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Nutrient, Tenderness, and Strip Loin Differences of Beef from Cattle Due to Mutation of  
the Myostatin Gene

By

Michelle E. Semler

A THESIS

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Master of Science

Major: Animal Science

Under the Supervision of Professor Chris R. Calkins

Lincoln, Nebraska

May, 2013

# Nutrient, Tenderness, and Strip Loin Differences of Beef from Cattle Due to Mutation of the Myostatin Gene

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University of Nebraska, 2013

Advisor: Chris Calkins

The objectives of this study was to determine tenderness, nutrient, and musculature differences of loins from cattle with variation of myostatin gene. Steers ( $n = 21, 22$ , and  $16$ ) and heifers ( $n = 19, 20$ , and  $20$ ) were genotyped as homozygous (MM; active myostatin gene), heterozygous (Mm; partially recessive myostatin) or homozygous (mm; recessive inactive myostatin), respectively. At 3 d post mortem *Longissimus dorsi* (LD) and *Semitendinosus* (ST) were collected. Loins were cut into 2.5 cm thick steaks evaluated for: total number steaks, total number vein steaks, total number non-vein steaks, and individual steak weight. Nutrient analysis steaks were cut 1.3 cm thick and trimmed to 0.3 mm of fat. Warner-Bratzler shear force (WBS) was conducted on steaks aged for 14 d. Steaks from ST of MM steers had greater ( $P < 0.001$ ) WBS values (3.6 vs. 3.1 kg) compared to mm. No differences in WBS values for the ST of heifers and LD of steers and heifers. Fat content from mm steaks decreased ( $P < 0.001$ ) while moisture ( $P < 0.001$ ) and protein increased ( $P < 0.001$ ) compared to MM and Mm. Calories decreased with increasing copies of recessive gene ( $P < 0.001$ ). Steaks from mm had greater concentrations of cholesterol ( $P < 0.001$ ), decreased levels of saturated fatty acids ( $P < 0.001$ ), monounsaturated fatty acids ( $P < 0.001$ ), polyunsaturated fatty acids ( $P < 0.001$ )

and trans fatty acids ( $P < 0.001$ ) than MM. Fat thickness decreased ( $P < 0.001$ ) for heterozygous and homozygous recessive cattle compared to homozygous dominant cattle. Weight and percentage of veins steaks did not differ for steers. Loins from Mm heifers had a decreased percentage of vein steaks ( $P = 0.01$ ) compared to MM and mm steaks from heifers. In conclusion, steaks from mm cattle had decreased fat content, greater cholesterol concentrations, and greater protein concentrations compared to MM. Loins of steers exhibited no difference in proportion of weight and percentage of vein steaks.

**Key Words:** myostatin, vein steaks, tenderness

## ACKNOWLEDGMENTS

Throughout the course of my graduate career there are numerous professors, colleagues, and loved ones I would like to thank. I first would like to thank Dr. Sherry Olsen from Iowa State University for spurring my interest in meat judging and meat science, prior to coming to Nebraska. Your guidance as not only a coach but also a mentor has led me toward a career in education and agriculture that I am truly passionate about. Also, thank you to my fellow meat judging team members and those students I had the opportunity to coach while working on my undergraduate degree at Iowa State University.

Second, I would like to thank those members serving on my committee: Dr. Chris Calkins, Dr. Steven Jones, Dr. Gary Sullivan, and Dr. Ty Schmidt. I would especially like to thank Dr. Chris Calkins for serving as my advisor and providing me the opportunity to further my education as well as provide me with travel opportunities and attendances at conferences I otherwise would not have experienced.

