Maintaining Meat Quality through the Beef Supply Chain

by

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Abstract

The studies presented here were designed to evaluate the impacts of increased time periods during two key phases of the beef supply chain: pre-harvest transportation and post-processing storage. In the first study, the objective was to determine the relationship between gut microbial community and pathogen presences in cattle under different levels of transportation stress. Cattle were transported for either 2.5 or 12 hours, with samples collected before and after transportation to evaluate microbial shedding, microbiome, temperature, weight, and exit velocity. Under longer transportation, there was greater weight loss compared with short transportation along with a decrease in microbial diversity, which was associated with an increase in the fecal shedding of *Escherichia coli*. It is likely that long transportation times are associated with increased risks to food safety due to this dysbiosis.

The second study objective was to determine the impacts of low temperature storage followed by secondary retail storages for long time periods on hamburger patty quality and shelf-life. Hamburger patties we packaged using modified atmosphere packaging and stored using a novel low temperature storage method for either 16, 20, or 30 days, after which they were kept in dark simulated retail storage for either 7, 10, or 14 days. Microbial growth fluctuated depending on storage time, but never exceeded acceptable limits. Similarly, the consensus of panelist's sensory scores decreased especially under the retail storage time, but never below acceptable limits. Overall, using this storage regimen, the shelf-life of beef hamburger patties can be extended to at least 30 days without freezing. This thesis demonstrates that it is practical and beneficial to extend the storage time in post-processing phases but increasing the time in pre-processing phases may have negative impacts to the beef supply chain.

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List of Abbreviations

APC	Aerobic Plate Count
ASV	Amplicon Sequence Variants
AT	After transportation
BT	Before transportation
CFU	Colony Forming Unit
COMb	Carboxymyoglobin
Dmb	Deoxymyoglobin
ECC	*Escherichia coli*/coliform
GIT	Gastrointestinal Tract
GSF	Golden State Foods
HS	Hyperbaric Storage
HUS	Hemolytic Uremic Syndrome
LAB	Lactic Acid Bacteria
LTM	Low Temperature Management
MAP	Modified Atmosphere Packaging
MMb	Metmyoglobin
Omb	Oxymyoglobin
PVC	Polyvinyl Chloride
STEC	Shiga Toxin-producing *E. coli*
TCC	Total Coliform Count
USDA	United States Department of Agriculture

Chapter 1: REVIEW OF LITERATURE

1.1 Ground Beef Production Management

Introduction

Ground beef reigns as a ubiquitous processed meat, featured in plethora of dishes and culinary traditions across diverse cultures. Within certain sectors of the food industry, such as food service, ground beef is a large contributor to the supply (Peel, 2021). According to the Economic Research Services of the United States Department of Agriculture, ground beef consumption has continued to rise since 2020. The Economic Research Services also stated that total US beef consumption has increased and exceeded 12 billion kilograms, approximating 26 kilograms per person, in 2020. The increasing demand of red meat production leads to concerns due to the recent statistics in beef production. When observing trends in beef production, the USDA reports that as of January 31, 2024, there is a 2% decrease in cattle inventory (88.8 million head) since the previous year (National Agriculture Statistics Service et al., 2024). A reduction in beef production is likely to exacerbate challenges in meeting the demand for red meat consumption, potentially leading to shortages and higher prices as consumers seek alternative options. This adversity encourages researchers to continue to advance methods of preservation and shelf-life improvement of meat to satisfy all sectors of beef consumption as well as not be wasteful with the resources. Food waste is a major issue that impacts food security and sustainability. According to the United Nations Environment Program's Food Waste Index Report of 2021, approximately 931 million tons of food waste was generated in 2019 at all stages of the food supply chain (United Nations Environment Program, 2021).

One of the greatest challenges in the meat industry is finding ways to suit consumer needs for fresh quality meat while simultaneously producing suitable storage methods and packaging methods to fit consumer demand. With this demand, researchers face obstacles such as producing, distributing, and storing meat within the meat supply chain (Nychas et al., 2008). Professionals within the meat supply chain encounter difficulties in consistently preserving meat over longer periods of time especially when the supply chain can have disruptions. With this knowledge, there is a need for a unique combination of the method of storage and method of packaging to extend the shelf-life of ground beef for food services that will ensure the freshness and safeness of the product. The objective of this review is to give context to current shelf-life contributions to the beef supply chain and how there is a need for innovative technology.

Ground Beef Production

Demand for animal products and meat resources is increasing as the global population increases. Ground beef is one of the highest-demand sources of nutrients in cuisine in the United States to fulfill the needs of the nutritious diet for its citizens. In fact, ground beef represents about 45% of the total beef consumption in the United States (Peel, 2021). The United States' beef supply is the product of a multi-segmented industry that continues to trend towards larger production units and is continuing to be characterized by vertical alignment among the industry segments (Drouillard, 2018). Beef packers are responsible for harvesting animals and fabricating the carcasses into wholesale products. Wholesale products include the primal (shank, chuck, brisket, rib, plate, loin, flank, round) and subprimal cuts. In addition to this, the majority of the wholesale beef products go through varied processing activities that add value to the product or create product that does not end up being a retail cut (Peel, 2021). These beef products that are not retail cuts are referred to as beef trimmings. Beef trimmings are the supply of multifaceted

processing activities that occur downstream. Most beef trimmings come from less valuable areas like the chuck or round of the animal, but the rib and loin are not excluded. One activity that beef products undergo is comminution, when particle size is decreased in the meat for incorporating raw meat materials into finished products most commonly through grinding (Berger et al., 2022). Making ground beef includes four main processing steps: pre-grinding, mixing, grinding, and forming (Berger et al., 2022). Each step is crucial for the ground beef product because these steps allow the beef product to get to the product size it needs to be, and it distributes the lean and fat particles. Starting here, the beef product can be either sold as ground beef, or it can proceed to the next stage in the process where it undergoes forming to hamburger patties and other related products.

In the ground beef industry, there are two different but equal market channels that the final product is facilitated through: retail and food service. With retail, ground beef is regularly part of supply chains that specialize in case ready products and processing (Peel, 2021). Meat grinding is a popular process of retail. Many Americans use ground beef as a product of lower cost to feed people and themselves. As for food service, ground beef is commonly provided by specialized grinders that utilize unique sets of inputs such as lean trimmings and different portions of fat trimmings (Peel, 2021). The specialized trimmings usually are bought by grinder facilities that form products that are either produced directly as a ground beef product, or the beef that is ground is incorporated in a certain food product. Some of the most popular restaurants in the world are known for their ground products like the quarter pounder from McDonalds or the Dave's Double from Wendy's. However, due to the nature of producing ground products, it is important to note the different factors that influence the meat to achieve the most superior product. Ground beef is mostly made up of water followed by protein, fat, and

some minerals. The quality of the beef, in general, is centralized around the breed of the animal, the sex, the age, the fat content (Poveda-Arteaga et al., 2023). These factors take the amount of connective tissue and fat amount into consideration due to the nature of the primals in beef; however, what is distinct about ground beef is that ground beef can veil a lower quality beef carcass unlike the wholesale cuts that come from it. Different sensory factors that are considered in ground beef tasting are tenderness, juiciness, ground beef flavor, intensity, connective tissue presence, and mouth coating effect (Kerth et al., 2015). These factors help determine and tailor ground beef production to meet consumer preferences and understand consumer acceptability (Berger et al., 2023).

Grinding is the process by which meat is reduced to a particle size for incorporation of raw meat materials into finished products which is also known as comminution. Equipment that is commonly used to achieve comminution includes the meat grinder, bowl chopper, emulsion mill, and flaking machine. The most common piece of equipment that is utilized is the meat grinder. Using this different equipment, especially the meat grinder, comes with risk which makes it is important to carefully consider microbial contamination within ground beef. Beef trimmings must remain in constant monitoring through the fabrication process, and there are many opportunities for it to be contaminated microbially. When the meat is ground, the surface area is increased due to a reduction of the particle size; meanwhile, the mixing of the meat gives more opportunity for contamination to other portions of the ground material that were previously not contaminated. Additionally, each time that meat goes through grinding, the pressure of the grinder walls, feeding screw, grinder blade, and plate all increase the temperature of the meat which can create a more favorable environment for microbial growth and lead to quality deterioration. When meat goes through the grinding process, it typically goes through two

different grindings before it is formed (Berger et al., 2022). During earlier years, researchers inoculated beef trimmings to determine the extent of how long contamination can be prolonged in ground beef. Five 907 kg combo bins of beef trimmings were processed into 4.54 kg chubs of raw ground beef, wherein the second combo was contaminated with a green fluorescent protein - expressing strain of *E. coli* (Koohmaraie et al., 2015). Koohmaraie et al. discovered through two facilities producing the 4.54 kg chubs, strains could not be detected after 26.5% more material (500 lb or 227 kg) and 87.8% more material 835 kg followed the contaminated combo at each establishment (2015). They found that the green fluorescent protein-expressing strain could not be detected postprocessing in any residual meat or fat collected from the equipment. Therefore, contamination of beef trimming product used in a grinding facility can broaden the exposure. It is important to ensure all environmental parameters are monitored through official documents and controlled by temperature to ensure the least amount of microbial load and contamination. The ending point of ground beef production will set the bar for the shelf-life of ground beef.

Shelf-Life

Shelf-life is the maximum time under environmental conditions that food or meat has good quality and is safe to consume. A long shelf-life is a phenomenon that many meat scientists and meat industry companies prioritize to ensure good quality meat is provided and maintain no risk to consumer health. Since meat is a highly perishable resource, it is imperative to understand the different factors that influence the shelf-life of fresh meat. Multiple factors influence shelf-life: type of storage, duration of storage, accumulation of spoilage organisms, type of packaging, and cooked versus raw meat. All these factors are interconnected in various ways, thus closely

interacting in research, and influencing its outcomes. The following paragraphs in this section will discuss the interaction of some of the major factors listed above.

The storage duration of meat and accumulation of spoilage organisms share a direct relationship with each other. Several shelf-life studies have included looking at the influence of retail display time on microbial loads. Early work shows that as time in storage increased for meat, spoilage organism counts increased especially with distinct lean-to-fat ratios (Berry et al., 1980). Berry and other researchers found that the shelf-life characteristic of off-odor smell was influenced by the increased ratio of fat in the ground beef formulation (1980). Moreover, later researchers later agreed with this trend by observing minced meat under the effects of sodium lactate and sodium chloride in vacuum packages stored at 2 °C. They concluded that over a storage period of 21 days, Aerobic Plate Counts (APC) were continuing to increase over time, but the treatments retarded the growth compared to the control sample (Patsias et al., 2006). Furthermore, a study conducted in 2014 showed the shelf-life of the 81:19 lean-to-fat ratio of ground beef was influenced by varied packaging and temperature elements over a 20-day storage period. Results showed that non-pathogenic microbial counts for APC, psychrophilic APC, coliforms, and *Lactobacillus* increased during display and temperature abuse (Rogers et al., 2014). As expected, more recent work has generally agreed upon that spoilage organism counts increase as the simulated retail display period extends over a 20-day storage period. Per Smith et al., they discovered that eclectic vacuum packaging material influenced the shelf-life characteristics of the ground beef; however, microbial counts persisted in growth over time (2021). Similarly, a recent study utilizing cassia glauca leaf extract concentrations showed the pattern of aerobic bacterial counts increasing as the 15-day storage period occurred. In the control, the log CFU/g increased at a steep rate compared to the minced beef treated with the leaf

extract which is a method of a natural antimicrobial (Ghoneim et al., 2023). Microbially, slower spoilage occurred with the leaf extract which illuminates another method of preventing spoilage. There are ways to mitigate this type of interaction by utilizing different types of storage and types of packaging and other antimicrobial elements while also utilizing the right combination to achieve the best option for extended shelf-life of ground beef.

The type of storage, type of packaging, and accumulation of spoilage organisms all have a relationship with each other when it comes to extending shelf-life of meat. The different storage types (refrigerating, low temperature management, freezing) and types of packaging (overwrap, modified atmosphere packaging (MAP), vacuum packaging) can influence the length of time the quality of a certain meat product can remain. Mechanical frozen storage has been utilized since the late 1800's, to maintain meat quality and food safety (Qian et al., 2021). Freezing meat is a great storage method for the purpose of broad lengths of storage time while ensuring there is not a drastic decrease in the quality of meat (Wang et al., 2021). As Wang et al. states, they discovered this by allotting beef rolls made up of *Longissimus lumborum* into ten package by temperature (-12 °C or -18 °C) treatment groups (2021). In the end, optimal results of the meat depended on the desirable length of storage time at -12 °C: within 30 days/overwrap, 30-90 days/ 60% O2 + 40% N2 MAP, 30-180 days/ CO MAP (F. Wang et al., 2021). For -18 °C, overwrap storage was recommended for within 90 days of storage, and 90–180 days, 60% O2 + 40% N2 MAP or CO MAP (F. Wang et al., 2021). Since meat is primarily made up of water, it is important to note that there is a key interaction with water within meat and how it maintains the muscle structure.

In recent research, 30 beef longissimus dorsi muscles were cut into 50 mm x30 mm x 30 mm pieces (50 ± 0.5 g) and divided into five equal portions (150 total) and then assigned

randomly into five groups (stored at −1, −6, −9, −12, and −18 °C). Beef samples were vacuum packaged and allocated to six freezers with temperatures monitored (Qian et al., 2021). Results of this study indicated that meat cannot stay in prolonged frozen storage because of how it influences changes in the myofibrillar protein, as shown by looking at different chemical components such as sulfhydryl content, ionic bonds and hydrogen bonds, Ca^{2+}-ATPase activity, surface hydrophobicity, and carbonyl content from myofibril protein extraction (2021). Over the 168 day-storage period, in general, these components encountering a higher storage temperature decreased and the surface hydrophobicity increased. Qian et al inferred that the denaturation of proteins induced by freezing is milder when the temperature of freezing is lower (2021). Further, researchers have found that the quality of frozen product is affected by freezing rate, storage time, storage temperature, product composition, and type of package (Bhattacharya et al., 1988). Bhattacharya et al., discovered that there was an interaction with drip loss being affected by the packaging type, composition of meat, freezing storage temperature, and the length of time in storage (1988). Researchers prepared samples of ground beef containing 15% or 30% fat, and before beef trimmings were ground, they were chilled overnight in a cooler at 1-2 °C. Quarter pounders (113 g) were made, frozen at -35 °C for 4-5 hours then split into two groups where one was bagged in polyethylene bags and vacuum packaged. Samples were then allotted to three different storage temperatures: -12.2 °C, 23.3 °C, 34.4 °C. Drip loss was the highest at -12.2 °C and the lower temperatures had no interaction (Bhattacharya et al., 1988). This was due to the denaturation of protein while being frozen, but it was not proven that a lower temperature causes more damage. These factors can be adjusted to prevent meat quality from decreasing. However, there have been studies that have shown positive progress with the method of frozen storage. Some researchers have found that within the range of -12 °C and -18 °C, meat can have extended

shelf-life up to 30 days in overwrap packaging and 90 – 180 days MAP (Qian et al., 2021). While looking into refrigerated storage, one of the most significant hinderances to the quality of meat in fresh storage is microbial growth. However, packaging methods such as MAP and vacuum packing are resources to help combat microbial growth. In a study dealing with different packaging systems having an effect on the shelf-life of refrigerated ground beef, Conte-Junior et al. used seven different treatments of MAP including ambient air and vacuum packaging where they collected data on daily O_2 levels and CO_2 levels, pH, filtration time, total volatile basic nitrogen, aerobic mesophilic heterotrophic bacteria, and aerobic psychotropic heterotrophic bacteria over 20 days at 2°C (2020). Results showed that MAP packaging was able to extend the shelf-life of ground beef within refrigerated storage using different combinations of gas mixtures (Conte-Junior et al., 2020). The packaging methods of MAP and vacuum packaging were able to have an antimicrobial effect due to depriving the needed elements to prolong the lag phase of the microorganisms as well as reduce the microbial load below the standard shelf-life spoilage threshold (7.0 log CFU/g), and it allowed extension of shelf-life by 3-5 more days than the control group only having ambient air (100% O_2) (Conte-Junior et al., 2020). As observed, there are options to extending the shelf-life of fresh meat; however, not as much as frozen meat. New technology needs to be evaluated in different storage types to provide customers with fresh meat without having to freeze it or be wasteful due to short shelf-life.

