do not always result in larger ribeye areas (Moore et al., 2012).

CONCLUSION

Bison meat production continues to increase steadily over the years in the United States. Most of the bison stakeholders want the industry to continue to expand and grow. Animal handling, bison behavior, employee training, facility design and transportation duration were identified as the most critical factors that impact animal welfare in the bison production system. Moreover, the stakeholders understood that animal welfare is a critical component for bison production and that it directly affects meat quality. Approximately half of the participants agreed that a quality grading system would benefit the U.S. bison industry; however, a relatively high proportion of participants were neutral (neither agree nor disagree) with this statement. Future work could benefit from asking more detailed questions about the resources necessary for industry growth, such as industry funds for marketing, consumer insight research, and new product development.

This study benchmarks the live animal and carcass characteristics of bison. Multiple live animal characteristics and process facility factors such as distance traveled, season, number of head bumps in the chute, sex class, live weight, fat thickness, and ribeye area can influence meat quality parameters of bison. The current study did not measure animal age and diet, indicating a need for further research to understand other factors that could affect bison dressing percentage and meat color. Lastly, further investigation is needed to examine the factors that affect the ribeye area and its impact on meat quality attributes, such as tenderness. This comprehensive approach will ensure the industry's continuous growth while maintaining high standards of animal welfare and meat quality.

Table 1. Summary of respondent demographics (n = 110).

Description	Percent (n)
Time in the industry	
Less than a year	2 (3)
1-5 years	19 (21)
6-10 years	16 (18)
More than 10 years	60 (66)
Gender	
Men	73 (80)
Women	24 (27)
Prefer not to answer	1 (2)
Industry Location	
Alaska or Hawaii	1 (2)
Midwest	40 (45)
Northeast	4 (5)
West	36 (40)
Southeast	5 (6)
Southwest	10 (12)
Industry Sector	
Cow calf producer	69 (76)
Finisher	38 (42)
Processor	12 (13)
Retailer	26 (29)
Other	25 (28)
Race	
Hispanic or Latino	>1 (1)
Native American	2 (3)
Prefer not to answer	8 (9)
White	87 (93)
Average age	53

[1] Midwest (IA,IL,IN,KS,MI,MN,MS,ND,NE,OH,SD,WI), Northeast (CT,DE,MA,ME,MD,NH,NJ,NY,PA,VT,RI), Southeast (AL,AR,FL,GA,LA,KY,MS,NC,SC,TN,VA,WV), Southwest (AZ,NM,OK,TX) and West (CA,CO,ID,MT,NV,OR,UT,WA,WY).

[2] Most of the respondents were affiliated with more than one sector of the industry, therefore, many individuals selected more than one sector.

Table 2. Likert scale summary of responses regarding respondent perceptions of the value implications of bison welfare during production and harvest (n = 110).

Statement	Strongly agree, % (n)	Agree, % (n)	Neither agree nor disagree, % (n)	Disagree, % (n)	Strongly disagree, % (n)	Don't know, % (n)	No response, % (n)
Animal welfare is a critical component of the bison production system	77 (85)	19 (21)	2 (3)	0.9 (1)	0 (0)	0 (0)	0 (0)
Animal welfare impacts bison meat quality	69 (76)	29 (32)	0 (0)	0.9 (1)	0 (0)	0 (0)	0.9 (1)
Transportation of bison to the slaughter plant is an area of concern due to its impacts on animal welfare.	32 (36)	28 (31)	21 (24)	9(10)	1 (2)	6 (7)	0 (0)

Table 3. Likert scale summary of responses regarding respondent perceptions respecting bison meat quality (n = 110).

Question or statement	Strongly agree % (n)	Agree % (n)	Neither agree nor disagree % (n)	Disagree % (n)	Strongly disagree % (n)	Don't know % (n)	No response % (n)
There are quality differences between grass and grain finished bison.	41 (46)	37 (41)	14 (16)	2 (3)	0 (0)	3 (4)	0 (0)
Grass-finished bison should be marketed as a premium product.	25 (28)	25 (28)	22 (25)	14 (16)	10 (11)	1.8 (2)	0 (0)
A carcass grading system for bison would benefit the U.S. bison industry.	22 (24)	32 (35)	29 (32)	7 (8)	3 (4)	4 (5)	1.8 (2)
Bison is marketed appropriately based on its unique characteristics.	20 (23)	62 (69)	8 (9)	4 (5)	2 (3)	0.9 (1)	0 (0)

Table 4. Antemortem and postmortem measurements obtained from bison in three separate processing plants included in the study.

	Mud Score	n	% of Total
Antemortem	1	2088	91.46%
	2	192	8.41%
	3	3	0.13%
	Grunt		
	No	2276	99.65%
	Yes	8	0.35%
Postmortem	**Blood Splash**		
	No	2185	95.67%
	Yes	99	4.33%
	Bruised [b]		
	No	71	3.11%
	Yes	2213	96.89%
	Multiple [c]		
	No	66	2.98%
	Yes	2147	97.01%

[a] scale from 1 to 3 (1- less than 1/3 mud coverage, 2- between 1 and 2/3 mud coverage, 3- more than 2/3 mud coverage)

[b] Percent of bison that had at least one bruise with a size at least between 0.1 and 7.62cm in the whole carcass.

[c] Percent of bison that had more than one bruise with a size at least between 0.1 and 7.62 cm in the whole carcass.

Table 5. Means of antemortem and postmortem measurements obtained from bison in three separate processing plants included in the study.

	Attribute	n	Mean	Std Dev	Min	Max
Antemortem	Distance Traveled (km)	2284	823.7	583.6	39.7	2159.6
	Live Weight (kg)	2280	455.8	75.1	173.6	978.2
	Number of Head Bumps [a]	2284	0.7	1.5	0.00	14.0
Postmortem	Hot Weight (Kg)	2283	276.4	49.7	73.0	622.3
	Cold Weight (Kg)	2183	271.0	49.1	73.0	617.2
	Dressing %	2279	60.5	3.3	32.2	70.9
	Fat Thickness (cm)	2274	1.4	1.1	0.0	8.1
	Ribeye Area (cm^2)	2274	62.6	9.8	14.8	93.5
	*L**	2275	32.92	2.85	18.52	57.02
	*a**	2275	18.41	2.28	9.90	33.29
	*b**	2275	12.75	1.94	1.99	29.95

[a] Percentage of bison that forcefully and intentionally hit the inner surface of the chute at least one time.

Table 6. Total percentage of bruises in bison categorized by carcass location, size, and sex class. Bruises were categorized into a 1-4 ordinal scale based on 7.62 cm increments and further separated by sex class (bull, cow, and heifers).