Thanks are also extended to my fellow meat science graduate student, both present and past for their assistance with my research. Thank you for answering questions in regards to coursework, statistical analysis, and any other questions I may have asked of you. Aside from research, the camaraderie and conversations within the office are something I will always cherish and never forget. I am also grateful for the coordinating and assistance from Tommi Jones throughout taste panels and scheduling of the kitchen hours. As well, thank you to Calvin Schrock for cooperating through multiple fabrication times through the meat lab. Finally, I would like to thank Sherri Pitchie for your help in

making photocopies, proofreading of posters, and numerous other tasks you assisted with. Thank you for being a friendly face that was always welcoming and willing to listen. As well I would like to thank Lone Creek Cattle Company for funding this research and providing individuals to help gather samples during multiple collection dates.

Special thanks are given to numerous friends from childhood and beyond. Your open ears and words of encouragement through times of stress and difficulties are greatly appreciated. Lastly, I would like to thank my family for always being there for me and supporting me through my college career at both Iowa State and the University of Nebraska. You pushed me to the best of my abilities and without you I would not be the person I am today.

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## INTRODUCTION

It has been reported that an inactivated myostatin allele is responsible for the double muscling phenotype seen within Piedmontese cattle. The inactive myostatin allele leads to an increase in overall muscle mass due to hyperplasia, or an increase in muscle fiber numbers. As well, double muscled cattle produce leaner carcasses with less overall fat content compared to normal commercially produced animals. These animals produce a heavier weight of the most desirable cuts of meat such as the strip loin than conventionally raised cattle. Fatty acid and cholesterol content may also be altered with an increase in cholesterol content and polyunsaturated fatty acid concentration due to the increase in muscle fiber numbers and decrease in overall fat content. The focus of this research was to determine musculature differences of strip loins, and to determine tenderness and nutrient differences, of cattle genotyped as homozygous dominant (MM), heterozygous (Mm), or homozygous recessive (mm) for the myostatin allele. Cattle genotyped as homozygous dominant possess an active myostatin gene, while heterozygous genotypes possess a partially recessive active myostatin gene, and cattle homozygous recessive (mm) possess an inactive myostatin gene.

Multiple studies have been conducted comparing tenderness of beef from double muscled cattle to homozygous dominant cattle (active myostatin gene), although many studies have only included the *Longissimus* (Wheeler et al., 2001). It was hypothesized within this current study that beef from cattle which are homozygous recessive for the myostatin gene would be equal in tenderness for the strip loin and eye of round compared to cattle homozygous dominant and heterozygous for the myostatin gene even though the

beef from homozygous recessive cattle is leaner. Furthermore, with the increase in overall muscle mass of the most desirable cuts, such as the strip loin and rib roll, the muscular differences of strip loins were analyzed to determine the number and percentage of vein steaks within each strip loin. The vein steak is a steak removed from the strip loin that contains both the *Longissimus dorsi* and *Gluteus medius* muscle and possess a piece of connective tissue separating the two muscles. Vein steaks are considered to be less tender and thus have a decreased value compared to strip steaks without the *Gluteus medius*.

The objectives of this study were to: 1. Determine differences in nutrient composition of beef from cattle with different myostatin genotypes 2. Determine tenderness differences of cattle with different genotypes and 3. Determine if homozygous recessive cattle with an inactive myostatin gene produce a greater number and percentage of vein steaks from the strip loin compared to cattle homozygous dominant or heterozygous for the myostatin gene.

## REVIEW OF LITERATURE

### I. Causes of Double Muscling

Double muscling is an inherited condition that leads to an increase in muscle mass due to the homozygous recessive allele of the myostatin gene. Myostatin is a member of the transforming factor  $\beta$  superfamily of secreted growth and differentiation factors, is a negative regulator of myogenesis (Aberle et al., 2001; McPherron and Lee, 1997) and is important in the regulation of skeletal muscle mass. Due to years of selections, part of the sequence for the myostatin gene has been mutated or lost (Aberle et al., 2001). When myogenesis is not controlled, animals with a greater number of muscle fibers (hyperplasia) are produced (Aberle et al., 2001).

