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Impact of Postweaning Management Strategies on Beef Carcass Characteristics and Meat Quality

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IMPACT OF POSTWEANING MANAGEMENT STRATEGIES ON BEEF CARCASS CHARACTERISTICS AND MEAT QUALITY

BY

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This dissertation is approved as a creditable and independent investigation by a candidate for the Doctor of Philosophy degree and is acceptable for meeting the dissertation requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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ABSTRACT

IMPACT OF POSTWEANING MANAGEMENT STRATEGIES ON BEEF CARCASS CHARACTERISTICS AND MEAT QUALITY

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The overall objective of this dissertation was to determine the impact of selected post weaning management strategies on beef carcass characteristics and meat quality. The specific objectives were 1) determine the effect of a rumen-protected long chain fatty acids fed during finishing on live performance, serum fatty acid profile, carcass traits, and predicted carcass composition, and 2) determine effects of feeding brassica-based cover crops to cattle during backgrounding on live animal performance, carcass characteristics, tenderness, juiciness, and flavor of strip steaks, and case life of ground beef and strip steaks.

Results from both objectives indicate post weaning management strategies do impact carcass characteristics and meat quality. Specifically, the supplementation of rumen-protected long chain fatty acids during finishing increased hot carcass weight and altered blood serum fatty acid concentrations without impacting other meat quality attributes such as marbling score or tenderness. Additionally, feeding steers a backgrounding diet containing brassicas can increase tenderness in early in the aging period and alter case life color stability without impacting subjective palatability or carcass characteristics.

CHAPTER 1: Review of Literature

Introduction

The United States is second in the world for per capita beef consumption. However, the national average has been on the decline; steadily dropping from 30.6 kg per capita to 26.1 kg over the last 20 years (OECD, 2020). Conversely, the demand for poultry has slowly risen over the same time frame (OECD, 2020). One of the reasons poultry consumption has increased while beef has decreased is because poultry is a less expensive alternatives to beef. Therefore, to entice consumers to choose beef products when shopping, the products they buy must be high quality and consistent.

Beef quality is perceived by consumers in a variety of ways: tenderness, color, juiciness, marbling, and flavor; all of which have been extensively studied. Color has been shown as the most important factor consumers evaluate prior to purchasing (Mancini and Hunt, 2005) and palatability is important after purchasing. Palatability is a multifaceted term to describe the overall taste and texture properties of meat including tenderness, juiciness, flavor, and aroma (Aberle et al., 2001). Marbling, the fat within muscles also known as intramuscular fat, while associated with palatability, is the most variable in consumer preferences with some consumers preferring more marbling and some preferring leaner products. Management strategies for growing and finishing beef animals have shown to impact all of these quality traits. The influence of these strategies on carcass characteristics and meat quality have been widely studied; several of which are discussed in this literature review.

Tenderness

Beef tenderness has been extensively studied since 1991 with the first National Beef Tenderness Survey conducted by Morgan et al. (1991) and subsequent audits published in 2000, 2007, 2013, and 2017 (Brooks et al., 2000; Voges et al., 2007; Guelker et al., 2013; Martinez et al., 2017). While research has shown that tenderness variation has decreased, and overall beef tenderness has improved since the 1991 survey, there is still room for improvement. Shackelford et al. (1991) identified a Warner-Bratzler shear force tenderness threshold, and found that steaks with a shear value of 3.9 kg of force should have a 68% chance of being considered slightly tender or better and steaks with a shear value of 4.9 to have a 50% chance of being considered slightly tender. Miller et al. (2001) further evaluated the tenderness threshold and found that it was present between 4.3 to 4.9 kg of shear force. Unsurprisingly, previous National Beef Tenderness Surveys have found the cuts from the round to be less tender, less juicy, and less desirable than steaks from other parts of the carcass (Brooks et al., 2000; Guelker et al., 2013; Martinez et al., 2017). Conversely, in the most recent tenderness survey, over 95% of porterhouse, top loin, and top blade steaks fell into the very tender category, and as a result, received desirable sensory ratings (Martinez et al., 2017). Commonly, beef palatability is discussed with the attributes of tenderness, juiciness, and flavor (Koohmaraie, 1995). These attributes are considered to be related under a halo effect where an improvement in one attribute can make up for the deterioration of another (Nyquist et al., 2018). The production practices common for raising beef in the United States do not result in much variation in juiciness and beef flavor and as a result the best

way to improve palatability is likely through improving tenderness (Koohmaraie, 1995). Thus, an understanding of the factors that influence tenderness is necessary.

Factors that impact tenderness

Tenderness is a complex trait that is influenced by a large variety of factors. Most of these factors can be influenced by one of three primary mechanisms: proteolysis of myofibrillar proteins, sarcomere length, and connective tissue (Koohmaraie et al., 2002). The extent that each of those three aspects can impact the tenderness is variable depending on a variety of influences to be discussed later. However, in order to more completely discuss tenderness formation, it is first necessary to be familiar with muscle structure. Whole muscles are made up of muscle bundles, and muscle bundles are made up of muscle fibers (Aberle et al., 2001). The muscle fibers are comprised of small contractile units, called sarcomeres, that are lined up in a boxcar like fashion (Aberle et al., 2001). The length of a sarcomere is measured as the distance from one Z-disk to the next and spans about 2.5μm (Aberle et al., 2001). The proteins that are found in the sarcomere can be divided into three categories: cytoskeletal (provide structure and support to the sarcomere), regulatory (regulate the ability of the sarcomere to contract), and contractile (responsible for the mechanism of contraction) (Aberle et al., 2001). Several of the proteins found in sarcomeres are important for the development of tenderness in meat. Desmin, nebulin, and titin are categorized as cytoskeletal proteins that hold sarcomeres in alignment within the myofibers as well as within themselves (Aberle et al., 2001). Titin is the largest protein found in animal tissue and spans from the Z-disk to the M-line of the sarcomere and is responsible for maintaining structural alignment of the sarcomere during contraction (Aberle et al., 2001; Huff-Lonergan et al.,

2010). Nebulin is located near the thin filament of the sarcomere and spans from the Z-disk to the end of the thin filament, providing support to the thin filament (Aberle et al., 2001; Huff-Lonergan et al., 2010). Desmin is located around the Z-disk and connects myofibrils together (Aberle et al., 2001; Huff-Lonergan et al., 2010). These cytoskeletal proteins all play large roles in the development of tenderness because in event of their degradation, the structural integrity of the muscle fibers begins to weaken (Huff-Lonergan et al., 1996). Consequently, proteins such as desmin are routinely evaluated during the study of the proteolysis of muscle fibers.

Connective tissue

Connective tissue functions in living muscle as a support system for muscle fibers and connecting various tissues within the body. The three main layers of connective tissue associated with muscle are the epimysium, perimysium, and endomysium. The epimysium surrounds the whole muscle, the perimysium surrounds muscle bundles, and the endomysium surrounds the muscle fibers (Aberle et al., 2001). The amount of connective tissue within each individual muscle is dependent on muscle location, specie, breed, and animal age (Purslow, 2005; Calkins and Sullivan, 2007). Connective tissues within muscle are primarily composed of collagen and elastin surrounded by a proteoglycan matrix (Aberle et al., 2001; Purslow, 2005). The overall impact of connective tissue on tenderness is influenced by both the amount of connective tissue present and its solubility (Calkins and Sullivan, 2007). Additionally, the collagen in the connective tissue within the muscles of older animals has an increased amount of crosslinking, which is the linking together of two or three collagen molecules (McCormick, 1994). The presence of connective tissue within meat results in background

toughness (Purslow, 2005); which is relatively unchangeable with the exception of solubilization of collagen through moist heat cookery methods such as braising, pot roasting, and stewing.

Proteolysis of myofibrillar proteins

Several proteinase systems have been suggested to have roles in tenderness development including the ubiquitin proteasome, caspase, lysosome, and calpain systems. The ubiquitin proteasome system plays a large part in antemortem degradation of cellular proteins (Tanaka, 2009). However, up to 400 molecules of ATP are necessary for protein degradation by proteasome (Goll et al., 2008). As ATP is depleted during rigor mortis, it is unlikely that proteasome plays a large part in postmortem protein degradation. The caspase system is instrumental in apoptosis in antemortem muscle tissue (Goll et al., 2008). However, Du et al. (2004) indicated that while caspase-3 cleaves actomyosin, it is likely an upstream activator for proteasome. Additionally, Mohrhauser et al. (2011) concluded that caspase-3 is not directly responsible for degradation of nebulin, troponin T, desmin, and titin in a postmortem muscle system. Therefore, caspase is not likely the main proteinase system responsible for tenderness formation. The lysosomal system functions by engulfing proteins, and intact myofibrillar proteins are too large for lysosomes to overtake (Goll et al., 2008). Thus, it is believed that the functionality of the lysosomal system in protein degradation is to degrade extracellular proteins resulting from macrophage invasion of cells, not postmortem protein denaturation (Lowell et al., 1986). The calpain system is made up of isoforms of the proteolytic enzyme calpain and its inhibitor calpastatin. It is believed to be responsible for the majority of postmortem protein degradation (Koohmaraie, 1988; Huff-Lonergan et al., 1996; Huff-Lonergan et

al., 2010). The main calpain isoforms characterized in skeletal muscle are known as calpain-1 and calpain-2. While both isoforms of calpain degrade myofibrillar proteins including desmin, troponin-T, nebulin, and titin, they have different requirements for activity (Huff-Lonergan et al., 1996; Huff-Lonergan et al., 2010). Calpain-1, formerly known as μ-calpain requires between 5 and 65 μM of calcium for half maximal activity while calpain-2 (formerly m-calpain) requires between 300 and 1000 μM of calcium for the same activity (Goll et al., 1992). It is believed that calpain-1 is responsible for the majority of postmortem protein degradation as it is activated with calcium levels present in postmortem muscle (Geesink et al., 2006). The overall length of activity of calpain is still unclear. Boehm et al. (1998) indicated by 1 d postmortem, the activity of calpain-1 is only 20% of its at-death activity, and by day 7, activity is less than 4%. Similarly, Koohmaraie et al. (1987) indicated low calcium requiring calcium-dependent protease (calpain-1) and calpastatin degraded rapidly during postmortem storage. This means that calpain-1 autolyzes rapidly postmortem, but the rate of autolysis slows over time. Conversely, Geesink and Goll (1995) suggested autolysis occurs much slower, but calpain-1 associates with insoluble muscle fractions resulting in the inability to detect the active protease. However, the most rapid changes in tenderness of longissimus muscle occur within the first 72 hours postmortem (Wheeler and Koohmaraie, 1994), and Koohmaraie et al. (1987) observed the greatest rate of change in myofibril fragmentation index during the first 24 hours postmortem. While there is a plethora of evidence to suggest the calpain system plays a major part in altering postmortem muscle structure, further research is still warranted to determine the implications other protease systems, or

currently unknown factors within the calpain system, may have on tenderness formation after the first 72 hours postmortem.

Sarcomere length

Two important structures within the sarcomere are the thick and thin filaments, primarily made up of contractile proteins. The thin filament is primarily made up of actin, and the thick filament is primarily made up of myosin (Aberle et al., 2001; Huff-Lonergan et al., 2010). When muscles contract, the actin and myosin proteins create cross bridges pulling thick and thin filaments past one another; described by Huxley and Hanson (1954) and Huxley and Niedergerke (1954) as the sliding filament theory. The sliding of the filaments pulls the Z-disks together, shortening the sarcomere and overlapping the proteins (Aberle et al., 2001). Once a muscle has undergone rigor mortis, the actomyosin cross bridges formed during contraction become permanent and sarcomere length becomes fixed. The shorter the sarcomere is at completion of rigor, the more protein overlap there will be. More protein overlap results in increased force needed to shear through the sarcomere, thus decreasing tenderness (Locker, 1960). Therefore, shorter sarcomeres are associated with increased shear force values. The length of sarcomeres can be altered through several exogenous factors. Rapid postmortem chilling rates have been shown to cause a condition known as cold shortening where sarcomere lengths of affected muscles are reduced causing decreased tenderness in comparison to carcass chilled at a slower rate (Locker, 1960; Locker and Daines, 1976; King et al., 2003). The sarcomere length of some muscles can also be altered by alternative carcass hanging methods. Hostetler et al. (1972) explored different methods of carcass positioning including the commonly used Achilles tendon suspension, laying the carcass

horizontally on a table, and aitch bone suspension, later known as the Tenderstretch method. The Tenderstretch method was effective at increasing the length of sarcomeres in the longissimus muscle (Hostetler et al., 1972). Another method to increase sarcomere length is known as the Tendercut™ which involves suspending carcasses by the Achilles tendon and then severing the ischium of the pelvic bone, the junction of the 4th and 5th sacral vertebrae, and the connective tissues of the round/loin region to improve round muscle tenderness (Wang et al., 1994) or severance of the 12th thoracic vertebrae and the ischium to improve longissimus muscle tenderness (Claus et al., 1997). However, these alternative hanging methods are not widely used in the United States as the carcasses suspended using the Tenderstretch method take up more room when they are hung. Additionally, the Tendercut™ method requires additional personnel to make the cuts required and could result in unintentional damage to the muscles surrounding the severed bones.

Dietary impacts on tenderness

Dietary supplements have been explored as a means to improve meat tenderness. Swanek et al. (1999) evaluated the impact of dietary vitamin D supplementation of beef steers and observed improvements in tenderness. Dietary vitamin D can activate the increase of plasma calcium through stimulating intestinal calcium absorption, mobilizing calcium from bone, or increasing renal absorption of calcium (Nicolaysen, 1937; Carlsson, 1952; Sutton and Dirks, 1978). Swanek et al. (1999) hypothesized the increase in plasma calcium concentrations activated the calpain system thus improving tenderness. Montgomery et al. (2000) administered dietary vitamin D for nine days prior to slaughter and also observed improved tenderness of strip steaks at two weeks postmortem but no

other aging periods, which they also attributed to activation of calpain. However, (Scanga et al., 2001) observed increases in serum calcium but no impact on tenderness was detected when the authors supplemented steers with dietary vitamin D.

The addition of β-agonists to feedlot diets has also been shown in decrease tenderness formation in whole muscle meat products. Strydom et al. (2009) evaluated the impact of zilpaterol-HCl, ractopamine-HCl, and clenbuterol when fed during the last 30 days of finishing and observed an increase in calpastatin activity and subsequent increase in Warner-Bratzler shear force values in steaks from the β-agonist treatments compared to a control. Similarly, Scramlin et al. (2010) compared ractopamine-HCl and zilpaterol-HCl against a control and also observed increased shear force values for the ractopamine-HCl treatment compared to the control at 3 and 7 days postmortem and no differences at day 14 and 21. Conversely, the zilpaterol-HCl treatment demonstrated increased shear force values throughout all aging periods. However, Scramlin et al. (2010) did not evaluate calpastatin activity, nor hypothesize a mechanism to support differences in tenderness. It is worth noting that zilpaterol is no longer marketed in the United States and clenbuterol is illegal to feed in the United States, however, ractopamine-HCl is still regularly used at this time.

Dietary antioxidants can also impact meat tenderness. Vitamin E, a common antioxidant is known for aiding in color stability (to be discussed later). However, it has also been shown to improve tenderness of beef steaks. Harris et al. (2001) evaluated dietary supplementation of vitamin E in conjunction with a calcium chloride injection on meat quality of the longissimus muscles and observed increases in the rate of tenderness formation and proteolysis for the treatment supplemented with vitamin E and injected

with calcium chloride. The authors attribute those results to greater calpain activity brought on by both increased available calcium to active calpain as well as reduced calpain oxidation due to the antioxidant properties of vitamin E (Harris et al., 2001). Similar results were obtained by Carnagey et al. (2008) who evaluated the impact of administration of a bolus of vitamin D to heifers 7 days prior to slaughter, a top dress of vitamin E for 104 days, a combination of both, or a control receiving neither vitamin and observed improvements in tenderness in steaks from heifers administered vitamin E compared to the control.

While debate continues regarding the mechanisms involved, most researchers can agree on is that tenderness is a complex topic with many factors contributing to the tenderization process. While the three main mechanisms of proteolysis of myofibrillar proteins, sarcomere length, and connective tissue are widely accepted, the factors impacting those mechanisms still require further research.

Meat color

Consumers view meat color as an indication of freshness and wholesomeness and heavily rely on appearance for purchasing decisions (Carpenter et al., 2001; Mancini and Hunt, 2005). Nearly 15% of meat in a retail setting receives a price discount due to product discoloration resulting in over one billion dollars in revenue loss in the United States annually (Smith et al., 2000). The color muscle from properly exsanguinated muscle, and subsequently meat, is very largely determined by a protein within the muscle structure called myoglobin (Livingston and Brown, 1981; Mancini and Hunt, 2005). Myoglobin is a monomeric heme protein containing iron (Suman and Joseph, 2013). The iron can accept six electrons; four are pyrrole groups of the heme porphyrin ring and one

is associated with histidine (Suman and Joseph, 2013). The sixth position can bind with small ligands such as oxygen, carbon monoxide, or nitric oxide (Suman and Joseph, 2013). Whatever is liganded to the sixth position is what determines the redox state and pigment of the myoglobin (Mancini and Hunt, 2005; Suman and Joseph, 2013). The main pigments of fresh meat are deoxymyoglobin, oxymyoglobin, and metmyoglobin (Mancini and Hunt, 2005). Deoxymyoglobin is a purplish-red color, commonly associated with vacuum packaged meat, and is the result of no ligand attached to the heme iron (Suman and Joseph, 2013). Oxymyoglobin is the result of oxygen liganded to the heme iron and is responsible for the iconic bright cherry red color associated with fresh beef (Mancini and Hunt, 2005). Metmyoglobin is the result of oxidation of the ferrous form of iron to a ferric state in addition to a water molecule bound to the heme iron and is observed as the brownish green color associated with discolored meat (Suman and Joseph, 2013). Discoloration is generally referred to as the amount of surface area covered by metmyoglobin (Suman and Joseph, 2013).

