DISSERTATION

PRE-HARVEST AND POSTMORTEM METHODS OF DECREASING THE INCIDENCE AND SALVAGING VALUE OF NON-CONFORMING BEEF CARCASSES

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY PHILLIP DEAN BASS ENTITLED PRE-HARVEST AND POSTMORTEM METHODS OF DECREASING THE INCIDENCE AND SALVAGING VALUE OF NON-CONFORMING BEEF CARCASSES BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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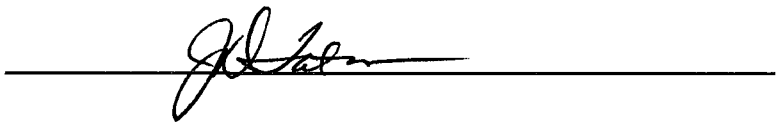

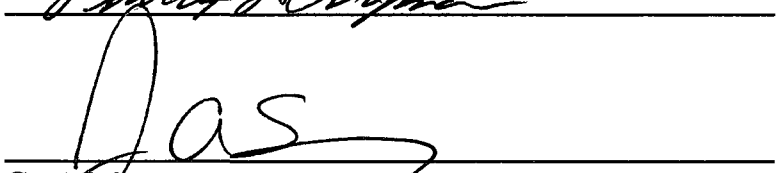

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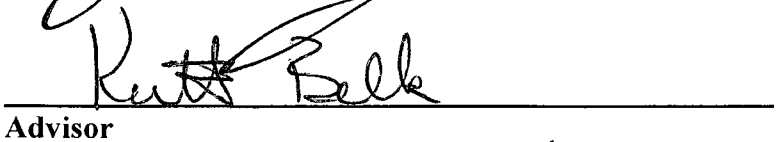

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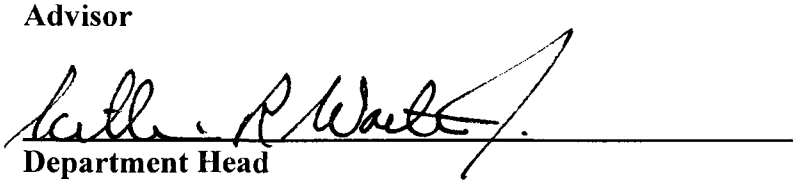

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ABSTRACT OF DISSERTATION

Pre-harvest and Postmortem Methods of Decreasing the Incidence and Salvaging Value of Non-Conforming Beef Carcasses

It has been stated that the number of non-conforming carcasses in the beef industry cost the industry millions of dollars yearly in lost revenue. The objectives of this research were to identify muscles in dark-cutting beef carcasses that were not affected by the dark-cutting condition, identify relationships between the portion size of individual muscles in the beef carcass and the carcass ribeye area, and finally to investigate the effects of dietary magnesium (Mg) supplementation on the quality characteristics of beef cattle subjected to stress-inducing environments. Carcasses in the dark cutter study were divided into three classes; 1/3, 1/2, and full degree of dark cutting (DEGDC). The ultimate pH of individual muscles within the carcasses resulted in 7, 9, and 5 muscles having mean pH values considered normal for 1/3, 1/2, and full DEGDC carcasses, respectively. A nation wide survey was conducted to identify the acceptable color range of fresh beef muscles for food service chefs and retail meat merchandisers. Muscles that were within an acceptable color value range for food service chefs had the potential to add between $42.29 and $26.44 per side when valued at Choice prices compared to commodity discounted prices. Muscles that were within an acceptable color range value for retail meat merchandisers had the potential to add between $30.30 and $16.74 per side when valued at Choice prices compared to commodity discounted carcasses prices. The

portion size study evaluated 14 muscles (triceps brachii long head, infraspinatus, chuckeye complexus, pectoralis profundus, longissimus thoracis, latissimus dorsi, gluteus medius, longissimus lumborum, tensor fasciae latae, psoas major, semimembranosus, biceps femoris, semitendinosus, and vastus lateralis). Seven of the 14 muscles were found to have no relationship between individual muscle portion size and ribeye area. A nationwide survey was conducted with foodservice chefs and retail meat merchandisers to evaluate the acceptability of portion sizes from carcasses varying in ribeye area size. Results of the survey demonstrated that the portion size of many muscles were still acceptable to retail meat merchandisers and foodservice chefs even though the ribeye area of a carcass may be non-conforming. The study involving the Mg supplementation to beef cattle (N = 144) indicated that the Mg was taken up in the blood stream of the cattle fed the supplement, but no effect on tenderness or reduction in the incidence of quality defects (e.g., dark cutting) were observed. The results of these studies demonstrate methods of returning value to non-conforming beef carcasses, however, other methods of preventing non-conformity are necessary to research.

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DEDICATION

To Mom and Dad, your unending support, love, and encouragement will forever be with me.

To my wife Tina, this is OUR Ph. D. Thank you for your love, support, tolerance, and understanding.

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CHAPTER I

Introduction

It has been postulated that carcasses considered non-conforming, or not fitting the target specifications for what is considered ideal for the industry, cost the beef industry millions of dollars each year in lost income (Savell, 2007). Non-conforming carcasses will cause monetary loss to the industry in the form of devaluing saleable product due to quality defects, or disallowing of product into premium branded programs due to potentially undesirable portion sizes, therefore preventing some carcasses from reaching their full value potential. Some of the non-conformities that constantly plague the industry are dark cutting carcasses and carcasses that produce cuts that are too large due to oversized muscles (Savell, 2007).

The beef industry, as a whole, tries to provide carcasses that conform to ideal industry specifications; however, when non-conforming carcasses are produced it is critical that the industry find ways to salvage the value of those carcasses. The National Cattlemen's Beef Association has provided funding to assess different ways of either preventing or capitalizing on carcasses that are considered by the industry to be non-conforming.

The objectives of this dissertation were to identify what muscles in the beef carcass are in fact affected by the dark cutting condition of the ribeye muscle, what muscles have portion sizes and dimensional properties that are influenced by the size of

the ribeye muscle, and are there pre-harvest feed management strategies that can help prevent stress-induced quality defects (e.g., dark cutting, muscle toughening).

CHAPTER II

Review of the Literature

Non-conforming Beef Carcasses

Non-conforming beef carcasses, known in industry as "out carcasses," are carcasses that do not conform to ideal industry specifications (Savell, 2007). Non-conformities in a beef carcass can come in many forms such as insufficient quality grade, poor yield grade (i.e., USDA yield grades 4 and 5), incorrect phenotype, weight (e.g., too heavy/light), muscling (too much, too little), and color challenges (e.g., dark-cutters). There are many factors that may contribute to the non-conformity of a beef carcass; however, the one thing in common with non-conforming beef carcasses is the decrease in value or loss in potential value that is incurred as a result of the non-conformity.

Value-based pricing grids are being used more and more frequently for the marketing of fed beef cattle for slaughter (Tatum et al., 2006). Cattle considered to be non-conforming will be discounted greatly when marketed on a value-based pricing grid (Doherty et al., 1999). Although a producer can achieve great premiums using the value-based pricing grids, the benefits to using the grids can be lost when an over abundance of non-conforming beef carcasses are marketed as such (Doherty et al., 1999). As more and more cattle continue to be marketed through value-based pricing grids, it is essential to investigate ways of returning value to those carcasses that do not conform to ideal industry specifications.

pH

In live animals, pH balance must be controlled for maintaining optimal enzymatic and biochemical activity necessary for sustaining life (Murray et al., 2006). Likewise, changes to muscle tissue upon death are very dependent upon pH. Ultimate postmortem muscle pH has been demonstrated to affect tenderness (Purchas, 1990; Watanabe et al., 1996; Wulf and Page, 2000), water holding capacity (Offer, 1991; Page et al., 2001; Huff-Lonergan and Lonergan, 2005), and lean color (Hunt, 1980; Faustman and Cassens, 1990; Page et al., 2001). For the reasons listed above, it is imperative that researchers have a good understanding of muscle pH to ultimately be able to control the changes of pH in muscle tissues.

Normal muscle pH of live meat animals is near and around 7.0 (McLoughlin, 1970; Aberle et al., 2001). After exsanguination, the ability for a living system to continue to produce adenosine triphosphate (ATP) aerobically is no longer possible due to the loss of blood to carry oxygen to the body cells. As a result of the loss of blood, and thus cessation of cellular oxygen delivery, a living system will result to anaerobic glycolysis to continue producing ATP (Murray et al., 2006). Under normal aerobic conditions, glycolysis converts glucose into pyruvate via several biochemical steps; pyruvate enters into the mitochondria of the cell where it is converted to acetyl CoA and subsequently begins the Krebs Cycle which produces factors (NADH, FADH) that are used in the mitochondrial membrane to produce ATP via the electron transport chain where oxygen is the final electron receptor. When oxygen is unavailable, the living system will convert from aerobic energy metabolism to anaerobic energy metabolism where pyruvate is converted into lactic acid instead of acetyl CoA. The ultimate result of

anaerobic glycolysis is the production of 2 ATP (versus 38 ATP via aerobic glycolysis) and a corresponding decrease in muscle pH due to lactic acid accumulation (Dransfield, 1981; Murray et al., 2006).

Under normal conditions, accumulation of lactic acid from anaerobic glycolysis will ultimately be transported by the blood to be metabolized by the liver and kidney: however, after exsanguination, there no longer is a functioning circulatory system to remove accumulated lactic acid from skeletal muscle and, hence, lactic acid will continue to be produced and amassed until no more glycogen remains for metabolism, or the pH drops to a point where metabolism can no longer occur (Seideman et al., 1984; Murray et al., 2006). The accumulation of lactic acid postmortem causes a normal decrease in beef muscle pH to between 5.40 to 5.79 (Lawrie, 1958; Tarrant and Mothersill, 1977; Zhang et al., 2005).

The normal decrease in muscle pH has been demonstrated to be essential for obtaining the appropriate fresh meat color that is so desired by consumers (Walters, 1975). The ability for muscle to bind water, as previously mentioned, is somewhat dependent upon pH (Huff-Lonergan and Lonergan, 2005). At elevated muscle pH, the water in muscle is bound more tightly to proteins (net protein change is increased as pH moves away from the isoelectric point for muscle protein) and thus does not reflect light as much as if the water was less tightly bound; this results in a darker colored lean (Page et al., 2001). Alternatively, at a lower than normal muscle pH, water in muscle is less tightly bound to proteins and can reflect more light than normal, thus resulting in a more pale colored lean (Walters, 1975). As color has been identified as one of the most important factors in the selection of a meat product (Kropf, 1980; Aberle et al., 2001), it

is imperative that the factors contributing greatly to the determination of meat lean color are understood and controlled.

As mentioned above, ultimate muscle pH has been observed to have a relationship with muscle tenderness (Purchas, 1990; Watanabe et al., 1996; Wulf and Page, 2000). Purchas (1990) observed early on that Warner-Bratzler shear force (WBSF) values had a quadratic relationship with ultimate muscle pH such that, as ultimate muscle pH increased from the lower normal value of 5.4 up to approximately 6.0, so did the WBSF values; however, a decrease in WBSF was observed as ultimate muscle pH increased from 6.0 to around 7.0. Purchas and Aungsupakorn (1993) and Watanabe et al. (1996) later confirmed the quadratic relationship between ultimate muscle pH and WBSF. It is currently unknown as to why the quadratic relationship exists between ultimate muscle pH and WBSF.

Color

The ideal color for fresh beef lean was suggested by Allen (1968) to be "cherry red," but later adjusted by Jeremiah et al. (1972) to be slightly more-pale than the previously suggested "cherry red." It was noted by Billmeyer and Saltzman (1966) that the ideal color of beef lean may differ from person to person. Regardless, it has been suggested by Dunsing (1959), Jeremiah et al. (1972), and Kropf (1980) that color is the largest deciding factor as to whether or not a customer will purchase a meat product. Fresh meat color can be influenced by ultimate pH (Hunt, 1980; Faustman and Cassens, 1990; Page et al., 2001), concentration and redox state of muscle pigments (Ledward et al., 1974; Aberle et al., 2001), and muscle type (Aberle et al., 2001), among several other factors. Of the factors influencing the color of fresh meat, the control of ultimate muscle

pH is one of the most simple, inexpensive, and most used means of controlling and manipulating muscle color as many of the other factors are essentially fixed (i.e., pigment concentration, muscle type, etc.).

For marketing purposes, color is used as one of the factors for subjectively determining the USDA Quality Grade of a beef carcass in addition to identifying lean color quality defects (USDA, 1997; AMSA, 2001). Subjective color evaluation, however, can introduce variation regardless of how well an individual is trained (Okerman and Cahill, 1969; AMSA, 1991). Therefore, it is recommended that objective color evaluations be conducted on lean muscle to minimize variation associated with lean color evaluation (AMSA, 1991). One popular means of objective meat color evaluation is the use of a portable spectrophotometer that identifies color as a three-dimensional subject (Morgan et al., 1997; AMSA, 1991; HunterLab, 1996). According to AMSA (1991) and HunterLab (1996), color can be broken down into three main tristmulus components: L*, a*, and b*. L* is rated on a scale of 0-100 where 0 equals black and 100 equals white. The values of a* are rated on a scale of -60 to 60 where -60 equals green and 60 equals red. The values of b* are also rated on a scale of -60 to 60 where -60 equals blue and 60 equals yellow. Each color component (L*, a*, and b*) can thereby be quantified and ultimately analyzed statistically as a result of the quantification. It is good practice, however, to not only evaluate the tristimulus components individually, but also as a three dimensional subject which can be accomplished by identifying the first principle component of the three components and subsequently analyzing the resulting principle component, which is a single orthogonal value that is the best estimate of the independent multivariate association among all three tristimulus components.

Enzymatic Post Mortem Meat Tenderization

Tenderness is likely one of the most important eating attributes of a fresh whole muscle meat product (Boleman et al., 1995; Miller et al., 2001; Destefanis et al., 2008). Fresh meat tenderness can be influenced by many factors including, but not limited to, muscle pH (Purchas, 1990; Watanabe et al., 1996; Wulf and Page, 2000), muscle rigor state (Aberle et al., 2001), ante mortem animal activity (Sensky et al., 1996; Ferguson et al., 2001; Sensky et al, 2006; Warner et al., 2007), sarcomere length (Koohmaraie et al., 1988a), age of animal (Shimokomaki et al., 1972; Aberle et al., 2001), electrical stimulation (Takahashi et al., 1987; Simmons et al., 2008), and live animal hormonal implant management strategies (Platter et al., 2003) among many other factors. While there are many factors that ultimately affect consumer perceptions of beef tenderness, a primary management factor is post mortem aging.

Post mortem aging is the process of utilizing naturally occurring enzymes in the muscle that elicit degradation of certain muscle proteins over time. Though there are a few additional endogenous enzymes naturally found in muscle tissue capable of breaking down myofibrillar proteins, the primary tenderizing enzymes of interest are the calcium dependent proteases known as calpains (Koohmaraie, 1988; Koohmaraie, 1992; Koohmaraie 1994). There are two main types of calpains commonly studied as the primary post mortem proteolytic enzymes. One of the types of calpains is the μ-calpain, also known as calcium dependant protease-I (CDP-I), which requires a small amount of calcium (~5-65 μM) for half maximal activity (Cong et al., 1989; Barrett et al., 1991; Huff-Lonergan and Lonergan, 1999). The second calpain isoform is m-calpain, also known as calcium dependent protease-II (CDP-II), which requires a greater amount of

calcium (~300-1000 μM) for half maximal activity (Cong et al., 1989; Barrett et al., 1991). Calpains have been observed to weaken the structural myofibrillar proteins in muscle, thereby contributing to increased tenderization (Koohmaraie, 1994; Huff-Lonergan and Lonergan 1999). Some of the structural myofibrillar proteins degraded by calpains include: titin (maintains sarcomere longitudinal integrity), nebulin (anchors thin filaments to the Z-line), Troponin-T (subunit of troponin), filamin (crosslinks actin filaments), and desmin (surrounds the Z-line) among several other minor structural proteins (Pearlstone and Smillie, 1982; Robson and Huiatt, 1983; Wang and Wright, 1988; Huff-Lonergan and Lonergan, 1999). By degrading the above mentioned proteins, calpains can compromise the integrity of the muscle cellular ultrastructure resulting in improved tenderization. However, inherent compounds, however, do exist to counter the catabolic effects of the calpains.

Calpastatin is the primary inhibitor of calpain activity (Dransfield, 1993). Calpastatin inhibits calpains by binding to calpains and forming a calpain-calpastatin complex which prevents the calpains from continuing any proteolytic activity (Dransfield, 1993; Wendt et al., 2004; Melloni et al., 2006). Averna et al. (2006) demonstrated that conformational changes in calpains occur upon interaction with calpastatin. Cottin et al. (1981) observed a decrease in calpain inhibition by calpastatin as pH declined. This phenomenon may explain some of the reasons why aged fresh beef with a lower pH (~5.5) tends to be more-tender than aged fresh beef with a higher pH (~6.0). Calpastatin has been observed to occur at higher levels in *Bos indicus* cattle which are known to have more excitable temperaments than *Bos taurus* cattle (O'Conner et al., 1997). Temperament can contribute greatly to ultimate tenderness of beef; more excitable cattle

generally will produce meat with higher shear force values than calmer cattle (King et al., 2006). A likely reason for the observance of higher shear force values in excitable cattle is due to the increase in catecholamine levels which result in an increase in circulating calpastatins (Sensky et al, 1996; Sensky et al., 2006). Therefore, it is essential for cattle managers to practice methods for controlling cattle temperament to minimize excitability which has been demonstrated to have an effect on meat quality.

Dark Cutting Beef

Dark cutting beef is characterized as a stress-induced quality defect that produces an undesirable dark color in the fresh lean of the beef carcass ribeye (USDA, 1997). Munns and Burrell (1966) stated that even though the nutritive value of dark cutting meat is not compromised, the consumer recognizes the lean as being of lesser quality and will refuse to purchase the product solely based on the color of the lean. Additionally, dark cutting beef often is associated with undesirable detectable off flavors such as sour and bitter (Wulf et al., 2002) in addition to a decrease in overall beef flavor (Dransfield, 1981). For these reasons, dark cutting beef is often assigned a dramatic discount on the carcass basis.

Dark cutting beef is a result of an over expenditure of glycogen in the muscle of beef cattle immediately prior to harvest commonly associated with prolonged stress (Lawrie, 1958; Dransfield, 1981; McVeigh and Tarrant, 1982; Shackelford et al., 1994). Glycogen is the storage form of glucose in mammalian skeletal muscle (Murray et al., 2006) and, when exhausted, will not allow normal reductions in muscle pH to occur. Page et al. (2001) have demonstrated that carcasses with an ultimate pH of 5.87 or greater will result in a dark cutting ribeye whereas Apple et al. (1995) characterized dark cutter

carcasses as having ultimate pH greater than 6.0. Regardless of ultimate pH, dark cutting carcasses will generally have an elevated ultimate pH that is greater than normal beef carcass ribeyes, commonly between 5.40 to 5.79 (Lawrie, 1958; Tarrant and Mothersill, 1977; Zhang et al., 2005).

Factors contributing to dark cutting carcasses include, but are not limited to, intense prolonged ante mortem stress (Apple et al., 1995), severe and extended transportation stress (Warriss et al., 1990), large changes in temperature and environment (Scanga et al., 1998), heifers exhibiting estrus behaviors (Kenny and Tarrant, 1988), aggressive hormonal implant strategies (Scanga et al., 1998), and comingling of animals from differing/foreign social groups (McVeigh and Tarrant, 1983; Ferguson et al., 2001). The common relationship amongst all of the factors stated above is that each has the potential to increase the physiological stress of the animal resulting in glycogen deficiency upon slaughter, preventing the normal production of lactic acid and subsequent decline in pH (Lawrie, 1958).

As the dark cutting condition continues to be an issue in the beef industry, it is wise for packers to attempt to make the most of a bad situation. Dark cutting beef has been utilized in either a grinding operation or a further processing environment to either hide the undesirable color attributes or exploit the high water holding capacity that coincides with the high ultimate pH (Moiseev and Cornforth, 1999). Currently, dark-cutting carcasses are marketed in extremely discounted whole muscle programs (e.g., "No Roll"). It would be beneficial for the meat industry to identify alternatives to the current practices of extremely discounting the dark cutting carcasses.

Importance of Pre-harvest Stress Management

Quality control for fresh beef products begins while animals are alive (Tatum et al., 1999). Besides the essential processes of genetic selection and nutritional strategies, pre-harvest animal stress management is necessary for preventing stress-induced quality defects from occurring in the meat (i.e., dark cutting and lean toughening).

Stresses, perceived or physical, can disrupt an animals homeostasis causing the animals body to adjust for maintaining or restoring the balance in the biological system which involves slight changes in behavior, or complex changes in neurological and endocrinal function (Ferguson et al., 2001). Upon excitation in response to external stimuli, the hypothalamic-pituitary-adrenal axis is activated as a survival mechanism which stimulates several metabolic systems to mobilize energy stores in the event that an animal has to fight or run from its fear-inducing stimuli (King et al., 2006).

Intense physiological stress in an animal prior to harvest can result in the dark cutting condition as has been demonstrated, and widely accepted, by many researchers over the years. By handling animals gently and with minimal excitement prior to slaughter, the incidence of dark cutting carcasses can be minimized and thus result in more valuable product. Dark cutting beef, depending on ultimate pH, has at times been demonstrated to be a more tender than normal beef despite the poor aesthetic value (Purchas, 1990). Stress can induce other quality defects, however, other than the familiar and obvious dark cutting condition.

Animals that are stressed prior to harvest have been associated with decreases in lean tenderness (Voisinet et al., 1997a; King et al., 2006). Upon activation of the hypothalamic-pituitary-adrenal axis, a sympathetic nervous response causes an increase in circulating catecholamines (i.e., epinephrine and norepinephrine) from the adrenal

gland which in turn causes an up-regulation of β-adrenoceptors (Hossner, 2005). The up-regulation of the second messenger system, stimulated by the up-regulation of β-adrenoceptors, has been observed to increase the production of calpastatin, the inhibitor of the proteases known as calpains (Sensky et al., 1996; Sensky et al., 2006). Inhibition of the calpains can cause a dramatic decrease in post-mortem proteolysis and subsequent whole muscle tenderness. Therefore, it is imperative that pre-harvest animal stress management is practiced to insure that the subsequent carcass achieves its maximum quality potential.

Pre-harvest stress can be controlled in a variety of ways. First and foremost, it is essential to handle the livestock in a low impact, stress-free setting where the amount of psychological stresses are minimized and the animal is not in a state of fear (Grandin, 1997). Unfortunately, it would be nearly impossible to prevent all types of fear-inducing stressors from coming into contact with all livestock; therefore, scientists have looked for other ways of preventing the physiological effects induced by a stressful environment.