		0	**1**	**2**	**3**	**4**
		No Bruise	(0.1-7.62cm)	(7.62-15.24cm)	(15.24-30.48cm)	(>30.48cm)
R1	**Bull**	93.37	3.27	1.82	1.27	0.27
	Cow	85.93	2.51	5.03	2.51	4.02
	Heifer	88.41	5.08	2.85	2.34	1.32
R2	**Bull**	87.65	4.09	5.00	2.72	0.05
	Cow	84.42	5.53	4.52	1.51	4.02
	Heifer	84.15	6.50	5.39	3.15	0.81
R3	**Bull**	86.65	5.09	4.18	3.27	0.82
	Cow	73.87	5.03	8.54	7.54	5.03
	Heifer	75.30	9.76	6.61	6.00	2.34
R4	**Bull**	74.21	11.63	7.81	5.63	0.73
	Cow	58.29	9.05	12.06	11.06	9.55
	Heifer	49.80	18.70	16.87	9.76	4.88
R6	**Bull**	36.97	26.07	30.06	6.63	0.27
	Cow	23.12	16.08	36.68	20.60	3.52
	Heifer	41.46	25.10	26.32	6.91	0.20
R7	**Bull**	83.56	8.45	5.09	2.45	0.45
	Cow	74.87	9.05	9.55	4.52	2.01
	Heifer	69.82	12.80	11.38	4.78	1.22
R8	**Bull**	87.38	5.54	4.27	1.63	1.18
	Cow	63.82	5.53	12.56	9.05	9.05
	Heifer	75.20	7.11	6.30	6.00	5.39
5A	**Bull**	61.49	12.99	9.99	9.17	6.36
	Cow	76.88	5.53	9.05	4.02	4.52
	Heifer	49.49	11.99	12.40	12.09	14.02
5B	**Bull**	54.59	3.00	3.45	9.63	29.34
	Cow	78.39	3.52	4.02	3.52	10.55
	Heifer	63.62	2.64	4.37	6.91	22.46
5C	**Bull**	44.41	3.91	5.45	13.71	32.52
	Cow	70.85	4.52	5.53	8.04	11.06
	Heifer	49.09	3.15	7.01	12.80	27.95
L1	**Bull**	90.10	4.90	2.45	2.18	0.36
	Cow	88.44	2.01	3.52	4.02	2.01
	Heifer	86.89	5.69	3.66	2.54	1.22
L2	**Bull**	88.74	3.09	4.72	2.27	1.18
	Cow	81.41	4.52	6.03	5.03	3.02
	Heifer	83.13	7.22	4.37	3.05	2.24
L3	**Bull**	86.56	4.36	3.09	4.54	1.45
	Cow	70.35	6.03	5.03	9.55	9.05
	Heifer	71.04	12.09	7.42	6.20	3.25
L4	**Bull**	78.84	8.45	6.99	4.54	1.18
	Cow	55.28	8.04	13.07	9.05	14.57
	Heifer	55.89	13.01	14.43	11.28	5.39
L6	**Bull**	42.14	24.52	27.34	5.90	0.09
	Cow	23.12	17.59	38.69	19.10	1.51
	Heifer	43.29	26.63	22.97	6.71	0.41
L7	**Bull**	82.11	8.36	6.99	2.18	0.36
	Cow	75.38	5.53	8.54	7.04	3.52
	Heifer	66.67	11.99	12.50	6.91	1.93
L8	**Bull**	88.19	4.45	3.81	2.45	1.09
	Cow	75.38	4.02	4.52	7.54	8.54
	Heifer	74.19	7.22	7.62	5.79	5.18

Table 7. Linear regression parameter estimates for dressing percentage, ribeye area, and fat thickness of bison.

Term	**Estimate**	**Std Error**	***P*-Value**
	Dressing percentage		
Intercept	53.2402	0.5189	<.0001
Ribeye Area	0.5975	0.0376	<.0001
Fat Thickness	1.2480	0.1463	<.0001
Bruise Score	0.50021	0.2471	0.0431
Sex Class [Heifers]	*Referent*		
Sex Class [Bull]	-0.0280	0.1580	0.8593
Sex Class [Cow]	-0.9921	0.2435	<.0001
Plant [1]	*Referent*		
Plant [2]	1.5395	0.4157	0.0005
Plant [3]	-0.5974	0.3398	0.0845
	Ribeye area		
Intercept	4.22426	0.2676	<.0001
Sex Class [Heifers]	*Referent*		
Sex Class [Bull]	0.4237	0.0752	<.0001
Sex Class [Cow]	-0.5233	0.1131	<.0001
Live Weight	0.00503	0.0002	<.0001
Plant [1]	*Referent*		
Plant [2]	-0.175	0.1469	0.2385
Plant [3]	0.60061	0.1201	<.0001
	Fat thickness		
Intercept	-0.6591	0.0754	<.0001
Sex Class [Heifers]	*Referent*		
Sex Class [Bull]	-0.3451	0.0215	<.0001
Sex Class [Cow]	0.0873	0.0332	0.0088
Live Weight	0.00125	6.41E-05	<.0001

Table 8. Linear regression parameter estimates for objective color measurements of bison.

Term	**Estimate**	**Std Error**	***P*-Value**
	L*		
Intercept	30.3097	0.5145	<.0001
Ribeye Area	0.2643	0.0453	<.0001
Fat Thickness	1.13862	0.1741	<.0001
Sex Class [Heifers]	*Referent*		
Sex Class [Bull]	-0.1634	0.1676	0.3301
Sex Class [Cow]	-0.4381	0.2461	0.0762
Number Head Bumps	0.113	0.0344	0.001
Distance Traveled	-0.0011	0.0004	0.014
Plant [1]	*Referent*		
Plant [2]	0.0061	0.2893	0.9832
Plant [3]	-0.679	0.2282	0.0044
	a*		
Intercept	13.3461	0.4752	<.0001
Ribeye Area	0.2349	0.0353	<.0001
Fat Thickness	1.0406	0.1346	<.0001
Sex Class [Heifers]	*Referent*		
Sex Class [Bull]	-0.6964	0.1337	<.0001
Sex Class [Cow]	-0.0183	0.1886	0.9225
Number of Head Bumps	0.0589	0.0246	0.017
Live Weight	0.0023	0.0004	<.0001
Plant [1]	*Referent*		
Plant [2]	0.3686	0.2333	0.1196
Plant [3]	-0.6233	0.1922	0.0019
	b*		
Intercept	8.8879	0.4144	<.0001
Ribeye Area	0.1761	0.0313	<.0001
Fat Thickness	0.8123	0.1197	<.0001
Sex Class [Heifers]	*Referent*		
Sex Class [Bull]	-0.6732	0.1191	<.0001
Sex Class [Cow]	0.2286	0.1665	0.1708
Number of Head Bumps	0.0647	0.0219	0.0032
Live Weight	0.0017	0.0004	<.0001

Table 9. Logistic regression odds ratios for bruising and blood splash in bison.

Term	Estimate	Std Error	Odds Ratio	95% Confidence Interval Low	95% Confidence Interval High	*P*-value
			Bruising			
Intercept	1.8144	1.0644	6.1375	0.7823	51.4091	0.0883
Live Weight	0.0033	0.0011	1.0033	1.0012	1.00554	0.0025
Sex Class [Heifer]	*Referent*					
Sex Class [Bull]	-1.2089	0.2958	0.2985	0.1517	0.5058	<.0001
Sex Class [Cow]	1.2702	0.4873	3.5617	1.6046	12.0054	0.0091
Plant [1]	*Referent*					
Plant [2]	1.4451	0.4887	4.2425	1.9004	14.321	0.0031
Plant [3]	-0.9873	0.2753	0.3725	0.1947	0.601	0.0003
Season [Winter]	*Referent*					
Season [Spring]	0.5623	0.2957	1.7547	1.0094	3.2652	0.0573
Season [Summer]	-0.0806	0.2749	0.9224	0.5473	1.6279	0.7691
Season [Fall]	-1.2435	0.2159	0.2883	0.1856	0.4348	<.0001
			Blood Splash			
Intercept	-6.2296	0.8145	0.0019	0.0003	0.0096	<.0001
Live Weight	0.0018	0.0006	1.0018	1.0004	1.0031	0.0086
Number of Head Bumps	0.1277	0.0526	1.1362	1.0174	1.2528	0.0152
Plant [1]	*Referent*					
Plant [2]	0.8001	0.233	2.2258	1.4592	3.7404	0.0006
Plant [3]	0.7062	0.2255	2.0264	1.356	3.3721	0.0017
Season [Winter]	*Referent*					
Season [Spring]	0.566	0.3082	1.7612	1.026	3.8238	0.0663
Season [Summer]	1.3079	0.2899	3.6984	2.2722	7.8787	<.0001
Season [Fall]	0.4022	0.3014	1.4952	0.887	3.2225	0.182