Measuring meat color

Meat color can be assessed instrumentally or subjectively. Instrumental color can be taken using colorimeters or spectrophotometers using one of several color systems including Hunter, CIE, and tristimulus (Mancini and Hunt, 2005). Hunter and CIE color variables are commonly used to determine meat color and include L*, a*, and b* measurements (Mancini and Hunt, 2005). The L*, a*, b* color space is a three dimensional measurements of color with L* measuring brightness (0 = black, 100 = white), a* measuring green (negative a* values) to red (positive a* values), and b* measuring blue (negative b* values) to yellow (positive b* values) (AMSA, 2012). In a

study evaluating pork longissimus muscle color, Lindahl et al. (2001) reported approximately 86% of color variation was due to pigment content, myoglobin form, and internal reflectance. Additionally, 86% and 90% of the variation of L* and a* values respectively were influenced by those same factors, while b* values were mostly explained by myoglobin forms with no variation explained by pigment content (Lindahl et al., 2001). Evaluating meat color on an L* and a* basis is relatively easy to comprehend as most people would consider meat on a red color scale and are familiar with brightness. However, b* values tend to be more difficult to apply as the b* values of blue to yellow are not intuitively related to meat color and trained panelists generally associate b* values with brown (O'Sullivan et al., 2003a).

Subjective color sensory panels may also be used to evaluate meat color and can either be trained or untrained. Trained panels tend to result in more normal distributions of data as the panelists are more familiar with the attributes being evaluated and have been exposed to reference samples (O'Sullivan et al., 2003b). Although untrained panels may result in skewed distributions and increased variation, they are still important when evaluating meat in a mock retail setting as they may provide insight closer to normal consumer preferences (O'Sullivan et al., 2003b; Mancini and Hunt, 2005).

Factors affecting meat color

Many endogenous factors impact meat color including pH, muscle source and fiber type, antioxidants and lipid oxidation, mitochondrial activity, live animal management, diet, and genetics (Faustman and Cassens, 1990; Mancini and Hunt, 2005). One of the most studied factors impacting color is muscle source. Muscles throughout the body have different functions and location, and therefore require different metabolic

processes, consequently resulting in different meat color chemistry (Hunt and Hedrick, 1977). Muscles composed primarily of red fiber types contain more myoglobin, thus are darker, than muscles composed of white muscle fiber types (Cassens and Cooper, 1971). Additionally, meat with an increased pH tends to be darker than meat with a decreased pH. Mitochondrial activity also impacts color as mitochondria compete with myoglobin for oxygen resulting in increased deoxymyoglobin (Ramanathan et al., 2009).

Live animal diets have also been shown to impact meat color. For example, mixed results have been reported when feeding distiller's grains during the finishing phase of beef cattle. Roeber et al. (2005) observed negative effects on color stability with the inclusion of 40% and 50% diet dry matter inclusion of distiller's grains but no impact when fed at 10% or 25%. Gill et al. (2008) evaluated the impact of corn or sorghum distiller's grains compared to steam flaked corn across two separate slaughters. In the first slaughter group, they found no differences in color between diets containing either type of distiller's grain compared to the steam flaked corn diet. However, in the second slaughter, they observed steaks from the steam flaked corn treatment were darker, more red, and more yellow than the distiller's grain counterparts. The addition of the antioxidant Vitamin E to beef cattle diets has also been shown to increase the duration of color stability of beef steaks (Bloomberg et al., 2011; Harsh et al., 2018). Antioxidants such as vitamin E within diets provide color stability by functioning as free radical scavengers and reduce peroxidation of fatty acids in plasma membranes (Buttriss and Diplock, 1988).

Exogenous factors impacting meat color include packaging type, antioxidants, and tricarboxylic acid (TCA) cycle metabolic intermediates (Suman et al., 2014).

Packaging type plays a crucial role in meat color. Vacuum packaging involves removing the headspace from packages resulting in no oxygen available to bind to myoglobin resulting in the formation of the deoxymyoglobin pigment and a purplish color (Suman and Joseph, 2013). Packaging with carbon monoxide or oxygen allowed in the head space, also known as modified atmosphere packaging, allows oxygen or carbon monoxide to bind to myoglobin, resulting in a bright cherry red color (Suman and Joseph, 2013). A non-comprehensive list of food grade antioxidants including erythrobate, rosemary extract, ascorbate, and lactate have all been demonstrated to improve color stability and shelf life (Mancini et al., 2001; Sepe et al., 2005; Suman et al., 2005; Lee et al., 2006; Nassu et al., 2012). The use of TCA cycle metabolic intermediates, such as lactate, have been intensely studied over the past decade (Suman et al., 2014). The incorporation of lactate into beef products primarily via injection has been shown to improve color stability by generating NADH to prevent formation of metmyoglobin (Lawrence et al., 2004; Kim et al., 2006; Kim et al., 2009). Similar improvements to color stability have been observed with the introduction of succinate (Ramanathan et al., 2011) and pyruvate (Ramanathan et al., 2011; Ramanathan et al., 2012).

Factors impacting meat quality and palatability continue to be researched. As mentioned, live animal management can play a significant role in meat quality. Most research on beef quality involving live animals focuses on the finishing phase. However, there are studies that suggest meat quality can be impacted earlier in life such as during the backgrounding phase.

Backgrounding

Backgrounding cattle is a diverse practice that has become increasingly popular within the beef industry. Backgrounding involves feeding weaned calves a diet designed to manipulate body composition or calf weight for a period of two to six months (Klopfenstein et al., 1999a). This enables producers to meet specific markets and allows them to sell heavier calves at a later point in their development. Therefore, backgrounding provides flexibility for producers to sell their calves at a time that makes the most economical sense (Block et al., 2001). Moreover, some feedlots prefer backgrounded calves because the risk of morbidity is lower and a more consistent rate of gain is already established (SAFRR, 2003). This strategy also allows producers market a group of calves that is more uniform in their composition compared to calves placed into feedlots directly after weaning, known as calf-feds. (Vaage et al., 1998). Additionally, backgrounding can be used as a period of nutrient restriction that allows feedlots to utilize compensatory gain, which is a period of rapid growth upon transition to a higher plane of nutrition, adding value to the purchased animals. While backgrounded calves tend to consume more feed and gain weight faster than their calf-fed counter parts upon entry into a feedlot, they are less efficient in their feed conversions (Klopfenstein et al., 1999b). Producers most interested in backgrounding systems are those who have access to low cost roughages, housing facilities or grazing land, and a desire to hold weaned calves for up to an extra 200 days before marketing or transitioning to a finishing diet.

Backgrounding dietary impact on carcass characteristics and meat quality

A variety of backgrounding studies have been conducted to evaluate the influence of backgrounding diet on carcass characteristics and meat quality (Laborde et al., 2002;

McCurdy et al., 2010; Pordomingo et al., 2012; Lancaster et al., 2014; Cox-O'Neill et al., 2017). Lancaster et al. (2014) conducted a meta-analysis evaluating the categories of calf-fed vs yearling production systems, levels of dietary starch during backgrounding, and rate of gain during backgrounding. When evaluating calf-fed vs yearling production, Lancaster et al. (2014) reported increased body weight and average daily gain and decreased gain to feed efficiency for yearlings compared to calf-feds. Additionally, 12^{th} rib back fat thickness and percent kidney pelvic heart fat were increased for calf-feds compared to yearlings (Lancaster et al., 2014). A meta-analysis of dietary starch level (categorized as high starch vs medium starch and high starch vs low starch) and rate of gain showed no differences among treatments for live performance or carcass characteristics. (Lancaster et al., 2014). McCurdy et al. (2010) evaluated the impact of the backgrounding diets of ad libitum high concentrate, wheat pasture, sorghum silage, or program fed high concentrate. This study achieved the goal of similar gains for all treatments during the backgrounding and observed no differences in carcass characteristics among treatments when evaluated immediately after backgrounding (McCurdy et al., 2010). However, overall live performance differed, with the backgrounding ad libitum high concentrate treatment resulting in a lower final body weight; translating into a smaller ribeye area, increased 12^{th} rib back fat thickness, and increased yield grade (McCurdy et al., 2010). Pordomingo et al. (2012) backgrounded heifers on one of four diets containing 40%, 70%, or 100% alfalfa hay or pasture grazing. The 100% alfalfa hay diet resulted in the smallest ribeye area, least back fat, and least amount of intramuscular fat (Pordomingo et al., 2012). Additionally, the 100% alfalfa diet resulted in the most cook loss of strip steaks and decreased juiciness scores compared

to all other treatments, which the authors attributed to the reduced intramuscular fat (Pordomingo et al., 2012). Laborde et al. (2002) evaluated the impact of a high concentrate high moisture corn diet compared to alfalfa silage and observed no differences in carcass characteristics, with the exception of ribeye area, as well as no differences in longissimus muscle shear force and sensory attributes evaluated by a trained panel. Additionally, Fehrman (2016) and (Nenn, 2017) evaluated the impact of grazing turnips for 29 days prior to transitioning to a dry lot diet containing haylage, dry rolled corn (DRC), and dried distillers grains with solubles (DDGS; cover crop treatment) during backgrounding compared to a dry lot diet containing corn stover, haylage, DRC, and DDGS without a grazing period (Control). Nenn (2017) observed decreased backgrounding average daily gains (ADG) for the cover crop treatment compared to the control. However, live animal performance and carcass characteristics did not differ among treatments upon completion of the finishing phase (Nenn, 2017). Interestingly, Fehrman (2016) observed decreased Warner-Bratzler shear force (WBSF) values, increased overall liking and texture liking, and improved tenderness ratings in a consumer sensory panel in strip steaks from steers on the cover crop treatment compared to control. Finally, Cox-O'Neill et al. (2017) conducted a two-year study evaluating the impact of the backgrounding diets of a midwestern dry lot, grazing corn residue, or grazing an oat / brassica cover crop. The cover crop backgrounding system resulted in increased body weights and hot carcass weight compared to the other two treatments (Cox-O'Neill et al., 2017). The increased live and hot carcass weights observed by Cox-O'Neill et al. (2017) and lack of differences in other carcass characteristics led to the

conclusion that backgrounding on brassica mixture cover crops is a viable backgrounding strategy.

Cover crops

Backgrounding on cover crops has been a growing practice within the agriculture industry over the past decade. The number of acres seeded into cover crops was an estimated 10.3 million acres in 2012 with an estimated growth of several million acres by 2017 (USDA, 2014; SARE, 2016). However, little research has been conducted to evaluate the efficacy of cover crops as a backgrounding diet, or the potential impacts on beef carcass characteristics or meat quality. Cover crops are usually planted after the harvest of cash crops such as oats, corn, or wheat and have become an integral part of sustainable agriculture. Two of the main purposes of planting cover crops include soil conservation and feed for grazing livestock (SARE, 2016). Economically, cover crops can benefit farmers and ranchers by providing a low-cost forage to extend the grazing season for ruminants in addition to improving crop yields by improving soil health and reducing soil compaction (Ball et al., 2008; Drewnoski et al., 2018).

Brassica cover crops in beef diets

Brassicas are a cold hardy crop that can be ready for grazing as little as 60 days after planting and include kale, forage rape, turnips, and radish (McCartney et al., 2009). Forage rape, turnip, and forage radish are highly digestible and have been shown to provide over 4300 kg of dry matter per hectare and the crude protein levels generally hold steady from October to December when seeded by mid-June (McCartney et al., 2009; Villalobos and Brummer, 2015). Grazing weaned calves on cover crops such as brassicas, clover, and grasses can be cost effective alternatives to purchasing hay or other

feedstuffs in the late fall and early winter (Cox-O'Neill et al., 2017). It is recommended that ruminants be limit grazed to acclimate them to the feed slowly to avoid digestive issues such as polioencephalomalacia or nitrate poisoning (Ball et al., 2008; Villalobos and Brummer, 2015; Drewnoski et al., 2018). The most common use of brassicas in beef cattle diets is the grazing of mature cows during the late fall and early winter. Very limited research has been conducted to evaluate the impact of brassica cover crops on live animal performance or meat quality, both of which include the development of adipose tissue.

Adipose tissue growth

Fat depots

Adipose tissue develops in four general depots in mammals: abdominal (perirenal, mesenteric, and omental), intermuscular, subcutaneous, and intramuscular (Pethick and Dunshea, 1996; Bonnet et al., 2010; Du et al., 2013). Each fat depot develops in a unique fashion compared to the others. Bruns et al. (2004) conducted an experiment to determine the rate and extent at which marbling develops in serially slaughtered steers as well as changes in carcass composition. The experiment was designed for hot carcass weight to increase linearly; the researchers documented a quadratic increase of subcutaneous rib fat and linear increases in intramuscular fat and percent kidney, pelvic, heart fat (KPH) (Bruns et al., 2004). Bruns et al. (2004) disagreed with previously published literature that established an ordered development of fat depots first starting with abdominal (measured in beef carcasses at % KPH), subcutaneous (backfat or 12^{th} rib fat in beef carcasses), intermuscular (seam fat between muscles), and finally intramuscular (marbling) (Andrews, 1958). While these fat depots begin hyperplasia (increase in cell

numbers) at different points of prenatal development, there is overlap in development and growth with all tissues undergoing hyperplasia and hypertrophy (increase in cell size) to some extent throughout fetal and early postnatal growth (Du et al., 2013). Although, several studies have reported a plateau of marbling scores with days on feed (Moody et al., 1970; Butts Jr et al., 1980; Greene et al., 1989). Bruns et al. (2004) did not observe the plateau in their published data but admitted with change in the method of data analysis, marbling scores showed a quadratic response meaning as days on feed increased, the rate of marbling development slowed. These data observed by Bruns et al. (2004) are not surprising as animals have a genetic potential for marbling development; meaning regardless of nutrition and management, they will eventually meet a limit to the amount of marbling they can develop (Park et al., 2018).

Adipogenesis

Adipose tissue growth is necessary throughout the body. Abdominal fat protects vital organs and subcutaneous fat plays a large role in thermoregulation. However, from a production and profitability standpoint, adipogenesis is desirable in intramuscular fat depots as it is a large factor in determining quality grade, but less desirable in other depots as increases in subcutaneous, abdominal, and intermuscular adipose result in product waste and increased production costs (Du et al., 2013).

Adipoblasts develop from common mesenchymal progenitor cells and differentiation of adipoblasts begins in the prenatal stage of development (Du et al., 2013). Two main families of transcription factors are responsible for adipocyte differentiation: peroxisome proliferator activated receptor (PPAR) γ and CCAAT/enhancer binding protein (C/EBP) (Gregoire et al., 1998; MacDougald and

Mandrup, 2002; Avram et al., 2007; Du et al., 2013). The process of adipogenesis is accomplished through several waves of transcription factors. The first set of transcription factors necessary for adipogenesis are C/EBPβ and C/EBPδ (Gregoire et al., 1998; MacDougald and Mandrup, 2002). The presence of these two C/EBP isoforms activate the expression of PPARγ and C/EBPα, which are two integral transcription factors necessary for adipocyte development (Spiegelman and Flier, 1996; Gregoire et al., 1998; MacDougald and Mandrup, 2002; Rosen and MacDougald, 2006).

Factors that impact adipogenesis

Adipogenesis is a complicated process that can be altered by the presence or absence of a variety of factors. Many factors have been shown to suppress adipogenesis including Wnt glycoproteins, inflammatory cytokines, growth hormone, and other growth factors (MacDougald and Mandrup, 2002). Retinoic acid, the bioactive form of vitamin A, has also been suggested to block adipogenesis (Schwarz et al., 1997; Berry et al., 2012; Wang et al., 2017). Therefore, diets high in vitamin A, such as diets containing fresh dark green plants, may limit adipogenesis and could possibly reduce marbling deposition in beef carcasses. Factors known to stimulate adipogenesis are IGF-1, glucocorticoids, and macrophage colony-stimulating factor (MacDougald and Mandrup, 2002). Another factor that can stimulate adipogenesis is the inclusion of long-chain saturated and unsaturated fatty acids in the diet (MacDougald and Mandrup, 2002; Smith, 2002). Diascro et al. (1998) noted that fatty acids are ligands for PPARγ and can partially be responsible for adipocyte differentiation. Gaillard et al. (1989) further proposed arachidonic acid promoted the differentiation of adipocytes by promoting cyclic AMP, which signals C/EBPβ and C/EBPδ induction by prostacyclin. To understand how dietary

fatty acids promote adipogenesis, it is first necessary to become familiar with how ruminants digest lipids.

Ruminant digestion of lipids

The ruminant digestive system is comprised of a four-compartment stomach, with each compartment responsible for a different phase of digestion. The reticulum is responsible for collecting small digesta particles and transporting them to the omasum and allowing large particles to travel to the rumen; the rumen is the fermentation vat that allows for microbial fermentation by the rumen microorganisms that digest cellulose; the abomasum is the true stomach that produces hydrochloric acid and digestive enzymes; and the omasum absorbs water and some nutrients from feed (Parish et al., 2017).