Over the years, different types of feed/food supplements have been investigated to evaluate their efficacy in preventing a physiological stress response in animals and humans (Schaefer et al., 1990; Adeola and Ball, 1992; Henry et al., 1996; Apple et al., 2000; Schaefer et al., 2001; Shimosawa et al., 2004). Among the studies investigating feed/food supplements that have demonstrated some promise to have beneficial physiological effects is magnesium (Mg).

D'Souza et al. (1998) fed supplemental Mg, in the form of magnesium aspartate, to finished market hogs for 5 d prior to harvest. A decrease in circulating blood nor-epinephrine levels were observed in the hogs fed the Mg supplement compared to the

control group. Additionally, other meat quality traits were observed to be improved in the hogs fed the Mg supplement. Gardner et al. (2001) observed similar results in sheep fed supplemental magnesium oxide.

Magnesium, the second most abundant cation in the blood (Wacker and Parisi, 1968), is essential for numerous enzymatic activities in the body (Rude, 1989). Magnesium also is essential for blood pressure, catecholamine, and corticosteroid regulation (Delpiano and Altura, 1996; Murasato et al., 1999). A deficiency of Mg will cause the blood pressure to elevate, and in severe cases, heart failure can occur (Murasato et al., 1999). Magnesium deficiencies also induce hyperactivity and irritability, likely a result of the reduced control of circulating catecholamines (Fiset et al., 1996). According to Shimosawa et al. (2004), Mg is employed in controlling catecholamine release by physically blocking the N-type voltage gated calcium channels. By preventing calcium from entering through the N-type calcium channels, calcium-induced calcium release cannot be accomplished in chromaffin cells which consequently will not allow for the ionic changes in chromaffin cells, thereby preventing release of catecholamines stored in chromaffin granules (Barrett, 2005).

Further research should be conducted to evaluate the physiological effects of Mg supplementation to control stress induced quality defects in the effort to prevent non-conforming carcasses.

CHAPTER III

Recovering value from beef carcasses classified as dark cutters by United States Department of Agriculture graders

INTRODUCTION

Quality of beef is determined by a complex combination of appearance, cooking, and eating characteristics (Dransfield, 1981). Color and appearance of fresh product has been characterized as the largest deciding factor in relation to whether or not a customer will purchase a cut of beef at retail (Dunsing, 1959; Jeremiah et al., 1972; Kropf, 1980). "Dark-cutting" lean color is an issue during beef carcass grading and is a result of elevated pH values in valuable beef cuts, especially the ribeye (Berg and Butterfield, 1976). In value based cattle pricing systems, carcasses that are considered less desirable with respect to color of the ribeye (i.e., dark cutters) generally do not return full value to producers (USDA, 1997). Discounted prices are extremely detrimental to producers who market cattle using grid-pricing systems, by which approximately 34% of beef in the US are marketed (Smith et al., 2005). Smith et al. (2005) reported that the dark-cutting condition is found in 1.5% of the carcasses in the US; as a result, the dark cutting condition has potential for a large negative economic impact on the beef industry.

By identifying beef muscles that are not affected by ribeye lean color, it may be possible to capture un-realized value for dark-cutting carcasses by aggregating the

individual values of acceptable muscles that can be merchandised at full price. This study was conducted to identify ideal ranges of color for various beef muscles and to determine which muscles within a carcass classified by USDA graders as a "dark cutter" should be valued at full price.

MATERIALS AND METHODS

Carcass Dissection and Muscle Analysis

Between December 2006 and February 2007, alternating sides of beef carcasses classified as having ribeyes exhibiting 1/3, 1/2, or full degree of dark cutting (**DEGDC**) by USDA graders at chain speeds (USDA, 1997) were purchased from two commercial beef packing companies and transported under refrigerated conditions to the Colorado State University Meat Laboratory for fabrication and dissection; carcass chill times were within 36-48 hours. After weighing to determine chilled carcass weight, carcass sides were sequentially fabricated into primal cuts (IMPS #'s 113 and 117 chuck/shank, IMPS # 120 brisket, IMPS # 103 rib, IMPS # 172 loin, IMPS # 158 round, IMPS # 121 plate, and IMPS # 193 flank) and subprimal cuts (IMPS # 114E clod heart-triceps brachii long head, IMPS # 114E clod heart-triceps brachii lateral head, IMPS # 114D top blade-infraspinatus, IMPS # 116B mock tender-supraspinatus, IMPS # 114F petite tender-teres major, IMPS # 116D chuck eye roll-complexus/longissimus dorsi/spinalis dorsi/multifidus, IMPS # 120A brisket flat-pectoralis profundi, IMPS # 112 ribeye roll-complexus/longissimus thoracis/spinalis dorsi/multifidus, IMPS # 109B lifter meat-latissimus dorsi, IMPS # 184E top sirloin butt-gluteus medius/biceps femoris, IMPS # 180 1 × 0 strip loin- longissimus lumborum, IMPS # 185A flap meat-obliquus abdominis interni, IMPS # 185D tri-tip-tensor fasciae latae, IMPS # 189A tenderloin-psoas major,

IMPS # 193 flank steak-rectus abdominis, IMPS # 169 top round-semimembranosus/adductor, IMPS # 170A bottom round heel out-biceps femoris/semitendinosus, IMPS # 167A knuckle-vastus lateralis/vastus medialis/vastus intermedius/rectus femoris).

Individual weights of subprimal cuts were recorded. Subprimals then were fabricated into individual muscles, weighed, cut at the halfway point of the longitudinal axis of the muscle, and the face of each cut was sliced using a meat slicer (Model 2712, Hobart, Troy, OH) to obtain a 0.254-cm sample for pH analysis. After a 20 minute bloom time, lean color was measured in the face of the remaining muscle half (L*, a*, and b*) using a portable spectrophotometer with a port size of 1.27 cm, a D-65 illuminant, and calibrated using a black and a white tile (Miniscan XE Model 45/0-L, Hunter Laboratories, Reston, VA). Final color values were the mean of three measurements per muscle. After measuring lean color, the remaining muscle half was vacuum packaged, aged for 14 d postmortem, and stored at -20°C to be used for further sensory and tenderness analysis.

Each 0.254-cm sample slice, obtained from individual muscles, was diluted 10:1 with double-distilled de-ionized water and homogenized (Model 1120 Waring Blender). The pH of the homogenate was determined using a pH meter (Orion 2 Star pH Benchtop, Thermo Electron Corp.).

Steak Preparation

Steaks (2.54-cm thick) for sensory panel evaluation and Warner-Bratzler shear force (**WBSF**) measurement were cut in the frozen state using a band saw (Model 400, AEW-Thurne, AEW Engineering Co. Ltd., Norwich, England) under refrigerated

conditions (4°C). A representative 14 muscles were evaluated in the study (individual muscles making up the majority of the weight of their respective subprimals) and were further analyzed for WBSF and sensory panel analysis. Beef muscles evaluated for sensory characteristics and WBSF included: longissimus lumborum (**SLLD**), longissimus thoracis (**RLD**), gluteus medius (**GM**), infraspinatus (**IN**), tensor fasciae latae (**TF**), vastus lateralis (**VL**), round biceps femoris (**RBF**), psoas major (**PM**), semimembranosus (**SM**), semitendinosus (**ST**), pectoralis profundi (**DP**), triceps brachii—long head (**TBL**), latissimus dorsi (**LFT**), and chuck complexus (**CHCOM**). Steaks from the teres major (**TM**) were cut for WBSF evaluation only. Steaks were cut such that the first steak from the face was designated for WBSF and the second steak was designated for sensory panel analysis.

Steaks were individually labeled, vacuum packaged, and stored under frozen conditions (-20°C). Steak samples remained frozen during the entire fabrication process and were never subjected to temperature abuse.

Sensory Panel

Panelists were subjected to conditions approved by the Human Use in Research Committee of Colorado State University. Before initiation of sensory panel analysis, panelists were trained by the procedures outlined by Meilgaard et al. (1991), AMSA (1995), and Meisinger (2005). Panelists were screened to ensure that they could identify overall tenderness, overall juiciness, beef flavor intensity, and the following off-flavors: sour, bitter, metallic, liver, serum, and oxidized. Panelist screening was conducted using a modified version of the triangle test. Correct selections of sensory attributes and off-

flavors greater than 70% were required for potential panelists to be selected for the actual panel.

Panel sessions were conducted for 5 weeks until all 60 panel sessions were completed. A maximum of three sessions per day were conducted and panelists were rotated in and out so that one panelist would not participate in more than two panel sessions in a day. Panels included 8 trained panelists in each session, and panelists evaluated 14 samples per panel session, each sample representing one of the 14 muscles chosen for sensory evaluation. Each DEGDC (1/3, 1/2, and full) was represented in each panel session. Every panel session contained 5 steaks from 2 of the DEGDC and 4 steaks from the remaining DEGDC. The random assignment of steaks to panels was conducted in a rotating manner resulting in each DEGDC being represented by 4 steaks every third session.

Before cooking, steaks for sensory evaluation were tempered for 24 h at 2°C. Steaks were cooked on an electric grill (Salton Clamshell Grill Model No. GR39A, Salton, Inc., Lake Forest, IL) to a target internal temperature of 70°C. Initial temperature, peak off-temperature, and cooking loss were recorded for each steak. Initial and cooked temperatures were recorded using a type K thermocouple (Model HH-21 Handheld Microprocessor Digital Thermometer, Omega Engineering, Inc., Stamford, CT). Following cooking, steaks were cut into cubes (1.3-cm x 1.3-cm x thickness of cooked steak) and care was taken to ensure that no large amounts of connective tissue or cooked edges were contained in the sample cubes. Samples were placed in ceramic bowls, covered with foil and clearly identified with a letter code before being placed in a

warming oven at 54°C until presentation to the trained sensory panel; samples did not remain for more than 20 min in the warming oven before panel evaluation.

Panelists used an 8-point, end-anchored rating scale (AMSA, 1995) to evaluate overall tenderness, overall juiciness, and beef flavor intensity (1 = extremely tough, extremely dry, extremely bland; 8 = extremely tender, extremely juicy, extremely intense). A two point scale (1 = detectable, 0 = not detectable) was used to determine prevalence of off flavors. Panelists were separated by partitions in the sensory evaluation room. Red incandescent lights were used as the lighting source for each panelist. Unsalted crackers (Premium Saltine Crackers, Kraft Foods Global, Inc. Northfield, IL) and distilled water (Big K, Inter-American Products, Inc, Cincinnati, OH) were used by panelists to cleanse their palettes between samples (minimum of one minute between samples).

Warner-Bratzler Shear Force

Shear force steaks were randomly assigned to each shear force data collection day so that each muscle and DEGDC (1/3, 1/2, and full) subclass was represented equally. Shear force steaks were tempered at 2°C for 36 h before cooking. Steaks were cooked using the methods previously described for sensory panel evaluation. Initial temperature, peak off-temperature, and cooking loss were recorded for each steak. After cooking, each steak was allowed to equilibrate to room temperature (22°C). Once the steaks cooled to room temperature, 4 (very small steaks only) to 10 cores (1.27-cm diameter) were removed from each steak parallel to the orientation of the muscle fibers. Each core then was sheared once perpendicular to the muscle fiber orientation using an Instron Testing Machine (Model 4443, Instron Corp., Canton, MA) fitted with a Warner-Bratzler

shear head (cross speed of 200 mm/min). Peak shear force measurements were recorded and averaged to obtain a single shear force value for each steak (Gruber et al., 2006a).

Survey

A combination telephone and internet-based survey was used to determine acceptability thresholds for subprimals in relation to fresh beef lean color. Interviewees were subject to conditions approved by the Human Use in Research Committee of Colorado State University. Color swatches were produced from digital images of the face of the individual muscles from which lean color was measured. Using the principle component procedure (PROC PRINCOMP) of SAS (SAS Inst., 2004), correlations between color scores were assessed in a three dimensional analysis, weighted, and then ranked. First principle component (Eigenvalue = 2.196) values of L*, a*, and b* (Eigenvectors = 0.467, 0.595, and 0.654, respectively) allowed the researchers to select appropriate incremental color images of muscles to use as a survey for participants to answer questions concerning ideal colors for lean beef muscle. Six color swatches were chosen and each color swatch was assigned a letter. First principle component values for each color swatch used in the survey are located in **Table 3.1**.

A standardized script was developed for use in conducting the survey to ensure unbiased and concise results. Those surveyed were asked, over the phone, to view the lean color swatches (via a temporary website) and determine which color swatches were acceptable, and then unacceptable to their customer base.

A list of the top 50 retail food outlets was obtained (Staff, 2006), and of those who were found to sell fresh whole-muscle beef products, the beef merchandisers of those companies were subsequently called and asked to participate in the survey.

Additionally, in-person interviews were conducted when available (9 retailers, 6 chefs). Of the 195 individuals contacted representing retail food outlets located around the United States, 34 completed the survey. Similarly, 105 chefs from around the United States who use beef in their cuisine were contacted to participate in the survey; of the chefs, 33 completed the survey.

Salvage Value Analysis

Previous research suggested that a probability of 0.50 or greater, when data are analyzed logistically, is required to attain favorable odds that end users will find a product acceptable (Platter et al., 2005). Muscles that were determined to have lean color first principle component values exceeding 0.50 probability of acceptance for retail meat merchandisers and foodservice chefs were assigned a monetary value to determine the salvageable value per carcass. Salvageable carcass value was achieved by utilizing the results from the survey; the weights of individual muscles with acceptable color values were aggregated and assigned a monetary value based on average boxed beef values for USDA grade (Choice, Select, No Roll) from 2006 (USDA, 2006).

Statistical Analysis

Color attributes (L*, a*, and b*), pH, WBSF, and the 8-point sensory characteristics were compared by DEGDC (1/3, 1/2, and full) and muscle using repeated measures and analysis of variance (ANOVA). Computations were performed using SAS PROC MIXED (SAS Inst., 2004). The model included main effects for DEGDC group and muscle, DEGDC by muscle interaction, and a random effect for carcass nested within the DEGDC group. For the evaluation of cooked beef traits (e.g., WBS and sensory scores), peak off-temperature was added to the model as a covariate when significant (P

< 0.05). When no interaction was detected ($P > 0.05$), DEGDC groups were compared, averaging over muscles, and muscles were compared averaging over DEGDC. When the interaction was significant, DEGDC groups were compared separately for each muscle, and muscles were compared separately for each DEGDC. For pH and color attributes (i.e., no covariate), comparisons were made using the LSD method ($\alpha = 0.05$); for other variables, mean separation was performed using pairwise t-test comparisons of least squares means. ANOVA assumptions were evaluated using a combination of residual plots, normal plots, and tests of normality.

Relationships between the lean color of the ribeye and the lean color of other muscles were evaluated using linear regression. Muscle color from the three color attributes (L*, a*, and b*) was summarized by its first principle component. The ribeye first principle component was the independent variable in the regression. Computations were performed using SAS PROC PRINCOMP and PROC REG (SAS Inst., 2004).

Sensory panel data (off-flavor detection) were analyzed using a repeated measures logistic model. Computations were performed using SAS PROC GLIMMIX (SAS Inst., 2004). Effects included in the model, as well as in comparisons of main effect and interaction least squares means, were performed in the same manner as in the repeated measures ANOVA model. For variables with very low frequency of "Yes" responses (i.e., bitter, sour, oxidized off-flavors), the DBLDOG option was used to aid the convergence.

Acceptability (yes/no) of color swatches of beef lean, that were evaluated by foodservice chefs and retail meat merchandisers were modeled as a function of the first principle component of the color attributes using quadratic logistic regression. The fitted

model was used to estimate the first principle component value at which probability of acceptance exceeds 0.50. Computations were performed using SAS PROC GLIMMIX (SAS Inst., 2004).

RESULTS AND DISCUSSION

Color and pH

A muscle × DEGDC interaction ($P < 0.001$) was detected for L*, a*, b* and pH. The least squares means of pH for the muscle × DEGDC interaction are presented in **Table 3.2**. Mean pH values for 1/3, 1/2, and full DEGDC RLD were 5.72, 5.76, and 6.06, respectively. Within DEGDC category, pH for RLD and SLLD muscles did not differ ($P > 0.05$). Previous research has demonstrated that "normal" ultimate pH of LM is within the range of 5.40 to 5.79 (Lawrie, 1958; Tarrant and Mothersill, 1977; Zhang et al., 2005). More recent research has shown that a reasonable approximate threshold pH for dark cutting carcasses was 5.87 in the LM (Page et al., 2001). In the present study, regardless of DEGDC category, no muscles had mean pH values of less than 5.60.

Within a muscle, 1/3 DEGDC pH values differed ($P < 0.05$) from 1/2 DEGDC for only 4 of the 29 muscles (GM, serratus ventralis, vastus intermedius, and VL). The pH values for serratus ventralis, vastus intermedius, and VL removed from 1/3 DEGDC were greater than the same muscles removed from the 1/2 DEGDC carcasses. Numerically, 1/3 DEGDC muscles had greater pH values than those of the 1/2 DEGDC muscles. These results contradicted expectations; when the pH of the muscle increases, the degree of dark cutting increases concomitantly (Lawrie, 1958; Page et al., 2001). Consistent with expectations, the mean 1/3 DEGDC GM pH value was less than the mean 1/2 DEGDC GM pH value ($P < 0.05$; **Table 3.2**).

Within a muscle, 1/2 DEGDC pH differed ($P < 0.05$) from full DEGDC pH for 13 of the 29 muscles (adductor, chuck spinalis dorsi, rectus abdominis, obliquus abdominus interni, IN, rectus femoris, RLD, SLLD, SM, serratus ventralis, and TM). As expected, mean pH values for several muscles (adductor, chuck spinalis dorsi, rectus abdominis, obliquus abdominis interni, IN, rectus femoris, RLD, SLLD, SM, serratus ventralis, and TM) removed from the 1/2 DEGDC carcasses were lower than that for corresponding muscles removed from full DEGDC carcasses (**Table 3.2**). Across all muscle and DEGDC categories, 24% of the muscle × DEGDC subclasses had mean pH values lower ($P < 0.05$) than a 5.87 threshold (Page et al., 2001) for dark cutting carcasses. Several of the LM mean pH samples from the strip loin and the ribeye roll, in addition to all of the mean LM pH samples from the chuck eye roll, were less than pH 5.87 (**Table 3.2**).

A total of 7 muscles (adductor, LFT, RLD, sirloin biceps femoris, ST, triceps brachii lateral head, and TBL) from carcasses classified by USDA graders as 1/3 DEGDC, 9 muscles (adductor, DP, RBF, rectus femoris, rib spinalis dorsi, sirloin biceps femoris, SM, triceps brachii lateral head, TBL, and VL) from carcasses classified by USDA graders as 1/2 DEGDC, and 5 muscles (GM, RBF, triceps brachii lateral head, TBL, and VL) from carcasses classified by USDA graders as full DEGDC would have fallen within a calculated 95 percent confidence limit of pH values considered "normal" for those respective muscles (NCBA, 2000); calculations not shown. Variation in pH values among and within muscles was explained using previous scientific literature. Presence of non-homogeneously stored glycogen residues throughout the animal surely contributed to such variance (Howard and Lawrie, 1956). A study conducted by Tarrant and Johnson (1980) found that pH is most frequently affected in the LM compared to 13 other muscles.

If pH is associated with color (Egbert and Comforth, 1986; Wulf et al., 1997; Wulf and Wise, 1999; Page et al., 2001; Wulf et al. 2002), then the color should be more frequently affected in the LM as well, when compared to other muscles. This logic helps explain why there was so much variation within and between muscle color values of dark-cutting carcasses; the most variable muscle (LM) was the muscle used to classify (i.e., grade) the carcass thereby inadvertently adding variation to the color results of the remaining muscles within the carcass. This indicated that LM is not the best muscle to use when predicting the pH condition of all of the remaining muscles in a carcass.

Beef lean color least squares means for the muscle × DEGDC interaction subclasses are reported in **Table 3.3**. L*, a*, and b* values of beef lean color for dark-cutting carcass muscles by DEGDC were numerically lower than the color values reported for the same muscles from normal carcasses of a previous study (NCBA, 2000). The high amount of color variation in muscles dissected from dark-cutting carcasses was demonstrated to be much greater than the amount of variation in color from the same muscles that were considered normally colored in a previous study (NCBA, 2000). Wulf and Wise (1999) discovered a greater relationship of b* values to dark cutters than L*. Additionally, several studies demonstrated high correlation between a* and b* values (Wulf and Wise, 1999; Page et al. 2001), thus explaining drastic differences due to the dark-cutting condition in a* values compared to "normal" carcass a* values of a previous study (NCBA, 2000). Furthermore, the larger size of the spectrophotometer port used in the previous NCBA (2000) study on "normal" carcass lean (2.54 cm vs. 1.27 cm diameter) may have allowed "normal" carcass lean color reflectance values to be

greater as a result of the larger opening, compared to the current study, which would have included flecks of marbling causing color scores to be higher.

Greater variability in color scores of muscles dissected from dark-cutting carcasses in the present study, compared to muscle colors from a previous trial that were considered to be "normal" (NCBA, 2000), can be explained by the subjectivity in visual evaluation of a color (i.e., the subjective determination of degree of dark-cutting of a ribeye). Previous studies have noted changes and variation between visual panels when attempting to assess color differences between beef muscles (Okerman and Cahill, 1969; AMSA, 1991). Therefore, a result of the subjectivity of the USDA graders evaluation of ribeye color may have resulted in even greater variation of the remaining muscles within each respective carcass. Moreover, Hood (1980) described how muscles can vary in color within animal and within muscle; therefore, color variation may have been dependent upon whether or not the location of the color measurement was a location that happened to be inadvertently more-dark than the rest of that particular muscle.

The three-dimensionality of color (AMSA, 1991, Morgan et al., 1997) prompted the use of principle component analysis. By using the first principle component (explains the greatest amount of variability orthogonally in space) of each muscle, a single value could be assigned to each muscle that was representative of all three color values (L*, a*, and b*) concomitantly (**Table 3.4**) (SAS, 2004). The relationship between the first principle component of the ribeye lean color and the first principle component of color measures for other muscles were investigated using linear regression (**Table 3.5**). Results of linear regression showed that the first principle component values for lean L*, a*, and b* of 18 of the 29 muscles (DP, chuck complexus, chuck longissimus dorsi,

chuck spinalis dorsi, IN, PM, rectus femoris, rib complexus, rib spinalis dorsi, sirloin biceps femoris, serratus ventralis, TBL, triceps brachii lateral head, TF, vastus intermedius, VL, and vastus medialis) were not related ($P > 0.05$) to the first principle component values for lean color of the ribeye face, once again indicating that color of the carcass ribeye is not an accurate indicator of lean colors for all remaining muscles within a carcass.