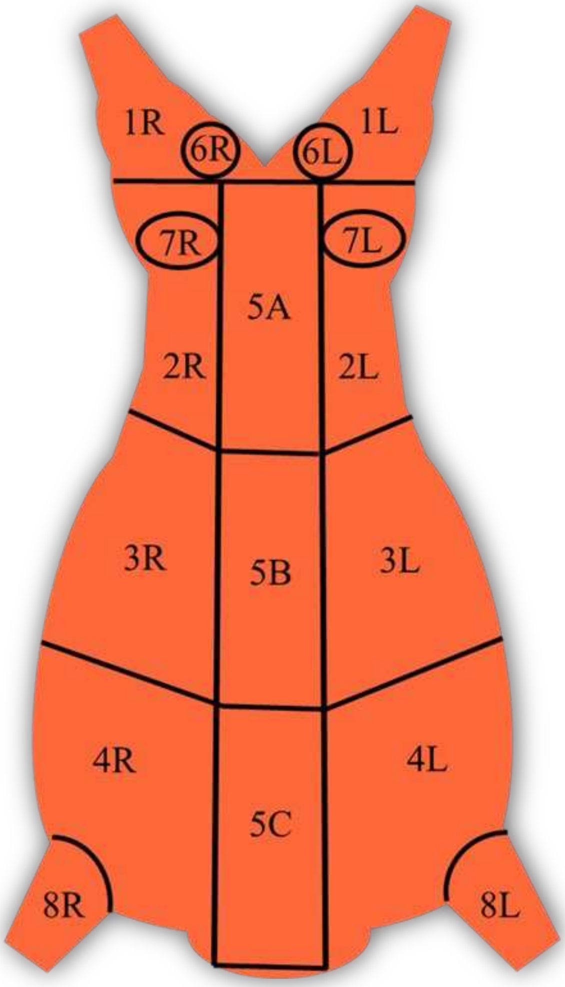

Figure 1 Carcass bruising map used in the current study adapted from Strappini et al. 2012.

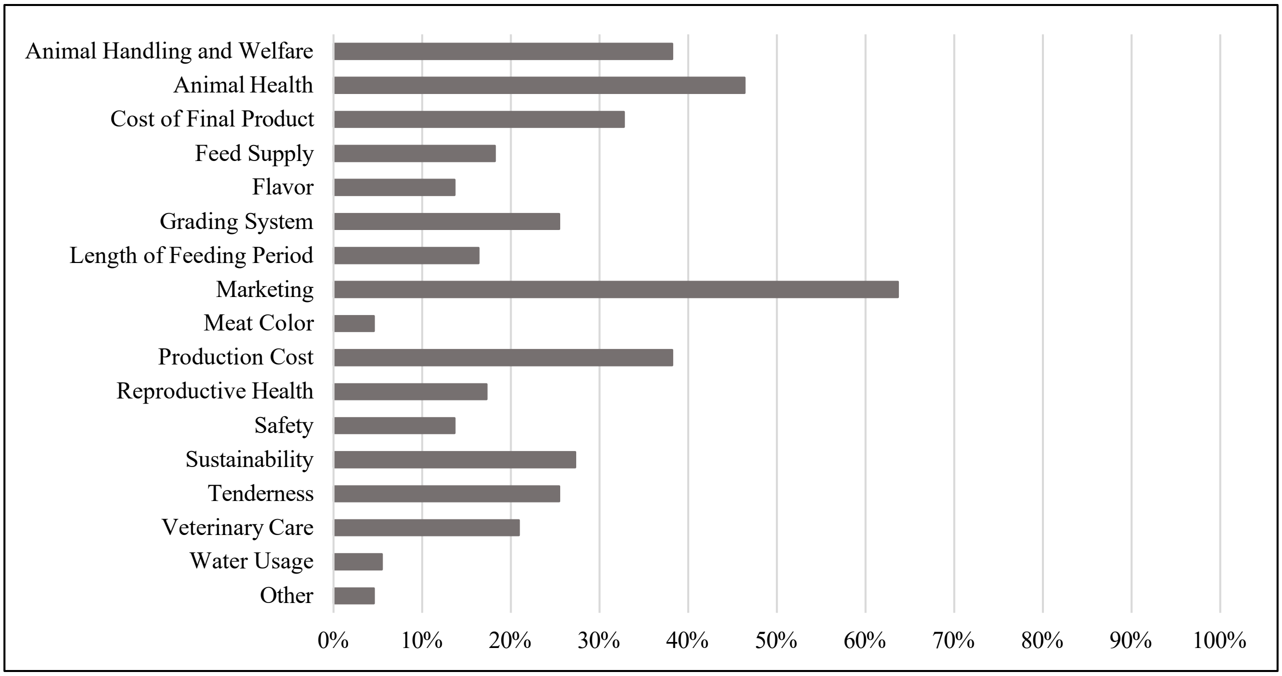

Figure 2. Percent selected responses regarding attributes of bison that would benefit from enhancements or improvements. Multiple answers allowed.

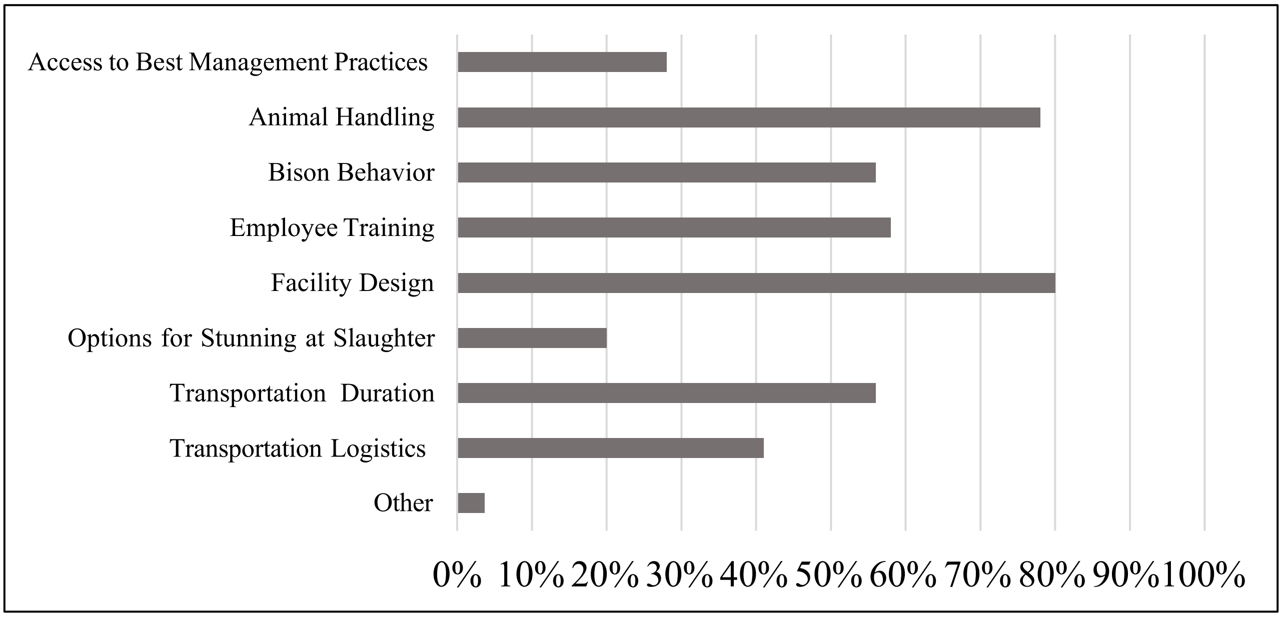

Figure 3. Percentage of selected responses collected from respondents concerning factors that impact animal welfare within the bison industry. Multiple answers were allowed.