As a result of the different stomach structure of a ruminant, the way they digest feed is much different than the monogastric. The microorganisms in the rumen play a large role in the nutrients absorbed by the animal. One of the nutrients that is absorbed in a manner unique to ruminants is dietary lipid. Unlike with monogastrics, triglycerides consumed by ruminants are extensively modified by rumen microbes before they are absorbed by the animal (Byers and Schelling, 1988). When an animal consumes a feed containing triglycerides, the microbes split the triglycerides into a glycerol backbone and three fatty acids (Van Soest, 1982). The fatty acids then undergo biohydrogenation, which is the process of adding hydrogens to fatty acids with double bonds with the goal of converting all double bonds to single bonds, fully saturating the fatty acid (Byers and Schelling, 1988). The fatty acids then enter the intestine as highly saturated unesterified fatty acids (Byers and Schelling, 1988). The low pH of the abomasum and proximal small intestine solubilize the fatty acids that were not soluble in the rumen (Byers and Schelling, 1988).

The fatty acids are then absorbed by the small intestine (Van Soest, 1982). The absorbed lipids are turned into lipoproteins, commonly chylomicrons and very low density lipoproteins, and travel through the lymph where they then enter the blood stream (Byers and Schelling, 1988). The lipoproteins then come into contact with apoproteins which activate lipoprotein lipase in tissues such as skeletal muscle, adipose, or mammary tissue, which allows the cells to take up the fatty acids for use (Byers and Schelling, 1988).

Rumen protected fatty acids

The development of rumen bypass fatty acids has allowed for the absorption of long chain fatty acids in higher concentrations than what can occur naturally. Rumen bypass fatty acids are generally coated in a calcium salt that is not soluble into the rumen, allowing the fatty acids to enter the small intestine in their original form (Zinn et al., 2000). Extensive research has investigated the influence of rumen protected fatty acids fed to dairy cows on milk profiles and reproduction (Perfield II et al., 2002; Kitessa et al., 2004; Pappritz et al., 2011).

The ability of rumen protected fatty acids to impact the fatty acid profile of meat has also been studied. Schlegel et al. (2012) fed two levels of rumen protected conjugated linoleic acid to heifers during the early growing period and observed a dose dependent increase in trans-10, cis-12 CLA in longissimus muscle and subcutaneous fat composition but saw no differences in beef quality characteristics. Additionally, a study conducted on early weaned steers evaluated the impact of a dietary rumen undegradable fat source on meat quality and observed increased serum fatty acid levels along with improved marbling scores for the supplemented treatment group (Mangrum et al., 2016). Moreover, Tipton et al. (2017) evaluated length of lipid supplementation on carcass quality of young

steers and observed increased ether extract of longissimus muscle in steers supplemented with a rumen bypass lipid compared to the control. The improved marbling scores and increased ether extract values observed by Tipton et al. (2017) and Mangrum et al. (2016) may be explained by the composition of the rumen bypass lipid supplement. The supplement utilized in both studies contained long chain fatty acids, which as noted earlier, can help upregulate the expression of PPARγ which would increase adipogenesis.

Summary

In conclusion, post-weaning management practices have been shown to have a variety of impacts on beef live animal performance, carcass characteristics, and meat quality. The utilization of backgrounding management practices allows producers to grow their cattle until it makes economic sense to sell them. Many producers have begun to plant cover crops to use as a feedstuff for weaned calves and for wintering cows with the added benefit of improving soil health. The use of brassica mixture cover crops as a backgrounding diet for weaned calves and the potential implications on carcass characteristics and meat quality is not well characterized and warrants further investigation. In addition, finishing phase management may also alter end product quality. Rumen protected long chain fatty acids have been shown to improve marbling scores compared to non-supplemented counterparts potentially by activating PPARγ to increase adipogenesis of intramuscular fat cells. Most research considering the impacts of RPFAs has been conducted on dairy cows or backgrounding calves. Little research has been conducted to evaluate RPFAs as part of a finishing diet as a means to improve marbling scores and meat quality.

Therefore, to better understand the impacts of post-weaning management strategies, the objectives of this dissertation are:

1. Determine the effect of a rumen-protected LCFA fed during finishing on live performance, serum fatty acid profile, carcass traits, and predicted carcass composition.
2. Determine effects of feeding brassica-based cover crops to cattle during backgrounding on live animal performance, carcass characteristics, tenderness, juiciness, and flavor of strip steaks, and case life of ground beef and strip steaks.

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CHAPTER II: EFFECTS OF RUMEN PROTECTED LONG CHAIN FATTY ACID SUPPLEMENTATION DURING THE FINISHING PHASE OF BEEF STEERS ON LIVE PERFORMANCE, CARCASS CHARACTERISTICS, BEEF QUALITY, AND SERUM FATTY ACID PROFILE [1]

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2020

[1] Reprinted with permission: C. E. Bakker, A. D. Blair, J. K. Grubbs, A. R. Taylor, D. W. Brake, N. M. Long, K. R. Underwood. Effects of rumen protected long chain fatty acid supplementation during the finishing phase of beef steers on live performance, carcass characteristics, beef quality, and serum fatty acid profile. Translational Animal Science. 2019. Volume 3. Issue 4. Pages 1585-1592. By permission of Oxford University Press.

Abstract

The effect of a rumen-protected long chain fatty acid (LCFA) supplement on live performance, meat quality, blood serum fatty acid profile, and predicted carcass composition was evaluated in this study. Angus steer calves (n = 99) were fed a low energy diet for 77 days prior to finishing. Prior to study initiation, the steers were separated into 12 pens with 8 or 9 steers per pen. Steers were transitioned from the low energy forage-based diet to a high concentrate diet containing high moisture ear corn, corn silage, dry rolled corn, soybean meal, and a liquid supplement containing monensin across 21 days. Megalac-R (RPFA) was fed to 6 pens at 2% of the diet dry matter. Control pens (CON; n = 6) received an additional 2% of diet dry matter as dry rolled corn and soybean meal. The final finishing diet net energy for gain (NE_g) was 1.20 and 1.19

mega calories.kg^{-1} of dry matter (DM) for RPFA and CON treatments respectively. Steers were weighed every 28 days. Growth performance data including average daily gain (ADG), gain to feed ratio (G:F), and DM intake (DMI) were calculated as both monthly and overall data. After a 147-day finishing phase, steers were transported to a commercial abattoir for slaughter. After a 28-hour chilling period, carcass data were obtained by trained personnel. Final live weights were greater ($P = 0.01$) for RPFA than CON cattle. Overall ADG and overall G:F was increased ($P = 0.02$; $P = 0.01$ respectively) for RPFA cattle. Ribeye area, backfat thickness, kidney pelvic heart fat, marbling score, and yield grade did not differ ($P > 0.05$) between treatments. Predicted percent carcass fat was increased for RPFA cattle ($P = 0.05$). Conversely, predicted percent carcass protein ($P = 0.07$) and bone ($P = 0.06$) tended to be greater for CON cattle. Long chain fatty acid supplementation during the finishing phase did not increase marbling scores of the steers in this study but did increase final live weight, HCW, and predicted total body fat. These results suggest RPFA supplementation has the potential to increase adipose tissue development. However, it is likely that animal age during supplementation and duration of supplementation impact the effect RPFAs have on carcass characteristics.

Key Words: beef, fatty acid profile, finishing diet, meat quality, rumen protected fatty acids

Introduction

Marbling, a primary factor used for quality grading in the United States beef industry has a strong relationship to beef carcass value (Boykin et al., 2017; USDA, 2018). Consumers are willing to pay a premium for beef with increased marbling (Platter

et al., 2005). Marbling positively influences beef flavor and juiciness; two major attributes that contribute to palatability (Mcbee and Wiles, 1967; Behrends et al. 2005; Brewer et al., 2007; Felderhoff et al., 2007; Cashman et al., 2019).

A key transcription factor regulating adipose development and differentiation is peroxisome proliferator gamma (PPARγ) (Saladin et al., 1999; Feve, 2005; Ebrahimi et al., 2018). Kern et al. (2014) reported PPARγ is correlated to marbling development throughout different growth stages in beef steers. There are many activators of PPARγ including long chain fatty acids (LCFA) (Smith, 2002; Sauma et al., 2006). The supplementation of rumen-protected LCFA's (Mangrum et al. 2016) and rumen protected polyunsaturated fatty acids (Cooke et al., 2011) have increased marbling in carcasses from early weaned steers and feeder cattle when compared to non-supplemented controls. However, there is limited research to determine how a rumen-protected LCFA fed during the finishing phase affect predicted carcass composition. Therefore, the objective of this study was to determine the effect of a rumen-protected LCFA fed during finishing on live performance, serum fatty acid profile, carcass traits, and predicted carcass composition. We hypothesized supplementation of a rumen-protected LCFA during the finishing phase would increase marbling scores, alter blood serum fatty acid profiles towards the LCFA composition of the supplement, and increase predicted carcass fat of beef carcasses.

Materials and Methods

Animal procedures were reviewed and approved by the South Dakota State University Institutional Animal Care and Use Committee (approval number 15-081E). Angus steers (n = 99; initial body weight 361± .61 kg) from a single source were received at the South Dakota State University Ruminant Nutrition Center and fed for 77 days to

achieve a weight gain of 1.2 $kg.day^{-1}$. Steers received an anabolic implant containing 200 mg progesterone propionate and 20 mg estradiol benzoate (Synovex-S; Zoetis, Parsippany, NJ) upon receiving. On day 78, steers were adapted to a grain-based diet or a grain-based diet that contained (dry matter-basis; DM) 2% Megalac-R (Church and Dwight Co., Inc. Ewing, NJ) by replacing 1.75% dry-rolled corn and 0.25% soybean meal (Table 2.1) so that 6 pens received supplement long-chain fatty acids from Megalac-R (RPFA) and 6 pens of cattle did not (CON). Diets were formulated to provide approximately 1.3 mega calories net energy for gain (NE_g).kg^{-1} of dry matter for RPFA and CON treatments respectively. Due to feed availability, diet ingredients were changed slightly at the beginning of weeks 4 and 9 of the finishing phase (Table 2.1). At day 28 of the finishing phase, received an anabolic implant that contained 120 mg trenbolone acetate and 24 mg estradiol (Revalor-S; Merck Animal Health, Madison, NJ). Growth performance data including average daily gain (ADG), gain to feed ratio (G:F), and DM intake (DMI) were calculated as both phase and overall data. Each phase encompassed the time frame between weight collections. Phase 1 was day 0-28, phase 2 was day 29-44, phase 3 was day 45-72, phase 4 was day 73-100, phase 5 was day 101-128 and Phase 6 was day 129-147.

After a 147-day finishing phase, steers were transported to a commercial abattoir for slaughter. Hot carcass weight (HCW) was collected prior to chilling the carcasses. After a 28-hour chilling period, carcasses were ribbed between the 12^{th} and 13^{th} rib. Ribeye area (REA), backfat thickness (FT), and kidney, pelvic, heart fat (KPH), and marbling scores were measured by trained personnel. USDA yield grades were calculated using HCW, REA, FT, and KPH.

Proximate analysis and carcass composition calculation

A subset of carcasses (n = 24, 2 per pen) were selected for carcass composition analysis using 9-10-11 rib dissections and analyzed using equations as described by Hankins and Howe (1946). Subset selection was conducted by choosing the carcasses of the two steers with initial body weights, recorded at the beginning of the finishing phase, closest to the average initial weight of the pen. Soft tissue was separated from bone and both were weighed and recorded. The soft tissue was homogenized using a bowl chopper (Model CM-14, Mainca, St. Louis, MO). A 1 kg sample was packaged and stored at -20^{o}C for determination of moisture, protein, fat, and ash through proximate analysis.

The homogenized proximate analysis samples were prepared by freezing in liquid nitrogen and then powdered using a Waring commercial blender (Model 51BL32, Waring Products Division, New Hartford, CT) to produce a homogenous sample. Proximate analysis of the soft tissue was conducted to determine water, fat, crude protein, and ash content of the samples. To determine water content, approximately 5.5 g of sample was weighed, placed in pre-weighed foil pans, covered in pre-weighed filter paper, and placed in an oven (Thelco Laboratory Oven, Precision Scientific, Winchester, VA) for 24 hours at 101^{o}C. After drying and reweighing, dried samples were extracted with petroleum ether in a side arm soxhlet (method 960.39; AOAC, 2000) for 60 hours. Excess ether was allowed to evaporate from samples under the fume hood prior to drying at 101^{o}C for 4 hours and subsequent reweighing. Fat content was calculated as the difference between dried and extracted sample weight. Crude protein was determined by wrapping approximately 200 mg of sample in nitrogen free foil sheets and inserting samples into a nitrogen analyzer (Rapid N III, Elementar, Hanau, Germany). To determine ash content,

3 g of sample was placed in a pre-weighed crucible, dried for 24 hours at 101^{o}C to determine water content, and ashed for 16 hours at 500^{o}C in a muffle furnace (Isotemp Programmable Muffle Furnace, Fischer Scientific, Waltham, MA) and reweighed following cooling in a desiccator.

Hankins and Howe (1946) equations for steers were used to predict composition of the carcass soft tissue from the chemical composition of the 9-10-11 rib section soft tissue using the following equations:

Carcass water = 16.83 + 0.75 (9-10-11 rib water content)

Carcass fat = 3.49 + 0.74 (9-10-11 rib fat content)

Carcass protein = 61.9 + 0.65 (9-10-11 rib protein content)

Total carcass values were calculated from the previously calculated values for soft tissue composition by equations for the proportion of carcass bone and soft tissue outlined by Hankins and Howe (1946) and described by Kern et al. (2014).

Serum fatty acid profile

Blood samples were collected on day 140 of finishing by jugular venipuncture in vacutainer tubes at 1200 hours, 4 hours post feeding, to determine serum fatty acid composition. Samples were allowed to clot and centrifuged at 4^{o}C at 2000 x g for 20 minutes. Serum was collected and transferred into 2mL Eppendorf tubes and frozen until analyzed as described by Park and Goins (1994). Briefly, duplicate 1 mL samples of serum were lyophilized (LabConco, Kansas City, MO) and trans methylated. Samples were analyzed using a Shimadzu 2014 gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a Shimadzu AOC-20S automatic sampler. Separations were completed using a 60-m capillary column (Agilent Technologies, Santa Clara, CA). Identification of

fatty acids was achieved by comparing retention times of known standards. An internal standard, methyl tricosanoic (C23:0) acid, was incorporated into every sample during methylation in order to quantify the sample as a percentage of weight of total fatty acids.

Warner-Bratzler shear force (WBSF)

Strip loins (IMPS 180) were collected from the carcasses chosen as a subset for carcass the prediction of carcass composition. The strip loin was faced and then one 2.54-cm steak was cut, vacuum packaged, and aged for 14 days prior to freezing. Steaks were thawed for 24 hours at 4°C and then cooked to a target internal temperature of 71°C using an electric clam shell grill (George Foreman, Model GR2144P, Middleton, WI). Peak internal temperatures were recorded for each steak using a digital thermometer (Atkins Aqua Tuff NSF Series, Cooper-Atkins Corporation, Middlefield, CT). Steaks were then stored overnight at 4°C. Steaks were removed from refrigeration and equilibrated to 20°C before 6 cores (1.27 cm-diameter) were removed parallel to the muscle fiber direction. Cores were sheared perpendicular to the direction of the muscle fibers using a Warner-Bratzler shear machine (G-R Electric Manufacturing Company, Manhattan, KS) fitted with a Mecmesin 500N basic force gauge (Mecmesin Ltd. West Sussex, United Kingdom) and peak force (kg) was recorded for each core. Shear force value was determined by averaging the peak force values for all 6 cores for each steak.

Intramuscular fat content

The portion of each strip loin that was removed when the strip loins were faced was designated for ether extraction. Steaks were allowed to thaw slightly and trimmed of visible connective tissue and other muscles leaving only the longissimus muscle. Steaks were then minced, snap frozen in liquid nitrogen, and powdered using a Waring

commercial blender (Model 51BL32, Waring Products Division, New Hartford, CT), and placed back in a -20°C freezer until lipid extraction. Lipid extraction was conducted as previously described for carcass composition.

Statistical analysis

Live animal performance, rib composition data, fatty acid profiles of serum, WBSF, and ether extract were analyzed using PROC Mixed of SAS with fixed effect of treatment. Pen was used as the experimental unit. Live performance data were analyzed separately by phase in addition to overall performance. Significance was determined at $P \leq 0.05$ and a trend was declared at $0.05 < P < 0.10$.

Results and Discussion

Animal performance data are presented in Table 2.2. Overall average daily gain was increased ($P = 0.02$) by RPFA compared to CON as well as during phase 3 ($P = 0.05$). Overall gain to feed ratio was also increased ($P = 0.01$) by RPFA vs CON and during phase 3 ($P = 0.02$). Live weight tended to be increased by RPFA compared to CON during phase 4 ($P = 0.07$) and was increased for phases 5 and 6 ($P < 0.01$ and $P = 0.01$ respectively).