Logistical teams within the food industry face many challenges with sourcing fresh meat materials due to its easily perishable nature. Not to mention, there are market factors that are evolving which causes adjustment to the beef supply. Low temperature management is a new patented technology that is a “never frozen” process which maintains a storage environment in which the internal product temperature never falls below 0°C and includes certain storage factors

that contribute to the technology. This method could potentially provide an industry solution for storing meat products in non-frozen conditions, extending shelf-life of the products, and sustaining the quality while delivering the freshest product to customers. These factors form the perfect combination that can achieve longer shelf-life while keeping the meat fresh and not frozen. In recent years, other innovated technology for new refrigerated storage such as Hyperbaric storage (HS) has shown to improve the shelf-life of raw meat as well. Hyperbaric storage is a food preservation methodology that relies in food storage under pressure, usually between 50 and 220 MPa at variable uncontrolled room temperature. Echoing Santos et al., they compared raw fresh meat of minced beef and pork in pieces preserved by HS at room-like temperatures (75 MPa/25°C) and HS at cold temperatures (60 MPa/10°C) for up to 60 days and compared it to refrigeration (4°C) (2020). It was presented that HS extended the shelf-extension due to the log CFU/g not exceeding over 1.00 log CFU/g, and overall, there was an increase in pH over 30 days as well as lower and higher values for drip loss and moisture content were found compared to standard refrigeration (Santos et al., 2020). For this technology, physicochemical analyses performed did not reveal a clear tendency for better results at 60 MPa/10 °C compared to HS at 25 °C (Santos et al., 2020). This concludes that more factors besides pressure and variable temperature should be considered to extend the storage period without compromising the quality of the fresh meat, but the factors similar low temperature management show promising advances to storage of fresh meat.

Influences of Microbiology on Quality

The intricate interplay between microbiological factors and meat quality profoundly shapes the overall characteristics and safety of meat products. The meat's chemical composition

favors microbial growth due to its available nutrients, which in turn encourages meat spoilage (Doulgeraki et al., 2012). The muscle of healthy animals is a sterile environment until it is introduced to outside elements. Skin, hide, and feathers protect the muscles from any contamination. When introduced to outside elements, the microbiological quality of meat depends on the physiological status of the animal at slaughter, the exposure the carcass gets during slaughter and processing, the temperature and other conditions of storage and distribution (Nychas et al., 2008). Of course, there are different intrinsic and extrinsic factors and parameters that influence the microbial load on meat. One of first efforts to reduce or prevent microbial growth during slaughter is utilizing different intervention techniques such as a hot wash, organic spray wash, and rapid chilling. The initial interaction with microbes will determine the final microbial load of the product which then influences the shelf-life and safety of the meat.

Storage temperature, moisture, and oxygen are the factors that have the greatest influence on growth of microorganisms in fresh meat (Addis, 2015). Since microbes can be classified into different habituating temperature categories such as psychrophiles, mesophiles, and thermophiles, it is important for meat processers and consumers to understand the proper temperatures for the storage of meat. Research has shown that the cooler the temperature is, the lower the growth of the most dominant spoilage organisms. In earlier years, Hernandez-Herrero et al. saw this trend while working with beef livers (1999). As days of storage progressed with temperatures 0 °C and 3 °C in aerobic conditions, growth was seen first in 3 °C storage, and it was more intense than in 0 °C temperature conditions (Hernández-Herrero et al., 1999). Ground meat allows microorganisms to proliferate more than whole cut meat, and the high moisture content of this and all meat products provides an environment conductive to microbial growth and proliferation on meat, as water is a critical factor for microbial proliferation. Because of this,

the oldest preservation techniques included drying or desiccation. In a ready-to-eat meat trial, researchers found that while using new technology for inactivating Lactic Acid Bacteria (LAB) was relatively successful, the results of the technology working was dependent upon the water activity in the emulsion process of preparing the ready-to-eat product (Ferreira et al., 2023). Furthermore, in earlier studies dealing with 12 industrial fresh pork sausages in storage conditions of 4 °C to 42 days, researchers monitored the microbial activity, pH, and water activity (a_w) of the meat (Dias et al., 2013). Drawing from Dias et al., they discovered that as stroage time increased, more a_w was available in the sausage; therefore, the microbial presence increased, but the LAB out competed the mesophilic bacteria by day 28 and so on (2013). Higher moisture content in fresh products, especially meat, influences the microorganism life cycle and growth patterns. This phenomenon dictates spoilage in the meat and explains why drying meat is popular and common for preserving meat.

Exploring microbial activity in ground beef versus whole cut meat reveals nuanced differences in contamination levels and preservation techniques, underscoring the importance of understanding microbial dynamics in meat processing and storage. There is a unique interaction of microbes in ground beef versus whole cuts of meat. Some characteristics of why this is such has been previously stated; however, in an early study looking at spoilage of ground beef, researchers took four samples of fresh ground beef and stored it at 5-7 °C for up to 28 days (Jay et al., 2003). According to Jay et al., they found that all samples spoiled by day 9 essentially in the same ways as assessed by aerobic, psychotropic, and Gram-negative counts as well as extract-release volume and pH values, with *Pseudomonas* spp. as the dominant organism in the spoiled samples (2003). Similar, in a later study, researchers examined ground beef and how different antimicrobial methods like utilizing a post magnetic field could influence spoilage

organisms (Goldschmidt Lins et al., 2017). Goldschmidt Lins et al. discovered that spoilage was not prohibited in ground beef, using the antimicrobial element and spoilage was seen on day 12 with varying values below the spoilage threshold with the treatments (2017). Following, in the next few years, researchers were utilizing *Moringa oleifera* leaves powder to improve the nutritional properties and inhibit lipid oxidation and the proliferation of microorganisms during cold storage 4 ± 1 °C over a 15 day period (Mashau et al., 2021). As Mashau et al. states, they found that although nutritional properties of the meat increased, microbial composition also increased; unsurprisingly, the control sample spoiled within the first 5 days of storage, and unless there was a higher concentration of the *Moringa oleifera* leaves powder formulation within the ground beef, the spoilage threshold was still surpassed (2021). Due to the nature of ground beef, it is easier for microbes to infect the meat because of the processing method which allows existing microbes to be incorporated better through out the product due to the mixing steps in further production. Contrastingly, whole cuts of beef have a different interaction with microbes. In a study using beef hind quarters, researchers submitted them to dry aging at 1 °C for 12-36 days and they assessed the effectrs on intrumental, chemical and microbilogical characterisitics of beef loin muscles (Hulánková et al., 2018). Hulánková et al. discovered that water losses amounted around 3.0% up to 21 days of aging, and while that was occurring, researchers found that the fresh beef had good microbiological quality with Total Viable Count (TVC), psychrotrophic and lactic acid bacteria of 2.6, 2.5 and 1.04 logCFU per cm^2 of the surface; however, the mean values for the TVC and psychrotrophic microorganisms reached approximately 5 logCFU/cm^2 after the 14 and more days (2018). This is still remarkable based previous literature discussed in this section and the fact that it did not surpass the spoilage threshold of 7 log CFU/g. Further, researchers also dealing with whole cut meat (boneless strip

loins) under different storage conditions (dry aging vs. vacuum packaging) , were subjected 26 days of storage in a cooler, and the meat was assessed for chemical, instrumental, and microbiological data (Khazzar et al., 2023). Echoing Khazzar et al., they found that compared to the control, the prolonged ageing raised both the peroxide value and the total microbial count, especially in the dry aged samples; however, both remained within the recommended limits (2023). Additionally, in more recent research, investigators utilized forty strip loins (*Longissimus lumborum*) that were collected from left half-carcasses of steers fattened on a high-concentrate diet, and were subjected to different packaging and storage treatments such as vacuum packaging *versus* vacuum packaging with antimicrobial agent and two storage conditions of meat (chilled for 120 days at 1.38 ± 0.21 °C *versus* chilled for 28 days and then frozen at −20 °C for 92 days) where 10 strip loins corresponded to each treatment (Luzardo et al., 2024). It was found that Luzardo et al. observed storage condition having the greatest impact on the microbial counts, and with the different combinations of storage, chilled conditions was the only condition to either approach or surpass the spoilage threshold at day 90 to day 120 (2024). Considering all the literature, it's crucial to recognize how the preparation and type of meat product can impact microbial interactions.

Meat Color

Meat color is one of the most important characteristics of meat products for the perception of meat freshness and wholesomeness (X. Wang et al., 2021;Suman and Joseph, 2013;King et al., 2023). In fact, it is the utmost importance in meat marketing, since it is one of the first quality attributes seen by consumers (Troy and Kerry, 2010). Meat color entails a complex process during which, under refrigerated, aerobic display conditions, fresh meat color

changes cause consumers to discriminate discolored meats which causes up to a billion dollars in annual revenue losses for the meat industry (Smith et al., 2000). Myoglobin is the main protein pigment that is associated with meat surface color. To illuminate the process more specifically, gases bind to the heme iron portion of the myoglobin protein which then determines the outcome of the meat color. Myoglobin is typically found in three forms: oxymyoglobin (OMb), deoxymyoglobin (DMb), metmyoglobin (MMb) (X. Wang et al., 2021), but also has an additional chemical state which is carboxymyoglobin (COMb). Figure 1 demonstrates the different color states of meat when myoglobin is bound with different molecules and undergoes oxidation and reduction.

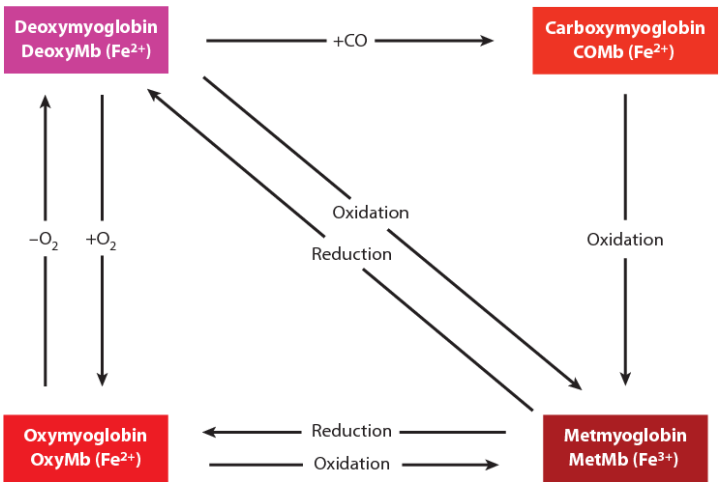

Figure 1 Myoglobin redox forms in fresh meats (Suman and Joseph, 2013)

There are different factors that affect meat color as well besides their myoglobin protein states. Different factors that can affect meat color include pre-harvest events, post-harvest events, and lighting. When looking at the pre harvest stage in an animal's life, methods that were conducted before slaughter have shown to influence meat color. The type of feed used, the duration of food withdrawal, and age of animal before harvest influenced the lightness (L*) of

the meat (Tomasevic et al., 2021). Acevedo-Giraldo et al. utilized two hundred and forty pigs were allotted to different lairage times (1, 8, 12, 24 h) and on farm periods of 0 h or 8 h (2020). Researchers found that the decreased time between withdrawing feed from pigs and their time to be slaughtered influenced the carcass traits in having higher L* values in the carcass (Acevedo-Giraldo et al., 2020). Additionally, Silva et al. evaluated the effect of castration on carcass and meat quality traits of Nellore cattle harvested after 0, 100, or 200 days on feed (2019). Researchers found that increasing the time on feed in general (more intermuscular fat) correlated with increased lightness (L*) and increased yellowness (b*) due to different pigments in the meat (Silva et al., 2019). Furthermore, post-harvest events affect meat color. Once meat is obtained from the carcasses, different factors like temperature can influence consumer's perception of quality and freshness (Schulte et al., 2019). As Schulte et al. states, they used aging and post-aging freezing of pork loins for 14 days and evaluated them with Purge, objective color, pH, subjective color and marbling score, cook loss, SP, and WBS at each aging period (2019). It was found that frozen pork loin chops had an increased redness (a*) value at the 21-day period versus the day 1 period of the freezing portion of the experiment (Schulte et al., 2019). Moreover, temperature is a crucial element that influences the stability of the myoglobin structure (Tomasevic et al., 2021). To reduce the modification in characteristic color of fresh meat, low temperatures that are below 4 °C are imperative (Schulte et al., 2019). Contrastingly, other researchers have found that frozen temperature ranges do not affect the stability of color. Per Alvarenga et al., they determined the effect of aging/freezing sequencing on meat quality, oxidative stability and biochemical attributes of beef muscles by vacuum packaging 10 beef carcasses that were cut into 3 sections and subjected to aging/freezing treatments (2019). It was found that there was no effect statistically in the aging/freezing sequence which could have been

due to the type of packaging that stabilized the color (Alvarenga et al., 2019). Comparatively, Wang et al. found that temperature fluctuations (between −18 to −17 °C, −18 to −15 °C, and −18 to −13 °C) during the freezing period of fresh meat cuts from the hind leg of yellow cattle can alter the color of meat (2020). They found that there was a decrease in redness (a*) and an increase in yellowness (b*) which infers that the meat was becoming oxidized (Wang et al., 2020). This coincides with what researchers know about thawing of meat and the release of free water due to protein degradation over time. Therefore, it is important to consider all pre- and post-harvest factors when identifying ways to prevent discoloration of meat in different settings.

Conclusion

There are many factors that influence shelf-life, and different methods of storage influence meat quality and ensure food safety. Shelf-life can be a solution of many problems in the meat industry. One pressing issue in the meat industry is battling food security to a growing population that is moving exponentially. Within the growing population, food shortages, food waste, and sustainability are areas that the meat industry are dedicating their time and efforts to focus on. Improvements of different storage methods such as low temperature management can help alleviate consumer demand for delivering fresh meat products while reaping the benefits of frozen storage meat quality without freezing the meat. This is a great method for food services to request and utilize for their wholesale products because it will help their business satisfy the demands of their customers. It is important for the meat industry to continue to expand on different storage methods for food services so that they can provide a way to deliver fresh meat products to customers.