The myostatin protein is encoded by the myostatin gene which is specifically expressed within muscle tissue. Myostatin is involved in the communication that regulates proliferation and differentiation of myoblasts (Kambadur et al., 1997) both pre and postnatal. Rehfeldt et al., (2005) reported that the hypermuscularity observed due to the homozygous recessive allele of the myostatin gene results in fiber hyperplasia rather than hypertrophy, and from an increase in myonuclear proliferation and protein accretion. Myofiber hyperplasia occurs early in pregnancy and results in homozygous recessive calves having twice the number of fibers at birth compared to homozygous dominant for the myostatin gene (Swatland and Kieffer, 1974). With the total muscle fiber numbers determined during pre-natal development, the inactivation of the myostatin gene allows for exponential development of muscle fibers (Wegner et al., 2000). Homozygous recessive cattle that exhibit double muscling have a higher proportion of Type IIB muscle

fibers and a decreased proportion of Type I and Type IIA (Aberle et al., 2001; Wegner et al., 2000). Type IIB muscle have the ability to work at a wider pH range and thus are more prone to oxidation. Kambadur et al. (1997) reported that the expression of myostatin gene is detected early in myogenesis prenatal and continues through to adult skeletal muscle, and may control muscle fiber size and fiber number during embryonic and fetal myogenesis and fiber size postnatal.

With the loss of myostatin an increase in skeletal muscle mass postnatal of about 20% is typically observed due to muscle hyperplasia (Grobet et al., 1997). Double muscled cattle not only have an increase in skeletal mass but will also produce leaner carcasses compared to homozygous dominant cattle (Casas et al., 1998). Kambadur et al. (1997) described the myostatin gene as “partially recessive” because there was some noticeable difference in animals carrying the heterozygous allele compared to animals carrying the homozygous dominant allele. However, for the double muscling phenotype to be observed the animals must be homozygous recessive for the myostatin gene (Kambadur et al., 1997). Cattle possessing a homozygous recessive or heterozygous allele of the myostatin gene will yield a carcass with a greater amount of closely trimmed retail products; due to the increase muscle mass and reduced fat content, as well as increased lean growth efficiency and improved feed conversion (Keele and Fahrenkrug, 2001). However, the increase in weight of double muscled cattle cannot solely be attributed to increased muscle mass. The weight of skin, adipose tissue, digestive tract size, and internal organs is reduced in double muscled cattle (Kumbadur et al., 1997) when compared to non-double muscled cattle.

The Piedmontese is a breed of cattle that are homozygous recessive for the myostatin gene and this have an increase in muscle mass in relation to non-double muscled cattle (McPherron and Lee, 1997). The specific mutation of the myostatin mutation identified in Piedmontese cattle results in a substitution of tyrosine for cysteine in the mature region of the myostatin protein (McPherron and Lee, 1997). Similarly, Kambadur et al. (1997) reported that the mutation identified within the Piedmontese breed is affected by a conserved cysteine of the myostatin that affects the function of myostatin. The nonfunctional myostatin protein observed in Piedmontese cattle is thus responsible for the loss of control of muscle growth. These cattle will also exhibit a 20 % increase (on average) in muscle mass for some of the most desirable beef cuts, which are also typically also leaner than those from non-double muscled cattle (Casas et al., 1998; Kambadur et al 1997).

Wheeler et al. (1996) conducted a research trial to compared carcass traits from mating Hereford and Angus cows to Hereford, Angus, Charlois, Gelbvieh, Pinzgauer, Shorthorn, Galloway, Longhorn, Nellore, Piedmontese, or Saler sires. Wheeler et al., (1996) noted that Piedmontese-sired steers possessed the largest *Longissimus* area and were the most muscular when compared at a constant carcass weights across all breeds evaluated. Wheeler et al., (1996) also reported that Piedmontese steers possessed greater dressing percentages, the lowest adjusted fat thickness, and more desirable numerical USDA yield grade when cattle were compared at constant age. The percentage of lipids was decreased in Piedmontese-sired steers for both raw and cooked *Longissimus* steaks when compared to all other sire breeds. Furthermore, percent protein was greatest when

chemical composition was performed on raw *Longissimus* steaks for Piedmontese-sired steers compared to all other breeds studied (Wheeler et al. 1996).