Increased final live weight translated to an increased HCW ($P = 0.04$; Table 2.3). Ribeye area, FT, KPH, marbling score, and yield grade did not differ ($P > 0.05$) between treatments. Carcass characteristics of the subsample group were similar to the characteristics of the experimental groups meaning the subset carcasses can be considered a representative sample for meat quality characteristics (Table 2.4). In addition to similar carcass traits, ether extract and WBSF values of steaks for the subset carcass group were not different between RPFA and CON carcasses ($P > 0.05$). The

increase in HCW was likely the result of an increase in weight distributed throughout the carcass resulting in no differences in measurable carcass characteristics. Conversely, Mangrum et al. (2016) fed a rumen protected fatty acid to early weaned steers (weaned at 150 ± 5 d) and did not observe a difference in final live weights or hot carcass weight. However, Mangrum (2016) did see an increase in marbling scores in the RPFA treatment group compared to steers not provided a fatty acid supplement when fed over the course of a 110-day backgrounding phase then transitioned to a 176-day finishing period. A comparison of Megalac to two other rumen-protected lipid supplements rich in PUFAs resulted in an increase in intramuscular fatty acid content for the Megalac diet, high in palmitic acid, compared to a diet containing a protected lipid supplement high in 18:2n-6 and 18:3n-3 (Scollan et al., 2003). Additionally, the fatty acid profile of the lipid supplement showed increases in the proportion of 18:2n-6 and 18:3n-3 in muscle compared to the Megalac treatment (Scollan et al., 2003). It is possible that the increase in intramuscular fat reported by Scollan (2003) was not observed in the present study for two reasons. First, the cattle used by Scollan (2003) were Charolais, which is a terminal breed not known for their marbling potential as opposed to the Angus influenced cattle in the present study (Herring, 2006). Second, the dietary fat content provided by Scollan (2003) was twice the level of dietary fat provided in the current study. Both of these reasons suggest level of supplement and genetic potential for marbling of cattle may affect the efficacy of a rumen-protected supplement. Adipogenesis occurs differently among the four main fat depots. Bruns et al. (2004) observed that subcutaneous fat and KPH increased in a quadratic fashion while intramuscular fat increased linearly when evaluated in carcasses ranging from 208 to 380 kg. Moreover, intramuscular adipocytes

are thought to undergo hyperplasia from the late fetal stage until about 250 days of age, while subcutaneous adipose tissue hyperplasia continues through weaning (Hood and Allen, 1973). The observation of differences in marbling scores by Mangrum et al. (2016) and the lack thereof in this study can potentially be explained by the timing of supplementation. The steers in Mangrum et al. (2016) began treatment at approximately 150 days of age. Therefore, they were well within the "marbling window" proposed by Du et al. (2013) that spans from early weaning to approximately 250 days of age. This is supported by the work of Tipton et al. (2017) that found that supplementation of Megalac-R at either 150 or 210 days of age for either 45 or 90 days resulted in increased marbling at the end of supplementation in beef steers.

Although there was no difference in marbling scores between treatments in the present study, predicted carcass composition was altered with a rumen protected LCFA supplement. Predicted percent carcass fat was increased for RPFA cattle ($P = 0.05$; Table 2.5). Conversely, predicted percent carcass protein ($P = 0.07$) and bone ($P = 0.06$) tended to be greater for CON cattle. The increase in carcass fat could likely be attributed to general adipocyte growth, spurred by either the RPFA supplement or the slightly increased NE_g of the RPFA diet, instead of a focused increase in intramuscular adipocytes. The decrease in predicted carcass protein and bone was likely the inverse reaction to increased carcass fat. The results of our study in combination with previous data (Du et al., 2013; Mangrum et al., 2016) seem to suggest that physiological age is a critical factor in the effectiveness of RPFA supplementation on marbling scores.

The increase in phase 6 live weight and HCW is likely the result of increased growth throughout the carcass as evidenced by a lack of differences in any carcass

characteristic. The increase in predicted carcass fat indicates adipogenesis was increased in RPFA cattle. However, the lack of differences in backfat and marbling scores suggests the increased adipogenesis was distributed across the body instead of primarily within intramuscular fat or subcutaneous fat depots. While PPARγ is widely known as a transcription factor that upregulates adipogenesis and can be activated by long chain fatty acids, it is also thought to stimulate lipogenesis (Saladin et al., 1999; Kersten, 2001; Sauma et al., 2006). The majority of adipocyte hyperplasia (cell proliferation) occurs until approximately 8 months of age in beef cattle (Hood and Allen, 1973). Although, evidence supports that some hyperplasia can occur after this time when existing cells reach lipid capacity and the majority of adipocyte tissue growth is the result of hypertrophy (Robelin, 1981; Cianzio et al., 1985; Du et al., 2013). Therefore, the RPFA supplement could have increased adipocyte hypertrophy and lipogenesis throughout the carcass as evidenced by the increase in predicted carcass fat in this study. However, adipocyte size was not evaluated and therefore we cannot conclude this difference was due to increased adipocyte size.

Serum fatty acid concentrations are reported in Table 2.6. Steers fed the RPFA treatment had greater amounts of palmitic (16:0; $P < 0.01$), stearic (18:0; $P = 0.02$), vaccenic (18:1n7; $P < 0.01$), linoleic (18:2; $P = 0.02$) and total fatty acids ($P = 0.01$) in response to increased dietary fatty acid content.

Inclusion of a RPFA into finishing diets altered blood serum fatty acid profile shown by increased serum fatty acids included in the LCFA supplement. The results of this study are similar to Mangrum et al (2016) with increases in palmitic, stearic, linoleic, and total fatty acids. These results were expected as RPFA is protected from rumen

biohydrogenation which results in greater absorption of the unsaturated fatty acids contained in RPFA into the bloodstream (Zinn et al., 2000). Differences in serum fatty acid profile did not translate into increases in intramuscular fat content. These results differ from Oliveria et al., (2012) where Nellore bulls were fed Megalac-E for 96 days, and an increase in linoleic acid and total omega-6 fatty acids was observed compared to a control diet without oil supplementation. The discrepancy between the two studies can be explained by the different fatty acid profile of the two supplements (Megalac-R vs Megalac-E) and the increased DM inclusion of Megalac-E compared to Megalac-R (4.5% vs 2%). This suggests that with more time, the differences in serum fatty acid profile could translate to differences in the fatty acid profile of the meat, but this research has not yet been conducted on finishing cattle supplemented with the Megalac-E product.

Conclusions

Previous research has shown the impact of LFCA supplementation on carcass characteristics and meat quality; however, results appear to be dependent on a myriad of factors. This study determined that HCW and final weight was increased for steers fed the RPFA treatment compared to the control. Furthermore, the fatty acid profile of blood serum reflects increases in the concentrations of fatty acids present within the Megalac-R product. This indicates that while marbling scores did not differ between experimental treatments, the fatty acids from the RPFA supplement were absorbed into the blood stream. Therefore, further research is warranted to determine if feeding rumen protected long chain fatty acids at a different stage of development such as the time frame from weaning to finishing or a longer feeding period than what occurred in the current study would result in increased marbling scores and improved USDA quality grades. Also, it

would be important to determine if this feeding strategy is best utilized for cattle with traditionally lower marbling scores.

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Table 2.1. Finishing diet for steers fed a control diet (CON) or control diet with 2% rumen protected long chain fatty acids (RPFA)[1]

Ingredient	**CON**	**RPFA**	**CON**	**RPFA**	**CON**	**RPFA**
Diet Identification[2]	1		2		3	
Weeks on Diet[2]	3		5		12	
High moisture ear corn	34.52	34.52	34.77	34.75	35.15	35.22
Corn silage	-	-	-	-	8.25	8.25
Oatlage	11.57	11.57	5.80	5.80	-	-
Dry rolled corn	35.11	33.60	46.59	44.34	43.84	41.60
Megalac-R[3]	-	2.00	-	2.03	-	1.99
Soybean meal	-	-	7.78	8.03	7.71	7.96
DDGS	13.32	12.66	-	-	-	-
Liquid supplement[4]	6.15	5.65	5.06	5.06	4.98	4.98
Nutrient Composition						
NE_g[5], Mcal / kg	1.31	1.33	1.32	1.34	1.33	1.35
Crude Protein	12.48	15.50	13.84	13.76	13.19	13.14
Fat	3.68	3.61	2.97	2.88	2.95	2.87
NDF	15.40	21.10	15.40	15.23	14.22	14.05

[1] Percent inclusion: calculated on a dry matter basis

[2] Diet was altered to accommodate feed ingredient availability. Steers were fed diet 1 for 3 weeks, diet 2 for 5 weeks, and diet 3 for 12 weeks.

[3] Church and Dwight Co., Inc. Ewing, NJ

[4] Westway Feed Products, Tomball, TX; contained 45.17% protein, 1.13% fat, 51.86% ash, and 588 g/T monensin

[5] Net Energy for Gain, mega calories / kg

Table 2.2. Live performance data of steers fed a control diet (CON) or diet with 2% rumen protected long chain fatty acids (RPFA)[1,6]

Variable	CON	RPFA	SEM	*P*-value
Phase 1[2]				
Weight, kg	412	414	1.40	0.52
ADG[3], kg/d	1.78	1.81	0.05	0.65
DMI[4], kg/d	7.44	7.45	0.01	0.49
G:F[5]	0.238	0.243	0.01	0.59
Phase 2[2]				
Weight, kg	435	436	2.09	0.91
ADG[3], kg/d	1.53	1.46	0.14	0.74
DMI[4], kg/d	9.30	9.26	0.14	0.83
G:F[5]	0.163	0.158	0.01	0.78
Phase 3[2]				
Weight, kg	487	493	2.36	0.11
ADG[3], kg/d	1.83	2.02	0.06	0.05
DMI[4], kg/d	9.83	9.79	0.11	0.80
G:F[5]	0.187	0.208	0.01	0.02
Phase 4[2]				
Weight, kg	531	540	3.11	0.07
ADG[3], kg/d	1.59	1.70	0.06	0.23
DMI[4], kg/d	9.94	10.00	0.10	0.68
G:F[5]	0.160	0.170	0.01	0.29
Phase 5[2]				
Weight, kg	577	587	1.97	<0.01
ADG[3], kg/d	1.64	1.68	0.06	0.62
DMI[4], kg/d	10.27	10.46	0.12	0.26
G:F[5]	0.160	0.162	0.01	0.86
Phase 6[2]				
Weight, kg	606	616	2.37	0.01
ADG[3], kg/d	1.52	1.50	0.05	0.81
DMI[4], kg/d	10.60	10.78	0.16	0.44
G:F[5]	0.143	0.143	0.01	1.00
Overall[2]				
ADG[3], kg/d	1.62	1.68	0.02	0.02
DMI[4], kg/d	9.51	9.57	0.07	0.54
G:F[5]	0.170	0.176	0.001	0.01

[1] Least square means

[2] Phase 1: d 0-28, Phase 2: d 29-44, Phase 3: d 45-72, Phase 4: d 73-100, Phase 5: d 101-128, Phase 6: d 129-147, Overall: d 0-147

[3] Average Daily Gain

[4] Dry Matter Intake

[5] Gain : Feed Ratio

[6] Percent inclusion: calculated on a dry matter basis

Table 2.3. Carcass data of steers fed a control diet (CON) or diet with 2% rumen protected long chain fatty acids (RPFA)[1,3]

Variable	**CON**	**RPFA**	**SEM**	***P*-value**
HCW, kg	362.92	368.38	1.07	0.04
REA[4], cm^2	81.57	82.78	1.03	0.18
Backfat[4], cm	1.19	1.14	0.05	0.32
KPH, %	1.85	1.85	0.23	0.97
Marbling Score[5]	406	404	10.75	0.87
Yield Grade	3.14	3.09	0.09	0.57

[1] Percent inclusion: calculated on a dry matter basis

[3]Least square means

[4] Ribeye area and backfat measured between the 12^{th} and 13^{th} rib

[5] Marbling Score: 300 = $Slight^0$, 400 = $Small^0$, 500 = $Modest^0$

Table 2.4. Carcass and meat quality data of subset of steers (n = 24) fed a control diet (CON) or diet with 2% rumen protected long chain fatty acids (RPFA)[1] used for predicted carcass composition and meat fatty acid analysis[3]

Variable	**CON**	**RPFA**	**SEM**	***P*-value**
HCW, kg	360.89	371.30	4.89	0.04
REA[4], cm^2	82.19	83.48	2.58	0.61
Backfat[4], cm	1.17	1.22	0.08	0.60
KPH, %	1.83	1.85	0.06	0.79
Marbling Score[5]	382	404	23.36	0.36
Yield Grade	3.04	3.09	0.16	0.80
WBSF[6], kg	3.50	3.48	0.10	0.91
Ether Extract[7], %	4.39	5.05	0.52	0.39

[1] Percent inclusion: calculated on a dry matter basis

[3]Least square means

[4] Ribeye area and backfat measured between the 12^{th} and 13^{th} rib

[5] Marbling Score: 300 = $Slight^0$, 400 = $Small^0$, 500 = $Modest^0$

[6]WBSF: Warner-Bratzler shear force of longissimus muscle; aged 14 d

[7]Ether Extract of longissimus muscle

Table 2.5. Predicted carcass composition of subset of steers (n = 24) fed a control diet (CON) or diet with 2% rumen protected long chain fatty acids (RPFA)[1,2,3]

Variable	**CON**	**RPFA**	**SEM**	***P*-value**
Bone, %	14.59	13.78	0.39	0.06
Fat, %	23.88	25.52	0.72	0.05
Moisture, %	47.35	46.75	0.51	0.26
Protein, %	13.79	13.56	0.14	0.07

[1] Percent inclusion: calculated on a dry matter basis

[2] Calculated according to Hankins and Howe (1946)

[3] Least square means

Table 2.6. Serum fatty acid profile (µg/ml of serum) of steers fed a control diet (CON) or diet with 2% rumen protected long chain fatty acids (RPFA)[1,2]

Fatty Acid	**CON**	**RPFA**	**SEM**	***P*-value**
14:0	13.67	15.74	2.26	0.38
16:0	139.88	176.60	8.46	<0.01
18:0	237.11	281.69	15.91	0.02
18:1n9	22.42	27.95	5.69	0.35
18:1n7	92.69	120.01	7.00	<0.01
18:2	605.93	801.17	51.11	0.02
20:4n6	25.32	30.20	2.92	0.13
20:4n3	42.41	45.88	3.21	0.31
Total Fatty Acids	1249.68	1532.93	94.29	0.01

[1] Percent inclusion: calculated on a dry matter basis

[2] Least square means

CHAPTER III: IMPACT OF FEEDING COVER CROP FORAGE CONTAINING BRASSICAS TO STEERS DURING BACKGROUNDING ON LIVE ANIMAL PERFORMANCE, CARCASS CHARACTERISTICS, AND MEAT QUALITY

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Abstract

Brassica cover crops are an option for producers to plant to improve soil health and utilize as a feedstuff for cattle. While brassica cover crops have been widely used for grazing cows, their use as a backgrounding feedstuff is relatively unknown. The objective of this study was to determine the impact of feeding a brassica cover crop mixture during backgrounding on carcass characteristics and beef tenderness. Thirty Angus-based steers were assigned to one of two dietary treatments during backgrounding 1) ad libitum access to freshly cut brassica cover crop forage (CC) containing radish, turnip, rapeseed, rye grass and liquid supplement or 2) common midwestern dry lot backgrounding diet containing silage, soybean meal, grass hay, and liquid supplement (CON). Steers were assigned to electronic feed bunks (Insentec, Hokofarm Group; Netherlands) for collection of individual feed intake. Diets were formulated to be nutritionally similar on a dry matter basis. Steers were paired by weight across treatments and pair fed. Dry matter intake was calculated daily for steers in the CC treatment and the following day CON steers were allowed access to an equal amount of dry matter. Steers were weighed weekly and backgrounded for 44 days before transitioning to a common finishing diet and weighed every 28 d. Steers were harvested at an estimated average backfat thickness of

1.02 cm. Standard carcass data were measured, striploins collected, and steaks were aged 3, 7, 14, or 21 d for analysis of Warner-Bratzler shear force (WBSF), autolysis of calpain-1, proteolysis of desmin and troponin-T, and subjective tenderness of 14 d aged steaks evaluated by a trained sensory panel. Data were analyzed using PROC Mixed of SAS with fixed effect of treatment, aging d, and their interaction where appropriate. Proteolysis, calpain-1 autolysis, and shear force data were deemed repeated measures. Significance was considered at $P < 0.05$. Treatment did not influence live animal growth or carcass characteristics ($P > 0.05$). A treatment by d interaction was observed for WBSF values ($P = 0.02$). Control steaks were less tender than CC steaks at d 3 and 7 but did not differ on d 14 and 21. Also, WBSF values were increased for CON steaks at d 3 compared to d 7. Cover crop steaks had increased WBSF values at d 3 compared to d 7, 14, and 21. No treatment differences were detected for calpain-1 autolysis, degradation of desmin and troponin-T, or subjective tenderness ($P > 0.05$). An increased abundance of autolyzed calpain-1 (76 kDa subunit) and decreased abundance of the active (78 kDa) and inactive (80 kDa) subunits was detected with increasing aging d ($P < 0.001$). The abundance of intact desmin and troponin-T decreased with aging d ($P < 0.0001$). Feeding a brassica mixture cover crop during the backgrounding phase of production did not impact live growth, carcass characteristics, subjective tenderness, autolysis of calpain-1, or proteolysis of desmin and troponin-T. However, steaks from the CC treatment reached their ultimate tenderness earlier postmortem than the CON treatment. Further investigation into the mechanisms regulating these differences in tenderness is warranted. However, producers can use cover crops to improve soil quality and be assured that

feeding them in backgrounding diets of beef steers will not negatively impact carcass quality.

Introduction

Post-weaning management practices can both positively and negatively impact palatability traits (Swanek et al., 1999; Montgomery et al., 2000; Roeber et al., 2005; Harsh et al., 2018). Few studies exist that evaluate the impact of backgrounding weaned calves on brassica cover crops on beef palatability and case life. A two-year study conducted on weaned calves during backgrounding allowed calves ad libitum intake of corn residue or an oat/brassica cover crop, or a limit fed silage-based dry lot diet (Cox-O'Neill et al., 2017). Following a common finishing phase, the authors observed increased body weight and hot carcass weight for the cover crop treatment in comparison to the other two treatments. Fehrman (2016) observed an improvement in strip steak tenderness in steaks from steers backgrounded on turnip cover crops compared to steers fed a dry lot diet containing corn stover. Given the limited research conducted to understand the effects of feeding cover crops in a backgrounding system on beef palatability and case life, the objective of this study was to determine effects of feeding brassica-based cover crops to cattle during backgrounding on live animal performance, carcass characteristics, tenderness, juiciness, and flavor of strip steaks and case life of ground beef and strip steaks. We hypothesize a brassica mixture cover crop diet during backgrounding would improve tenderness without impacting other palatability or case life attributes.