Survey and Cost Analysis

To determine the probability of acceptance of beef lean color, a survey was developed for evaluation by retail meat merchandisers and foodservice chefs. First principle component values were utilized to develop color swatches (**Table 3.1**) to be included in the survey. The probability of acceptance of the first principle component of lean color measures associated with the survey color swatches evaluated by foodservice chefs and retail meat merchandisers are presented in **Figure 3.1**. First principle component values corresponding to a ≥ 0.50 probability of acceptance were between 0.2047 to 2.0981 and 0.7713 to 2.9839 for foodservice chefs and retail meat merchandisers, respectively. The range of principle component values evaluated in the survey was 3.681 (from -0.0129 to 3.552) where the colors were representative of fully discounted dark cutting-like beef (low numbers) to pale pink veal-like lean (high numbers). The quality grade and associated value of a carcass can only be decreased as a result of the ribeye being too dark, not too light (USDA, 1997; AMSA, 2001); therefore, acceptability of lean color ultimately was determined from the lower acceptable first principle component value thresholds and up.

Based on color acceptability results from the survey, weights of individual muscles with acceptable color values were aggregated to determine salvageable carcass value by DEGDC subclass and quality grade. Individual muscle weights from carcass fabrication were used for estimating mean salvage values for dark-cutting carcasses on a per head basis. Mean salvage value (USDA, 2006) of muscles at a ≥ 0.50 probability of acceptance for lean color are stratified by end user (foodservice chef, retail meat merchandiser), quality grade (Choice, Select), and DEGDC (1/3, 1/2, and full) in **Table 3.6**, and represent the difference between the aggregate value of muscles with acceptable color values (probability ≥ 0.50) and the same muscles at un-graded commodity boxed beef prices (common pricing scheme for dark-cutting beef carcasses). Salvage value for both Choice and Select grades for chefs did not differ among DEGDC subclasses, although probabilities approached significance. Regardless of quality grade, responses from chefs showed that salvage amounts were numerically greater for 1/3 DEGDC carcasses than 1/2 and full DEGDC carcasses; salvage dollar amounts for 1/2 DEGDC were numerically greater than for full DEGDC. Survey responses collected from merchandisers indicated that Choice and Select salvage amounts were larger ($P < 0.05$) for 1/3 DEGDC than for full DEGDC carcasses. In general, as the DEGDC increased from 1/3 to full, there were fewer muscles with acceptable color values (Data not shown), which resulted in decreased mean salvage values. Nonetheless, all but 6 of the 60 carcasses had at least one muscle that was salvageable with regard to acceptable color.

As a result of variation among carcasses, as well as the lack of relatedness between ribeye color and color of other muscles, it would be difficult to determine which muscles would consistently have an acceptable lean color within each DEGDC. Analysis

of variance revealed that first principle component lean color values are independent of the DEGDC main effect ($P = 0.848$). First principle component lean color values corresponding to ≥ 0.50 probability of acceptance for meat merchandisers and foodservice chefs was 0.7714 and 0.2205, respectively. The number of muscles with acceptable mean first principle component color values (first principle component values greater than the 0.7714 and 0.2205 thresholds) for retail meat merchandisers and foodservice chefs was 4 (TBL, RLD, triceps brachii lateral head, and the rib complexus) and 12 (TBL, RLD, triceps brachii lateral head, rib complexus, SM, ST, rib spinalis, TM, serratus ventralis, IN, GM, and the PM) of the 29 muscles evaluated, respectively.

Warner-Bratzler Shear Force and Sensory Panel Evaluation.

No muscle × DEGDC interaction was detected for WBSF ($P = 0.213$); the main effect of DEGDC was not significant ($P = 0.233$), but muscle served as a significant source of variation ($P < 0.001$). Least squares means and percent CV for WBSF of individual muscles are presented in **Table 3.7**. Ranking of muscles by tenderness was in general agreement with previous reports (Gruber, 2006a); the PM and IN were among the most tender while the SM and RBF were among the least tender. Previous research suggests that high LM ultimate pH results in more tender WBSF values (Purchas, 1990). Muscles dissected from the dark-cutting carcasses compared to muscles dissected from carcasses considered "normal" (NCBA, 2000) were found to be numerically slightly more-tender, with the exception of the RBF, SM, and TFL; Dransfield (1981) confirms this stating that, on average, dark-cutting beef was determined to be only marginally more-tender than "normal" beef. Comparable amounts of variation were associated with

the means from both the dark cutting carcasses and those considered "normal" (NCBA, 2000).

No muscle × DEGDC interaction was detected for any sensory attributes evaluated ($P > 0.05$). The main effect of DEGDC did not impact any sensory attributes ($P > 0.05$). Sensory differences were detected among different muscles for overall tenderness, overall juiciness, and overall beef flavor intensity ($P < 0.05$; **Table 3.8**). Previous studies also have reported differences in sensory attributes between different beef muscles (Dransfield, 1981, Wulf et al., 2002, Meisinger et al. 2007). Findings for tenderness and flavor intensities of dark-cutting muscles in the present study were similar to sensory attributes for muscles from carcasses with "normally-colored" lean analyzed in previous studies (NCBA, 2000; Jones et al., 2004).

Differences were detected between muscles for the amount of detectable off-flavors (bitter, sour, metallic, liver, serum) expressed following cooking ($P < 0.05$; **Table 3.9**). No differences between DEGDC were reported for any off-flavors ($P > 0.05$); no interaction was detected for any off-flavors ($P > 0.05$). Off-flavors detected were similar to those found within the TM, VL, IN, rectus femoris, and triceps brachii previously investigated on "normal" beef carcasses (Meisinger, 2005). Detectable off-flavors can be attributed to the individual muscles themselves containing off-flavors that will be present regardless of whether or not the carcass is a dark cutter. Therefore, off-flavors detected should not cause any decrease in carcass value as they would likely be detected in "normal" carcasses as well. A sensory evaluation of muscles from dark cutters and muscles from normal carcasses, however, should be conducted in the future to further verify this suggestion.

Beef ribeye color did not prove to be a good indicator of lean color of other muscles in the carcass and, in fact, the color was highly variable throughout the LM. Evaluation of pH for the muscles dissected from dark-cutting carcasses in this study revealed that several are not affected by the dark-cutting condition in the ribeye and are, in fact, near what would be considered normal for those respective muscles. Many muscles exhibited lean color within an acceptable range to retail merchandisers and foodservice chefs that should allow those muscles to achieve full price when marketed, regardless of ribeye dark-cutting condition. Shear force and sensory evaluation of some of the muscles in the study did not appear to be affected by the dark-cutting condition. Further research should be conducted with control carcasses (non-dark cutting) to concomitantly evaluate the true differences between normal and dark-cutting carcasses with respect to sensory detection of off-flavors.

Table 3.1. L*, a*, b*, and first principle component values (Eigenvalue = 2.196; Eigen vectors of L*, a*, and b* were 0.467, 0.595, 0.654, respectively) of color swatches used in national survey of retail meat merchandisers and chefs to determine ideal color preferences in fresh beef lean

Color swatch[1]	L*	a*	b*	First principle component value[2]
A	37.07	14.66	13.63	-0.0129
B	38.18	16.76	14.18	0.6865
C	39.54	16.51	15.74	1.2023
D	35.68	21.04	16.25	1.6924
E	33.69	22.07	18.76	2.2342
F	34.09	24.27	20.95	3.2552

[1] Color swatches ranged from full-dark cutter appearance to pale pink veal appearance.

[2] First principle component value of the respective color values from n = 1779 observations; the first principle component accounted for 73.2% of the variation among the color measurements.

Table 3.2. Least squares means (CV%) of pH values[1] for muscles dissected from 1/3, 1/2, and Full degree dark cutting carcasses (DEGDC) (n = 20)[2]

Muscles	1/3	1/2	Full
Adductor	5.79 (8)	5.78 (8)	6.01 (8)
Pectoralis profundi	5.88 (8)	5.77 (8)	5.89 (8)
Chuck complexus	6.01 (8)	5.86 (8)	6.03 (8)
Chuck longissimus dorsi	5.85 (8)	5.77 (8)	5.81 (8)
Chuck spinalis dorsi	6.15 (8)	6.07 (8)	6.27 (7)
Rectus abdominis	6.03 (8)	5.94 (8)	6.12 (8)
Obliquus abdominis interni	6.07 (8)	5.99 (8)	6.20 (7)
Gluteus medius	5.60 (8)	5.94 (8)	5.79 (8)
Infraspinatus	6.05 (8)	5.91 (8)	6.09 (8)
Latissumus dorsi	5.75 (8)	5.80 (8)	5.91 (8)
Psoas major[3]	5.92 (8)	5.77 (8)	5.92 (8)
Round biceps femoris	5.62 (8)	5.66 (8)	5.69 (8)
Rectus femoris	5.90 (8)	5.75 (8)	5.94 (8)
Rib complexus	6.01 (8)	5.92 (8)	6.06 (8)
Longissimus thoracis	5.72 (8)	5.76 (8)	6.06 (8)
Rib spinalis dorsi	5.95 (8)	5.87 (8)	6.03 (8)
Sirloin biceps femoris	5.73 (9)	5.70 (9)	5.79 (8)
Longissimus lumborum	5.66 (8)	5.76 (8)	6.15 (8)
Semimembranosus	5.63 (8)	5.72 (8)	5.96 (8)
Serratus ventralis	6.20 (7)	6.02 (8)	6.31 (7)
Semitendinosus	5.73 (8)	5.72 (8)	5.84 (8)
Supraspinatus	5.88 (8)	5.81 (8)	5.94 (8)
Triceps brachii lateral head	5.78 (8)	5.71 (8)	5.79 (8)
Triceps brachii long head	5.76 (8)	5.67 (8)	5.78 (8)
Teres major	6.02 (8)	5.98 (8)	6.28 (7)
Tensor fasciae latae	5.82 (8)	5.69 (8)	5.78 (8)
Vastus intermedius	6.57 (7)	6.39 (7)	6.46 (7)
Vastus lateralis	5.90 (8)	5.71 (8)	5.72 (8)
Vastus medialis	6.07 (8)	5.96 (8)	5.97 (8)

[1] Ultimate pH measured 2 to 3 days postmortem.

[2] Within muscle x DEGDC, pH value LSD ($P < 0.05$) = 0.18, between muscle x DEGDC pH value LSD ($P < 0.05$) = 0.13.

Table 3.3. Least squares means (CV%) of L*, a*, and b*[1,2] for muscles dissected from 1/3, 1/2, and Full degree dark cutting carcasses (n = 20)

	L*[3]			a*[4]			b*[5]		
Muscles	1/3	1/2	Full	1/3	1/2	Full	1/3	1/2	Full
12-13 rib separation	33.09 (16)	32.08 (16)	30.00 (18)	17.40 (28)	16.64 (29)	13.19 (37)	13.50 (32)	13.26 (32)	10.38 (41)
Adductor	33.96 (15)	34.28 (15)	30.41 (17)	18.63 (26)	18.22 (27)	15.75 (31)	14.89 (28)	14.47 (29)	11.88 (35)
Pectoralis profundi	35.14 (15)	35.13 (15)	35.01 (15)	17.53 (28)	18.57 (26)	16.47 (30)	13.81 (30)	14.14 (30)	13.02 (32)
Chuck complexus	35.24 (15)	35.30 (15)	34.83 (15)	16.66 (29)	17.20 (28)	16.07 (30)	13.26 (32)	13.35 (31)	12.99 (32)
Chuck longissimus dorsi	35.64 (14)	36.37 (14)	35.57 (14)	18.25 (27)	17.08 (29)	17.23 (28)	15.02 (28)	14.47 (29)	14.40 (29)
Chuck spinalis dorsi	35.59 (14)	35.89 (15)	34.55 (15)	17.43 (28)	17.21 (28)	17.63 (28)	13.55 (31)	13.87 (30)	13.94 (30)
Rectus abdominis	35.07 (16)	34.77 (16)	34.00 (16)	17.26 (30)	17.94 (29)	17.52 (30)	14.27 (31)	14.74 (30)	14.47 (31)
Obliquus abdominis interni	35.16 (15)	36.47 (15)	34.18 (16)	16.72 (30)	15.51 (32)	15.48 (32)	12.31 (35)	12.31 (35)	11.80 (37)
Gluteus medius	36.49 (14)	35.98 (14)	33.06 (16)	18.74 (26)	18.73 (26)	17.04 (29)	15.33 (27)	15.34 (27)	13.38 (31)
Infraspinatus	35.24 (15)	35.71 (14)	34.60 (15)	16.79 (29)	16.17 (30)	15.79 (31)	13.54 (31)	12.98 (32)	12.65 (33)
Latissumus dorsi	35.24 (14)	35.49 (14)	35.63 (14)	17.66 (28)	16.43 (30)	16.33 (30)	14.22 (29)	13.49 (31)	13.84 (30)
Psoas major[7]	37.10 (14)	37.98 (14)	37.38 (14)	17.23 (29)	18.46 (27)	17.27 (29)	14.45 (29)	15.74 (27)	14.69 (29)
Round biceps femoris	38.51 (13)	37.15 (14)	36.17 (14)	17.85 (27)	18.79 (26)	17.53 (28)	15.05 (28)	15.27 (27)	14.18 (29)
Rectus femoris	36.27 (14)	36.55 (14)	36.10 (14)	15.66 (31)	16.01 (30)	15.32 (32)	13.21 (32)	13.37 (31)	13.10 (32)
Rib complexus	34.79 (15)	33.86 (15)	34.39 (15)	18.45 (26)	18.25 (27)	18.39 (27)	14.31 (29)	14.09 (30)	14.48 (29)
Longissimus thoracis	35.09 (15)	33.36 (15)	31.62 (16)	16.27 (30)	14.73 (33)	12.84 (38)	13.26 (32)	11.84 (35)	10.48 (40)
Rib spinalis dorsi	34.06 (15)	35.33 (15)	35.95 (14)	17.28 (28)	17.27 (28)	16.32 (30)	13.17 (32)	13.66 (31)	13.20 (32)
Sirloin biceps femoris	34.01 (15)	34.07 (15)	33.90 (15)	16.22 (30)	16.91 (29)	16.84 (29)	12.54 (33)	12.89 (32)	12.87 (33)
Longissimus lumborum	34.78 (15)	32.85 (16)	31.36 (17)	15.73 (31)	15.55 (31)	12.68 (38)	12.86 (33)	12.22 (35)	9.91 (43)
Semimembranosus	33.53 (15)	33.71 (15)	30.16 (17)	18.25 (27)	18.07 (27)	14.73 (34)	14.56 (29)	14.54 (29)	11.28 (37)
Serratus ventralis	34.47 (15)	34.65 (15)	33.92 (16)	17.94 (27)	17.10 (29)	16.19 (30)	13.25 (32)	13.01 (32)	12.43 (34)
Semitendinosus	38.19 (13)	37.92 (14)	36.25 (14)	16.73 (29)	16.77 (29)	15.32 (32)	15.96 (26)	15.66 (27)	14.07 (30)
Supraspinatus	33.64 (15)	34.53 (15)	34.23 (15)	16.36 (30)	16.77 (29)	16.69 (29)	12.78 (33)	13.06 (32)	13.16 (32)
Triceps brachii lateral head	34.06 (15)	34.20 (15)	34.03 (15)	15.95 (31)	16.12 (30)	16.15 (30)	12.68 (33)	12.89 (32)	12.97 (32)
Triceps brachii long head	34.77 (15)	34.89 (15)	34.57 (15)	16.27 (30)	17.34 (28)	16.28 (30)	13.06 (32)	13.53 (31)	12.98 (32)
Teres major	35.96 (15)	35.46 (15)	34.09 (15)	17.85 (28)	17.19 (29)	15.27 (32)	14.71 (29)	14.47 (29)	12.68 (34)
Tensor fasciae latae	37.66 (14)	38.00 (14)	38.09 (13)	16.63 (29)	17.88 (27)	16.31 (30)	14.28 (29)	15.39 (27)	14.48 (29)
Vastus intermedius	31.46 (16)	31.77 (16)	30.64 (17)	15.81 (31)	15.46 (32)	15.55 (31)	11.30 (37)	10.82 (39)	10.95 (38)
Vastus lateralis	34.18 (15)	35.31 (15)	34.59 (15)	16.24 (30)	16.83 (29)	16.26 (30)	12.98 (32)	13.63 (31)	13.01 (32)
Vastus medialis	32.06 (16)	32.43 (16)	31.77 (17)	14.81 (33)	14.98 (33)	15.33 (32)	10.72 (39)	10.83 (39)	11.29 (37)

[1] Objective color measurements taken after a 20-minute bloom time.

[2] L*- black to white, 0 to 100; a*-green to red, (-60) to 60; b*-blue to yellow, (-60) to 60.

[3] Within muscle x DEGDC, L* value LSD ($P < 0.05$) = 2.03, between muscle x discount L* value LSD ($P < 0.05$) = 1.43.

[4] Within muscle x DEGDC, a* value LSD ($P < 0.05$) = 1.87, between muscle x discount a* value LSD ($P < 0.05$) = 1.32.

[5] Within muscle x DEGDC, b* value LSD ($P < 0.05$) = 1.17, between muscle x discount b* value LSD ($P < 0.05$) = 1.14.

Table 3.4. Least squares means ± SEM of the first principle component values[1] of individual muscles[2] (n = 60)

Muscle	Mean First Principle Component Value Means[3]
12^{th} -13^{th} rib separation	-1.007 ± 0.172
Adductor	-0.555 ± 0.172
Pectoralis profundi	-0.161 ± 0.171
Chuck complexus	-0.465 ± 0.174
Chuck longissimus dorsi	-0.494 ± 0.174
Chuck spinalis dorsi	-0.593 ± 0.174
Rectus abdominis	0.087 ± 0.171
Obliquus abdominis interni	-0.399 ± 0.171
Gluteus medius	0.564 ± 0.171
Infraspinatus	0.546 ± 0.175
Latissumus dorsi	-0.566 ± 0.174
Psoas major	0.704 ± 0.171
Round biceps femoris	-0.474 ± 0.171
Rectus femoris	-0.633 ± 0.171
Rib complexus	1.517 ± 0.171
Longissimus thoracis	1.183 ± 0.171
Rib spinalis dorsi	0.330 ± 0.174
Sirloin biceps femoris	0.062 ± 0.171
Longissimus lumborum	-0.389 ± 0.171
Semimembranosus	0.207 ± 0.171
Serratus ventralis	0.439 ± 0.171
Semitendinosus	0.209 ± 0.171
Supraspinatus	0.058 ± 0.171
Triceps brachii lateral head	1.419 ± 0.171
Triceps brachii long head	0.775 ± 0.171
Teres major	0.408 ± 0.171
Tensor fasciae latae	-1.300 ± 0.171
Vastus intermedius	-0.969 ± 0.171
Vastus lateralis	0.029 ± 0.171
Vastus medialis	-0.649 ± 0.178

[1] First principle component values for the combined L*, a*, and b* color space.
[2] Mean principle component values averaged over degree of dark cutter.
[3] Between muscles, first principle component value LSD ($P < 0.05$) = 0.3404.

Table 3.5. Simple linear regression of the dependent variable of the first principle component value of individual muscles and the independent variable of the first principle component of the LM at 12th and 13th rib separation (LMPC)

Muscle	*P*	Intercept	B_1(LMPC)	r^2	RMSE[1]
Adductor	<0.001	0.516	0.722	0.238	1.845
Pectoralis profundi	0.082	0.451	0.202	0.053	1.223
Chuck complexus	0.262	0.103	0.114	0.022	1.078
Chuck longissimus dorsi	0.065	0.810	0.235	0.060	1.333
Chuck spinalis dorsi	0.694	0.341	0.038	0.003	1.029
Rectus abdominis	0.010	0.692	0.239	0.135	0.899
Obliquus abdominis interni	0.014	-0.156	0.266	0.108	1.096
Gluteus medius	<0.001	1.220	0.674	0.344	1.330
Infraspinatus	0.084	0.020	0.160	0.053	0.970
Latissumus dorsi	0.017	0.426	0.214	0.097	0.936
Psoas major	0.102	1.177	0.284	0.048	1.793
Round biceps femoris	0.001	1.375	0.422	0.183	1.273
Rectus femoris	0.171	0.122	0.208	0.033	1.602
Rib complexus	0.275	0.600	0.119	0.021	1.149
Longissimus thoracis	<0.001	-0.364	0.734	0.495	1.059
Rib spinalis dorsi	0.164	0.175	0.121	0.034	0.915
Sirloin biceps femoris	0.803	-0.282	0.027	0.001	1.152
Longissimus lumborum	<0.001	-0.459	0.711	0.528	0.951
Semimembranosus	<0.001	0.322	0.744	0.394	1.319
Serratus ventralis	0.204	0.006	0.159	0.029	1.301
Semitendinosus	<0.001	1.390	0.637	0.404	1.105
Supraspinatus	0.068	-0.053	0.189	0.058	1.085
Triceps brachii lateral head	0.877	-0.307	0.016	0.001	1.089
Triceps brachii long head	0.485	0.002	0.074	0.009	1.119
Teres major	0.019	0.616	0.420	0.096	1.822
Tensor fasciae latae	0.157	0.949	0.170	0.035	1.269
Vastus intermedius	0.117	-1.120	0.169	0.043	1.135
Vastus lateralis	0.314	0.026	0.156	0.018	1.641
Vastus medialis	0.237	-1.188	0.130	0.025	1.130

[1] Root mean square error.

Table 3.6. Least squares mean ± SEM estimated salvage value per carcass of 1/3, 1/2, and full degree of dark cutting conditions (DEGDC); [1, 2, 3, 4]

	Chefs			Merchandisers		
DEGDC	Choice	Select	n	Choice	Select	n
1/3	$42.29 ± 4.96[a]	$14.71 ± 1.96[a]	20	$30.39 ± 3.43[a]	$10.37 ± 1.33[a]	18
½	$34.68 ± 4.96[ab]	$10.62 ± 1.96[ab]	20	$25.91 ± 3.43[ab]	$7.50 ± 1.33[ab]	18
Full	$26.44 ± 5.09[b]	$8.11 ± 2.01[b]	19	$16.74 ± 3.34[b]	$5.03 ± 1.29[b]	19
P	0.09	0.07		0.02	0.02	

[a,b] Within a column, means without a common superscript differ ($P < 0.05$).

[1] Differences of mean aggregate value of muscles with color values corresponding to probability of acceptance greater than or equal to 0.50 for foodservice chefs and retail meat merchandisers at Choice and Select prices versus the same muscles at un-graded boxed beef prices.