1.2 Transportation-Associated Stress

Introduction

Within the beef supply chain there are many aspects that contribute to the final product of meat being placed on a retail shelf. One area that has not been critically investigated entirely is the process of transporting cattle. Transporting cattle to their next point in the supply chain is a critical portion of beef production. This is a necessary process of the production cycle that occurs during transition changes of bovine life events. There are different avenues that cattle can travel by for the different purposes of beef production: road, rail, sea and air (Tarrant, 1990). With little question, travel by road is the most notorious mode of transportation, besides sea, for cattle.

Transportation for cattle is the period where their physiological state is the most vulnerable due to related environmental effects. These effects contribute to substantial economic loses for producers in many fashions such as digestibility of feed and function of the rumen (Deng et al., 2017). Furthermore, the distance of travel can also have consequences to the cattle's body. However, more research needs to be conducted on different factors such transportation distance and their physiological effects on cattle.

Animal Stress

Stress is a broad term that implies to a threat or circumstance in which the animal's body needs to adjust (von Borell, 2001). This can be induced by its environment, lack of water, weather, feed access, motion, and injuries (EFSA Panel on Animal Health and Welfare et al., 2022). Cattle are prey animals, so they have a specific flight zone that triggers them to flee when they feel stressed or threatened. This makes them more prone to situations in which stress is induced. Animals that experience stress can face physiological consequences such as shrinkage,

immune modulation, inflammatory responses, and changes in their different microbiotas. Further, stress can be measured and observed in terms of physiological and behavioral differences that might be indicative for the individual's state of homeostasis (von Borell, 2001). In one early study, researchers evaluated the effect of transport time up to 14 hours on cattle and the effects of vehicle design on animal welfare, stress, and meat quality using measurements of blood serum parameters, heart rate monitoring, behavior recording and occurrence of carcass bruising (Honkavaara et al., 2003). Honkavaara et al. found that heart rates were generally higher during smaller lengths of travel, cattle that were in groups of three or more induced more stress due to the presence of bruises on the hips, and muscle glycogen was lowest in the short times of transport (2003). Furthermore, other parameters such as behavioral differences can be used to suggest an animal's composition of stress. In early research, investigators evalutated the relationship of the temperament appraisals with serum concentations of cortisiol while also comparing temperament assesments utilizing yearling Brahman bulls over a 60 day interval (Curley et al., 2006). According to Curley et al., they discovered that temperamnet measurements (exit velocity, chute score, pen scores) were positvely correlated with one another, and exit velocity was positively correlated with serum cortisol values within day 0 and 120 (2006). They concluded that measures of exit velocity can be a valuable tool for assement of cattle and a possible predictor of temperament and stress responsiveness. AIthough these studies show some episodes where stress can be induced and measured, other situations sush as weaning, environmental temperature, restraint, socail isolation/mixing, and feed deprevation can also cause stress to arise in cattle (Chen et al., 2015). It is important for producers to understand the impact of stressors in the animal's body especially if the immune system is affected. In an earlier study, researchers investigated sixteen castrated male calves to investigate the effect of weaning

stress on total leukocyte and differential counts, neutrophil functional activity, lymphocyte immunophenotypes, and acute phase protein response (Lynch et al., 2010). Drawing from Lynch et al., they found that abrupt weaning (most stressful condition for calves) resulted in increased neutrophil counts and impaired trafficking and phagocytic function which illuminates how stressors can suppress the immune system of the animal (2010). Furthermore, other researchers investigated the same production process of weaning utilizing male and female beef calves to characterize the immune response to weaning stress in bovine leukocytes at the physiological and molecular levels (O'Loughlin et al., 2011). O'Loughlin et al. discovered that weaning elicits an immediate and short-lived acute stress response in the calves, and it heightened the inflammatory response and cellular mobilization (2011). In a worst-case scenario, a compromised immune system could potentially result in health complications severe enough to endanger the animal.

While numerous articles and studies have explored stress and its interaction with cattle and other animals, reaching conclusive findings remains a challenge due to the complexity of the system and the individuality of each animal. For instance, an animal can have stressed based on how hot their environment is. Factors of their environment, in the summer particularly, are exposure to high ambient temperatures, direct and indirect solar radiations, and humidity. Heat stress is a billion-dollar global problem (Gupta et al., 2023) In a study in earlier years, scientist used two groups of cattle, one in cool loading conditions and the other group in non-cooled conditions, to determine the effect of heat on their mammary gland enzymatic activity and other factors (Adin et al., 2009). They found that heat stress made biological factors of the cow in the dry period suffer compared to cattle that were cooled and showed a significant increase in milk production (2009). Furthermore, in a recent study, researchers utilized two groups of cows,

cooled or heat stress group, to investigate alterations in the mammary proteome and phosphoproteome, a catalog of proteins containing a phosphate group as posttranslational modification, during lactation as a result of dry period stress (Skibiel et al., 2022). Per Skibiel et al., they discovered that 251 proteins and 224 phosphorylated proteins were differentially abundant in the lactating mammary gland of heat stressed cattle versus cooled cattle, and the top functions of the differentially abundant proteins and phosphoproteins affected were related to immune function and inflammation, amino acid metabolism, reactive oxygen species production and metabolism, tissue remodeling, and cell stress response (2022). Animal stress is important to understand due to the negative effects it can have on an animal. Ultimately, it is the hinderance to profit in the animal supply chain. Transportation stress, specifically, is the main source of conflict for people involved in the beef supply chain especially those who are farther from the next stage in production. Transportation stress is a phenomenon that is introduced to animals when they are participating in assembly, loading, confinement and motion, unloading, and penning (Tarrant, 1990). These events are necessary to continue animals, especially cattle, to the next stage of their production cycle whether its new destination is a feedlot or an abattoir. Cattle can experience an overwhelming event when various physical and emotional stimuli occur simultaneously. Different stimuli during long distance transportation can include, but is not limited to rough handling during loading, deprivation of food and water, poor vehicle design, poor road conditions, extremes of temperature and humidity, overcrowding, mixing different species and age groups, high air velocity, noise, motion, vibration, and length of the journey (Minka and Ayo, 2009). These stimuli, which induce stress in cattle, can produce negative effects to the animal and carcass. In the 2022 National Beef Quality Audit, it was reported that 57.2% of market cows experienced major bruising followed by market bulls having 41.4% of

major bruising (National Cattlemen's Beef Association, 2023). This is due to cattle being in an unfamiliar environment while transported to the next stage in production all the while in close proximities with other cattle and handling systems not adequate for animal size and number. In addition to the increased bruising which compromises the yield of carcasses, transportation stress also can affect the carcass quality. In a recent study, researchers studied 298 cattle in commercial slaughter and 96 cattle were studied that were herded to a mobile abattoir (Hultgren et al., 2022). Per the research of Hultgren et al., they observed a decrease in tenderness with cattle traveling larger distances using the Warner-Bratzler shear force as their testing method compared to the animals that walked to the mobile abattoir (2022). Unfortunately, there is a lack of resources as of recent that can speak on carcass quality specifically looking at transportation stress in the United States; however, in another study, researchers obtained data from a South African abattoir based on 100 cattle of different genotypes to evaluate the effects of distance travelled, lairage duration and number of stunning shots on the plasma levels of bovine heat shock protein 70, cortisol and glucose and their relationship with beef quality (Chulayo et al., 2016) . As stated by Chulayo et al., they discovered that the presence of a stress biological indicator negatively correlated with meat quality due to pre-slaughter stress (2016). In conclusion, as an industry, it is crucial that the interaction of transportation stress and meat quality is studied more due to previous knowledge and the gap of knowledge.

Effects of Stress on Microbiology

Efforts to decrease stress in an animal is an emergent point to current cattle industry. Previously stated, stress in an animal can alter its body physiologically. Additionally, microbiomes can be altered when environmental change is occurring, and different factors such

as diet, seasons, temperature, breed of animal can change how it composes itself. Cattle transport stress is causing significant problems to the beef cattle industry such that researchers have been focusing on how nutrition-metabolism, hormone secretion levels, and immune competence are imbalanced.

In recent studies, researchers were able to isolate the rumen microbiome of cattle to observe how stress altered the microbiome. They concluded that when observing 18 male beef cattle that traveled for 6 hours, the stress of transport affects the microbial flora by decreasing the relative abundance of Bacteroidetes, increasing Firmicutes at the phyla level, and changing metabolites of the microorganism (Li et al., 2019). This is a significant effect due to the importance of the microbes task in aiding rumen fermentation (Wetzels et al., 2015; Jolazadeh et al., 2015; Walsh et al., 2009). Similarly, another researcher observed 8 Xianan beef cattle that were transported over 1000 km. Their study concluded that stress did affect the rumen microbiome due to the changes in volatile fatty acid production as well as influencing the degradation of cellulose (Deng et al., 2017). Cattle's ability to digest is imperative for growth. The beef industry continues to investigate the effects on stress in the cattle's rumen microbiome due to the serious nature of the consequences that can arise with beef production.

The direct influence of stress from transportation on the gut microbiome of cattle has recently become the new highlight of the beef industry due to the impact it has on food safety. Although there are not many studies that have been conducted on the overall effect that it has on the microbiome, some studies have shown whether cattle are more prone to shedding pathogenic microbes than others that do not experience stress. In a study involving 200 steers and heifers, animals were swabbed for hide and fecal samples before and after transport. Results showed that stress influences the prevalence of *Salmonella* spp. in fecal samples by increasing the percentage

two-fold (Barham et al., 2002). Contrastingly, another study of 150 weaned steers and heifers was conducted to determine the effect transportation stress had on the shedding of *E. coli* O157:H7. Researchers found that cattle were observably more excitable had a smaller prevalence of the pathogenic microbe than calm cattle in later points when their feeding period occurred (Schuehle Pfeiffer et al., 2009). However, researchers seem to struggle with the potential bias of factors that can influence the prevalence of the pathogenic microbe, number one being the state of the infection, which makes it difficult to determine if there is a difference with transport stress induced in the animal. Future studies are needed to continue to observe this interaction and create methods that could eliminate more bias to get true results of stress on the gut microbiome of cattle.

Foodborne Illness

Ensuring the safety of the food supply chain is crucial in preventing the spread of foodborne illnesses, protecting consumers from harmful contaminants, and maintaining trust in the food industry. Unsafe food can lead to a range of health issues, from mild discomfort to severe illnesses and, in some cases, fatalities. For perspective, based on a recent statistic, the world population is around 7.8 billion people, and about 56 million people die every year (World Health Organization, 2015). Of those people, 7.96% of people experience foodborne diseases, and 7.5% of annual deaths were attributed to foodborne illness in the world (World Health Organization, 2015). With the foodborne cases, 10.4%-14.1% were caused by food-producing animals between 1999 and 2017 (World Health Organization, 2015) where bacteria are the culprit of most foodborne illnesses followed by viruses and parasites (Lee and Yoon, 2021).

Food animals are the major reservoirs for many foodborne pathogens such as *Campylobacter* species, non-typhi serotypes of *Salmonella enterica*, Shiga toxin-producing

strains of *E. coli*, and *Listeria monocytogenes* (Heredia and García, 2018). The issue at hand is the ineffective interventions that eliminate them from animals and foods. However, the real concern lies where it is hard for the industry to control the presences of the pathogenic microbes.

Salmonella spp. is one of the most common pathogenic microbes that can create Salmonellosis which is one of the most common foodborne diseases in the world (Eng et al., 2015). *Salmonella* is a member of the *Enterobacteriaceae* family, and it includes Gram-negative, flagellated, non-sporulating, and facultative bacteria that well with average human body temperature (37°C) (Foley et al., 2013). The bacterium is capable of inducing localized gastroenteritis in humans and some animals, but the range of infection is influenced by factors like immunity and bacterial virulence factors (Heredia and García, 2018). Although cattle are not known for infecting people with *Salmonella*, certain anatomy, like lymph nodes, can introduce the microbe into the meat supply (Wottlin et al., 2022). Sometimes, cattle's lymph nodes are included in beef trimmings, and these trimmings can be utilized for comminuted products like ground beef. The risk of this food borne disease comes from the final cook temperature of the meat before it is consumed. Failure to cook the meat to 71 °C degree of doneness can lead to risk of salmonellosis.

In general, coliforms are Gram-negative bacteria which some mentionable examples include genera *Enterobacter*, *Escherichia* and *Klebsiella*, and can be utilized in microbial evaluations to indicate if product or equipment is unsanitary (Tatini and Kauppi, 2002). *E. coli*, the main focus in microbial evaluations due to potential foodborne illness, is the dominant nonpathogenic flora of the human intestine with the exception of anaerobic bacteria which helps in the production of vitamins, and aids in battling pathogenic microbes (Feng, 1995). However, some strains have evolved to creating foodborne diseases that affect the gastrointestinal, urinary,

or central nervous system by the virulence factors that have allowed them to adapt to new niches (Farrokh et al., 2013). *E. coli* is a Gram-negative, facultative anaerobe, non-sporulating rod within the *Enterobacteriaceae* family (Feng, 1995). There are a few pathotypes of *E.coli* that cause harm to the human body; however, Shiga toxin producing strains of *E.coli* are the most common of the pathotypes especially O157:H7 (Croxen et al., 2013). Shiga-toxigenic *E. coli* (STEC) usually causes severe hemorrhagic colitis in humans which comes with symptoms like abdominal cramping and vomiting; however, the main concern with STEC strains are the public health aspect due to their association with large outbreaks and hemolytic uremic syndrome (HUS) which is a small percentage of cases (Feng, 1995). There are different modes of how transmission of the bacteria can infect humans, but the focus in perspective of the beef industry is how it can be transmitted from animal to the food supply to humans. Contamination of meat can come from two major sources of the animal: the hide and directly from the rectum. This creates a problem for packing plants and forces attention to detail to ensure further contamination to sterile meat surfaces to combat further exposure after processing. The risk of food borne diseases caused by STEC comes from failure to cook ground beef to at least 71 °C degree of doneness can lead to risk of HUS or other known diseases. This is due to contaminated meat being used in comminuted products where the mechanical process allows meat to warm up temporarily caused by the friction and pressure of the grinder.

Pathogenic bacteria will continue to cause outbreaks and deaths throughout the world due to the exposure from food-animals. Researchers should continue to investigate how to enhance our production systems to minimize the entry of pathogens into the food chain.

Microbiomes

Microbiomes are a microbial ecosystem that encompasses different elements that are interactive, and dynamic based on the different elements within it like genetic elements, structures, and metabolites of characteristic microbiota (Khalil et al., 2022). Microbiomes previously investigated in cattle include the respiratory tract, gastrointestinal tract (GIT), reproductive tract, and the skin. These different regions have specific niches and different microorganisms like bacterial and fungal species while providing a unique footprint in the animal body.