Piedmontese cattle have been reported to have improved tenderness when compared to cattle homozygous dominant for the myostatin gene. Previous research by Wheeler et al. (2001) compared the contribution of Piedmontese breed composition and myostatin genotypes on tenderness of four major beef cuts (*Longissimus thoracis*, *Gluteus medius*, *Semimembranosus*, and *Biceps femoris*) using a trained sensory panel. Cows were bred to produce cattle that were homozygous dominant, heterozygous, and homozygous recessive for the myostatin gene to investigate the impact of the myostatin genotype on tenderness. Inheritance was studied by producing cattle with 25:75, 50:50, or 75:25 ratio of Piedmontese: Herefords (or Piedmontese: Angus). Beef from homozygous recessive cattle was more ( $P < 0.05$ ) tender than beef from heterozygous cattle. Beef from cattle possessing the heterozygous allele were more tender ( $P < 0.05$ ) than beef from cattle possessing the homozygous dominant allele. Wheeler et al. (2001) also reported that the Piedmontese breed composition within the three myostatin genotypes data indicated that breed composition had no effect on tenderness and that the myostatin genotype was responsible for the observed effect on tenderness. These results of Wheeler et al. (2001) is in agreement with those reported by Wheeler et al. (1996) where shear force values of *Longissimus* of Piedmontese (homozygous recessive) tended ( $P < 0.05$ ) to be less than the shear force values of multiple other breeds. Furthermore, sensory panels rated beef from Piedmontese with greater scores than that of other breeds, possibly indicating a more tender product. Wheeler et al. (2001) also observed that the *Gluteus*

*medius* of cattle possessing the homozygous recessive and heterozygous alleles were more tender than the *Longissimus* of homozygous dominant cattle. All muscles evaluated from cattle possessing the heterozygous allele were more tender when compared to muscles from cattle possessing the homozygous dominant allele. Wheeler et al., (1996) concluded these results imply that producing Piedmontese cattle with possessing the homozygous recessive or heterozygous allele of myostatin improves the tenderness of not just valuable cuts (strip loin and rib roll), but also of lower-valued cuts, which could result in increased carcass value.

## **II. Fatty Acid Composition**

Fat and fatty acids play integral roles in the nutritional value of meat. Fatty acid composition is influenced by both genetic and environmental factors, with the double muscling gene in cattle being an example of a genetic effect (De Smet et al., 2004). Fatty acid composition of an animal is impacted by overall fat and muscle content due to the composition of neutral lipids and phospholipids (Wood et al., 2008).

Within cattle, microbial biohydrogenation within the rumen degrades fatty acids within feedstuff to monounsaturated and saturated fatty acids (Wood et al., 2008). Jenkins (1992) reported that unsaturated fatty acids within the rumen have short shelf lives as the microbes convert these fatty acids into more saturated end products resulting in increased amounts of saturated fatty acids and few polyunsaturated fatty acids. However cattle conserve long chain polyunsaturated fatty acids in the form of phospholipids within muscle (Wood et al., 2008).



Phospholipids play a large role within cell membranes and that large proportions of phospholipids are rich in polyunsaturated fatty acids (Rule et al., 1997). Within double muscled animals the polyunsaturated content of the phospholipid fraction tends to be greater, even though double muscled animals have a decreased concentration of total lipids (De Smet et al., 2004; Wood et al., 2008).

