Understanding Beef Quality Development and Different Feeding Regimes

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ABSTRACT

Consumption of beef is expected to increase as the world approaches nearly 9 billion inhabitants by the year 2050, adding unprecedented challenges to the future beef industry. Even so, maintaining quality will still be of utmost importance for producers for two reasons. First, the majority of US cattle are sold on the "grid", which offers both premiums and financial penalties based on quality grades. Second, consumers demand quality. Herein, we explored alternative, cost-saving feeding strategies that impact muscle biochemistry and ultimate meat quality in an effort to determine the most feasible management responses during times of sporadic markets.

Our results show that reducing feed inputs from intensive feeding (grain-finished) regimes to maintenance diets of forage or grain up to 60 d had minimal effect on metabolic properties of muscle, thus preserving both quality and yield grades. Specifically, muscle metabolism remained largely unchanged, as indicated by lack of significance in oxidative and glycolytic proteins such as succinate dehydrogenase (SDH), citrate synthase (CS), lactate dehydrogenase (LDH), and phosphofructokinase-1 (PFK-1). Additionally, because maintenance rations were fed, we found no difference in non-esterified fatty acid (NEFA) concentration, or O-linked- β-N-acetylglucosamine (O-GlcNAc) protein abundance suggesting a longer or more aggressive feeding approach may be required to evoke such nutrient based muscle and quality differences.

Because quality is important and is a factor for optimal pricing at market, intensive feeding practices are often needed to meet such standards. Still, as the cost of feeding increases, producers struggle to balance quality and profitability. To that end, we evaluated carcass quality of cattle subjected to a reduction of time on feed by 30 d, and found quality, yield and color were similar to that of cattle intensively fed for 120 days. Although, little differences were noted between

indicators of postmortem metabolism, short-fed (SF) cattle showed a trend for greater adenosine monophosphate deaminase 1 (AMPD1), and significant difference in the expression of myosin heavy chain isoform (MyHC) IIX suggesting muscle of SF cattle is transiting away from that of grass-fed (CON) cattle. Even so, SF cattle proved to have similar color and quality to cattle fed for 120 days, or more traditionally fed.

While we failed to detect differences in muscle between days on feed compared to that of CON (grass-fed), we observed differences in quality and yield between long fed and grass-fed cattle. These data suggest variances in quality may be a result of underlying mechanisms yet to be explained. Therefore, we explored the hallmark biomarkers credited for beef quality development. Despite significant differences in quality and yield grades, data resulted in no differences in myoglobin, oxidative or glycolytic proteins, or calpain-1 and calpastatin between varying phases of growth. However, based on our complementary transcriptomics data, we found linear trends in gene expression related to adipogenesis and muscle hypertrophy, implying these differences may simply be a result of growth rather than muscle function.

When taken together, our data suggests severe nutrient restrictions may be required to evoke such a shift in muscle that leads to exacerbated differences in quality. A greater understanding of those mechanisms that drive meat quality development from a conventional grain feeding perspective may prove impactful for the future of our industry.

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General Audience Abstract

As the demand for beef continues to increase, so does the demand for quality. Generally, consumers prefer beef that is bright-cherry red, possess adequate marbling, and offers a tender bite when consumed. These quality attributes are influenced by many factors, however plane of nutrition, or more simply nutrient energy source (grass vs grain) have shown to play a rather impactful role in quality development. Therefore, high-energy intensive feeding systems (feedlot) have been widely adopted by the US beef industry as a means of producing beef. Even so, the cost of gain for cattle is steadily increasing making profitability challenging from a producer standpoint. Therefore, the aim of this dissertation was to investigate low-input feeding strategies and the influence they have on beef quality.

First, we tested nutrient availability's effect on muscle plasticity of mature market ready steers. Our results show that reducing feed inputs from intensive feeding (grain-finished) regimes to maintenance diets of forage or grain up to 60 d had minimal effect on metabolic properties of muscle, thus preserving both quality and yield grades.

Next, we challenged varying degrees and plane of nutrition on finishing steers to better understand the extent to which intensive feeding has on ultimate beef quality. To that end, we evaluated carcass quality of cattle subjected to a reduction of time on feed by 30 d, and found quality, yield and color were similar to that of cattle intensively fed for 120 days. Although, little differences were noted between hallmark indicators of muscle fiber type, Short-fed (90 d) cattle proved to be transiting away from that of grass-fed (CON) cattle, aligning closer to consumer expectations of quality.

Taken together, our data suggest variances in quality may be a result of underlying mechanisms yet to be explained. Therefore, we explored the hallmark biomarkers credited for beef quality development. Despite significant differences in quality and yield grades, data resulted in no differences in myoglobin, oxidative or glycolytic proteins, or calpain-1 and calpastatin between varying phases of growth. However, based on our complementary transcriptomics data, we found linear trends in gene expression related to adipogenesis and muscle hypertrophy, implying these differences may simply be a result of growth rather than muscle function.

In conclusion, our data suggests severe nutrient restrictions may be required to evoke such a shift in muscle that leads to exacerbated differences in quality. A greater understanding of those mechanisms that drive meat quality development from a conventional grain feeding perspective may prove impactful for the future of our industry.

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Table of Contents

List of Figures

Chapter 3

Chapter 4

List of Tables

Chapter 3

Chapter 4

Chapter 5

Chapter 1. Introduction & Literature Review–Muscle energy metabolism, growth, and meat quality in beef cattle*

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Abstract

World meat production must increase substantially to support current projections in population growth over the next 30 years. However, maximizing product quality remains a focus for many in the meat industry, as incremental increases in product quality often signal potential increases in segment profitability. Moreover, increases in meat quality also address concerns raised by an ever-growing affluent society demanding greater eating satisfaction. Production strategies and valued endpoints differ worldwide, though this makes the global marketing of meat challenging. Moreover, this variation in production schemes makes it difficult for the scientific community to precisely understand those mechanisms controlling beef quality. For example, some cattle are produced in low-input, extensive, forage-based systems. In contrast, some producers raise cattle in more intensive operations where feeding programs are strategically designed to maximize growth rates and achieve significant fat deposition. Yet, others produce cattle that perform between these two extremes. Fresh meat quality, somewhat like the variation observed in production strategies, is perceived differently across the globe. Even so, meat quality is largely predicated on those characteristics visible at the retail counter, namely color and perceived texture and firmness. Once purchased, however, the eating experience is a function of flavor and tenderness. In this review, we attempt to identify a few areas where animal growth may impact postmortem energy metabolism, thereby altering meat quality. Understanding how animals grow and how this affects meat quality development is incumbent to all vested in the meat industry.

Introduction

World population is projected to reach 9 billion inhabitants by the year 2050. This dramatic growth in population will require an increase in all agricultural food commodities. Correspondingly, beef production will need to increase by nearly 60% [1] in order to feed the burgeoning global population. This increase in production will likely occur in the Southern hemisphere where production systems have the greatest capacities to increase production, either through increased capacities to add ancillary resources needed to expand the beef industry or simply by adding greater land masses [2]. However, cattle reared in the Southern hemisphere are typically managed differently and this production paradigm may lead to differences in end-product quality. Specifically, the bulk of beef produced in South American countries and Australia are fed high forage-based diets. Even when more intense feeding programs are attempted, these systems are less aggressive compared to cattle fed under more intensive feeding paradigms. Therefore, cattle growth and meat quality should be explored to better understand the potential opportunities and challenges in expanding beef production worldwide.

While there have been a myriad of exhaustive reviews on beef cattle production and meat quality [3,4], the focus of this short review is to identify a couple of areas where knowledge gaps exist. These will better enable the community of meat scientists to offer reliable assistance to our colleagues attempting to maximize beef production systems across the globe. It is important to understand, however, that various production systems throughout the world have evolved largely in response to a myriad of factors including, but not limited to, cultural, societal, and political pressures. To suggest, imply, or otherwise advocate that one system should be adopted as the norm for meat production worldwide would be imprudent and cavalier.

Muscle Growth

Muscle formation begins early in fetal development. Russell and Oteruelo [5] determined that the majority of skeletal muscle fibers begin to develop within the first two months of embryonic development in cattle and this continues well into the seventh month of gestation [6]. Yet, when a calf is born, it is no longer able to create new muscle fibers [7]. Rather, the existing muscle fibers continue to grow through hypertrophic mechanisms [8], largely yet to be understood [9]. Nonetheless, muscle is a heterogeneous collection of muscle fibers that vary in their ability to function and metabolize energy [10]. At the same time, these fibers experience differing abilities to grow in volume [11]. Briefly, muscle fibers are broadly characterized into fast and slow-contracting fibers, which are largely based on the type of myofibrillar and regulatory proteins in each muscle fiber [12]. Fibers are also categorized by the relative differences in the predominant type of metabolism existing within the fiber as compared against other muscle fibers across a given muscle. As mentioned, speed of contraction is not simply a function of the myosin molecular structure [13], but often for the sake of simplicity and gross estimation, fibers are largely classified by the predominate myosin heavy chain [14]. To that end, slow-contracting fibers consist mainly of type I myosin heavy chains (MyHCs), while fast-contracting fibers consist of either type IIA, type IIX, and type IIB MyHCs, and correspondingly, vary in their abundance of glycolytic-based enzymes and substrates (IIA < IIX(D) < IIB) [14]. It is important to understand that while this approach provides great insight into the nature of various factors that affect meat animal production, the mechanisms responsible for these differences are occasionally clouded by the overall lack of crispness in defining muscle fiber type or even muscle type. Further, the muscle studied is somewhat limited in its use for understanding the changes in overall production efficiency of an animal. Regardless, while animals are generally born with a higher proportion of slower-contracting, type I fibers, the composition of fibers within a muscle generally begins to

shift collectively from a more oxidative to a glycolytic nature of metabolism during the process of hypertrophy [14,15]. This shift in the cues responsible for this change in muscle heterogeneity may be an interesting area of future exploration.

While this review is mainly focused on beef cattle, the best success story for improving growth efficiency is the modern-day broiler (chicken). Today, most integrators produce meat chickens in a third of the time required to achieve the same weight in 1957, with only a third the amount of feed. While remarkable, what are the biological mechanisms responsible for achieving such progress? The *pectoralis major* muscle tends to consist predominately of type IIB muscle fibers that are large in diameter and more glycolytic in nature. At the same time, cattle tend to have more oxidative muscle fibers [16]. In fact, cattle muscle lacks any discernable type IIB fibers, yet the gene resides in the cattle genome [15]. Therefore, are fast-contracting, glycolytic fibers required for improvements in growth efficiency? If so, what are the cellular mechanisms for these changes?

Nutrition

We have known for some time that maternal restriction of nutrients during the early stages of pregnancy compromises embryonic and fetal growth. Restriction of nutrient intake through any number of different forms during the early stages of pregnancy reduces the number of muscle fibers, thereby reducing muscle mass and postnatal performance [17,18]. Similarly, a reduced supply of nutrients during critical stages of development may impact the formation of adipocytes, which largely form the basis for intramuscular fat deposition and meat palatability [17]. Therefore, feed restriction of pregnant cows can have a detrimental impact on the postnatal growth rate and meat quality.

During postnatal growth, providing appropriate levels of nutrients throughout all stages of an animal's life is critical to achieve an optimal growth rate and produce the highest quality product possible. However, in some feeding paradigms, maximal growth rate is not achieved and may not even be the goal. For example, in less intensive, low-input pasture-based production systems, feed restriction often occurs, and nutrient intakes are well below the requirements needed for optimal growth [6]. In fact, nutrient intakes are often well below requirements, affecting animal performance, lean tissue deposition, and composition, as well as variation in meat quality traits depending on the stage of life that it occurs in the animal [19]. In pasture-based systems, energy intake above that needed for maintenance is used for tissue deposition (muscle and fat) but is generally limited, leading to slower growth rates and fat deposition when compared with feedlot-finished animals. This slower growth rate is generally associated with lowered meat quality depending on the benchmarks used to assess and define quality. However, this nutrient restriction can also affect performance in different ways, depending on the severity and the stage in which it occurs.

Differences in the nutritional plane also influence muscle fiber type composition. Specifically, pasture-fed animals were shown to have a higher frequency of slow-twitch oxidative fibers and a lower frequency of fast-twitch glycolytic fibers compared to feedlot-finished animals [20]. Furthermore, Gagaoua et al. [21] showed a switch to a more oxidative fiber type (MyHC-IIA) at the expense of fast-twitch glycolytic fibers (MyHC-IIX) in grass-fed animals compared to those fed hay or haylage. In contrast, an increase in metabolizable energy intake led to an increase in live weight gain and induced a higher frequency of fast-twitch glycolytic fibers [22]. In addition, studies have confirmed a positive correlation between dietary energy level and the proportion of glycolytic fibers in cattle [23,24,25]. Moreover, increasing nutrient intake after a period of dietary

restriction shifts muscle metabolism toward a more glycolytic type [26], arguing that a positive relationship exists between growth and fast-contracting fibers. However, when cattle go through a period of energy restriction, a decrease in muscle fiber size is observed on all fiber types, especially on fast-twitch glycolytic fibers [11]. Therefore, understanding the effects of feeding paradigms on muscle fiber type and composition and growth is warranted. Of course, these changes in muscle fiber type composition have some impact on meat quality traits, such as color and tenderness. Oxidative muscles are known to have a decreased rate and extent of postmortem pH decline and lightness [27,28] and inherently have an increase in redness due to a higher myoglobin concentration [29], thus resulting in darker meat when compared to glycolytic muscles.

The relationship between muscle fiber type and tenderness is still quite controversial. An increase in meat tenderness was observed as the frequency of type I fibers increased along with a decrease in the percentage of type IIX in cattle [30]. In contrast, Kovanen et al. [31] reported that slow-contracting muscles contain more collagen, which plays an important role in the binding of muscle fibers and decreases meat tenderness. Renand et al. [32] showed that bovine muscles with larger fibers, especially type IIX fibers, exhibited tougher meat than muscles with smaller muscle fibers, such as those of oxidative fibers. However, a positive correlation between tenderness and fast-glycolytic fiber frequency has been noted in cattle [33], which may be due to a higher calpain/calpastatin ratio in fast-twitch glycolytic muscles, partly explaining the faster rate of aging in glycolytic muscles [34]. Either way, considerable information is lacking in the area of meat tenderness (covered more below) and muscle growth and fiber type composition.

Postmortem Metabolism

Following stun and exsanguination, muscle labors to maintain ATP homeostasis. However, ATP turnover is quite high postmortem and, in an effort, to regulate ATP loss, the phosphagen system immediately activates postmortem [35]. Phosphocreatine (PCr) re-phosphorylates ADP to ATP using the enzyme creatine kinase (ADP + phosphocreatine → ATP + creatine). In addition to maintaining ATP levels, creatine kinase consumes hydrogen ions (H^+), thereby partially buffering pH decline postmortem. However, the phosphagen system is incapable of maintaining ATP homeostasis for an extended time. Once 70% of PCr is consumed, ATP decreases rapidly in the muscle tissue [36]. This decrease in ATP, or more specifically, increase in ADP, triggers glycolysis in an effort to create more ATP and allows the muscle to stay in a relaxed state [37].

During this entire process, ATP is continually hydrolyzed, releasing H^+ ions and inorganic phosphate (P_i). Similarly, H^+ ions accumulate in muscles during a bout of exercise, but these ions are partially consumed by the formation of lactate and its removal by the circulation. Ultimately, these substrates (carbons) are made available to the muscle in the form of glucose through the Cori cycle [38]. In postmortem muscle, however, conversion to lactate remains the sole source of buffering hydrogen ion accumulation in muscle, but with time, these ions ultimately lower muscle pH from 7.0 to 5.7–5.5 within 24 h. Acidification of muscle is absolutely mandatory for the development of the typical color and textural properties of fresh beef. When abbreviated, fresh beef appears dark and has a firm and dry (DFD) texture. Yet, if metabolism is accelerated postmortem, carcass temperatures are elevated and the pH decline is greater [39,40]. This combination of low pH and high temperature results in excessive protein denaturation and a product with impaired water binding ability and color, leading to an inferior product, though its occurrence is rare in the transformation of cattle into beef [41].

Conversion of muscle to meat has traditionally been thought to be an anerobic process due to the inability to deliver oxygen to the mitochondria, yet mitochondria function postmortem [42,43]. Scheffler et al. [44] first proposed the possibility of mitochondria influencing postmortem metabolism using an in vitro system [45]. Specifically, these investigators found that addition of mitochondria to glycolyzing reactions increased the rate of ATP loss and attributed this to the F_1F_0-ATP synthase functioning in reverse and acting as an ATPase. Matarneh et al. [46] attributed the role of mitochondria, in part as the mechanism for pH breaching the normal set points of the ultimate pH of muscle. This flux in pH is because F_1F_0-ATP synthase, or complex V of the mitochondria, disassociates postmortem [47] and allows the F_1 subunit to hydrolyze ATP at levels below those environments normally permissible for most myofibrillar ATPases [48]. While the exact role the mitochondria plays postmortem remains obscure, these data argue that the mitochondria may affect ATP homeostasis postmortem and may affect pH decline and, ultimately, meat quality attributes, perhaps even in those cattle fed in differing finishing systems across the globe.

Grazing animals possess lower concentrations of muscle glycogen at the time of slaughter, leading to an inadequate carcass pH drop after slaughter, impairing meat color, tenderness, and shelf life. Immonen et al. [49] found a lower muscle glycogen content in cattle fed only hay compared to cattle fed a high grain diet. Administration of a short-term, high-energy diet is an effective strategy to reduce glycogen loss prior to slaughter and improve the final pH [50]. Knee et al. [51] also proposed that the supplementation of cattle fed low-energy forage with a grain-based feed for three weeks prior to slaughter reduces the incidence of DFD due to an increase in muscle glycogen. In addition, McGilchrist et al. [52] reported that a higher rate of growth,

achievable by the administration of a high-energy diet, may reduce the incidence of DFD due to an increased glycogen content.

Meat Quality

Consumers use lean color as an indicator of freshness and quality [53]. Though meat color and quality are not well-correlated [54], consumers consider beef color to be one of the most important attributes when purchasing it [55]. Yet, 15% of all retail beef cuts fail to meet the expectations associated with the bright cherry red lean designation [55,56]. This lack of acceptable lean color costs the industry nearly $1 billion dollars annually in the United States alone [55]. While lean color is key in making purchasing decisions, beef tenderness has shown to be the most important quality attribute when consuming beef [57], and similar to undesirable beef color, 25% of commercial beef does not meet consumer expectations in regard to tenderness [58].

Color

Variations in fresh beef quality are impacted by a number of inherent physiological aspects of the animal including, age, sex, breed, growth rate, and nutrition [59]. However, meat color is heavily predicated by the abundance of the pigment protein myoglobin [60]. Myoglobin is a water-soluble protein responsible for transporting and storing oxygen from the blood to the muscle [61]. Due to muscle variation in metabolism and energy demand, the myoglobin concentration differs not only between species, but also between muscles [62]. Endurance muscles and muscles that are more fatigue resistant, such as muscles located near the bone, need oxygen, as they tend to be rich in mitochondria and utilize oxidative metabolism as a source for energy production. Due to the muscles' need for oxygen, myoglobin is in high abundance and causes the muscle to have a deeper red color [63]. Glycolytic muscles are typically muscles used for quick bursts of energy, and because oxygen is not required for their function, myoglobin abundance is lessened [64], giving the muscles a lighter or paler appearance.

In general, beef and other ruminants produce meat that is darker than their differing counterparts—monogastric animals. This difference has been largely attributed to differences in myoglobin content, or its lack thereof [65]. Curiously, beef from cattle predominately fed grass diets produce even darker lean meat than their concentrate-fed counterparts [20,66,67,68]. While many argue that a lack of glycogen metabolism leads to a modified dark cutting beef phenomenon, grass-fed cattle have more oxidative muscle than those cattle finished on a concentrate diet [23]. Moreover, glycolytic flux (glycolysis) appears to stop earlier in redder, more oxidative muscles and thereby results in a higher-than-normal ultimate pH, independent of glycogen availability [64]. As muscle grows and experiences hypertrophy, it becomes more glycolytic or less oxidative, although fiber type composition is highly variable between individuals of the same breed reared under similar nutritional and environmental conditions [12]. Indeed, cattle that are fed high forage diets grow slower than cattle fed high concentrate diets [69,70], suggesting that lean meat from grass-fed cattle differs from that of high-concentrate fed cattle, and raises the argument that the latter possess muscle that is more glycolytic and therefore more resistant to generating dark color lean meat. Alternatively, this also argues that darker beef originates from cattle lacking sufficient energy intake, or kind, to change their muscle to a more glycolytic type.

Tenderness

Meat tenderness is impacted by pH [71,72,73,74,75]. In fact, when coupled with temperature, the rate and extent of pH has shown to create an ideal "window" for meat tenderization in beef. This ideal relationship is optimized when a carcass maintains a pH greater than 6.0 while the carcass temperature remains elevated above 35 °C. Further, as a carcass begins to chill, the pH must drop below 6.0 prior to temperatures falling below 12 °C [76]. Although it is well established that meat tenderization is a result of Ca^{2+} activated calpain proteases and their ability to degrade myofibrillar proteins along the Z disks, this ideal "window" is likely related to μ-calpain activation

under conditions of high pH. Maddock et al. [77] determined that μ-calpain activation is highest at pH 6.5. While pH 6.5 cannot be maintained during normal pH decline, Hwang and Thompson [78] determined that calpain activity is optimized when an intermediate pH decline is achieved with the pH reaching 6.0 at 1.5 h postmortem. Furthermore, Lomiwes [74], compared proteolysis between high and low ultimate pH (pH_u) myofibrillar proteins. *Longissimus dorsi* muscle, isolated from bulls 24 h postmortem and aged, showed differences in proteolysis. The results illustrated that high (pH_u) beef underwent rapid tenderization in the early postmortem period, whereas low (pH_u) beef underwent later degradation of myofibrillar proteins. This difference in the rate of proteolysis is likely attributed to the increased activation of μ-calpain at a high pH, while μ-calpain influences the initial proteolysis of titin and nebulin in the early postmortem period. Much of the tenderization in low (pH_u) beef comes from residual μ-calpain activities during the aging process. However, a rapid decline in pH inactivates μ-calpain as well as other key enzymes due to extreme denaturation, ultimately inhibiting postmortem proteolysis [79].

The calpain system also comprises an additional protein, calpastatin. Calpastatin is a specific inhibitor of μ-calpain and can blunt proteolysis. The calpain to calpastatin ratio alters the rate and limit of meat tenderization [80], making it difficult to produce consistent beef as calpastatin levels vary across breeds [81,82], muscle types [83], and the presence of growth promotants such as beta-agonists [80,84].

While pH and μ-calpain influence beef tenderization, it is difficult to control because so many factors can alter the rate and extent of proteolysis, specifically feeding regimes. Currently, there is much debate in the literature regarding concentrate and forage-finished cattle and the ensuing tenderness of meat. Bruce et al. [85] compared differing extensive feeding strategies (124 d or 175 d) of concentrate and forage diets and found that steers fed high energy diets produced carcasses

with increased tenderness, regardless of the days given feed compared with forage-fed beef. These data are in agreement with many studies [86,87], yet others have shown no difference in meat quality between grain and forage-finished beef, including tenderness [88,89]. At the same time, however, others have reported lower shear force values in meat from grass-fed animals than from those fed with concentrate [90, 91]. It has been hypothesized that the greater vitamin E content in meat of pasture-fed cattle increases the collagen turnover due to greater expression of matrix metalloproteinases, which improve meat tenderness [92]. Validation of this hypothesis remains forthcoming.

It is important to note that the effects of different feeding systems may not influence the tenderness of all muscles in a similar way [93]. While diet may influence beef tenderness at times, it is typically confounded by other factors that have also shown to influence tenderness, such as age, growth rate, carcass weight, and external fat cover. This suggests that diet may not independently influence tenderness. In addition, the combination of degree of physical activity and feeding strategy affect muscles of different metabolic properties to a different extent [93]. Regardless, further investigation into the exact role of how nutrition, age, growth, and exercise influence beef quality is well warranted.

Conclusions

A variety of beef production systems have emerged across the globe for a number of reasons. However, animal performance targets differ based on the production paradigms implemented. Whether those differences in beef quality characteristics observed, regardless of the metrics used, are simply a result of divergent management schemes or are truly indicative of underlying mechanisms resulting from differences in growth rate warrants further exploration. To anticipate and respond to projected increases in the global demand of beef, we must understand,

in detail, those mechanisms responsible for optimizing lean beef production and maximizing its quality, regardless of where it is produced.

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Chapter 2. The intricate role of growth and nutrition as related to beef quality

Abstract

Understanding and improving beef quality has been forefront of meat science research for decades. While incredible advancements have been made in improving efficiency, quality, and consumer acceptance, the beef industry still struggles to produce consistent quality beef across the globe. Divergence in breed, age, environment and management practices create huge variability in beef quality and results in a proportion of product that does not meet consumers expectations for color, marbling, and tenderness. Consequently, the industry loses untold millions of dollars annually on products that are either discarded or heavily discounted. As the global population is expected to exceed over 9 billion inhabitants, resources needed for beef production will continue to shrink. Wasted or discarded sources of protein must cease to occur. Thus, there is a critical need to better understand the mechanisms driving beef quality so that targeted approaches may be employed to reduce inputs yet maintain high yielding beef with quality that consumers prefer. Therefore, the intent of this review is to highlight the interaction between the plane of nutrition and muscle growth as it relates to beef quality.

Introduction

In recent years, the global consumption of beef has steadily risen, reaching a record high in 2020, with over 58.9 billion kg of beef consumed across the world (National Chicken Council, 2022). Still, the global demand for beef is expect to increase further as the population breaches over 9 billion inhabitants by the year 2050 (Wicks et al., 2019). This surge in consumer interest will undoubtedly put pressures on beef production in ways never experienced before. Beef producers will be charged to do more with less (Godfray, et al. 2010). Despite the constraints surrounding the future of beef production, beef quality will remain vital to the globe's food-producing portfolio (Liu, Ellies-Oury, Stoyanchev, & Hocquette, 2022). Quality attributes such as color, marbling and tenderness have guided consumer purchasing decisions for decades, setting a precedent for the industry and its future.

Due to the significant economic value beef quality offers the industry, quality attributes have been heavily investigated. Collectively, decades of research suggest beef that meets or exceeds "traditional" meat consumer expectations is a direct result of cattle that have been subjected to intensive grain finishing systems (Gómez et al., 2022). However, the beef industry is complex, amassed of diverse breeds, environments, management systems, and feeding regimes, making it difficult to produce consistently high-quality beef. Moreover, the range in cattle and management practices across the globe mask the underlying mechanisms controlling quality development, yielding the momentum of the industry. Still there is strong evidence to suggest that growth and nutrition play an intricate role in the development of fresh beef color, marbling and tenderness (Wicks et al., 2019). Therefore, this review aims to provide an overview of the interrelationship of nutrition and growth as it relates to postmortem metabolism and ultimate beef quality.

Growth and Nutrition

Postnatally, muscle experiences hypertrophic growth, allowing for cells to increase in size (cross-sectional area, CSA), ultimately leading to improved yield performance of meat producing animals. However, for hypertrophy to occur, the muscle must undergo the ongoing and energy-demanding process of protein turnover (protein synthesis, protein degradation). During the growth phase, the rate of protein synthesis is high, overriding protein degradation, and increasing protein accretion (Koohmaraie, Kent, Shackelford, Veiseth, & Wheeler, 2002). This phase of lean growth is variable and largely regulated by a synergetic collaboration of muscle fiber type, nutrient availability and exercise (Wicks et al., 2019; Blaauw, Schiaffino & Reggiani, 2013; Oksbjerg and Therkildsen, 2017).

Muscle fibers are classified by contractile speed (slow or fast) and metabolic properties (oxidative or glycolytic). However, nutrition (substrate availability) and exercise (function), strongly dictate fiber type differentiation, shifting from I ↔ IIA ↔ IIX(D) ↔ IIB. This topic has been extensively reviewed by Lefaucheur and Gerrard (2000). Briefly, muscle cells require energy to maintain homeostasis and contractile function, relying on the production of adenosine triphosphate (ATP). The phosphorylation of adenosine diphosphate (ADP) to ATP production can be produced through two main metabolic pathways; oxidative phosphorylation and glycolysis. Oxidative metabolism takes place in the matrix of mitochondria and begins with the catabolism of amino acids, acetate or pyruvate that is then converted to acetyl-CoA, initiating the production of ATP through the citric acid cycle and electron transport chain (ETC). On the other hand, glycolysis consists of glycogenolysis, or the conversion of glycogen to glucose. In this reaction, glucose residues are removed from glycogen as glucose 1-phosphate, which is later converted to glucose 6-phosphate then subjected to a string of reactions called glycolysis, which depending on the starting material results in either two or three ATP molecules of ATP. Because muscle is a

collection of heterogenous fiber types, both modes of energy production are used, however plane of nutrition and substrate availability have shown to greatly influence muscle metabolism and muscle fiber type (Picard & Gagaoua, 2020). This notion is supported by Vestergaard et al. (2000), who reported extensively fed bulls possessed muscle with an oxidative phenotype and greater abundance of type I fibers compared to intensively fed bulls. Additionally, Seideman and Crouse (1986) found the same to be true in steers subjected to extensive feeding regimes as well. Furthermore, Johnston et al. (1975) reported an increase in more glycolytic fibers as dietary energy levels were increased, yet diminished when protein content was limited. This response in muscle plasticity from low energy diets curbs the potential for hypertrophic growth and lean muscle accretion. In fact, Beline et al. (2021) found cattle with increased type I fibers have slower growth rates and produce lighter weight carcasses and muscle with smaller muscle fibers (CSA). Slower-twitch fibers have greater rates of protein synthesis and protein degradation compared to more glycolytic fiber types, making them less efficient in terms of growth (Lefaucheur & Gerrard, 2000). Although IIX and IIB fibers have a lower rate of protein synthesis, Greising, et al. (2012) suggest their rate of protein degradation must be even lower in order to maintain contractile speed, fiber size, or in the case of growth, increased hypertrophy.

Postmortem Metabolism

Just as muscle fiber type is critical for growth, it is equally as important for meat quality development postmortem. The biochemical process in which muscle is converted to meat is tightly regulated by the rate and extent of pH decline postmortem. As previously stated, living tissue (muscle) requires energy (ATP) to maintain normal contractile function. However, at death, muscle does not immediately cease to function, rather it labors to maintain homeostasis, first by the phosphagen system. As a form of anaerobic metabolism, the phosphagen system breaks down

phosphocreatine to creatine, donating a phosphate to ADP, thus rephosphorylating ATP. Because this process functions to maintain an energy equilibrium, it is reversible, consuming H^+ in the process of rephosphorylation and aiding in the buffering of pH early postmortem. Although a rapid process, this system cannot sustain ATP production for long periods, forcing muscle to utilize anaerobic glycolysis.

As the muscle undergoes the metabolic process of anaerobic glycolysis, a cascade of ten reactions ensues, producing 2 ATP, and 2 lactate per 1 molecule of glucose. Additionally, glycolysis produces two H^+, and without a functioning circulatory system, H^+ begins to accumulate, initiating the acidification process. However, the rate and extent of pH decline postmortem varies, and is largely dependent on both glycogen concentration and buffering capacity of the muscle. Muscle requires 53 mmol of glycogen per kg of tissue in order to reach what is considered to be ultimate pH (pH_u) of 5.5 – 5.7 within 24 h (Henckel et al., 2002). However, when glycogen levels are low, glycolysis is abbreviated resulting in an elevated pH_u, consequently altering the meat quality attributes such as color and texture (Henckel et al., 2002). Conversely, the inverse does not appear to be true. In fact, Henckel et al. (2002) found excess glycogen (> 53 mmol) does not correlate with extended pH decline. Moreover, Van Laack et al. (2001) reported that glycogen concentration, though critical in the conversion of muscle to meat, only accounts for 40% of variation in pH_u, suggesting the acidification process is more complex and regulated by other factors associated with postmortem metabolism.