From a food safety perspective, the GIT is the most important microbiome to investigate. The GIT includes all segments that connect the mouth of the animal to the anus. These components include pre-gastric (mouth, pharynx, tonsils and esophagus), fermentation and gastric compartments (reticulum, rumen, omasum, and abomasum), and the small and large intestines (Durso et al., 2017). The GIT has differentiated microbiomes which are dependent on the nature of their environment and the microbes that proliferate in that region. In a contemporary study, researchers were able to determine the divergence in composition along the GIT and were able to focus on the lower-gut microbiome (small intestine and hindgut regions) (Mao et al., 2015). Based on Mao et al., they utilized dairy cattle GIT and observed that 542 genera belonging to 23 phyla was distributed through the GIT while also observing Firmicutes, Bacteroidetes Proteobacteria predominating (2015). Similarly, another study observed two contemporary steer groups to analyze samples from the jejunum of the small intestine (Myer et al., 2016). According to the findings of Myer et al., they found that the phylum Firmicutes accounted for up to 90% of the populations within all of the samples and was dominated by the families Clostridiaceae and Ruminococcaceae (2016). Nonetheless, when an infection of the lower gut arises, there is a reaction within the microbiome. In a recent study, researchers wanted

to evaluate the persistence of an *E. coli* O157:H7 infection in the recto-anal junction (Mir et al., 2020). Per Mir et al., differences were based upon the collection techniques of a swab sample collection verses a fecal sample, but Firmicutes to Bacteroidetes ratio was higher in swab samples from O157 colonized animals and influenced the consistent yet decreased O157 colonization (2020). However, the concern of the abundance lies with the risk it brings to food if the microbiome is overcome by pathogenic organisms. This event presents itself when an animal is encountering an infection of a certain microbe that causes the microbiome to become unbalanced (Kim et al., 2017). Other research suggests that it may not be the certain diversity of the microbiome itself that allows other microbes to proliferate, but the environment (diet) can influence infection (Kim et al., 2014). Kim et al. conducted a study where fecal samples were collected from 426 cattle fed 1 of 3 diets typically fed to feedlot cattle: 1) 143 steers fed finishing diet (83% dry-rolled corn, 13% corn silage, and 4% supplement), 2) 147 steers fed late growing diet (66% dry-rolled corn, 26% corn silage, and 8% supplement), and 3) 136 heifers fed early growing diet (70% corn silage and 30% alfalfa haylage) (2014). It was found that Firmicutes and Bacteroidetes were dominant phyla in all fecal samples, but depending on the starch, fat, and protein values of the particular diet, the dominant genera (Oscillibacter, Turicibacter, Roseburia, Fecalibacterium, Coprococcus, Clostridium, Prevotella, and Succinivibrio) and unclassified groups differed ($P < 0.001$) with diets (Kim et al., 2014). In a contemporary study, researchers wanted to examine if the diet of cattle utilizing wet distillers' grains with solubles created an environment in which the diversity of the microbial community would change (Durso et al., 2012). Durso et al. concluded that at the genus level, Prevotella (Gram negative) and Anaerobacter (Gram positive) were the most frequently occurring bacteria in our beef cattle fecal samples which suggests that in addition to previously observed changes in *E. coli* O157:H7, the

entire bacterial community structure is different for animals with the 40% corn based WDGS compared to traditional corn finishing diets (2012). Ultimately, more investigations are needed that target other stressors that can perturb the microbiome of cattle in the distal large intestine and rectum.

Cattle are the main reservoir for the Shiga Toxin-producing *E. coli* (STEC) and are able to harbor the pathogen in their gastrointestinal tract without developing clinical disease (Matthews et al., 2023). Specifically, the pathogenic microbe colonizes in the colon and persists in the rectum (Larzábal et al., 2020). The gut microbiome is critical for the regulation and signaling of the immune response and for preventing colonization (Kamada et al., 2013). Within the cattle's gastrointestinal microbiome, non-pathogenic *E. coli* can account for up to 1% of the bacterial population of the gut (Callaway et al., 2009). When observing the interaction that *E. coli* has with microbiomes, certain elements allow the microbe to proliferate in the environment. Diet, temperature, and season as well as industrialized animal husbandry practices have a profound effect on STEC prevalence and the native gut microbiome composition (Sapountzis et al., 2020). In a recent study, investigators were able to identify that cattle with a higher prevalence of STEC in their gut microbiome were characterized to have a higher forage diet (Vasco et al., 2021). Following, the researchers concluded that STEC carriage in cattle is favored by highly diverse microbiota profiles which is also associated with fore-dominant diets (Vasco et al., 2021). Understanding the influence of diet on the animal's gut microbiota is important due to overwhelming evidence of its interaction with *E. coli* (Kudva et al., 1997; Krause, 2003; Van Baale et al., 2004; Sheng et al., 2013). This is the dominant material of research when looking at the interaction of pathogenic *E. coli* and its effect on the gut microbiome. Further studies should investigate other elements like stress or genetics that could potentially lead to better

understanding of how this pathogenic microbe colonizes and survives in higher quantities in one animal versus the other.

Salmonella spp. is not often associated with cattle due to its strong correlation with chicken. However, cattle are not excluded from hosting *Salmonella*. This microbe is a significant food safety concern in commercial beef production, and it mainly contaminates through the inclusion of *Salmonella*-infected peripheral lymph nodes that are sometimes included in ground beef (Wottlin et al., 2022). Research has proven differences in the prevalence of *Salmonella*-positive cattle based on the season, region, and cattle source (Webb et al., 2017; Nickelson et al., 2019). However, cattle can still host *Salmonella* in their fecal matter as well as host a high prevalence of different serotypes (Samuel et al., 1981). Based on recent research with 1,840 fecal samples, concentrations of *Salmonella* ranged from 1.0 log10 CFU/g to 6.2 log10 CFU/g, with 72% of positive samples' concentrations equal to 1.0 log10 CFU/g (Wottlin et al., 2022). Variation is due to all of the different elements that influence of proliferation as stated earlier, but nonetheless, still remarkable that *Salmonella* can co-habit with other pathogenic microbes like *E.coli*.

In all, microbiomes of cattle are temperamental based on their environmental conditions. Therefore, certain combinations of the breed of animal, diet, season, commensal bacterial environment, and other factors alike can influence how the gut microbiome of the animal is composed which is relevant based on the consequences of pathogenic bacteria that can reside there and be exposed.

Conclusion

Delving into the research of transportation stress in cattle is not merely an academic pursuit but a crucial endeavor with far-reaching implications for the beef industry and

consumers. The welfare of cattle before and during transportation directly impacts the quality of meat and overall sustainability of the livestock industry. Investigating transportation stress and how it affects microbiomes in the animal can lead to better understanding of the risk of food pathogens and the reaction of the microbiome. By mitigating and understanding transportation stress, researchers and producers can enhance the ethical treatment of animals, reduce economic losses related to compromised meat quality, and contribute to a more sustainable approach to livestock production. Ultimately, the insights gained from such research efforts hold the potential to reshape the industry by promoting animal welfare and address broader concerns related to food safety.

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Chapter 2: FECAL SHEDDING OF POTENTIALLY PATHOGENIC ORGANISMS AND FECAL MICROBIOME DYSBIOSIS OF CATTLE EXPOSED TO DIFFERENT LEVELS OF TRANSPORTATION STRESS

Abstract

Transportation stress is a well-known problem facing the beef cattle industry especially in the southeastern United States, where cattle must often travel large distances to reach feeding and harvest facilities. This stress can impact fecal shedding of pathogens and, through that, meat safety, but causes of this are still unclear. The objective of this study was to determine the relationship between gut microbial community and pathogen presence in cattle under different levels of transportation stress to clarify food safety risks. Behavioral, physiological, and biological samples were collected before and after the transportation event (2.5- or 12-hour travel time) from 17 Angus-based heifers. Microbiome samples were collected via sterile double headed swabs and were subjected to DNA extraction to be further analyzed. Overall, the main factors impacted by the difference in transportation time were body weight, *Escherichia coli* shedding, and microbiome structure. There was a difference in the shrinkage (decrease in body weight) between the 2.5-hour (-12.7 kg) and 12-hour transport (-21.3 kg; $P < 0.05$) and there was a difference in the proliferation of *E. coli* (0 log CFU/g to 2.14 log CFU/g) between the 2.5-hour and 12-hour transport event ($P = 0.006$). Additionally, the microbiome analysis demonstrated that animals under longer transportation had a decrease fecal microbial diversity ($P < 0.05$). This shift was associated with a change in the Firmicutes to Bacteroidotas ratio, which has been shown as a marker of dysbiosis in human health. Overall, the results of this study suggest that transportation stress can influence cattle's physiological and biological levels as well as influence the fecal microbiome.

Introduction

Transportation stress is a critical concern facing farmers today due to the potential deleterious effects to the animal as well as their carcass merit (National Cattlemen's Beef Association, 2023). Every journey that the cattle undertake, whether to the market or between pastures, presents challenges that can profoundly impact the welfare of cattle and, ultimately, the total profit for farmers. Understanding the complexities of transportation stress and implementing proactive procedures is essential for maintaining the health and productivity of the herds.

Transportation stress is likely to occur when animals undergo a series of events to relocate from one location to another including assembly, loading, confinement during motion, unloading, and penning processes (Tarrant, 1990). Various stimuli encountered during long-distance transportation include, but are not limited to, rough handling during loading, deprivation of food and water, inadequate vehicle design, poor road conditions, extreme temperatures and humidity, overcrowding, mixing different species and age groups, high air velocity, noise, motion, vibration, and the duration of the journey (Minka and Ayo, 2009). Cattle that experience stress through this process could encounter physiological consequences including shrinkage, immune modulation, inflammatory responses, and alterations in their microbiome. These can be measured and observed through physiological measures, such as weight and cortisol levels, and behavioral changes, such as exit velocity, indicative of their homeostatic state (von Borell, 2001). It has been previously established that behavioral cues such as exit velocity correlate with cortisol concentrations (Curley et al., 2006; Chen et al., 2016; Lima et al., 2018). Furthermore, studies have linked transportation stress to influencing pathogen shedding such as *Escherichia coli* and *Salmonella* spp. (Barham et al., 2002; Schuehle Pfeiffer et al., 2009). However, there is

still a knowledge gap surrounding how different lengths of time under transportation stress impact the amount of pathogen shedding, and, further, how this stress impacts the other commensal microbes in the gastrointestinal microbiome. Knowing the relationship between stress, microbiome structure, and pathogen shedding can help answer key economic and food safety questions related to carcass exposure to these pathogens within the abattoir.

Ensuring stringent food safety measures within the beef industry is paramount, with robust processes meticulously designed and rigorously enforced to safeguard against any compromise in food quality or safety. The objective of this study was to determine the relationship between gut microbial community and pathogen presence in cattle under different levels of transportation stress to clarify food safety risks.

Materials and Methods

All animals used in this study were owned by Auburn University. All procedures with animals were performed in accordance with the protocols approved by Institutional Animal and Care and Use Committee at Auburn University, protocol 2023-5340.

Animals. A total of 17 commercial Angus-based heifers with no more than 1/4 Brahman influence (12 months of age) were sampled during November 2023 at the Agricultural Experiment Station Beef Unit E. V. Smith Research Center Beef Unit (Shorter, AL). Animals were not habituated to transportation or human-acclimated prior to the experiment. Heifers were housed on a bermudagrass and fescue pasture and were fed free choice bermudagrass hay with supplemental soyhull/corn gluten pellets which was fed in the amount of 1% of body weight, daily. Cattle were not fasted before transportation.

Sample collection. Samples for the experiment were collected immediately before (BT) and after (AT) the animals underwent transportation. Heifers were restrained in a hydraulic restraint system, where body weights were collected from the EziWeigh7i scale (Tru-Test Datamars, Auckland, New Zealand), calibrated by Michelli Weighing & Measurement for collection. For microbiological culture analysis, at least 10 g of feces matter were collected *per rectum*, placed in 532 mL Whirl Pak bags (Pleasant Prairie, WI) and stored on ice until transportation to the laboratory. Animals were then rectally swabbed with a BBL Culture Double Swab (Becton, Dickinson and Company, Sparks, MD, USA) for DNA sequencing. Swabs were inserted approximately 10 cm into the anus of the animal and twisted to contact the sides of the surface of the swab with fecal matter. Swabs were stored in their original sterile storage container and placed in a cooler of ice for transport. Body temperature was measured using a rectal thermometer (Sharptemp-V, Dallas, TX). When the animals were released from the restrainer, exit velocity was recorded using the SKILZ Speed Gates (Impulse Footcare, LLC, Durham, NC). Exit velocity was determined by the rate at which the animal left the chute over a fixed distance (1.6002 m.). The infrared sensors triggered the timer to begin and stopped once the animal triggered the second set of infrared sensors. After BT samples were taken, animals were loaded as a group onto a 7.32 m x 2.13 m gooseneck cattle trailer with a 1000 kg truck for transportation.

The first transport duration was 2.5 hours traveling from the E.V. Smith Research Center (Shorter, AL.) to West Point, GA then to Montgomery, AL. then back to E.V. Smith utilizing the I-85 corridor (245.42 km). For weather conditions of the 2.5-hour and 12-hour transport, data was acquired through the Weather Link Auburn source through the Wx.Medius website (*Weather Exchange*, 2024). During handling and initial data collection of the 2.5-hour transport,

temperatures averaged 12.6 °C to 15.1 °C with a relative humidity decreasing from 87.2% to 83.5%. During the 2.5-hour transport, temperatures ranged from 15.1 °C to 16.7 °C with a relative humidity decreasing from 82.4% to 78.2%. When cattle arrived back to the facility after the 2.5-hour transport for data collection, temperature remained at 16.7 °C with a relative humidity increasing from 78.5% to 80.6%. After transport, animals were unloaded, and sample collection was duplicated. This represented the short distance of travel and was selected using the mean reported transport time as listed in the 2022 National Beef Quality Audit (National Cattlemen's Beef Association, 2023). Two weeks after the short transport time, the same animals were used for the long transport evaluation. The animals traveled the same route as the 2.5-hour transport, but it was repeated six times to fulfill the 12-hour transport time (1,472.55 km). The sample collection process was repeated as described above for the long transport time of 12 hours (a measurement based on the scale of the experiment and consideration of the maximum value of the 2022 National Beef Quality Audit) (National Cattlemen's Beef Association, 2023). For the 12-hour transport, the handling and initial data collection temperatures averaged -2.7 °C to -0.8 °C with a relative humidity increasing from 95.2% to 96.1%. During the 12-hour transport, temperatures ranged from -0.4 °C to 14.2 °C with a relative humidity fluctuating from a minimum 36.7% to a maximum 95.6%. When cattle arrived back to the facility after the 12-hour transport for data collection, temperature decreased from 3.6 °C to 2 °C with a relative humidity increasing from 85% to 91.9%. Following each transportation event, animals were released back into their normal pasture with freely available forage and water.

Microbial Evaluation. Fecal samples were transported to the Food Safety Microbiology laboratory located within the Department of Animal Sciences at Auburn University (Auburn, AL) and stored at -18 °C until processing. For processing, samples were thawed, and 1 g of fecal

matter was weighed and mechanically stomached for 30 s in 10 mL of peptone water (Lot: 2293112, Becton, Dickinson and Company, Sparks, MD). Samples were serially diluted and plated onto Total Plate Count Compact Dry plates (APC; Lot: 007303, Hardy Diagnostics, Santa Maria, CA.), *Escherichia coli* and coliform Compact Dry plates (Lot: 011303, Hardy Diagnostics, Santa Maria, CA) and *Salmonella* Compact Dry plates (Lot: 016303, Hardy Diagnostics, Santa Maria, CA). *Salmonella* plates were incubated at 42 °C for 24 h and *Escherichia coli* and APC plates were incubated at 37 °C for 24 or 48 h, respectively. All plates were then stored at 4 °C until counting.