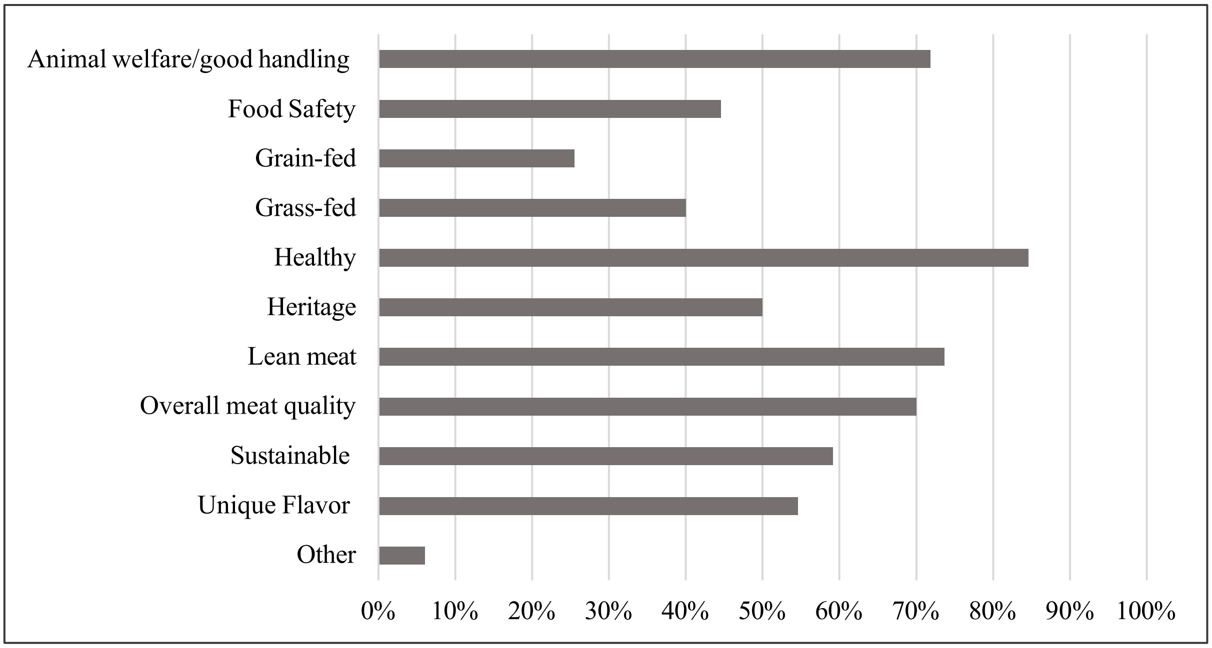

Figure 4. Percentage of selected responses collected from respondents concerning attributes of bison that provide added value to consumers. Multiple responses were allowed.

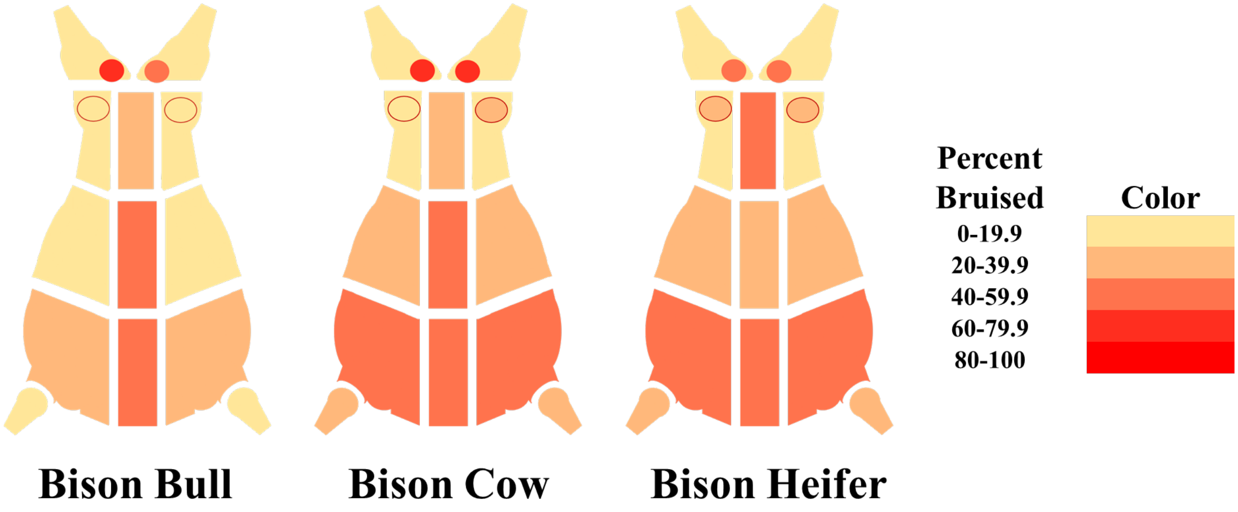

Figure 5. Bison bruise heat map with redder sections representing higher percentage that had at least one bruise with a minimum size between 0.1 and 7.62cm.

CHAPTER 3: MITOCHONDRIAL RESPIRATION AND ELECTRON TRANSPORT CHAIN COMPLEX ABUNDANCE OF BISON AND BEEF

Lay Summary

Previous research has indicated that mitochondria can influence multiple meat quality parameters. However, no studies have compared the mitochondrial metabolism of bison with beef. Therefore, the objective of this study was to compare mitochondrial oxygen consumption rate (JO_2), hydrogen peroxide production, citrate synthase content, and electron transport chain complex protein abundance of beef and bison. The left side masseter muscle of crossbred Angus steers (n = 12) and bison (n = 12) were collected between 30- and 60 minutes postmortem. The JO_2 of permeabilized muscle fibers at specific respiration states was evaluated utilizing the Oroboros O_2K high-resolution respirometry system. The electron transport chain protein abundance and citrate synthase content of bison and beef muscle were measured utilizing gel electrophoresis. All mitochondrial data were analyzed as mixed models with species as the fixed effect and day as the random effect. No differences were found in JO_2 between bison and beef under baseline, LEAK respiration (LEAK oxygen flux; $P = 0.8813$), ROT (rotenone respiration; $P = 0.1071$), and CCCP (carbonyl cyanide m-chlorophenyl hydrazone respiration; $P = 0.7502$) respiration states. Bison permeabilized muscle fibers had a higher ($P = 0.0016$) JO_2 during max OXPHOS (+D) and produced more hydrogen peroxide ($P = 0.0234$) during this respiration state when compared to beef. Respiration control rate (RCR) did not differ ($P = 0.2928$) between beef and bison permeabilized muscle samples. Citrate synthase content was not different between species ($P = 0.4650$). Bison muscle samples contained lower relative protein abundance of the electron transport chain complexes II ($P = 0.0057$) and III ($P = 0.0020$) than beef. Overall, the results from this study provide a comparison between bison and beef mitochondrial activity, which could explain the differences in production efficiency and quality between the two species.