[2] Muscles evaluated from each carcass include: adductor, pectoralis profundi, chuck complexus chuck longissimus dorsi, chuck spinalis dorsi, rectus abdominis, obliquus abdominis interni, gluteus medius, infraspinatus, latissimus dorsi, psoas major, round biceps femoris, rectus femoris, rib complexus, longissimus thoracis, rib spinalis dorsi, sirloin biceps femoris, longissimus lumborum, semimembranosus, serratus ventralis, semitendinosus, supraspinatus, triceps brachii long head, triceps brachii lateral head, teres major, tensor fasciae latae, vastus intermedius, vastus lateralis, and vastus medialis.

[3] Dollar amounts based off of the USDA Annual Meat Trade Review (2006) average value for boxed beef cuts.

[4] Value based on actual weights of acceptable muscles.

Table 3.7. Least squares means (CV %) for Warner-Bratzler shear force (kg) of muscles averaged over 1/3, 1/2, and full USDA dark-cutting discount carcasses (N = 60)

Muscles	Dark cutters[1]
Chuck complexus	4.48 (22)[d]
Pectoralis profundi	5.36 (19)[bc]
Gluteus medius	5.23 (19)[bc]
Infraspinatus	3.08 (33)[h]
Latissumus dorsi	4.53 (22)[d]
Psoas major	3.05 (33)[h]
Round biceps femoris	5.46 (18)[b]
Longissimus thoracis	4.40 (23)[de]
Longissimus lumborum	4.33 (23)[de]
Semimembranosus	6.04 (17)[a]
Semitendinosus	4.53 (22)[d]
Triceps brachii long head	4.06 (25)[ef]
Tensor fasciae latae	3.74 (27)[fg]
Teres major	3.45 (29)[g]
Vastus lateralis	5.03 (20)[c]

[a—h] Means, within a column, without a common superscript, differ ($P < 0.05$).

[1] No significant muscle × dark-cutting discount was detected ($P = 0.213$); no significant dark cutting discount main effect was detected ($P = 0.233$); significant muscle main effect was detected ($P < 0.001$).

Table 3.8. Least squares means ± SEM of overall tenderness, juiciness, and beef flavor intensity of individual muscle sensory characteristics found within dark cutting carcasses (n = 60)[1]

Muscle	Overall Tenderness	Overall Juiciness	Overall Beef Flavor Intensity
Chuck complexus	5.40 ± 0.12^{c}	5.75 ± 0.09^{a}	5.38 ± 0.06^{ab}
Pectoralis profundi	3.80 ± 0.12^{hi}	5.10 ± 0.09^{cd}	5.09 ± 0.06^{cd}
Gluteus medius	4.72 ± 0.12^{e}	4.72 ± 0.09^{f}	5.10 ± 0.06^{cd}
Infraspinatus	6.31 ± 0.12^{b}	5.81 ± 0.09^{a}	5.48 ± 0.06^{a}
Latissimus dorsi	4.09 ± 0.12^{gh}	5.13 ± 0.09^{cd}	5.12 ± 0.06^{cd}
Psoas major	7.05 ± 0.12^{a}	5.48 ± 0.09^{ab}	5.18 ± 0.06^{c}
Round biceps femoris	3.95 ± 0.12^{ghi}	5.08 ± 0.09^{cd}	5.24 ± 0.06^{bc}
Longissimus thoracis	5.35 ± 0.12^{cd}	5.20 ± 0.09^{c}	5.11 ± 0.06^{cd}
Longissimus lumborum	5.21 ± 0.12^{cd}	4.93 ± 0.09^{def}	5.13 ± 0.06^{cd}
Semimembranosus	3.68 ± 0.12^{i}	4.48 ± 0.09^{g}	4.98 ± 0.06^{de}
Semitendinosus	4.32 ± 0.12^{fg}	4.28 ± 0.09^{g}	4.84 ± 0.06^{e}
Triceps brachii long head	5.14 ± 0.12^{d}	5.05 ± 0.09^{de}	5.17 ± 0.06^{c}
Tensor fasciae latae	5.50 ± 0.12^{c}	5.30 ± 0.09^{bc}	5.00 ± 0.06^{de}
Vastus lateralis	4.57 ± 0.12^{ef}	4.83 ± 0.09^{ef}	5.08 ± 0.06^{cd}

[a—i] Within a column, means without a common superscript letter differ ($P < 0.05$).

[1] No differences between 1/3, 1/2, and full dark cutting carcasses were determined ($P < 0.05$) and no interaction between muscle and degree of dark cutting were detected ($P < 0.05$).

[2] Eight point sensory panel scale (8 = extremely tender, extremely juicy, extremely intense beef flavor; 1 = extremely tough, extremely dry, extremely bland beef flavor).

Table 3.9. Mean probability ± SEM of off-flavors of individual muscle sensory characteristics found within dark cutting carcasses (n = 60)[1,2]

Muscle	Bitter	Sour	Metallic	Liver	Serum	Oxidized	Detectable Off-flavor
Chuck complexus	0.101 ± 0.014^{bc}	0.027 ± 0.008^{cd}	0.098 ± 0.014^{cd}	0.128 ± 0.018^{b}	0.101 ± 0.014^{cd}	0.053 ± 0.010	0.449 ± 0.025^{bcd}
Pectoralis profundi	0.101 ± 0.014^{bc}	0.022 ± 0.006^{cd}	0.080 ± 0.013^{d}	0.076 ± 0.013^{def}	0.082 ± 0.013^{de}	0.041 ± 0.009	0.379 ± 0.024^{ef}
Gluteus medius	0.048 ± 0.010^{ef}	0.062 ± 0.008^{a}	0.142 ± 0.016^{ab}	0.076 ± 0.013^{def}	0.138 ± 0.016^{bc}	0.031 ± 0.008	0.452 ± 0.025^{bcd}
Infraspinatus	0.081 ± 0.013^{cd}	0.001 ± 0.016^{d}	0.106 ± 0.014^{abcd}	0.185 ± 0.022^{a}	0.145 ± 0.016^{ab}	0.035 ± 0.008	0.492 ± 0.025^{ab}
Latissimus dorsi	0.158 ± 0.017^{a}	0.030 ± 0.009^{bcd}	0.089 ± 0.013^{cd}	0.080 ± 0.013^{def}	0.063 ± 0.011^{e}	0.036 ± 0.009	0.413 ± 0.025^{cde}
Psoas major	0.034 ± 0.010^{f}	0.045 ± 0.008^{abc}	0.120 ± 0.015^{abc}	0.094 ± 0.015^{bcde}	0.078 ± 0.012^{de}	0.027 ± 0.009	0.379 ± 0.025^{ef}
Round biceps femoris	0.056 ± 0.011^{def}	0.056 ± 0.008^{ab}	0.147 ± 0.016^{a}	0.121 ± 0.017^{bc}	0.190 ± 0.018^{a}	0.039 ± 0.009	0.519 ± 0.025^{a}
Longissimus thoracis	0.101 ± 0.014^{bc}	0.042 ± 0.011^{abcd}	0.098 ± 0.014^{cd}	0.071 ± 0.013^{def}	0.071 ± 0.012^{de}	0.031 ± 0.008	0.382 ± 0.024^{ef}
Longissimus lumborum	0.124 ± 0.015^{ab}	0.035 ± 0.010^{abcd}	0.103 ± 0.014^{bcd}	0.063 ± 0.013^{ef}	0.058 ± 0.011^{e}	0.019 ± 0.007	0.382 ± 0.025^{ef}
Semimembranosus	0.045 ± 0.010^{ef}	0.061 ± 0.008^{a}	0.112 ± 0.015^{abcd}	0.101 ± 0.015^{bcd}	0.121 ± 0.015^{bcd}	0.024 ± 0.007	0.447 ± 0.025^{bcd}
Semitendinosus	0.042 ± 0.009^{f}	0.033 ± 0.009^{bcd}	0.119 ± 0.015^{abc}	0.055 ± 0.011^{f}	0.060 ± 0.011^{e}	0.019 ± 0.006	0.306 ± 0.023^{g}
Triceps brachii long head	0.075 ± 0.012^{cde}	0.035 ± 0.01^{abcd}	0.089 ± 0.013^{cd}	0.101 ± 0.016^{bcd}	0.101 ± 0.014^{cd}	0.024 ± 0.007	0.394 ± 0.025^{def}
Tensor fasciae latae	0.053 ± 0.010^{ef}	0.032 ± 0.007^{bcd}	0.076 ± 0.012^{d}	0.086 ± 0.014^{cdef}	0.094 ± 0.013^{de}	0.023 ± 0.007	0.341 ± 0.024^{fg}
Vastus lateralis	0.091 ± 0.013^{bc}	0.018 ± 0.007^{d}	0.122 ± 0.015^{abc}	0.122 ± 0.017^{bc}	0.138 ± 0.016^{bc}	0.028 ± 0.008	0.464 ± 0.025^{abc}

[a–g] Within a column, means without a common superscript letter differ ($P < 0.05$).

[1] No significant differences or interaction between 1/3, 1/2, and full dark cutting carcasses were determined ($P < 0.05$).

[2] Off flavor detection (0 = not detected, 1 = detected).

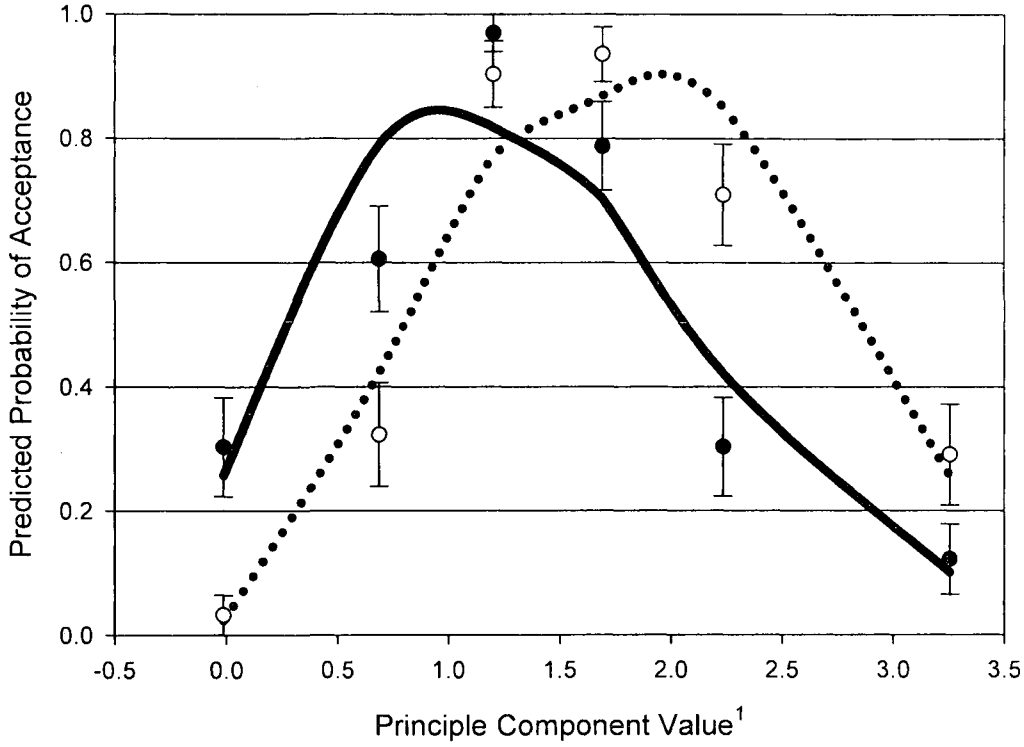


Figure 3.1. Least squares means ± SEM and predicted acceptable probability regression lines for the first principle component value chosen to be acceptable for retail meat merchandisers (○) and foodservice chefs (●). Regression equation for retail meat merchandisers (• • •) [$P_i = \exp(-3.6573 + (5.9671 * PC) + (-1.5890 * PC^2) / 1 + \exp(-3.6573 + (5.9671 * PC) + (-1.5890 * PC^2)]$. Regression equation for Chefs (▬) [$P_i = \exp(-0.9907 + (5.5303 * PC) + (-3.4955 * PC^2) + (0.5171 * PC^3) / 1 + \exp(-0.9907 + (5.5303 * PC) + (-3.4955 * PC^2) + (0.5171 * PC^3)]$. Fit statistics of χ^2/degrees of freedom for chef and merchandiser lines are 1.03 and 1.08 respectively (Values close to 1.00 are a closer fit).

[1] Principle components take into account L*, a*, and b* values. Weights are assigned to each value and then ranked. Colors from this ranking range from fully discounted dark cutting-like beef (low values) to pale pink veal-like (high values) lean color.

CHAPTER IV

Associations between portion size acceptability of beef cuts and ribeye area of beef carcasses

INTRODUCTION

In 2005, the National Beef Quality Audit (**NBQA**) showed that inconsistency in fresh beef product size and weight was a concern to the industry as the inconsistency in whole muscle product led to an inconsistency in portioning (Smith et al., 2005). Beef carcasses with LM areas at the 12th and 13th rib interface (**LMA**) of less than 71.0 cm^2 or greater than 103.2 cm^2 may be considered by some to be non-conforming (Savell, 2007). In 2000, the NBQA reported that the percentage of carcasses with an LMA less than 71.0 cm^2 or greater than 103.2 cm^2 were 7.9% and 5.3%, respectively (Smith et al., 2000). In 2005, the NBQA data demonstrated that the percentages of carcasses with an LMA less than 71.0 cm^2 or greater than 103.2 cm^2 were 7.6% (Savell, 2007) and 7.8% (L. G. Garcia, Texas A&M University, College Station, TX, personal communication), respectively.

As a result of the large percentages of carcasses with non-conforming ribeyes, there was interest in determining if the size of muscles in the remainder of the carcass correspond to the size of the LMA; and if so, are those muscles still within an acceptable size range for merchandising in both the foodservice and retail sectors. Therefore, the first objective of this study was to determine if portion-size of muscles in the beef carcass

can be predicted by LMA of the carcass. The second objective was to measure acceptability of differing portion-sizes for muscles in which size was related to carcass LMA.

MATERIALS AND METHODS

Meat samples were obtained from 2 federally inspected beef processing facilities in Colorado.

Carcass Dissection and Muscle Analysis

Between December 2006 and February 2007, beef (non-dairy type) sides from individual carcasses (n = 60) with varying ribeye sizes (ranging from 67.74 to 166.13 cm^2) were purchased from two commercial beef packing companies and transported, under refrigerated conditions (2 ± 1°C), to the Colorado State University Meat Laboratory for fabrication and dissection. Upon arrival at the Meat Laboratory, carcass sides were weighed to determine chilled carcass weight and fabricated into the following subprimal muscle cuts: NAMP # 114E clod heart, triceps brachii long head (**TBL**); NAMP #114D top blade, infraspinatus (**IN**); NAMP # 116H chuckeye, complexus (**CHCOM**); NAMP # 120A brisket flat, pectoralis profundus (**DP**); NAMP # 112C ribeye, longissimus thoracis (**RLD**); NAMP # 109B lifter meat, latissimus dorsi (**LFT**); NAMP # 184B top sirloin, gluteus medius (**GM**); NAMP # 180 1x0 strip loin, longissimus lumborum (**SLLD**); NAMP # 185 tri-tip, tensor fasciae latae (**TFL**); NAMP # 189A tenderloin, psoas major (**PM**); NAMP # 169A top round, semimembranosus (**SM**); NAMP # 171B outside round, biceps femoris (**RBF**); NAMP # 171C eye of round, semitendinosus (**ST**); and NAMP # 167F knuckle tip, vastus lateralis (**VL**).

Dimensions and weights of each subprimal muscle cut were recorded; dimensional measurements included maximum length (longitudinal axis of the muscle),

maximum width (perpendicular to the longitudinal axis of the muscle), and maximum depth of the muscle (measured as the highest point with the cut lying on a flat, level, surface). Individual muscles were bisected at the midpoint along the longitudinal axis, and the cross-sectional surface of the resulting muscle face was measured for maximum depth and width. The cross-sectional surface was then traced to obtain an area measurement. After all dimensional measurements had been obtained, a steak (1.27-cm thick) was removed from each muscle and weighed.

Survey

An internet-based survey was used to determine purchaser portion-size acceptability thresholds for the 14 subprimals dissected from the 60 beef carcass sides. Interviewees were subjected to conditions approved by the Human Use in Research Committee of Colorado State University. Using the SAS statistical package (SAS Inst. Inc., Cary, NC), regression was used to determine whether muscles had a portion size (mass/unit thickness; grams/1.27-cm thick steak) that varied linearly with the LMA of its respective carcass. Similarly, regression was used to assess the relationship between LMA and the cross-sectional surface area measurement of the midpoint of each muscle evaluated. The LMA of each carcass side was regressed against the afore-mentioned dependent variables (portion size and cross-sectional surface area) on the 14 muscles dissected from the carcasses. These muscles were chosen as representatives of their respective larger subprimals when multiple muscles were included in a specific subprimal (e.g., CHCOM—largest single muscle located within the chuck eye roll; NAMP # 116D). Results of simple linear regression are provided in **Table 4.1**. From these regression analyses, it was determined that SLLD, RLD, DP, ST, RBF, TBL, LFT, IN and GM were

to be further evaluated as their portion size or cross-sectional surface area was associated ($P < 0.05$) with the independent variable of LMA.

Once the muscles requiring further evaluation were identified, simple linear regression equations were utilized to obtain the estimated length and width of the face of the respective muscles at four sizes of LMA (67.74, 83.87, 100.00, and 116.13 cm^2), assuming LMA was an accurate predictor of the muscles. After the dimensional parameters were obtained, a website was developed to display general images of the muscles and the cross-sectional surface of the muscle along with descriptive characteristics associated with each muscle. The general image of the muscle was to aid the participant in orientation with what the muscle looked like and by no means was the image of the muscle to be evaluated by the participant. The portion weight characteristics being evaluated were listed as portion-size weights from steaks cut at 1.27 cm, 1.91 cm, and 2.54 cm in thickness. Portion size weights of steaks cut at 1.91 cm thick and 2.54 cm thick were predicted from the 1.27 cm thick portion steak cut during the carcass dissection phase of the study. Additional descriptive characteristics included surface area of the face of a steak cut from the midpoint of the longitudinal axis of the subprimal – laterally, the length of the face, and the width of the face. Carcass LMA sizes 67.74, 83.87, 100.00, and 116.13 cm^2 were assigned a corresponding letter in ascending order from A to D, respectively. All measurements were displayed in English system units which were more familiar to those participating in the survey than were metric units of measure. An example of a muscle portion-size evaluation web page used to conduct the survey is provided in **Figure 4.1**.

A standardized script was developed for the survey to ensure unbiased and concise results. The survey involved contacting the participants by telephone and having them go to a designated website at which the survey was conducted. Those surveyed were asked to state whether or not (yes or no) they merchandised the beef muscles listed on the survey website; the common names of the muscles were used for ease of understanding of the muscles being examined. The list of common muscle names included bottom round (RBF), eye of round (ST), top sirloin butt (GM), striploin (SLLD), lifter meat (LFT), ribeye (RLD), chuck clod heart (TBL), top blade (IN), and brisket flat (DP). Of the subprimals that received a "yes" response, the survey participant was then asked to evaluate the portion size characteristics listed under each letter of the respective muscle, and to determine from those characteristics whether a portion (identified by a letter) was "acceptable" or "unacceptable" for their customer base.

A list of the top 50 retail food outlets was obtained (Progressive Grocer, 2006) and, of those that were determined to sell fresh whole muscle beef products, the beef merchandisers of those companies were called and asked to participate in the survey. Additionally, in-person interviews were conducted when available. Of the 195 individuals contacted, representing retail food outlets located around the United States, 34 completed the survey. Similarly, 105 chefs from around the United States who use beef in their cuisine were contacted to participate in the survey. Of the chefs contacted, 33 completed the survey.

Statistical Analysis

Simple descriptive statistics were calculated using PROC MEANS (SAS Inst., 2004). Linear regression analysis of the independent variable (LMA) and the dependent

variables, portion size (mass/unit thickness; grams/1.27 cm thick steak) and surface area of the face at the midpoint-cut of a muscle (cm^2), were conducted using PROC REG (SAS Inst., 2004). To be certain that all subprimals for which portion size may be associated with LMA were included in the survey, significance level of the regression was set at $P < 0.05$. Simple correlation coefficients (r) between carcass LMA and dimensional parameters of various muscles were determined using PROC CORR (SAS Inst., 2004). Descriptions of strength of correlation are in accordance with Devore and Peck (2005). Curvilinear relationships were evaluated, but were detrimental to not be applicable.

Frequency data (survey muscle acceptability results) were analyzed using the GLIMMIX procedure of SAS. Options specified in the analyses were binomial distribution and logit link function. Estimated parameters to fit logistic regression lines to survey results (muscle sizes) based on the equation $P_i = \exp(\beta_o + \beta_i x_i) / [1 + \exp(\beta_o + \beta_i x_i)]$ were derived using the GLIMMIX procedure of SAS (SAS Inst., 2004). Carcass LMA was used as the independent variable in each regression equation estimating acceptability of muscle size and the interaction between muscle size and user (chef or merchandiser).

RESULTS AND DISCUSSION

Simple descriptive statistics of dimensions and portion characteristics of muscles evaluated are presented in **Table 4.2**. The aggregate mean ± SD of all carcass chilled side weights and LMA were 175.3 ± 15.8 kg and 86.6 ± 9.4 cm^2, respectively. Results of simple linear regression of LMA on the dependent variables of muscle portion weights (g/1.27-cm steak) and muscle midpoint cross-sectional surface area (cm^2) (**Table 4.1**)

revealed that portion weight for seven of the 14 muscles (CHCOM, DP, PM, SM, TFL, TBL and VL) were not associated with LMA at $\alpha = 0.05$ ($P = 0.138$, 0.101, 0.312, 0.074, 0.723, 0.189and 0.394 respectively). Similarly, portion cross-sectional surface are as for 7 muscles (CHCOM, IN, LFT, PM, SM, TFL, and VL) were not related to LMA at $\alpha = 0.05$ ($P = 0.725$, 0.106, 0.431, 0.089, 0.155, 0.523, and 0.239 respectively). Data showed no association between LMA and portion characteristics for five of the 14 muscles (CHCOM, PM, SM, TFL, and VL) when either of the dependent variables (muscle portion weights, midpoint cross-sectional surface area) were evaluated. Results suggested that LMA is not a good predictor of individual muscle portion sizing characteristics. Griffin et al. (1999) found similar results when they predicted muscle subprimal yields by use of the LMA of the carcass; they found weak correlations for which many were not different from zero ($P > 0.05$).

Simple correlations between muscle dimensional characteristics and LMA are displayed in **Table 4.3**. As expected, portion characteristics for RLD had moderate to lowly-moderate correlations with carcass LMA. Alternatively, portion characteristics for the SLLD had weak to very weak correlations with carcass LMA. Furthermore, most dimension parameters had rather weak correlations with carcass LMA. Previous research has demonstrated that significant variation in size, eating quality, and other muscle characteristics can and do exist within individual muscles of a beef carcass, specifically the LM (Reuter et al., 2002, Sweeter et al., 2005; Bass et al., 2008).