DNA Extraction and Sequencing. Rectal swab samples were transported to the Food Safety Microbiology Laboratory at Auburn University prior to collection. Samples were stored in a freezer (FKFH21F7HW Frigidaire, Charlotte, NC.) at -18 °C until DNA extraction took place. Total DNA was extracted from one swab head of all samples (n = 68 and one negative control) using the ZymoBIOMICS DNA Miniprep Kit (Lot No. 228944, Irvine, CA) following manufacturer instructions. DNA samples were sent to Novogene Corporation Inc. (Sacramento, CA) for amplicon sequencing of the V3-V4 region of the rRNA gene at their China lab location (Guangzhou City, Guangdong Province, China). Libraries were prepared using 341f/806r primer set, and sequencing was conducted on the Illumina NovaSeq platform with paired-end 250bp chemistry.

Microbiome Data Analysis. After sequencing, microbial rRNA gene amplicons were analyzed using QIIME2 version 2024.2 (Bolyen et al., 2019). Sequences were denoised and paired reads were joined using DADA2 (Callahan et al., 2016). Taxonomy was then classified using the SILVA 138 99% database using the QIIME2 feature-classifier plugin with a pre-trained classifier (Bokulich et al., 2018; Quast et al., 2013). Assigned taxa were used to filter

non-microbial DNA from the dataset, including chloroplast and mitochondria. Data were rarefied to 144,444 ASVs/sample and diversity analysis was conducted using the core metrics pipeline. Alpha diversities (observed features and Shannon's diversity) were statistically compared using a Kruskal-Wallis test with a Benjamini-Hockberg multiple testing correction (Kruskal, 1952). Beta diversities were calculated using the Bray-Curtis dissimilarity metric and differences were determined using the PERMANOVA test with multiple testing correction (Anderson, 2017). Additionally, changes in the microbial diversity between BT and AT samples taken from the same animal were calculated using the QIIME2 longitudinal plugin and paired distances were statistically analyzed using a Kruskal-Wallis test with multiple test correction (Bokulich et al., 2018). PCoA analysis was conducted using QIIME2 Emperor. For all tests, significance was assigned at $\alpha = 0.05$.

Statistical Analysis. This experiment was analyzed as a completely randomized design. Statistics were analyzed in a student's t-test using R-Studio software, version 4.2.2, with the emmean, tidyverse, and ggplot2 packages (R Core Team, 2022; Lenth, 2024; Wickham et al., 2019; Wickham, 2016). Delta values for weight, body temperature, exit velocity, *Salmonella* plate counts, *Escherichia coli* plate counts, and APCs were calculated by subtracting BT from AT values The α-level for mean differences was set at 0.05. Tendencies were reported with an α-level greater than 0.05 and less than 0.1.

Results

The Impact of Long Transportation on Animal Welfare Markers. Cattle under long-time (12h) transportation stress exhibited greater weight loss and faster exit velocities. Initial comparisons were made between the before transportation (BT) values for both transportation times to determine whether the 2-week rest period was sufficient. There was no difference in other animal welfare measurements such as temperature ($P = 0.88$) and exit velocity ($P = 0.25$) when the BT values from both transportation events were compared, indicating the rest period was sufficient to restore the animals to their homeostatic level. However, there was a tendency for a difference in the body weight ($P = 0.08$) in the BT values, likely due to normal animal growth. To evaluate the main effect of transportation time, the delta values (differences between AT-BT) were evaluated. There was a difference ($P < 0.05$) in the change in weight from the 2.5-hour transport and 12-hour transport. Cattle weight loss was smaller in the short-transport animals (-12.7 kg vs. -21.3 kg; Table 1). Further, when comparing the delta values of the 2.5-hour and 12-hour transport, there was a tendency for the cattle's exit velocity to increase after the long transport ($P = 0.09$).

The Long Transportation Effects on Microbiology Assessments. There was a difference ($P = 0.006$) in the delta log CFU/g of *Escherichia coli* from the 2.5. hour transport and 12-hour transport (Table 1). The longer transport event had a larger difference in *E. coli* log CFU/g compared to the shorter transport event (0.00 vs. 2.14). Other factors such as *Salmonella* ($P = 0.27$) and Aerobic Plate Counts ($P = 0.53$) did not show a difference in the change between the 2.5 hour and 12-hour transport.

Table 1 Behavioral, physiological, and biological measurements changing with transportation time and their differences.

	Before 2.5 hours		After 2.5 hours		Before 12 hours		After 12 hours		Delta 2.5 hours		Delta 12 hours		*P*-value
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	
Weight (kg)	402.4	9.21	389.7	9.90	413.7	10.16	392.4	10.57	-12.7	1.66	-21.3	2.39	2.7e-07***
Temperature (°C)	39.2	0.24	39.2	0.21	39.3	0.21	39.3	0.17	1.7e-15	0.29	-5.29e-2	0.17	0.88
Exit Velocity (m/s)	1.87	0.206	1.83	0.184	2.22	0.222	1.94	0.178	-0.04	0.163	-0.51	0.219	0.09**
Salmonella (log CFU/g)	1.80	0.337	2.03	0.300	2.58	0.326	3.51	0.379	0.23	0.250	0.93	0.570	0.27
Escherichia coli (log CFU/g)	0	0	0	0	0.32	0.320	2.46	0.739	0.00	0.00	2.14	0.723	0.006***
Aerobic Plate Count (log CFU/g)	5.37	0.337	5.85	0.104	5.69	0.039	5.85	0.387	0.48	0.343	0.16	0.369	0.53

Values represent means and standard error margins for each measured category.
Values of Salmonella, Escherichia coli, and Aerobic Plate Count were represented in log CFU/g.
** indicates a tendency for significance of the means in the row.
*** indicates significance of the means in the row of delta values.

Microbiome Sequencing. A total of 69 samples, including 68 fecal swab samples and one negative extraction control, were sequenced for the V3-V4 region of the 16S rRNA gene. A total of 13,829,500 forward and 13,829,500 reverse reads were obtained from all samples, with an average quality score of 37. These reads were denoised into 17,431 amplicon sequence variants (ASV). After the removal of ASV that assigned to non-microbial origins (chloroplasts and mitochondria), there were 17,410 ASV remaining that were included in the analysis. The negative control did not contain any identifiable sequences and was filtered from the dataset.

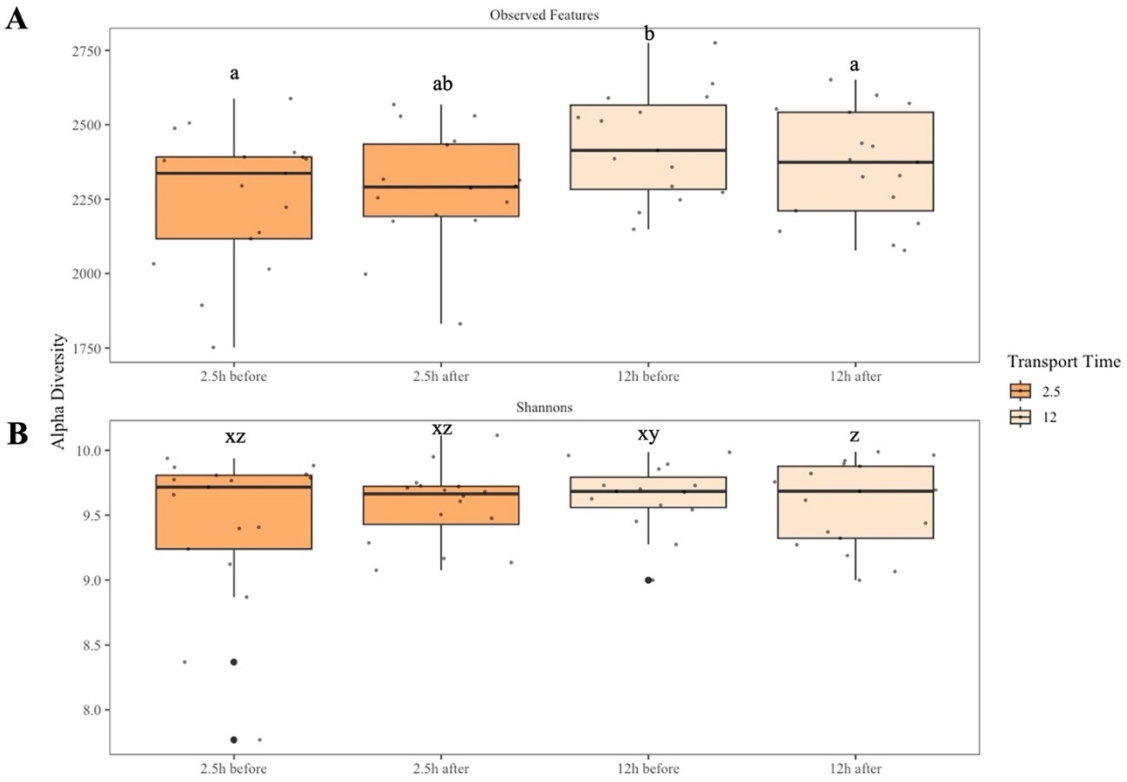

Figure 1. Alpha diversity calculated using the observed features (A) and Shannon's diversity (B) metrics. Samples represent fecal microbiomes collected from cattle before and after 2.5 or 12 hours of transportation. Colors indicate the transportation time.
[abc, xyz] boxes with different letters indicate a difference ($P < 0.05$).

Microbial Diversity. The data represented in this study comes from fecal samples BT and AT of each transportation travel time of 2.5 h or 12 h. Differences were identified ($P = 0.01$) in the within-sample alpha diversity across the different sampling points (Figure 1A and 1B). The diversity remained similar between the BT and AT sampling points in the 2.5-h transportation period for both metrics (observed features, Shannon's diversity). Conversely, there was a decrease in the alpha diversity between the BT and AT samples for the 12-h transport time from approximately 2,500 to approximately 2,270 observed features, ($P = 0.007$ for observed features (Figure 1A) and approximately 9.8 to approximately 9.2 ($P = 0.004$ for Shannon's diversity (Figure 1B). There was a slight separation of samples and observable shift based on beta diversity between sampling points (Figure 2A). Furthermore, there was a greater change in the 12-hour transport time of the individual animal's beta diversity (distance) between before and after transportation samples within an animal (Figure 2B; $P < 0.05$). Visualization of this shift is displayed in PCoA (Figure 2C & D). While overall there is little separation between all four groups, within a transportation time group there was a shift in the overall community composition between the BT and AT sampling times and was further compared with their distance in the BT and AT samples with the 2.5-hour and 12-hour transport in each individual animal.

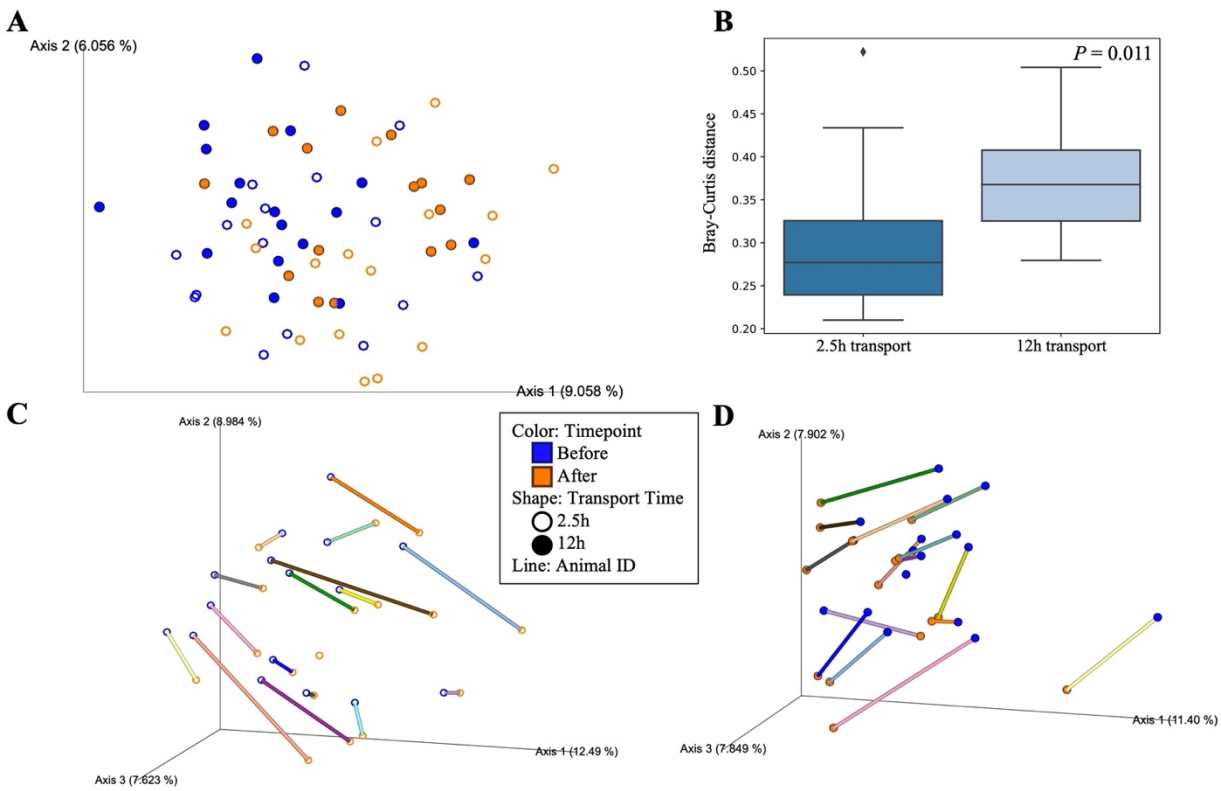

Figure 2. Beta diversity of cattle fecal microbiomes collected before and after transportation for 2.5 or 12 hours. Beta diversity was calculated using the Bray-Curtis metric. A) PCoA analysis of all samples. B) Paired sample analysis indicating the change in an individual animal's beta diversity (distance) between before and after transportation samples within an animal. The graph represents the statistical difference in these paired distances between transportation times. Paired distances were compared using a Kruskal-Wallis test with multiple test correction and boxes with different letters were statistically different ($P < 0.05$). C) PCoA of only 2.5h transportation samples. The lines connect samples from the same animal before and after transportation. D) PCoA of only 12h transportation samples. The lines connect samples from the same animal before and after transportation.

Taxonomic Classification. The fecal microbiomes of cattle before and after transportation stress contained bacterial phyla and bacterial classes with changes in composition (Figure 3). Of these, the class Clostridia was the most relatively abundant in all samples, followed by Bacteroidia. Interestingly, Gammaproteobacteria tended to be overrepresented in the AT samples compared to the BT samples for both transportation times. The microbiome data analysis revealed a dysbiosis in the gut microbiome with the 12-hour transportation, as indicated by an increase ($P < 0.05$) in the Firmicutes:Bacteroidotas ratio which was essentially 2:1. Relative

frequency of Firmicutes and Bacteroidota showed a slight change in microbiome composition of the 2.5-hour transport of BT and AT; however, when observing the 12-hour transport of BT and AT, there is a shift in the relative frequency of Firmicutes and Bacteroidotas.