Materials and Methods

Animals and experimental diets

Animal procedures were reviewed and approved by the South Dakota State University Institutional Animal Care and Use Committee (approval number 18-010A). Angus-based steers (n = 30; initial body weight 315 ± 25kg) were obtained from a local producer. Three d after arrival at the South Dakota State University Cow Calf Education Research Unit (CCERF), steers were vaccinated for prevention of Bovine Rhinotracheitis, Parainfluenza 3, Bovine Respiratory Syncytial Virus, *Mannheimia haemolytica*, and Bovine Viral Diarrhea Types 1 and 2 (Inforce™3 and ONE SHOT® BVD, Zoetis Inc, Kalamazoo, MI) administered a dewormer (Safe-Guard®, Merck Animal Health, Madison, NJ) and an insecticide (Clean-Up™ II; Bayer Healthcare LLC, Shawnee Mission, KS), weighed, and provided an electronic identification tag. Steers were stratified into treatments by initial body weight. The control treatment (**CON**) received a traditional midwestern backgrounding diet consisting of corn silage, grass hay, soybean meal and a liquid supplement containing monensin (Table 3.1). The cover crop treatment (**CC**) received a backgrounding diet of freshly cut brassica cover crop foliage including annual ryegrass (64.50%), radish (15.08%), Trophy rape seed (9.42%), and purple top turnip (9.40%) and the same liquid supplement as the CON treatment (Table 3.1). After determining treatment, steers were assigned to automated feed bunks that monitored and controlled individual intake (Insentec RIC, Hokofarm Group; Netherlands). Bunk assignments were made based on treatment and initial body weight. Steers were blocked by bodyweight into light, middle, or heavy groups for each treatment. Within each treatment, one steer from each bodyweight block was assigned to

each bunk. Steers were allotted 4 weeks to become acclimated to the feeding system. All steers received a common diet of grass hay and corn silage for the duration of the acclimation process.

After acclimation was complete, steers were fed their experimental diets for 44 d beginning on October 15, 2018. On d 15 of backgrounding, the diets were altered slightly to accommodate a change in liquid supplement inclusion (Table 3.1). The tops of the cover crops were cut daily using a sickle bar mower and collected using a forage harvester. Cover crops were transported to the CCERF within one h of being harvested. Diets were formulated to be isocaloric and isonitrogenous on a dry matter basis based on feed samples taken prior to study initiation. Daily feed intakes were recorded by the feeding system. Steers were pair fed to achieve a similar nutritional profile between treatments. Pairs were stratified by body weight. To accomplish a pair feeding system, the steer in the CC treatment was allowed *ad libitum* access to feed, and the following day the CON steer was allowed the same amount of dry matter that his pair consumed the previous day. Cover crop dry matter (DM) was evaluated weekly and the diet was adjusted accordingly. Body weights were collected every 7 d for the duration of the backgrounding phase. The backgrounding phase was ended on d 44 due to inclement weather that prevented proper harvesting of the cover crop forage.

Finishing phase, harvest, and product collection

Upon completion of the backgrounding phase, all steers were transitioned to a common finishing diet for 187 d (Table 3.2). During the finishing phase steers were weighed every 28 d. Once steers were fully adapted to the finishing diet, they received an anabolic implant containing 200 mg trenbolone acetate and 28 mg estradiol benzoate

(Synovex-Plus; Zoetis, Parsippany, NJ). Steers were ultrasounded on d 164 for prediction of slaughter date to target an entire study group average of 1.02 cm of backfat.

For both the backgrounding and finishing diets, feed samples were obtained weekly and frozen prior to analysis. Samples were composited by month for each feeding phase. Monthly diet analysis can be found in Appendix A.

Steers were transported approximately 240 km to a commercial abattoir for harvest. Standard carcass data and instrumental longissimus color were recorded (Chroma Meter CR-410; Konica Minolta, INC. Osaka, Japan) by trained personnel at 28 h postmortem. Untrimmed shoulder clods (IMPS 114) and strip loins (IMPS 180) were collected and transported under refrigeration to the South Dakota State University Meat Laboratory for fabrication.

Strip loin fabrication

Three d postmortem, strip loins were trimmed of external fat and the anterior end was faced to obtain an even cut surface prior to slicing 2.54-cm steaks. The portion removed when facing the strip loins was frozen and utilized for proximate analysis. The first, second, third, and fourth steaks were aged for 3, 7, 14, or 21 d postmortem respectively and then frozen to be used for Warner-Bratzler shear force (WBSF) evaluation. The fifth steak was aged for 14 d postmortem and frozen for a trained sensory panel. The sixth steak was used for case life, evaluated by objective color analysis. The seventh steak was aged for 14 d postmortem and frozen for an extra sample if further analysis was warranted. The eighth steak was quartered; each piece was designated for proteolysis and aged for 3, 7, 14, or 21 days.

Proximate analysis

Proximate analysis samples were trimmed of external fat and connective tissue and prepared by freezing in liquid nitrogen, and then powdered using a Waring commercial blender (Model 51BL32, Waring Products Division, New Hartford, CT) to produce a homogenous sample. Proximate analysis was conducted to determine moisture, fat, crude protein, and ash content of the samples. To determine moisture content, approximately 5.5 g of sample were weighed, placed in preweighed foil pans, covered in preweighed filter paper, and placed in an oven (Thelco Laboratory Oven, Precision Scientific, Winchester, VA) for 24 h at 101°C. Moisture content was calculated as the difference between wet and dried weight and expressed as a percentage of wet weight.

After drying and reweighing, dried samples were extracted with petroleum ether in a side arm soxhlet (method 960.39; AOAC, 2000) for 60 h. Excess ether was allowed to evaporate from samples under the fume hood prior to drying at 101°C for 4 h and subsequent reweighing. Fat content was calculated as the difference between pre- and post-extracted weight and expressed as a percentage of pre-extracted weight.

Crude protein was determined by weighing approximately 250 mg of powdered sample into a crucible. Samples were analyzed using the Dumas method (method 992.15; AOAC 1996) with a protein analyzer (rapid MAX N exceed, Elementar, Langenselbold, Germany).

To determine ash content, 3 g of sample was placed in a preweighed crucible, dried for 24 h at 101 °C, and ashed for 16 h at 500°C in a muffle furnace (Isotemp Programmable Muffle Furnace, Fischer Scientific, Waltham, MA) and reweighed

following cooling in a desiccator. Ash content calculated by dividing the ashed weight by the wet weight and is reported as a percentage.

Strip steak instrumental color

Strip steaks chosen for shelf life color evaluation were wet aged until 6 d postmortem before they were overwrapped in black 21.6cm x 16.5cm x 2.54cm polystyrene trays (Dyne-A-Pak, Quebec, Canada) with oxygen permeable polyvinyl chloride (15,500 – 16,275 cm^3/m^2/24 h oxygen transmission rate). Samples were placed into a cooler under previously described conditions and instrumental color was evaluated as described above for instrumental ground beef color. Lux was evaluated daily at 12 locations and averaged to determine light intensity over the samples. Average light intensity was 1651 lux throughout the 10-d case life evaluation.

Cook loss and Warner-Bratzler shear force

Steaks designated for WBSF were thawed for 24 h at 4°C. Prior to cooking, steaks were weighed (Model MWP, CAS, East Rutherford, NJ) and initial weight was recorded for cook loss. Steaks were cooked to a target internal temperature of 71°C using an electric clam shell grill (George Foreman, Model GR2144P, Middleton, WI). Peak internal temperatures were recorded for each steak using a digital thermometer (Atkins Aqua Tuff NSF Series, Cooper-Atkins Corporation, Middlefield, CT). When steaks were cooled to room temperature, they were weighed again to determine cook loss (Scale Model MWP, CAS, East Rutherford, NJ). Steaks were stored overnight at 4°C. The next morning, steaks were removed from refrigeration and equilibrated to room temperature before six cores (1.27-cm diameter) were removed parallel to the muscle fiber direction. Cores were sheared perpendicular to the direction of the muscle fibers using a texture

analyzer (EZ-SX, Shimadzu Corporation, Kyoto, Japan) fitted with a Warner-Bratzler shear force head with a crosshead speed of 20 cm/min. Peak force was recorded for each core, and shear force value was determined by averaging the peak force values for all six cores for each steak.

Protein extraction

Samples designated for proteolysis were powdered as previously described to produce a homogenous sample. Protein samples for gel electrophoresis and Western Blots were prepared as described by Melody et al. (2004) with several modifications. Briefly, 0.45 to 0.50 grams of sample was weighed and homogenized using an overhead stirrer (model RZR1; Heidolph, Schwaback Germany) in 10 mL of whole muscle buffer (2% sodium dodecyl sulfate [SDS], 10 nM sodium phosphate) to extract myofibrillar and sarcoplasmic proteins. Homogenized samples were centrifuged for 20 min at 3000 RPM (1,700 x g) at 25°C. Protein concentration of the supernatant was determined by diluting in duplicate samples using a 1:20 dilution in double distilled deionized water. Protein concentrations were determined using a Lowry assay kit (DC Protein Assay Kit; Bio-Rad Laboratories, Hercules, CA) and analyzed using a spectrophotometer at a wavelength of 750 nm (SpectraMax 190; Molecular Devices, Sunnyvale, CA) and related software (SoftMax Pro 6; version 6.2.1; Molecular Devices, Sunnyvale, CA) that evaluated protein concentration in relation to a standard curve. Gel samples were prepared to a concentration of 4 mg/mL and stored frozen until analysis. Prior to western blot analysis, gel sample protein concentration was checked using 15% SDS polyacrylamide separating gels (SDS-PAGE; acrylamide:N,N'-bis-methylene acrylamide = 100:1, 0.1% SDS, 0.05% TEMED, 0.5% ammonium persulfate [APS], and 0.375 M Tris HCl, pH 8.8) with

5% stacking gels (acrylamide:N,N′- bis-methelyene acrylamide = 100:1, 0.1% SDS, 0.125% TEMED, 0.075% APS, and 0.125 M Tris HCl, pH 6.8) to ensure proper dilution of the gel sample. Gels were run using a mini gel electrophoresis unit (model SE-260; Hoefer Scientific, Holliston, MA) at 120 v for 390 v h. Gels were visualized using a Coomassie blue stain (40% methanol, 7% glacial acetic acid, 0.1% Coomassie brilliant blue R-250) for 24 h. Gels were destained in 40% methanol and 7% glacial acetic acid. Protein profiles were visually analyzed for similarities across samples (FlourChem M multiflour imaging system; ProteinSimple, Santa Clara, CA) using auto-exposed white light.

Western blot analysis

Desmin and troponin-T degradation along with calpain-1 autolysis were determined using 40 µg of protein sample run on 15%, 15%, and 8% SDS-PAGE gels respectively at 120 v for 3 h. Each sample was run in duplicate. After electrophoresis, gels were transferred to a polyvinylidene difluoride (Immobilon, Darmstadt, Germany) membrane with a pore size of 0.45 µm using a TE-22 transfer unit (Hoefer Scientific, Holliston, MA) for 90 min at 90 v. Transfer buffer (24 mM Tris, 186 mM glycine, and 15% methanol) was maintained at 4°C using a refrigerated water bath (IsoTemp, model 6200 R28; Thermo Fischer Scientific, Asheville, NC) Once transfer was complete, membranes were blocked using a 0.5% non-fat dry milk solution for 1 h. Primary antibodies (1:80,000 Rabbit anti-desmin; Iowa State University, Lonergan Lab for desmin, 1:15,000 JLT-12 goat anti-mouse for troponin-T, Sigma, St. Louis, MO, and 1:10,000 Mu-Calpain Monoclonal Antibody #MA3-940; Thermo Fisher Scientific, Asheville, NC for calpain-1) were applied to the membranes and incubated at 4°C

overnight. Bots were equilibrated to room temperature for 1 hr prior to washing with PBS-Tween (66 mM sodium phosphate, 0.1 M NaCl, and 0.1% Tween-20) three times for 10 min each time. Secondary antibody was applied to each membrane (1:20,000 goat anti-rabbit horseradish peroxidase; Thermo Fischer Scientific, Asheville, NC for desmin, 1:20,000 goat anti-mouse horseradish peroxidase for troponin-T, and 1:10;000 goat anti-mouse horseradish peroxidase, Thermo Fisher Scientific, Asheville, NC for calpain-1) and incubated for 1 h. Blots were, again, washed three times for 10 minutes each with PBS-Tween following incubation. Membranes were developed using an ECL Prime detection kit (GE Healthcare, Lafayette, CO). Images were obtained using chemiluminescence with the imaging system previously described. Bands were quantified using AlphaView SA software (ProteinSimple; San Jose, CA). Intact desmin, intact troponin-T, and degraded troponin-T were analyzed as a ratio to an internal standard used across all western blots to control for inter gel variation. Calpain-1 autolysis of intact (80 kDa), active (78 kDa), and autolyzed (76 kDa) calpain-1 was analyzed as a percentage of detected calpain-1 in each sample.

Trained sensory panel

Twelve sensory panelists were trained to evaluate tenderness, juiciness, and beef flavor intensity of strip loin steaks according to AMSA training guidelines with modifications appropriate for the study (AMSA, 2016). Panelists evaluated the attributes on an anchored unmarked line scale with the far-left point indicating extremely tender, extremely juicy, or extremely bland beef flavor and the far-right point representing extremely tough, extremely dry, or extremely intense beef flavor. A copy of the trained sensory panel evaluation sheet can be found in Appendix B. Steaks were cooked on an

electric clamshell grill (George Foreman, Model GRP1060B, Middleton, WI) to a target peak internal temperature of 71 °C (Atkins Aqua Tuff NSF Series, Cooper-Atkins Corporation, Middlefield, CT), and held at 63 °C in a warming oven (Metro HM2000, Wilkes-Barre, PA). Approximately 15 min prior to sensory evaluation, steaks were trimmed of external fat and connective tissue and portioned into 2.54 cm x 1cm x 1 cm cubes. Two cubes were placed into a prelabeled lidded 2 oz plastic cup and returned to the warming oven until they were administered to panelists.

Evaluations were performed according to AMSA guidelines (AMSA, 2016). Ten samples were evaluated in each session, one session per d, for a total of 3 sessions. Samples evaluations were alternated by treatment to reduce first and last order bias. Panelists were secluded by partitioned booths with red lighting and separated from the steak preparation area.

Chuck clod processing and ground beef color evaluation

Chuck clods were trimmed of subcutaneous fat and ground twice through a 0.476 cm plate (4822 Hobart Mfg. Co., Troy, OH). Ground beef was allocated into five 0.454 kg portions. One portion was vacuum sealed and frozen immediately. The remaining portions were placed on white 14 cm x 14 cm x 1.27 cm polystyrene trays (Dyne-A-Pak, Quebec, Canada), overwrapped with oxygen permeable polyvinyl chloride (15,500 – 16,275 cm^3/m^2/24 h oxygen transmission rate), and assigned a three-digit identification code. Trays were placed in a cooler at 4°C with fluorescent lighting (F32 T8, 2975 lumens, 2.54cm diameter fluorescent bulbs; General Electric, Boston, Mass). Lux was measured in 8 locations of the cooler daily and averaged to calculate light intensity (Digital Lux Meter; Model LX1330B, Dr. Meter, London, England). Average light

intensity through the duration of the color panel was 1445 lux. Samples were rotated daily to eliminate a cooler location effect on sample color.

Subjective color evaluation was conducted by eight trained panelists between 1400 and 1600 h daily for 8 d. On d 0, panelists evaluated ground beef color on a scale of 1 to 8 with 1 indicating “Bleached Red” and 8 indicating “Very Dark Red” (Appendix C). Color evaluations on d 1 through 7 were evaluated on a scale of 1 to 8 with 1 indicating “Very Bright Red” and 8 indicating “Tan to Brown” (Appendix D). Discoloration for all d was evaluated on a scale of 1 to 6 with 1 indicating 0% discoloration and 6 indicating 81 to 100% discoloration (Appendix C and D). Panelists were allowed to evaluate lean color in 0.5-point increments and discoloration in 1-point increments. Beginning on d 3, panelists were asked to indicate if they considered the samples were acceptable for display in a retail setting. The panel was terminated on d 7 when all panelists considered at least 90% of samples unacceptable for retail.

Instrumental color was evaluated at 1600 h daily for the duration of the trained color panel. Instrumental L*, a*, and b* values were measured with a colorimeter (Chroma Meter CR-410; Konica Minolta, INC. Osaka, Japan) at 3 locations on each ground beef sample and averaged to obtain daily color values.

Statistical analysis

All data were analyzed using the MIXED procedure of SAS 9.4 (SAS Institute Inc., Cary, NC) with the fixed effect of treatment. Animal was considered to be the experimental unit. Live animal performance data were analyzed by diet phase (backgrounding or finishing) as well as overall. Cook loss, WBSF, case life color measurements, and Western Blot proteolysis data were analyzed as repeated measures.

Peak internal temperature was used as a covariate for WBSF and cook loss using a Toeplitz covariance structure. Significance was declared at $P < 0.05$. Treatment by day interactions were evaluated where appropriate and are reported when significant.