INTRODUCTION

Mitochondria are crucial organelles found in nearly all eukaryotic organisms. They are responsible for oxidative phosphorylation, involving the addition of a high-energy phosphate to adenosine diphosphate (ADP), producing adenosine triphosphate (ATP). This is possible via an electrochemical gradient between the intermembrane space and the mitochondrial matrix. Mitochondria use proteins embedded in the inner membrane to create the electrochemical gradient through the electron transport chain (ETC; Chaban et al., 2014). The five supercomplexes found in the inner mitochondrial membrane include: complex I (NADH-dehydrogenase), complex II (succinate-dehydrogenase), ubiquinone, complex III (cytochrome c oxidoreductase), complex IV (cytochrome c oxidase) and complex V (ATP-synthase; Huttemann et al., 2007). These complexes utilize NADH and $FADH_2$ generated from the tricarboxylic acid (TCA) cycle to generate the electrochemical gradient used to generate ATP (Watt et al., 2010).

In the context of mitochondrial metabolism, efficiency can be calculated by measuring the ATP-to-oxygen ratio (Salin et al., 2019). However, protons can pass the intermembrane without going through ATP-synthase (proton leak), and mitochondrial respiration may occur without ATP synthesis in uncoupled systems (Brand et al., 1994). Therefore, the respiratory control ratio (RCR) is often used to determine the degree of uncoupling in the mitochondria. The RCR measures the ratio between mitochondrial respiration state 3 (substrate-supported respiration in the presence of ADP) and state 4 mitochondrial respiration (substrate-supported respiration in the absence of ADP; Fernandez et al., 2020), serving as an indicator of mitochondrial efficiency.

Although the United States is one of the largest beef-producing countries in the world (OECD/FAO, 2023), other alternative red meat, such as bison, has been growing (USDA, 2024). Bison meat products are typically recognized as natural, lean, and expensive (Popoola et al., 2021).

However, bison meat exhibits a darker color compared to beef (Galbraith et al., 2014) and undergoes rapid discoloration under aerobic packaging (Pietrasik et al., 2006; Galbraith et al., 2011; Sood et al., 2020). This is interesting, considering that bison and beef myoglobin have the exact amino acid sequence (Joseph et al., 2010). Consumers select fresh meat products based on their visual characteristics, using color as the major indicator of freshness and wholesomeness (Mancini and Hunt, 2005; Suman et al., 2014). There is limited information regarding the extent of economic loss for the bison industry due to discoloration. However, Ramanathan et al. (2022) reported that the beef industry suffers from an annual financial loss of approximately $3.73 billion due to fresh meat discoloration. Multiple studies have indicated that mitochondria can influence fresh meat color (Tang et al., 2005; Mancini et al., 2018; Ramanathan and Mancini, 2018; Kiyimba et al., 2022) using measurements of mitochondrial activity such as oxygen consumption rate (OCR). Understanding mitochondrial activity differences between bison and beef could elucidate reasons for the color stability differences between species. Therefore, the objective of this study was to compare multiple mitochondrial metabolic processes of bison and beef that could subsequently explain differences in their meat quality parameters.

MATERIALS AND METHODS

Sample Collection

Both bison and beef samples were collected from a commercial meat processing facility in Northern Colorado over 4 collection days. The skin and the outer connective tissue of the left side masseter muscle of Angus crossbred beef steers (n = 12) and bison (n = 12) were removed, and muscle biopsy samples were collected between 30- and 60 minutes postmortem. A 0.5g sample was collected and placed into ice-cold BIOPS containing 10 mM Ca-EGTA (0.1 µM free calcium), 20 mM imidazole, 20 mM taurine, 50 mM K-MES, 0.5 mM DTT, 6.56 mM $MgCl_2$, 5.77 mM ATP, and 15 mM phosphocreatine (pH 7.1) for respirometry analysis. Additionally, approximately 10 g of masseter muscle was collected separately, snap-frozen, and stored at -80°C for Western Blot analysis. Samples for respirometry assays were kept on ice and transported to the Center for Meat Safety and Quality at Colorado State University. Samples were placed into fresh ice-cold BIOPS and were kept refrigerated overnight.

Muscle Fiber Permeabilization

Muscle fiber permeabilization was performed as reported by Zhai et al. (2022). Briefly, needle tip forceps and miniature dissection scissors were used to trim off connective tissue and gently tease apart muscle fiber bundles while immersed in ice-cold BIOPS solution (same composition as above). Samples were incubated in 50 µg/mL saponin for 20 minutes on ice while gently rocking, permeabilizing the cells but maintaining mitochondrial membrane integrity. Fibers were then rinsed with two 15-minute washes with ice-cold mitochondrial respiration medium (MiR05) containing 0.5 mM EGTA, 3 mM $MgCl_2$ hexahydrate, 60 mM lactobionic acid, 20 mM taurine, 10 mM KH_2PO_4, 20 mM HEPES, 110 mM sucrose, and 0.1% BSA (pH 7.1). Approximately 2 mg of permeabilized fiber was gently dried using ashless Whatman filter paper (Sigma, #WHA1001325) and weighed before being added to each chamber of the Oxygraph-2k high-resolution respirometer (Oroboros Instruments, Innsbruck, Austria) chamber containing 2 mL of respiration medium (MiR05).

High-Resolution Respirometry

Approximately 2 mL of pure oxygen was added to all chambers until the oxygen concentration reached approximately 400 µM O_2. Mass-corrected muscle oxidative capacities of beef and bison samples were assayed in MiR05 medium at 37°C by monitoring real-time changes in the oxygen concentration signal (JO_2) after the addition of exogenous metabolic substrates. The following substrates and concentrations were added to determine specific respiration states. LEAK (L) state was achieved via the addition of 5 µL of malate (1 mM), 5 µL of pyruvate (5 mM), 10 µL of glutamate (10 mM), and 20 µL of succinate (20 mM) to facilitate proton movement across the mitochondrial membrane without phosphorylation of ADP. OXPHOS (+D) state respiration was measured after the addition of 10 µL of ADP (2.5 mM). To measure the partial relative contribution of Complex I and Complex II to the OXPHOS state, 1 µL of rotenone (ROT; 1 mM; Complex I-inhibitor) was titrated until no more change in flux was observed. Then, 1mM of uncoupler (electron donor) carbonyl cyanide m-chlorophenylhydrazone (CCCP) was titrated to measure reserve Complex IV capacity, after which 2.5 µL of exogenous cytochrome c (4 mM) to measure mitochondrial membrane integrity. Lastly, 10 µL of N, N, N', N'-Tetramethyl-p-phenylenediamine dihydrochloride (TMPD; 1 mM) and 10 µL of ascorbate (4 mM) were added to measure maximal Complex IV activity.

Western Blotting

Approximately 50 mg of frozen muscle was homogenized on ice in lysis buffer containing 150 mM NaCl, 20mM sodium fluoride, 5mM sodium pyrophosphate, 1mM EDTA, 1 mM EGTA, 1 mM sodium orthovanadate added to mammalian protein extraction reagent (MPER; Pierce#78501) with supplemental protease inhibitor cocktail (Sigma Aldrich#P8340). After centrifugation at 14,000g for 10 minutes, the protein concentration of the supernatant collected was determined via bicinchoninic acid (BCA) assay (Thermo Fisher, #23227) using a colorimetric microplate reader (VersaMax, Molecular Devices).