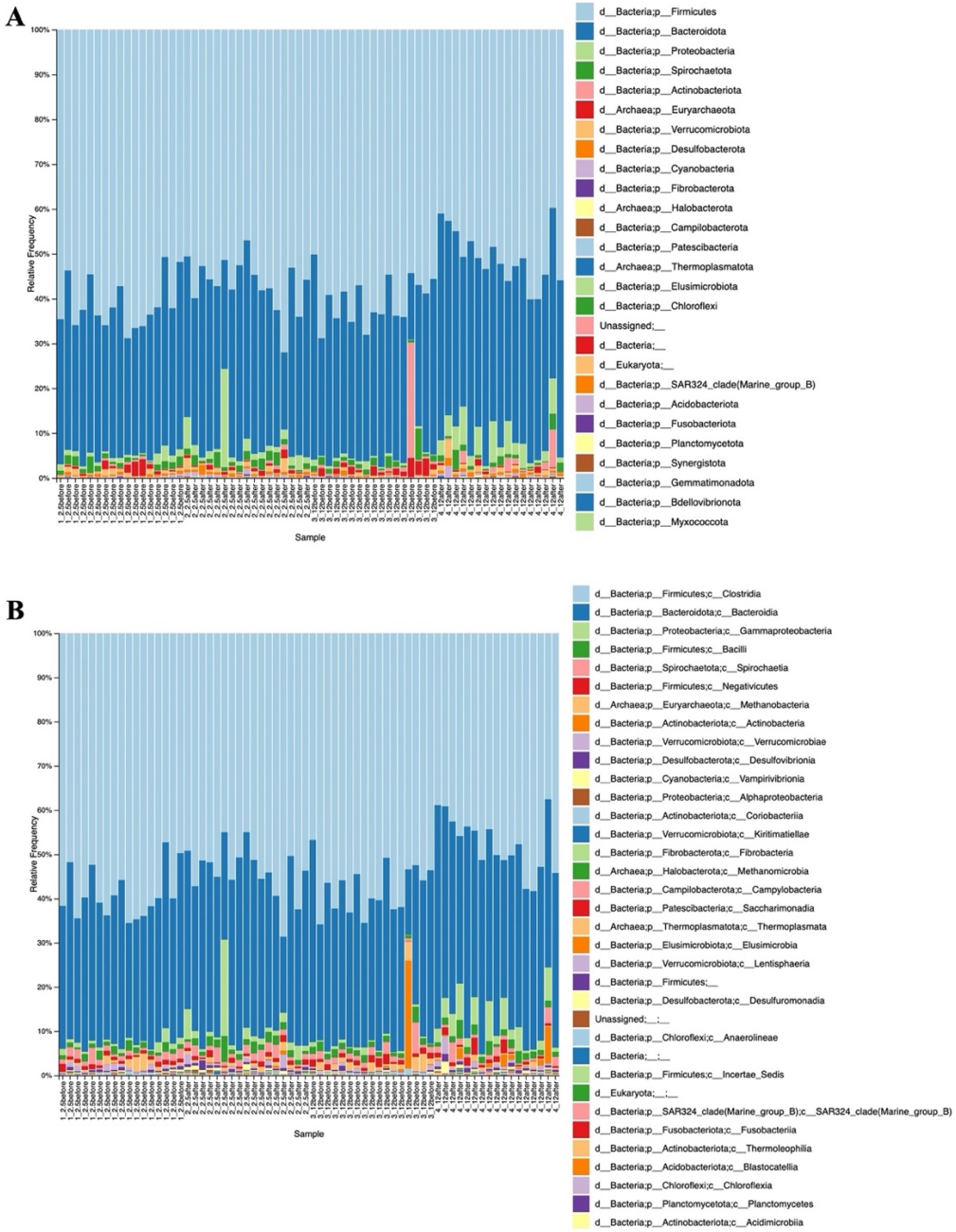

Figure 3. The relative of abundance of microbial taxa at the A) phylum level and B) class level. Taxonomy is associated with transportation and timepoint group. Colors represent different microbial taxa, and within a different sample from the animal, the bars are ordered by sampling event.

Discussion

There are multiple parameters from cattle that can be used to measure stress. Researchers are able to investigate this by evaluating behavioral assessments like exit velocity, feeding behavior, standing or lying down, and gait score, and physiological assessments such as weight and rectal temperature, blood samples, morbidity, and mortality (Meléndez et al., 2021).

Behavioral assessments. The changes in exit velocity due to transportation stress, represented by the delta values, had a tendency for an increase in exit velocity after the 12-hour transportation period which was expected. Because of the design of the exit chute route, which involves the cattle taking a quick left turn after traveling across the infrared lasers, this could give cause to the tendency of an increase in exit chute speed. When animals are experiencing an event that induces stress like handling or transportation, their instinct would be to flee which is activated by their "fight or flight" response; however, these data indicate a trend for an increased exit velocity after the long travel period (Grandin, 1997). It was expected to see a faster velocity in BT values due to the naivety of the animals which was not observed when comparing the BT values. This contrasts with a study that reported that there were more intermediate (2.27 m/s; $P < 0.001$) or excitable (3.60 m/s, $P < 0.001$) than calm cattle in the pre-shipment evaluation as shown by greater exit velocities (Schuehle Pfeiffer et al., 2009). However, in the 2.5-hour transport time, the changes in value were small, indicating the cattle's velocity had minimal change before and after that transport session compared the values in the 12-hour transport. Another study evaluated exit velocity based on poor corral and handling and modified corral and calm handling (stressful vs. less stressful event), and found that cattle that were experiencing more stress, they moved faster out of the chute which would be expected (Lima et al., 2018). This study is similar to current results; however, a greater difference of time between the BT and

AT values of the 12-hour transport session was hypothesized. Another explanation for this could be due to travel exhaustion of the cattle after they arrived from the different long duration of travel as well as habituating with process due to non-aversive events (Peischel et al., 1980). When the animals arrived back, their exit velocity had a tendency to be faster than it was prior to transport.

Physiological assessments. In the BT samples of the 2.5-hour transport and the 12-hour transport body weights in this study, there was a tendency for an increase in weight which was expected due to natural growth occurring during the 2-week rest period. When observing the difference in before and after values of the two travel times, cattle experienced, on average, 5% shrinkage which is not favorable, but it is not out of range of expectations. In a study with a similar traveling time of nine hours using 36 animals of different breeds, cattle incurred a 10% decrease in body weight after their travel session which shows an event of shrinkage (Sporer et al., 2008). This demonstrates the effect of transportation stress on the animal's body which demonstrates possible muscle deterioration which was not observed in this current study. Furthermore, another study investigating the effect of transportation and commingling on newly weaned calves indicated how the stress of being transported can cause more shrink than the anticipated amount of 5% (Arthington et al., 2003). At its maximum, there was a 7% decrease in body weight. Moreover, a more recent study utilizing newly weaned beef calves were transported for 15 hours in a livestock trailer on two separate hauls 1-week apart to evaluate the effect of rest stop duration on the indicators of calf welfare (Marti et al., 2017). Marti et al. found contrasting results in the control group without the rest compared to this current study's results (2017). This could be due to their cattle having access to food and water as well as being newly weaned which can thereby impact the amount of gastrointestinal fill and potential to lose weight

compared to older cattle (Schwartzkopf-Genswein et al., 2007). Overall, the cattle in this current study experienced shrinkage which can be caused by the stress of transportation; however, the cattle in this current study most likely lost their gut fill which contributed to their weight loss percentages since they did not exceed 5%. In addition, when weather was recorded on the day of data collection and transportation, mild cold stress was considered as an element to the experience of the animal during the 12-hour transport time due to temperatures ranging from -2.7 °C to 14.2 °C with added wind chill due to the high air velocity during the travel period. A study indicates that dairy cattle experiencing mild cold stress with low temperature (-11.1 °C) and air velocity within a free-standing barn experienced a lower milk-yield compared to other treatments groups (Angrecka and Herbut, 2015). However, researchers concluded that the animals were not vulnerable to the cold temperatures and air velocity, yet there is still opportunity to find the favorable conditions for the cattle. Furthermore, another study aimed to evaluate the effects of a long-term cold environment on growth performance, physiological behavior, biochemical blood indexes, and hormone levels in thirty Simmental cattle. It was indicated that animals that were in the winter cold temperature group, which averaged -14.2 °C over a 42-day period, experienced long-term cold stress and biological results suggested that low temperatures may inhibit the digestive function of Simmental cattle and enhanced the body's energy metabolism and stress hormone imbalance which ultimately damaged the normal growth and development of the cattle's body (Wang et al., 2023). Therefore, because of the high air velocity and lower temperatures that the cattle experienced during the 12-hour transport time of this current study, it could have contributed to the metabolism of the animal.

Microbiology assessments. A difference was found in the *E. coli* counts between the before and after travel times of the 2.5-hour transport time and the 12-hour transport time. At the

2.5-hour transport, there was no *E. coli* detected, but in the 12-hour transport, there was a mean of 2 log CFU/g *E. coli* in the fecal samples. A longer duration of transport stress showed an increase in *E .coli* shedding from the gastrointestinal tract. Although the presence of *E. coli* is seen, it is not clear if it is pathogenic. In an early study comparing microbial amounts of *E. coli* and *Salmonella* spp. before and after transport from the feed yard to the slaughter facility, there results were less obvious on whether transportation stress effected the prevalence of these microbes (Barham et al., 2002). This could be due to their complex sampling pattern besides just observing the feces that were directly in the rectal anal junction. Contrastingly, another study found that cattle that were more excitable (stressed) had a lower pathogenic presence of *E. coli* than cattle in later points of the study when they were able to feed (Schuehle Pfeiffer et al., 2009). Yet, researchers grapple with the potential bias of factors influencing the prevalence of pathogenic microbes, among them being the state of infection and feed type, rendering it challenging to discern any variance attributable to transport-induced stress in cattle.

Microbiome assessment. This study is novel in its inclusion of microbiome data along with the microbial enumeration, and thus has few sources that are available for comparison. The data presented here are a contribution to further discussion of the alterations of cattle fecal microbiomes with an influence of transportation stress. It was predicted to observe a microbiome shift with certain organisms becoming more dominant than others. When comparing the BT of the 2.5-hour and 12-hour transport length, there is a visual shift in the observed features and richness of the microbiomes; it was expected to see the richness and observed features decrease from the BT of the 12-hour travel time to the AT of the 12-hour. However, because cattle were naïve to the process of handling and loading it was interesting to see the initial richness and observed features in the 2.5-hour transport time have a lower diversity on average compared to

the BT of the 12-hour transport time. This alludes to cattle having new exposure to different stimuli combined with the transportation aspect in comparison to the BT 12-hour event. In a study related to microbiome communities and stressful events for cattle such as dehorning and castrating, researchers found that the cattle's gut microbiome was more diverse, using the Shannon diversity metric, in animals that did not experience dehorning compared to animals that experienced dehorning, and animals that experienced dehorning and castrating resulted in a decrease in the microbiome diversity (Mir et al., 2019). This aids in explanation with this current study because both studies illustrate new stimuli to cattle, and this type of interaction shows how new stimuli can affect the diversity in the cattle's gut microbiome like the initial diversity in the 2.5-hour microbiome behavior in this current study. Furthermore, it was intriguing to observe the lack of clustering in beta diversity analysis; however, there were defined regions of the BT and AT samples in which a shift was observed. There was a difference in the distances in the BT and AT microbiomes of the 12-hour transport which was expected; however, it was interesting to see how much they shifted which was visually represented. This concludes that longer transport times for cattle induced not only diversity within each sample, but it also illuminated the change in diversity between the BT and AT microbiome in one individual.

With classifying samples, it was intriguing to see the shift in the phyla in the BT and AT samples showing Firmicutes becoming more dominant after transport. As the ratio of Firmicutes to Bacteroidota has been hypothesized as an important marker of gut health, this was also analyzed in the current dataset. The shift in the ratios of Firmicutes and Bacteroidota can indicate that there is dysbiosis in the intestinal microbiome and can lead to inflammatory responses and introduce immunosuppressive properties which is seen in human medicine (Stojanov et al., 2020). Further, the class that appeared to dominate the most was the class Clostridia followed by

Bacteroidia. The Clostridia class was more dominant in the shorter transport time compared to the longer transport time. When the animals were approaching the time to be transported, the class Clostridia had a higher relative frequency compared to Bacteroidia, but then a shift happened after the animals were transported which made Bacteroidia have a stronger presence within the microbiome. At the end of the 12-hour AT, samples showed a greater relative abundance of other microbes compared to the 12-hour BT. In a similar study, researchers observed cattle fecal material in the field over time and discovered that Clostridia, Bacteroidia, and Sphingobacteria were dominant classes of bacteria in fresh cowpats (Wong et al., 2016). This aligns with what was observed in this current study; interestingly, though, Sphingobacteria were not observed here. Another study observing the periparturient cattle gut microbiome and the onset of *Salmonella* shedding showed that individual cow fecal microbiomes, predominated by Bacteroidetes, Firmicutes, Spirochaetes, and Proteobacteria phyla, changed before and after parturition (Muñoz-Vargas et al., 2018). Muñoz-Vargas et al. concluded that, although there were differences in some bacterial taxa between *Salmonella* positive and negative samples, there were not differences in the fecal microbial diversity or structure for cows with and without the onset of *Salmonella* shedding (2018). Even though noticeable changes have been seen in the taxonomy with the different events of transportation, more research should be conducted to obtain a clear picture of the relationship of transport stress and the fecal microbiome.

Conclusion

According to this current study's results, these findings can offer cattle producers valuable guidance on managing transportation stress. Transportation stress influences weight loss shrink levels, and it influences the proliferation of potential pathogenic microbes. From a microbiome standpoint, there were notable shifts in the diversity of the communities based on

the length of travel, and certain organisms became dominant more than others. By implementing strategies to minimize stress during transition, such as proper handling techniques, habituation, adequate provision of food and water, and optimization of transport conditions, the industry can safeguard the well-being of cattle. Through continued research, it would be interesting to see how transportation stress can influence the presence of pathogenic microbes on carcasses within the abattoir due to the presence of these microbes on hides. It would also be intriguing to have more samples of fecal matter over the course of travel to see if there is rapid change at a certain point in time. Transportation stress not only influences physiological and biological elements of cattle, but it also changes the composition of fecal microbiomes that can lead to dysbiosis of the gut and increased shedding of potentially pathogenic organisms.