Meat merchandisers and foodservice chefs across the nation were surveyed to assess relationships between LMA and retail portion size acceptability of the 9 remaining individual muscles (IN, LFT, DP, RBF, RLM, SLLD, GM, TBL, ST) for which portion

characteristics were related to carcass LMA ($P < 0.05$). The percentage of "Yes I merchandise" responses for each subprimal cut evaluated in the survey by foodservice chefs and retail merchandisers are reported in **Table 4.4**. Results from the survey were used to analyze the effects of user (chef or retail merchandiser) and LMA sizes on individual muscle portion size acceptability.

No significant main effects or interactions between user and LMA sizes were reported for muscles IN and LFT ($P > 0.05$; data not shown). No differences between different sizes of LMA were reported for probability of acceptance of portion sizes from muscles IN and LFT ($P > 0.05$; data not shown); this suggests that LMA size does not affect the probability of acceptance of the portion sizes generated from IN and LFT muscles.

Least squares means ± SEM for probability of acceptance of the retail portion sizes by foodservice chefs and retail meat merchandisers from muscles ST, DP, RBF, and TBL generated from carcasses with LMA sizes of 67.74, 83.87, 100.00, and 116.13 cm^2 are reported in **Figure 4.2,** along with predicted non-parametric fitted regression lines. Least squares means ± SEM for probability of acceptance of the retail portion sizes by foodservice chefs and retail meat merchandisers for RLD, SLLD, and GM generated from carcasses with LMA sizes of 67.74, 83.87, 100.00, and 116.13 cm^2 are reported in **Figure 4.2,** along with predicted non-parametric fitted regression lines.

According to Platter et al. (2005), a probability of 0.50 or greater is required to attain favorable odds that end-users will find a product acceptable. For a USDA premium-branded program with a minimum LMA requirement of 70.97 cm^2, 5 of the remaining 7 muscles examined in the survey (GM, RBF, SLLD, ST, and TBL) still

produced acceptable portion sizes (0.50 or greater probability of acceptance) for both chefs and merchandisers from carcasses with LMA that are outside the minimum specification (USDA, 2007a,b). Based on USDA branded carcass programs with maximum LMA sizes of 103.23 cm^2, muscles not being fully valued that would otherwise be considered acceptable (0.50 or greater probability of acceptance) by both chefs and retail merchandisers included DP, RLD, SLLD, and TBL (**Figures 4.2**). The muscles GM and ST would produce portion cuts that would be at or above the 0.50 probability of being acceptable from carcasses with LMA up to 109.68 cm^2 for retail merchandisers and foodservice chefs (**Figures 4.2**). The muscles ST and RBF had fitted prediction regression lines that demonstrated a trend of increasing probability of acceptance as the carcass LMA became smaller when marketed to retail merchandisers; a similar trend was observed with the RBF and GM when those muscles were marketed to foodservice chefs. The CHCOM, SM, PM, TFL, and VL portion sizes and acceptability were not related to carcass LMA size. There was a quadratic relationship between the acceptability of the portion sizes from the muscles DP, TBL, RLD, SLLD, and GM and the LMA of the carcasses when marketed to retail meat merchandisers. Similarly, there was a quadratic relationship observed between the acceptability of the portion sizes from muscles ST, DP, TBL, RLD, and SLLD and the LMA of the carcasses when marketed to foodservice chefs indicating a range of acceptable size for those cuts.

Although the 2005 NBQA (Smith et al., 2005) makes note that non-conforming ribeye area size (too large or too small) is of concern to the beef industry, the data from the current study's survey results demonstrate a wide range of ribeye area sizes that would produce acceptable portion sizes from many muscles within the beef carcass.

Furthermore, research conducted by Sweeter et al. (2005) observed no difference in consumer preference between ribeye steaks cut from carcasses with extremely small to extremely large LMA (61 cm^2 to 119 cm^2), concluding that LMA size was either not a factor or consumers existed for each size of LMA. Dunn et al. (2000) determined ideal carcass LMA to be between 77.4 and 96.6 cm^2 for foodservice, and stated that tenderness, cooking time, and consistency were optimized in steaks produced from animals falling within the aforementioned LMA range. Results from the current study would somewhat agree with the Dunn et al. (2000) findings; however, the highest points of the fitted quadratic-shaped regression lines of the current study tended to be located above the slightly larger carcass LMA's demonstrating either a new trend in larger portion size preference or simply that different muscles will result in different portion-size preferences. The Dunn et al. (2000) study included only the longissimus lumborum muscle.

Results of the current study demonstrate that beef carcass ribeye area does not accurately predict the size and dimensions, and therefore ultimately the portion-size, of the chuck complexus, semimembranosus, tensor fasciae latae, or vastus lateralis muscles. Natural variation, in addition to breed, sex, live animal management and maturity level of the animals (and subsequent carcass), may contribute to the lack of relationship between carcass ribeye area and other muscle size, portion size, and dimension parameters. Furthermore, the desirability of the remaining muscles in a beef carcass does not necessarily coincide with the size of the beef carcass ribeye. These results indicated that carcass ribeye area is not an accurate measurement when determining the ultimate value

of a beef carcass and should therefore not be a determinant to the value of other muscles in the carcass.

Table 4.1. Simple linear regression of dependent variables, muscle portion weights (g/1.27-cm steak) and muscle cross-sectional surface area (cm^2), and the independent variable, ribeye area.

	Portion Weights				Surface area			
Muscle	*P*	r	r^2	RMSE[1]	*P*	r	r^2	RMSE[1]
Biceps femoris	0.003	0.378	0.143	31.196	< 0.001	0.470	0.221	21.594
Complexus	0.138	0.194	0.038	27.268	0.725	0.047	0.002	15.253
Pectoralis profundus	0.101	0.214	0.046	36.548	0.036	0.272	0.074	15.125
Gluteus medius	0.006	0.351	0.123	55.211	< 0.001	0.461	0.213	28.289
Infraspinatus	0.024	0.294	0.086	22.102	0.106	0.213	0.045	9.272
Latissimus dorsi	0.002	0.403	0.162	26.688	0.431	0.106	0.011	12.157
Longissimus lumborum	0.045	0.260	0.068	30.842	< 0.001	0.558	0.311	11.000
Longissimus thoracis	< 0.001	0.487	0.237	25.031	< 0.001	0.515	0.265	10.206
Psoas major	0.312	0.133	0.018	20.318	0.089	0.222	0.049	9.766
Semimembranosus	0.074	0.234	0.055	42.179	0.155	0.186	0.035	26.504
Semitendinosus	0.004	0.369	0.136	19.909	0.055	0.249	0.062	16.795
Tensor fasciae latae	0.723	-0.047	0.002	38.857	0.523	0.084	0.007	19.157
Triceps brachii	0.189	0.172	0.030	43.702	0.037	0.272	0.074	23.054
Vastus lateralis	0.394	0.112	0.013	44.073	0.293	0.138	0.019	21.052

[1]Root mean squared error

Table 4.2. Simple descriptive statistical means (CV,%) of weight and dimensional characteristics for whole muscle, cross-sectional faces of the midpoint, and portion steaks of 14 beef muscles evaluated (n = 60).

Muscle	Whole Muscle Weight (kg)	Whole Muscle Height (cm)	Whole Muscle Width (cm)	Whole Muscle Length (cm)	Face Length (cm)	Face Width (cm)	Face Surface area (cm^2)	1.27-cm thick portion steak weight (g)
Biceps femoris	6.01 (14)	10.80 (9)	26.33 (8)	51.56 (12)	19.92 (12)	10.51 (12)	172.29 (14)	254.32 (13)
Complexus	1.09 (32)	4.32 (24)	16.40 (16)	32.88 (14)	12.15 (23)	2.74 (27)	34.06 (40)	58.06 (47)
Pectoralis profundus	2.45 (19)	4.10 (17)	30.25 (10)	36.93 (15)	25.03 (15)	3.39 (18)	74.43 (21)	110.37 (34)
Gluteus medius	3.24 (14)	8.04 (13)	26.32 (6)	22.96 (12)	25.71 (8)	7.67 (14)	218.76 (14)	270.49 (22)
Infraspinatus	2.06 (13)	6.52 (10)	14.16 (8)	37.57 (11)	13.67 (8)	5.41 (13)	71.92 (13)	104.79 (22)
Latissimus dorsi	1.64 (16)	3.93 (16)	30.25 (9)	33.00 (14)	25.32 (12)	2.73 (22)	58.04 (21)	86.26 (34)
Longissimus lumborum	5.54 (14)	8.83 (10)	21.49 (10)	42.41 (111)	19.07 (8)	7.63 (12)	134.32 (15)	190.06 (17)
Longissimus thoracis	2.92 (15)	6.71 (11)	16.41 (8)	39.45 (11)	14.41 (7)	6.07 (12)	85.86 (14)	138.95 (20)
Psoas major	2.74 (13)	6.14 (10)	16.34 (9)	60.32 (11)	13.16 (12)	5.76 (14)	65.66 (15)	106.22 (19)
Semimembranosus	4.88 (14)	11.43 (10)	21.67 (10)	35.22 (12)	19.88 (11)	11.06 (10)	189.68 (14)	274.62 (16)
Semitendinosus	2.56 (16)	9.33 (10)	12.97 (9)	37.50 (11)	12.30 (8)	7.63 (14)	82.47 (13)	130.89 (16)
Tensor fasciae latae	1.18 (17)	4.87 (16)	23.15 (15)	23.73 (22)	20.82 (14)	4.44 (18)	89.04 (20)	121.56 (32)
Triceps brachii	2.00 (29)	6.33 (14)	18.99 (16)	29.44 (18)	18.83 (18)	6.00 (12)	102.36 (23)	159.06 (28)
Vastus lateralis	1.81 (15)	7.47 (10)	20.15 (12)	25.19 (12)	18.56 (11)	6.70 (14)	122.26 (17)	173.88 (25)

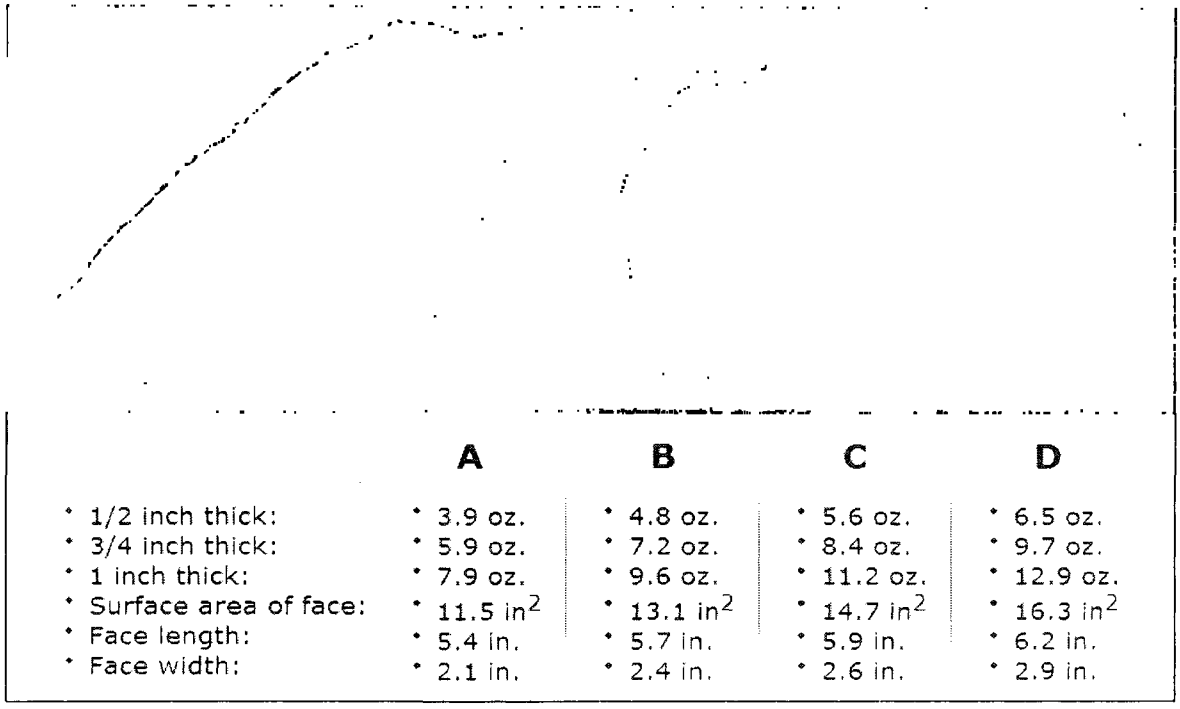
Table 4.3. Simple correlation coefficients and levels of significance of muscle dimension characteristics with ribeye area

Muscle	Whole Muscle Max Height	*P*	Whole Muscle Max Width	*P*	Whole Muscle Max Length	*P*	Face Length[1]	*P*	Face Width[1]	*P*
Biceps femoris	0.366	0.004	0.151	0.253	0.340	0.008	0.433	0.001	0.279	0.031
Complexus	0.048	0.718	0.079	0.550	0.012	0.927	0.138	0.293	0.136	0.305
Pectoralis profundi	0.285	0.029	0.302	0.019	0.019	0.884	0.191	0.152	0.122	0.363
Gluteus medius	0.479	< 0.001	0.285	0.027	0.154	0.245	0.271	0.036	0.493	< 0.001
Infraspinatus	0.459	< 0.001	0.299	0.021	- 0.002	0.988	0.094	0.477	0.198	0.130
Latissimus dorsi	0.425	< 0.001	- 0.057	0.669	0.028	0.831	- 0.032	0.811	0.211	0.108
Longissimus lumborum	0.230	0.021	0.250	0.054	0.015	0.909	0.214	0.103	0.143	0.274
Longissimus thoracis	0.573	< 0.001	0.370	0.004	0.006	0.963	0.376	0.003	0.537	< 0.001
Psoas major	0.226	0.089	0.112	0.397	- 0.070	0.598	0.059	0.661	0.156	0.239
Semimembranosus	0.270	0.037	0.386	0.003	0.285	0.028	0.232	0.075	0.250	0.054
Semitendinosus	0.223	0.090	0.427	< 0.001	0.155	0.238	0.311	0.016	0.222	0.089
Tensor fasciae latae	0.147	0.261	0.114	0.386	- 0.114	0.387	- 0.139	0.292	0.303	0.020
Triceps brachii	0.307	0.018	0.263	0.044	- 0.027	0.827	0.069	0.601	0.264	0.044
Vastus lateralis	0.218	0.094	0.032	0.807	- 0.086	0.515	- 0.066	0.616	0.055	0.674

[1] Dimensions of the cross-sectional surface cut from the longitudinal midpoint of the respective muscle.

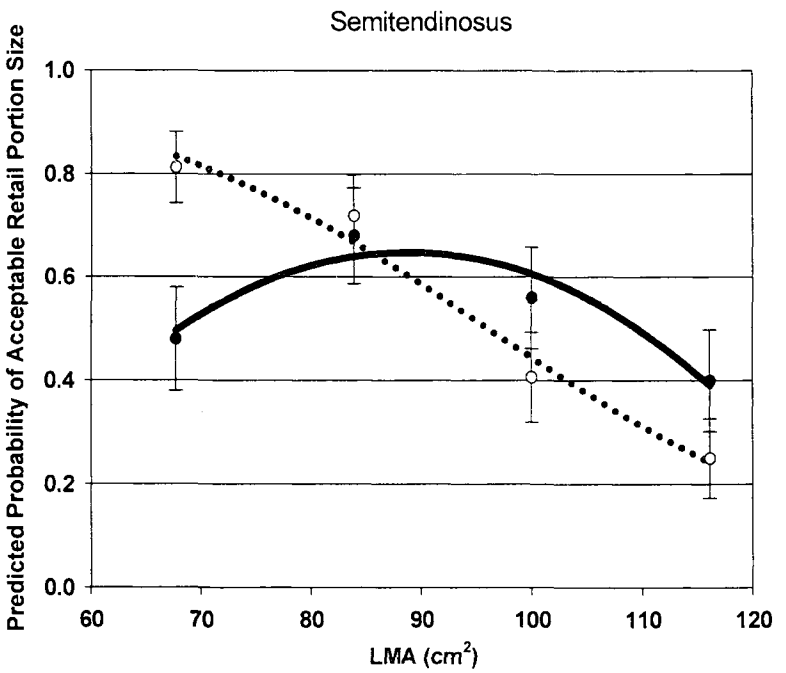
Table 4.4. Percentage of "Yes" responses when asked, "Do you merchandise the following beef cuts," for beef muscles evaluated by foodservice chefs and retail meat merchandisers who completed a national Internet-based survey.

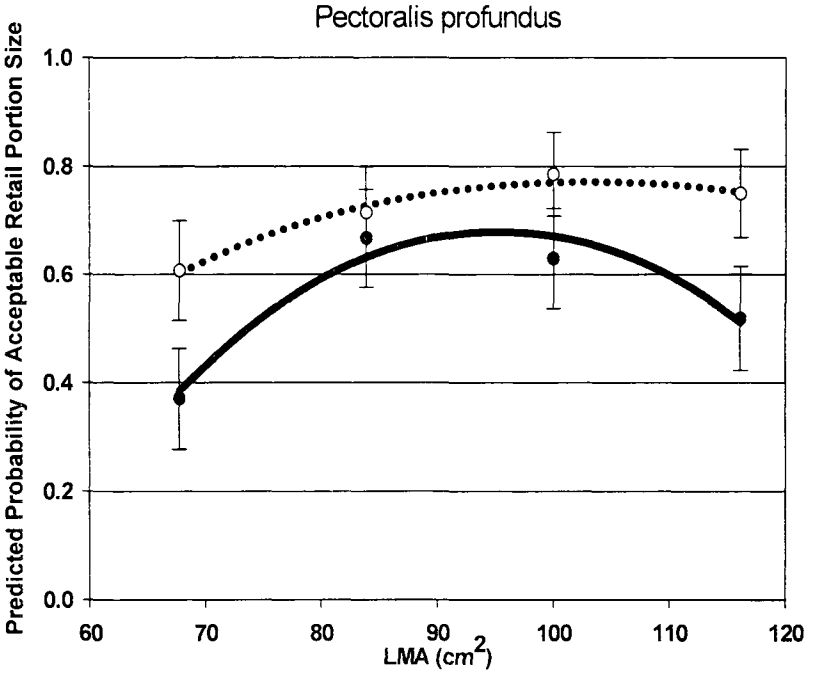
Subprimal	Chef (n = 33)	Merchandiser (n = 34)
Bottom Round	73%	97%
Eye of Round	76%	94%
Top Sirloin	97%	97%
Strip Loin	97%	97%
Lifter Meat	39%	35%
Ribeye	97%	100%
Chuck Clod	64%	91%
Top Blade	55%	79%
Brisket Flat	82%	82%



	A	B	C	D
• 1/2 inch thick:	• 3.9 oz.	• 4.8 oz.	• 5.6 oz.	• 6.5 oz.
• 3/4 inch thick:	• 5.9 oz.	• 7.2 oz.	• 8.4 oz.	• 9.7 oz.
• 1 inch thick:	• 7.9 oz.	• 9.6 oz.	• 11.2 oz.	• 12.9 oz.
• Surface area of face:	• 11.5 in^2	• 13.1 in^2	• 14.7 in^2	• 16.3 in^2
• Face length:	• 5.4 in.	• 5.7 in.	• 5.9 in.	• 6.2 in.
• Face width:	• 2.1 in.	• 2.4 in.	• 2.6 in.	• 2.9 in.

Figure 4.1. Example of a web page used during a nationwide Internet-based survey to evaluate portion-size characteristics of 9 beef muscles. Participants evaluated the portion-size characteristics below the letters in the figures to determine which portions (identified by letters) would be considered acceptable or unacceptable to their customer base in relation to portion-size. Portion-size characteristics were displayed in English system units which were much more familiar to the participants than were metric units of measure. The letters corresponded to carcass LM areas. The data received from the survey were used to evaluate muscles in relation to carcass LM areas; participants were unaware of the carcass LM areas that corresponded to the letters. The image of the muscle in the figure was to aid the participant in orienting themselves with what the muscle looked like and by no means was the image to be evaluated by the participant.

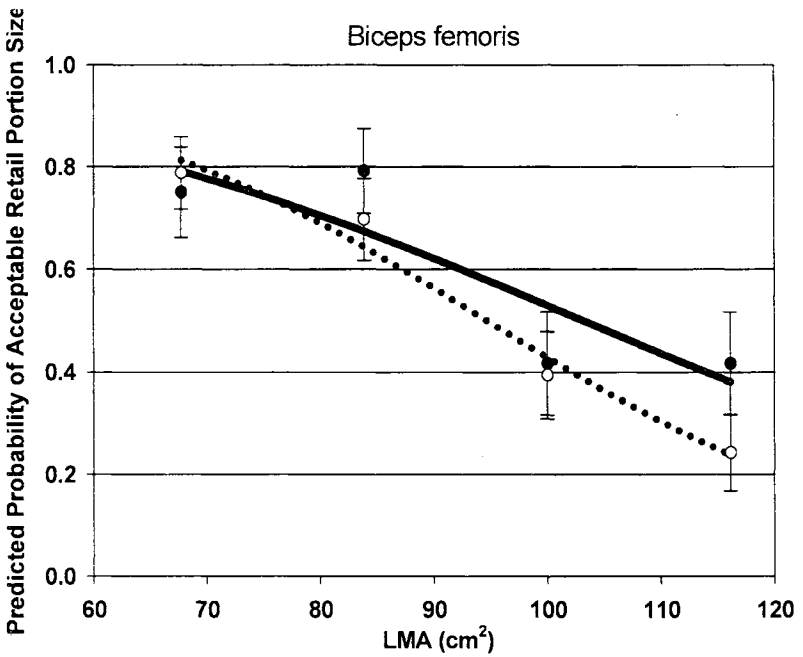


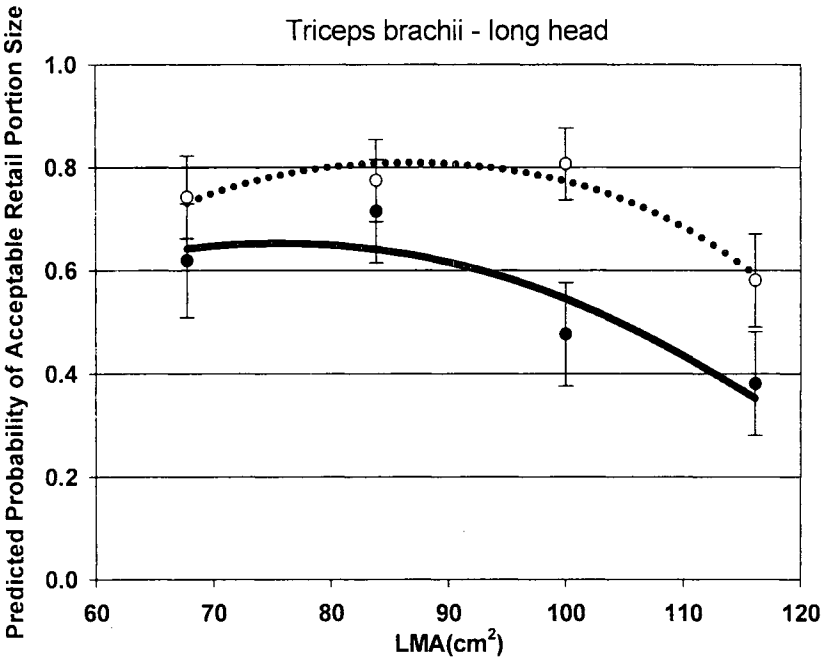


Characteristic	A	B	C	D
2.54 cm thick steak (g)	116.2	127.6	141.7	155.9
1.91 cm thick steak (g)	172.9	192.8	212.6	232.5
1.27 cm thick steak (g)	229.6	258.0	283.5	311.8
Surface area (cm^2)	75.5	83.2	90.3	98.1
Face Length (cm)	11.7	12.2	12.7	13.2
Face Width (cm)	7.1	7.6	7.9	8.4

A	B	C	D
93.6	107.7	121.9	136.1
141.7	161.6	181.4	204.1
189.9	215.5	243.8	269.3
65.8	72.9	80.6	87.7
23.9	24.9	25.9	26.9
3.3	3.3	3.6	3.6

Figure 4.2.