Honikel and Hamm (1974) stated hydrolysis of ATP during anaerobic glycolysis accounts for 90% of H^+, while the remaining 10% results from hydrolysis of ATP present in the muscle at the time of death. Still, the rate and accumulation of H^+ in muscle postmortem is regulated by the muscle's ability to buffer H^+ (buffering capacity). Removal or consumption of H^+ can be achieved

by several means, however inorganic phosphates (P_i) significantly contribute to this process. As ATP is consumed, a P_i is released making it available to bind to H^+, thus protecting the cell from rapid hydrogen accumulation. This process cannot be maintained in dying muscle, as it requires continuous availability of adenonucleotides to maintain an energy equilibrium and buffering capacity. Recall, ATP is depleted postmortem, however, in effort to maintain an energy balance, adenylate kinase (AK) is activated, converting 2 ADP back to ATP, and adenosine monophosphate (AMP). AMP slows the rate of ATP production, signaling AMPD to remove AMP. This is an irreversible reaction, resulting in inosine monophosphate (IMP). IMP is further broken down into inositol by IMP-GMP 5'-nucleotidase (cN-II) in the purine cycle, making it unable to contribute to ATP synthesis (Ipata &; Pesi, 2018). As IMP increases, H^+ begins to exceed the rate to that of which it can be consumed, leading to pH decline postmortem.

White muscle has greater buffering capacity than that of red muscle (Lefaucheur & Gerrard, 2000), yet more glycolytic muscles produce lower pH_u (Apaoblaza et al., 2020). Red muscle is rich in mitochondria, and greatly depends on aerobic metabolism for muscle function, therefore postmortem metabolism was traditionally thought to be an anaerobic process. However, Tang et al. (2005) reported mitochondria function for several hours postmortem and contribute to the conversion of muscle to meat. In fact, Scheffler et al., (2015) reported that adding functioning mitochondria to an *in vitro* model reduced the rate at which ATP is lost. Moreover, Matarneh et al. (2018) showed mitochondria increase the rate of ATP hydrolysis through the F_1 ATPase, aiding in buffering capacity. Still, Apaoblaza et al., (2020) found cattle with more oxidative metabolism resulted in higher pH and had lower levels of IMP, suggesting that oxidative muscle may result in higher pH simply from lacking sufficient levels of nucleotides. However, Matarneh et al. (2018) suggests the difference in pH between oxidative and glycolytic muscle may be explained through

difference in phosphofructokinase (PFK) activity and glycolytic flux. While there is still need for further understanding of mechanisms controlling postmortem metabolism between oxidative and glycolytic muscle, it is clear that the energy state of the muscle at death heavily influences the rate and extent of pH decline and furthermore development of meat quality.

Meat Quality

Color

Myoglobin is a protein found in striated and cardiac muscle that primarily functions to transport oxygen from the blood to the mitochondria. Additionally, myoglobin is the primary pigment in muscle and is responsible for development of lean color. While all muscles need oxygen, muscles that are built for endurance and fatigue resistance are highly oxidative requiring greater amounts of oxygen for ATP production, thus myoglobin is increased and pigment changes (Hocquette, Ortigues-Marty, Pethick, Herpin, & Fernandez 1998) Even so, meat color is influenced not only by pigment concentration, but also by the biochemical state of myoglobin and muscle structure (Renerre, Anton, Gatellier, 1992; Abril, Campo, Önenç, Sañudo, Albertí, Negueruela, 2001).

Postmortem, pH gradually declines from 7.0 to approximately 5.6, however this process can be altered by a number of factors and if abbreviated, results in elevated pH that is further from the isoelectric point of muscle proteins and therefore, greater water is bound in the tissues resulting increased water-holding capacity (Ertbjerg & Puolanne, 2017). When water is tightly bound, the surface of meat appears dry and light absorption results in a dark lean (Wicks et al., 2019). Conversely, if the rate and extent of pH decline is increased, proteins are denatured, altering binding ability of the proteins, freeing water. Consequently, greater light is scattered rather than absorbed and meat appears more pale in color (Wicks et al., 2019). Both elevated and low pH extremes are possible and result in quality defects referred to as either dark, firm and dry (DFD)

when pH is high, or pale soft and exudative (PSE) when pH is nearing the isoelectric point (Boles & Pegg, 2010), respectively.

Although pH greatly influences the color of meat, the initial (bloom) and sustained (stability) color of meat is a function of the biochemical state of myoglobin. Myoglobin exists in three forms in fresh beef: deoxymyoglobin (DMb), oxymyoglobin (OMb) and metmyoglobin (MMb). In anoxic conditions, myoglobin remains in a DMb state resulting in a deep purple color. However once oxygenized beef will transition to OMb, the meat appears bright-cherry red. Finally, myoglobin can be oxidized (Fe^{3+}) to the MMb and appear brown in color (Mancini, Belskie, Suman, & Ramanathan, 2018). The transition from DMb to OMb, and moreover the ability for tissue to maintain color stability over time, is dependent on both the oxygen consumption rate (OCR) and metmyoglobin reducing activity (MRA; Lanari & Cassens, 1991). Because mitochondria are still viable postmortem (England et al., 2018), the affinity for oxygen favors the mitochondria, resulting in increased OCR and delayed bloom (Mancini et al., 2018). Therefore, muscles with increased abundance of mitochondria, such as that from slow growing or older animals, produce meat with a delayed bloom, while glycolytic muscles have the potential for a more rapid bloom (reviewed by: Jacob, 2020). NADH is a key regulator in MRA and color stability. Although processes for increasing NADH are continuously depleted postmortem, both muscle types are capable of stabilizing color through enzymatic and non-enzymatic mechanisms. Metabolites such as lactate, succinate, malate, and pyruvate have all shown to replenish NADH, allowing for increased MRA and color stability. While bloom and sustained color can be influenced by pH decline, temperature and aging; generally, meat with delayed bloom will have an increased color stability, while the inverse is true for meat that blooms more rapidly (reviewed by Ramanathan and Mancini 2018).

Marbling

Marbling is a term used to describe the flecks of adipose deposited intramuscularly between muscle fibers and is highly correlated with improved palatability in beef (Hocquette et al., 2010). However, like many aspects encompassed within the realm of meat quality, marbling is influenced by many factors such as age, sex, breed, and genetics. Still, the degree of marbling is arguably most influenced by diet and nutrient intake, as marbling can only occur when energy exceeds requirements for maintenance, storing it as fat (Hocquette et al., 2010). Intramuscular fat results from the synthesis and degradation of triglycerides (TG). TG are a type of fat derived from the diet and synthesized through fatty acid (FA) *de novo* synthesis often occurring in adipose tissue in ruminants. Both acetate and glucose can be used as substrate for FA synthesis, and though acetate is the primary substrate in ruminants, glucose concentrations can be augmented with carbohydrate rich diets, increasing gluconeogenesis as propionate becomes more readily available in high concentrate diets (Smith & Crouse, 1984). Rhoades et al. (2007) showed cattle subjected to cereal grain finishing produced greater IMF, while pasture-based feeding may contribute more to subcutaneous fat accumulation. The authors suggest glucose is the preferred substrate for IMF compared to acetate, as IMF has increased glucose transport 4, which is responsible for glucose uptake in adipose. For this reason, many cattle are finished on high energy diets, and though the preferred degree of marbling varies across the globe, deepening our understanding of the underlying mechanisms controlling IMF deposition has allowed for more targeted management strategies for producing beef with desired level of quality.

Tenderness

Beef tenderness is highly regarded in terms of consumer acceptance and is a leading factor for consumers' repeat purchasing habits (Miller, Carr, Ramsey, Crockett, & Hoover, 2001). Generally, tenderization relates to both the myofibrillar (muscle) and a connective tissue

component (collagen) of meat. However, the extent of tenderization is widely thought to be the result of calpain-mediated proteolysis. Specifically, the Ca^{2+} activated cysteine protease, calpain-1 is thought to greatly contribute to postmortem proteolysis by degrading muscle proteins thus increasing tenderization. In fact, Geesink et al. (2006) found no degradation of structural proteins of calpain-1 knockout mice, even when aged for 3 days, suggesting calpain-1 drives proteolytic degradation and thus increasing tenderness.

In the presence of Ca^{2+}, calpains are autolyzed, undergoing a conformational change and activating the protease for enzymatic degradation of proteins (Koohmaraie, 1992). However, calpains are regulated by the endogenous inhibitor calpastatin (Goll, Thompson, Taylor, & Christiansen, 1992) creating a calpain:calpastatin ratio. Slow oxidative fibers have a higher concentration of calpains than their fast-twitch counterparts. Even so, oxidative muscle also has an increased abundance of calpastatin, limiting the extent of proteolysis (Koohmaraie, 1996). It is often thought that the calpain:calpastatin ratio controls the extent of postmortem proteolysis, and while accurate, more recent data has indicated that meat tenderness results from a holistic interaction encompassing sarcomere length, collagen, pH decline, and most recently the potential of mitochondria influencing postmortem proteolysis.

Following the natural biochemical reactions required for the conversion of muscle to meat, pH will decline and ATP will be depleted, resulting in an irreversible cross-bridge linking of actomyosin. This contractile state known as *rigor mortis*, results in the shortening of sarcomeres. Indeed, the overlapping and the loss of extensibility increases toughness, and can only be mitigated through elongation of fibers (England et al., 2012) or through the naturally occurring proteolytic calpain system (Koohmaraie, 1996).

England et. al (2012), found the longer sarcomeres of hip-suspended carcasses improved proteolysis, thus increasing tenderness of the *longissimus* muscle. However, the practice of carcass hip-suspension requires increased labor and reduces valuable rail space making it unappealing to most processors. Therefore, proteolysis and aging have been areas largely studied in the realm of meat tenderness. Although calpains are activated by Ca^{2+}, they are closely regulated by pH decline postmortem and have shown to function best at a pH of 6.5 (Maddock, Huff-Lonergan, Rowe, & Lonergan, 2005). However, Ca^{2+} and pH levels cannot be maintained postmortem, thus altering calpains' proteolytic capacity. Fast fibers have increased glycolytic flux and more extensive sarcoplasmic reticulum, capable of sequestering high levels of Ca^{2+} compared to slow fibers and is thought to promote proteolysis as there is increased free Ca^{2+} for calpain activation. In fact, Dang et al. (2020), reported an increase of calpain-1 activation when mitochondrial Ca^{2+} uptake was inhibited. Moreover, the authors reported increased cytosolic Ca^{2+} levels at 24 h, consequently increasing proteolysis and tenderness. Additionally fast-twitch muscles tend to have a more gradual pH decline, while more oxidative muscles have been linked to decreased rate and extent of pH, leading to increased sarcomere shortening and decreased proteolysis. Even so, calpain-1 loses proteolytic effect 24 h postmortem, yet proteolysis continues during the aging process, increasing tenderness. Chen et al. (2018) suggested mitochondria may in part contribute to proteolytic aging through the induction of apoptosis and caspase-mediated proteolysis. While the full scope of proteolysis and the interaction of contributing factors is yet to be fully understood, there is evidence to suggest that tenderness is improved in fast-twitch muscle (Seideman, Crouse, & Cross, 1986) and strengthens the argument for intensive finishing system, producing beef with increased IIX fibers.

Conclusion

Holistically, the development of beef quality is a complex process. Driven by the integration of a robust set of intrinsic and extrinsic co-factors, the development of beef quality can widely vary, making consistency of global beef production challenging. Even so, the state of muscle at the time of death plays a significant role in postmortem metabolism and ultimate meat quality and is subject to plane of nutrition and growth rate. However, with an ever-growing population and projected constraints surrounding conventional production practices, further investigation is warranted into developing low-input management strategies aimed at producing high quality beef.

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Chapter 3. Determining muscle plasticity and meat quality development of low-input extended fed market-ready steers.

Abstract

In March 2020, the World Health Organization declared COVID-19 a pandemic, which ultimately led to many meat processors temporarily shutting down or reducing processing capacity. This backlog in processing capacity forced many feedlots to retain cattle for longer periods of time and assume the risk of major market fluctuations. The aim of this study was to understand how a dietary insult affects meat quality and muscle metabolism in market-ready steers (590 kg). Sixteen market-ready (590 kg) commercial Angus-crossbred steers were subjected to a maintenance diet of either forage or grain for 60 d. *Longissimus lumborum* (LL) muscle samples were collected immediately postmortem and processed for characteristics reflecting the underlying muscle fiber type and energy state of the tissue. Despite cattle being subjected to a 60 d feeding period, there were no detectable differences in carcass characteristics, color of lean, or ultimate pH (pH_u). Moreover, our data show that muscle plasticity is rather resilient, as reflected by lack of significance in oxidative and glycolytic enzymes, myosin heavy chain isoforms (MyHC), myoglobin, and mtDNA contents. These data show that market ready steers are capable of withstanding a low-input feeding strategy up to 60 d without dramatically impacting underlying muscle characteristics and meat quality development.

Introduction

Historically, consumers prioritized intrinsic cues, such as nutrition and safety when making decisions at the meat counter (Schroeder, Tonsor, & Mintert, 2013). Today, consumers make purchasing decisions with added focus on socially conscious benefits, such as origin, animal welfare, and sustainability claims (Aboah & Lees, 2020). Despite the addition of these novel cues, meat quality is still important to consumers seeking a positive eating experience (reviewed by: Liu, Ellies-Oury, Stoyanchev, & Hocquette, 2022). In fact, Platter et al. (2005), found that consumers are willing to pay a premium for tender beef, a quality characteristic ranked highest among eating satisfaction and repeat purchasing (Banović, Grunert, Barreira, & Fontes, 2009). However, tenderness is a post-purchasing attribute that cannot be easily evaluated prior to procuring, forcing consumers to use visual cues when making purchasing decisions (Krystallis & Arvanitoyannis, 2006). Visual indicators such as meat color and degree of marbling are often leading factors for consumers when making purchasing decisions (Aboah, & Lees, 2020). Consumers prefer beef that is bright cherry-red, as it is perceived as wholesome and fresh (Faustman, & Cassens, 1990). Moreover, consumers use degree of marbling as a visual indicator of predicted eating experience (Cheng, Cheng, Sun, & Pu, 2015). Specifically, consumers prefer to purchase beef with increased marbling expecting increased tenderness and flavor (Testa, Grigioni, Panea, & Pavan, 2021). Although correlations between degree of marbling and tenderness have been debated (Wulf, O'Connor, Tatum, & Smith, 1997; Tatum, Smith, & Carpenter, 1982; Henrickson and Moore, 1965; Breidenstein, Cooper, Cassens, Evans, & Bray 1968; Parrish, Olson, Miner, & Rust, 1973; McBee & Wiles 1967; Campion, Crouse, & Dikeman, 1975), flavor is highly correlated to degree of marbling (Wheeler, Cundiff, Koch, 1994) and is increasingly important to meeting consumer satisfaction (Felderhoff, et al., 2020). However, due to the variability of production systems, coupled with both environmental and economic influences

(reviewed by: Sakowski, et al., 2022), generating quality beef that meets these consumer expectations is challenging, and at times, unavailing.

The U.S. Department of Agriculture (USDA) created Beef Grading Standards in 1927. This program was designed to evaluate beef carcasses based on age, lean color, and degree of marbling in effort to segregate beef into more uniform classifications (USDA-AMS, 2017). Additionally, this system was an attempt to brand various levels of quality in effort to better advise consumers on predicted palatably, as well as create a unified pricing system for producers, processors, and consumers. Carcasses that achieve the grade of Prime or Choice are often a result of intensive feeding strategies, and while easily marketable to consumers, it requires greater inputs. Even so, the beef industry is a consumer driven market. Therefore, cattle that are adequately fed and achieve optimal quality, or qualify for branded programs such as "All Natural" are rewarded with premiums (USDA-AMS, 2023), ultimately incentivizing producers to produce "fed" cattle. Conversely, producers are penalized from producing cattle that are older, possess lower quality, or yield dark lean. This system of purchasing beef carcasses is commonly referred to as 'grid' pricing (Schroeder, Hogan, & Anderson, 2009). Despite the financial incentives grid pricing provides, markets fluctuate, creating both 'bull' and 'bear' markets. However, during periods of bear market, producers are often faced with selling assets at inopportune market prices, or continue to increase feeding cattle on expensive diets, waiting to sell during a more profitable market. Unfortunately, due to grid pricing, the latter option results in increased yield grade as well as heavier carcass weight, which can result in added discounts, only further exacerbating feedlot marketing decisions.

Once the World Health Organization declared COVID-19 a pandemic (World Health Organization, 2020), much of the US went into immediate lockdown. The increase need for social distancing forced many meat processors to shut down, or reduce processing capacity to stem plant

outbreaks (Marchant-Forde, & Boyle, 2020). Harvesting beef in the US from April to June 2020 was reduced nearly 25% - 35% compared to the year prior (Lusk, Tonsor, & Schulz, 2021). This backlog in processing forced many feedlots to retain cattle for longer periods of time, increasing their days on feed (DOF; Halfman, 2020). In fact, feedlots with capacity of 1,000 head or more reported an increase of 252% of cattle on feed for greater than 180 days (Schulz, 2020). This increase in retention required producers to alter normal production strategies in effort to mitigate financial loss and slow growth rate. Even so, average carcass weights increased nearly 25% (Martinez, Maples, & Benavidez, 2021), with carcasses approaching 408 kg, qualifying for a discount in grid pricing (USDA-AMS, 2023). Granted, the degree of marbling in retained cattle also significantly increased (USDA-AMS, 2021). Still, with carcasses dressing over 408 kg, premiums for quality grade are often not sufficient to overcome discounts on hot carcass weight and yield grade due to excessive trim and oversized retail cuts (Reiman, 2020). This 'holding' of finished cattle, though necessary at the time, ultimately cost the feedlot sector over $3 billion within the first month of the declared pandemic (Peele, et. al., 2020).

While the pandemic was unforeseeable, and nearly impossible to circumvent, it exposed the US to serious vulnerabilities in our food supply. Furthermore, it forced producers across the industry to shoulder the financial burden of retained ownership and assume the risk of major market fluctuations. Although global pandemics are the exception rather than the rule, fluctuations in the markets still occur that cause operations to sell cattle at inopportune times. Therefore, there is a need to understand better the resilience in market-ready steers (590 kg) so operations have more flexibility in marketing their assets.

Materials and Methods

Animals and Sample Collection

Twenty-two commercial Angus cross-bred steers of similar age were conventionally raised and finished to a weight of ~590 kg. Six steers (~590 kg) were randomly selected and harvested. This subset of cattle served as a baseline for carcass and quality assessments, as well as color and ultimate pH (pH_u) of typical market-ready cattle. Remaining steers (n = 16) were randomly assigned to a maintenance diet of either forage (grass, haylage; n = 8) or a conventional grain-based finishing diet (n = 8) for 60 d. Following the 60 d feeding period, steers were weighed and transported to the Virginia Tech Meat Center for harvesting using standard protocols. Animals were allowed lairage and access to water before being harvested. Steers were rendered unconscious with a captive bolt and exsanguinated. Blood was collected during the exsanguination process, and muscle samples were collected from the *longissimus lumborum* (LL) muscle immediately following exsanguination. Samples were snap frozen in liquid nitrogen and stored at -80 C for further analyses. Hot carcass weight (HCW) was recorded and all carcasses entered a conventional chilling cooler at approximately 45 min postmortem. Following a 24 h chilling period, an additional LL muscle sample was taken. Regardless of time point, muscle samples were diced, snap frozen in liquid nitrogen and stored at −80 °C until further analysis.

Carcass Evaluation and Color Analysis

Following a 24 h chilling period (2 ± 1°C) carcasses were ribbed between the 12th and 13th rib for carcass evaluation, as described by American Meat Science Association (AMSA) yield and quality grading standards (AMSA, 2001). Ribeye area (REA), 12th rib fat thickness (FT), estimated

percent kidney, pelvic and heart fat (KPH), and hot carcass weight were measured and used to calculate carcass yield grade. Carcass maturity and marbling scores were used to determine carcass quality grade. Ribbed carcasses were allowed to bloom for 30 min prior to objective color analysis. Triplicate color measurements were taken using a Konica Minolta CR-400 colorimeter (Ramsey, NJ, USA), Illuminant D, 0º observer angle. Averaged color values were expressed as Commission Internationale de l'Éclairage (CIE) L* (lightness), and a* (redness) b* (yellowness).

pH Analysis

Muscle pH was measured as outlined by Bendall, (1973) with modifications. Finely ground tissue was homogenized in a buffer (1:8 w/v) containing 5 mM Na-iodoacetic acid and 150 mM KOH using a Qiagen Tissuelyser for 2 min at 25 1/s frequency. Once homogenized, samples were heated at 25°C for 5 min, centrifuged for 5 min at 13,000 x g, and placed back on the heating block at 25°C for 1 min. The pH was measured using a calibrated Orion Ross Ultra pH electrode (Thermo Scientific, Pittsburgh, PA).

Blood Collection and NEFA Analysis

Blood was collected using BD Vacutainer Plastic Serum tubes (Fisher Scientific Cat Number: 23-021-018). Blood samples were allowed to clot at ambient temperature for 1 h and then centrifuged at 4 °C for 30 min at 1,100 × g. Serum was separated into 1-mL aliquots and stored at −80 °C for subsequent analyses of non-esterified fatty acid (NEFA). NEFA concentration was determined using a commercial quantitative colorimetric assay kit (Wako Diagnostics, Richmond VA), measured using a 96-well microplate and read at 550 nm. Final NEFA concentration was equated using the following equation:

Sample Concentration = Standard Concentration X (Sample Absorbance) (Standard Absorbance), and reported as mmol/L.

Protein Extraction and Determination

One hundred mg of frozen powdered tissue (LL, heart, masseter (MS), cutaneous trunci (CT), liver) was homogenized in 8 M urea, 2 M thiourea, 3% SDS (w/v), 75 mM DTT, 0.05 M Tris–HCl (pH 6.8), and heated at 95°C (Warren, Krzesinski, & Greaser, 2003). Homogenized samples were diluted 1:20 and used for total protein quantification using Reducing Agent and Detergent Compatible Protein Assay (Bio-Rad Laboratories, Hercules, CA, USA), according to manufacturer's specifications. Samples were diluted in extraction buffer (Warren et al., 2003) containing 0.05% bromophenol blue to a final concentration of 3 mg/mL. All samples were stored at −80°C until further analysis.

Gel Electrophoresis and Immunoblotting

Muscle proteins and controls were separated by SDS-PAGE (10%, 15% or 18%), transferred to nitrocellulose membranes, and blocked at room temperature with either Prometheus™ OneBlock™ Blocking buffer (Genesee Scientific Corporation, El Cajon, CA) or 5% non-fat dry milk in Tris-buffered saline solution with 0.1% tween-20 (1× TBS-T) added. Following blocking, membranes were incubated overnight at room temperature with primary antibodies specific for phosphofructokinase-1 (PFK, Santa Cruz Biotechnology, Inc, SC-166722 at 1:1000 dilution), calpain-1 (CAPN1; Thermo-Fisher 9A4H8D3 at 1:1000 dilution), calpastatin (CAST; Thermo-Fisher 1F7ED10 at 1:1000 dilution), citrate synthase (CS; Santa Cruz Biotechnology, Inc, SC-390693 at 1:1000 dilution), succinate dehydrogenase-A (SDH-A; Abcam ab14715 at 1:1000 dilution), lactate dehydrogenase-A (LDH; Novus NBPI48336 at 1:30000

dilution), O-GlcNAc (Abcam ab2739 at 1:1000 dilution), and myoglobin (Santa Cruz Biotechnology, Inc, SC-25607 at 1:1000 dilution). IRDye fluorescent secondary antibodies (LI-COR Biosciences, Lincoln, NE) were used for visualization of bands, and protein abundances were normalized to total protein (Revert 700 Protein Stain, Li-Cor Inc., Lincoln, NE). All blots were imaged using a LI-COR Biosciences Odyssey Infrared scanner (Li-Cor, Inc., Lincoln, NE, USA) and band intensity was measured using Image Studio lite (Li-Cor, Inc., Lincoln, NE, USA) with protein abundance reported as arbitrary units (AU).

Gene Expression

Total RNA was extracted using the Direct-zol RNA Mini Prep Kit (Zymo Research, Irvine, CA). Twenty ng/μL of total RNA was reversed transcribed using the High-Capacity cDNA Reverse Transcriptase Kit (Applied Biosystems, Waltham, MA). Two μL of cDNA was used for amplifying gene specific primers (Table 3-1) and SYBR chemistry in a 7500 Fast Real-Time PCR System (Applied Biosystems, Waltham, MA) for the quantification of myoglobin and myosin heavy chain isoforms. Relative gene expression was calculated by the $2^{-\Delta\Delta Ct}$ method.

Mitochondrial DNA Content

Total DNA was purified using a DNAeasy mini spin columns according to manufacturer's recommendation (Quigen, Germantown, MD) and quantified by optical density at 260 nm (Nanodrop 2000 spectrophotometer, ThermoScientific, USA). Mitochondria (mtDNA) and genomic DNA (gDNA) quantification was performed as previously described (López-Andreo, Lugo, Garrido-Pertierra, Prieto, & Puyet, 2005). Briefly, 25 ng of total DNA was amplified (TaqMan™ Fast Advanced Master Mix Applied Biosystems ™) with organelle-specific DNA primers (500 nm each) and 250 nM MGB probe for 40 cycles of 20 s at 95 °C and 30 s at 60 °C for

40 cycles. Total mtDNA quantity (ng/µl) was inferred from the standard curve and normalized to the gDNA total quantity and presented as a ratio of the two as the fold difference.

Statistical Analysis

Data were analyzed using the Proc Mixed procedure using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). Carcass was the experimental unit and the statistical model included the fixed effects of treatment, with harvest day as a random variable. Means were compared using Tukey-Kramer Multiple Comparison Test if a significant effect was detected. Data on graphs are least square means ± standard error means (SEM), and differences were considered significant at $P < 0.05$, or unless otherwise stated.

Results

Carcass Validation and Carcass Evaluation

To investigate the impact of low-input extended feeding on muscle plasticity and ultimate meat quality, independent of muscle growth and/or age, a maintenance diet of either forage or grain was fed to market ready (590 kg) steers for 60 d. Bodyweight remained unchanged between treatments throughout the duration of the feeding trial (Fig.3-1a, $P = 0.787$; 3-1b, $P = 0.763$). Additionally, HCW (Fig. 3-1c, $P = 0.507$), DP (Fig. 3-1d, $P = 0.553$), REA (Fig. 3-1e, $P = 0.339$), 12th rib FT (Fig. 3-1f, $P = 0.800$), KPH (Fig. 3-1g; $P = 0.019$) yield grade (Fig. 3-1h, $P = 0.757$), and marbling score (Fig. 3-1i, $P = 0.133$) were evaluated, with no differences noted between treatments.

Color and pH

No differences were noted in lightness (L*; Fig.3-2a, *P = 0.467*), redness (a*, Fig.3-2b, *P = 0.107*) or yellowness values (b*) (Fig. 3-2c, *P = 0.323*). Moreover, there was no differences in pH at 24 h (Fig. 3-3b; *P = 0.220*) between treatments.

NEFA Concentration and Protein Abundance

Despite being subjected to difference in nutrient intake for 60 d, no significant differences in NEFA concentrations were noted (Fig. 3-4; *P = 0.117*). Underlying muscle characteristics were analyzed using western blotting assays. Overall, protein abundance tended to follow an expected outcome but no significant differences were noted in O-GlcNAc (Fig. 3-5, *P =0.501*), SDH-a (Fig. 3-6a; *P = 0.737*), CS (Fig. 3-6b; *P = 0.119*), LDH (Fig. 3-7a; *P = 0.321*), PFK-1 (Fig. 3-7b; *P = 0.374*), myoglobin (Fig. 3-8 *P = 0.354*), calpain-1 (Fig. 3-9a; *P = 0.934*), or CAST (Fig 3-9b; *P = 0.527*).

Gene Expression and mtDNA

To understand the role of nutrient availability on muscle plasticity, gene expression of MyHC isoforms were evaluated. Furthermore, mtDNA abundance, and myoglobin gene expression were compared between treatments in effort to determine whether the diet changed muscle fiber type, as reflected by its oxidative markers. No differences were observed between treatments for MyHC-I (Fig. 3-10a; *P = 0.656*), MyHC-IIA (Fig. 3-10b; *P = 0.161*), MyHC-IIX (Fig. 3-10c; *P = 0.537*), mtDNA abundance (Fig. 3-11a; *P = 0.901*), or myoglobin (Fig. 3-11b, *P = 0.173*).

Discussion

Beef quality is a complex result of a number of different biological and biochemical processes that independently or together can affect the final product. At the same time, however, beef quality remains relatively stable as data reported herein suggest. Management strategies aimed at genetic selection, feeding regimes, and growth rate account for a number of differences in factors known for dictating ultimate quality. Specifically, breed, sex, plane of nutrition, and age can impact finishing endpoint, which is largely influenced by muscle type, energy metabolism, and fat deposition (reviewed by: Wicks et. al., 2019). As muscle grows it shifts from oxidative to glycolytic, or more simply, from red to white (reviewed by: Picard & Gagaoua, 2020) This shift in fiber type alters the mechanisms in which energy is 'handled' in the cell for function. Red, oxidative fibers rely on aerobic metabolism for energy production (ATP), while white fibers generate ATP through anerobic glycolysis. This discrepancy in energy metabolism heavily influences both the rate and extent of pH decline postmortem. Oxidative muscles have shown to have equivalently similar glycolytic potential to that of glycolytic muscles (Kirkpatrick et. al., 2023), still muscle pH_u remains elevated (England et al., 2016), partially explaining the noted color differences between red and white muscles, and even between species. Moreover, proteolysis is highly correlated with both muscle fiber type (Bhat, Morton, Mason, & Bekhit, 2018; Totland, Kryvi, & Slinde, 1988) as well as pH decline (Hwang & Thompson 2001; Bhat et al., 2018; Hopkins & Geesink, 2009; Lonergan, Zhang, & Lonergan, 2010; Melody et al., 2004), lending some further understanding to the disparity tenderness between muscle phenotype (Ramos et al., 2020). Although pH decline does not directly influence intramuscular fat deposition, marbling favors a glycolytic phenotype (Hocquette et al., 2012).

Currently, the industry has determined that cattle fed high-energy cereal grain diets produce the most consumer acceptable product as it allows for increase in type II fibers, efficient growth

rate, and acceptable fat accumulation. However, muscle plasticity is responsive to both nutrient availably and restriction. Early weaning (Scheffler et al., 2014), compensatory gain (Sainz, De la Torre, & Oltjen,1995) and back-feeding cull cows (Matulis, McKeith, Faulkner, Berger, & George 1987, Pritchard and Berg, 1993) are prime examples of how nutrient energy source can alter muscle, postmortem metabolism, and ultimate quality. Even so, much of the current literature surrounding feeding regimes and meat quality center around growing muscle, and are confounded by days on feed, growth rate, or finishing weight (reviewed by: Muir, Deaker, & Bown,1998; Apaoblza et al., 2020; Gómez et al., 2022). Although we do not argue the influence plane of nutrition has on muscle metabolism and meat quality, Yambayamba and Price (1991), found no difference in fiber type between cattle subjected to *ad libitum* feeding compared to cattle subjected to 2 months of restricted feeding. While cattle subjected to 4 months of restricted feeding possessed a greater abundance of oxidative fibers, compared to other treatments. These data argue a 'window of time' may exist where cattle can be subjected to restricted or low energy diets without shifting from glycolytic to oxidative muscle fiber type or losing yield and quality standards. Therefore, we chose to investigate the effects of protracted feeding of either forage or grain-based maintenance diets to market ready steers (590 kg) for 60 d in effort to understand better its effect on meat quality attributes and the cellular mechanism controlling them.