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Chapter 3: SHELF-LIFE AND QUALITY DETERIORATION PATTERNS IN GROUND BEEF PATTIES STORED UNDER LOW TEMPERATURE MANAGEMENT AND RETAIL STORAGE CONDITIONS

Abstract

There is a need in meat and food service industries to improve the shelf-life of comminuted meat products to secure a safe and good-quality food supply. The recent development of low temperature management (LTM) technologies provides an option to improve shelf-life while maintaining industry standards of quality in food service. The objective of this study was to determine the impacts of LTM followed by secondary storage on hamburger patty quality and shelf-life. To accomplish this, eight cases of hamburger patties were allocated to nine treatment groups in a completely randomized with a 3 × 3 factorial treatment structure containing three LTM storage periods (16, 20, or 30 d) and three secondary retail storage times (7, 10, or 14 d), with a baseline treatment collected on the day of packaging. Response variables of interest were microbiological growth, packaging integrity, and sensory deterioration. Microbiological markers demonstrated a decrease in Aerobic Plate Counts from day 20 to day 30 ($P = 0.01$), and an increase in growth from day 7 to day 10 in dark secondary retail storage ($P < 0.0001$). Lactic Acid Bacteria grew from day 7 to day 10 in secondary retail storage but decreased day 14 ($P < 0.0001$). Coliform Counts decreased in LTM storage ($P = 0.04$) and dark secondary retail storage ($P = 0.005$). Sensory deterioration was observed in sear, texture defects, and aroma/flavor defects under LTM storage and in juiciness, seared flavor, and aroma/flavor defects ($P < 0.05$) for secondary storage. It is evident that LTM can increase the maximum storage time of ground beef patties and is an option for improving product shelf-life in the food service industry.

Introduction

Meat products, especially those ground or otherwise comminuted, are highly perishable foods with generally short shelf-lives. Ground beef is a historically important contributor to the American diet that is still considered a staple today (Agarwal and Fulgoni, 2022). It plays an important role as a nutritious protein source for a rapidly increasing population, but its use is limited by the rapid onset of spoilage that reduces the options for marketing and storage. Spoilage is a complex, multifaceted process that is driven by the enzymatic breakdown of muscle tissues, oxidation of proteins and lipids, and microbial growth (Mortazavi et al., 2023). These result in significant sensory changes to the product including discoloration, texture change, sliminess, and the production of off-odors and flavors (Miller et al., 2022; Rogers et al., 2014).

The quick service restaurant industry is disproportionately impacted by spoilage outcomes as these restaurants are held to a high standard of product consistency by their customers and rely on a complicated supply chain. The COVID-19 pandemic revealed a need to update the mechanisms for product preservation in the supply chain, so these restaurant systems are prepared in the event of sudden changes in the supply of raw materials (Martinez et al., 2021). Clusters of issues occurred in different parts of the food supply chain which resulted in a crisis to maintain production and food safe products. During the COVID- 19 lockdown, purchasing anxiety led to the formation of new purchasing habits among consumers, including buying products they had not previously encountered (Galanakis et al., 2021). The difficulty in managing product shelf-life has been further exasperated by a trend toward “fresh never frozen” products in quick service dining. These factors have created a bottleneck effect in the industry which is ultimately driving innovation for storage methods (Peel, 2021). The U.S. consumption

of hamburger patties has been increasing since the 1950's when fast food was penetrating the food market (Song, 2016). As of 2020, ground beef represented about 45% of total domestic beef consumption (Ishmael, 2020). Ground beef consumption will continue to grow as it is an easy and inexpensive nutritional resource. Therefore, these combined factors have revealed a need for novel product storage systems to maintain shelf-life without freezing the product or otherwise affecting sensory perceptions.

The development of the proprietary low temperature management (LTM) storage system presents a potential solution to these challenges. Under LTM, products are held at consistent low temperatures for long time periods with limited spoilage development. The prospective applications for this technology are numerous, with the storage of hamburger patties for quick service dining being an excellent candidate. Therefore, the objective of this research was to determine the impacts of LTM storage followed by secondary retail storage for long time periods on hamburger patty quality and shelf-life.

Materials and Methods

Preparation of beef patties. The production of raw materials was conducted at Golden State Foods (GSF; Opelika, AL). Ground beef patties were formed after coarse grinding using a 1.27-cm plate with carbon steel blade (Provisur, Chicago, IL.), mixing (Provisur), and fine grinding using a 2.4-mm plate and carbon steel blade (Provisur) using an industrial patty former (Tomahawk Manufacturing, Plymouth, WI.). Patties weighed approximately 113 g and were made in stacks of five with a sheet of paper between using the Interlever system patty stacker machine (Pac Pro Inc. Model # 200IDLXP126, Souderton, PA.). Three stacks of five patties traveled by conveyer to the Multivac R245 machine (Kansas City, MO) to be packaged into

modified atmosphere packaging sleeves (MAP; 35% CO_2:5% N_2). The packaging film composition was 80 microns nylon/ethylene vinyl alcohol/enhanced polyethylene coextrusion – easy peel (WINPAK, Minneapolis, MN.), and oxygen transmission rate was 0.9 cm^3/m^2/ 24h/ 23 °C dry and moisture vapor transmission was 8.0 g/sq. m/ 24hrs/ 37.8 °C at 90% relative humidity. Sleeves of patties were placed into cases with four total sleeves per case (N = 70 cases). There was 35.93 kg of ground beef patties per treatment with a lean point of 75.7/24.3. When boxes were complete, they were separated and stacked on their own pallet to be microbially cleared and sent to the designated location. The sleeves of hamburger patties remained in their box and boxes remained unopened until the end of the total storage period concluded, and sample collection occurred when they arrived back to GSF.

Eight cases were allotted into nine treatment groups following a completely randomized design with a 3 × 3 factorial treatment structure containing three LTM storage times and three secondary retail storage times, with a baseline treatment collected on the day of packaging. Cases were stored under LTM conditions for either 16, 20, or 30 days at the iQ Foods LTM facility (Atlanta, GA). Storage conditions of LTM are proprietary and generally protected by the parent company, but, broadly, product is stored at temperatures of -3.3 °C or higher and humidity is monitored but not managed. The system is also characterized by specific air flow rates, air flow patterns, and pallet orientations. After removal from LTM, the cases were then transported 230 km in a refrigerated truck kept at 4 °C and monitored by temperature loggers, to the Lambert-Powell Meat Laboratory at Auburn University (Auburn, AL) for secondary retail storage at 4 °C for either 7, 10, or 14 days (MLT4220DA, Jacksonville, FL).

Sealability, Tensile Strength, and Gas Evaluation. Each sleeve (n = 4) from the case was tested in the FlexPak Leak Detector (Ontario, Canada). The chamber full of water and added gas

pressure (10 psi) inflated the MAP which caused indication of any leaking packages due to observable bubbles leaving the packaging. Sleeves were within the chamber at least 15 seconds. The sealability measurement was conducted before the gas evaluation. If the sleeves passed, they received an A (acceptable) and if they failed, they would receive a U (unsatisfactory). Sleeves belonging to chambers 1 and 4 of the Multivac machine were tested for tensile strength (N) using the Starrett® BLC-100 (MiSumi, Schaumburg, IL.).

Gas samples were collected from each sleeve using the Dansensor Checkmate 3 machine (Mocon, Brooklyn Park, MN) to generate four replicates of CO_2 and O_2 concentration in the packaging. A 21-gauge x 3.81 cm INE-JECT® needle (Medsitis, Hattiesburg, MS) was used to pierce the packaging leaving no room for oxygen to fluctuate and collected the atmosphere data. The machine detected gas concentration percentage values which were displayed on the screen.

Microbial Analysis. Ten sleeves from each treatment group were selected for microbial analysis by collecting one sleeve from each case and randomly selecting an additional two sleeves from the treatment group. Five of the fifteen patties were randomly selected from each sleeve for sample collection (~385 g). Samples were stored in a Whirl Pak bags (Pleasant Prairie, WI) and were refrigerated at 4.4 °C until picked up at 23:30. Microbiological tests were conducted at Food Safety Net Services (Atlanta, GA) to evaluate Aerobic Plate Counts (APC), Lactic Acid Plate counts (LAB), coliform plate counts, and *Escherichia coli* plate counts using nonselective, selective, and selective/different petrifilms (3M, St. Paul, MN) respectively.

Sensory Assessment. One randomly selected sleeve from each case was opened and used for sensory analysis by a trained sensory panel consisting of four GSF experts. The panel reported the consensus value for each measured attribute. This consensus, based on the McDonald's standard, uses the mode of the sensory score numbers reported by the panelist and

round it to the nearest whole number for each attribute. All panelists were within one quality index value between the highest and the lowest score per attribute. If the range was greater than one, panelist would recalibrate before continuing evaluation.

Samples were allowed to bloom in the quality lab with a room temperature range of 18.3 °C to 21 °C once the sleeve was opened before cooking. Samples were cooked on an industrialized clamshell grill (Taylor United Technologies Series C850, model C85223GW00, Rockton, IL) following standard industry methods for 79 seconds, resulting in endpoint temperatures ranging from 81.5 °C to 87.4 °C (mean = 79.7 °C). Once patties were cooked, panelists evaluated the patties using the McDonald's Fresh Beef Sensory Evaluation method. Each sample was evaluated for 11 attributes that were assigned to three categories: (1) Appearance: sear evenness, sear color, appearance defects (2) Texture/mouth feel: initial bite, crumbliness, juiciness, chewiness, texture defects (3) Aroma/flavor: seared flavor, beef flavor, and aroma/flavor defects. A sensory score was given for each attribute based on a numeric scale of 1to 9 with 5 being the target value (Appendix A). Then, a quality index value was assigned for the quality category. The category was represented by the attribute score furthest from the target of 5, which is then represented as a percentage, with 100% being the target. Each number in the sensory score away from 5 in either direction represented a cumulative deduction from the target of 15%, 25%, 35%, and 25%. For example, if the worst sensory score in a category was 7 it received a 40% deduction (15% + 25%) and that category received an overall score of 60%. Finally, an overall product quality score was calculated by taking the mean of all three category scores. Samples were considered acceptable if the quality index for all categories were above 60%, unacceptable if the quality index for any category falls below 60%.

Statistical Analysis. Data were analyzed using SAS v. 9.4 (SAS Institute, Cary, NC). All response variables were analyzed using the generalized linear mixed models procedure (PROC GLIMMIX) is SAS. The fixed effect was treatment, and the random effect was sleeves. Denominator degrees of freedom were adjusted using the Kenward-Roger approximation method (Kenward and Roger, 1997). The α-level for mean differences was set at 0.05. Means separations were performed based on *F*-protected *t*-tests using the Tukey-Kramer's HSD (Kramer, 1956). The sensory panel scores were reported as consensus values; therefore, means were reported without additional statistical analysis.

Results & Discussion

This study was designed to assess the impacts of LTM storage followed by secondary retail storage on the quality and shelf-life of hamburger patties in conditions that simulated a quick food service supply chain. To accomplish this, ground beef patties were formed, packaged, and transported to a LTM storage facility, then to a secondary storage facility before analysis. The ambient temperatures during transportation to secondary storage was measured with temperature loggers (Emerson, St. Louis, Missouri). After ambient temperatures dropped following initial loading onto the refrigerated truck, the critical limit for the patties of 4.4 °C was not exceeded during transportation to secondary retail storage. During secondary retail storage, the product was maintained at 4 °C.

Packaging integrity analysis

The packaging and gas mixture integrity can explain underlying features that can contribute to the overall quality of comminuted meat. In the treatment group of 16 days LTM

and 14 days of dark secondary retail storage, there was a failure in the testing of the O_2% concentration; therefore, the treatment was removed which would otherwise affect statistical analysis. As expected, no differences were identified in the concentrations of CO_2 ($P = 0.25$) and O_2 ($P = 0.05$) under the LTM storage period (Table 1). Another study found that when keeping beef in a uniform storage environment (dark and 0 °C or 4.4 °C), the headspace gas composition in the packaging had a small range in fluctuation and remained consistent in concentration values over a 10-d period (Daly and Acton, 2004). Researchers have also observed that the gas composition as well as the headspace ratio of the packaging can show consistent results of constant gas concentration in certain storage environments (Coventry et al., 1998; Bingol and Ergun, 2011). In contrast, in the current study, there was a difference ($P < 0.05$) in the CO_2 concentration levels between secondary retail storage periods following LTM storage. Similarly, a study focusing on headspace in MAP packages observed changes in the gas composition when met with different storage temperatures (Limbo et al., 2010). This suggests that a greater incorporation of CO_2 in the gas mixture allows LAB to thrive and therefore, further alter the gas composition in the packaging. The different temperature storage allowed microbes to grow which similarly occurred in this current study while cases were in secondary retail storage (Coventry et al., 1998). Following, over day 7, 10, and 14 of secondary retail storage, there was an increase in CO_2 concentration ($P < 0.001$). This is potentially due to the metabolism of O_2 by spoilage microbial activity in this period which was also found in a MAP study observing the same behavior with the gas concentration (Conte-Junior et al., 2020).

There was a difference in tensile strength of the MAP on day 16 of LTM storage ($P < 0.001$; Table 1). In a study that looked at antimicrobial properties that were combined with different packaging materials, it was observed that the tensile strength (yield strength), among

other mechanical properties, influenced the shelf-life of chilled meat by keeping the integrity of the film together; packaging with a higher yield strength extended the shelf-life up to 20 days (Dong et al., 2017). However, there is a balance to be acknowledged for practical purposes in the industry. Indeed, the packaging should be strong enough to stay sealed and not produce a leak for meat to get exposed to more oxygen, but recipients of the meat in the food service industry should be able to open the packaging with some ease.

Table 1. Main effects of storage type and length on gas levels and packaging integrity after the end of dark secondary retail storage. At the end of the combined Low Temperature Management (LTM) and retail storage periods all four sleeves in a case were sampled for gas evaluation and package strength yield testing. All values within and across cases were used for statistical analysis.

	LTM Storage (days)					Dark Secondary Retail Storage (days)				
	16	20	30	SE	*P*-value	7	10	14	SE	*P*-value
CO_2 conc. (%)	18.14	17.99	18.43	0.187	0.18	17.40^{z}	18.09^{y}	19.07^{x}	0.187	<0.0001
O_2 conc. (%)	-0.06	0.08	0.45	0.155	0.05	0.28	0.25	-0.06	0.155	0.29
Tensile Strength (N)	1.91^{b}	2.62^{a}	2.78^{a}	0.053	<0.0001	2.36	2.46	2.49	0.053	0.13

$^{abc, xyz}$ means with different superscripts in the same main effects group (LTM storage, retail storage) are significantly different ($P < 0.05$).

Microbiological evaluation

Aerobic Plate Counts (APC) were used to identify the level of microbial spoilage, with a threshold of 7 log CFU/g used to represent the spoiled state (ICMSF, 1986). The main effects for the microbiological evaluations are presented in Table 2. There was no difference in APC values between day 16 and day 20 of LTM storage, but counts were lower ($P = 0.01$) after day 30 compared to day 16. After entering secondary dark retail storage, the APC increased from day 7 to day 10, then decreased on day 14. These results indicate that most differences in the aerobic spoilage organisms were driven more by days under secondary retail storage than under LTM. The unexpected patterns may be driven by the MAP gasses causing a shift in microbial

competition (Ercolini et al., 2006). These depressed growth levels may be due to the production of antimicrobial compounds by the anaerobic organisms in the environment as well However, there was no point where the products contained greater than 7 log CFU/g which indicates that the hamburger patties were under the threshold of spoilage from a microbial standpoint (ICMSF, 1986). The APC results agree with another study that focused on storage length (7, 14, 21, or 28 d) and storage conditions (2.3 or -1.7°C; Martin et al., 2013). As time increased, regardless of temperature, log CFU/g increased as well for APC, but not over the spoilage threshold. It is expected for slow aerobic bacteria growth due to the environment of MAP and the environment of refrigeration, but compared to the current study, the LTM storage was able to further extend this shelf-life period when observing the APC values (Hur et al., 2013).