Results and Discussion

Live animal performance

Live animal performance data are presented in Table 3.3. No differences were observed in body weight, average daily gain (ADG), or dry matter intake (DMI) throughout the study ($P > 0.05$). Gain to feed (G:F) ratio did not differ in the backgrounding or finishing phase ($P > 0.05$). Overall G:F was increased for the CON treatment compared to CC (0.143 vs 0.134; $P = 0.02$). This is likely due to the numeric increases observed in G:F for both the backgrounding and finishing phases. Similarly, Nenn (2017) observed no differences in overall ADG or final body weight. Conversely, in a comparison between a brassica / oat grazing diet compared to a dry lot diet, Cox-O'Neill et al. (2017) observed an increase in backgrounding phase ADG and a decrease in backgrounding phase DMI as well as an increased final live weight and finishing phase daily DMI for the brassica diet compared to the dry lot diet.

Carcass characteristics and proximate analysis

Carcass characteristics and longissimus color recorded at the time of grading did not differ between treatments ($P > 0.05$; Table 3.4). The lack of difference in backfat thickness was not unexpected as cattle were harvested at a common backfat thickness predicted with ultrasound. Similar to the current study, Fehrman (2016) did not observe differences in carcass characteristics in the comparison of a backgrounding diet including turnips to a dry lot diet. Additionally, Cox-O'Neill et al., (2017) did not observe a

treatment effect in backfat thickness or calculated yield grade. However, they did observe a decrease in REA and HCW for the dry lot treatment compared to the cover crop treatment. No differences were observed in the proximate analysis of longissimus steaks between treatments ($P > 0.05$; Table 3.4). These results support the carcass data as no differences in marbling scores were detected, thus no difference in percent fat was expected.

Strip steak color analysis

No differences in instrumental L* values were observed for strip steaks between treatments (46.61 ± 0.12 vs 46.88 ± 0.12; $P = 0.110$; CC vs CON respectively) or by d of case life ($P = 0.986$; Figure 3.1). A treatment by d interaction was observed for a* values ($P < 0.0001$; Figure 3.2). No statistical differences were observed between treatments on any day. However, steaks in the CC treatment presented numerically increased a* values compared to CON steaks throughout the case life period. Additionally, a treatment by d interaction was also observed for b* values ($P < 0.0001$; Figure 3.3). Steaks from the CC treatment displayed increased b* values compared to steaks from the CON treatment on d 2 (8.43 vs 7.78; $P = 0.042$) and 5 (7.86 vs 7.23; $P = 0.049$), while remaining numerically increased on all other d ($P > 0.05$). Fehrman (2016) also evaluated instrumental color during case life on strip steaks over 8 d and observed no treatment effects for L*, a*, or b* values. To our knowledge, no other studies have reported the impact of backgrounding on cover crops on ground beef case life.

Interestingly, the a* values for ground beef are the opposite compared to the steaks. The ground beef a* values were numerically increased for the CON treatment while steak a* values were increased for the CC treatment. The differences in behavior

of the two types of sample could be attributed to differences in lipid content, muscle type, or mitochondrial activity, all of which impact meat color (Cassens and Cooper, 1971; Hunt and Hedrick, 1977; Ramanathan et al., 2009).

Warner-Bratzler shear force and cook loss

A treatment by d interaction was observed for WBSF values ($P = 0.017$; Figure 3.4). Steaks from the CON treatment were less tender than CC steaks at d 3 (3.41 vs 2.89 ± 0.14; $P = 0.011$) and 7 (3.16 vs 2.47 ± 0.14; $P = 0.0007$) before becoming similar on d 14 and 21 ($P > 0.05$). Additionally, WBSF values were increased for CON steaks at d 3 compared to d 7 ($P = 0.045$), 14 ($P < 0.0001$), and 21 ($P < 0.0001$) as well as values for d 7 were increased compared to d 14 ($P = 0.0001$) and 21 ($P = 0.001$). Steaks in the CC treatment presented increased WBSF values at d 3 compared to d 7 ($P = 0.0007$), 14 ($P = 0.015$), and 21 ($P = 0.044$). These data suggest steaks from steers backgrounded on brassica cover crops reach ultimate tenderness at d 7, while steaks from the traditional backgrounding diet reached ultimate tenderness at d 14. Similar results were observed by Fehrman (2016) as steaks from steers backgrounded on brassica cover crops were more tender at d 5 compared to steaks from steers backgrounded on corn stover.

Treatment did not impact cook loss ($P = 0.114$; Table 3.5). However, cook loss increased with length of postmortem aging ($P = 0.042$; Table 3.6). Steaks aged 21 d experienced greater amounts of cook loss than steaks aged for 3 (19.36 ± 0.59 vs 17.82 ± 0.59%; $P = 0.017$) or 7 d (19.36 ± 0.59 vs 17.27 ± 0.58%; $P = 0.010$ respectively). Similar results were observed by Shanks et al. (2002) when evaluating cook loss over 35 d postmortem. Increases in cook loss over time may be the result of damage to cellular

membranes, which would enable a greater amount of moisture to leak out of the muscle during cooking (Shanks et al., 2002).

Western blot analysis of desmin, troponin-T, and calpain-1

No treatment effects were observed for intact desmin, intact troponin-T, degraded troponin-T, inactive (80 kDa) calpain-1, active (78 kDa) calpain-1, or autolyzed (76 kDa) calpain-1 ($P > 0.05$; Table 3.5). These data suggest while differences in WBSF exist, the proteins and time points chosen for evaluation do not adequately explain the differing rates of tenderness development. Given the differences in tenderness were observed when the experimental diets were fed for only 44 d with a 147 d finishing phase prior to slaughter, the mechanisms responsible for those differences is likely quite complex. Huff-Lonergan et al. (1996) observed an increase in the rate of desmin degradation for steaks with lower WBSF values and concluded that calpain-1 was likely partially responsible for the degradation. Calpain-1 activates and autolyzes quickly postmortem. Boehm et al. (1998) suggested by 1 d postmortem, calpain-1 has only 20% of its at-death activity. Therefore, it is possible that the conflicting data for WBSF and proteolysis were due to a faster rate of calpain autolysis prior to d 3 sampling for the CON treatment compared to CC. Troponin-T is categorized as a regulatory protein that regulates contraction. It is generally believed to provide little structural support to myofibers and is used as an indicator of tenderness as opposed to causing the physical disruption of myofibers needed for tenderness formation (Koohmaraie, 1992; Huff-Lonergan et al., 2010). As troponin-T degradation has been established as a good indicator of tenderness formation, it is unclear why differences in intact and degraded troponin-T were not observed in this study. However, while not statistically significant, the proteolysis data for desmin and troponin-

T numerically support the WBSF data. The data from the CC treatment indicate numeric decreases in intact desmin and troponin-T and a numeric increase in degraded troponin-T compared to the CON treatment. Thus, it is possible that an additive effect of proteolysis of the proteins evaluated can partially explain the observed differences in WBSF.

A day effect was observed for desmin, troponin-T, and calpain-1 ($P < 0.001$; Table 3.6). These data are not surprising as it is well documented that myofibrillar proteins degrade during postmortem storage (Koohmaraie et al., 1984; Huff-Lonergan et al., 1996; Boehm et al., 1998).

Trained sensory panel

No treatment differences were observed for tenderness, juiciness, or flavor ($P > 0.05$; Table 3.5) as evaluated by a trained sensory panel. Notably, the panel results support d 14 WBSF and cook loss results as no differences were observed for those data. Thus, differences in tenderness and juiciness were not expected. Similarly, Duckett et al. (2007) evaluated the impact of winter stocker growth on palatability and observed no differences in flavor were detected by a trained panel. Therefore, it is likely that any impact backgrounding diet had on flavor was dissipated via a common finishing period.

Ground beef color analysis

During evaluation of initial ground beef color, the trained color panelists tended to rate CC ground beef closer to a cherry red color compared to CON (4.03 vs 3.82 ± 0.08; $P = 0.073$; Figure 3.5). No treatment by d interactions were observed for trained color panel scores for d 1 through 7 ($P > 0.05$). However, trained panel color scores were increased for CC ground beef samples compared to CON samples (5.79 vs 5.48 ± 0.029; $P < 0.001$; Figure 3.6). These values indicate the CC treatment was closer to a reddish tan

/ brown color while the CON treatment was closer to a moderately dark red color. Additionally, color scores were increased from d 1 to d 7 ($P < 0.001$; Figure 3.7). Color scores increased linearly from d 1 to d 5, and from d 6 to d 7. The change in color over time was expected as the myoglobin state of meat changes from oxymyoglobin (bright cherry red) to metmyoglobin (brown) as it oxidizes when exposed to oxygen and light (Mancini and Hunt, 2005; Suman and Joseph, 2013). A treatment by d interaction was observed for trained panel discoloration scores when evaluated from d 0 to d 7. Treatments discolored similarly from d 0 to d 3 before the rate of discoloration increased for the CC treatment compared to CON ($P < 0.001$; Figure 3.8). The increased color scores coupled with the increased rate of discoloration for CC compared to CON treatments are likely due to an increase in metmyoglobin formation. Suman and Joseph (2013) noted that discoloration is generally referred to as the amount of surface area covered by metmyoglobin. Therefore, it can be inferred that the CC treatment could have resulted in an increased oxidation rate of ground beef. The evaluation of the impact of backgrounding diets on ground beef case life is largely unstudied. However, Fehrman (2016) reported a treatment by day interaction for color scores evaluated by a trained panel and noted an increase in color scores for the cover crop treatment compared to the control on d 1 to 4 of case life. Additionally, trained panelists found samples from the cover crop treatment to be less desirable than the control on d 1 to 4 (Fehrman, 2016). Fehrman (2016) did observe a treatment by day interaction for discoloration scores of ground beef. However, unlike the current study, the authors reported differences in discoloration on d 2 to 4 before all treatments became similar for d 5 to 7 (Fehrman, 2016).

Instrumental L* values of ground beef did not differ between treatments (48.08 ± 0.13 vs 48.33 ± 0.12; $P = 0.144$; CC vs CON respectively) or d of case life ($P = 0.984$; Figure 3.9). A treatment by d interaction was observed ($P < 0.0001$; Figure 3.10) for redness (a*). While no differences were observed between treatments on any d, values for CON samples were numerically increased throughout the observation period. A treatment by d interaction was also observed for yellowness (b*) values ($P < 0.0001$; Figure 3.11). Yellowness values were increased for both treatments on d 0 compared to d 1 and d 2. Then, values remained similar from d 2 to d 5. Day 6 values were increased ($P < 0.05$) compared to d 2 and 3 and similar to d 4, 5, and 7. The instrumental color results of this study coincide with the trained panel observations. As the panel went on, the panelists indicated the color of the samples became more brown, which would be associated with decreasing a*. O'Sullivan et al. (2003) noted that panelists generally associated b* values with brown colors, thus the increasing b* values after day 3 are consistent with their findings. It is possible that the differences in observed color were due to differences in oxidation rate of the samples as lipid oxidation has been shown to impact color (Faustman and Cassens, 1990; Mancini and Hunt, 2005). However, oxidation of the samples was not evaluated in this study and no evidence supporting this hypothesis could be generated.

Conclusion

The present study has shown that dietary management during the backgrounding phase has the ability to influence meat color and tenderness, even after a common finishing diet. The rate of discoloration of ground beef was increased for the CC treatment. Tenderness as measured by WBSF was improved more rapidly in steaks from

the CC treatment, but differences were not detected after 14 d of postmortem aging, and no treatment effects were observed for proteolysis data. As color and tenderness are important quality attributes to consumers, additional research is warranted to continue to evaluate the impacts of dietary brassica cover crop forages during backgrounding on meat quality.

Appendix A: Feed analysis of monthly composite samples (dry matter basis)

Analysis conducted by Servi-Tech Laboratories, Hastings, NE

Backgrounding	Month	CF[1]	NDF[2]	ADF[3]	CP[4]	Ash	TDN[5]	NEM[6]	NEG[7]
SBM	All	0.9	10.3	7.5	53.2	6.6	83	0.92	0.62
Hay	All	1.3	67.9	43.5	8.3	9.7	54.3	0.51	0.25
Cover crop	All	0.9	46.0	37.9	12.4	11.3	60.6	0.60	0.34
Silage	All	2.3	38.8	24.3	7.1	4.4	70.7	0.75	0.48
Finishing	**Month**	**CF[1]**	**NDF[2]**	**ADF[3]**	**CP[4]**	**Ash**	**TDN[5]**	**NEM[6]**	**NEG[7]**
DRC	Jan	3.4	7.8	2.5	8.3	1.5	86.6	0.97	0.67
Hay	Jan	1.1	62.3	38.7	8.2	10.1	59.7	0.59	0.33
Earlage	Jan	2.8	19.8	10.6	7.7	2.1	79.7	0.88	0.59
DDGS	Jan	7.6	32.9	14.4	32.4	6.1	77.6	0.85	0.56
DRC	Feb	3.4	7.8	2.6	7.3	1.5	86.6	0.97	0.67
Hay	Feb	1.2	65.1	41.8	9.6	10.4	56.1	0.54	0.28
Earlage	Feb	2.4	17.4	9.5	7.8	1.9	80.8	0.89	0.60
DDGS	Feb	6.9	33.3	15.1	32.4	5.5	77.6	0.85	0.56
DRC	March	3.3	7.8	2.6	7.0	1.5	86.6	0.97	0.67
Hay	March	1.4	66.6	41.9	7.3	9.0	56.1	0.54	0.28
Earlage	March	3.2	17.1	8.9	7.2	1.8	80.8	0.89	0.60
DDGS	March	6.5	33.8	15.0	32.7	5.9	77.6	0.85	0.56
DRC	April	3.2	7.6	2.9	7.5	1.6	85.7	0.93	0.66
Hay	April	1.3	65.5	41.2	7.8	9.0	56.1	0.54	0.28
Earlage	April	3.2	18.2	9.2	7.7	2.0	80.8	0.89	0.60
DDGS	April	6.5	34.4	15.0	32.8	6.8	77.6	0.85	0.56
DRC	May	3.1	8.1	2.7	7.5	1.6	86.6	0.97	0.67
Hay	May	1.6	62.2	41.5	8.6	8.6	56.1	0.54	0.28
Earlage	May	3.2	18.7	9.4	8.6	2.5	80.8	0.89	0.60
DDGS	May	7.6	33.9	15.0	31.6	6.0	77.6	0.85	0.56

[1] Crude fat

[2] Neutral detergent fiber

[3] Acid detergent fiber

[4] Crude protein

[5] Total digestible nutrients

[6] Net energy, maintenance

[7] Net energy, gain

Appendix B: Trained sensory panel evaluation sheet

Sample ID_____

Extremely Tender — Extremely Tough

Extremely Juicy — Extremely Dry

Extremely Bland Beef Flavor — Extremely Intense Beef Flavor

Sample ID_____

Extremely Tender — Extremely Tough

Extremely Juicy — Extremely Dry

Extremely Bland Beef Flavor — Extremely Intense Beef Flavor

Appendix C: Initial ground beef trained panel scale sheet

Day 0 Color and Discoloration Scores

Color*

1 = Bleached red

2 = Very light cherry red

3 = Moderately light cherry red

4 = Cherry red

5 = Slightly dark red

6 = Moderately dark red

7 = Dark red

8 = Very dark red

Discoloration**

1 = No discoloration; 0%

2 = Slight discoloration; 1 – 20%

3 = Small discoloration; 21 – 40%

4 = Modest discoloration; 41 – 60%

5 = Moderate discoloration; 61 – 80%

6 = Extensive discoloration; 81 – 100%

* Use 0.5 point increments
** Use 1 point increments

Appendix D: Ground beef trained panel scale sheet

Day 1-7 Color and Discoloration Scores

Color*

1 = Very bright red

2 = Bright red

3 = Dull red

4 = Slightly dark red

5 = Moderately dark red

6 = Reddish tan / brown

7 = Dark reddish tan / brown

8 = Tan to brown

Discoloration**

1 = No discoloration; 0%

2 = Slight discoloration; 1 – 20%

3 = Small discoloration; 21 – 40%

4 = Modest discoloration; 41 – 60%

5 = Moderate discoloration; 61 – 80%

6 = Extensive discoloration; 81 – 100%

* Use 0.5 point increments
** Use 1 point increments

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Table 3.1. Backgrounding diet composition for steers backgrounded on a cover crop mixture including brassicas (CC) or a common midwestern backgrounding diet (CON) prior to transitioning to a common finishing diet[1]

Ingredient	**CC**	**CON**	**CC**	**CON**
Diet composition	d 0 - 14		d 15 - 44	
Cover crop mixture, %	95.06	-	96.39	-
Corn silage, %	-	54.43	-	58.11
Ground hay, %	-	18.83	-	20.25
Soybean meal, %	-	14.99	-	16.85
Liquid supplement[2], %	4.94	11.75	3.61	4.16
Nutrient composition[3],				
ADF[4], %	36.03	22.54	36.53	24.47
NDF[5], %	43.73	35.45	44.34	38.46
Ether extract, %	0.87	1.66	0.87	1.77
Crude protein, %	13.31	17.02	13.06	16.10
Ash, %	10.74	5.21	10.89	5.56
NE_M[6], Mcal/cwt	60.15	71.62	60.11	72.35
NE_G[7], Mcal/cwt	34.34	44.93	34.25	45.26

[1] Calculated on a dry matter basis

[2] Contains 512 g/ton (DM) of monensin; Dakotaland Feeds, Huron, SD

[3] Analyzed by Servi-Tech Laboratories, Hastings, NE

[4] Acid detergent fiber

[5] Neutral detergent fiber

[6] Net energy, maintenance

[7] Net energy, gain

Table 3.2. Common finishing diet composition for steers backgrounded on a cover crop mixture including brassicas or a common midwestern backgrounding diet[1]