Both nutrient restriction and over-feeding causes differences in body weight, growth rate, energy metabolism, and ultimate quality (Sami, Augustini, & Schwarz, 2004), as does differences in nutrient energy source (Gómez et al., 2022). However, variability in carcass and quality dissipate when weight and growth rate are held constant regardless of plane of nutrition (Gómez et al., 2022). Our data confirms the latter construct, as we found no difference in weight (Fig. 3-

1a, b), or carcass yield and quality (1c-i) between low-input extended feeding regimes and suggests growth may be required for shifts in muscle metabolism, and ultimate quality development.

Moreover, plane of nutrition has proven to influence meat color. However, we were unable to detect significant differences in L* values between treatments (Fig. 3-2a). These data are in conflict with those of Shibata et al. (2009), Vestergaard et al. (2000b), Apaoblaza et al. (2020), Bidner et al. (1981) and Gómez et al. (2022), all of which found forage-based feeding regimes to produce darker lean. Even so, those studies differ from the present study because we allowed for maximal growth to finishing, rather than limited growth to final weight. Moreover, those studies had differing endpoints in bodyweight, fat thickness, marbling, age, or even combinations of these variables. Such discrepancies can alter the intrinsic biology of muscle, subsequently influencing ultimate meat color (Hughes, Clarke, Purslow, & Warner, 2020; Ramanathan, Suman, & Faustman, 2020). Gómez et al. (2022) found darker lean in beef finished on pasture-based feeding regimes finished to similar weights, however, the authors acknowledged color inconsistencies between grass and grain-fed beef may have be exacerbated by growth rate and also be influenced by marbling (Hughes et al., 2020). Moreover, Apaoblaza et al. (2020) noted darker lean in grass-fed beef compared to conventional intensive feeding regimes as well. However, these steers were fed for 127 d, allowing for increased growth, marbling and shifting of muscle metabolism with grain-fed steers possessing a more glycolytic phenotype, suggesting discrepancy in color may be a result of difference in muscle metabolism. However, in a comparably short feeding trial (85 d), similar to the present study, French et al. (2000) found no difference in L* when bodyweight and fat thickness were equal at time of slaughter, Additionally, Daly et al. (1999), reported no difference in lean color of restricted grain-fed steers compared to similar growth rate of grass-fed

steers, suggesting that an interaction of growth and plane of nutrition must occur in order to create difference in lean color of grass and grain-fed beef.

Still, L* is highly correlated to pH with higher pH resulting in darker lean (Qiao, Fletcher, Smith, & Northcutt, 2001). Moreover, L* tends to shoulder that of b* values, which is also highly correlated with pH decline (Meadus and MacInnis, 2000). Abril et al. (2001) found b* values, yellowness to be a useful indicator for discriminating between the two pH groups, correctly segregating samples 86–95% of the time. However, we found no difference in pH (Fig. 3-3) or b* values (Fig. 3-2c) suggesting muscle metabolism was not influenced by differing planes of nutrition at 60 d.

Even so, grass-fed beef can still appear dark in color despite having no difference in pH_u compared to grain fed beef (Moloney, O'Riordan, Monahan, and Richardson, 2022). Yet, even when difference in pH_u is noted and lean of grass-fed beef is darker; pH_u is still well within what is considered optimal and normal pH (Briskey,1964), and suggests other factors aid in color discrepancy between grass and grain-fed beef. Apaoblaza et al. (2020), reported increased myoglobin concentrations and darker lean in beef from grass-fed cattle compared to that of grain-fed cattle. Myoglobin is highly correlated to redness (a*), however the authors also reported that grass-fed beef was less red. Interestingly, we found no difference in myoglobin abundance (Fig. 3-8; Fig 3-11b), or a* values between treatments (Fig. 3-2b). While myoglobin abundance is imperative for increased pigment, the redox state is equally as vital. As oxygen penetrates the muscle it binds to deoxymyoglobin (DMb) converting it to oxymyoglobin (OMb) shifting the pigment from purple to red. To that end, redness values (a*) are positively correlated with myoglobin reductase activity (MRA) and negatively associated with relative content of MMb% (Wang et al., 2021). This well-regarded construct is often a result in difference in mitochondria

abundance (fiber type) or more simply, muscle metabolism (Apaoblaza et al., 2020; Vestergaard et al., 2000a). While we found no difference in a* values in the present study, we also found no difference in expression of various muscle fiber type-specific contractile proteins (MyHC-I, MyHC-IIA, MyHC-IIX; Fig. 3-10 a-c) or mtDNA compared to genomic DNA and may explain vary results compared to current literature.

Muscle plasticity refers to a muscle's ability to alter both structural and functional properties, in response to extrinsic factors such as growth or plane of nutrition. In fact, as muscle grows it experiences hypertrophy and results in muscle shifting from red to white. This shift in fiber type also requires a shift in the way a muscle produces energy, moving from oxidative metabolism to a glycolytic metabolism (reviewed by: Picard & Gagaoua, 2020). Even so, metabolism must have an adequate nutrient source to sustain such energy requirements. For example, oxidative metabolism is largely a function of the mitochondria (TCA cycle), requiring acetate in the form of acetyl-CoA to begin the process. On the other hand, energy can also be produced through anerobic glycolysis, which through a cascade of reactions, converts glycogen to lactate. Cattle receive 70% of their nutrient energy from short chain fatty acids (SCFA) in the form of acetate, propionate, or butyrate (Bergman, 1990). The ratio of SCFA is largely dictated by plane of nutrition and is well documented that pasture or grass-fed cattle have high proportion of acetate, compared to grain-fed cattle which have increase abundance of propionate and butyrate (Zhang, Ye, Liu, & Mao, 2017). This difference in nutrient availability can stimulate a shift in fiber type, with extensively fed cattle producing more Type I fibers, while intensively fed cattle produced more Type IIA, and IIB fibers (Vestergaard, Oskbjerg, & Henckle, 2000b). This increase in oxidative fiber type also results in decreased glycogen and increased citrate synthase activity and darker lean (Vestergaard, Oskbjerg, & Henckle, 2000b). Granted, this study allowed for

increased weight gain, and possible differences in voluntary exercise, which have also been linked to altering muscle metabolism and generating darker lean (Priolo, Micol, Agabriel, Prache, & Dransfield, 2002). Even so, there is strong evidence to support the notion that plane of nutrition influences ultimate lean quality, at least when metabolism is shifted between treatments (Apaoblaza et al., 2020). Therefore, we explored nutrient sensing indictors as well as proteins abundance of oxidative and glycolic enzymes.

Although VFA's were not measured in this study, there is substantial evidence to support that acetate is increased in cattle subjected to forage-based diets (Zhang et al. 2017) and lends to an increase of energy production through the citric acid cycle. However, cattle with oxidative metabolism, fueled by acetate, tend to be nutrient stressed and express higher NEFA concentration, when compared to high-energy grain-fed cattle (Blanco, Joy, Ripoll, Sauerwein, & Casasús, 2011). In fact, increased NEFA concentration is often an indicator of increased beta oxidation, which functions to convert fatty acids into acetyl-CoA, allowing for increased energy production by way of the Krebs cycle. Therefore, we measured serum NEFA concentration in effort to determine if differences in feeding regime resulted in differences in energy availability. While NEFA concentrations for forage fed cattle were numerically increased, we found no significant difference between treatments (Fig. 3-4). This is in agreeance with Blanco et al. (2011), who found significant increased NEFA concentration in young bulls subjected to pasture grazing compared to feedlot finishing. However, this significance was lost over duration of time on feed. Even so, our diets were formulated to meet maintenance energy requirements and may aid in explaining why significance was not observed.

Although we found no difference in serum NEFA concentration, steers were still subjected to two differing rations for 60 d, potentially leading to variance in acetate to propionate ratio

between treatments and influencing substrate availability. Apaoblaza et al. (2020) reported an increase in the nutrient sensor O-GlcNAc in grain-fed cattle. While still not fully understood, it has been reported that O-GlcNAc is highly correlated to substrate availability (Lazarus et al., 2012). To that end, Apaoblaza et al. (2020) also determined that grass and grain-fed cattle differ energetically, further supporting muscle's ability to sense and respond to nutrient availably. Still, cattle from this study were fed for twice as long as the cattle in the present study and suggests that longer feeding periods may be required, as we found no difference in the O-GlcNAc between treatments (Fig 3-5.).

Additionally, we analyzed both oxidative and glycolytic proteins in effort to better understand if we were able to shift metabolism through differing nutrient energy source. Despite 60 d feeding period, we were unable to detect any difference in oxidative proteins such as SDH-A, citrate synthase or the pigment protein myoglobin (Fig. 3-6a, b; 3-8, respectively). Moreover, we also found no difference in glycolytic metabolism surrogate proteins, LDH or PFK-1 (Fig. 3-7a, b) between forage and grain-fed diets. This of course, contradicts others who have reported differences in muscle fiber type and/or metabolism between intensively and extensively feed cattle (Apaoblaza et al., 2020; Vestergaard et al., 2000a). Furthermore, calpain-1 and CAST were evaluated as indicators of proteolysis (Ouali, & Talmant, 1990), but also predicators of tenderness, yet we found no difference between either protein (Fig. 3-9a, b). Protein similarity suggests that even with aging, proteolysis would not differ, resulting in comparable eating experiences, at least in regard to tenderness.

Conclusion

In conclusion, our data trend as expected and strengthens the argument that nutrient energy source can influence muscle metabolism and ultimate quality development. However, our data also

show that beef muscle is somewhat resilient to short-term nutrient insults, suggesting the creations of nutrient-induced differences in quality and yield require more aggressive feeding challenges. To that end, our data indicates that low-input feeding strategies, if strategically managed, can be used to leverage marketing of fed cattle for financial gains.

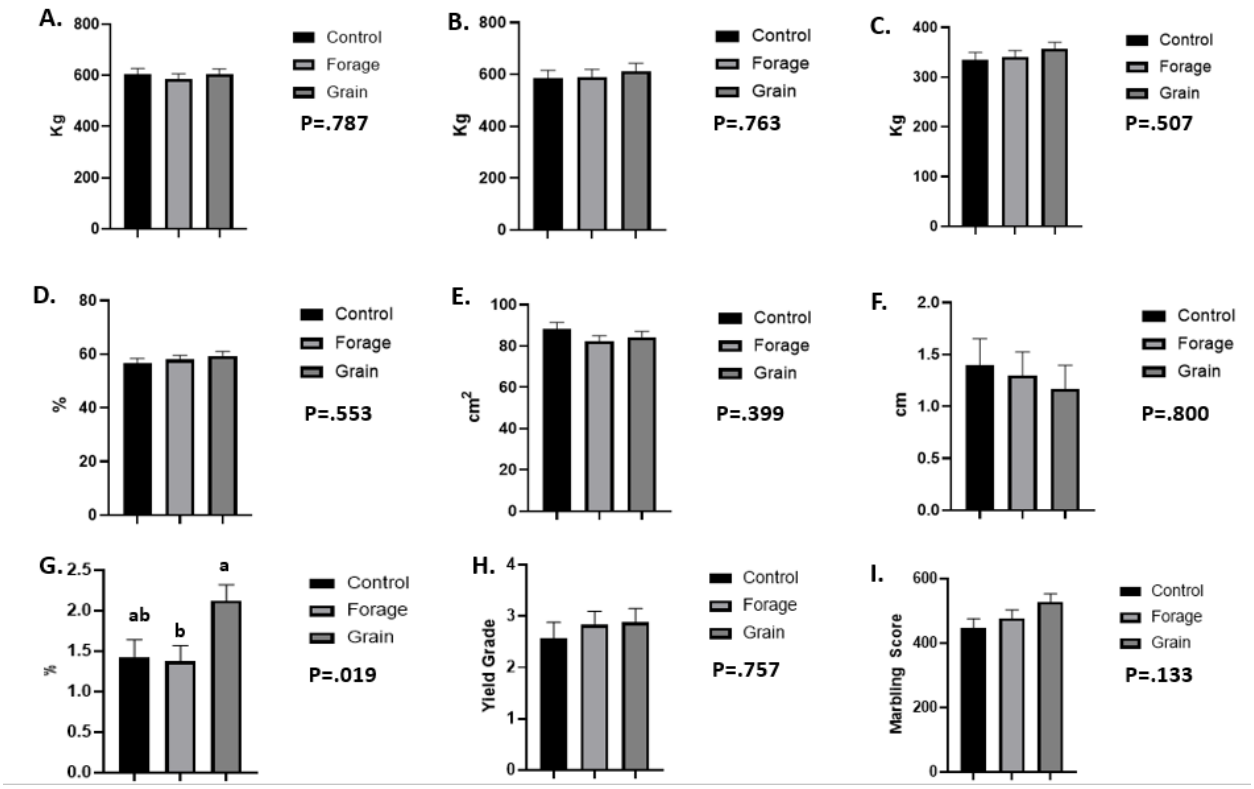

Figure 3-1 (A.) Means for initial body weight (kg) between treatments at start of feeding trial (0d) **(B.)** final body weight (kg) of steers at the end of a 60-d feeding period **(C.)** Means for hot carcass weight (kg) **(D.)** dressing percentage (%), **(E.)** ribeye area (cm2), **(F.)** 12th rib fat thickness (cm), **(G.)** estimated percent kidney, pelvic, and heat fat (KPH) **(H.)** yield grade, and **(I.)** marbling score (200 = traces, 300 = slight, 400 = small, 500 = modest, 600 = moderate) between treatments. Data represent LS means ± SE. Means are considered significantly different at $P < 0.05$.

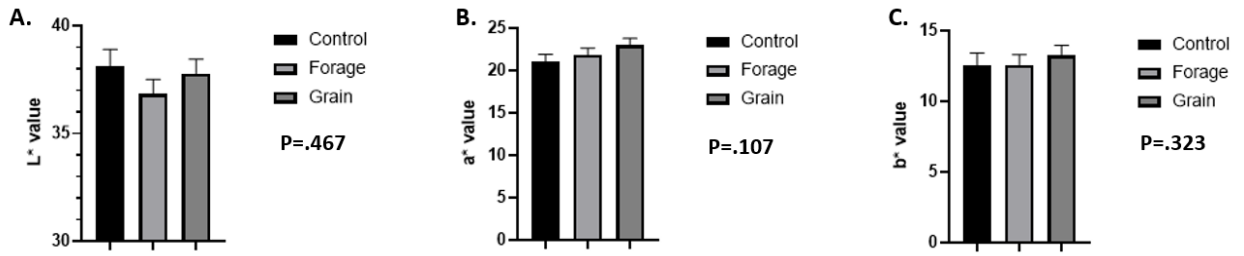

Figure 3-2 (A.) Means of lightness (L*), **(B.)** redness (a*), and **(C.)** yellowness (b*) values LL at 24 h with 30 min bloom. Means are considered significantly different at $P < 0.05$

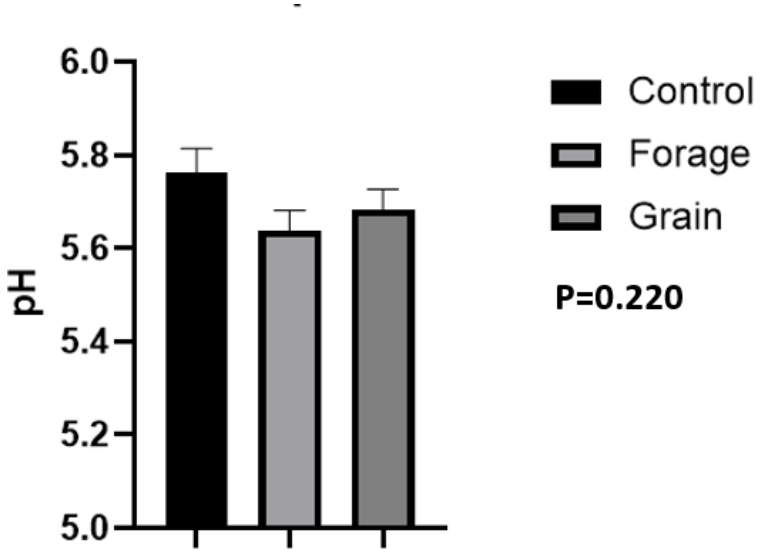

Figure 3-3. Means of pH values from the LL at 24 h postmortem. Data represent LS means ± SE. Means are considered significantly different at $P < 0.05$.

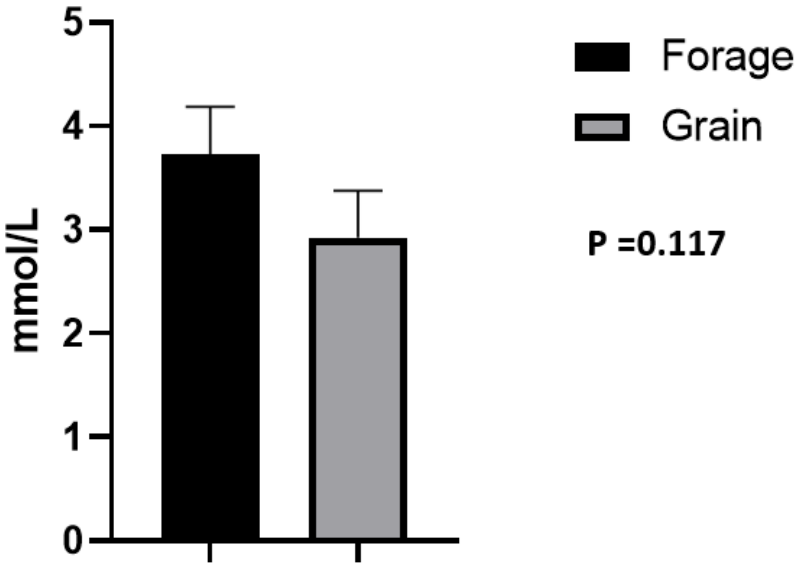

Figure 3-4. Means of non-esterified fatty acids (NEFA) concentration (mmol/L) between treatments post 60 d feeding trial. Data represent LS means ± SE. Means are considered significantly different at $P < 0.05$.

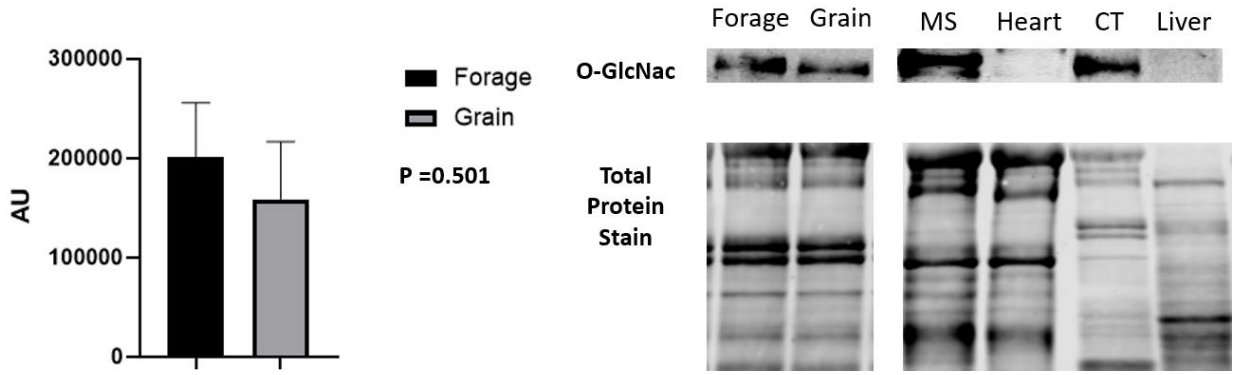

Figure 3-5. Figure 5. Relative abundance of O-GlcNAc in longissimus muscle (LL) of forage and grain-fed cattle post 60 d feeding trial. Data represent LS means ± SE. Means are considered significantly different at $P < 0.05$.

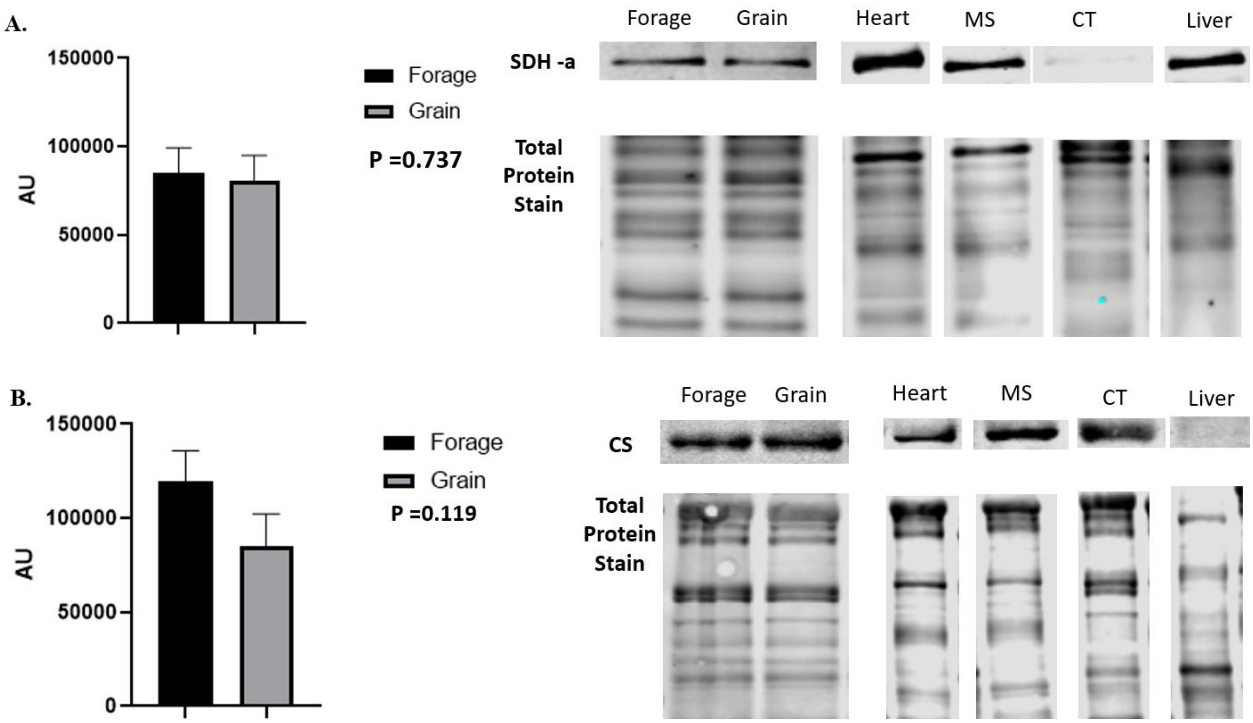

Figure 3-6. (A.) Relative abundance of succinate dehydrogenase-A (SDH-A), and **(B.)** citrate synthase (CS) in longissimus muscle (LL) of forage and grain-fed cattle post 60 d feeding trial. Data represent LS means ± SE. Means are considered significantly different at $P < 0.05$.

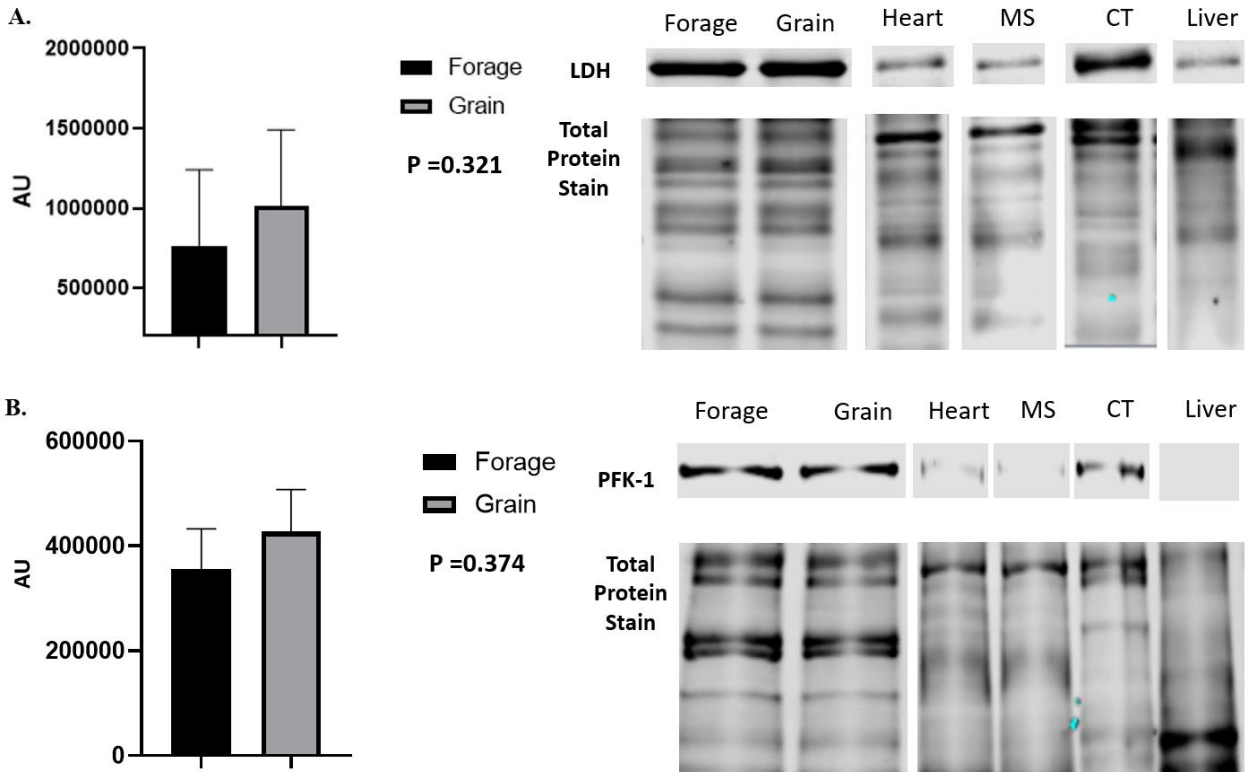

Figure 3-7. **(A.)** Relative abundance of lactate dehydrogenase (LDH), and **(B.)** phosphofructokinase-1(PFK-1) in *longissimus* muscle (LL) of forage and grain-fed cattle post 60 d feeding trial. Data represent LS means ± SE. Means are considered significantly different at $P < 0.05$.

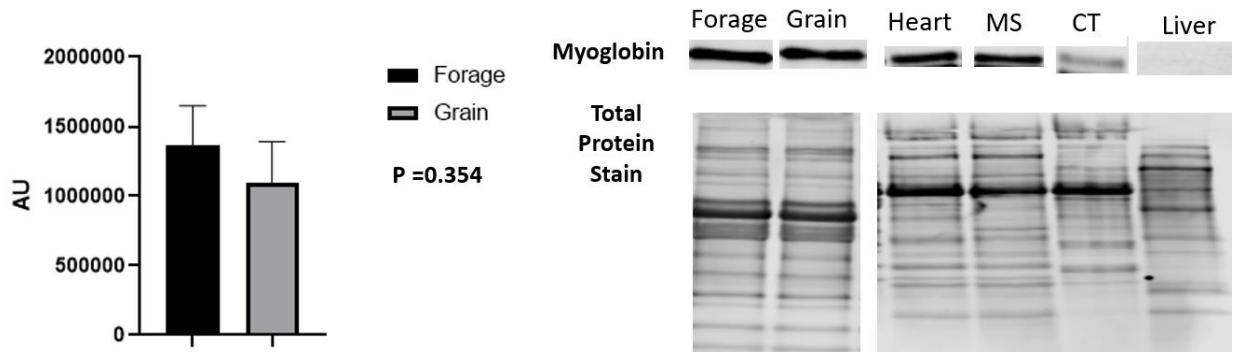

Figure 3-8. Relative abundance of myoglobin in *longissimus* muscle (LL) of forage and grain-fed cattle post 60 d feeding trial. Data represent LS means ± SE. Means are considered significantly different at $P < 0.05$.

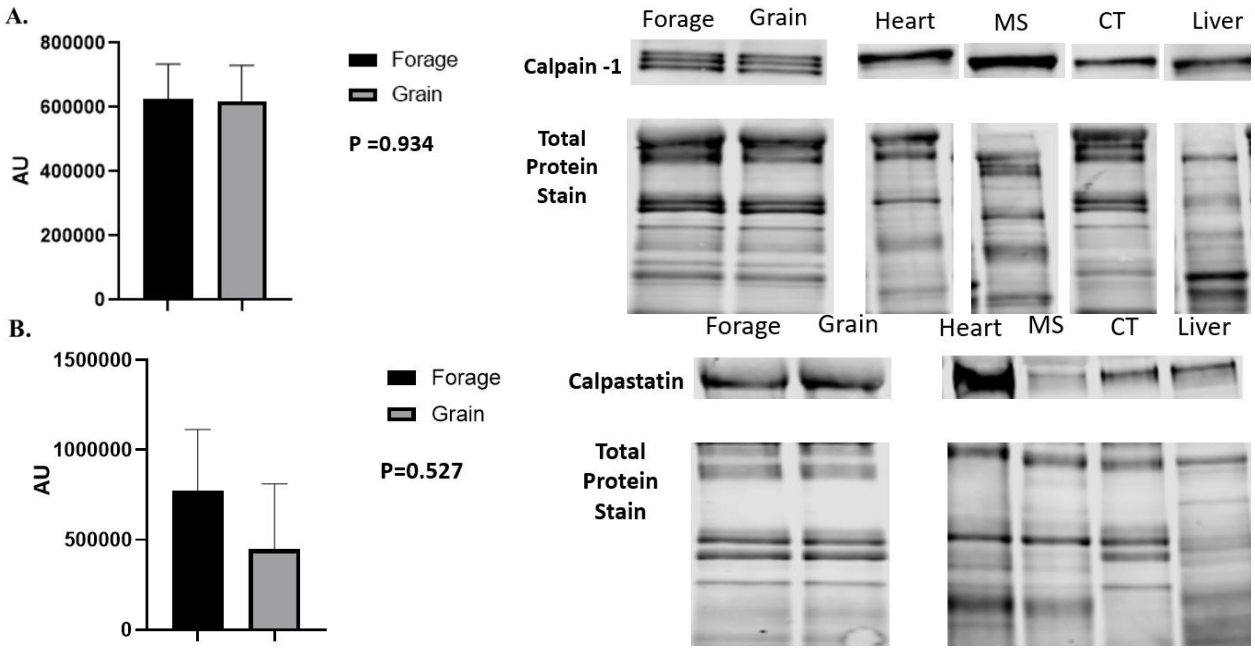

Figure 3-9. (A.) Relative abundance of calpain-1 and **(B.)** calpastatin in *longissimus* muscle (LL) of forage and grain-fed cattle post 60 d feeding trial. Data represent LS means ± SE. Means are considered significantly different at $P < 0.05$.

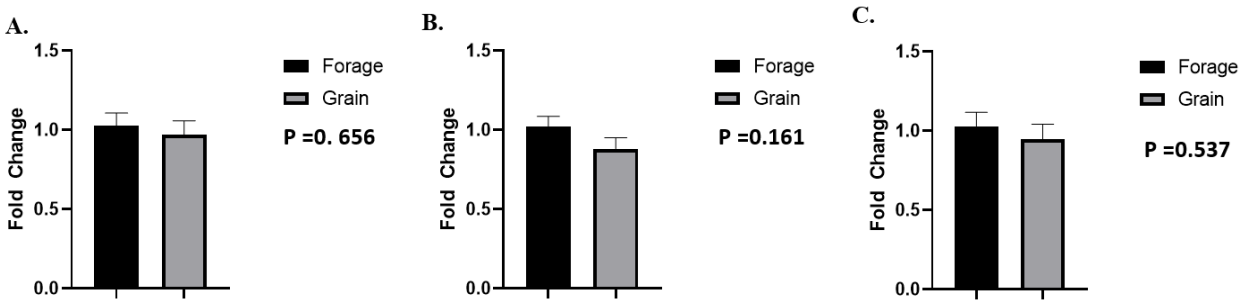

Figure 3-10. Means of gene expression of **(A.)** myosin heavy chain type I (MyHC-I), **(B.)** myosin heavy chain type IIA (MyHC-IIA), and **(C.)** myosin heavy chain type IIX (MyHC-IIX) between treatments and presented as fold differences. Data represent LS means ± SE. Means are considered significantly different at $P < 0.05$.