It is feasible that Lactic Acid Bacteria (LAB), which are more competitive in low-oxygen environments, may be reducing the ability of the aerobic organisms to grow during this period (Doulgeraki et al., 2010). The LAB levels were similar regardless of the time under LTM storage ($P = 0.5$) with mean values of 3.17 log CFU/g, 3.59 log CFU/g, and 3.24 log CFU/g for day 16, day 20, and day 30, respectively. The LAB levels increased during dark secondary retail storage between day 7 and day 10 storage (3.67 log CFU/g to 4.62 log CFU/g, $P < 0.0001$), then decreased to 1.71 log CFU/g by 14d dark retail storage ($P < 0.0001$). The pattern of LAB growth observed during the dark secondary storage time period diverged from that usually seen in extended shelf life studies, where a consistent trend of growth is usually observed (Ercolini et al., 2006; Jiménez et al., 1997; Fraqueza et al., 2008; Insausti et al., 2001). The increase from 7d to 10d storage agreed with results from previous work, especially studies describing LAB growth in MAP environments. Doulgeraki et al found that, over a period of storage time, the temperature of storage and the method of packaging (MAP -) influenced how LAB grew, and when samples

were collected during the initial, middle, final points in storage, LAB grew from 5.6 log CFU/g to 6.74 log CFU/g then to 7.24 log CFU/g (Doulgeraki et al., 2010). Additionally, over a 14-day storage period, MAP packaging a minced beef product under similar conditions to the current study using a gas mixture with a low percentage of oxygen with carbon dioxide and nitrogen increased the LAB growth from 3.00 log CFU/g to 4.20 log CFU/g (Esmer et al., 2011). Interestingly, the mean values in the current study shared similarities in Esmer et al, though the decrease was not identified there. Other studies have shown LAB growth plateau or decrease during extended vacuum or MAP storage (Jones, 2004; Leisner et al., 1995). Even though growth trends are made evident through these studies based the type of packaging in refrigeration, researchers are puzzled with the growth behavior in their studies; therefore, the patterns of LAB growth here in this current study may be due to an atypically shortened growth curve of the specific organisms, may be the result of some sublethal injury during the LTM, or may be due to the modification of the growth environment during growth.

The coliform count decreased from day 16 to day 20 under LTM and day 7 and day 14 in secondary retail storage ($P = 0.005$). This is also in contrast to previous work evaluating different packaging methods which included utilizing MAP gas mixtures with minced meat, and the MAP with a low oxygen environment composition (10% O2/30% CO2/60% N2) demonstrated that coliform counts increased in small increments from 1.58 to 2.27 log CFU g-1 in 4 °C storage (Degirmencioglu et al., 2012). Potentially, this study observed the decrease in coliform counts due to the competition of other organisms that were present. There were no *E. coli* colonies identified in any sample during this study, which was expected as the raw materials originate from a facility that has a zero-tolerance agreement with GSF.

Table 2**.** Main Effects of microbial plate counts (log CFU/g) of ground beef patties in modified atmosphere packaging collected after the end of dark secondary retail storage. Patties were collected at the end of the combined LTM and retail storage periods and a representative 385g was removed from a packaging sleeve for testing.

	LTM Storage (days)					Dark Secondary Retail Storage (days)				
	16	20	30	SE	*P*-value	7	10	14	SE	*P*-value
APC	5.33^{ab}	5.79^{a}	4.94^{b}	0.242	0.01	4.50^{y}	6.30^{x}	5.27^{y}	0.243	<0.0001
LAB	3.17	3.59	3.24	0.277	0.50	3.67^{y}	4.62^{x}	1.71^{z}	0.277	<0.0001
Coliform counts	2.32^{a}	1.33^{b}	1.46^{ab}	0.209	0.04	2.20^{x}	1.68^{xy}	1.22^{y}	0.210	0.005
E. coli counts	0	0	0		-	0	0	0		-

Abbreviations: LTM, Low Temperature Management; APC, Aerobic Plate Counts; LAB, Lactic Acid Bacteria counts

$^{abc,\ xyz}$ means with different superscripts in the same main effects group (LTM storage, retail storage) are significantly different ($p < 0.05$).

Sensory analysis

It was hypothesized that sensory characteristics would be maintained at baseline levels during LTM storage, then move away from acceptable during the dark retail storage. The main objective was to determine whether the sensory measurements would remain within the acceptable range, as defined by the primary customer, McDonalds, during extended storage. In this study the consensus score for patties approached but never passed the threshold for acceptability, but this was not able to be statistically confirmed. A pattern in quality deterioration was observed, but not confirmed. Sear evenness, texture defects, and aroma/flavor defects, based on the change in the consensus quality index values, suggest that those attributes were most detected from panelists (Table 3). When observing sear evenness between day 16 and day 20, there was an indication of change from the baseline attribute value. This is where the greatest value in change was seen within this characteristic from the target values. The texture defects attribute suggested that, between days 20 and 30 of LTM, the scores continued to rise above the

target value of the attribute. Notably, the biggest sensory impact was noticed from the panelist consensus in the identification of aroma/flavor defects, which rose the longer products were under LTM storage. Previous literature has demonstrated this observed pattern with beef in MAP packaging over time. Researchers noted that when beef is in an oxygen dominant environment, there was a significantly higher incidence of off-odor than other packaging methods such as vacuum packaging (Seideman et al., 1979; Jääskeläinen et al., 2016). These findings conclude that the longer beef stays in storage, the more defects that become present. It is also important to note from previous literature that odor ratings of beef in higher concentrations of CO_2 corresponded with beef in vacuum packaging (Seideman et al., 1979; Hou et al., 2023). This is consistent with the current study due to the original gas mixture composition which was dominant in CO_2 which can explain the alterations in the sensory attributes. Overall, as time elapses, it is expected to observe some aroma/flavor defects due to the nature of meat and how microbe proliferation and muscle tissue consumption alters the environment. Other characteristics including sear color, appearance defects, initial bit, crumbliness, juiciness, chewiness, seared flavor, beef flavor suggest that minimal sensory deterioration was noticed from the consensus of the panelists.

In the secondary retail storage days, the panelist consensus of the quality index values in the juiciness, seared flavor, and aroma/flavor defects were highlighted as altered attributes. When observing the juiciness attribute, the panelists suggested patties became more dry over time, and this was seen between day 7 and day 14 (-0.36 to -0.79) of secondary retail storage. Following, seared flavor was indicated by the consensus of panelist that over time, there was less of the seared taste, especially between day 7 and day 14. It was also suggested that within the aroma/flavor defects of day 7 in secondary retail storage, the panelist noticed sensory

deterioration. Prior research noted that when observing consumers, it was found that consumers likability of cooked beef was higher the lower the oxygen concentration was over a 12-day period (Zakrys et al., 2009). Other previous literature reported that ground beef stored in low oxygen environments (dominant in CO_2) does in fact delay strong and unpleasant off odor, but it does not inevitably prevent odor from occurring within the first 15 days of the storage (Luño et al., 1998; Hai et al., 2021). Following, previous literature highlighted that, under similar conditions to this current study, there were slight changes in the off odor, tenderness, and overall acceptability of beef, but it did not show a difference in the juiciness and flavor (Hur et al., 2013). This illuminates possibilities as to why there was a shift in perception of the panelist's consensus in juiciness, aroma and flavor defects, and other minor differences in sensory characteristics. In general, the increased LTM storage time suggested that the texture and aroma were noticeable by panelists, while the secondary retail storage additionally were noticeable with the flavor and juiciness attributes. These indications from the panelist's consensus were all expected with increased storage time, and products did not become unacceptable at any point during the experimental period. Meaning, LTM storage could be used, and customers could potentially taste a little amount of deterioration in the hamburger patties if they were to be held in storage to that extended time frame.

Table 3. Main effects of storage type and length on sensory outcomes after the end of dark secondary retail storage. Ground beef patties were sampled at the end of the combined Low Temperature Management (LTM) and retail storage periods. One of four sleeves per case was randomly selected for sensory analysis. Patties were cooked for 79 seconds before analysis following industry standards. Values represent a consensus based on most prevalent value from four panelist scores* within a storage group. Delta values were calculated by subtracting a baseline value taken on the day of production from sensory scores at the end of storage.

	LTM Storage (days)			Dark Secondary Retail Storage (days)		
	16	20	30	7	10	14
Δ Sear evenness	0.15	0.38	0.28	0.16	0.33	0.31
Δ Sear color	-0.48	-0.17	-0.53	-0.36	-0.33	-0.48
Δ Appearance Defects	-0.05	-0.08	0.04	-0.04	-0.04	-0.01
Δ Initial Bite	-0.22	-0.29	-0.08	-0.25	-0.21	-0.14
Δ Crumbliness	-0.20	-0.13	-0.21	-0.21	-0.13	-0.20
Δ Juiciness	-0.54	-0.63	-0.60	-0.36	-0.63	-0.79
Δ Chewiness	0.60	0.33	0.53	0.44	0.33	0.68
Δ Texture Defects	0.68	0.67	1.13	0.75	0.92	0.80
Δ Seared Flavor	-0.72	-0.75	-0.86	-0.57	-0.79	-0.97
Δ Beef Flavor	-0.04	-0.33	-0.13	-0.09	-0.17	-0.25
Δ Aroma/Flavor Defects	0.316	0.708	1.373	0.368	0.833	1.196

*Scores were assigned based on the McDonald's sensory score system. A 9-point scale determines the distance from target of the tested sample, with a 5 representing the target. Moving from 5 toward 1 represents the trait becoming less pronounced and moving from 5 toward 9 represents the trait becoming more pronounced. The sample fails with a score of 1, 2, 8, or 9.

Conclusion

Overall, LTM storage does increase shelf-life of hamburger patties to a certain extent while creating a specific storage time range for secondary retail storage. Once the meat reached the 20-day storage time of LTM, we observed deterioration in product quality by the consensus of sensory panelists, though all indicators, including sensory and microbial levels were still acceptable by all product endpoints. Generally, this indicates that all products were acceptable for human consumption, though they may not achieve the industry goals of an entirely consistent final product. Quality deterioration was suggested more by time under secondary retail storage following LTM storage, indicating that LTM may serve to delay the final spoilage outcomes until the secondary retail storage period. Future studies should include sampling timepoints after

LTM but before secondary retail storage to fully indicate spoilage traits to the specific storage type. In summary, LTM storage could extend the shelf-life of ground beef patties up to a month, demonstrating that this is an effective method to optimize the quick service beef patty supply chain.

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Conclusion

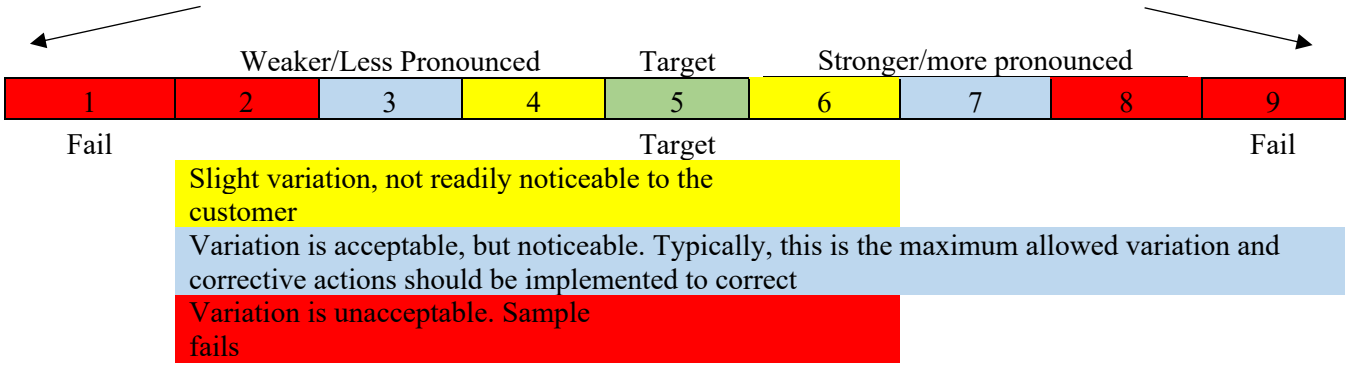
Ensuring optimal meat quality throughout the beef supply chain is imperative for meeting consumer expectations and safeguarding public health. From the farm to the consumer, various factors such as animal welfare, handling practices, transportation methods, and processing methods can impact meat quality. Producers can benefit from the transportation stress study by the illuminating evidence that shows the influence of transportation stress on cattle. It was found that there is an effect on cattle when it comes to physiological and biological measurements. Continuously, it was also found that microbial communities within the fecal samples were influenced with different transport periods. The longer the cattle were on the trailer, the less diverse their fecal microbial communities were structured. This shows presence of dysbiosis which can lead to adverse effects to the animal.

Processing facilities of raw materials can derive advantages from the shelf-life study focusing on low temperature management technology. Results show that the low temperature management was able to extend the usage of the ground beef patties that usually succumb to deterioration up to a month's time; however, sensory measurements and microbial evaluation remained in acceptable ranges. The low temperature management technology is an excellent example of how meat shelf-life can be extended to end the cycle of food waste is an effective method to optimize the quick service beef patty supply chain.

The beef supply chain is multifaceted and requires detailed attention to each step that the animal and meat product goes through. Continuous improvement of these stages in the industry will not only strengthen resources available to consumers, but it will also alleviate waste and contribute to the sustainable industry that is agriculture.

Appendix

Appendix A: Fresh Beef Sensory Evaluation



The 9 point "Degree of Difference" scale is used to determine how far away from Target the test sample is and if that difference is great enough to reject the sample

Typically, the center of the scale "5" is Target (what the sample should achieve in the aspects being considered. For a beef patty, the attributes are usually Appearance (Shape and Color), Texture (how the patty performs in the mouth) and Aroma

The further away from Target a sample is, the less acceptable it becomes. Based on tolerances and critical characteristics of the sample; "Failure" can occur as early as a 3 or 7 but no further away than 1 or 9.

The scale can move in either direction for most characteristics: A beef patty could be tough to chew (moving to the right) or too soft and mushy (moving to the left).

The general categories (Appearance, Texture, Aroma) may have multiple defects and therefore, the scale will move in only 1 direction (to the right). Either patty is Target or it is not, the more defects associated with a category, the further away from target the sample is scored.

A single defect in the general category may be significantly "less than target" to result in a failure (wrong shape, strong off odor, bad taste, wrong color, etc.)

Usually, when using the 9-point scale, multiple samples are evaluated and against the target. This prevents a single sample from having too much influence on the score. If the evaluation involves 6 patties and 1 has a bad shape or off color and the other 5 do not, the overall score of the 6 patties may be a 6 while the single patty may be a 7 or 8. However, if all patties have the same bad shape or color, then the score may be an 8 or a 9, depending on the significance of the variation.