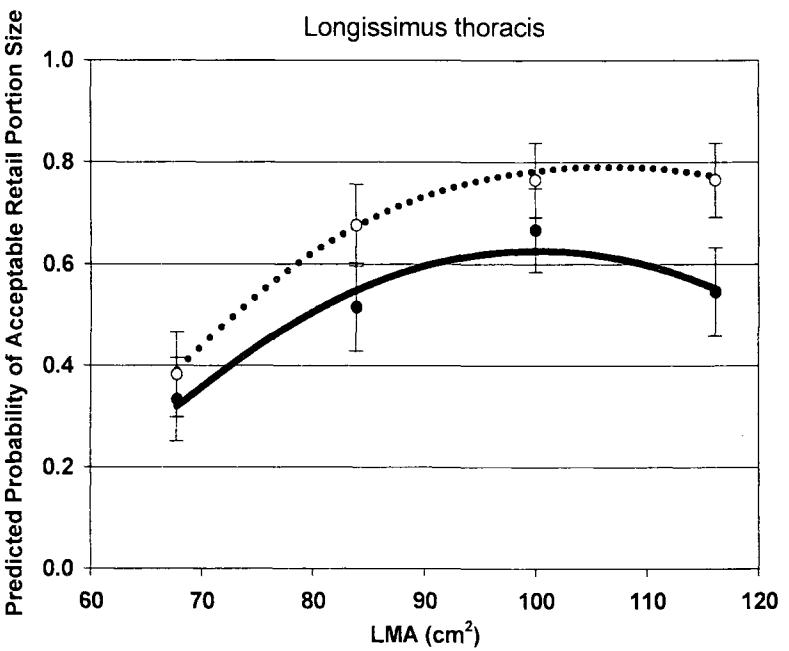


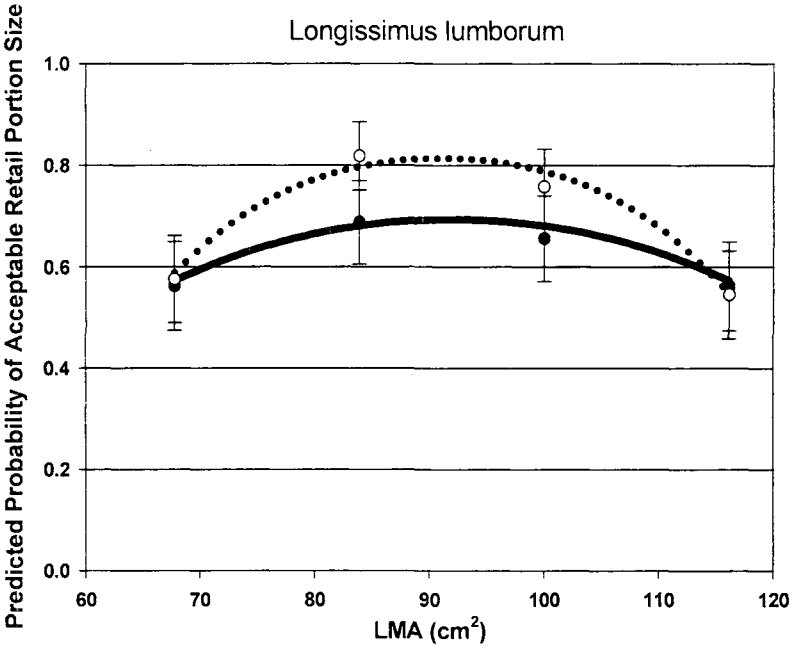


Characteristic	A	B	C	D
2.54 cm thick steak (g)	229.6	249.5	272.2	294.8
1.91 cm thick steak (g)	343.0	377.0	408.2	442.3
1.27 cm thick steak (g)	459.3	501.8	544.3	586.8
Surface area (cm^2)	149.7	169.0	188.4	208.4
Face Length (cm)	17.8	19.6	21.3	23.1
Face Width (cm)	9.9	10.4	10.9	11.7

A	B	C	D
144.6	155.9	170.1	181.4
215.5	235.3	255.1	275.0
289.2	314.7	340.2	365.7
89.0	100.6	111.6	122.6
18.3	18.8	19.0	19.6
5.6	5.8	6.3	6.6

Figure 4.2 continued.

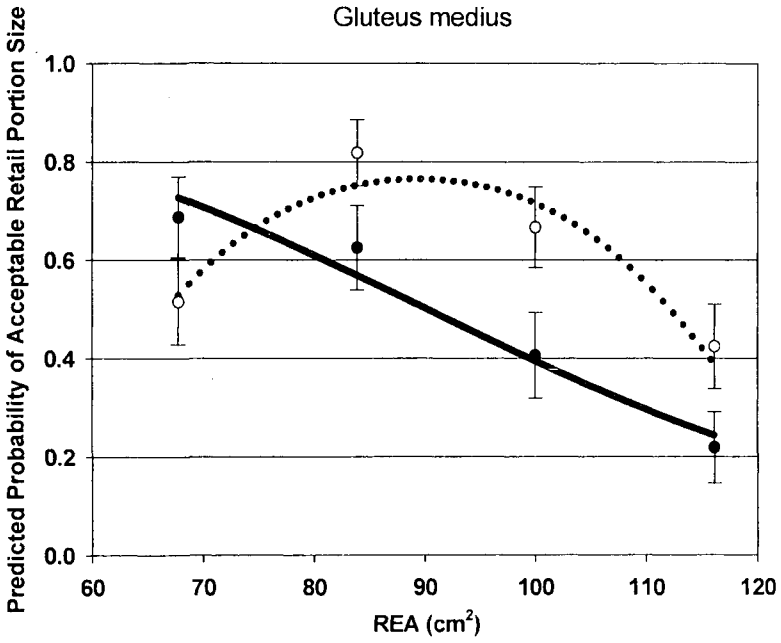




Characteristic	A	B	C	D
2.54 cm thick steak (g)	110.6	136.1	158.8	181.4
1.91 cm thick steak (g)	167.3	201.3	238.1	275.0
1.27 cm thick steak (g)	221.1	269.3	317.5	365.7
Surface area (cm^2)	73.5	83.9	94.8	105.2
Face Length (cm)	13.7	14.2	15.0	15.7
Face Width (cm)	5.3	5.8	6.6	7.4

A	B	C	D
172.9	187.1	201.3	215.5
260.8	280.7	303.3	323.2
345.9	374.2	402.6	430.9
77.4	89.7	102.6	114.8
18.3	19.0	19.6	20.1
7.4	7.6	7.9	8.1

Figure 4.2continued.



Characteristic	A	B	C	D
2.54 cm thick steak (g)	229.6	263.7	300.5	334.5
1.91 cm thick steak (g)	343.0	396.9	450.8	501.8
1.27 cm thick steak (g)	459.3	530.1	601.0	669.0
Surface area (cm^2)	189.0	214.2	239.4	263.9
Face Length (cm)	24.6	25.7	26.4	27.4
Face Width (cm)	6.6	7.6	8.4	9.4

Figure 4.2 continued.

Figure 4.2. Least squares means ± SEM and predicted acceptable probability regression lines for the carcass ribeye area at the 12th and 13th rib interface (LMA) chosen to be acceptable for retail meat merchandisers (○) and foodservice chefs (●) based on muscle portion-size characteristics. Predicted acceptable probability regression line for retail meat merchandisers (• • •) and for foodservice chefs (▬) are based on the non-parametric regression equation of $[P_i = \exp(\beta_o + \beta_i x_i) / 1 + \exp(\beta_o + \beta_i x_i)]$. Data points representative of retail portion cuts from the respective muscle, at the midpoint of the longitudinal axis of the respective muscle, from a carcass with the corresponding LMA. The carcass LMA represented by the letters were: A = 67.74 cm^2, B = 83.87 cm^2, C = 100.00 cm^2, and D = 116.13 cm^2. Portion-size characteristics of each muscle are listed immediately below each figure and correspond with the data points located directly above each letter representing the portion-size characteristics. Fit statistics of χ^2/degrees of freedom (values closer to 1.00 indicate a closer fit) for the predicted lines of retail meat merchandisers were 1.02, 1.07, 1.02, 1.03, 1.02, 1.01, and 1.02 for the muscles semitendinosus, pectoralis profundi, biceps femoris, triceps brachii – long head, longissimus thoracis, longissimus lumborum, and gluteus medius, respectively. Fit statistics of χ^2/degrees of freedom for the predicted lines of foodservice chefs were 1.03, 1.07, 1.02, 1.04, 1.02, 1.01, and 1.02 for the muscles semitendinosus, pectoralis profundi, biceps femoris, triceps brachii – long head, longissimus thoracis, longissimus lumborum, and gluteus medius, respectively.

CHAPTER V

Attenuating stress induced quality defects using magnesium oxide feed supplementation

INTRODUCTION

It is well documented that exposure of cattle to psychological or physical stressors before slaughter increases the incidence of meat quality defects (Ferguson and Warner, 2008). Stress-induced quality defects have been linked to activation of the sympatho-adrenal (SA) system. When cattle are exposed to adverse stimuli, SA activation causes release of catecholamines (epinephrine and norepinephrine) into the circulatory system, causing a number of physiological responses including increased heart and respiration rates, elevated body temperature, decreased protein degradation, and increased hepatic and muscle glycogenolysis (Ferguson et al, 2001; Knowles and Warris, 2007). Catecholamine-induced depletion of muscle glycogen stores have been implicated as the primary cause of high-pH, dark firm and dry muscle characteristics (Apple et al., 2005). In addition, elevation of plasma epinephrine concentration has been shown to increase calpastatin activity in muscle (Sensky, 1996), which may lead to reduced postmortem tenderization and increased meat toughness (Warner et al., 2007). The inability to completely avoid stress during pre-slaughter shipment of livestock has led to the investigation of strategies for lessening the effects of stress on glycogen depletion and meat tenderness.

Research conducted using swine and sheep suggests that dietary magnesium (Mg) supplementation may counteract some of the effects of stress on meat quality, by reducing catecholamine secretion (D'Souza et al., 1998; Dunshea et al., 2005) and decreasing muscle glycogen loss (Gardner et al., 2001). Dietary Mg supplementation of cattle to prevent beef quality defects, to our knowledge, has not been investigated. Therefore, the objective of this study was to evaluate the effectiveness of short-term dietary magnesium oxide supplementation of finishing steers and heifers for attenuating the effects of acute pre-harvest stress on beef quality characteristics.

MATERIALS AND METHODS

The experiment was conducted at the Colorado State University Eastern Colorado Research Center (CSU-ECRC) and the Colorado State University Agricultural Research Development and Education Center (CSU-ARDEC) by trained personnel using procedures approved by the Institutional Animal Care and Use Committee at Colorado State University.

Animals and Experimental Design

The experimental sample consisted of spring-born (calved March through May, 2007) male and female calves (herd-contemporaries) produced by mating British crossbred cows to Charolais (n=2) or SimAngus (n=1) bulls in a terminal crossbreeding system at the CSU-ECRC near Akron, CO. After weaning (September 2007), steer and heifer calves were implanted (Revalor-IS and Revalor-IH, respectively; Intervet, Inc., Millsboro, DE), placed in the CSU-ECRC feedlot, and fed conventional, corn-based finishing diets. All cattle were re-implanted (Revalor-IS, Revalor-IH; Intervet, Inc., Millsboro, DE) on December 6, 2007. On April 23, 2008, when the cattle were 12 to 14 mo old (approximately 4 to 6 wk before their

projected marketing dates), 72 steers and 72 heifers were selected for use in the study and transported to the CSU-ARDEC research feedlot near Fort Collins, CO. Individual BW were recorded shortly after arrival at the CSU-ARDEC feedlot. At receiving, BW for steers ranged from 491 to 688 kg, whereas BW for heifers ranged from 405 to 602 kg. Animals representing each sex class then were sorted into sire groups, blocked by BW (light, medium, and heavy), and assigned randomly to 4 treatment groups on an as-fed basis: A) Control, 0% added dietary Mg, B) 0.25% added dietary Mg, C) 0.50% added dietary Mg, and D) 0.75% added dietary Mg. Sires were represented equally among treatments. Cattle representing each sex × block × treatment subclass were penned together for the experiment, resulting in 24 pens with each pen consisting of 6 animals (**Table 5.1**). One animal died in the pen due to bloat prior to the termination of the trial.

Diets. On the day of arrival at the ARDEC feedlot, cattle were fed alfalfa hay (1 kg/hd) and allowed unlimited access to fresh water. Once the hay had been completely consumed, cattle were placed on a transitional diet for 3 d at a daily rate of 8.0, 9.5, and 10.6 kg/animal on d 1, 2, and 3, respectively (**Table 5.2**). Following the 3-d transitional period, cattle were provided ad libitum access to a conventional, high concentrate finishing diet (**Table 5.2**) for the remainder of the trial.

Dietary treatments were initiated on 29-April (heavy block), 4-May (medium block), and 12-May (light block). Dietary Mg was provided in the form of magnesium oxide (MgO) for the final 14-d of the finishing period. During each 14-d MgO supplementation period, feed was dispensed once daily from a feed mixer truck. Supplemental MgO was incorporated into the diet using a ground corn carrier, which was hand mixed into each pen's

daily feed issue. Ground corn supplements, prepared separately for each of the 4 treatment groups using a small ribbon mixer (Dough Mixing Machine, Model-FS.28.8 RPM, Champion Machinery Co., Joliet, IL), were formulated to provide 0%, 0.25%, 0.50%, or 0.75% additional Mg (expressed as % of total mixed ration) when included in the diet at a daily rate of 0.23 kg supplement/animal. Results of lab analyses of ingredients were used to adjust supplement inclusion rates to make certain that experimental diets contained the specified levels of supplemental Mg. On d 1 of the supplementation period, cattle were weighed prior to feed issue (on-test BW) and returned to their respective pens. Daily feed consumption of each pen was recorded during the 14-d experimental feeding period and refusals were collected and weighed at the end of the test period.

Pen Behavior Assessment. On d 13 of the trial, 2 experienced evaluators briefly walked through each pen (40 m × 6.1 m) of cattle independently assessing each animal's behavior and assigning scores for pen behavior using a 15-cm semi-structured line scale (Gruber et al., 2006b). The line scale was divided into 5 equally-sized sections representing behaviors described as: 1) calm – docile, undisturbed, calm, with a small flight zone; 2) restless – quiet, not easily disturbed, slightly restless; 3) nervous – nervous, easily disturbed, large flight zone; 4) flighty – fearful, easily excited or agitated, moves to furthest point in pen; and 5) aggressive – very fearful, easily excited or agitated, exhibiting aggressive behavior. The 2 evaluators' scores were averaged to obtain a single pen behavior score for each animal.

Off-Test Measurements. Cattle were weighed individually (off-test BW) on 13-May (heavy block), 18-May (medium block), and 26-May (light block) at the conclusion of each

test period. After final weights were recorded, animals comprising the entire block (steers and heifers from all 4 treatment groups) were comingled in a large pen to induce a physiological stress (Ferguson, 2001). After 8 h, each animal was restrained in a hydraulic squeeze chute (Commercial Pro Model Silencer, Moly Manufacturing, Inc., Lorraine, KS) and blood samples were obtained via jugular venipuntcure using a 20 gauge needle (10 mL of blood was collected in a lavender top BD Hemogard sterile plastic Vacutainer tube, Fisher Scientific Reference No. 366643, containing spray-dried K_2EDTA for plasma catecholamine analysis and 6 mL of blood was collected with the same 20 gauge needle in a royal blue top BD Hemogard sterile plastic Trace Mineral Vacutainer tube, Fisher Scientific Reference No. 368380, containing clot activator for serum Mg analysis). Following collection, blood tubes immediately were placed on ice.

While in the hydraulic chute, cattle were scored for chute behavior, following application of light pressure to the animal using the squeeze feature of the chute, using a 150-mm semi-structured line scale, similar to the scale used for pen behavior (descriptors for flight zone were not considered in chute scoring). Also, while in the chute, rectal temperature, heart rate, and respiration rate were recorded for each animal. Upon release from the hydraulic squeeze chute, cattle were subjectively scored based on their exit velocity using a 4 point scale (1 = walk, 2 = trot, 3 = moderate run, 4 = fast run). After blood samples had been collected and chute scores had been assigned, the cattle were once again comingled in a single, large holding pen.

Within 30 min of collection, plasma samples were centrifuged at 2,500 x *g* for 25 min and plasma subsequently was harvested using sterile, single-use 3 mL transfer pipettes.

Serum samples were allowed to clot and subsequently centrifuged at 2,500 x *g* for 25 min; serum was removed using sterile 3-mL transfer pipettes. Plasma and serum samples were frozen and stored at -80°C until further analysis.

Blood analyses

Blood serum and plasma samples were transported on dry ice to Warren Analytical Laboratories in Greeley, Colorado where serum magnesium levels were measured using atomic absorption spectroscopy (Willis, 1959) and plasma catecholamines (epinephrine, norepinephrine, and dopamine) were measured using a 3-CAT ELISA kit (Labor Diognostika Nord GmbH & Co. KG, Nordhorn, Germany). Blood plasma catecholamine levels were reported in ng/dL. Because of the cost related to the blood serum magnesium assays, a subsample of 3 animals from each pen were analyzed; blood serum magnesium levels were reported in mg/dL.

Slaughter Measurements and Carcass Data Collection

The morning after the cattle were mixed and off-test parameters were assessed, cattle were loaded onto trucks and transported 72 km to a commercial harvest facility. Cattle in the heavy, medium, and light blocks were slaughtered on 14-May, 19-May, and 27-May, respectively. Cattle were humanely harvested using modern commercial slaughter methods. Individual animal and carcass identification were maintained throughout the harvest process.

Immediately after exsanguination, blood from each animal was collected in a clean 88 mL disposable paper cup and the collected blood was poured into a 10 mL Monoject glass blood collection tube (Fisher Science Reference No. 352788) containing powdered Potassium Oxalate and Sodium Flouride. Blood tubes were placed on ice until the samples

could be measured to determine blood lactate levels. Blood lactate levels were measured within 1 h post collection using the Lactate Scout lactate meter and Lactate Scout Test Strips (Sports Resource Group, Inc., Hawthorn, NY); lactate was recorded in mM/L.

At approximately 30 min postmortem, prior to electrical stimulation, a tissue sample (3 ± 1 g) was excised from the Longissimus lumborum from between the second and third lumbar vertebrae. Muscle tissue samples were placed in sequentially numbered 532 mL Whirl-Pak bags. Numbers on the bags corresponded with the identification number of the carcass from which the tissue sample was excised. Within 15 min, the tissue samples were wrapped in wax paper, wrapped again in aluminum foil, labeled with the corresponding carcass number, and snap frozen in liquid nitrogen. Muscle tissue samples were transported on dry ice to Colorado State University where they were stored at -80 °C for measurement of glycolytic potential at a later date.

Prior to chilling, carcasses were electrically stimulated by traveling through four zones (17 s each zone) of 60 Hz of electricity with varying voltage levels: 25, 25, 45, and 55 V (1 s on, 1 s off). Carcasses were spray chilled (2 ± 1 °C) for 8 h and then air chilled (2 ± 1 °C) for 16 ± 1 h. After 24 ± 1 h postmortem chilling, carcasses were transferred from the chill cooler to the sales cooler where they were ribbed at the 12^{th} and 13^{th} ribs and allowed to bloom for 20 min. Following the 20 min bloom time, ribeye color was evaluated objectively using a portable spectrophotometer with a 6 mm diameter port size, a D-65 illuminant, and calibrated using a black and a white tile (Miniscan XE Model 45/0-L, Hunter Laboratories, Reston, VA). Final color values were reported as the mean of 3 color measurements and were expressed in the form of L*, a* and b* (black to white, green to red, and blue to yellow,

respectively). During the final kill period there was a malfunction with the portable spectrophotometer and the color values from that kill group were deemed unusable and removed from the analyses. Carcass maturity (skeletal and lean), marbling score, and the presence and severity of dark cutting beef characteristics were evaluated by official USDA graders. Experienced Colorado State University beef carcass evaluators also evaluated the carcasses and recorded values for estimated % KPH, preliminary yield grade (measured and adjusted), ribeye area, and recorded HCW.

Preparation and Collection of Meat Sample. Each carcass was fabricated and the striploin (NAMP # 180) from the right side of each carcass was obtained and transported under refrigeration (2 ± 1 °C) to the Colorado State University Meat Laboratory. Upon arrival at the Colorado State University Meat Laboratory, striploins were placed in refrigeration (2 ± 1 °C) until the following morning.

Within 48 h postmortem, each striploin was fabricated into 5.08-cm thick sections and the resulting LM sections were then randomly assigned to 1 of 5 aging periods (3, 7, 14, 21, and 28 d), vacuum packaged, and aged for the appropriate designated period of time. Additionally, during striploin fabrication, a small sample of lean tissue was excised (approximately 10 g) and ultimate pH was determined using the procedures outlined in Bass et al. (2008). After aging, LM samples were frozen and stored at -20 °C, and thereafter, fabricated into 2.54-cm thick steaks using a band saw (Model 400, AEW-Thurne, AEW Engineering Co. Ltd., Norwich, UK) in a refrigerated fabrication room (4 °C). After fabrication, the 2.54-cm thick steaks were individually vacuum packaged and placed back

into frozen storage (-20 °C); steak samples remained frozen during the entire fabrication process and were never subjected to temperature abuse.