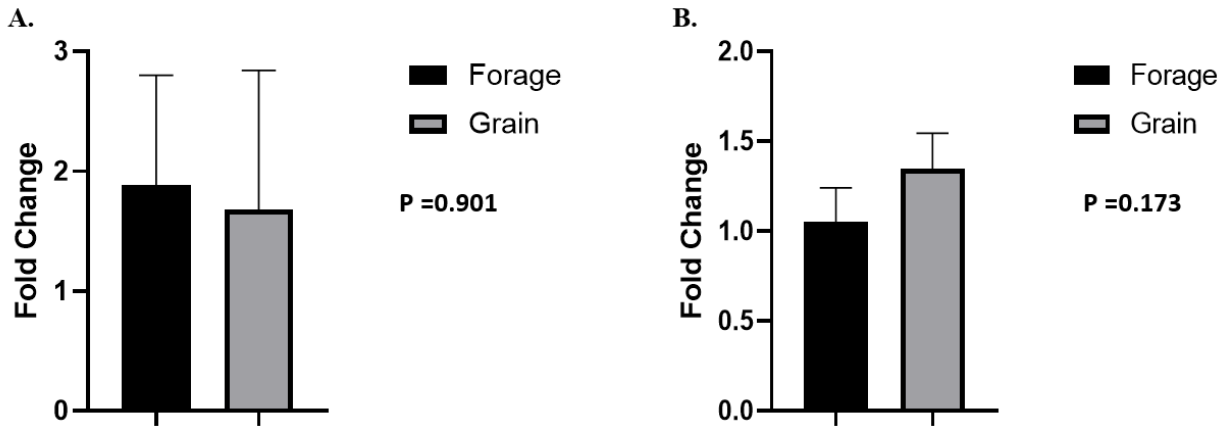

Figure 3-11. **(A.)** Mitochondrial (mt) DNA copy number relative to genomic (g) DNA copy number and **(B.)** myoglobin gene expression between treatments and presented as fold differences. Data represent LS means ± SE. Means are considered significantly different at $P < 0.05$.

Table 3-1. Primer sequence used in quantitative reverse transcription – PCR assays.

Gene Name	Sequence
MHC 1	F5: AAA-GCT-AGC-CCA-GCT-GAT-TAC R5: CTC-TCT-CCT-CTC-CAC-CAT-CTT
MHC IIA	F1: TCT-GAA-CTC-TGC-TGA-CCT-ACT-C R1: CTG-CAT-TGG-TTA-CCT-GCT-CTA-C
MHC IIx	F5: AAA-GCT-AGC-CCA-GCT-GAT-TAC R5: CTC-TCT-CCT-CTC-CAC-CAT-CTT
Myoglobin	F1: CAG-GCT-CTT-CAC-AGG-TCA-TC R1: CTT-CAT-CTC-AGC-CTC-TGT-CTT-C
S18	F5: GCG-AGT-ACT-CAA-CAC-CAA-CAT-C R5: CCT-CAA-CAC-CAC-ATG-AGC-ATA-TC

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Chapter 4. Reducing extensive feeding by 30 days does not reduce yield or quality in finishing steers.

Abstract

The price of corn and distiller grains has steadily increased, pushing the cost of gain near record highs, making profitability of intensive feeding difficult. Therefore, there is a need to better understand the degree to which days on feed influences carcass quality and yield. We chose to test whether reducing intensive feeding by 30 d can reduce inputs, while still producing beef that meets consumer's expectations. Thirty Angus commercial cross-bred steers were randomly assigned either pasture finishing (CON), short (SF), and long feeding (LF) The latter two treatments consisted of 90 or 120 d of an ad libitum high concentrate feeding program, respectively. Carcass evaluation and *longissimus lumborum* (LL) samples were collected 24 h postmortem, while color was measured over time. SF cattle were comparable to that of LF cattle in regards to yield and quality. Moreover, 90 d of intensive feeding increased lean color (L*, a*). Though unable to determine the difference between treatments in muscle metabolism and ultimate pH, two hallmark indicators for difference in quality and color, our data does tend to follow that of expected results, and shows an increase in IIX fibers of SF cattle. This suggests that metabolism is beginning to shift in response to feeding regime and improving lean quality. Overall, the data reported herein argue that reduction of the feeding period to 90 d could lower financial inputs but not compromise final carcass and product quality.

Introduction

Currently, chicken (broiler) is the most consumed animal protein in the US, nearly doubling the per capita consumption of beef (National Chicken Council, 2022a). Consumer's decision to purchase chicken over beef has been driven both the versatility and value (National Chicken Council 2022b). Genetic selection and nutritional management have catapulted the poultry industry forward, allowing modern day broilers to reach a finishing endpoint 1/3 the amount of feed compared to broilers in 1957 (Zuidhof, et al., 2014). This efficient growth rate significantly reduces cost of production, creating a nutritious and affordable source of protein. Conversely, the process of producing beef requires both increased time and inputs for cattle to reach market weight, making it difficult to compete from a price per pound standpoint. The cattle industry has countered this relatively slower growth rate with intensive cereal grain feeding. This method of beef production is more efficient in means of average daily gain (ADG) and days on feed (DOF) especially compared to that of cattle reared on pasture-based feeding systems (Carrillo, et al., 2021). Moreover, cattle reared in this system are more inclined to produce beef that meets consumers expectations for appearance and eating quality. Specifically, consumers have ranked beef color and degree of marbling among the highest attributes when making purchasing decisions preferring beef to be bright-cherry red and of a higher quality grade (Aboah, & Lees, 2020). Furthermore, decades of research have shown that sensory attributes such as flavor and tenderness greatly increase when cattle are subjected to intensive feeding regimes (reviewed by: Wicks et. al., 2019). These characteristics are just as vital to consumers and their probability for repeated purchasing (Aboah, & Lees, 2020).

The feeding period for grain-fed cattle spans 90 to 300 days depending on weight, feeding conditions, and desired finishing endpoint (ERS-USDA, 2022). The price of corn, distiller grains and other by-products have steadily increased, pushing the cost of gain near record highs, making

profitability of intensive feeding difficult (Dennis, 2022). It is currently estimated that the cost of gain is $143/CWT, a steep increase from $127/CWT just a year prior (Langemeier, 2023). Cattle require approximately 2.72 kg of dry matter for 0.45 kg of gain. In a feedlot setting, cattle are capable of gaining 1.36 kg per day, and when extrapolated over a mild feeding period of 120 days, the cost would be nearly $520/head to reach market readiness. While seemingly negligible to a $36.4 billion industry, nearly 15 million cattle are intensively fed in the US annually (NASS-USDA, 2022). Based on these statistics, reducing DOF by even 30 days could save the feedlot industry over $2 billion annually. Still, cattle must be fed adequately in order to meet consumer expectations of quality. Therefore, there is a need to better understand the degree to which days on feed influences carcass quality and yield. Thus, we chose to test whether reducing intensive feeding by 30 days can reduce inputs while still producing beef with characteristics comparable to that of longer feeding periods.

Materials and Methods

Animals and Harvesting

Thirty Angus commercial cross-bred steers of similar age were weaned and placed on pasture for grazing until an average weight of 453 kg was achieved. Cattle were then randomly assigned to 120 d diet consisting of either pasture grazing (CON, n = 10); a short feed (SF, n = 10), consisting of 30 days of pasture grazing followed by 90 d of grain finishing diet, or a long feeding (LF, n = 10, Fig. 4-1) of 120 d of grain finishing diet. Following the 120 d feeding period, steers were weighed and transported to the Virginia Tech Meat Center for harvesting. Harvesting occurred over the course of 3 weeks. An equal number of cattle from each treatment were randomly selected for harvest each week, with 10 cattle being harvested per week. Regardless of harvest day, all cattle were harvested under Virginia Department of Agriculture Consumer Services (VDACS)

meat and poultry inspection. Standard harvesting procedures were used and all carcasses entered a conventional chilling cooler (2 ± 1°C) approximately 50 min postmortem.

Sample Collection

Tissue from the *longissimus lumborum* (LL) was excised immediately following exsanguination (0 min) as well as at 3, 6, 12, and 24 h postmortem. Samples were diced, snap frozen in liquid nitrogen, and stored at −80 °C until analysis. In addition to time point sampling, whole bone-in loins were collected from all carcasses and aged for 21 d, 2.54 cm thick steaks were pulled on day 1, 3, 7, 10, 14, and 21. Steaks were subjected to 72 h display for objective color analysis.

Carcass Evaluation

Following a 24 h chilling period (2 ± 1°C) carcasses were ribbed between the 12th and 13th rib for carcass evaluation as described by the American Meat Science Association (AMSA) yield and quality grading standards (AMSA, 2001). Ribeye area (REA), 12th rib back fat thickness (BFT), estimated percent kidney, pelvic and heart fat (KPH), and hot carcass weight (HCW) were measured and used to calculate carcass yield grade. Carcass maturity and marbling scores were used to determine carcass quality grade.

Color Analysis

Color was measured over time using aged steaks (Fig. 4-2). Briefly, bone-in loins were removed from carcasses placed in chilling cooler (2 ± 1°C) and dry aged for 21 days. One 2.54 cm thick steak was randomly collected from each loin on designated aging timepoint (1, 3, 7, 10, 14,

and 21 d). Steaks were overwrapped using oxygen permeable film, stored in refrigerated cooler (2 ± 1°C), kept out of direct light and subjected to 72 h display for color analysis over time. Using a Minolta CR-400 colorimeter (Ramsey, NJ, USA), Illuminant D, 0° observer angle, triplicate color measurements were taken at 0, 1, 24, 48, and 72 h for each age steak. Averaged color values were expressed as Commission Internationale de l'Éclairage (CIE) L* (lightness), and a* (redness) b* (yellowness).

pH Analysis

To measure pH the iodoacetic method was used, as described by Bendall (1973) with some modifications. Powdered tissue and buffer (1:8 w/v) were homogenized using a Qiagen TissueLyser II (2 min at 25 Hz). The buffer contained 5 mM Na-iodoacetic acid and 150 mM KOH. Once homogenized, samples were heated at 25°C for 5 min, centrifuged for 5 min at 13,000 x g, and placed back on the heating block at 25°C for 1 min. pH was measured using a calibrated Orion Ross Ultra pH electrode (Thermo Scientific, Pittsburgh, PA).

Protein Extraction and Determination

Finely powdered tissue was homogenized (TissueLyzer II; Qiagen, USA; 2 min 25 Hz) with a buffer containing 8 M urea, 2 M thiourea, 3% SDS (w/v), 75 mM DTT, 0.05 M Tris–HCl (pH 6.8) heated at 95°C (Warren, Krzesinski, & Greaser, 2003). Samples were then diluted 1:20 and used for total protein quantification using Reducing Agent and Detergent Compatible Protein Assay (Bio-Rad Laboratories, Hercules, CA, USA), according to manufacturer's specifications. Finally, samples were diluted to a final concentration of 3 mg/mL in extraction buffer (Warren et al., 2003) containing 0.05% bromophenol blue. An additional tube of 100 mg tissue was

homogenized with an extraction buffer containing 50 mM Tris HCL, 150 mM NaCl, 1% NP, .25% sodium deoxycholate. Samples were centrifuged at 10,000 x g for 10 mins and supernatant were collected. Homogenates were diluted 1:20 and protein concentration was determined using BCA protocol. Samples were then diluted in extraction buffer and lamelli buffer to a final concentration of 3 mg/mL. All samples were allocated and stored at −80°C until further analysis.

SDS-Page and Immunoblotting

Samples were heated at 60°C for 10 min prior to being subjected to SDS-Page. A stacking gel of 5% was used for all proteins, however separating gels varied on protein of interest. A 15% polyacrylamide separating gel was used to detect succinate dehydrogenase (SDH; Abcam ab14715 at 1:1000 dilution), and lactate dehydrogenase (LDH; Novus NBPI48336 at 1:30000 dilution). For detection of citrate synthase (CS; Santa Cruz Biotechnology, Inc, SC-390693 at 1:1000 dilution), phosphofructokinase (PFK, Santa Cruz Biotechnology, Inc, SC-166722 at 1:1000 dilution), adenosine monophosphate deaminase 1 (AMPD1; Abcam Ab72541 at 1:1000 dilution) and calpain-1 (CAPN-1; Thermo-Fisher 9A4H8D3 at 1:1000 dilution), a 10% gel was used. An 8% gel was used for the detection of calpastatin (CAST; Thermo-Fisher 1F7ED10 at 1:1000 dilution), while an 18% gel was used for myoglobin (Santa Cruz Biotechnology, Inc, SC-25607 at 1:1000 dilution). Gels were run at room temperature at 60V for 20 min, and then 120V for 120 min (Bio-Rad Laboratories, Hercules, CA, USA) for SDH, LDH CS, CAPN1, and CAST. PFK and AMPD1 were also run at room temperature; AMPD1 and PFK gels were run at 50V for 25 min, and then 100V for 150 min and myoglobin was run at 60V for 20 min and 200V for 50 min (Bio-Rad Laboratories, Hercules, CA, USA). Separated proteins were then transferred to nitrocellulose membranes at 70V for 50 min at 4°C using a Bio-Rad (Bio-Rad Laboratories, Hercules, CA, USA) and a transfer buffer containing 50 mM Tris, 0.38 M glycine, 0.01% (w/v) SDS, and 10% (v/v)

methanol. Membranes were blocked overnight at room temperature with either Prometheus™ OneBlock™ Blocking buffer (Genesee Scientific Corporation, El Cajon, CA) or 5% non-fat dry milk in Tris-buffered saline solution with 0.1% tween-20 (1X TBS-T) added. Primary antibodies specific for SDH, LDH, CS, PFK, AMPD1, CAPN1, CAST, and myoglobin were diluted in respective blocking buffer, and membranes were incubated over night at room temperature. Membranes were then washed with TBS-T three times for 5 mins each before being incubated for 1 h at room temperature with secondary antibody diluted in TBS-T. Goat anti-mouse florescent antibody (LI-COR Biosciences, Lincoln, NE) was used for SDH, while goat anti-rabbit florescent antibody (LI-COR Biosciences, Lincoln, NE) was used for LDH. Membranes were washed an additional three times for 5 mins each. All membranes were reversibly stained with Revert 700 Protein Stain (Li-Cor Inc., Lincoln, NE) for visualization of bands and proteins were normalized to total protein. All blots were imaged using a LI-COR Biosciences Odyssey Infrared scanner (Li-Cor, Inc., Lincoln, NE, USA) and band intensity was measured using Image Studio lite (Li-Cor, Inc., Lincoln, NE, USA) with protein abundance reported as arbitrary units (AU).

Gene Expression

Direct-zol RNA Mini Prep Kit (Zymo Research, Irvine, CA) was used to extract total RNA from LL muscle. Twenty ng/µL of total RNA were reverse transcribed using the High Capacity cDNA Reverse Transcriptase Kit (Applied Biosystems, Waltham, MA). Two µl of cDNA was amplified using gene specific primers (Table 4-1) and SYBR chemistry in a 7500 Fast Real-Time PCR System (Applied Biosystems, Waltham, MA) for the quantification of calpain-1, calpastatin myoglobin and myosin heavy chain isoforms. Relative gene expression was quantified by the $2^{-\Delta\Delta Ct}$ method.

Mitochondrial DNA Content

Total DNA was purified using a DNAeasy mini spin columns according to manufacturer's recommendation (Quigen, Germantown, MD and quantified by optical density at 260 nm (Nanodrop 2000 spectrophotometer, ThermoScientific, USA). Mitochondria (mtDNA) and genomic DNA (gDNA) quantification was accomplished as previously described (López-Andreo,et al., 2005). Briefly, 25 ng of total DNA was amplified (TaqMan™ Fast Advanced Master Mix Applied Biosystems ™) with organelle-specific DNA primers (500 nm each) and 250 nM MGB probe for 40 cycles of 20 s at 95 °C and 30 s at 60 °C for 40 cycles. Total mtDNA quantity (ng/µl) was inferred from the standard curve and normalized to the gDNA total quantity presented as a ratio of the two.

Statistical Analysis

Data were analyzed using the Proc Mixed procedure using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). Carcass was the experimental unit and the statistical model included the fixed effects of treatment, with harvest day as a random variable and repeated statement used for pH. Reported color data was also analyzed using the SAS Proc Mixed function. However, the fixed effects included treatment, aging time (day), and display time (time), with loin as random variable. The repeated statement was used for steak. Means were compared using Tukey-Kramer Multiple Comparison Test if a significant effect was detected. Data on graphs are least square means ± standard error means (SEM), and differences were considered significant at $P < 0.05$ unless otherwise stated.

Results

Carcass Evaluation

Cattle subjected to grain finishing diets resulted in heavier final bodyweights, and while LF was similar to SF, LF steers were significantly heavier ($P = 0.036$) than that of the CON (Fig. 4-3a). HCW was increased ($P < 0.001$) with days on feed (Fig. 4-3b), however, dressing percentage (DP) only differed ($P = 0.06$) between CON and LF (Fig. 4-3c) making SF comparable to LF. Carcass evaluation measurements followed the same trend with LF resulting in increased REA (Fig. 4-4a; $P = 0.019$), FT (Fig. 4-4b; $P = 0.005$), yield grade (Fig. 4-4c; $P = 0.016$), and marbling score (Fig. 4-4d; $P = 0.002$) compared to CON, and though not significant, SF was increased in all carcass evaluation measurements compared to CON but did not differ from LF. However, we found no differences in KPH between treatments (data not shown).

Color and pH

Lightness increased with time of bloom regardless of treatment, however an interaction between treatment and bloom time ($P = 0.062$) was noted (Fig. 4-5a), with SF beef equal to LF throughout display time. Both SF and LF reached peak lightness by 24 hr, and sustained that level of lightness through 72 hr, while CON required 48 hr bloom to achieve similar values. Additionally, an interaction between treatment and time of bloom was noted in a* (redness) and b* (yellowness) (Fig. 4-5b; $P < 0.001$; Fig. 4-5c; $P = 0.009$). Both a* and b* increased between 0 and 24 h of display but was similar across treatments.

Aging improved lightness and redness in beef of fed cattle resulting in highest L* values (Fig. 4-6a; $P < 0.001$) with SF and LF achieving peak lightness values by day 10, and sustained through day 21. However, CON was unable to reach similar lightness values as days of aging was

increased. Still, CON and SF resulted in the highest a* values for days 3, 10, 14, and 21 (Fig. 4-6b; *P < 0.001*). While treatments did not vary within aging day for yellowness (b*), there was a significant difference between day 7 and all other aging days (Fig. 4-6c; *P = 0.05*). Curiously, however, both L* and a* values followed that same trend, generating lean that was comparable to 1 d aged lean or in some cases even darker, less red, and yellow.

An interaction for aging and bloom time existed for L*, a* and b* (Fig.4-7a; *P < 0.001*; Fig. 4-7b; *P < 0.001*; Fig. 4-7c *P < 0.001*, respectively). Lightness was increased with bloom time, regardless of aging time, however, L* had similar lightness values from day 10 through day 21. Conversely, a* values were highest on days 3 and 21, while b* values were highest on days 14 and 21.

Despite color variation between treatments and times, ultimate pH (pH_u) was similar for all treatments. Even so, rate of pH decline differed with CON being lower at 12 h postmortem (Fig. 4-8; *P = 0.04*).

Protein Abundance

An increase in oxidative proteins such as SDH, CS, and myoglobin have been shown to increase in cattle fed lower energy diets, resulting in increased ultimate pH (pH_u) and darker lean (Gómez et al., 2022a; Apaoblaza et al., 2020). However, we were unable to detect any differences in SDH (Fig. 4-9a; *P = 0.281*), CS (Fig. 4-9b; *P = 0.750*), or myoglobin (Fig. 4-9c; *P = 0.892*) despite differences in feeding regimes. Additionally, we were also unable to distinguish any differences LDH (Fig. 4-10a; *P = 0.772),* PFK (Fig. 4-10b; *P = 0.338*), or AMPD1 (Fig. 4-10c; *P = 0.07*) all indicators of a more glycolytic metabolism, Finally, no differences were noted between treatments for CLPN1 (Fig. 4-11a; *P = 0.837*) or CAST (Fig. 4-11b; *P = 0.392*).

Gene Expression and mtDNA

Although we were unable to detect differences in protein abundance for myoglobin, though a significant difference in gene expression of SF cattle compared to CON (Fig. 12e; *P = 0.016*). Expression of MyHC differed between treatments. LF cattle had increased MyHC-I (Fig. 4-12a; *P = 0.002*), MyHC-IIA (Fig. 4-12b; *P = 0.005*), MyHC-IIX (Fig. 4-12c*; P = 0.001*) compared to CON, however, SF cattle did not differ from that of LF cattle. Still, no differences were noted in mtDNA (Fig. 4-12d*; P = 0.672*).

Discussion

Carbohydrates are fermented in the rumen and the resulting microbes yield volatile fatty acids (VFA). VFAs such as acetate, proportionate and butyrate fuel over 70% of a ruminant's energy supply (Bergman, 1990). Cattle fed high forage diets result in proportionally greater levels of acetate, while cattle fed concentrate diets have a relatively higher percentage of propionate (Zhang et al. 2017). Utilization of acetate and propionate for metabolized energy differ, giving rise to two different means of energy production. Acetate is a two-carbon short chain fatty acid (SCFA) and can be readily converted to acetyl-CoA and metabolized through the mitochondria to produce energy through the citric acid cycle (Yoshii, Furukawa, Saga, & Fujibayashi, 2015). On the other hand, propionate primarily functions to aid in gluconeogenesis, increasing substrate availability for ATP production through glycolysis (Aschenbach et al., 2010). However, as substrate availability shifts, so does muscle fiber type, metabolism, and potential for growth and meat quality.

Myosin heavy chain (MyHC) isoforms are often used to classify muscle fiber type, based by contractile speed (slow or fast-twitch) and metabolism in which the muscle produces energy

(oxidative or glycolytic; Blaauw, Schiaffino, & Reggiani, 2013). However, muscle is dynamic and capable of shifting from fast-to-slow or slow-to-fast as dictated by muscle function and substrate availability (reviewed by: Pette & Staron, 2000). Generally, meat animal production selects for increased growth rate, favoring a shift from slow-to-fast fibers. Muscle growth requires a unique balance of both protein synthesis and protein degradation, often referred to as protein turnover. Type I fiber have a high rate of protein turnover, limiting the extent of hypertrophic growth. However, fast-twitch fibers have increased hypertrophy and develop larger cross-sectional areas (CSA; Lefaucheur, & Gerrard, 2000). Still, the metabolism in which the fibers receive energy is also critical in overall growth and protein accretion. Slow-twitch, type I fibers rely on oxidative metabolism, and though efficient in ATP production, allows for the loss of carbons through the production of ATP. This results in inefficient growth (reviewed by: Inigo, Deja, & Burgess, 2021). However, fast-twitch muscle relies on glycolysis for energy production, which allows for both increased growth rate (Cooper, Cassens, Kastenschmidt, & Briskey, 1970) and intramuscular fat (IMF) accumulation (Frylinck, Strydom, Webb, & Du Toit, 2013; Mwangi et al., 2019; Wicks et al., 2019). Gómez et al. (2022a) reported that grain-finished cattle with increased growth rate generated heavier carcasses with increased REA, BFT, color and degree of marbling. Alternatively, cattle with reduced intake and slower growth rate required over 100 more days on feed to reach an equivalent target endpoint, and resembled growth and finish similar to that of pasture reared cattle. Similarly, Koch et al. (2019) found that grass-fed cattle weighed nearly 100 kg less than cattle subjected to a concentrate diet following a 127-feeding day period, consequently resulting in less quality finish, and darker lean. Apaoblaza et al., (2020), further investigated muscle samples from Koch et al. (2019), determining grass-fed beef to have a more oxidative muscle profile and produced inferior growth and quality compared to grain-fed cattle, which

possess a more glycolic profile. Correspondingly, Gómez et al. (2022b) and Antonelo et al. (2022) further analyzed muscle samples from Gómez et al. (2022a) determining both slower growing and pasture reared cattle possessed a great oxidative phenotype as well, strengthening the claim of Apaoblaza et al. (2020). The clear advantages that intensive feeding provides for producers are efficient growth and the increased likelihood of improved quality. However, the recent exponential cost of inputs has challenged such production strategies. Still, the demand for beef is expected to increase exponentially by the year 2050 (Alexandratos, & Bruinsma, 2012), warranting the exploration of cost saving, alternative feeding strategies, capable of producing beef that meets consumer expectations.

Data presented herein attempts to address these pressing concerns and provide some encouraging and novel insight. First, our results affirm the intensive feeding program used in this study resulted in intensively fed cattle (LF) achieved more favorable carcass scores compared to that of grass-fed cattle (CON). Although no significant differences were noted between CON and SF in terms of carcass evaluation, SF characteristics were numerically increased and did not differ to those of LF carcasses. The similarity in SF carcass composition to that of LF carcasses is likely due to some degree of compensatory gain. Following a period of restricted development, usually due to reduced nutrient intake; a physiological process occurs in which accelerated growth is evident in response to excess nutrients (Hornick, Van Eenaeme, Gérard, Dufrasne, & Istasse, 2000). Although compensatory gain can be achieved following protein restriction, it is more profound following nutrient energy restriction (Drouillard, Ferrell, Klopfenstein, & Britton, 1991), driving first protein synthesis and later adipose deposition (Hornick et al., 2000). Even so, a recovery of 100% is rarely observed and is dependent on duration and intensity of restriction (Ryan, 1990). However, Hornick et al. (2000) suggested that compensation is best when growth

restriction is limited to approximately 3 months and not too severe. Although performance data was not recorded, our results of final body weight and carcass measurement data align with the aforementioned construct of compensatory gain. Moreover, we observed a significant increase in IIX MyHC gene expression between muscle of SF cattle compared to that of CON, hallmark of compensatory gain (Picard, et al., 1995; Brandstetter, Picard, & Geay,1998, reviewed by: Picard, & Gagaoua, 2020). Granted, we were unable to show significant difference between gene expression for MyHC I or IIA between SF and CON. However, type I fibers remain rather constant postnatally, while shifts from IIA to IIX have proven to be more pronounced (Spindler, Mathias, & Cramer, 1980; Seideman, & Crouse, 1986; Solomon, West, & Hentges 1986). Still, MyHC of SF was similar to LF cattle across all three adult isoforms in cattle, showing the plasticity of muscle and also suggesting some of the increases may be related to increased growth rate.

Compensatory gain and concentrate feeding regimes are often accompanied by glycolytic metabolism (Cassar-Malek et al., 2004). Energy metabolism can be indicated by an abundance of metabolic properties known for aiding in glycolysis, or the citric acid cycle, in the case of oxidative metabolism (Hocquette et al., 1998). PFK is activated by high concentrations of AMP and functions as the key regulating enzyme in glycolysis. Known as the "committed" step of glycolysis, PFK catalyzes fructose 6-phosphate and ATP to fructose 1,6-bisphosphate and ADP, allowing for a cascade of reactions, yielding two pyruvates. However, in the instant of anaerobic glycolysis, like that used for energy production in type IIX fibers, pyruvate is converted by yet another metabolic indicator of glycolytic metabolism, LDH. This allows for the production of 2 ATP and 2 lactate molecules. On the other hand, oxidative metabolism is often indicated by increased abundance of SDH and CS. CS serves as the catalyzing enzyme combining acetyl-CoA with oxaloacetic acid to form the intermediate, citrate; initiating the first step of the citric acid

cycle. SDH is a dual enzyme, converting succinate to fumarate within the citric acid cycle, as well as aiding in the transferring of electrons within the electron transport chain. We investigated both glycolytic and oxidative proteins to determine energy metabolism of cattle subjected to varying feeding regimes. Despite intensive feeding for 90 or 120 days, we were unable to determine differences between fed cattle to that of CON. These data part from those of Apaoblaza et al. (2020), who reported a clear difference in protein abundance of SDH and LDH between grass and grain-fed beef. Additionally, Vestergaard et al. (2000) determined increased CS activity in extensively fed bulls, while intensively fed bulls had increased LDH. Even so, Hocquette et al. (1998) stated that enzymes qualitatively characterized as biomarkers for metabolism do not always correlate given they are not rate-limiting but rather substrate driven. Moreover, Ghosh et al, (2014) discussed the inconsistencies of qualitative protein analysis using immunoblotting, stating current methods for normalization are substandard. The utilization of housekeeping proteins (HKP) such as GAPDH or actin can be unreliable due to high expression, leading to overloading and inaccurate quantification. Additionally, Greer et al. (2010) showed that HKP proteins such as GAPDH and alpha tubulin change with cell density in cultured cells, making them unreliable and potentially misleading. While we do not argue the overall validity of Apaoplaza et al. (2020) findings, which are supported by a compelling set of data, it is important to note that the authors found significant difference between GAPDH of grass and grain-fed cattle. This contributes to Greer et al. (2010) construct of changes in HKP and potential for inadequate results. Technologies such as total protein staining allow for fluorescent signaling proportional to that of the total protein and has proven to serve as a better normalization control (Ghosh, Gilda, & Gomes, 2014). Although our protein abundance data does not reflect that of Apaoblaza, et al. (2020), proteins were normalized using the preferred method of total protein staining, accompanied by proven controls. This may

indicate that other methods, such as enzyme activity, may be more accurate (Pette & Straon, 2000, Vestergaard et al., 2000). Regardless, our data does quantitatively resemble that of expected results for both glycolytic and oxidative metabolism, and may also lack significance due to age, growth, insufficient sampling population, or days on feed.

The conversion of muscle to meat is influenced by many factors. Muscle metabolism is the ultimate dictator in pH decline, and largely responsible for meat color development (reviewed by: Ramanathan et al., 2020). We found no differences in ultimate pH, however our results showed a drastic decline between 6 and 12 h for CON, leading to significantly lower pH at 12 h compared to the fed groups. Although obscure, based on our substrate data, the difference in rate of pH decline seems to imply at least some level of difference in metabolism between CON and fed groups. Moreover, our data shows that, despite no difference in pH_u, CON appeared to remain rather constant between 12 and 24 h. This indicates a difference in muscle function and may contribute to the darker lean noted in CON at 0 and 1 h bloom. Typically, pH will gradually decline from a pH of approximately 7.0 to an ultimate pH of 5.5 - 5.6 giving beef the classic bright-cherry red color that consumers prefer (reviewed by: Boles, & Pegg, 2010). However, our data show pH_u to be slightly higher than 5.6 across all treatments, yet CON resulted in the darkest lean. It is often thought that dark lean arises from low glycogen due to stress or adverse handling, resulting in dark, firm, and dry (DFD) beef. Yet, not all dark beef is a result of extreme stress and improper handling. In fact, despite relatively normal pH, dark lean has been known to arise in bulls, older animals, and cattle subjected to low energy diets (Therkildsen et al., 1998; Boccard et al. 1979; Apaoblaza et al., 2020). Therefore, the central dogma surrounding dark-cutting beef has been challenged. Mahmood et al. (2017), recently reported a classification of dark beef, termed ‘atypical dark’ (AT) beef. AT beef does not follow the underpinning biology of traditional dark cutting beef and has

only a slightly higher pH_u (5.6 - 5.8). However, lean from AT beef is notably darker than that of normal beef. Kirkpatrick et al. (2023) sampled AT beef from both the US and Canada and determined it was more oxidative in nature, suggesting beef with an increasing oxidative profile may be more prone to produce darker lean. Additionally, England et al. (2016) concluded that oxidative muscle naturally results in a higher pH, despite substrate availability. This further supports Kirkpatrick et al.'s (2022) findings of dark lean arising from oxidative muscle. This construct aligns with both muscle metabolism and color discrepancies seen in intact males, older animals, and extensively fed cattle. Furthermore, if muscle in SF cattle is more glycolytic than CON, as evidenced by our pH data, this supports our findings.