Then, 30 µL of proteins were electrophoresed on 4-12% Bis-Tris mini protein gel (Thermo Fisher, # NW04122BOX), transferred to methanol-activated PVDF membranes, and blocked for

1 hour with 5% non-fat milk in Tris-buffered saline (TBS) at 24°C on a laboratory rocker. After washing thrice for 4 minutes each with Tris-buffered saline + 0.1% Tween (TBST), membranes were incubated with OXPHOS cocktail rodent western blot antibody (1: 5000 dilution; Thermo Fisher, #45-8099) at 24°C for 1.5 hours. For citrate synthase analysis, samples were incubated with rodent citrate synthase western blot antibody for 1 hour at 24°C (1: 3000 dilution; Thermo Fisher, #PA5-22126). After incubation, membranes were washed thrice for 4 minutes each with TBST, then incubated at room temperature for 1 hour with horseradish peroxidase-conjugated Goat Anti-Mouse secondary antibody (1:3000 dilution; Thermo Fisher, Abcam, # AB6789) for OXPHOS antibody and Goat Anti-Rabbit (1: 3000 dilution; Thermo Fisher Abcam, #A27036) for citrate synthase. Both secondary antibodies were diluted in 5% non-fat milk TBS and incubated on a laboratory rocker for 1 hour. Dura Super Signal (Thermo Scientific, #34076) was applied for 20 seconds before visualization using an Analytik Jena UVP ChemStudio (Upland, CA). AmidoBlack (Sigma, # A8181) total protein stain was used as a loading control. Quantification of band density was performed using ImageJ software (NIH, Bethesda, MD). Western blot samples (bison, n = 12 and beef, n = 12) were analyzed in two separate gels. Protein abundance was standardized to the total protein of both species present in the separate gels and presented as a protein abundance ratio of bison to beef.

Statistical Analyses

All mitochondrial parameters were quantitative, and data were analyzed as mixed models with species as the fixed effect and day as a random effect using statistical software JMP Pro 16 (Statistical Discovery, NC, U.S.A.). Significance level was set at $\alpha = 0.05$. The O_2K samples were read simultaneously in duplicate. Two bison and one beef sample were removed from the O_2K data analysis due to experimental error, resulting in n = 10 for bison and n = 11 for beef for the O_2K analysis.

RESULTS AND DISCUSSION

Mitochondrial Respirometry

Understanding the mitochondrial activity of bison and beef muscles is important as it directly impacts the quality and color stability of the products, which are significant factors in consumer preference and purchase decisions (Mancini & Hunt, 2005; Suman et al., 2014). In the current study, no significant difference was found in baseline respiration (*P* = 0.6674), which represents initial respiration in the chamber supported by naturally present-unknown concentrations of ADP and TCA substrates, and LEAK (L; *P* = 0.8813), which is respiration without ADP phosphorylation. Additionally, no differences were found in ROT respiration (*P* = 0.1071), which is the partial respiration attributed to the activity of Complex II in the absence of Complex I activity, and CCCP respiration after the addition of a protonophore that acts as a potent chemical uncoupler (*P* = 0.7502) between beef and bison (Figure 2.1). The mitochondrial respiration state LEAK (L) can be interpreted as the respiration that occurs without ATP production. No differences in this state would mean that both species have similar mitochondrial coupling. To further test this, the RCR [OXPHOS (+D)/ LEAK (L)] was analyzed, and no differences (*P* = 0.2928) were found (Figure 2.2), suggesting that both bison and beef have similar mitochondrial efficiency in terms of proton leakage. Previous research by Ramos et al. (2024) found increased LEAK respiration after rigor mortis when comparing Angus and Brahman breeds. However, in the current study, samples were collected before postmortem rigor.

Although no differences (*P* > 0.05) were found in most respiration states in the present study, OXPHOS (+D) was greater (*P* = 0.0016) in bison than in beef. The increased OXPHOS (+D) in bison could be because bison muscle had higher concentration of mitochondria per mg of tissue, or bison mitochondria possessed enhanced ATP-generating capacity per mitochondrion. Other studies have used citrate synthase as a biological marker of mitochondrial concentration (Vigelsø et al., 2014; Eigentler et al., 2015). In this study, citrate synthase protein content was analyzed for a subsample of bison (n = 8) and beef (n = 8) samples via Western blotting. However, there was no significant difference between the citrate synthase content of bison compared to beef (*P* = 0.4650), suggesting that bison and beef might have similar mitochondrial density per mg of masseter muscle tissue.

Reactive oxygen species (ROS), such as superoxide anions, are highly reactive molecules. At normal levels, ROS play a role in cell signaling, biosynthesis, and host defense, whereas in high concentrations they can be detrimental in both low and high concentrations, leading to a lack of signaling and host defense or cell damage, respectively (Brieger et al., 2012). Moreover, excess ROS can oxidize both cell proteins and lipids, potentially leading to rancidity and negatively affecting the sensory profile and functional properties of meat products (Huang & Ahn, 2019). Under OXPHOS (+D), mitochondria functions at its highest capacity, so the formation ROS is prone to happen (Mailloux, 2020). In mitochondria, ROS, such as superoxide radicals, are stabilized by the antioxidant enzyme superoxide dismutase (SOD) to generate other compounds such as hydrogen peroxide (Buettner, 2011). In this study, bison produced significantly higher (*P* = 0.0234) levels of hydrogen peroxide (Figure 2.3) during OXPHOS (+D) respiration. Hydrogen peroxide can react with antioxidant enzymes such as glutathione peroxidase to be converted into water (Haverly-Coulson and Boyd, 2010). Previous studies have demonstrated that hydrogen peroxide can also lead to lipid peroxidation (Siddique et al., 2013).

Relative Protein Abundance

Mitochondrial oxygen consumption can sometimes be influenced by differences in ETC complex abundance. The complexes embedded in the inner mitochondrial membrane play a fundamental role in oxidizing multiple metabolites produced by the tricarboxylic acid cycle (TCA), including nicotinamide adenine dinucleotide (NADH), flavin adenine dinucleotide ($FADH_2$), and acetyl coenzyme A (Acetyl-CoA). This study compared the ETC protein abundance of bison and beef. Preliminary experimentation showed a higher concentration of ETC proteins in beef (data not shown). Therefore, the protein concentration of beef was used as a standard, and the ETC protein concentration of bison was expressed as relative abundance to beef. No differences were found in the relative abundance of complexes I ($P = 0.1637$), IV ($P = 0.0959$), and V ($P = 0.2051$). However, bison contained less complex II ($P = 0.0057$) and complex III ($P = 0.0020$) compared to beef (Figure 2.5). The flow of electrons in normal cell conditions is through the ETC and is finalized with oxygen being reduced to form water (Buettner, 2011.). However, if there is an imbalance in ETC protein abundance, the electron transport flow can be disrupted. Reactive oxygen species such as superoxide can arise from electron leakage, where electrons from the ETC exit the system before they are reduced to form water (Herlein et al., 2011; Tabassum et al., 2020). Hypothetically, a possible reason for the increased hydrogen peroxide production of bison (compared to beef) could be the lower concentrations of complexes II and III, as they could become overcharged with electrons resulting in the formation of superoxide, which is ultimately converted to hydrogen peroxide by SOD (Brieger et al., 2012). Nevertheless, research investigating the concentration of Coenzyme Q is needed to further understand if electron leakage is responsible for the high hydrogen peroxide production during the respiration of bison mitochondria.