Ingredient	**Step 1**	**Step 2**	**Step 3**	**Step 4**	**Step 5**
Diet composition	d 45 - 72	d 73 - 91	d 92 - 98	d 99 - 105	d 106 - 231
Corn silage, %	58.11	-	-	-	-
Ground hay, %	20.25	34.97	28.81	18.94	10.66
Soybean meal, %	16.85	-	-	-	-
Liquid supplement[2], %	4.16	5.82	6.35	6.48	6.47
Earlage, %	-	44.34	30.44	20.89	11.62
Dry rolled corn, %	-	1.11	18.31	36.44	52.34
Dried distillers grains with solubles, %	-	13.77	16.09	17.24	18.90
Nutrient composition					
ADF[3], %	24.47	20.24	17.15	12.94	9.76
NDF[4], %	38.46	35.18	30.70	24.45	19.49
Ether extract, %	1.77	2.72	3.01	3.34	3.49
Crude protein, %	16.10	12.63	12.71	13.07	13.73
Ash, %	5.56	3.32	4.81	3.95	3.17
NE_M[5], Mcal/cwt	72.35	76.09	79.22	83.65	86.03
NE_G[6], Mcal/cwt	45.26	48.53	51.34	55.30	58.14

[1] Calculated on a dry matter basis

[2] Contains 512 g/ton (DM) of monensin; Dakotaland Feeds, Huron, SD

[3] Acid detergent fiber

[4] Neutral detergent fiber

[5] Net energy, maintenance

[6] Net energy, gain

Table 3.3. Live animal performance of steers backgrounded on a cover crop mixture including brassicas (CC) or a common midwestern backgrounding diet (CON) prior to transitioning to a common finishing diet[1]

Variable	**CC**	**CON**	**SEM**	***P*-value**
Backgrounding phase[2]				
Initial weight, kg	314	316	6.608	0.840
ADG[3], kg/d	0.33	0.41	0.063	0.397
DMI[4], kg/d	6.47	6.48	0.330	0.982
G:F[5]	0.051	0.062	0.009	0.423
Body weight[6], kg	329	334	5.482	0.503
Finishing phase[2]				
ADG[3], kg/d	1.49	1.52	0.034	0.578
DMI[4], kg/d	10.81	10.80	0.219	0.971
G:F[5]	0.138	0.141	0.002	0.450
Body weight[6], kg	607	618	9.148	0.433
Overall				
ADG[3], kg/d	1.27	1.30	0.028	0.367
DMI[4], kg/d	9.51	9.14	0.188	0.175
G:F[5]	0.134	0.143	0.003	0.022

[1] Least square means

[2] Backgrounding phase was 44 d; Finishing phase was 187 d

[3] Average daily gain

[4] Average daily dry matter intake

[5] Gain to feed ratio

[6] Body weight at the end of the feeding phase

Table 3.4. Carcass data, longissimus muscle color, and proximate analysis of steers backgrounded on a cover crop mixture including brassicas (CC) or a common midwestern backgrounding diet (CON)[1]

Variable	**CC**	**CON**	**SEM**	***P*-value**
Hot carcass weight, kg	385	395	4.906	0.210
Ribeye area[3], cm^2	88.05	92.83	2.704	0.222
Backfat[3], cm	0.93	1.01	0.062	0.352
Marbling score[4]	469	503	17.880	0.190
Yield grade	2.67	2.59	0.123	0.650
L*[5]	41.59	41.26	0.438	0.606
a*[5]	24.53	24.40	0.180	0.611
b*[5]	9.82	9.58	0.134	0.214
Moisture, %	72.32	71.85	0.327	0.315
Fat, %	5.48	6.08	0.411	0.313
Protein, %	21.18	20.85	0.154	0.138
Ash, %	1.05	1.04	0.009	0.322

[1] Least square means

[3] Ribeye area and backfat measured between the 12^{th} and 13^{th} rib

[4] Marbling Score: 300 = $Slight^0$, 400 = $Small^0$, 500 = $Modest^0$

[5] Measured on the longissimus muscle at time of carcass grading

Table 3.5. Cook loss, proteolysis of desmin and troponin-T, autolysis of calpin-1, and trained sensory panel attributes for strip steaks from steers backgrounded on a cover crop mixture including brassicas (CC) or a common midwestern backgrounding diet (CON)[1]

Variable	**CC**	**CON**	**SEM**	***P*-value**
Cook loss, %	17.56	18.78	0.528	0.114
Intact desmin[2]	0.9262	1.0076	0.047	0.215
Intact troponin-T[2]	0.8926	0.9117	0.032	0.673
Degraded troponin-T[2]	1.0589	0.9781	0.052	0.314
Inactive calpain-1[3], %	9.99	10.92	0.899	0.470
Active calpain-1[3], %	31.71	32.52	0.735	0.444
Autolyzed calpain-1[3], %	58.24	56.44	1.373	0.361
Tenderness[4]	68.24	74.00	4.389	0.346
Juiciness[4]	97.28	92.22	4.552	0.428
Flavor[4]	80.85	86.16	3.054	0.204

[1] Least square means

[2] Expressed as a ratio to an internal standard

[3] Expressed as a percentage of total calpain-1

[4] Evaluated on an anchored unmarked line scale where 0 = extremely tender, extremely juicy, or extremely bland beef flavor and 185 = extremely tough, extremely dry, or extremely intense beef flavor

Table 3.6. Impact of aging day on cook loss, proteolysis of desmin and troponin-T, and autolysis of calpain-1 of strip steaks[1]

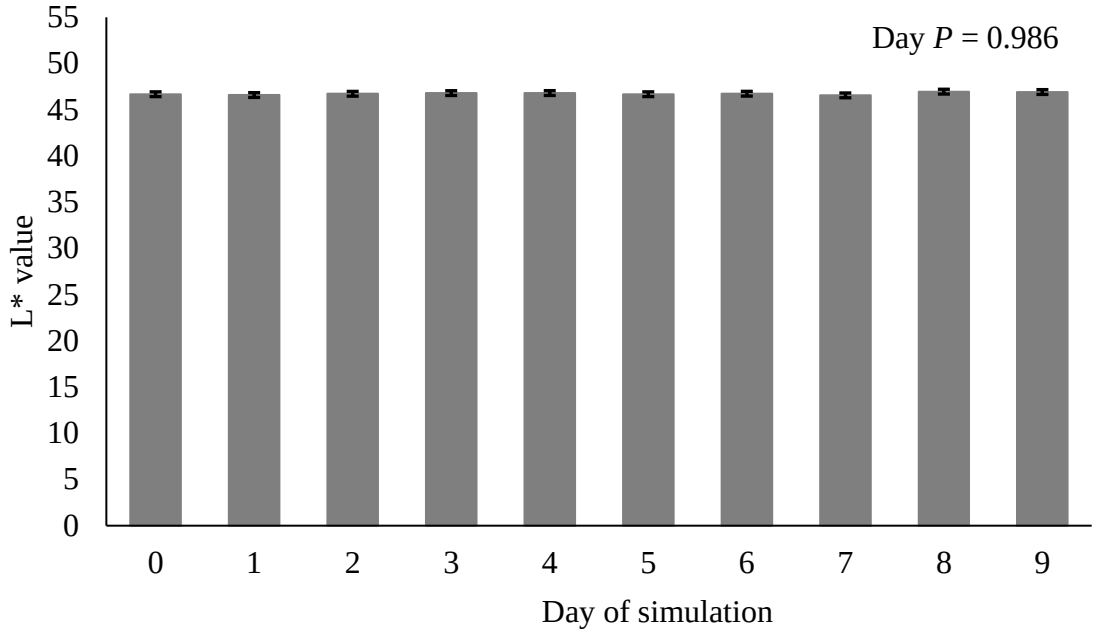
	3	7	14	21	SEM	*P*-value
Cook loss, %	17.857[b]	17.274[b]	18.210[ab]	19.363[a]	0.579	0.042
Intact desmin	1.4994[a]	1.0025[b]	0.7607[c]	0.6053[c]	0.064	< 0.0001
Intact troponin-T[2]	1.3437[a]	0.9993[b]	0.6769[c]	0.5887[c]	0.045	< 0.0001
Degraded troponin-T[2]	0.6758[c]	1.0216[b]	1.0786[b]	1.2881[a]	0.074	< 0.0001
Inactive calpain-1[3], %	13.46	7.45	-	-	0.899	< 0.0001
Active calpain-1[3], %	34.21	30.03	-	-	0.735	0.0004
Autolyzed calpain-1[3], %	52.22	62.47	-	-	1.373	< 0.0001

[1] Least square means

[2] Expressed as a ratio to an internal standard

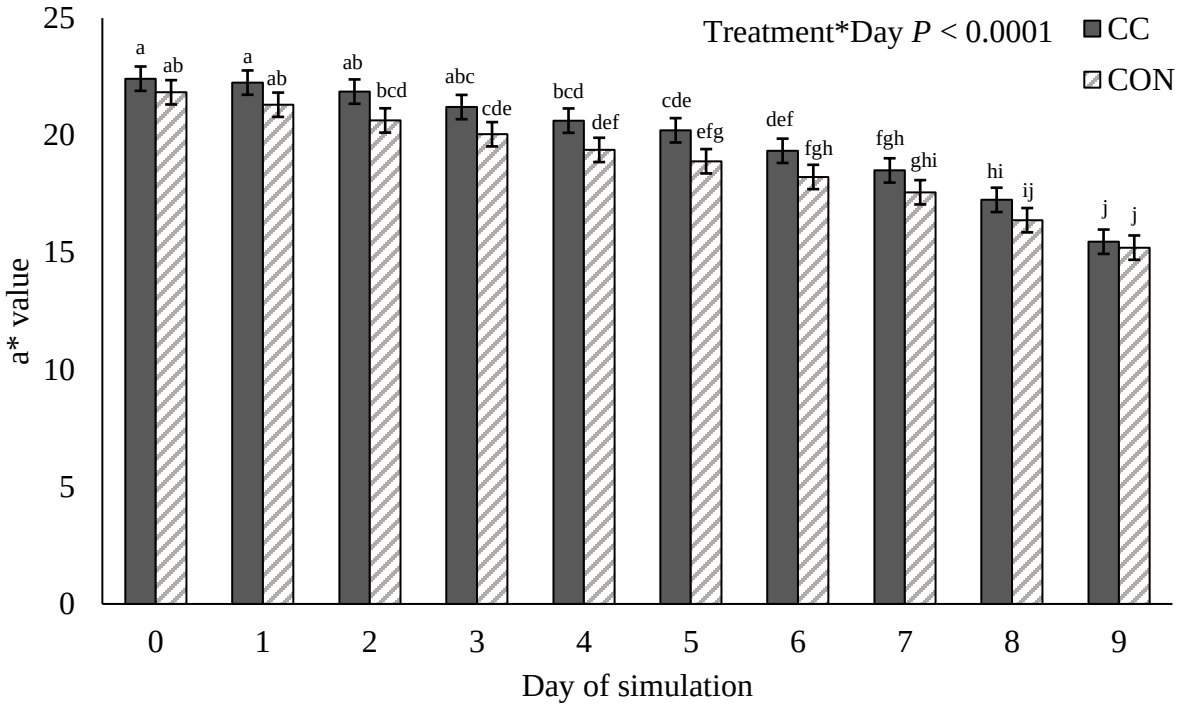
[3] Expressed as a percentage of total calpain-1

Figure 3.1. Impact of simulation day on instrumental L* values during a simulated retail display of strip steaks[1]



[1] Least square means

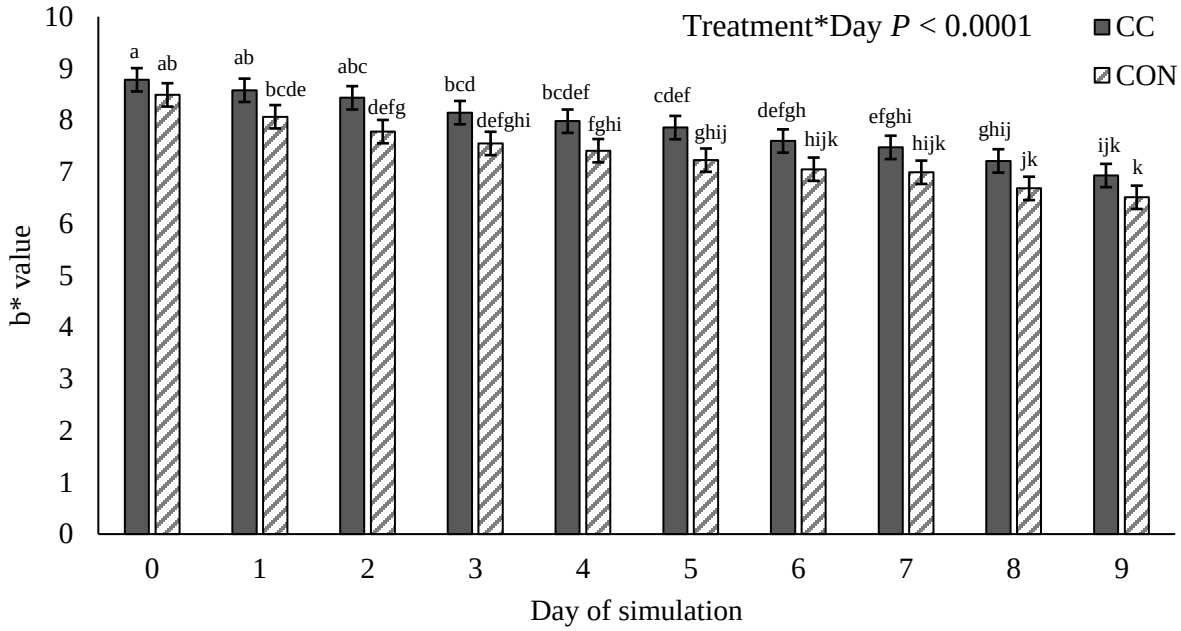
Figure 3.2. Impact of feeding a cover crop mixture including brassicas (CC) or a common midwestern backgrounding diet (CON) during backgrounding on a* (redness) color values during a simulated retail display of strip steaks[1]



[1] Least square means

[abcdefghij] Means lacking common superscripts differ $P < 0.05$.

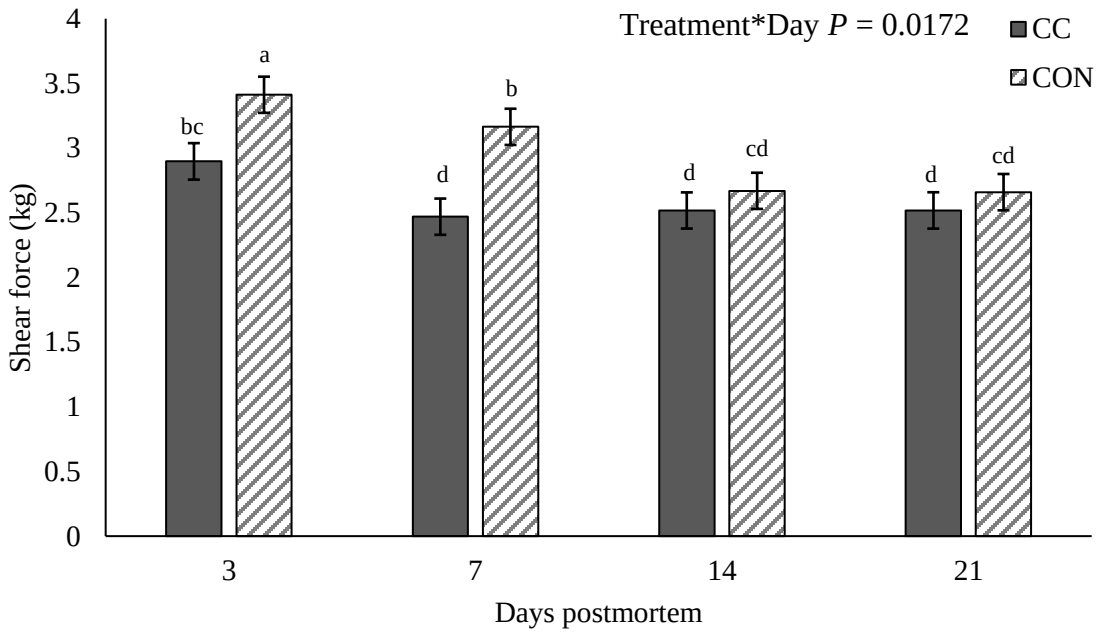
Figure 3.3. Impact of feeding a cover crop mixture including brassicas (CC) or a common midwestern backgrounding diet (CON) during backgrounding on b* (yellowness) color values during a simulated retail display of strip steaks[1]



[1] Least square means

[abcdefghijk] Means lacking common superscripts differ $P < 0.05$

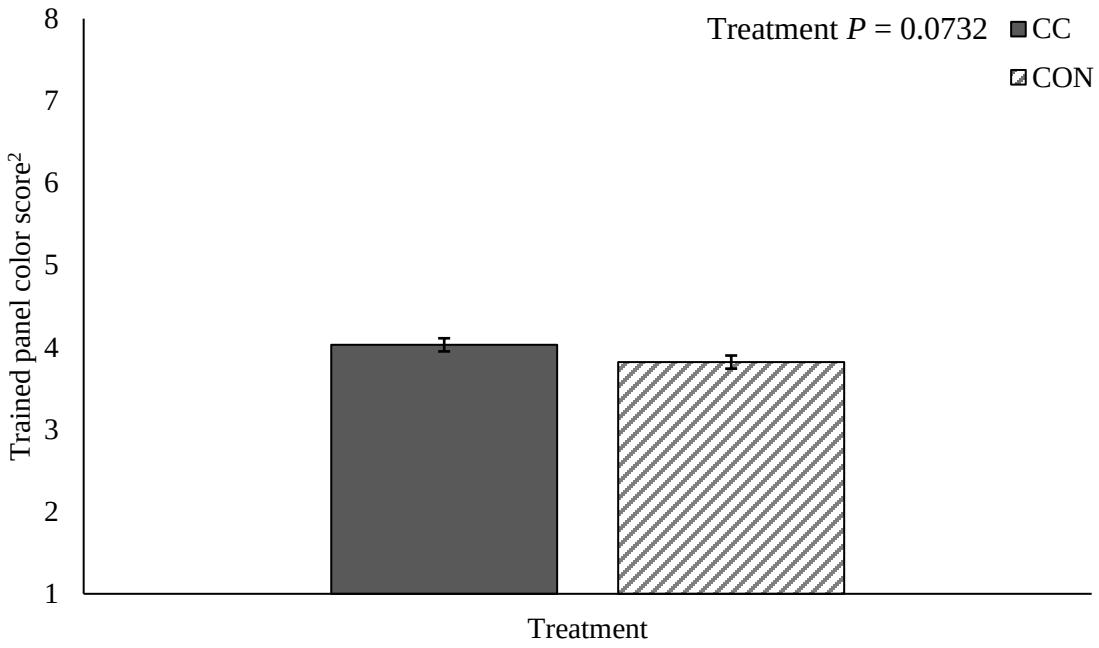
Figure 3.4. Impact of feeding a cover crop mixture including brassicas (CC) or a common midwestern backgrounding diet (CON) during backgrounding on Warner-Bratzler shear force values of strip steaks[1]



[1] Least square means

[abcd] Means lacking common superscripts differ $P < 0.05$.