Lean color is first evaluated on the rail and deemed acceptable or dark-cutting. Second, meat color is again heavily scrutinized by consumers at the retail counter, guiding their purchasing decisions (Aboah, & Lees, 2020). The blooming (initial) and stability (sustained) of lean color are driven by two competing mechanisms. Blooming occurs when oxygen diffuses from air into meat. As oxygen penetrates the muscle, it binds to deoxymyoglobin (DMb) converting it to oxymyoglobin (OMb), shifting the pigment from purple to red. However, because mitochondria are still viable postmortem and consuming oxygen (OCR; Lanari, & Cassens, 1991), there is competition with myoglobin, which limits potential for bloom intensity. On the other hand, color stability is dependent on metmyoglobin reduction activity (MRA) and relies heavily on mitochondria's ability to process both enzymatic and nonenzymatic NADH in order to maintain ferrous myoglobin forms. Because both initial and sustained color are of importance to the industry, we chose to measure color over a time frame that would be similar to that of an average retail display. Our data show lightness was significantly improved in fed cattle both initially and following a 1 h bloom compared to CON. This trend continued with sustained lightness being

comparable for both SF and LF over time. Even so, lightness of CON was improved over time, however 72 h blooming was required before reaching a level comparable to that of LF. These data suggest a difference in ORC and MMR activity. However, we found no difference in mtDNA between treatments, which coincides with Apaoblaza et al. (2020), who also found no differences in mtDNA between grass and grain-fed beef, yet still had found difference in color. Moreover, mitochondria function differently between competing energy metabolisms, despite difference in mtDNA (Pereyra et al., 2022). Additionally, myoglobin abundance is highly correlated to redness (a*; Mancini, & Hunt, 2005). Though myoglobin is typically increased in more oxidative muscle due to its working relationship with mitochondria, Apaoblaza et al. (2020) reported increased myoglobin in grass-fed cattle. However, a* (redness) was increased in grain-fed cattle, and Gómez et al. (2022a) also reported similar results. It is likely that redness is increased in fed cattle due to its availability to bind to oxygen, generating both a lighter and more red lean (Egbert & Cornforth, 1986). The data presented herein, shows redness increased over time, however, only marginable differences were noted between treatment over time unlike that reported in previous literature. Although our data showed an increase in gene expression in SF, our data failed to show differences in myoglobin protein abundance and may not differ as greatly in regards to metabolism as previous studies.

Dry aging of beef carcasses has been a long-established means of preservation, as well as an effective method to increase tenderness. Though once the norm in US beef processing, it has become increasingly scarce due to growth of technologies for several decades (reviewed by: Dashdorj et al., 2016). However, in recent years, dry aging has regained popularity, becoming a preferred method for many processors across the US. Therefore, initial and sustained color was also measured across aging carcass from 1-21 days. Our data show color (L*, a*, b*) is improved

with aging, regardless of treatment. However, our data reaffirm that of Gómez et al. (2022a), which showed that fed cattle excel in color compared to that of grass-fed cattle. Even so, our data are unique in that they show a threshold in which lean color can be improved. Our data found fed cattle to achieve ultimate lightness and redness by 10-d, while CON required 14 d to reach its ultimate lightness. However, regardless of aging time, CON still remained darker (L*) than that of fed cattle. As carcasses age, proteolysis ensues, altering muscle structure and increasing light scatter. This likely influenced much of the differences in L* values. Although color was sustained between 10 d for SF and LF and 14 d for CON, it also suggests that proteolysis plateaus at 10 d for fed cattle. This challenges the financial rationale behind longer aging periods.

Postmortem proteolysis increases meat tenderness, aiding in increased eating experience for consumers. This biological occurrence is tightly regulated by both the cysteine protease, calpain-1, as well as pH decline postmortem (Ramos, et al., 2020; Huff-Lonergan, et al., 1996; Dransfield, 1992). Although there are conflicting arguments as to how they control proteolysis, Hwang and Thompson (2001) determined calpain-1 is increasingly activated when pH reaches 6.0 within 1.5 h postmortem. However, Lomiwes et al. (2014), reported shear force increased in muscles with pH_u between 5.8 and 6.2, compared to that of normal pH_u beef (<5.8). This underscores the importance for complete and gradual pH decline. Still, calpains are inhibited by calpastatin, which have shown to be in greater abundance in more oxidative muscle (Ouali, & Talmant, 1990) and has been linked to tougher beef (Koohmaraie, 1992). We found no difference in either calpain-1 or CAST between treatments. Coupled with our pH data, these results suggest a very minimal difference between treatments fed to this extent.

Finally, degree of marbling is critical in determining quality and price point of beef both on the rail and at the retail counter. Marbling refers to the intramuscular fat streaking embedded

within the connective tissue matrix and is increased in response to net energy (Pethick and Harper, 2006). Precursors for marbling develop between 3-8 months, however expression of marbling is not observed prior to 200 kg, but linearly increases between 200-450 kg (Pethick, Harper, Hocquette, & Wang, 2006). While the fundamental principles surrounding marbling are understood, there is still much to be explored in regards to genetic factors controlling intramuscular adipogenesis. However, Thornton et al. (2017) reported that heifers with a high degree of marbling had an increase in AMPD1, compared to low-quality heifers. Additionally, Antonelo et al. (2022) reported similar results in grain-fed cattle with high growth rate compared to low growth rate. Although the role of AMPD1 in adipogenesis is not fully understood, it is highly expressed in skeletal muscle. In an effort to maintain ATP equilibrium, AMP is irreversibly converted into to IMP and ammonia in the purine nucleotide cycle (Hancock, Brault, & Terjung, 2006). Moreover, AMPD1 is found in increased levels in glycolytic type IIX fibers (Wang, et al., 2008) and likely gives rise to intramuscular fat deposits. This is due to its influence in insulin resistance and energy storage (Cheng, et al., 2014). Although we did not find significant difference in AMPD1, our data show considerable increases in SF compared to CON or LF. These data align well with both Thornton et al. (2017) and Antonelo et. al., (2022) who determined AMPD1 is highest in type IIX fibers. While still relatively low in terms of ultimate quality grade, averaging low choice, our data argue that a reduction of feeding for even 30 days, across even the mildest of feeding periods, can increase ultimate beef quality.

Conclusions

Reduction in the feeding period of cattle had little impact on carcass yield, quality, and color than that fed for 120 d. Though beef of the SF group closely parallels that of the grass-fed CON group, in terms of yield and quality, color was substantially improved in SF compared to

CON. Moreover, our findings reveal potential for optimizing lean color stability between intensive and extensive feeding regimes. These data suggest that fed beef reaches peak lightness following a 10 d aging period, however, grass-fed beef appears to need closer to 14 d. Still, further investigation into meat tenderization (proteolysis) is needed to better determine an optimal aging time that combines ideal color and tenderness. Even so, SF and LF cattle would likely receive comparable dollar/kg on rail, indicating greater profitability on a per kg basis as SF would significantly reduce inputs.

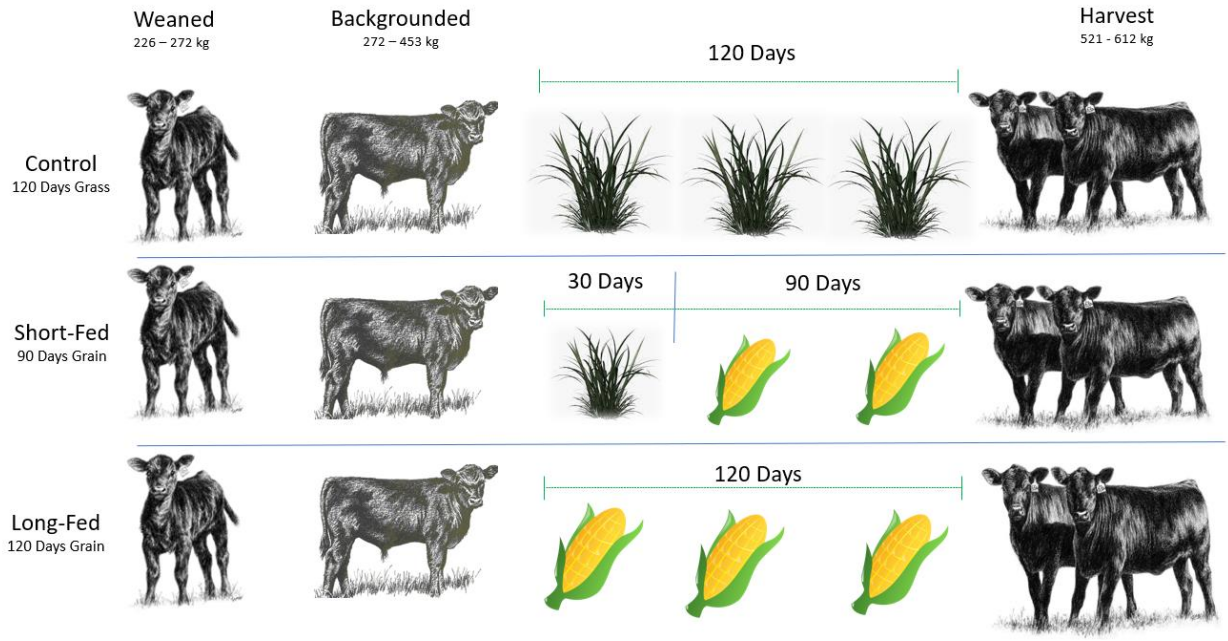

Figure 4-1. Schematic diagram of feeding regime for cattle subjected to control (CON), short-fed (SF), or long-fed (LF).

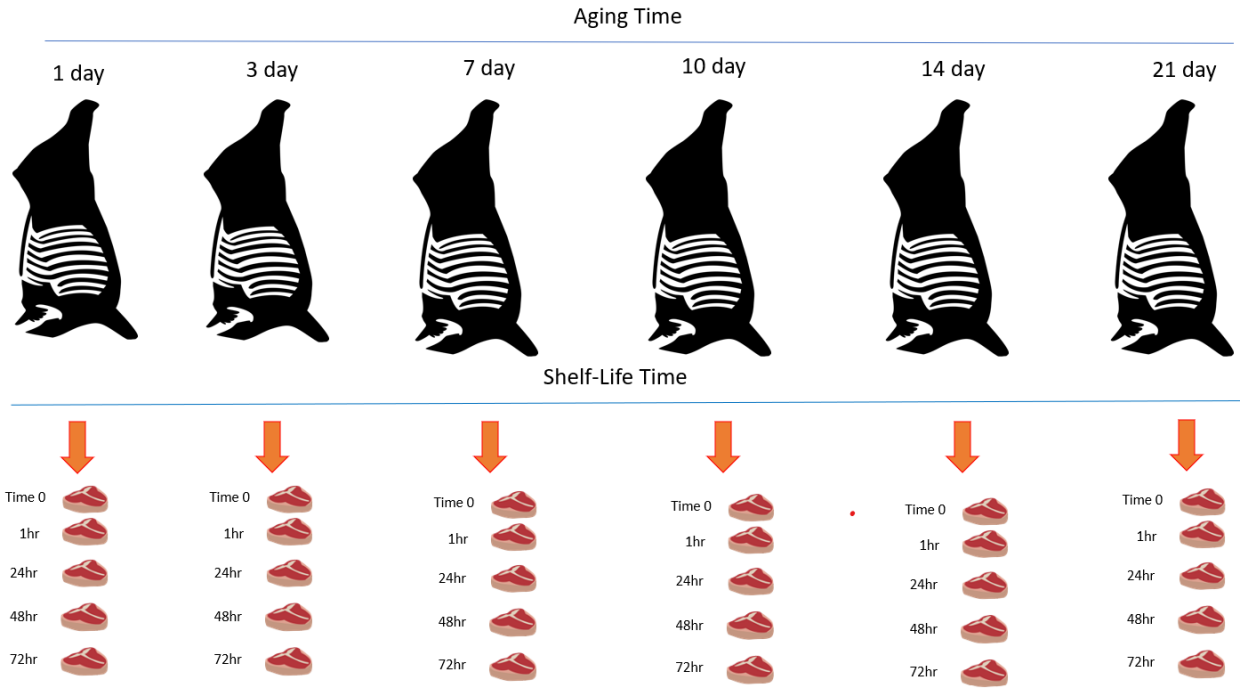

Figure 4-2. Schematic diagram of sampling time points across aging time and shelf-life display times in which color (L*, a*, and b*) were analyzed.

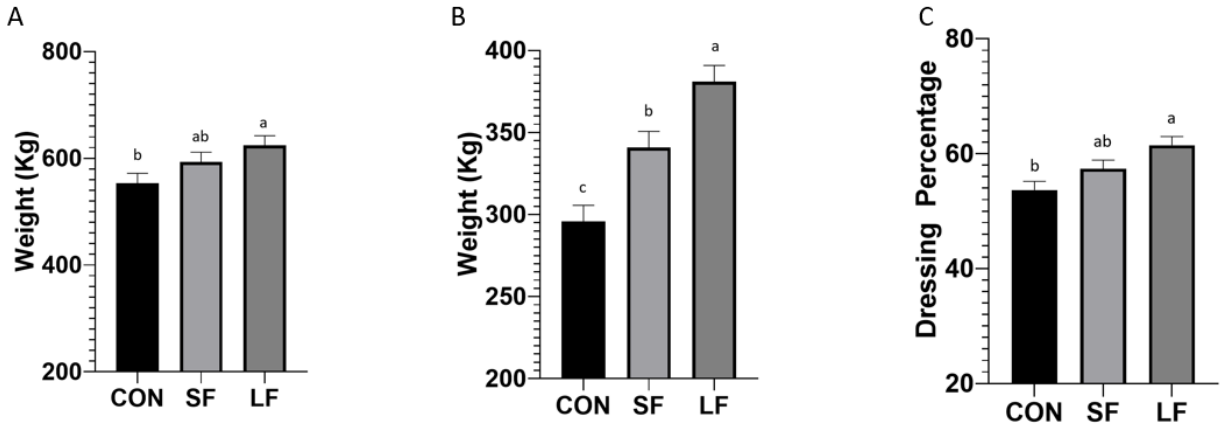

Figure 4-3. (A.) Means for final body weight (kg) of steers follow 120 d feeding period. between treatments at start of feeding trial (0d), and **(B.)** Means for hot carcass weight (kg), **(C.)** dressing percentage (%). Data represent LS means ± SE. Means are considered significantly different at $P < 0.05$. Means lacking common letters differ.

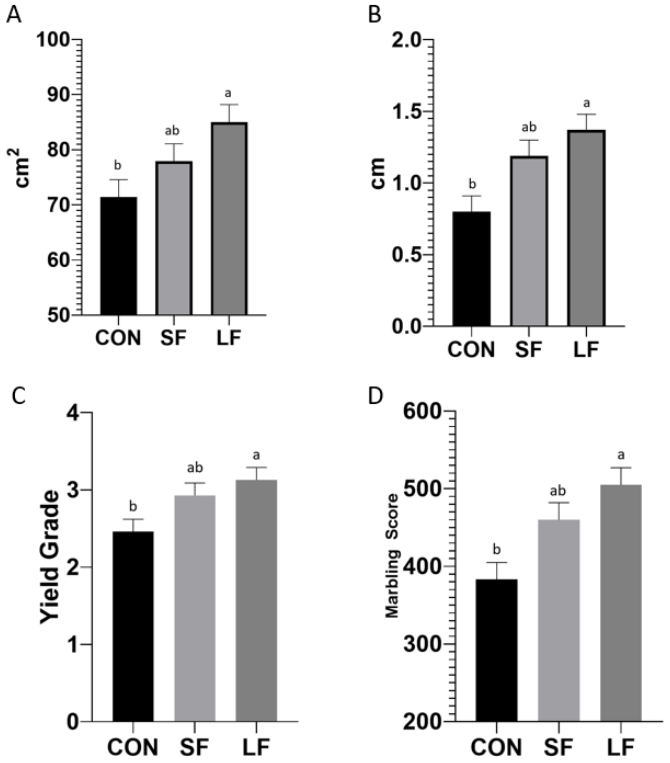

Figure 4-4. (A.) Means for ribeye area (cm2), **(B.)** 12th rib fat thickness (cm), **(C.)** yield grade, and **(D)** marbling score (200 = traces, 300 = slight, 400 = small, 500 = modest, 600 = moderate) between treatments. Data represent LS means ± SE. Means are considered significantly different at $P < 0.05$. Means lacking common letters differ.

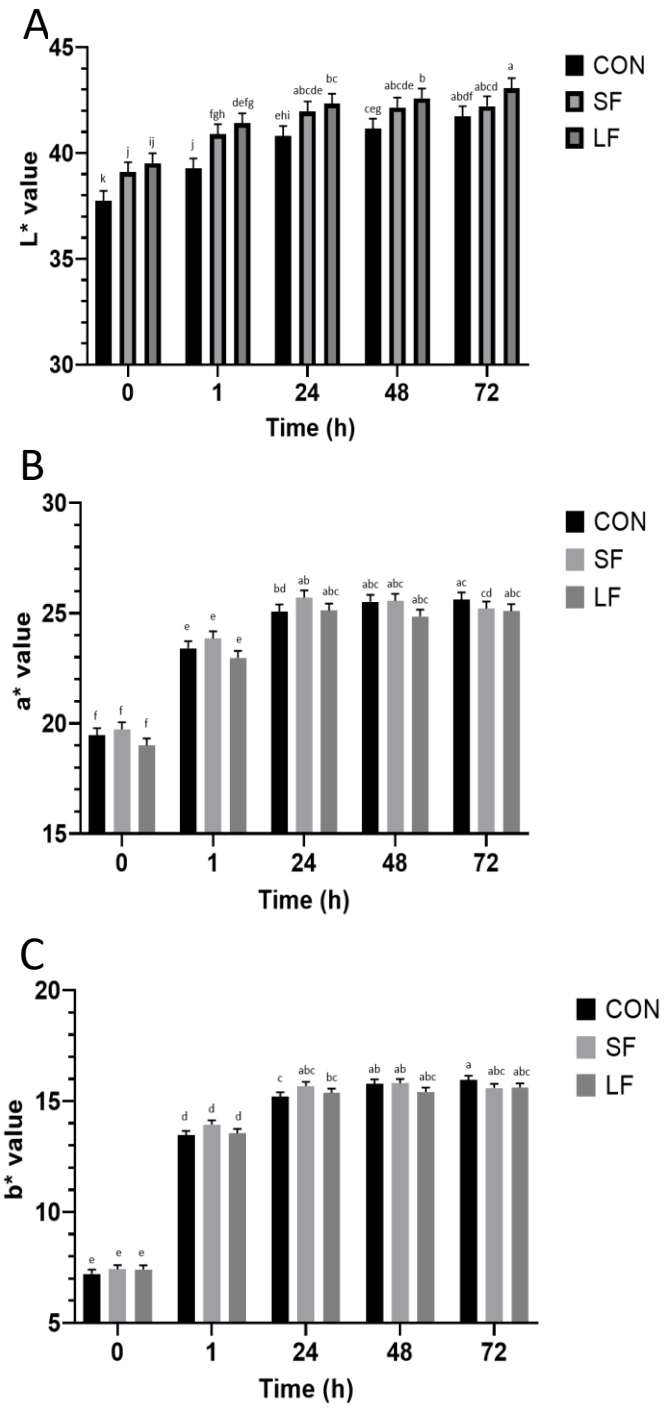

Figure 4-5. (A.) Means of lightness (L*), **(B.)** redness (a*), and **(C.)** yellowness (b*) values of LL at over 72 h display between treatments. Data represent LS means ± SE. Means are considered significantly different at $P < 0.05$. Means lacking common letters differ.

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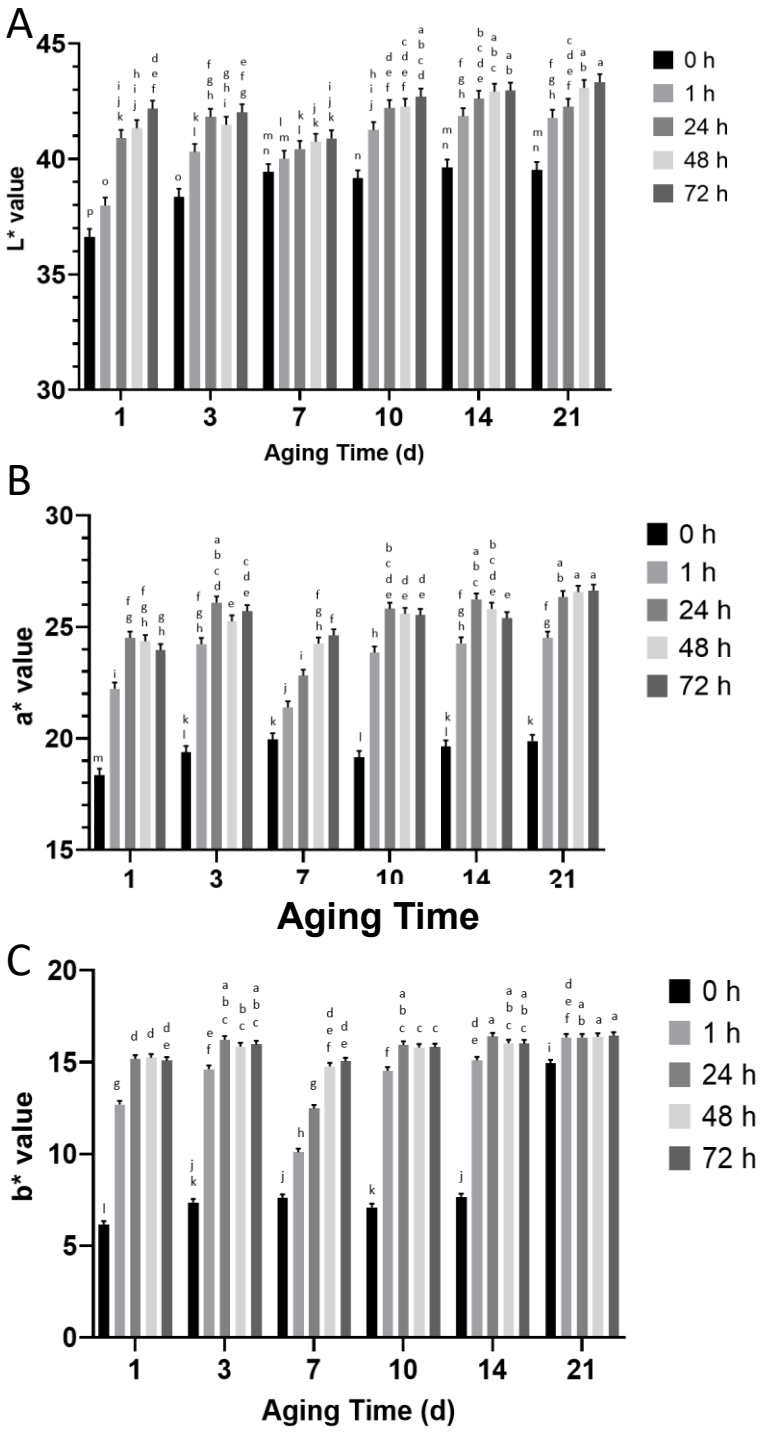

Figure 4-6. (A.) Means of lightness (L*), **(B.)** redness (a*), and **(C.)** yellowness (b*) values of LL over 21 d aging period between treatments. Data represent LS means ± SE. Means are considered significantly different at $P < 0.05$. Means lacking common letters differ.

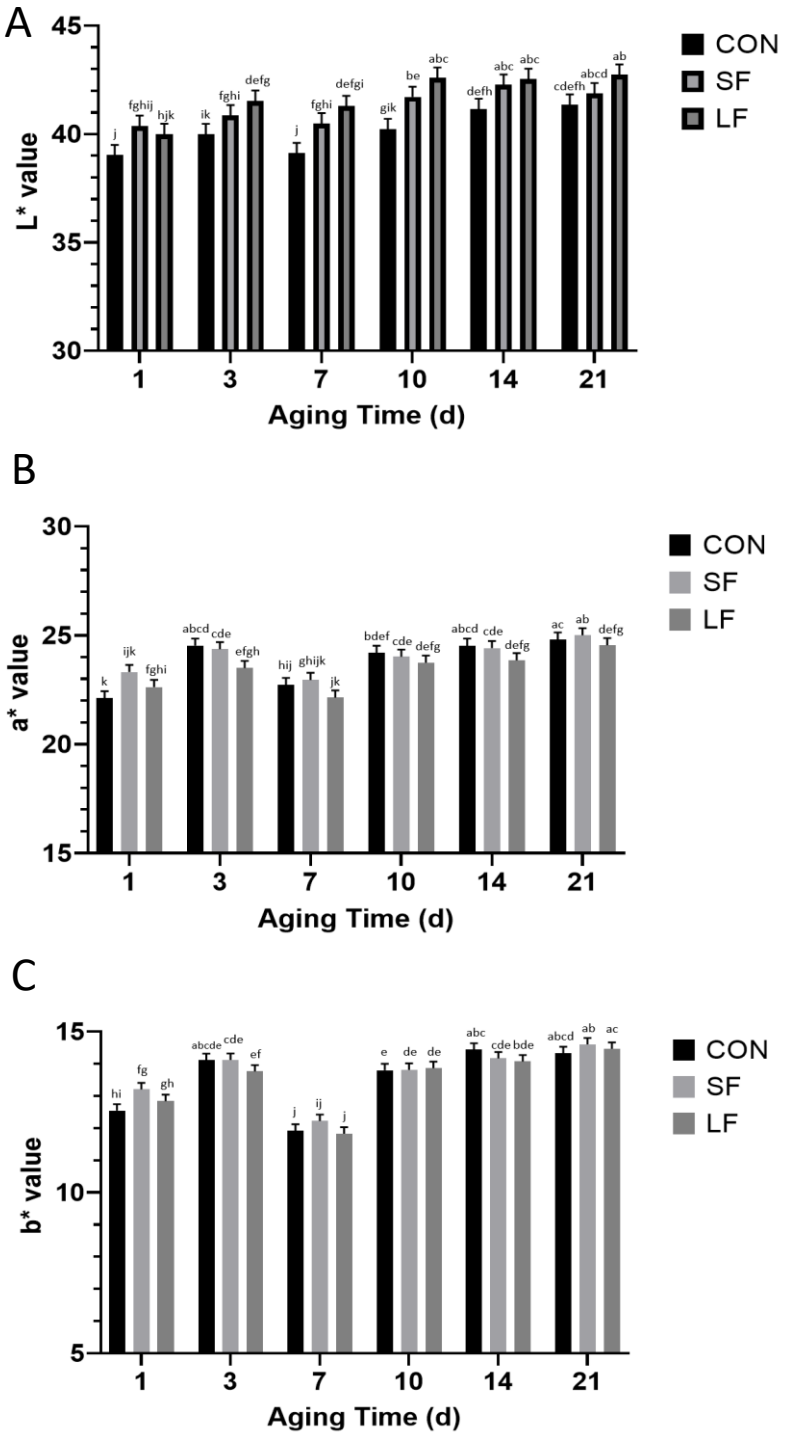

Figure 4-7. (A.) Means of lightness (L*), **(B.)** redness (a*), and **(C.)** yellowness (b*) values of LL at over 21 d aging period and 72 h display between treatments. Data represent LS means ± SE. Means are considered significantly different at $P < 0.05$. Means lacking common letters differ.

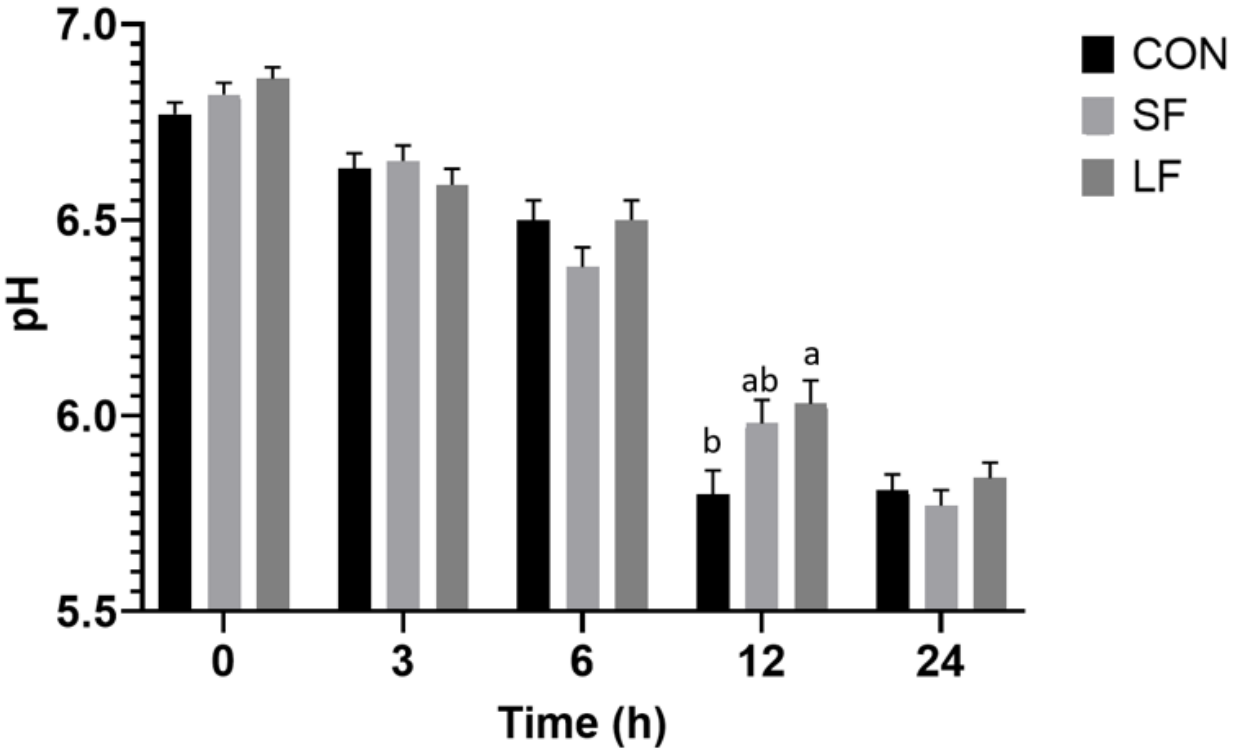

Figure 4-8. Means of pH values from the LL at across time postmortem. Data represent LS means ± SE. Means are considered significantly different at $P < 0.05$. Means lacking common letters differ.