CONCLUSION

Our results indicated that bison and beef did not differ in baseline, LEAK, ROT, and CCCP respiration rates. However, mitochondrial oxidative phosphorylation OXPHOS (+D) respiration capacity in bison was significantly higher compared to beef, and bison produced higher hydrogen peroxide during the OXPHOS (+D) respiration state than beef. The citrate synthase content between bison and beef was not significantly different indicating that bison and beef might have similar mitochondrial concentrations in the masseter muscle. Relative abundance of Complex I, Complex VI, and Complex V protein were not different between species, but Complex II and Complex III proteins in bison samples were higher compared to beef samples. These results can serve as the foundation for future research to further understand differences in mitochondrial activity between bison and beef.

Figures

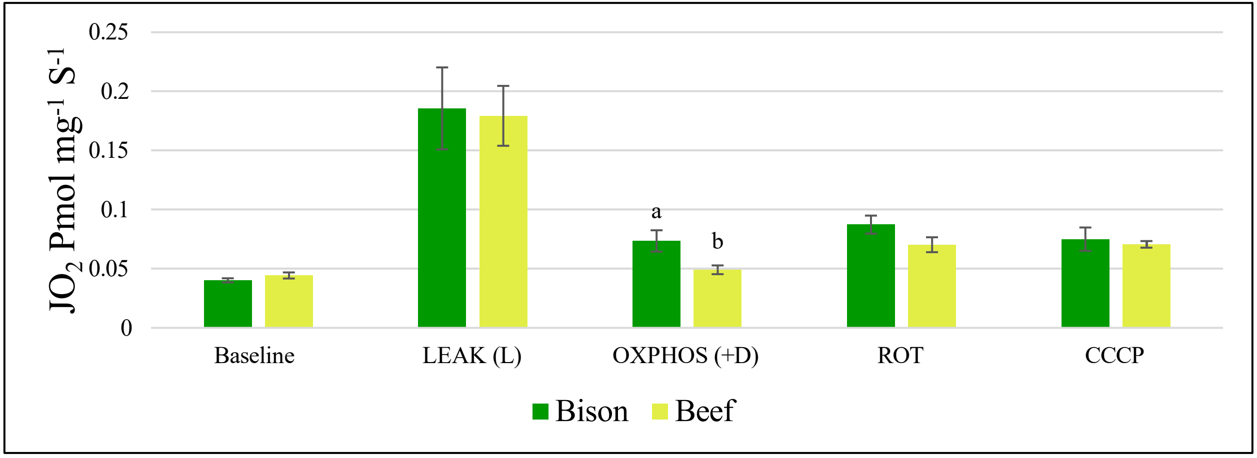

Figure 2.1. Permeabilized muscle fiber oxygen consumption (JO$_2$; average ± standard error) on specific flux states at approximately 1 h postmortem beef (n = 11) and bison (n = 10). Baseline: respiration supported by endogenous substrates and ADP. LEAK (L): respiration after the addition of 5 µL of malate (1 mM), 5 µL of pyruvate (5 mM), 10 µL of glutamate (10 mM), and 20 µL of succinate (20 mM), without phosphorylation of ADP. OXPHOS (+D): maximal respiration after addition of LEAK (L) substrates and 10 µL of ADP (2.5mM). ROT: respiration after 2x titrated addition of 1 µL of rotenone (1 mM; Complex I-inhibitor). CCCP: respiration after the addition of 1mM of uncoupler carbonyl cyanide m-chlorophenylhydrazone (uncoupler; CCCP).

$^{a-b}$ bars with different superscripts differ ($P < 0.05$).

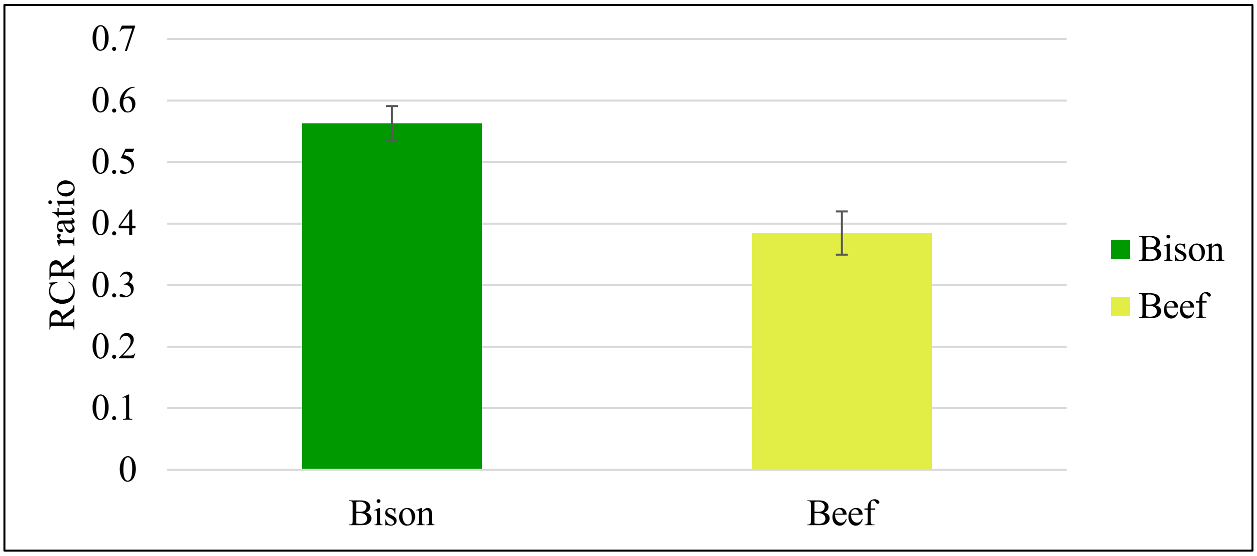

Figure 2.2. Respiratory control ratio (RCR; average ± standard error) between mitochondrial OXPHOS (+D) (substrate-supported respiration in the presence of ADP) and LEAK (L) (substrate-supported respiration in the absence of ADP) at approximately 1 h postmortem of bison (n = 10) and beef (n = 11) permeabilized muscle fibers.

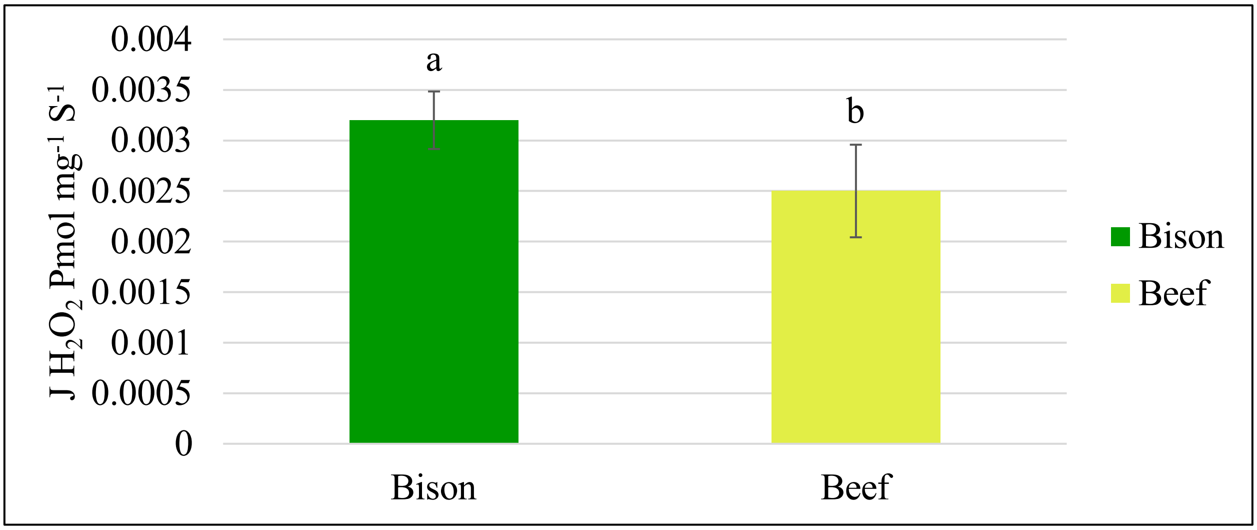

Figure 2.3. Hydrogen peroxide production during max OXPHOS (+D) respiration of approximately 1 h postmortem bison (n = 10) and beef (n = 11) permeabilized muscle fibers.