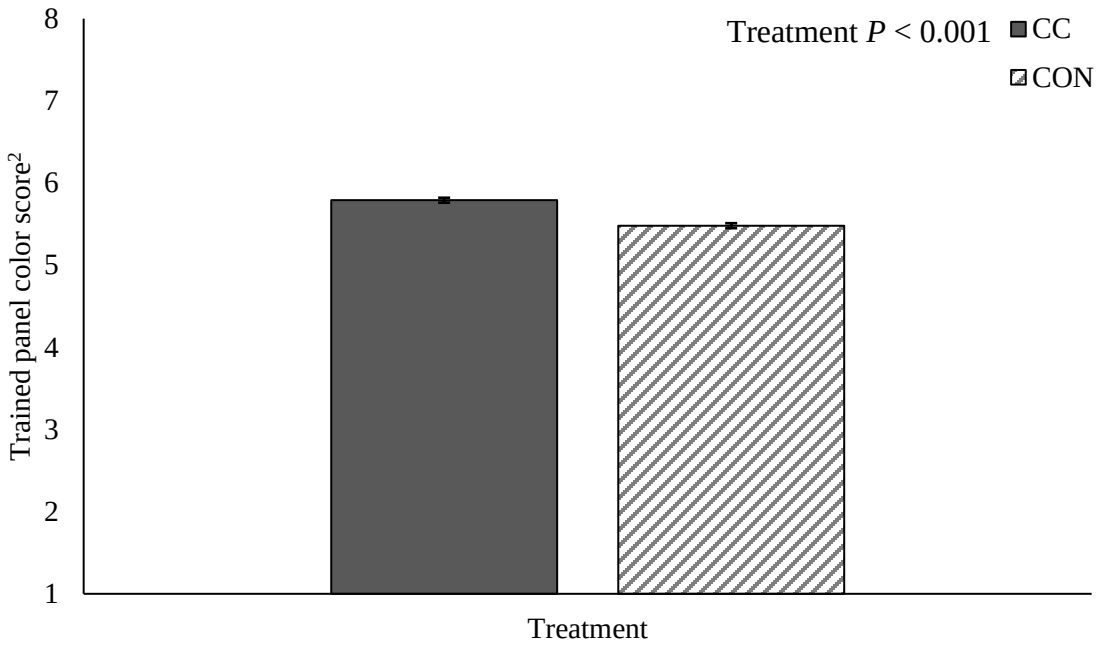
Figure 3.5. Impact of feeding a cover crop mixture including brassicas (CC) or a common midwestern backgrounding diet (CON) during backgrounding on initial trained panel color scores during a simulated retail display of ground beef[1]



[1] Least square means

[2] Trained panel color scores: 1 = Bleached red, 2 = Very light cherry red, 3 = Moderately light cherry red, 4 = Cherry red, 5 = Slightly dark red, 6 = Moderately dark red, 7 = Dark red, 8 = Very dark red

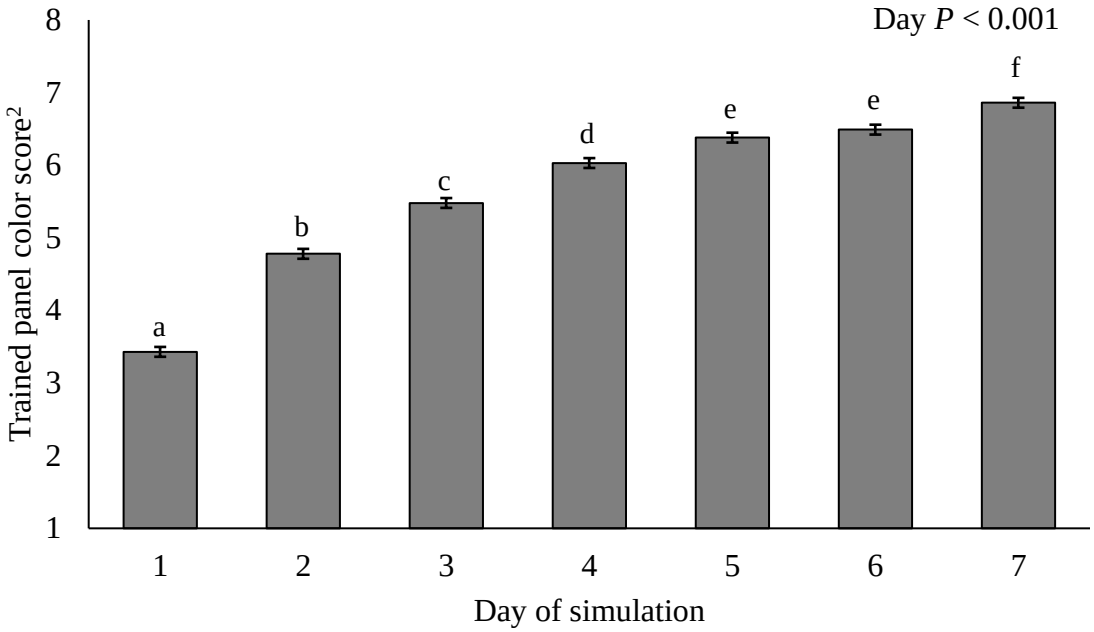
Figure 3.6. Impact of feeding a cover crop mixture including brassicas (CC) or a common midwestern backgrounding diet (CON) during backgrounding on trained panel color scores during a simulated retail display of ground beef[1]



[1] Least square means

[2] Trained panel color scores: 1 = Very bright red, 2 = Bright red, 3 = Dull red, 4 = Slightly dark red, 5 = Moderately dark red, 6 = Reddish tan / brown, 7 = Dark reddish tan / brown, 8 = Tan to brown

Figure 3.7. Impact of simulation day on trained panel color scores during a simulated retail display of ground beef[1]

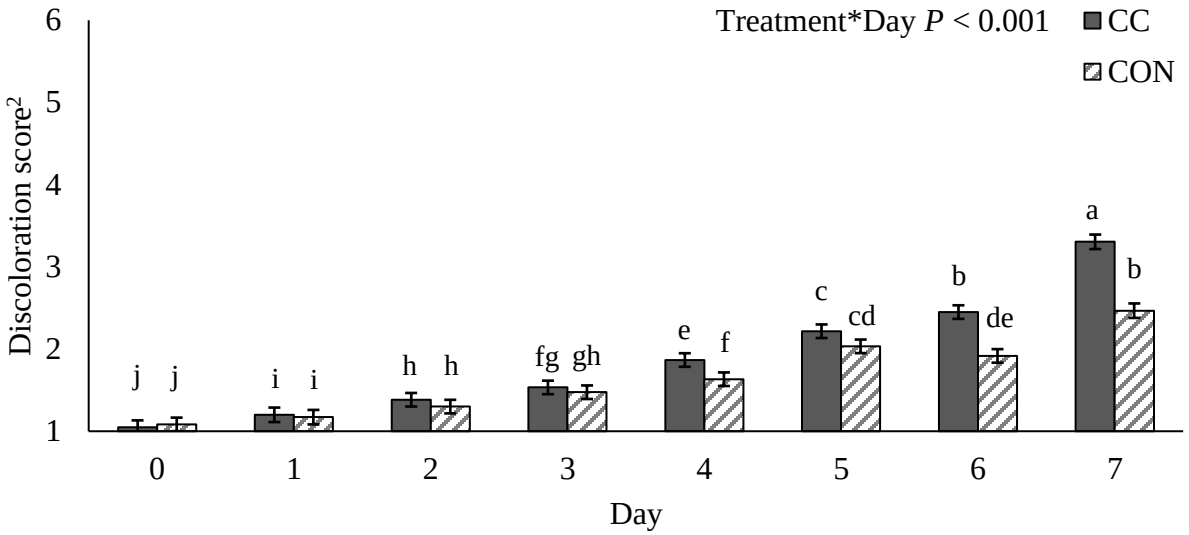


[1] Least square means

[2] Trained panel color scores: 1 = Very bright red, 2 = Bright red, 3 = Dull red, 4 = Slightly dark red, 5 = Moderately dark red, 6 = Reddish tan / brown, 7 = Dark reddish tan / brown, 8 = Tan to brown

[abcdef] Means lacking common superscripts differ $P < 0.001$.

Figure 3.8. Impact of feeding a cover crop mixture including brassicas (CC) or a common midwestern backgrounding diet (CON) during backgrounding on trained panel discoloration scores during a simulated retail display of ground beef[1]

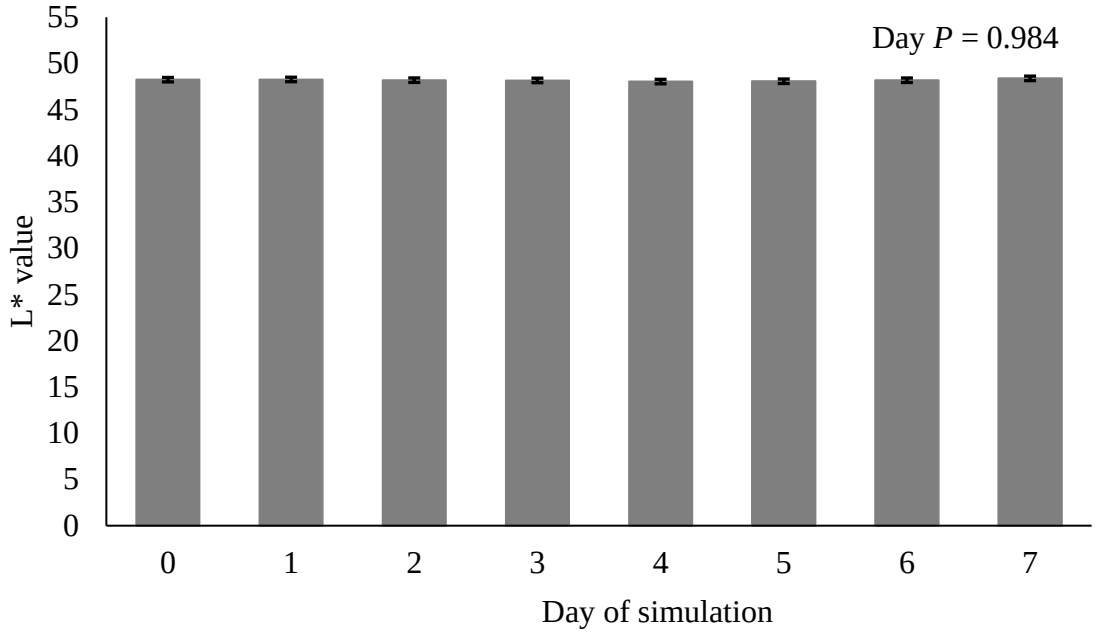


[1] Least square means

[2] Discoloration score: 1 = No discoloration, 0%; 2 = Slight discoloration, 1 – 20%; 3 = Small discoloration, 21 – 40%; 4 = Modest discoloration, 41 – 60%; 5 = Moderate discoloration, 61 – 80%; 6 = Extensive discoloration, 81 - 100%

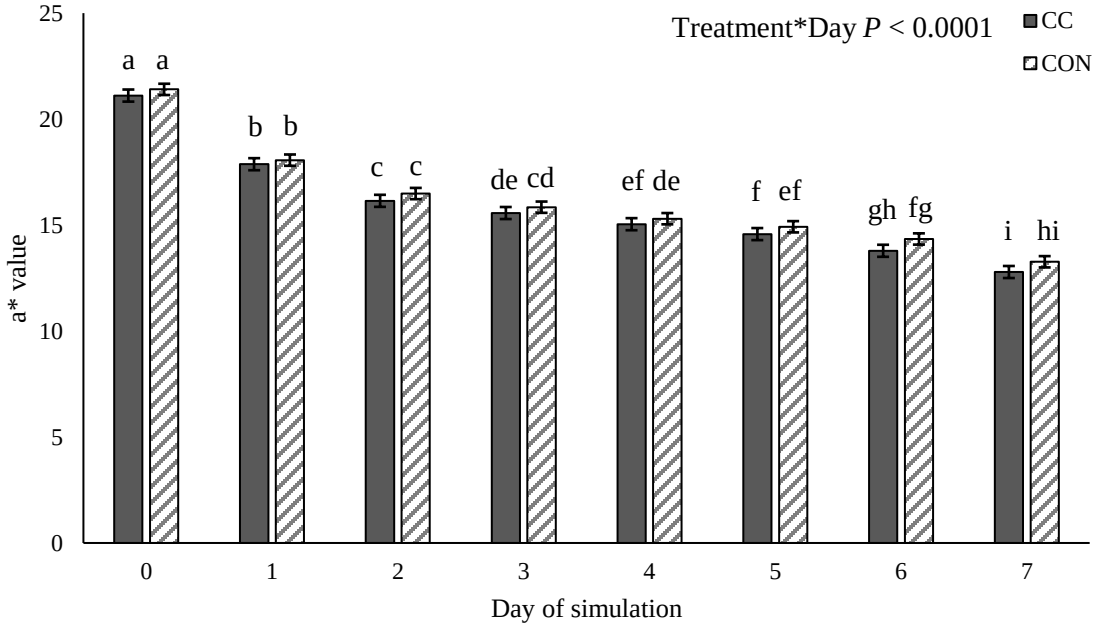
[abcdefghij] Means lacking common superscripts differ $P < 0.05$.

Figure 3.9. Impact of simulation day on instrumental L* values during a simulated retail display of ground beef[1]



[1] Least square means

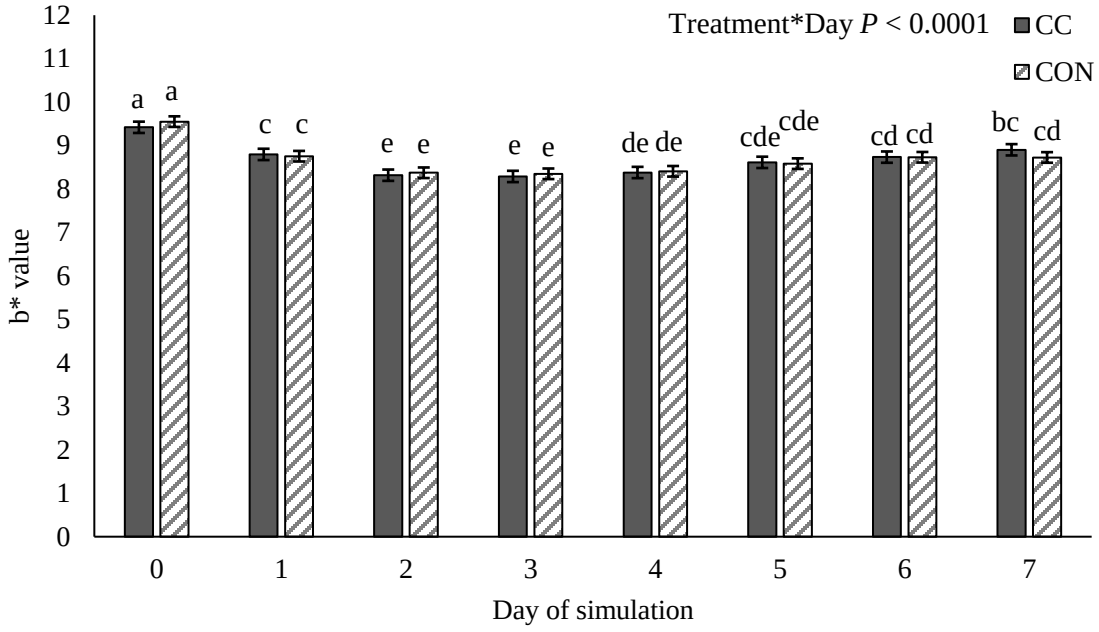
Figure 3.10. Impact of feeding a cover crop mixture including brassicas (CC) or a common midwestern backgrounding diet (CON) during backgrounding on instrumental a* (redness) color values during a simulated retail display of ground beef[1]



[1] Least square means

[abcdefghi] Means lacking common superscripts differ $P < 0.05$.

Figure 3.11. Impact of feeding a cover crop mixture including brassicas (CC) or a common midwestern backgrounding diet (CON) during backgrounding on instrumental b* (yellowness) color values during a simulated retail display of ground beef[1]



[1] Least square means

[abcde] Means lacking common superscripts differ $P < 0.05$.