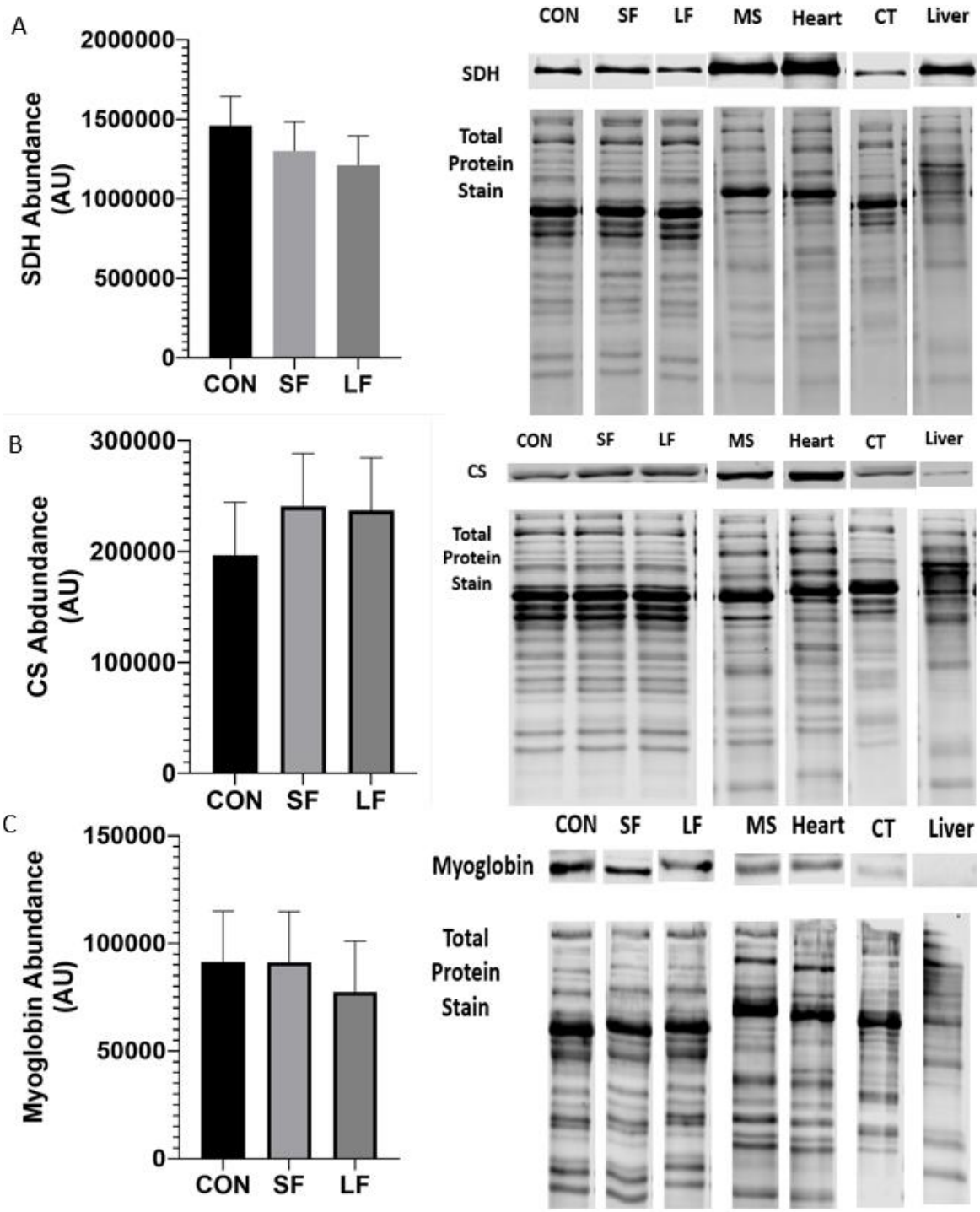

Figure 4-9. (A.) Relative abundance of succinate dehydrogenase (SDH), **(B.)** citrate synthase (CS) and **(C.)** myoglobin in *longissimus muscle* (LL) of steers subjected to CON, SF, and LF feeding regimes. Data represent LS means ± SE. Means are considered significantly different at $P < 0.05$.

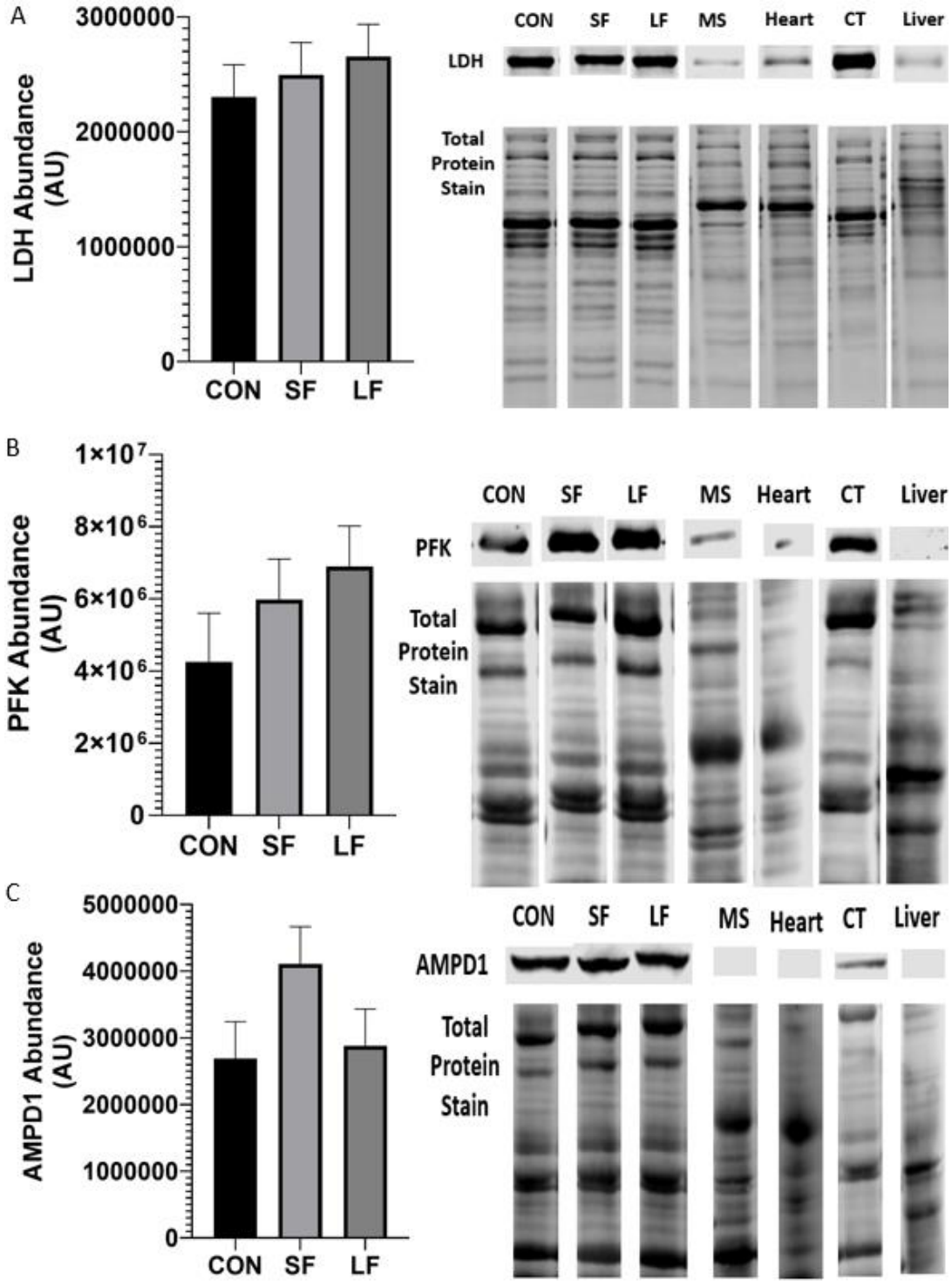

Figure 4-10. (A.) Relative abundance of LDH dehydrogenase (LDH), **(B.)** phosphofructokinase (PFK) and **(C.)** adenosine monophosphate deaminase 1 (AMPD1) in *longissimus muscle* (LL) of steers subjected to CON, SF, and LF feeding regimes. Data represent LS means ± SE. Means are considered significantly different at $P < 0.05$.

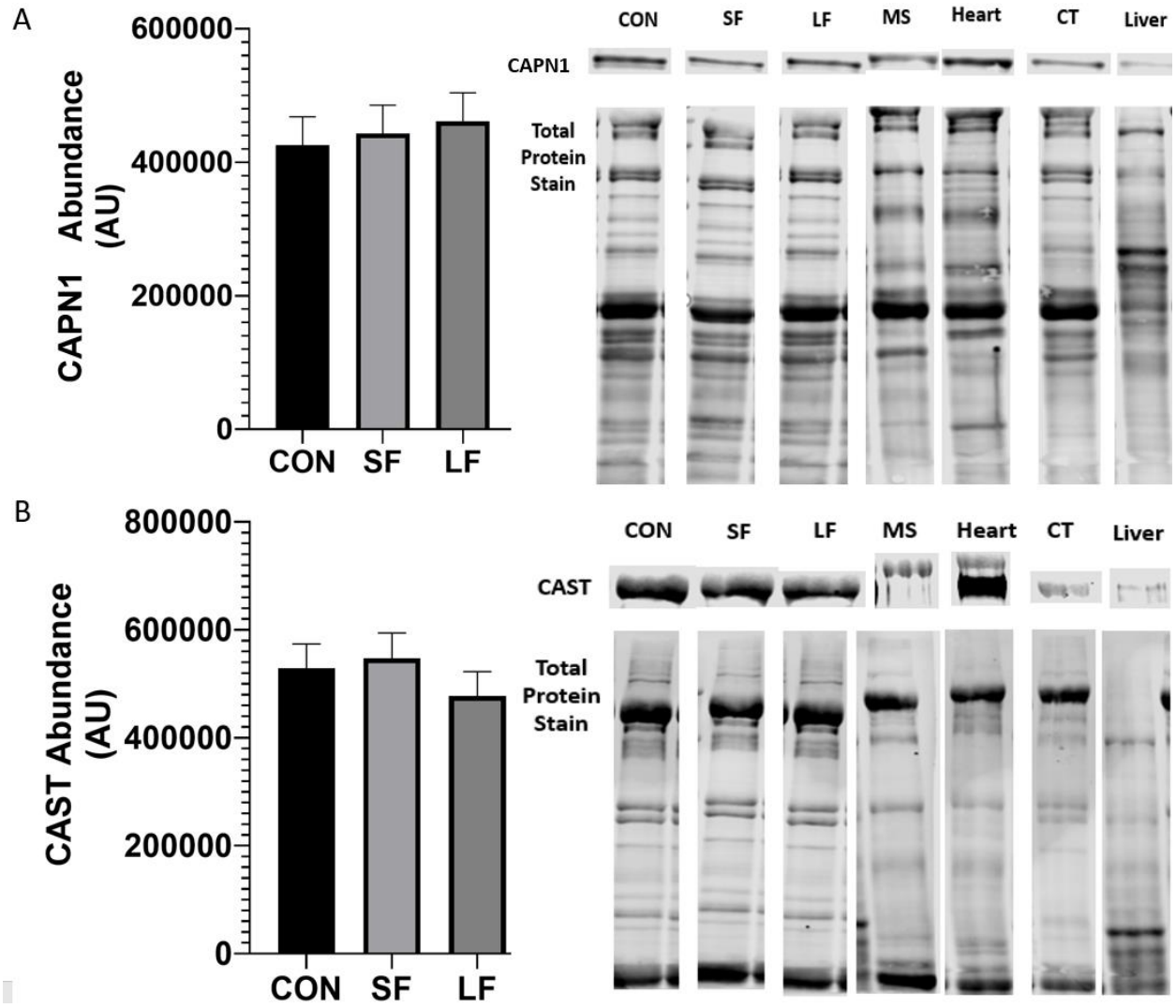

Figure 4-11. (A). Relative abundance of calpain-1 (CAPN1) and **(B.)** calpastatin (CAST) in *longissimus muscle* (LL) of steers subjected to CON, SF, and LF feeding regimes. Data represent LS means ± SE. Means are considered significantly different at $P < 0.05$.

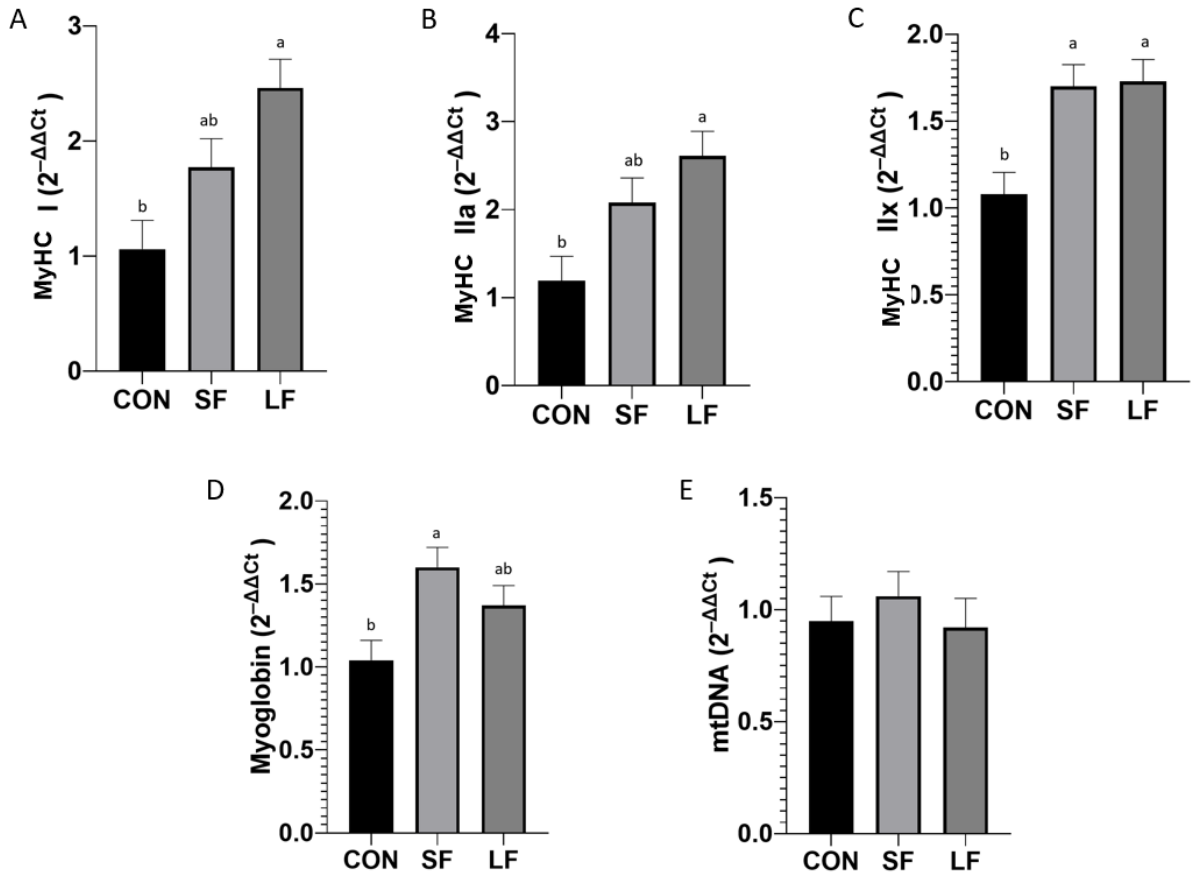

Figure 4-12. Means of gene expression of **(A.)** myosin heavy chain type I (MyHC-I), **(B.)** myosin heavy chain type IIA (MyHC-IIA), and **(C.)** myosin heavy chain type IIX (MyHC-IIX). **(D.)** Mitochondrial (mt) DNA copy number relative to genomic (g) DNA copy number and **(E.)** myoglobin gene expression between treatments and presented as fold differences. Data represent LS means ± SE. Means are considered significantly different at $P < 0.05$.

Table 4-1. Primer sequence used in quantitative reverse transcription – PCR assays.

Gene Name	Sequence
MHC 1	F5: AAA-GCT-AGC-CCA-GCT-GAT-TAC R5: CTC-TCT-CCT-CTC-CAC-CAT-CTT
MHC IIA	F1: TCT-GAA-CTC-TGC-TGA-CCT-ACT-C R1: CTG-CAT-TGG-TTA-CCT-GCT-CTA-C
MHC IIx	F5: AAA-GCT-AGC-CCA-GCT-GAT-TAC R5: CTC-TCT-CCT-CTC-CAC-CAT-CTT
Myoglobin	F1: CAG-GCT-CTT-CAC-AGG-TCA-TC R1: CTT-CAT-CTC-AGC-CTC-TGT-CTT-C
S18	F5: GCG-AGT-ACT-CAA-CAC-CAA-CAT-C R5: CCT-CAA-CAC-CAC-ATG-AGC-ATA-TC

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Chapter 5. Muscle characterization and meat quality of growing beef cattle

Abstract

Cattle reared under differing management practices, such as grain-finish versus grass-finished often differ in ultimate quality, as does beef derived from cattle of different ages, climates, and genetics. Even so, a large portion of the US cattle inventory are managed under similar conditions, yet variations in beef quality still persist. Therefore, further exploration of variation in beef quality of conventionally finished beef is needed, rather than isolating quality research to only extremes in spectrums. Using both targeted single variable methods, as well as transcriptomics, we aimed to identify underlying mechanisms controlling beef quality development in cattle from a conventional management approach. Thirty-six Angus crossbred steers were harvested at weights of 317 kg, 400 kg, 498 kg, or 589 kg (n = 9 each). Carcass yield, quality grades, and color were analyzed, as well as hallmark biomarkers known for their contribution in beef quality development. Moreover, RNA-Seq was conducted in effort to understand better those genes that aid in beef cattle growth and quality. No differences were observed in L* or pH, however a* increased beef from heavier cattle. Additionally, protein abundance of hallmark indicators credited for quality development such as myoglobin, CAPN1, and CAST, did not differ with weight. Moreover, metabolic indicators such as SDH, CS, and PFK also did not differ over weight gain. However, LDH was significantly decreased, sharing a negative relationship with weight gain. Expression of myosin heavy chain isoforms (MyHC I, IIA, and IIX) and myoglobin were impacted by growth. These data largely held when compared to transcriptomics data. Even so, 8 genes were identified to have linear relationships with weight gain, with CA3 and FLH resulting in positive relationship. When taken together, our data suggest minimal differences exist between hallmark biomarkers known for contributing to meat quality and suggest other factors play a role in disparity of quality attributes of cattle reared under similar conditions.

Introduction

Beef quality has been the focus of meat science research for some time, as quality proves to be an important factor in sustaining the industry (Andersen, Oksbjerg, Young, & Therkildsen, 2005). Remarkable breakthroughs have been made in understanding quality development, which have been successfully translated into practical application in both production management, and meat processing; greatly improving efficiencies, yields and quality for the industry. However, much of this discovery has been a result of single variable research focused on inequalities of extreme spectrums, such as grass versus grain-fed beef (Apaoblaza et al., 2020), pale soft and exudative meat (PSE) versus normal (Bowker, Wyneen, Grant, & Gerrard, 1999), or tough versus tender beef (Laville et al., 2009). Still, these studies offer insight into underlying mechanisms controlling quality development, such as energy metabolism, rate and extent of pH decline, as well as factors that contribute to postmortem proteolysis. Moreover, these studies and others like them have identified critical biomarkers that have become hallmark indicators for quality-based research, such as the abundance of oxidative versus glycolytic proteins, myoglobin for color, and calpain-1 for tenderness (Lee, Joo, & Ryu, 2010; Mancini and Hunt, 2005, Warner et al., 2022). There is strong evidence to support the aforementioned contributors can be vastly increased or decreased and alter meat quality. Still, a large percentage of beef produced in the US does not experience drastic conditions that would warrant adverse meat quality, yet 15% of beef does not meet consumer expectations of color (Killinger, Calkins, Umberger, Feuz, & Eskridge, 2004), while another 23% fails to achieve an acceptable level of tenderness (Gonzalez, & Phelps, 2018) These inconsistencies in quality are costly to the industry and waste valuable resources, as much of this product is heavily discounted or even discarded (Zerby et al., 1999). However, with a growing population that must be fed, coupled with the escalating cost of feeding cattle, producing

beef that meets or exceeds consumer expectations is pivotal, warranting further efforts in beef quality research.

Great effort has been made to ensure a consistent high-quality beef, however, beef production is complex and is influenced by an ever-growing range of interactions. In an attempt to unravel or study such a wide variety of variables, foodomics methods have been employed over recent years. The term “foodomics” encompasses metabolomics, lipidomics, proteomics and transcriptomics. When combined, these relatively new technologies allow for high-throughput analysis, outlining critical biomarkers and metabolic pathways that influence health, nutrition, and quality of meat (Balkir, Kemahlioglu, & Yucel, 2021, Valdés, et al., 2021). Specifically, transcriptomics has proven to be a useful tool as it depicts the transcription of DNA, which offers a real time “snapshot” of those genes in muscle tissues at a given time. After all, meat quality is largely predicated on the state of tissue at the time of death, as substrate availability and energy metabolism greatly influence meat quality development (Wicks et al., 2019). Technologies capturing the global expression of genes in muscle tissue at harvest may prove beneficial for gaining a deeper understanding of biochemical processes that drive both the growth of muscle and development of high-quality beef. Transcriptomics has the ability to identify the same pillar indicators traditionally measured in fresh beef quality studies, or identify novel genes yet to be fully explored. It is a promising tool and could be used to further understand the variability in product quality of cattle reared under conventional finishing practices. This line of research is essential for the future of the industry, as we strive to produce beef that meets consumers expectations in regard to color, marbling, and tenderness. Therefore, the objective of this project was to evaluate both the traditional hallmark biomarkers using standard methods of data collection and analysis, compared those data to data retrieved from transcriptomics, in effort to better

understand if traditional biomarkers are as influential in quality differences of conventionally fed-cattle as they are in explaining inequalities of extreme contrast.

Materials and Methods

Animals and Sample Collection

Thirty-six Angus crossbred steers were conventionally fed and 9 steers each were harvested at weights of approximately 317 kg, 400 kg, 498 kg, or 589 kg. Randomly selected steers were transported to the Virginia Tech Meat Center where they were humanely harvested under Food Safety and Inspection Service regulations. Immediately following exsanguination, muscle samples were taken from the *longissimus lumborum* (LL). Samples were diced, snap frozen in liquid nitrogen and stored at −80 °C until further analysis.

Carcass Evaluation and Color Analysis

After a 24 hr chill (2 ± 1°C), carcasses were ribbed between the 12th and 13th ribs and carcass data were gathered according to American Meat Science Association (AMSA, 2001) guidelines. Measurements of hot carcass weight (HCW), ribeye area (REA), 12th rib fat thickness (FT), and estimated percentage kidney, pelvic, and heart fat (KPH) were used to calculate yield, while marbling score, and carcass maturity were used to determine quality grade. Moreover, following a 30 min bloom period, subjective color was measured at the surface of the 12th rib. Using a Minolta CR-400 colorimeter (Ramsey, NJ, USA), Illuminant D, 0° observer angle, triplicate color measurements were taken and an average color value was expressed as Commission Internationale de l'Éclairage (CIE) L*(lightness), and a* (redness).

pH Analysis

Using a Qiagen TissueLyser II (2 min at 25 Hz), frozen powder tissue was homogenized in a 1:8 (w:v) buffer (5 mM Na-iodoacetic acid and 150 mM). Homogenized samples were heated for

5 min at 25°C, centrifuged at 13,000x g for 5 min and heated for 1 min at 25°C. Finally, pH was using a calibrated Orion Ross Ultra pH electrode (Thermo Scientific, Pittsburgh, PA).

Protein Extraction and SDS-PAGE and Immunoblotting

Approximately 100 mg of tissue (LL, heart, masseter (MS), cutaneous trunci (CT), liver) were solubilized in 1 mL of buffer as described by Warren et al. (2003). Homogenized samples were diluted 1:20 and used for total protein quantification using Reducing Agent and Detergent Compatible (RCDC) Protein Assay (Bio-Rad Laboratories, Hercules, CA, USA), according to manufacturer's specifications. Samples were diluted in an extraction buffer with 0.05% bromophenol blue added to a final concentration of 3 mg/mL. All samples were stored at −80°C until further analysis.

Samples were heated at 60 °C for 5 min and separated by SDS-PAGE (7%, 10%, 15% or 18% acrylamide). Proteins were transferred to nitrocellulose membranes, blocked overnight at room temperature, in either Prometheus™ OneBlock™ Blocking buffer (Genesee Scientific Corporation, El Cajon, CA) or 5% non-fat dry milk with Tris-buffered saline solution with 0.1% tween-20 (1× TBS-T) added. Additionally, primary antibodies were diluted in respective blocking buffer and allowed to incubate overnight at room temperature. Specific antibodies for lactate dehydrogenase-A (LDH; Novus NBPI48336 at a 1:30000 dilution), phosphofructokinase-1 (PFK; Santa Cruz Biotechnology, Inc, SC-166722 at 1:1000 dilution), succinate dehydrogenase-A (SDH-A; Abcam ab14715 at a 1:1000 dilution), citrate synthase (CS; Santa Cruz Biotechnology, Inc, SC-390693 at 1:1000 dilution), calpain-1(CAPN1; Thermo-Fisher 9A4H8D3 at 1:1000 dilution), calpastatin (CAST; Thermo-Fisher 1F7ED10 at 1:1000 dilution), and myoglobin (Santa Cruz Biotechnology, Inc, SC-25607 at 1:1000 dilution) used and normalized to total protein (Revert 700 Protein Stain, Li-Cor Inc., Lincoln, NE). Following washing (1× TBS-T) secondary antibodies

(IRDye α-IgG secondary antibody; LI-COR Biosciences, Lincoln, NE) were applied to blots and allowed to incubate for 1 h. All blots were imaged using a LI-COR Biosciences Odyssey Infrared scanner (Li-Cor, Inc., Lincoln, NE, USA). Protein band intensity was measured using Image Studio lite (Li-Cor, Inc., Lincoln, NE, USA) with protein abundance reported as arbitrary units (AU).

Gene Expression

Total RNA was extracted using the Direct-zol RNA Mini Prep Kit (Zymo Research, Irvine, CA). Twenty ng/μL of total RNA was reversed transcribed using the High-Capacity cDNA Reverse Transcriptase Kit (Applied Biosystems, Waltham, MA). Two μl of cDNA was for used for amplifying gene specific primers (Table 5-1) and SYBR chemistry in a 7500 Fast Real-Time PCR System (Applied Biosystems, Waltham, MA) for the quantification of myoglobin and myosin heavy chain isoforms. Relative gene expression was calculated by the $2^{-\Delta\Delta Ct}$ method.

RNA Extraction and Library Preparation for RNA Sequencing

RNA was extracted using Quick-RNA Mircoprep Kit (Zymo Research Irvine, CA, USA) per manufacturer's instructions. Sample nucleic acid quantity and quality was measured using the Nanodrop™ 2000 (Thermo Scientific, Pittsburg, PA, USA). All samples were diluted to a final concentration of 10 μg/ul of RNA and stored at −80 °C until shipped to Novogene. Libraries were prepared from messenger RNA purified by poly-T oligo attached to magnetic beads, followed by fragmentation. The first strand cDNA was synthesized using random hexamer primers, followed by the second strand cDNA synthesis using dUTP for the directional library (Parkhomchuk et al., 2009).

Alignment and Transcript Quantification

Sequences were aligned to the cattle reference genome (Bos_taurus.ARS-UCD1.2.105) obtained from Ensembl (Flicek et al., 2014) with hisat2 (v2.2.1; Kim, Langmead, & Salzberg, 2015) using parameters to increase sensitivity (--bowtie2-dp 2 --score-min L, 0, -1). Next, duplicates were removed using the function bammarkduplicates from biobambam2 (Tischler, & Leonard, 2014), and filtered alignments that were not primary, were less than 100 nucleotides long, and had more than 5% mismatches using samtools (Danecek et al., 2021). Lastly, fragments relative to the Ensembl cattle annotation (Bos_taurus.ARS-UCD1.2.105.gtf; Flicek et al., 2014) were counted using the function featureCounts from subread (v2.0.1; Liao, Smyth, & Shi, 2014).

Differential transcription abundance

A matrix was generated with fragment counts in R software (R Core Team 2021). Next, genes were filtered to retain only protein-coding genes and long non-coding genes for further analysis. Fragments per million per kilobase (FPKM) and counts per million (CPM) were enumerated using the functions 'rpkm' and 'cpm' from the R package 'edgeR' (Robinson, McCarthy, & Smyth, 2010). Genes that had more than one FPMK and one CPM in at least nine samples were filtered. For plotting transcript abundance, transcript per million (TPM) counted using the formula presented in Li, & Dewey (2011), accounting for a normalization factor obtained by the trimmed mean of M values (Robinson, & Oshlack, 2010).

Differential transcript abundance was enumerated by contrasting each group of samples using the quasi-likelihood F test in the 'edgeR' (Lun, Chen, & Smyth, 2016) R package, and the Wald method in the 'DESeq2' (Love, Huber, & Anders, 2014) R package. In both cases, nominal P value was adjusted using the false discovery rate (Benjamini, & Hochberg, 1995) method to account for multiple hypothesis testing. Statistically significant differential transcript abundance was inferred when FDR < 0.05 in both algorithms. Linear regression was developed using quasi-

likelihood F test in the 'edgeR' to identify genes with a progressive increase or decrease in transcript abundance in relation to their weight gain.

Gene ontology analysis

Enrichment or gene ontology categories were generated using "goseq" package (Young, Wakefield, Smyth, & Oshlack, A. 2010) in R software. Genes whose transcript abundances were estimated for the samples being tested as the background were used. Nominal P values were adjusted for multiple hypothesis testing using the family wise rate (FWER, Benjamini, & Hochberg, 1995).

Statistical Analysis

Carcass, color, pH, protein abundance, and gene expression generated using qPCR were analyzed using the Proc Mixed procedure using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). Carcass was the experimental unit and the statistical model included the fixed effects of treatment, with harvest day as a random variable. Means were compared using Tukey-Kramer Multiple Comparison Test if a significant effect was detected. Data on graphs are least square means ± standard error means (SEM), and differences were considered significant at $P < 0.05$.

Results

Carcass Evaluation Color, and pH Analysis

To evaluate differences in meat quality development over the growth phase of cattle, randomly selected groups of steers were harvested at varying weights during the growing and finishing phases. As such, final body weight and HCW differed with slaughter group (Fig. 5-1A; $P < 0.001$, Fig. 5-1B; $P < 0.001$, respectively). Surprisingly, the use of this model revealed that other carcass factors used in determining yield and quality do necessarily increase at the same rate as weight. Specifically, minimal differences were noted between 317 kg and 400 kg carcasses in

REA, with 400 and 489 kg following a similar trend. Still, REA increases from 317 and 498 kg cattle, with 589 kg showing the greatest increase compared to other weight groups. (Fig. 5-1d; $P < 0.001$). This same relationship was also noted in DP (Fig. 5-1c; $P < 0.001$). However, FT, KPH and yield grade separated into differences between lighter and heavier weight cattle, with greater values for heavier steers (Fig. 5-1e; $P < 0.001$, Fig. 5-2f; $P = 0.030$, Fig. 5-1g; $P = 0.004$, respectively). Marbling score showed improvement with weight (Fig. 5-1h; $P < 0.001$), 498 kg steers produced carcasses that averaged marbling scores between slight and small, compared 317 and 400 kg groups which remained in the traces category. As expected, 598 kg steers produced carcasses with the highest marbling, and entering into the modest category.

Following a 30 min bloom period, subjective color was measured. There was no difference in lightness (L* value; Fig. 5-2a; $P < 0.262$), however redness (a* value) significantly increased between lighter weight cattle (317 kg and 400 kg) and 589 kg weight group (Fig. 5-2b; $P < 0.001$). We found no difference in pH across treatments at 24 h (Fig. 5-3; $P = 0.535$).

Protein Abundance and Gene Expression

Although differences were established in growth, yield and quality grades (Fig. 5-1), only minor differences were observed in both oxidative and glycolytic proteins. Specifically, we found no significant differences, in PFK (Fig. 5-6b; $P = 0.429$), SDH (Fig. 5-6c; $P = 0.366$), CS (Fig. 5-6d; $P = 0.977$), myoglobin (Fig. 5-4a; $P = 0.361$), CAPN1(Fig. 5-7a; $P = 0.553$), or CAST (Fig. 5-7b; $P = 0.651$). Surprisingly, LDH showed a negative relationship with increased weight, and a significant difference between 317 and 589 kg steers was established (Fig. 5-6a; $P < 0.05$).