$^{a\text{-}b}$ bars with different superscripts differ ($P < 0.05$).

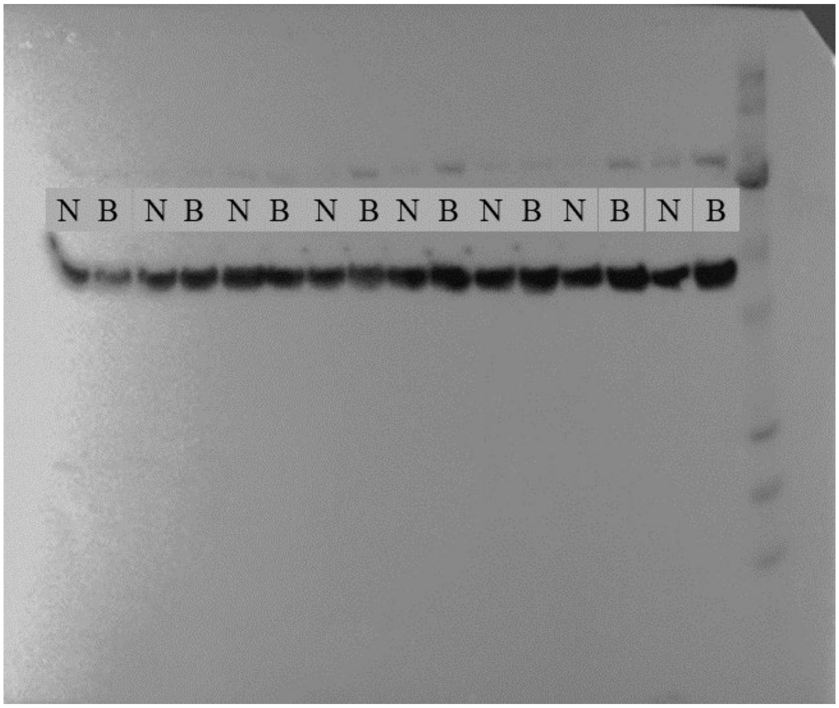

Figure 2.4. Representative Western blot with immunodetection of mitochondrial citrate synthase content in beef (B) and bison (N) masseter muscles.

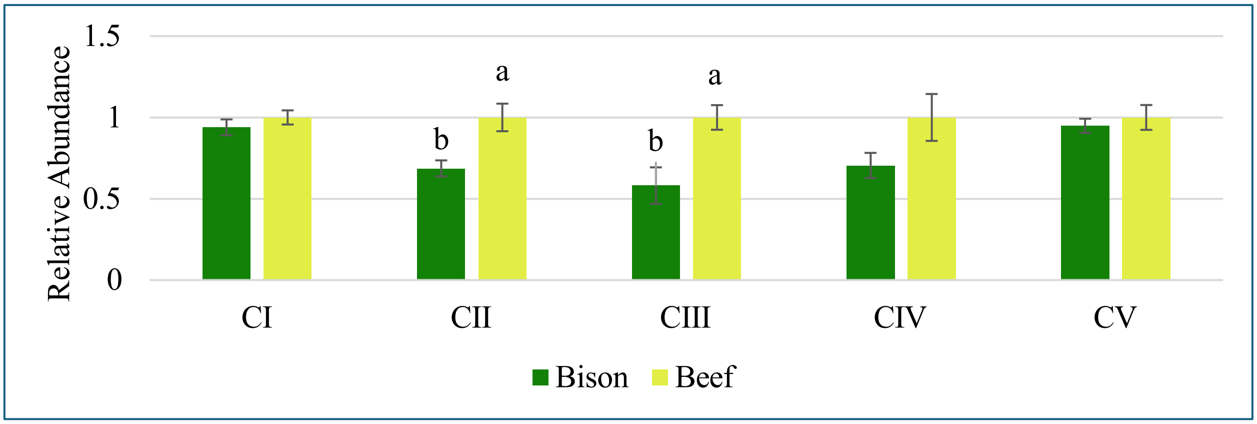

Figure 2.5. Electron transport chain protein relative abundance of bison (n = 12) and beef (n = 12) in mitochondria from muscle obtained approximately 1h postmortem. CI – NADH-ubiquinone oxidoreductase, CII – Succinate-ubiquinone oxidoreductase, CIII – Ubiquinol-cytochrome C oxidoreductase, CIV – cytochrome C oxidase, and CV – ATP synthase.

$^{a-b}$ bars with different superscripts differ ($P < 0.05$).

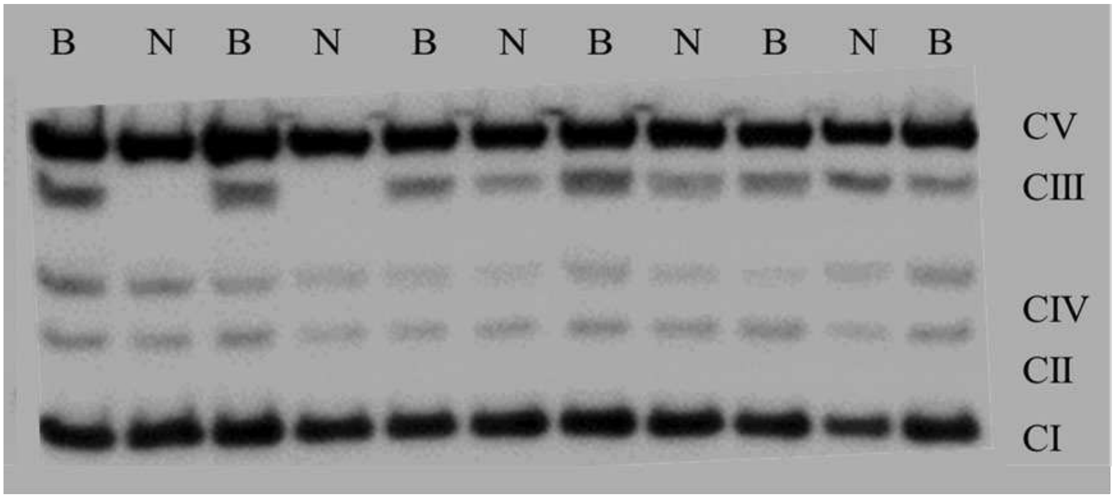

Figure 2.6. Representative Western blot with immunodetection of mitochondrial CI – NADH-ubiquinone oxidoreductase, CII – Succinate-ubiquinone oxidoreductase, CIII – Ubiquinol-cytochrome C oxidoreductase, CIV – cytochrome C oxidase, and CV – ATP synthase protein content in beef (B) and bison (N) masseter muscles.

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