Warner-Bratzler Shear Force Determination

Frozen steaks were tempered for 36 h at 2 ± 1 °C prior to cooking. Steaks were cooked on an electric conveyor belt grill (Model TBG-60, MagiGrill, MagiKitch'n Inc., Quakertown, PA) for a constant time of 6 min, 5 s at platen settings of 163 °C, to achieve a final internal target temperature of 71 °C. Peak internal temperatures were measured by inserting a Type K thermocouple (Model 39658-K, Atkins Technical, Gainesville, FL) into the geometric center of each cooked steak. Following cooking, steaks were allowed to equilibrate to room temperature (22 °C) and subsequently 6 to 10 cores (1.27-cm diameter) where removed parallel to the orientation of the muscle fiber. Each core was sheared once perpendicular to the orientation of the muscle fibers using an Instron testing machine (Model 4443, Instron, Corp, Canton, MA) fitted with a Warner-Bratzler shear head with a crosshead speed of 200 mm/min. Peak shear force measurements (kg of force) were recorded for each core and subsequently averaged resulting in a single shear force value for each steak.

Muscle Tissue Glucose and Lactate Determination

Frozen LM tissue samples, recovered 30 min postmortem, were tempered for 24 h at 2 ± 1 °C prior to homogenization. The tissue samples were prepared for glycolytic potential analysis using the methods outlined by McKeith et al. (1998). Briefly, a 1 g sample of tissue was weighed and homogenized (Ultra-Turrax T25 Basic) in a solution of perchloric acid to deproteinate the muscle tissue and extract the pertinent metabolites. Subsequently, amyloglucosidase from *Aspergillus niger* was added to the sample to break down the

available glycogen molecules into free glucose. Following deproteination and glycogen breakdown, samples were centrifuged at 7,000 × *g* for 5 min at 4°C and the supernatant were collected for further analyses. Glucose and lactate were quantified using commercially available assay kits (Stanbio Glucose Liqui-UV Proc. No. 1060, Stanbio Laboratory, Boerne, TX and Lactate Assay Kit II Cat. No. K627-100, BioVision Research Products, Mountain View, CA, respectively). Glycolytic potential was calculated using the formula stated by Monin and Sellier (1985): glycolytic potential = 2[glucose] + [lactic acid].

Statistical Analyses

The α–level of the study was set at 0.05. Analyses of the live cattle performance data were conducted using pen means because it was not possible to evaluate individual animal feeding performance.

Subjective live cattle behavior evaluation scores, live animal vital parameters, carcass characteristics, LM color attributes, pH, and blood analyses were compared by sex and supplemental Mg treatment using analysis of variance (ANOVA) in a 2 × 4 factorial design. The ANOVA computations were performed using PROC MIXED of SAS (SAS Inst., 2004). The model included main effects for sex, supplemental Mg treatment, sex by supplemental Mg treatment interaction, sire, and a random effect for pen nested within supplemental Mg treatment.

Warner-Bratzler shear force was compared by sex, supplemental Mg treatment, and aging period using repeated measures and ANOVA in a 2 × 4 × 5 design where aging period was the repeated measure. The model included main effects for sex, supplemental Mg treatment, aging period, all relevant two and three way interaction of the main effects, sire,

shear day, and peak temperature (covariate). When no interaction was detected ($P > 0.05$) sex was compared, averaging over supplemental Mg treatment, and supplemental Mg treatments were compared, averaging over sex. Mean separations were performed by pairwise t-test comparisons of least squares means using the PDIFF option at a significance level of $P < 0.05$. Assumptions of ANOVA were evaluated using a combination of residual plots and histograms.

Simple correlations among independent traits were calculated using PROC CORR (SAS Inst., 2004). Prevalence of dark-cutting carcasses between sexes and between supplemental Mg treatments were compared using PROC FREQ (SAS Inst., 2004) with the Fisher's exact test option due to some of the chi-square cells having numbers of observations lower than 5.

RESULTS AND DISCUSSION

Simple descriptive statistics, where animal is the experimental unit, characterizing behavioral and physiological stress responses are displayed in **Table 5.3**. Scores used to identify behavioral reactions of individual cattle to a) human presence in pen (pen behavior) and b) physical handling and chute restraint (chute behavior) indicated that most cattle (about 65% of the sample) remained calm during these events, whereas about 35% of cattle showed some visible evidence of stressful behavior (**Figure 1**). Steers and heifers exhibited similar ($P > 0.05$) behavioral reactions when scored for chute behavior (**Table 5.6**); however, heifers were more reactive ($P < 0.001$) than steers when confronted with human presence in the pen (mean pen behavior scores: heifer = 33.23, steer = 20.05).

Correlations among behavior scores (pen and chute) and various physiological stress indicators and meat quality traits, calculated within sex class, are shown in **Table 5.4**. For heifers, pen and chute scores were positively correlated with WBSF (r = 0.43 and 0.25, respectively); indicating that as heifers became more reactive (more nervous) beef tenderness decreased. Correlations for steers between chute score and WBSF were similar (r = 0.31), but pen score for steers was not ($P > 0.05$) correlated with WBSF. Previous research has demonstrated that more excitable animals have a tendency to produce LM steaks with higher shear force values (Voisinet et al, 1997a; King et al, 2006). Both heifers and steers had positive correlations among pen behavior score and blood plasma norepinephrine concentrations (**Table 5.4**). Both heifers and steers had positive correlations among chute behavior score and blood plasma epinephrine concentrations (**Table 5.4**). Positive correlations between behavioral scores and measured catecholamine concentrations indicated that increased physical stress response is associated with increased physiological stress indicators (epinephrine and norepinephrine) in the body as confirmed by previous research (Hossner, 2005). Blood plasma epinephrine concentration was moderately correlated (r = 0.40) with WBSF in heifers, but no correlations between blood plasma epinephrine and WBSF wasn detected ($P > 0.05$) in steers. Measures of WBSF from LM of steer carcasses were moderately correlated (r = 0.37) to muscle pH while only low correlations (r = 0.25) were detected between LM WBSF and pH in heifers. **Table 5.6** suggests that steer LM tended ($P < 0.10$) to have numerically lower mean glucose concentrations and mean glycolytic potential ($P = 0.093$ and 0.063, respectively) than heifers. Correlations between steer pH and WBSF, coupled with tendencies toward a drop in mean LM glucose and

glycolytic potential concentrations for steers versus heifers, indicated that steers may have exhibited a more acute stress response than heifers. Furthermore, although not measured, steers appeared to be more physically aggressive than the heifers when mixed in the common pen. The aggressive nature of the steers upon mixing indicated that the steers likely had a more acute stress event compared to the heifers.

Steer LM WBSF was negatively associated ($P < 0.05$) with measures of color (L*, a*, and b*). Alternatively, heifer LM WBSF marginally related (r = -0.29) to L* and not related to a* or b* (r = 0.01 and -0.08, respectively). Wulf et al (1997) observed a moderate negative correlation between LM WBSF and b* color values. The current study demonstrated that steer LM WBSF values were more highly correlated with color values than were heifer LM WBSF. This may have been due to the more acute stress response from the steers than heifers resulting from a greater usage of glycogen stores prior to harvest.

Of the behavioral stress indicators recorded in the current study, exit speed score seemed most effective for identifying individual differences in reactivity to stress. Data presented in **Table 5.7** showed that greater exit speed velocity was associated with increased ($P < 0.05$) plasma catecholamine concentrations and elevated ($P < 0.05$) rectal temperatures which were indicative of an acute adrenergic stress response (Shaw and Tume, 1992). In addition, greater exit speed was associated with increased LM WBSF (**Table 5.7**). These findings were consistent with those of previous studies (King et al., 2006, Burdick et al., 2008, Behrends et al., 2009) which have reported that the speed with which cattle exit a working chute (exit velocity) is associated with physiological symptoms of stress, as well as differences in cooked LM WBSF.

In unstressed cattle, with normal muscle glycogen reserves, muscle pH at 48 h postmortem normally ranges from 5.4 to 5.6, with a mean of approximately 5.5 (Young et al., 2004). Stress-induced muscle glycogen depletion before harvest results in muscle pH values greater than 5.6 and, in severe cases, produces dark-cutting lean characteristics (Immonen et al., 2000). Page et al. (2001) measured LM pH and muscle color characteristics of 1,000 beef carcasses selected to be representative of the U.S. population of grain-fed steers and heifers and reported a range in LM pH of 5.2 to 6.9 (mean = 5.5), together with a dark-cutting incidence of 2.6%. In the Page et al. (2001) study, more than 80% of carcasses had LM pH values of 5.4 to 5.59 (considered normal), 10% had LM pH values of 5.6 or greater, and 8% had pH values less than 5.4. In addition, most of the dark cutters in the Page et al. (2001) study had LM pH values greater than 5.87. In the current study, pre-harvest mixing of cattle, prior to handling and transport, produced a range in 48 h postmortem LM pH of 5.3 to 6.1 (mean = 5.5) and resulted in 8 carcasses (5.6% of the experimental sample) that were classified as dark cutters by USDA graders. The 5.6% dark-cutting incidence observed in the current study was twice as great as the dark-cutting incidence reported by Page et al. (2001) and nearly 3 times higher than the incidence of dark cutters (1.9%) recorded in the 2005 National Beef Quality Audit (Garcia et al., 2008). Percentages of carcasses in the current study with LM pH values of less than 5.4, 5.4 to 5.59, and $\geq$ 5.6 were 13%, 67%, and 20% respectively. Of the carcasses with 48 h LM pH values less than 5.4, all but 2 carcasses had final LM pH values that were within 0.05 pH units of 5.4, which is considered to be the lower limit for ultimate muscle pH under normal conditions (Immonen and Puolanne, 2000; Young et al., 2004). Carcasses classified as dark cutters in the current study had a mean LM

pH of 5.78, which is similar to the mean LM pH (5.76) reported by Bass et al. (2008) for carcasses classified by USDA graders as 1/2 degree dark cutters.

In the current study, of the 8 carcasses classified by USDA graders as dark cutters, 6 were from steers and 2 were from heifers. All of the supplemental Mg treatments contained at least one dark-cutting carcass; 0.00, 0.25, 0.50, and 0.75% supplemental Mg treatments had 3, 1, 2, and 2 dark cutting carcasses, respectively. The control treatment generated the greatest number of dark cutting carcasses, but there was no effect of Mg observed nor was there a sex effect observed ($P = 0.958$ and $P = 0.166$, respectively) based on the results from Fisher's exact test analyses. Therefore, the supplementation with dietary Mg was not enough to prevent the incidence of dark-cutting carcasses. However, numerically, the incidence of dark-cutting carcasses was lower among cattle that received supplemental dietary Mg compared to the control group (data not shown).

Data showing serum concentrations of Mg are summarized in **Figure 5.2**. Dietary supplementation of cattle with MgO produced a linear increase ($P = 0.002$) in serum concentrations of Mg (**Figure 5.2**). However, control cattle and cattle supplemented with 0.25% dietary Mg had nearly identical serum concentrations of Mg, suggesting that supplementation levels of 0.50% dietary Mg or higher were required to elicit an increase in circulating Mg concentrations (**Figure 5.2**). Control cattle and cattle supplemented with 0.25% dietary Mg (2.26 and 2.24 mg/dL, respectively) had lower concentrations of blood serum Mg than cattle supplemented with 0.75% dietary Mg (2.67 mg/dL; $P < 0.05$). Cattle supplemented with 0.50% dietary Mg did not have blood serum Mg concentrations that were different from any other Mg supplementation treatment (2.46 mg/dL; $P > 0.05$). Normal

cattle blood serum Mg concentrations have been found to be around 2.3 mg/dL (Lane et al., 1968) indicating that the control cattle and cattle fed the supplemental 0.25% Mg added diet were very close to the normal range of blood serum Mg concentration for cattle.

Data presented in **Table 5.5** show effects of sex and treatment on growth traits where pen is the experimental unit. Steers were heavier than heifers at the beginning and end of the trial and produced heavier carcasses. Performance for steers and heifers during the 14-d supplementation period (**Table 5.5**) did not differ ($P > 0.05$). Supplementation of cattle with MgO influenced ($P = 0.03$) DMI (**Table 5.5**); however, none of the supplemental groups (0.25%, 0.50%, or 0.75% Mg) differed ($P > 0.05$) from the control group with respect to daily intake. Cattle fed 0.25% Mg had greater mean daily intake during the 14-d supplementation period than cattle fed 0.75% Mg and no differences ($P = 0.728$) in ADG were observed among treatment groups (**Table 5.5**). Treatment had no effect ($P = 0.960$) on final BW (**Table 5.5**). These results suggest Mg may be fed at levels up to 0.75% of the total mixed ration for 14 d with no adverse effects on feedlot performance.

No sex × treatment interaction was observed ($P > 0.05$) for pen behavior score, chute behavior score, respiration rate, heart rate, rectal temperature, blood plasma epinephrine concentration, blood plasma norepinephrine concentration, blood lactate concentration, HCW, marbling score, muscle pH, muscle lactate concentration, muscle glucose concentration, muscle glycolytic potential concentration, L*, a*, b*, or WBSF. No significant sex or treatment main effects were observed ($P > 0.05$) for chute behavior score. A sex effect was observed for pen behavior score; heifers had increased ($P < 0.001$) pen scores compared to steers (**Table 5.6**). Previous research has confirmed that steers tend to be calmer than heifers

(Voisinet et al., 1997b; Wulf et al., 1997). A treatment effect also was observed ($P = 0.010$) among pen scores (**Table 5.6**). The control cattle treatment group generated the lowest pen behavior scores, while cattle fed 0.50% supplemental Mg diet generated the highest pen behavior scores (18.11 and 34.95, respectively; **Table 5.6**). Control cattle differed in behavior score compared to the cattle fed the 0.50% supplemental Mg diet ($P = 0.001$; **Table 5.6**), but no differences were observed ($P > 0.05$) between the cattle fed 0.25% or 0.75% added dietary Mg and any other treatment group (**Table 5.6**). It is unknown as to why the cattle fed the 0.50% added dietary Mg exhibited an increase in behavior scores compared to control cattle. It is difficult to determine if there was a behavioral change due to Mg treatment as no pre-treatment behavior scoring was conducted. It is therefore possible that the observed differences between treatment means were simply due to having a greater number of cattle with calmer dispositions randomly placed into the control treatment pens.

Sex had an effect on HCW ($P = 0.001$; **Table 5.6**). Previous studies have suggested overwhelming evidence that steers will result in greater HCW than heifers, given similar backgrounds and management schemes (Wulf et al., 1997; Choat et al., 2006; Boles et al., 2009). No treatment effect was observed ($P = 0.885$) between cattle HCW, further suggesting that Mg may be safely fed up to levels of 0.75% of the diet for 14 d with no adverse effects on carcass weight performance. Marbling score was effected by sex ($P = 0.006$) and heifers had a higher mean marbling score compared to steers. Previous research was confirmed that heifers deposit more marbling than steers (Wulf et al., 1997; Choat et al., 2006; Boles et al., 2009) at equal carcass composition. Regardless of the difference in

marbling score between steers and heifers observed in the current study, the category of degree of marbling (Slight) remained the same.

Steers generated an LM with increased ($P = 0.042$) ultimate pH compared to heifers (**Table 5.6**). The difference between steers and heifers was only 0.05 pH units, the magnitude of which was of no practical consequence. Additionally, the mean pH values presented in **Table 5.6** were well within the limits of what is considered normal for beef LM which is 5.40 to 5.60 (Lawrie, 1958; Tarrant and Mothersill, 1977; Zhang et al., 2005). Furthermore, no main effects were observed for the objective color evaluations of L*, a*, and b*, indicating that although a difference in ultimate LM pH was observed, it was not great enough to cause a change in the objective color scores. Objective color scores of L*, a*, and b* were found to be close to what many previous studies have found in normal beef (NCBA, 2000; Page et al., 2001).

No significant main effects (sex or treatment) were observed ($P > 0.05$) among the dependent variables of muscle glucose concentration, muscle lactate concentration, and muscle glycolytic potential from muscle tissue extracted 30 min postmortem (**Table 5.6**). Previous research has demonstrated similar results with regard to muscle glucose under normal early postmortem conditions (Kenny and Tarrant, 1988). The postmortem muscle glucose content observed in the current study helps to elucidate why no differences were observed between treatments among objective color values; if normal levels of glucose are available immediately post mortem, then normal muscle pH should be attained resulting in relatively normal muscle color. No main effects were observed ($P > 0.05$) for the dependent variables of circulating plasma epinephrine and norepinephrine concentrations. It was

interesting to find that, even though some subjective behavior scores were affected by sex or treatment, the circulating catecholamines were not observed to be affected by either sex or treatment.

No sex × treatment × aging period interaction ($P = 0.329$), no two-way interactions ($P > 0.05$) and no treatment effect was observed ($P = 0.881$) for LM WBSF. A sex effect was observed ($P = 0.013$) in which LM from steers had a greater mean WBSF than heifers. Some previous studies have demonstrated numerical increases in steer LM WBSF values when compared to heifers, however, none noted a significant difference ($P > 0.05$; Greathouse, 1985; Gruber et al., 2006b). Many studies demonstrate an increase in WBSF values in heifers rather than steers (Wulf et al., 1997; Choat et al., 2006; Boles et al., 2009). One possible reason for the decrease in WBSF of heifers when compared to steers may have been the slight increase in steer mean pH which has been demonstrated to cause an increase in WBSF (Watanabe et al., 1996). The mean LM WBSF values were still below threshold for obtaining favorable odds that a consumer would consider a steak as "tender" (Miller et al., 2001; Platter et al., 2003). An aging period effect was observed on LM WBSF ($P < 0.001$); least squares means ± SEM for WBSF of LM at the 7 aging periods (3, 7, 14, 21, 28 d) averaged over sex and treatment are displayed in **Figure 5.2**. The aging trend of the current study is similar to previous research indicating increasing tenderization up to 21 d of age (Gruber et al, 2006a).

Data indicated that supplementary Mg was consumed and that concentrations increased in the circulating blood serum of cattle for which dietary supplementation was highest. However, no behavioral, physiological, or tenderness benefits were obtained from

the increase in circulating blood serum Mg. Data demonstrated that an acute stress response was elicited as indicated by chute scores, high incidence of dark cutters, and the correlations observed with WBSF and increased catecholamine levels. If an attenuation of physiological stress response was to be achieved by the Mg supplementation, it should have been observed. The current study suggests that supplemental dietary Mg does not attenuate acute physiological stress.

Table 5.1. Experimental design of sex × supplemental magnesium factorial study

	Steers			Heifers			
Treatment	Light	Medium	Heavy	Light	Medium	Heavy	Row Total
	Number of animals						
Control	6	6	6	6	6	6	36
0.25% Mg	6	6	6	6	6	6	36
0.50% Mg	6	6	6	6	6	6	36
0.75% Mg	6	6	6	6	6	6	36
Column Total	24	24	24	24	24	24	144

Table 5.2. Transitional and Finishing diet ingredients and composition[1,2]

Ingredient	Transitional diet	Finishing diet
Alfalfa hay mid bloom	18.0	5.0
Corn silage 40% Gr	15.0	15.0
Corn grain flaked	35.5	48.0
Dry distillers grain	29.0	29.0
Finisher mineral mix[3]	2.5	3.0
Composition		
DM %	79.4	79.4
CP%	16.5	16.5
NEm Mcal/45.4kg	98.1	98.1
NEg Mcal/45.4kg	64.3	64.3
Calcium %	0.8	0.8
Phosphorus %	0.4	0.4

[1] Ration commercially formulated by Patterson Nutrition Co., Inc., Sterling, CO
[2] Ingredient amounts displayed on percentage as fed basis
[3] Formulated to provide the following: 570 ppm of Mn, 350 ppm of Zn, 230 ppm of Cu, 12.3 ppm of I, 0.80 ppm of Co, and 0.25 ppm of Se.