Myosin heavy chain isoforms (MyHC) I, IIA, and IIB differed between treatments revealing 589 kg cattle possessed the greatest expression of MyHC IIA (Fig. 5-5b; $P = 0.033$), yet the least amount of expression of MyHC I, and IIX (Fig. 5-5a; $P = 0.046$, Fig. 5-5C; $P = 0.006$, respectively). Expression for all MyHC isoforms remain relatively the same for between 317, 400,

and 498 kg weight groups. Similar differences were noted in myoglobin which revealed a significantly lower level of expression in tissue from 589 kg steers compared to other treatments (Fig. 5-4b*; $P < 0.001$*). In an effort to validate our protein and expression data, values from our RNA-Seq were evaluated for the aforementioned, as well as others (Table 5-2). Myoglobin was upregulated in all weights compared to 317 kg, while MYH7 (MyHC1) was greater in muscle of the heaviest group compared to that of smaller steers. Moreover, MYH2 (MyHC IIA) was down-regulated in 498 and 589 kg cattle compared to that of the 317 kg group.

Differential Transcription Abundance

After filtering, we retained an average of 8,564,182 fragments (± 4318951) per sample mapped to the genome and used for gene counting. An average of 6,152,144 fragments (± 3159343) were assigned to gene models and used for transcript quantification. After filtering lowly expressed genes, we quantified the transcript abundance of 11,884 genes (11,693 protein-coding genes and 191 long non-coding genes).

Altogether, there were 695 genes with differential transcript abundance in all five contrasts evaluated (*FDR < 0.05*, Fig. 5-8a). There was limited overlapping of genes with differential transcript abundance among the contrasts accounting for consecutive weight classes (Fig. 5-8b). Lastly, only ten genes overlapped when comparing the transcript abundance between the weights 400 kg, 498 kg, and 589 kg versus 317 kg (Fig. 5-8c). We also identified 236 genes with a trend (FDR < 0.01) to either increase (N = 46) or decrease (N = 190) transcript abundance with the increase in weight at sample harvest (see Table 3 for some examples). Interestingly, the following biological processes were significantly enriched (*FWER < 0.1*) among the 236 showing co-linearity with weight: ‘cellular response to fibroblast growth factor stimulus’, ‘chorio-allantoic fusion’, ‘electron transport chain’, ‘positive regulation of intracellular mRNA localization’, and

‘regulation of keratinocyte apoptotic process’ (Table 5-3). Notably other categories biologically relevant to muscle biology where also represented among those genes (i.e.: ‘positive regulation of myotube differentiation’, ‘regulation of stem cell proliferation’, ‘negative regulation of transcription by RNA polymerase II’, and ‘skeletal muscle satellite cell differentiation’; Table 5-3).

Discussion

As muscle experiences hypertrophy, fibers shift from slow oxidative to fast glycolytic (Blaauw, Schiaffino, & Reggiani, 2013; Lefaucheur, & Gerrard, 2000). This shift encompasses a change in function, consequently altering the abundance of regulatory proteins and use of metabolic pathways. This difference in muscle fiber type, which is largely classified by contractile speed, and energy metabolism, dictates postmortem glycolysis and the overall conversion of muscle to meat. Specifically, difference in energy metabolism alter the rate and extent of pH decline postmortem, consequently influencing meat quality development (Wicks et al., 2019; Wang, Matarneh, Gerrard, & Tan, 2021; Scheffler, & Gerrard, 2007). Oxidative muscle arrests glycolysis early, hindering color development (Mancini, Belskie, Suman, & Ramanathan, 2018), and has also shown to impede proteolytic activity postmortem, resulting in tougher meat (Thompson, 2002). On the other hand, muscles that are primarily glycolytic tend to have a more complete acidification, creating acceptable color and improving proteolytic function. Moreover, degree of marbling, which heavily influences overall quality grade, is generally increased in fed-cattle with more glycolytic fiber type (McIntyre et al. 2009; Gómez et al. 2022). These quality attributes are influenced by many extrinsic and intrinsic factors, however, the ideologies of meat quality are predicated on energy metabolism, and myoglobin and calpain-1 abundance (Wicks et al., 2019). Following lines of this central dogma, beef that meets or exceeds consumers

expectations of both visual quality and tenderness, should emanate from cattle possessing predominately glycolytic fibers, thus decreasing the need for higher levels of myoglobin, and greater CAPN1 to CAST ratios. Albeit, this is often the result of comparing cattle with extremes growth rates or quality characteristics (Apaoblaza et al., 2020, Gómez et al., 2022; Wright et al. 2018). However, in the most recent US Beef Quality Audit (Boykin et al., 2016), large variations in quality and yield exist among cattle of likely similar management conditions. Therefore, we need a better understanding of how differences in beef quality is possible during conventional finishing practices. To that end, the present study aimed to characterize growing muscle as it relates to beef quality, in effort to identify biomarkers responsible for growth and quality development.

Meat Color

Meat color is largely a function of the pigment protein myoglobin, and it is well understood that an increased abundance of myoglobin results in a redder lean (a*; Kim et al., 2010). Myoglobin is needed in all muscle to transfer of oxygen to the mitochondria, yet levels vary, with substantially more myoglobin found in oxidative muscles compared to that of glycolytic (Lawrie, 1952). Given our current understanding of muscle growth, which shifts towards IIX and IIB fibers, it would be expected to see decreased myoglobin and less red lean in growing tissue, which has been shown in both pork (Kim et al., 2010) and beef (Wegner et al., 2000). However, perceived color is complex and can be influenced by other factors other than myoglobin abundance alone (Purslow, Warner, Clarke, & Hughes, 2020). Rather, color can be significantly altered by the muscles' oxygen consumption rate (OCR), or the availability of oxygen to bind to myoglobin, which competes with mitochondria (Mancini, Belskie, Suman, & Ramanathan, 2018). To that end, our data show a significant increase in a* (redness; Fig. 5-2b; $P < .001$) in muscle of heavier

weight steers despite lighter weight cattle having increased expression of type I fibers compared to 589 kg group (Fig. 5-5a; *P = 0.046*). Similar results have been reported by Apaoblaza et al. (2020), Gómez et al. (2022) and Vestergaard et al. (2000), all of which found increased redness in heavier cattle, despite difference in energy metabolism and myoglobin abundance, which tended to favor extensively fed cattle. However, unlike the aforementioned, myoglobin abundance (Fig. 5-4a; *P = 0.316*) parallels that of a* in the present study. Albeit, not significant, still it strengthens the argument that differences in OCR, especially given that a significant difference exists in our qPCR myoglobin expression data, with 589 kg having an inverse relationship to that of protein abundance (Fig. 5-4a, b). Most times, transcription and translation are highly correlated in a positive manner. However, transcription occurs in the nucleus and translation occurs in the cytoplasm in eukaryotes, leading to a spatial and temporary disconnect that may explain the inverse relationship our data show between protein abundance of myoglobin and gene expression (Ralston, 2008). Our transcriptomics data show myoglobin decreases (Table 5-2) across all treatments when compared to 317 kg (baseline), which aligns with the aforementioned reasoning. If our theory is true and myoglobin transcription and translation share an inverse relationship, it aligns with the fundamental understanding that as cattle age, pigment is increased (Seideman, Cross, Smith, & Durland, 1984). Although this difference in color is more readily seen in cattle over 30 months, Kopuzlu et al. (2018) determined a* can significantly increase as early as 19 months and aligns well with our data.

Color is 3-dimensional and accounts for both redness and yellowness. However, arguably the most important in regards to beef color is lightness. Lightness, unlike redness, is minimally influenced by myoglobin concentration (MacDougall & Jones, 1981). Instead, lightness is a result of the degree in which light is scattered on the surface of meat, and is largely influenced by pH

decline postmortem. In fact, Hughes et al. (2017, 2018) found that muscle fibers shrink in diameter as pH declines. This disruption of the structural lattice allows for an increase in extracellular space between muscle bundles, thus increasing free water, and reflection of light. However, when pH remains high, muscle remains tightly bound, holding water and refracting light, giving meat a dark appearance. The latter is often the result in beef and has proven to cost the industry billions over decades. Although extreme cases of dark cutting have been fully explored and largely resolved with improvements in animal welfare, dark lean still remains, especially in areas of the world where extensive feeding systems are widely used (McGilchrist, Perovic, Gardner, Pethick, & Jose, 2014; del Campo et al., 2013). Moreover, anomalies such as atypical dark cutting beef, characterized by dark lean despite not having absurdly high pH, has becoming increasingly problematic (McKeith et al., 2016; Kirkpatrick et al., 2023; Mahmood, Turchinsky, Paradis, Dixon, & Bruce, 2018). This suggests that color development is yet to be fully understood. Others have studied this meat quality trait in an effort to understand such disparities (Ribeiro, Contreras-Castillo, Santos-Donado, & Venturini, 2021; Ponnampalam et al., 2017). Although our data show no difference in L*, our understanding of color development has improved. This is based on the correlation of pH and lightness, as well as the requirements needed to significantly alter L* values (lightness). Both rate and/or extent of pH decline must deviate from the normal acidification process (Briskey et al., 1966). The extent of pH decline is largely dictated by sufficient levels of glycogen (Henckel, Karlsson, Jensen, Oksbjerg, & Petersen, 2002), however when adequate glycogen levels are achieved, pH decline is regulated by maintaining PFK-1 activity. This functions as the "gatekeeper" to glycolysis, allowing glycogen to be readily converted into lactate and H^+, thus lowering pH (England, Matarneh, Scheffler, Wachet, & Gerrard, 2014). However, PFK-1 activity begins to lose function at a pH of approximately 5.9, with complete inactivation at

5.5 (England et al., 2014). Of course, other factors such as buffering capacity (Van Laack, Stevens, & Stalder, 2001) and depletion of adenine nucleotides (England et al., 2016) have shown to influence the conversion of muscle to meat. This suggests muscle fiber type is important. Therefore, we evaluated metabolism based on abundance of both oxidative (SDH, CS) and glycolytic proteins (LDH, PKF-1). Curiously, our data showed less abundance of LDH in heavier, higher-quality steers (589 kg) compared to that of 317 kg steers (Fig. 5-6a; $P < 0.007$). Results such as these are difficult to interpret. Much of the current literature would suggest higher quality cattle possess an increase in glycolytic fibers, typically complemented by a linear correlation with glycolytic biomarkers such as LDH (Apaoblaza et al., 2020, Antonelo, et al., 2022). However, LDH abundance in muscle is a bit of an anomaly. Oksbjerg et al. (2000) reported slow growth rate pigs, which possessed a more oxidative phenotype have increased LDH compared to pigs with increased growth rate and IIB fibers, yet still produced darker lean. While a full understanding of LDH is unclear, Brandstetter et al. (1998) found LDH decreased in aging steers beginning as early as 12 mo. and continuing until the completion of the trial (16 mo.).

Additionally, Hocquette et al., (2012) reported an increase in the oxidative enzyme isocitrate dehydrogenase (ICHD) as well as an increased abundance of MyHC I in heavier cattle. These data suggest that finishing cattle reach a plateau in growth and revert back to more oxidative muscle, as seen in mature (age) muscle. Our data, though not significant, follow closely that of Brandstetter et al. (1998), as we show increases in SDH (oxidative) with body weight. While the likelihood of fed-cattle possessing a greater glycolytic phenotype is undeniable, it is plausible that we do not fully understand this process. In fact, much of the current literature surrounding the topic is confounded by extremes in treatments, ultimately masking the entirety of muscle growth and metabolism. Therefore, due to the lack of extremes in our treatments compared to that of the

previously mentioned which tested growth rate, and bulls versus steers, respectively, it is not obscure to see such differences in LDH, which shows a decrease as cattle mature and gain weight, yet no difference in other proteins such as CS or PFK-1 (Fig. 5-6d; $P = 0.977$, Fig. 5-6b; $P = 0.429$) as tissue may simply not respond to the same degree when conventional approaches are used. With no difference in PFK-1, it is unsurprising that no difference in pH was noted between treatments, suggesting a greater difference in phenotype must exist for both pH and lightness to deviate from normal.

When comparing our single variable results with that of our transcriptomics, we find similar outcomes. Specifically, LDH, PFK-1, and CS, follow the same trends as our protein data. However, correlation between RNA-Seq data and that of our qPCR and western blot data is less apparent, especially regarding MyHC isoforms and SDH abundance. While one would expect all methods of analysis to show similar results, differences can emerge due to normalization, or specificity to primers or differences in muscles. Regardless of these slight inconsistencies, it does not change the scope of our data, which show lightness is minimally impacted as cattle mature using conventional finishing practices.

Marbling

Intramuscular fat, or marbling, refers to adipocytes dispersed between muscle fibers. The degree in which marbling occurs weighs heavily on carcass value and consumer preference. For consumers, increased marbling serves as a visual indicator of the level of 'quality' of beef and coincides with their expected eating experience (Testa, Grigioni, Panea, & Pavan, 2021). Although marbling has yet to explicitly predict eating experience (tenderness; Wulf, O'Connor, Tatum, & Smith, 1997), it is highly regarded by consumers, increasing the demand of well-finished cattle and offering premium incentives to producers. Marbling occurs later in tissue growth. Early in development, muscle accretion is greater than the rate of fat accretion. However, later in

development, muscle accretion rate slows, while fat accretion increases (Vernon, 1981). Adipocytes begin to form intramuscularly as early at 180 days into fetal development (Taga et al., 2011) with hyperplasia lasting as long as 250 days postnatally (Du et al., 2013). Following 250 days, IMF increases through hypertrophic growth, with the vast majority of expressed marbling occurring in carcasses weighing between 200-400 kg (Duckett, Wagner, Yates, Dolezal, & May, 1993). This understanding, coupled with strong evidence to support that marbling development is primarily derived from the energy substrate, glucose (Smith and Crouse, 1984), gives shape to the way in which much of the US cattle are fed.

Glucose transporter 4 (GLUT4), Peroxisome proliferator-activated receptor gamma (PPARγ) and sterol regulatory element transcription factor 1 (SREBF1) have all been linked to IMF development (Hocqutte et al. 2010; Maciel et al., 2022) and are widely recognized markers for analyzing glucose metabolism, and fat accretion (Lloyd, Steele, Valenzuela, & Dawkins, 2017; Zhou et al., 2022, Ladeira et al., 2019). Specifically, GLUT4 is an insulin-regulated glucose transporter which facilitates the uptake of glucose into muscle and adipose, giving rise to the preferred energy substrate needed for marbling. PPARγ, however, is largely responsible for insulin sensitivity, adipocyte differentiation and glucose metabolism (Ahmadian et al., 2013). On the other hand, SREBF1 is critical for lipogenesis (Shimano et al., 1999). When mTORC1 is activated by insulin, it stimulates the upregulation of the SREBF1 subunit, SREBF-1C. Upregulation of SREBF-1C allows for storage of excess nutrients such as triglycerides, thus promoting marbling (Li, Brown, & Goldstein, 2010; Gonzalez-Baró, Lewin, & Coleman, 2007).

Data presented herein reaffirm Duckett et al. (1993), which illustrate marbling is most prominent in carcasses weighing between 200 and 400 kg. Specifically, marbling remained largely unaffected between carcasses of 317 and 400 kg group steers (Fig. 5-1h; $P < 0.001$) which dressed

at a HCW of 179 and 223 kg, respectively (Fig. 5-1b; $P < 0.001$). Dressed carcasses from 498 kg group weighed 298 kg (Fig 5-1b) and were significantly increased from that of 317 and 400 kg treatment groups (Fig. 5-1b). Finally, steers from 589 kg grouped differed between all treatments achieving the highest marbling score (Fig. 5-1h).

With the current understanding of IMF development, coupled with the large difference in marbling score, our data are somewhat surprising. Although protein abundance was not measured for GLUT4, PPARγ, or SREBF1, we find little differences between increased weight groups when compared to 317 kg in our transcriptomics data set (Table 5-2). Granted, GLUT4 and SREBF1 were upregulated in all treatments compared to 317 kg cattle, with SREBF1 being significantly different in 589 kg steers to that of 317 kg steers. The increase in GLUT4 suggests an increase in glucose through diet, perhaps through greater feed intake. Moreover, 12th rib fat thickness (subcutaneous) does appear to deposit fat at the same rate as that of IMF, indicated by lack of significance between 498 and 589 kg steers. This again, suggests glucose is increased as Smith and Crouse (1984) proposed that marbling is mainly fueled by glucose, while acetate is primarily responsible for subcutaneous fat development. Still, all cattle were fed a carbohydrate rich diet, partially explaining the lack in significance, despite GLUT4 being upregulated. SREBF1 follows closely to that of our GLUT4 data. Recall, SREBF1 plays a key role in lipogenesis, and in the presence of glucose, the subunit SREBF-1c is upregulated, increasing storage of triglycerides. This offers insight into the significance of SREBF1 in steers from 589 kg weight group (Li et al., 2010) and is further supported by our marbling data.

Given the large body of literature claiming that PPARγ plays a significant role in adipocyte differentiation, as well as positive relationship with GLUT4, it would be expected to see increased expression of PPARγ, at least between the extremes in weights (317 vs 589 kg). However, our

results show the inverse, and though not significant, reveal PPARγ is downregulated in heavier, more marbled cattle. Tumor necrosis factor - alpha (TNF- α) is an inflammatory cytokine that aids in apoptosis (Holbrook, Lara-Reyna, Jarosz-Griffiths, & McDermott, 2019). Though not fully understood, it has been shown to suppress PPARγ. This is a bit perplexing, as PPARγ aids in an anti-inflammatory response. Still, Gregoire et al. (1998) proposed that TNF- α may supersede PPARγ's ability to suppress an anti-inflammatory response thus decreasing levels of PPARγ. While plausible, we found no difference in TNF- α across treatments (data not shown). However, our data revealed a consistent increase in the known binding receptor for TNF- α, tumor necrosis factor receptor 1 (TNFR1; data not shown) in heavier cattle. Moreover, TNFR1 was significantly increased between 317 and 498 kg steers. Although this increase in TNFR1 may partially explain the down regulation of PPARγ, further work is needed to better understand this complex relationship.

Meat Tenderness

Meat tenderness is central to consumer acceptability, driving eating satisfaction and repeat purchases by consumers (Grunert, Bredahl, & Brunsø, 2004). Therefore, the capability to consistently produce high-quality, tender beef is the top priority for all vested the beef industry. Numerous efforts have been made to improve strategies for effective meat maturation. Though it is yet to be fully understood, the literature suggests that CAPN1 is largely responsible for meat tenderization. CAPN1 is a cysteine and protease; when active, it targets and degrades myofibrillar proteins, ultimately disrupting muscle structure, thus improving tenderness. Although others have suggested CAPN2, CAPN3, and mitochondria may play a role in meat tenderness (Dang, et al., 2020; Ilian et al., 2001; Bhat, Morton, Mason, & Bekhit, 2018), Geesink et al. (2006) found that proteolysis was completely inhibited in CAPN1 knockout mice, affirming the essential role of CAPN1 in proteolysis. Efforts have been made to investigate the regulating mechanism of CAPN1,

namely its endogenous inhibitor CAST, and differences in fiber type, and pH decline. Oxidative fiber types have more CAPN1. However, red muscle also has the highest abundance of CAST, consequently blunting proteolysis (Ouali, & Talmant, 1990). Maddock et al. (2005) found CAPN1 to function best at a more neutral pH, however the authors also reported that CAST activity increases with rising ionic strength, indicating higher pH. When taken together, these data build a clearer understanding of the differences in limited proteolysis in red muscle. Although white muscle faces challenges in tenderization, it is better positioned for proteolysis due to its inverse relationship of the aforementioned. Additionally, white muscle sequesters Ca^{+2} more than that of red muscle (Swatland, 1977) giving rise to activation of CAPN1. Hwang and Thompson (2001) reported CAPN1 activity is heightened when an intermediate pH decline is achieved, making white muscle a more ideal contender for proteolysis and tenderization. Consistency in meat tenderness is yet to be resolved and warrants further exploration, but there is compelling evidence to indicate that CAPN1 and CAST are hallmark indicators of meat tenderness. They have been widely measured in studies of beef quality and sensory (Casas et al., 2006). Therefore, we chose to measure CAPN1 and CAST in growing tissue of conventionally finished, grain-fed cattle.

Data presented herein show no differences in CAPN1 or CAST as cattle move towards market weight (Fig. 5-7a; $P = 0.553$, Fig 7b; $P = 0.651$). This aligns with both Shi et al. (2011) and Dairoh et al. (2022) who found no relationship with CAPN1 and growth traits. Still a compelling set of evidence suggests that both muscle type and metabolism influence CLPN1 abundance and function (Ouali, & Talmant, 1990; Bhat et al., 2017). Data reported herein indicate a shift toward a more oxidative phenotype as cattle increase in weight, however, we find no substantial evidence that would suggest CAPN1 or CAST to differ. Additionally, extreme differences in muscle were essentially nonexistent and pH was unaffected. Moreover, these data

are reaffirmed by our transcriptomics data, which shows a general increase in both CAPN1 and CAST with weight, when compared to that of the 317 kg group.

Transcriptomic Analysis

Attributes such as color, marbling, and tenderness are foundational to consumers for determining quality. These traits are largely measured by fundamental biochemical indicators. However, development in quality attributes is not yet fully understood. Therefore, using technologies such as transcriptomics, which allows for entire genomes to be captured and analyzed has advanced our understanding of the underlying factors contributing to meat quality development (Hocquette Lehnert Barendse Cassar-Malek & Picard, 2007; Purslow, Gagaoua, & Warner, 2021). Even so, much of the literature is largely driven by extreme differences in treatments, seeking differences promoted by varying diets, growth rates, and breeds. Still, much of the US beef originates from similar management practices, warranting production strategies that integrate understanding of muscle growth and meat quality. To the best of our knowledge no model exists that explores these changes relative to weight gain. Therefore, we chose to explore genes of linear correlation to weight. Eight genes with varying biological pathways were identified.

CA3 and FHL1 were positively correlated with growth, while FGRT, GATM, RNF217, SOCS2, TGFBR2, and UKL1 were negatively correlated. CA3 catalyzes reversible hydration of CO_2 and promotes fatty acid synthesis (Yamamoto, Uramaru, Kawashima, & Higuchi, 2022). CA3 acts on CO_2 produced through the citrate acid cycle, generating bicarbonate (Yamamoto et al., 2022). Bicarbonate then functions as substrate for acetyl-Coa carboxylase, thus enabling conversion of acetyl-CoA to malonyl- CoA–which is then used for fatty acid synthesis (Lynch et al., 1995). FHL, however, has been linked to both myoblast fusion and myotube hypertrophy (Sowden, Smith, Morrison, & Edwards, 1998; Cowling et al., 2008), aiding lean growth and accretion. Together, the upregulation of both CA3 and FHL fit with our data as we see both a

continued increase in muscle growth (REA), and fat accumulation (IMF, FT). Upregulation in FHL suggests muscle accretion has yet to plateau even at weight as high as 589 kg, however, FHL has also been shown to correlate with fat deposition (Wang et al., 2009), and calcium regulation (Pillar et al., 2017). This may warrant further exploration into determining the contributing roles FHL plays in marbling and tenderness. Additionally, we observed a negative relationship between growth and RNF217 and ULK1, known promoters of apoptosis and autophagy, respectively (Jiang et al., 2021; Lazarus, Novotny, & Shokat, 2015). The downregulation in these genes could be an indication of shift toward a more glycolytic fiber type as some of our data may suggest, and logically aligns, as increased signaling of apoptosis and autophagy is more readily seen in red muscle as it experiences more protein turnover (Vincow et al., 2001; Basisty, Meyer, & Schilling, 2018). Moreover, our data also shows a negative relationship of TGFB2 in heavier cattle. TGFB2 has been linked to proliferation, differentiation, motility and apoptosis, which would suggest muscle generation through satellite cell incorporation, making our hypothesis of increased hypertrophy more plausible then regeneration of new, or at least not at the same rate. To that end, SOCS2 was also downregulated. SOCS2 is known to suppress cytokine signaling and growth hormone (GH), and when downregulated, growth is significantly increased (Li et al., 2022). Even so, our data suggest a negative correlation in GATM, which is influential in the generation of creatine from amino acids. Creatine is stored in the muscle and used as energy in anaerobic conditions, so a downregulation in GATM would likely lead to a more oxidative metabolism. On the other hand, it may also be that energy demands are being met by other facets such as diet, and creatine is not needed in the same capacity as it is in 317 kg steers. Finally, a downregulation in FCGRT was noted. FCGRT is largely responsible for the transfer of immunoglobulin G antibodies from the mother to the fetus (Laegreid et al., 2002). Though decreased for apparent reasons, it is

unlikely to have an influence on quality attributes. These data suggest a shift towards a more glycolytic metabolism, supported by increase in hypertrophic and adipogenic promoters (Lefaucheur and Gerrard, 2000). However, it is important to note that while linear, may not be significant between treatment groups.

Even so, other genes from muscle specific pathways prove to have a collinear relationship (see table 5-3). While many pathways are downregulated, we find an increasing trend in both COX1 and COX2, which aid in the transfer of electrons to cytochrome C in the electron transport chain (Cogliati, Lorenzi, Rigoni, Caicci, & Soriano, 2018), suggesting oxidative metabolism is on the rise. Many other biological processes are decreased as weight is increased, largely revolving around mRNA regulation of varying genes. This leaves much to be explored in terms of growth and meat quality.

Conclusion

Cattle subjected to cereal grain finishing will undoubtedly experience hypertrophy, protein and fat accretion, as well as a shift in muscle fiber type, which our data support. As expected, carcass measurements and quality parameters vary with weight. Traditional biomarkers known for influencing growth and quality remained largely unaffected when conventional management practices are used as compared to that of beef reared under varying production systems (reviewed by: Muir et al., 1998). Even so, our transcriptomics approach to understanding quality development in growing cattle revealed 8 genes with linear relationships to growth that may influence quality development. Further work is needed to determine their direct relationship to meat quality. Nevertheless, this robust set of data provides valuable knowledge about the biology of the tissue converted to beef. These data may be used to target feeding and management practices to augment muscle growth and meat color, marbling, and tenderness.

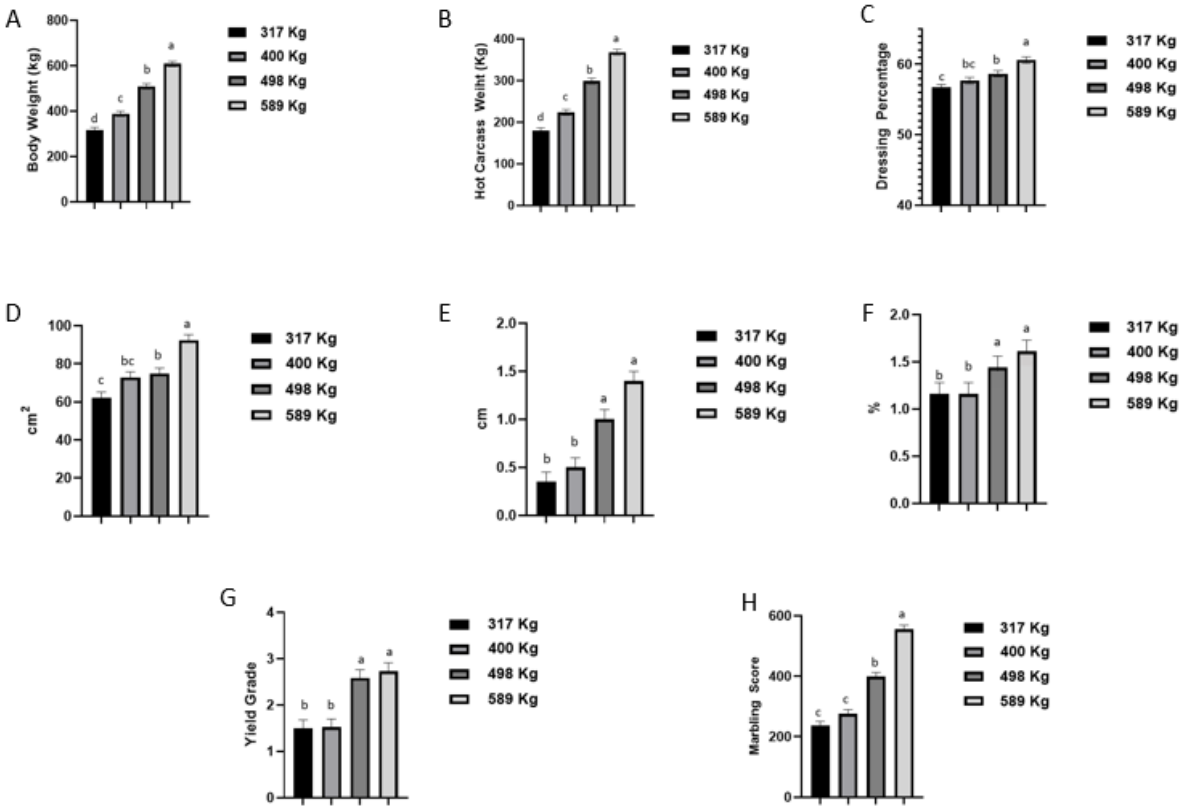

Figure 5-1. (A.) Means for final body weight (kg), **(B.)** hot carcass weight (kg), **(C.)** dressing percentage (%), (D.) ribeye area (cm2), **(E.)** 12th rib fat thickness (cm), **(F.)** estimated percent kidney pelvic heart fat (%), **(G.)** yield grade, and **(H.)** marbling score (200 = traces, 300 = slight, 400 = small, 500 = modest, 600 = moderate) between steers harvested at different weights. Data represent LS means ± SE. Means are considered significantly different at $P < 0.05$. Means lacking common letters differ.

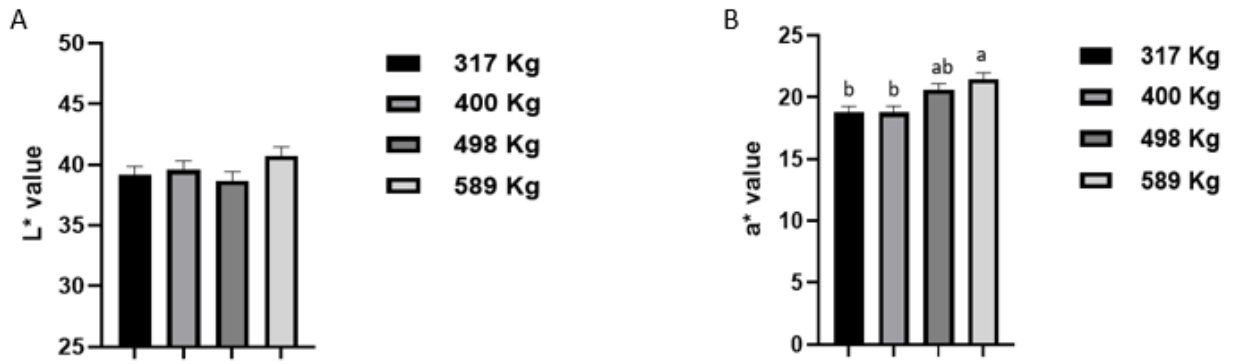

Figure 5-2. (A.) Means of lightness (L*), and **(B.)** redness (a*) values of LL of steers harvested at different weights. Data represent LS means ± SE. Means are considered significantly different at $P < 0.05$. Means lacking common letters differ.

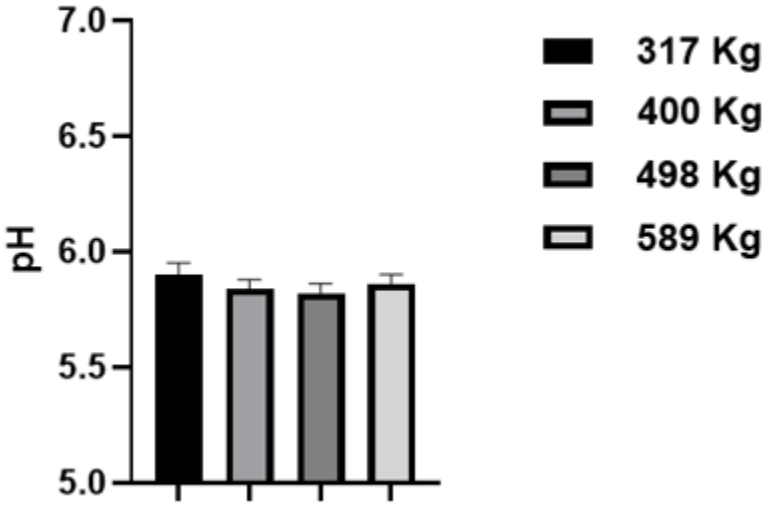

Figure 5-3. Means of LL pH values at 24 h from steers harvested at different weights. Data represent LS means ± SE. Means are considered significantly different at $P < 0.05$.

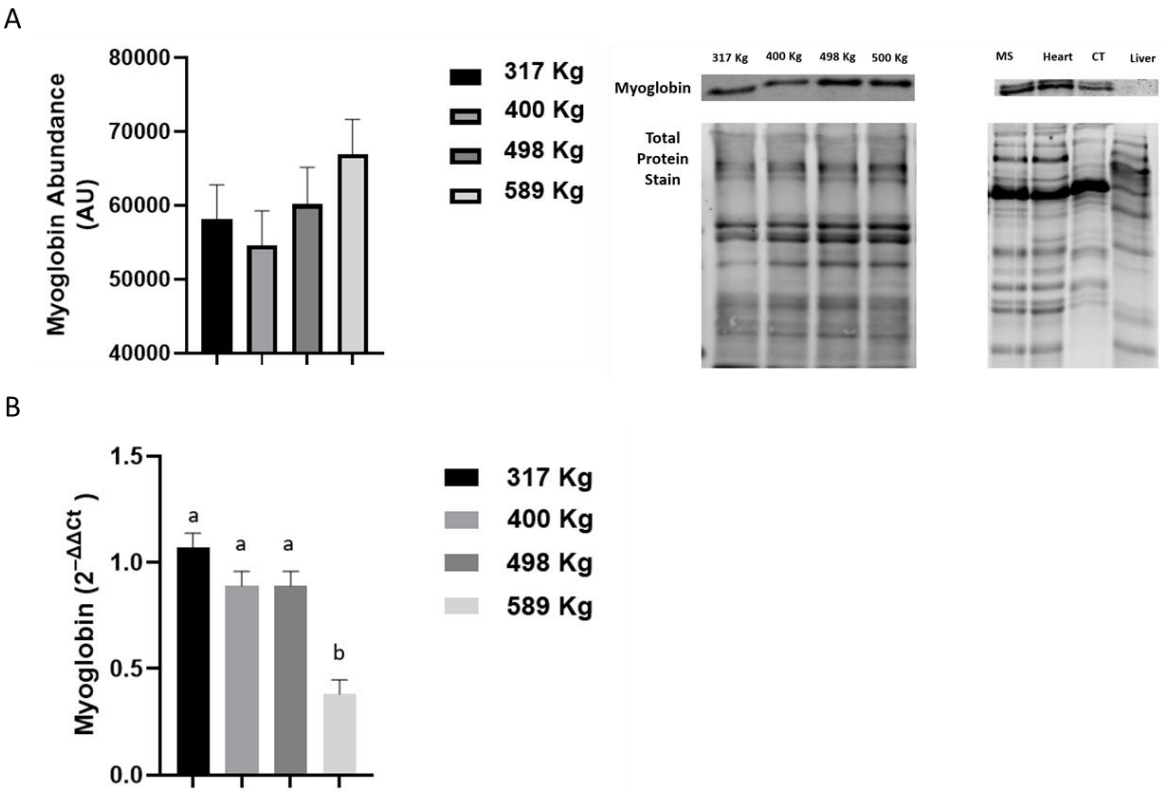

Figure 5-4. (A.) Relative abundance of myoglobin and **(B.)** means of gene expression of myoglobin between treatments and presented as fold change. Data represent LS means ± SE. Means are considered significantly different at $P < 0.05$. Means lacking common letters differ.

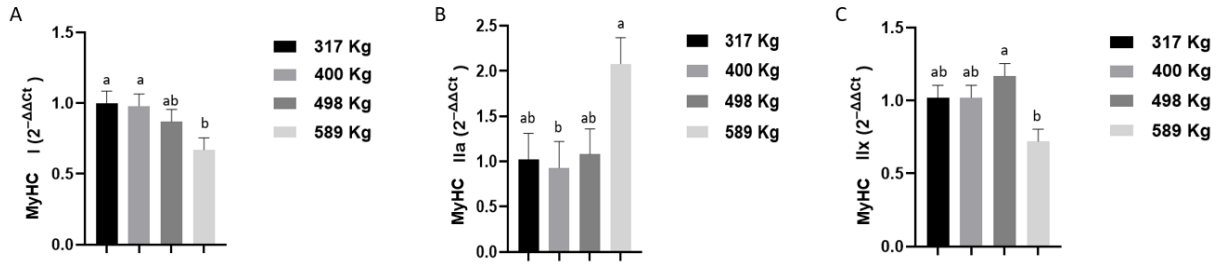

Figure 5-5. Means of gene expression of **(A.)** myosin heavy chain type I (MyHC-I), **(B.)** myosin heavy chain type IIA (MyHC-IIA), and **(C.)** myosin heavy chain type IIX (MyHC-IIX) in *longissimus* muscle (LL) of steers harvested at different weights and expressed as fold change. Data represent LS means ± SE. Means are considered significantly different at $P < 0.05$. Means lacking common letters differ.

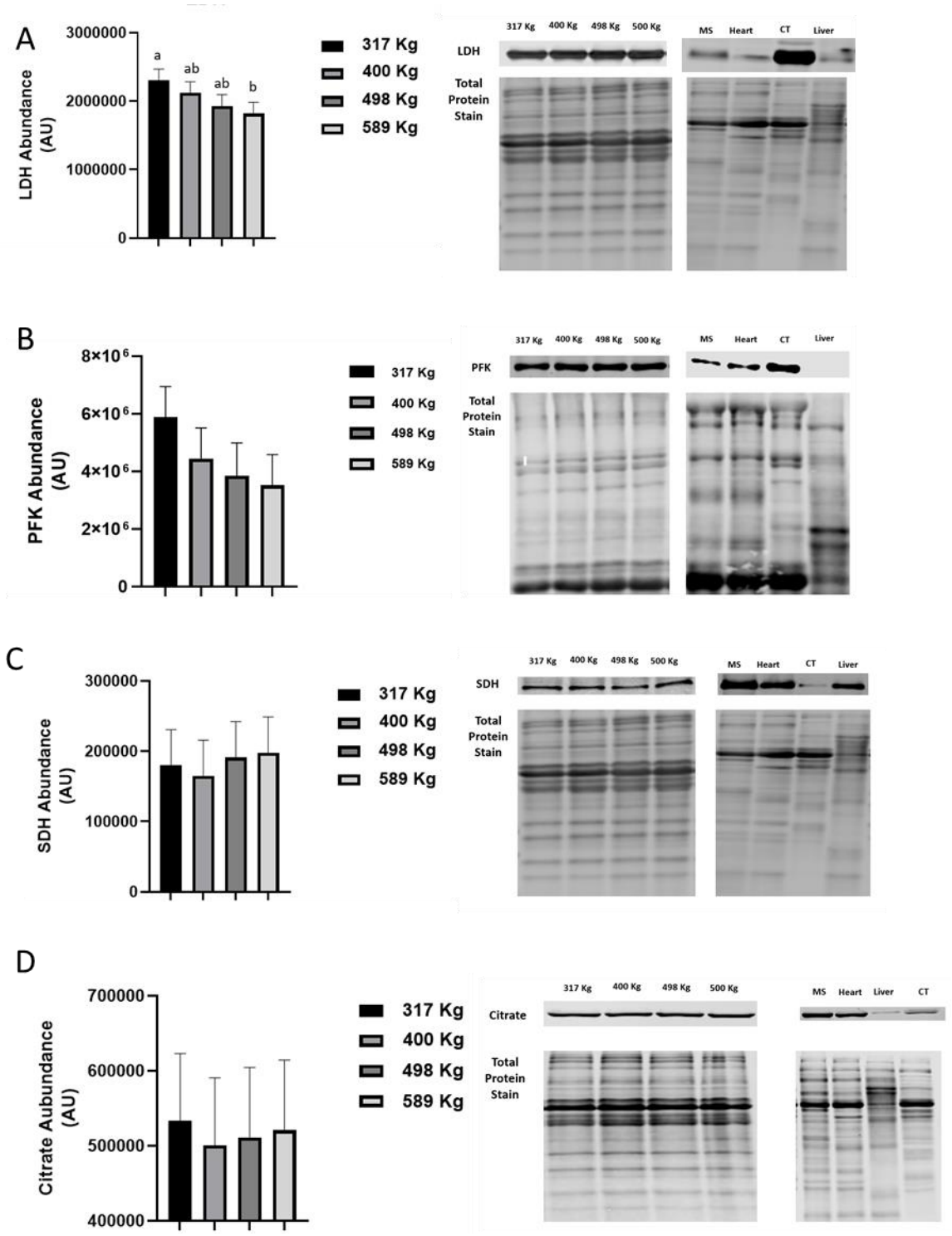

Figure 5-6. (A.) Relative abundance of LDH dehydrogenase-A (LDH), **(B.)** phosphofructokinase-1 (PFK) **(C.)** succinate dehydrogenase-A (SDH), and **(D.)** citrate synthase (CS) in *longissimus* muscle (LL) of steers harvested at different weights. Data represent LS means ± SE. Means are considered significantly different at $P < 0.05$. Means lacking common letters differ.

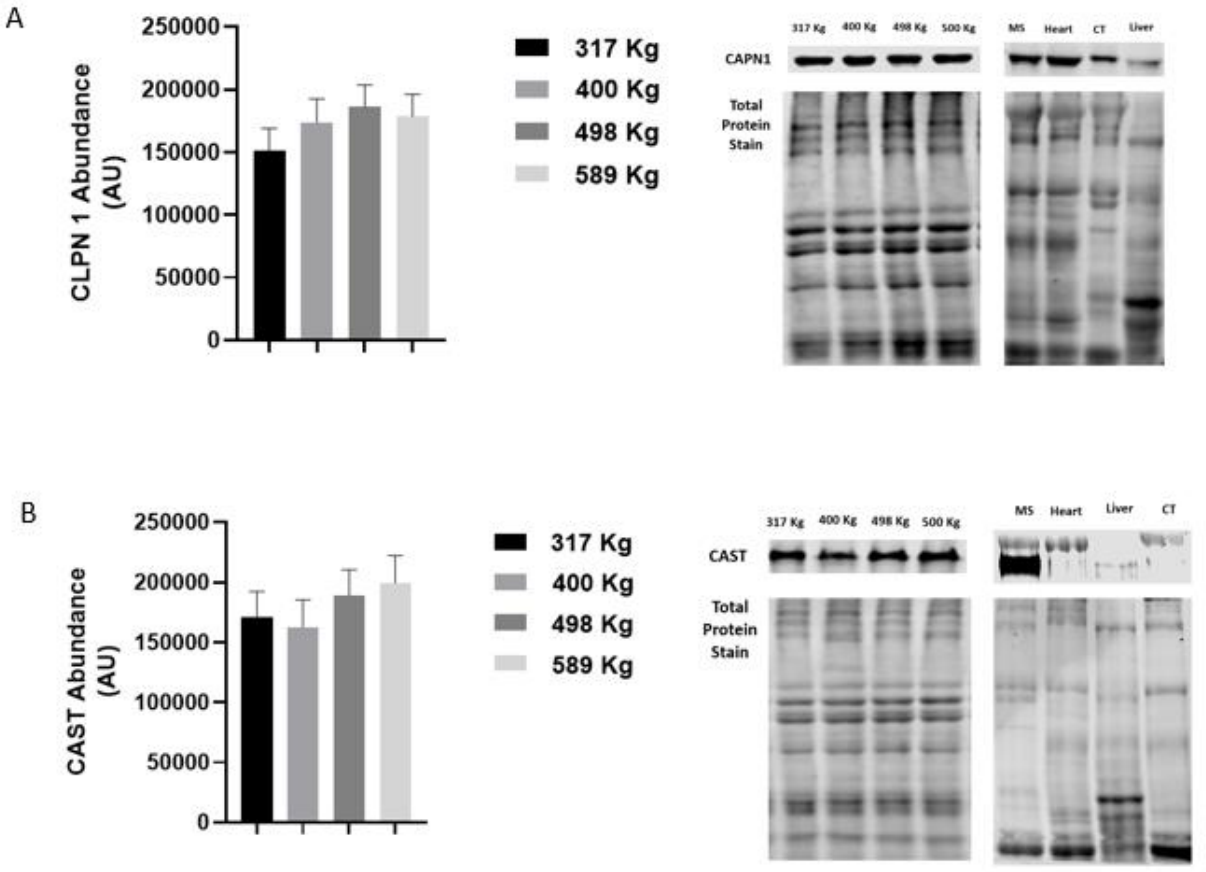

Figure 5-7. (A). Relative abundance of calpain-1 (CAPN1) and **(B.)** calpastatin (CAST) in *longissimus* muscle (LL) of steers harvested at different weights. Data represent LS means ± SE. Means are considered significantly different at $P < 0.05$.

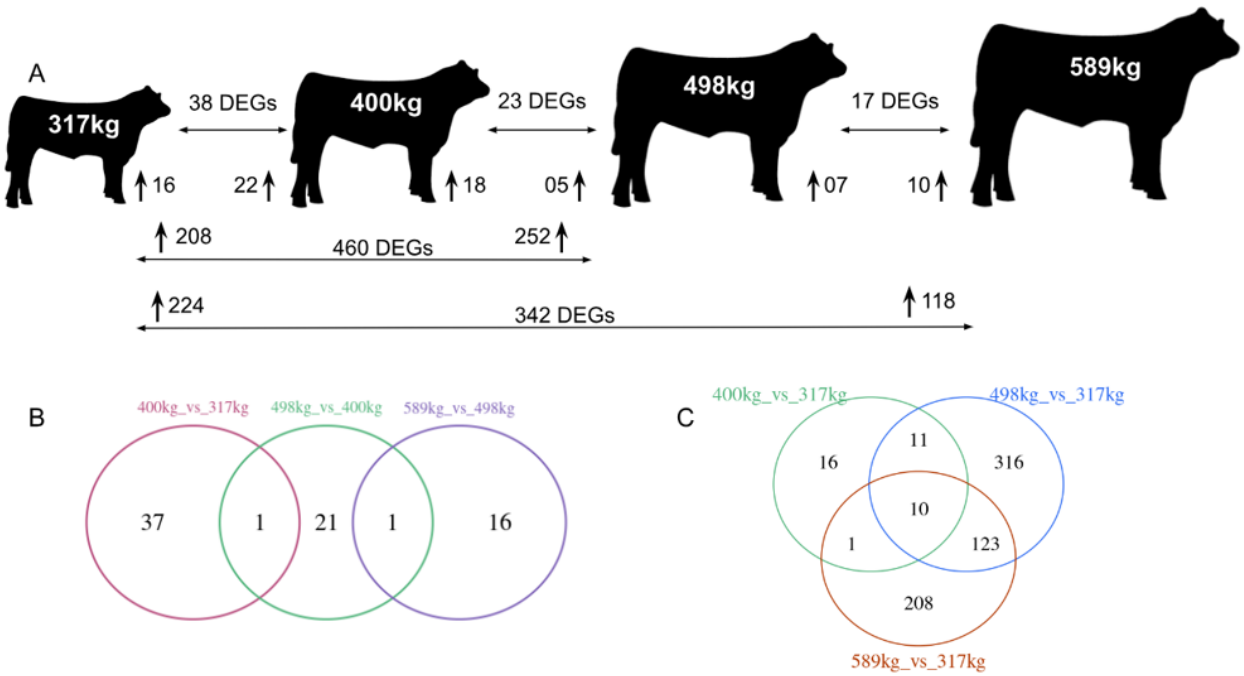

Figure 5-8. Differential transcript abundance in *longissimus lumborum* collected from steers of different weight classes. **(A.)** Number of genes with differential transcript abundance in each contrast. **(B.)** Number of genes with differential transcript abundance overlapping between each consecutive contrast. **(C.)** Number of genes with differential transcript abundance overlapping among the contrasts 400kg, 498kg, and 589kg versus 317kg.

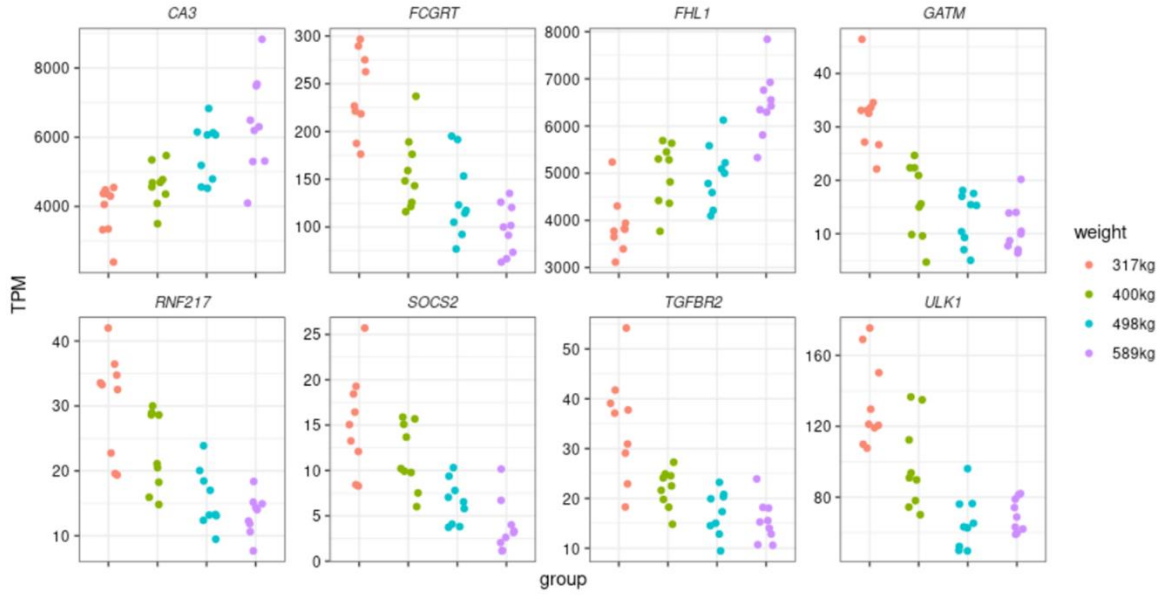

Figure 5-9. Linear relationship between weight at harvest and gene transcript abundance in *longissimus lumborum*.

Table 5-1. Primer sequence used in quantitative reverse transcription – PCR assays.

Gene Name	Sequence
MHC 1	F5: AAA-GCT-AGC-CCA-GCT-GAT-TAC R5: CTC-TCT-CCT-CTC-CAC-CAT-CTT
MHC IIA	F1: TCT-GAA-CTC-TGC-TGA-CCT-ACT-C R1: CTG-CAT-TGG-TTA-CCT-GCT-CTA-C
MHC IIx	F5: AAA-GCT-AGC-CCA-GCT-GAT-TAC R5: CTC-TCT-CCT-CTC-CAC-CAT-CTT
Myoglobin	F1: CAG-GCT-CTT-CAC-AGG-TCA-TC R1: CTT-CAT-CTC-AGC-CTC-TGT-CTT-C
S18	F5: GCG-AGT-ACT-CAA-CAC-CAA-CAT-C R5: CCT-CAA-CAC-CAC-ATG-AGC-ATA-TC

Table 5-2. Relationship between weight at harvest and gene transcript abundance in *longissimus lumborum*

Gene Symbol	*400 Kg Vs. 317 Kg*	*FDR*	*Adj. P-value*	*498 Kg Vs. 317 Kg*	*FDR*	*Adj. P-value*	*589 Kg Vs. 317 Kg*	*FDR*	*Adj. P-value*
MYH7 (I)	↑	0.248601	0.149237	↑	0.1213	**0.043003**	↑	**0.020397**	**0.002861**
MYH2 (IIa)	↓	0.708722	0.538659	↓	**0.005549**	**0.000368**	↓	**0.053235**	**0.023578**
MYH1 (IIx)	↓	0.33952	0.301833	↓	0.164938	0.090932	↓	0.803683	0.559994
MB	↓	**0.043078**	**0.002351**	↓	**0.002531**	**2.44E-05**	↓	**7.72E-05**	**2.99E-07**
LDHA	↓	0.247023	0.074381	↓	0.141871	**0.00836**	↓	0.458621	0.964897
SDHA	↓	0.674408	0.820054	↑	0.225717	0.758815	↓	0.777425	0.718534
PFKFB1	↓	0.577381	0.965873	↓	**0.037192**	0.422234	↓	**0.038397**	0.088936
CS	↑	0.092382	0.468109	↑	0.745164	0.395834	↑	0.185036	0.782479
CAPN1	↑	0.236996	0.731763	↑	**0.009352**	0.3149	↑	**0.048009**	0.258892
CAST	↑	0.874994	0.457388	↑	0.259645	0.883269	↑	0.88176	0.743667
SLC2A4 (Glut 4)	↑	0.894994	0.866315	↑	0.386869	0.136357	↑	0.119188	0.027795
PPARG	↓	0.338162	0.275292	↓	0.917592	0.752221	↓	0.063045	**0.022302**
SREBF1	↑	0.888973	0.8464	↑	0.5693	0.069809	↑	**0.013358**	**9.90E-06**

Table 5-3. Gene ontology enrichment analysis of genes with a significant colinearity with weight.

Biological Process	FWER	Fold Enrichment	Gene symbol	FDR	Trend
cellular response to fibroblast growth factor stimulus	0.0560	11.8	*CCL2*	0.0041	↘
			ZFP36	0.0053	↘
			NR4A1	0.0016	↘
			ZFP36L1	0.0080	↘
electron transport chain	0.0691	11.2	*ENOX2*	0.0043	↘
			COX1	0.0036	↗
			COX2	0.0008	↗
			ENSBTAG00000030735	0.0045	↗
chorio-allantoic fusion	0.0773	50.4	*CCN1*	0.0058	↘
			ZFP36L1	0.0080	↘
positive regulation of intracellular mRNA localization	0.0773	50.4	*ZFP36*	0.0053	↘
			ZFP36L1	0.0080	↘
regulation of keratinocyte apoptotic process	0.0773	50.4	*ZFP36*	0.0053	↘
			ZFP36L1	0.0080	↘
positive regulation of myotube differentiation	0.1172	16.8	*MAMSTR*	0.0023	↘
			RBM24	0.0006	↘
			CAV3	0.0100	↘
nuclear-transcribed mRNA catabolic process, deadenylation-dependent decay	0.1569	15.1	*ZFP36*	0.0053	↘
			ZFP36L1	0.0080	↘
			CNOT6L	0.0041	↘
regulation of stem cell proliferation	0.1586	16.8	*HMGB2*	0.0100	↘
			TGFBR2	0.0001	↘
			ZFP36L1	0.0080	↘
regulation of alternative mRNA splicing, via spliceosome	0.1990	6.3	*HNRNPL*	0.0007	↘
			HNRNPA1	0.0058	↘
			KHDRBS1	0.0002	↘
			RBM24	0.0006	↘
			RAVER1	0.0052	↘
nucleus localization	0.2202	33.6	*CDC42*	0.0047	↘
			CAV3	0.0100	↘
regulation of mRNA stability	0.2478	8.1	*ZFP36*	0.0053	↘
			RBM24	0.0006	↘
			PABPC4	0.0028	↘
			ZFP36L1	0.0080	↘
3'-UTR-mediated mRNA destabilization	0.2730	12.6	*ZFP36*	0.0053	↘
			RBM24	0.0006	↘
			ZFP36L1	0.0080	↘
negative regulation of transcription by RNA polymerase II	0.3894	2.0	*FOXD3*	0.0023	↘
			H1-2	0.0070	↘
			EID2	0.0018	↘
			HHEX	0.0088	↘
			JUND	0.0006	↘
			KLF5	0.0094	↘
			KLF2	0.0017	↘
			ZFP36	0.0053	↘
			PLAGL1	0.0006	↘
			JUN	0.0028	↘
			WWTR1	0.0079	↘
			HMGB2	0.0100	↘
			MED25	0.0079	↘
			TXNIP	0.0012	↘
			HOXD8	0.0051	↘
			PATZ1	0.0073	↘
			MAZ	0.0049	↘
			HDAC5	0.0079	↘
			SREBF1	0.0081	↗
			ZNF750	0.0006	↗
skeletal muscle satellite cell differentiation	0.4270	25.2	*KLF5*	0.0094	↘
			MEGF10	0.0046	↘

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Chapter 6. Summary & Implications

Beef quality is important as it sets a precedent for consumer demand and beef pricing structure. Therefore, deviations from what is considered 'normal' by industry can inhibit both producers and processors' bottom line. The scope of this work aimed to address both cost effective feeding strategies capable of achieving industry standards, as well as define causative agents that have generated disparities in color, marbling and tenderness in beef for decades. First, we tested muscle of market-ready steers and its ability to maintain metabolic properties and quality attributes given a reduction in energy intake for 60 d. Next, we subjected growing steers to either grass-fed (CON) for 120 d, short-feeding grain for 90 d (SF) or long grain feeding of 120 d (LF). Although different in design, both studies revealed muscle metabolism was largely uninfluenced regardless of treatment as indicated by lack of significance in oxidative and glycolytic proteins, as well as limited difference in pH. In general, quality, yield and color, were also consistent despite treatment. However, noticeable differences were seen between CON and LF cattle in regards to yield, marbling score and color, yet these findings were independent of differences in muscle metabolism. We hypothesized that more extreme differences in feeding and/or management practice must occur in order for muscle to deviate from normal muscle growth biochemistry. Otherwise, differences between beef quality and yield are minimal. Therefore, an additional study was conducted to evaluate conventional growth, as related to changes in hallmark indicators know for generating beef quality. The entirety of the genome was explored using a transcriptomics approach to determine underlying biomarkers that contribute to muscle metabolism and beef quality. Although significant differences in yield, quality and redness were reported, we failed to show differences in protein abundance for myoglobin, and energy metabolism indicating proteins, or calpain-1 and calpastatin. However, our transcriptomics data revealed upregulation in genes

related to hypertrophy and adipogenesis and shared a linear relationship to growth. This indicates quality differences that are likely a result of differences in muscle ultrastructure and fat deposition, rather than differences in metabolism, pH decline, myoglobin abundance or calpain-1 as often reported in the current literature. Methods commonly used in the field often test extreme spectrums of management, rather than more conventional methods of production.

Collectively, our data indicates muscle, although responsive to nutrient availability, remains rather resilient and capable of maintaining metabolic properties, suggesting differences in quality attributes require drastic changes in management and or environment. This is encouraging from a production standpoint as much of the US cattle inventory is finished using similar feeding practices. While, holding cattle for extended periods in an effort to sell during more lucrative markets is not a novel concept; our data does validate beef quality remains intact at least up to 60 d regardless of feeding forage or grain, provided maintenance requirements are met. This provides a viable option for both mid-size background/finishers as well as large scale feedlot operations. Additionally, with feeding costs reaching record highs, our data proves to be relevant, offering assurance to producers that cattle can still reach similar value and quality despites 30 fewer days on feed. Even so, additional work is needed to better understand the disparities in quality seen in that of conventionally reared cattle. The ability to pinpoint controlling mechanisms of muscle growth and deposition will allow for the development of targeted feeding strategies, ultimately reducing input costs, achieving preferred quality and increasing supply of beef preferred by consumers.

Appendix

Chapter 3:

Table 1. Least square means, standard error mean (±) and probabilities (*P*-value) of the effect of treatment on carcass traits.

Traits	*Control*	*Forage*	*Grain*	*P-value*
Initial Body Weight (kg)	603.28 ±23.63	586.27 ±20.46	604.70 ±20.46	0.787
Final Body Weight (kg)	586.64	590.35	613.11 ±29.10	0.763
Hot Carcass Weight (kg)	333.07 ±15.35	340.31 ±13.24	356.75 ±13.29	0.507
Dressing Percentage (%)	56.78 ±1.63	57.95 ±1.63	59.34 ±1.63	0.553
Ribeye Area (cm^2)	88.26 ±3.21	82.13 ±2.77	84.39 ±2.77	0.399
12^{th} Rib Fat Thickness (cm)	1.33 ±0.24	1.31 ±0.21	1.27 ±0.21	0.800
Estimated Percenter KPH (%)	1.41^{AB} ±0.11	1.38^{B} ±0.09	2.01^{A} ±0.09	0.019
Yield Grade	2.57 ±0.304	2.83 ±0.263	2.88 ±0.263	0.757
Marbling Score	446.67 ±29.56	477.50 ±25.60	527.50 ±25.60	0.133

Chapter 4

Table 2. Least square means, standard error mean (±) and probabilities (*P*-value) of the effect of treatment on carcass traits from.

Traits	*CON*	*SF*	*LF*	*SEM*	*P-value*
Final Body Weight (kg)	251.10^{B}	269.07^{AB}	283.06^{A}	± 8.27	0.036
Hot Carcass Weight (kg)	134.16^{C}	154.59^{B}	172.84^{A}	± 4.46	< 0.001
Dressing Percentage (%)	53.65^{B}	57.39^{AB}	60.64^{A}	± 2.61	0.06
Ribeye Area (cm^2)	460.58^{B}	502.58^{AB}	548.39^{A}	±2 0.58	< 0.019
12^{th} Rib Fat Thickness (cm)	2.05^{B}	3.02^{AB}	3.48^{A}	± 0.28	< 0.005
Estimated Percenter KPH (%)	1.25	1.30	1.55	± 0.09	0.190
Yield Grade	2.46^{B}	2.93^{AB}	3.13^{A}	± 1.42	0.016
Marbling Score	383.0^{B}	460^{AB}	505^{A}	± 13.29	0.002

Chapter 5

Table 3. Least square means, standard error mean (±) and probabilities (*P*-value) of the effect of treatment on carcass traits.

Traits	*317 kg*	*400 kg*	*498 kg*	*589 kg*	*SEM*	*P-value*
Final Body Weight (kg)	316.81^{D}	388.28^{C}	509.28^{B}	608.07^{A}	±11.49	< 0.001
Hot Carcass Weight (kg)	179.79^{D}	223.62^{C}	298.91^{B}	368.52^{A}	±6.77	< 0.001
Dressing Percentage (%)	56.69^{C}	57.68^{BC}	58.66^{B}	60.61^{A}	±0.45	< 0.001
Ribeye Area (cm^2)	62.32^{C}	73.03^{BC}	75.23	92.45^{A}	±2.90	< 0.001
12^{th} Rib Fat Thickness (cm)	0.36^{B}	0.53^{B}	1.04^{A}	1.42^{A}	±0.11	< 0.001
Estimated Percenter KPH (%)	1.16^{B}	1.16^{B}	1.44^{A}	1.61^{A}	±11.95	0.0307
Yield Grade	1.50^{B}	1.52^{B}	2.58^{A}	2.73^{A}	±0.18	< 0.001
Marbling Score	236.67^{C}	275.56^{C}	398.89^{B}	555^{A}	±14.06	< 0.001