Table 5.3. Simple descriptive statistics for behavior evaluations, physiological parameters, and meat quality characteristics

Trait	Mean	Minimum	Maximum	CV%
Pen behavior score[1]	26.47	5	84	59.9
Chute behavior score[1]	26.36	2	81	59.0
On-trial BW	1239.83	1000	1470	8.0
Off-trial BW	1282.83	1030	1545	8.2
Respiration, breaths/min	56.39	28	108	26.3
Heart rate, beats/min	78.00	52	144	21.6
Rectal temperature, °C	40.36	39.6	41.2	0.8
ADG	1.41	0.48	2.97	0.5
Blood serum Mg, mg/dL[3]	2.41	1.8	3.4	13.6
Blood lactate, mmol/L[4]	10.99	6.1	18.1	20.7
HCW	796.04	628	1001	9.2
Marbling Score[5]	374.72	180	540	15.6
Warner-Bratzler shear force, kg[6]	3.86	2.65	5.45	15.5
Muscle pH[7]	5.51	5.31	6.07	2.49
Muscle lactate, μmol/g[8]	58.01	27.35	81.81	19.4
Muscle glucose, μmol/g[8]	27.70	4.73	86.71	53.5
L*[9]	37.08	28.53	42.31	7.9
a*[10]	9.26	4.08	14.24	18.4
b*[11]	10.76	6.05	13.57	14.7

[1] Behavior scores assessed using a semi-structured continuous line scale (0 = Calm, 150 = Flighty)

[2] Exit speed score assessed using a semi-structured ordinal numbering scale system (1 = Walk, 2 = Trot, 3 = Run-moderate speed, 4 = Run-high speed)

[3] Serum concentrations determined from blood samples taken during routine processing through a hydraulic squeeze chute

[4] Blood lactate levels determined using a blood lactate meter from blood collected immediately after exsanguination

[5] 200 = Traces, 300 = Slight, 400 = Small, 500 = Modest, 600 = Moderate

[6] Warner-Bratzler shear force averaged across aging period (3, 7, 14, 21, and 28 d)

[7] Muscle pH at 48 h postmortem

[8] Muscle lactate and glucose measured from muscle tissue samples collected 30 min postmortem

[9] 0 = black, 100 = white (N = 95)

[10] -60 = green, 60 = red (N = 95)

[11] -60 = blue, 60 = yellow (N = 95)

Table 5.4. Correlation coefficients[1] showing linear relationships among behavior scores, physiological stress indicators, and meat quality traits within each sex class[2]

Variable	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.
1. Pen score[3]		0.11	-0.01	0.11	0.06	0.25	-0.01	0.04	0.01	0.09	0.03	0.11	0.03	0.00	0.18	0.08	0.07
2. Chute score[3]	0.20		-0.03	0.05	-0.25	-0.09	0.26	0.04	-0.04	0.09	0.01	-0.08	0.15	-0.32	-0.14	-0.18	0.31
3. Heart rate	-0.08	-0.01		0.50	-0.38	0.25	0.13	-0.22	0.25	-0.06	-0.03	-0.28	0.12	0.27	0.43	0.51	-0.26
4. Respiration rate	0.09	0.14	0.35		-0.29	0.12	0.19	-0.08	0.20	0.09	-0.18	-0.40	0.28	0.15	0.14	0.20	0.14
5. Rectal temperature	0.04	0.21	-0.33	-0.30		-0.02	-0.30	-0.01	-0.05	-0.08	0.04	0.22	0.00	-0.19	-0.22	-0.34	0.14
6. Plasma epinephrine	0.14	0.27	0.14	0.36	-0.06		0.13	0.11	0.00	0.13	0.20	0.04	-0.09	0.06	0.29	0.28	-0.02
7. Plasma norepinephrine	0.25	0.13	0.15	0.36	-0.18	0.49		0.12	0.01	0.16	0.00	-0.14	0.14	-0.08	0.12	0.02	0.11
8. Log LM glucose	-0.06	0.02	-0.31	-0.36	0.24	-0.24	-0.29		-0.18	0.84	-0.08	-0.18	-0.40	0.16	0.30	0.27	-0.22
9. Log LM lactate	0.05	0.05	0.14	0.15	-0.02	-0.04	-0.05	-0.13		0.27	0.01	-0.12	0.00	0.29	0.26	0.36	0.05
10. Log glycolytic potential[4]	-0.04	0.07	-0.22	-0.28	0.21	-0.29	-0.33	0.89	0.27		-0.08	-0.21	-0.36	0.20	0.35	0.37	-0.12
11. Blood lactate	-0.08	-0.11	-0.06	0.08	0.16	-0.02	0.00	0.16	0.02	0.13		0.00	0.10	-0.26	-0.04	-0.08	0.09
12. Lean maturity	0.16	0.03	-0.42	-0.31	0.20	-0.15	0.03	0.25	-0.11	0.18	-0.08		-0.03	-0.71	-0.17	-0.57	0.25
13. Muscle pH	0.08	0.03	0.08	0.14	-0.04	-0.16	-0.02	-0.16	0.16	-0.02	0.08	-0.05		-0.46	-0.36	-0.50	0.37
14. L*[5]	-0.17	-0.20	0.30	0.04	-0.28	-0.02	-0.11	-0.26	0.01	-0.21	-0.08	-0.47	0.01		0.44	0.73	-0.48
15. a*[6]	-0.02	0.19	0.32	0.25	-0.21	0.07	0.22	0.17	0.29	0.30	0.15	0.01	0.17	0.13		0.82	-0.55
16. b*[7]	0.06	0.09	0.16	0.17	-0.10	0.08	0.06	0.13	0.27	0.26	0.27	-0.36	0.06	0.51	0.68		-0.62
17. WBSF[8]	0.43	0.25	0.20	0.20	-0.06	0.40	0.23	-0.01	0.03	0.01	-0.13	0.05	0.25	-0.29	0.01	-0.08	

[1] For L*, a*, and b*, $|r| \geq 0.29$ differ from 0 ($P < 0.05$; N = 95); for all other traits, $|r| \geq 0.24$ differ from 0 ($P < 0.05$; N = 143).

[2] Coefficients for steers are above the diagonal and those for heifers are below the diagonal.

[3] Semi-structured continuous line scale

[4] Glyolytic potential = 2 ([glucose]) + [lactate]

[5] L*: 0 = black; 100 = white

[6] a*: Negative number = green; Positive number = red

[7] b*: Negative number = blue; Positive number = yellow

[8] Warner-Bratzler shear force, averaged across aging period (3, 7, 14, 21, and 28 d)

Table 5.5. LS Means showing effects of sex and treatment on growth traits

	Sex				Supplemental Mg[1]					
Item	Heifer	Steer	SE	*P*	0.00	0.25	0.50	0.75	SE	*P*
Number of pens	12	12			6	6	6	6		
Initial BW, kg	528	598	7.2	<0.001	567	562	560	563	10.2	0.973
Final BW, kg	546	619	6.9	<0.001	583	585	580	584	9.8	0.982
Daily DMI, kg	8.80	8.91	0.068	0.102	8.90^{ab}	9.10^{a}	8.90^{ab}	8.64^{b}	0.096	0.032
ADG, kg	1.29	1.53	0.221	0.450	1.13	1.6	1.39	1.53	0.312	0.728
G:F, kg	0.15	0.17	0.024	0.486	0.13	0.17	0.16	0.18	0.034	0.728

[1] Expressed as a percentage of total mixed ration

[a, b] Means without a common superscript letter differ ($P < 0.05$)

Table 5.6. LS Means showing effects of sex and treatment on subjective live cattle behavior evaluation scores, live animal vital parameters, carcass characteristics, blood analyses, and shear force evaluations.

	Sex				Supplemental Mg[1]					
Item	Heifer	Steer	SE	*P*	0.00	0.25	0.50	0.75	SE	*P*
Pen behavior score[1]	33.23	20.05	2.12	<0.001	18.11[b]	26.49[ab]	34.95[a]	27.01[ab]	3.03	0.010
Chute behavior score[1]	24.33	28.41	1.86	0.121	24.86	24.85	27.94	28.08	2.64	0.717
Respiration, breaths/min	57.00	55.70	2.70	0.739	56.33	52.30	59.00	57.78	3.83	0.636
Heart rate, beats/min	79.44	76.36	4.65	0.645	78.33	77.72	78.11	77.44	6.57	0.999
Rectal temperature, °C	40.42	40.29	0.07	0.243	40.31	40.42	40.32	40.36	0.10	0.868
Blood plasma epinephrine[2]	1.05	0.96	0.13	0.625	0.98	0.87	1.06	1.12	0.18	0.797
Blood plasma norepinephrine[2]	0.63	0.65	0.10	0.856	0.63	0.55	0.69	0.70	0.14	0.856
Blood lactate, mmol/L[3]	11.11	10.86	0.27	0.520	11.16	10.79	10.86	11.14	0.39	0.865
HCW	338.22	379.07	7.30	0.001	361.30	351.78	362.40	359.11	10.35	0.885
Marbling Score[4]	393.47	355.92	8.47	0.006	385.00	373.78	365.00	375.00	12.10	0.704
Muscle pH[5]	5.49	5.54	0.02	0.042	5.49	5.49	5.54	5.54	0.02	0.172
Muscle lactate, μmol/g[6]	56.95	57.78	2.28	0.793	56.39	57.69	56.33	59.07	3.18	0.912
Muscle glucose, μmol/g[6]	30.53	25.14	2.34	0.093	28.32	26.17	27.09	29.77	3.21	0.847
Muscle glycolytic potential, μmol/g[6]	117.30	107.02	4.30	0.063	112.82	108.17	109.45	118.20	5.93	0.575
L*[7]	37.60	36.58	0.51	0.196	36.99	37.32	37.36	36.70	0.73	0.902
a*[8]	9.70	8.82	0.33	0.097	8.94	9.27	9.37	9.45	0.47	0.871
b*[9]	11.20	10.33	0.34	0.108	10.26	10.91	10.84	11.05	0.48	0.678
WBSF, kg[10]	3.74	3.99	0.08	0.013	3.83	3.83	3.92	3.89	0.11	0.881

[a, b] Means without a common superscript letter differ ($P < 0.05$)

[1] Behavior scores assessed using a semi-structured continuous line scale (0 = Calm, 150 = Flighty)

[2] Blood plasma obtained post supplementary diet feeding period in a hydraulic squeeze chute

[3] Blood lactate levels determined using a blood lactate meter from blood collected immediately after exsanguination

[4] 200 = Traces, 300 = Slight, 400 = Small, 500 = Modest, 600 = Moderate

[5] Muscle pH at 48 h postmortem

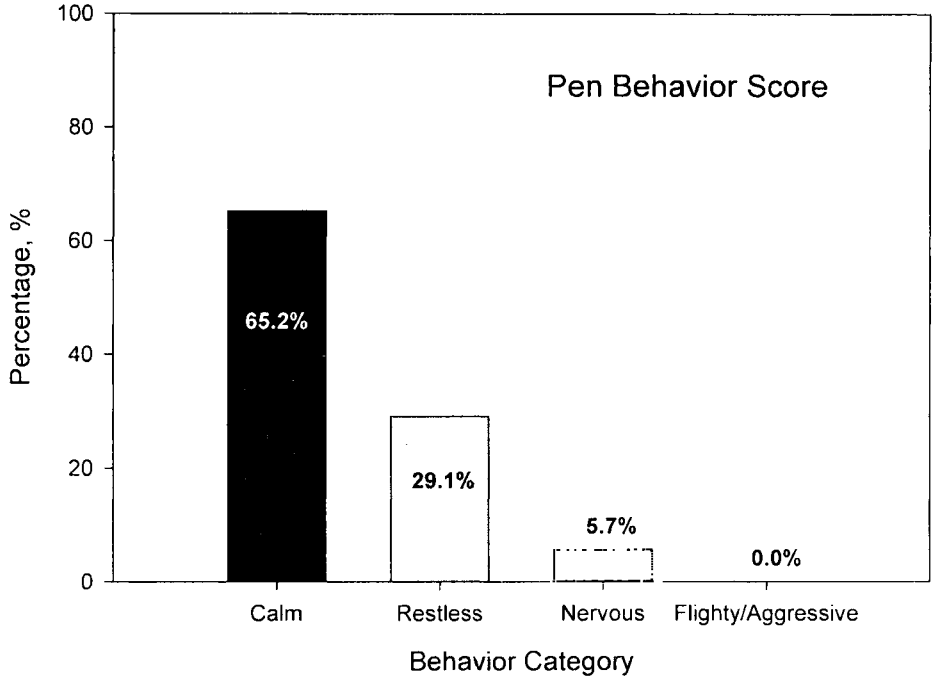
[6] Muscle lactate and glucose measured from LM tissue samples collected 30 min postmortem

[7] 0 = black, 100 = white

[8] -60 = green, 60 = red
[9] -60 = blue, 60 = yellow
[10] Warner-Bratzler shear force averaged across aging period (3, 7, 14, 21, and 28 d)

Table 5.7 Least squares means of physiological stress indicators categorized by exit speed velocities.

Item	Exit Speed				SEM	$P > F$
	Walk	Trot	Moderate Run	Fast Run		
Number of animals	70	44	22	7		
Respiration rate, breaths/min	52.3	50.2	51.7	54.6	6.94	0.855
Heart rate, beats/min	77.6	74.0	76.4	77.7	7.34	0.765
Rectal temperature, °C	40.7^{c}	40.8^{bc}	40.9^{ab}	41.1^{a}	0.14	0.002
Plasma epinephrine, ng/dL[4]	0.35^{d}	0.64^{c}	1.22^{b}	1.89^{a}	0.312	< 0.001
Plasma norepinephrine, ng/dL[4]	0.18^{c}	0.34^{bc}	0.62^{ab}	0.84^{a}	0.278	0.004
Blood lactate, mmol/L[5]	10.8	10.7	12.0	11.8	0.90	0.155
LM glycolytic potential, μmol/g[8]	106.5	114.7	109.1	117.6	12.06	0.542
LM lactate, μmol/g[8]	59.9	60.1	58.5	54.6	5.01	0.677
LM pH[7]	5.53	5.49	5.52	5.52	0.053	0.595
Warner-Bratzler shear force, kg	3.72^{b}	3.90^{b}	4.00^{b}	4.57^{a}	0.219	0.002


Pen Behavior Score
Percentage, %
100
80
60
40
20
0
65.2%
29.1%
5.7%
0.0%
Calm
Restless
Nervous
Flighty/Aggressive
Behavior Category

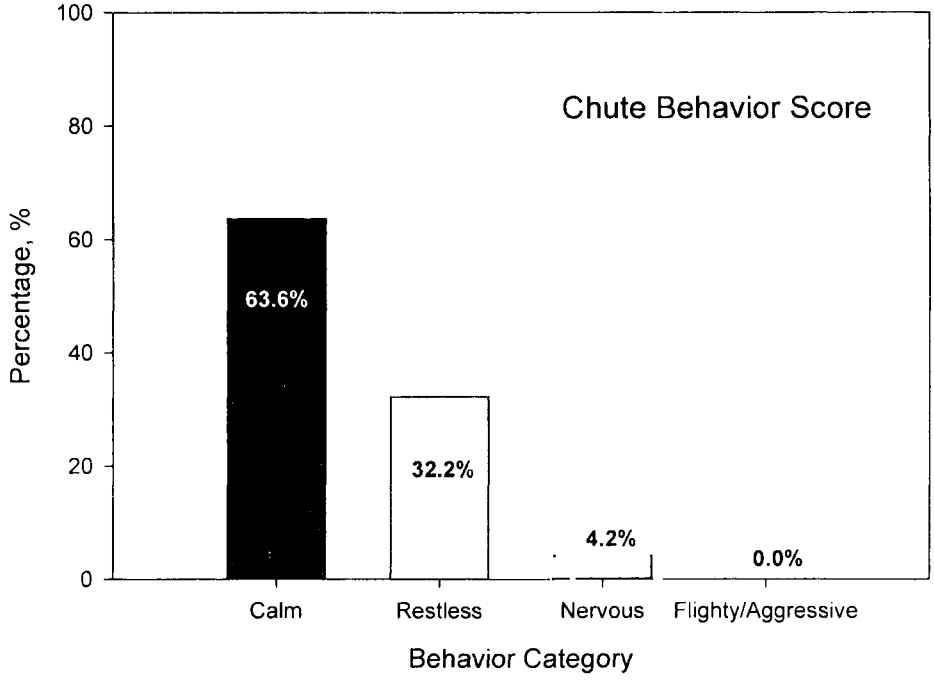

Chute Behavior Score
Percentage, %
100
80
60
40
20
0
63.6%
32.2%
4.2%
0.0%
Calm
Restless
Nervous
Flighty/Aggressive
Behavior Category

Figure 5.1

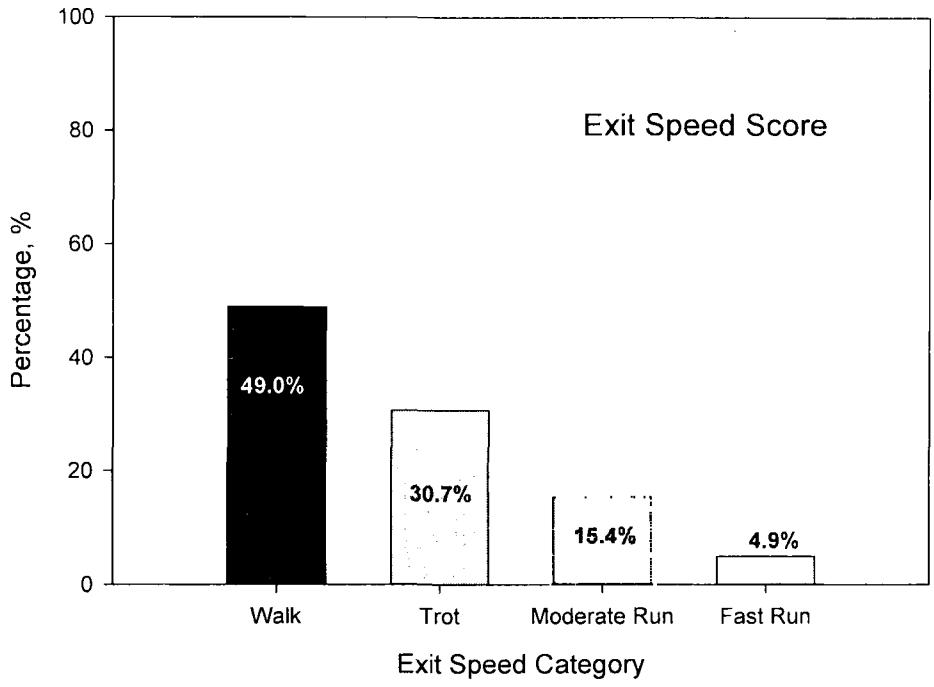


Figure 5.1 Frequency distribution of live animal behavior scores

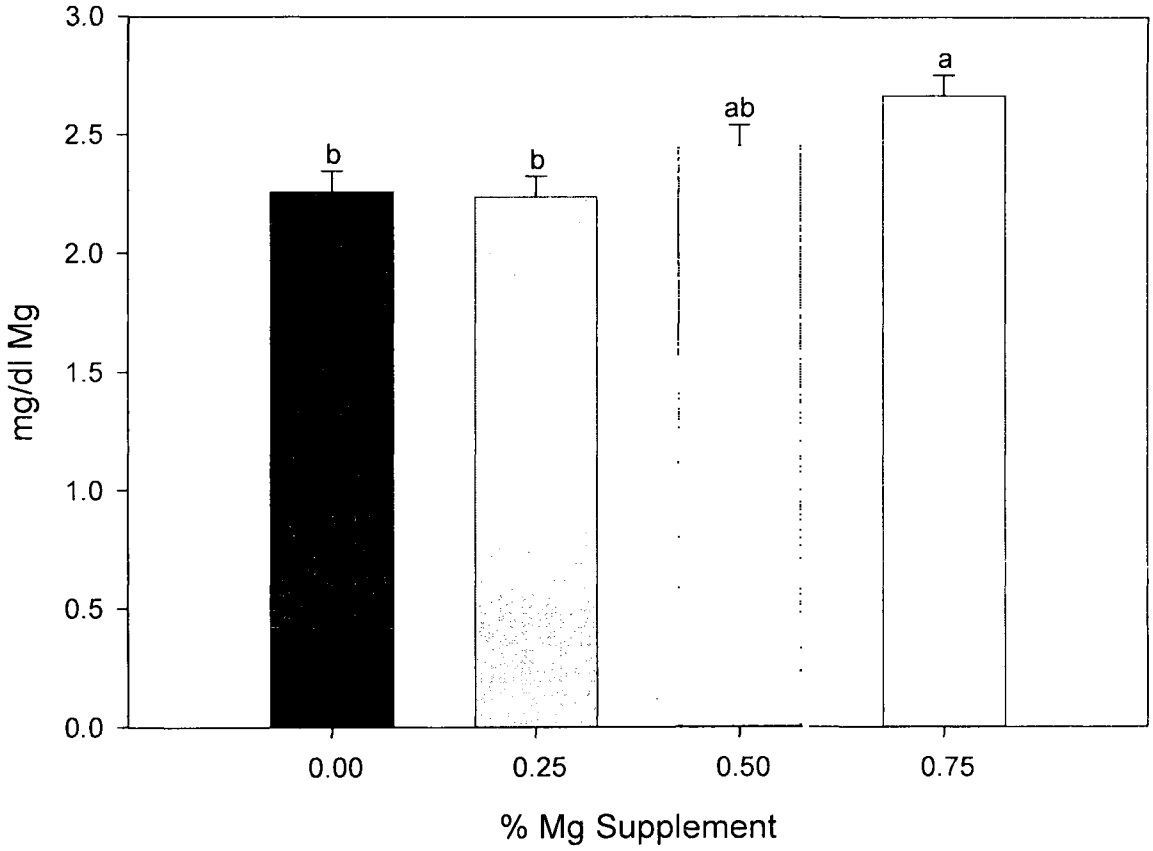


Figure 5.1. Least squares means of cattle blood serum magnesium content for the respective magnesium feed supplementation treatments fed to cattle for 14 d. Means lacking a common superscript letter differ ($P < 0.05$).

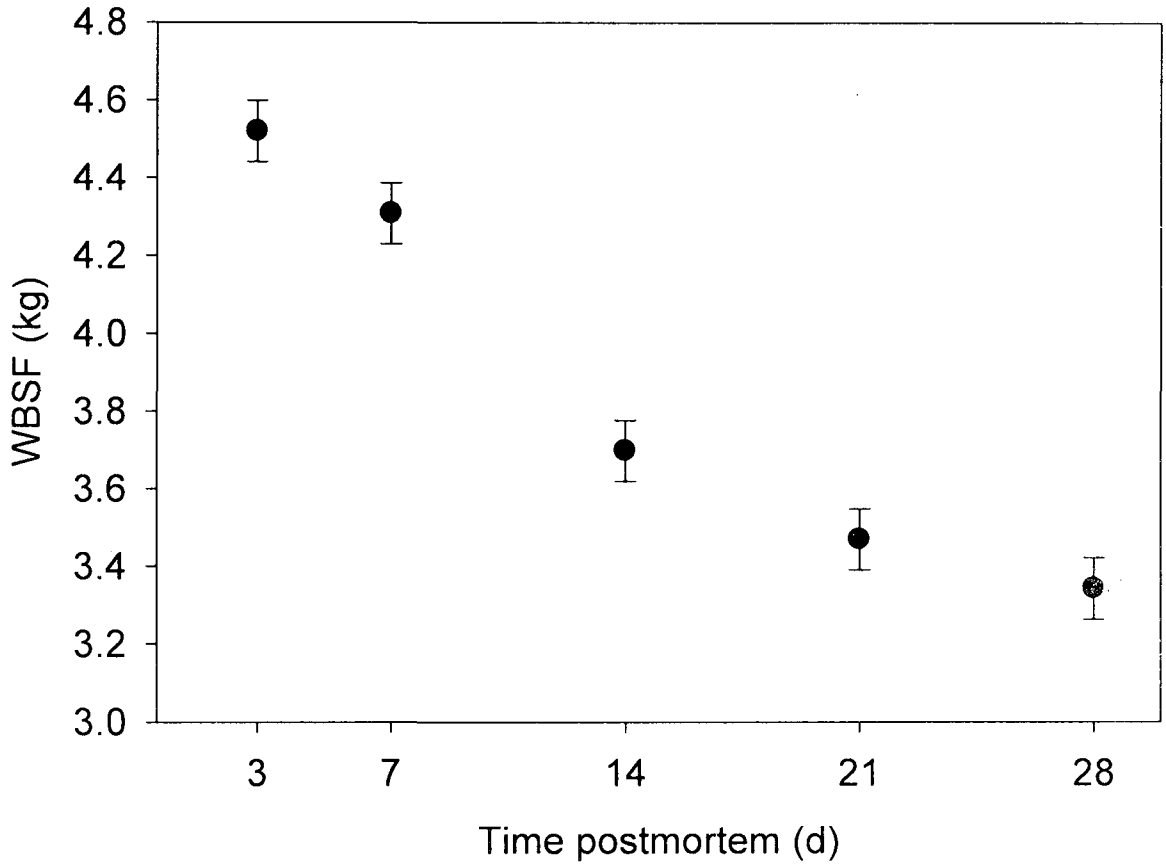


Figure 5.2. Least squares means of Warner-Bratzler shear force (WBSF) of beef LM at 7 aging periods (3, 7, 14, 21, 28 d) averaged over sex and supplemental magnesium treatment.

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