As livestock fattens, the concentrations of saturated fatty acids and monounsaturated fatty acid increase at a greater rate than concentration of polyunsaturated fatty acids (De Smet et al., 2004). Triacylglycerols deposited within the adipocytes as livestock fattens dilutes phospholipids concentration, thus explaining the decrease in polyunsaturated to saturated fatty acid ratio with increasing fatness (De Smet et al., 2004). The ratio of polyunsaturated to saturated fatty acid ratio increases as the intramuscular fat content decreases (De Smet et al., 2004). In a trial conducted by Dinh et al. (2009) designed to studied the intramuscular fat content and fatty acid composition of three breeds of cattle (Angus, Brahman, and Romosinauno); Dinh et al. (2009) observed that the proportion of monounsaturated fatty acids was greater ( $P \leq 0.005$ ) than saturated fatty acids regardless of cattle breed and that total fatty acid concentration was positively correlated with intramuscular fat content ( $P \leq 0.005$ ). However, differences in biohydrogenation in the rumen were not caused by differences within breed types (Dinh et al., 2009). The greater the fat content within a carcass the less the total water content due to fat containing limited amounts of water (Romans et al., 1994).

### III. Cholesterol

Cholesterol content in meat has led some consumers to perceive red meat and specifically beef in a negative image. Within the human diet an elevated intake of saturated fatty acids can lead to an increased concentration of low-density lipoprotein cholesterol resulting in an increased risk for coronary heart disease (Bohac and Rhee, 1988). In contrast, the consumption of polyunsaturated fatty acids decreases the amount of low-density lipoproteins (Bohac and Rhee, 1988).

Cholesterol has a primary role in stabilizing the membrane of cells and affects the fluid characteristics of the cell membrane as well as being a component of meat lipids (Du et al., 2009). Cholesterol within the membrane is associated with phosphatidyle choline, phosphatidyle choline is a major phospholipid within the cell membranes. This indicates an increase in cholesterol is correlated with an increase in polyunsaturated fatty acids as phospholipids are rich in polyunsaturated fatty acids (Rule et al., 1997). Stabilization of the cell membrane occurs when a hydroxyl group of cholesterol forms a hydrogen bond with the nearest phospholipid helping to immobilize the outer outside surface of the membrane (Berg et al., 2007). Rule et al. (1997) also stated that altering the concentration of cholesterol within muscle may redistribute the phospholipids in the cell and change the unsaturation of the membrane fatty acids. Genetic changes could lead to these differences in cholesterol concentration.

Taylor et al. (1990) evaluated the genetic differences between the Brahman and Hereford breeds as well as differences in cholesterol based on the type of feed (pasture vs. grain). Taylor et al., (1990) reported that there was no difference ( $P < 0.05$ ) in the

cholesterol content of muscles between the two breeds when weight, fat thickness, and feeding history were equivalent. Taylor et al., (1990) also reported that feed type and carcass fatness are not good indicators of cholesterol content. These results are in agreement with Wheeler et al. (1987) who reported that breed type had no effect on cholesterol concentration. Taylor et al. (1990) reported a correlation between intramuscular fat and cholesterol content within the Brahman group when intramuscular fat was greater than 3.6%, and concluded that as intramuscular fat content increased so did cholesterol content if the fat percentage was large.

Conducting a trial to compared pasture fed steers to feedlot steers, Lewis et al., (1993) reported results that were inconsistent with the findings of Taylor et al. (1990). Lewis et al., (199.) reported that the intramuscular fat content of raw steaks was related to cholesterol content in pasture-fed steers and feedlot steers. Differences were observed in the cholesterol content of *Longissimus* muscle when intramuscular fat was between 2.1 and 3.6% in steaks of pasture-fed cattle. However, no differences were noted in feedlot steers with 3.3 and 5.5% intramuscular fat.

Rhee et al., (1987) concluded that when cholesterol content was compared to degree of marbling for raw steaks, there was no difference among steaks with different amounts of marbling except for steaks that received a USDA marbling score of practically devoid. Steaks with a marbling score of practically devoid steaks contained less cholesterol than steaks from any of the other seven marbling scores (Rhee et al., 1982). Rhee et al., (1987) concluded that consumers should not be concerned about the amount of marbling in beef relative to cholesterol content.

When comparing muscle and subcutaneous fat to cholesterol content, Wheeler et al. (1987) reported that the cholesterol concentration in both muscle and adipose tissue did not differ when breed type, gender, or time on feed vary. Wheeler et al., (1987) results suggest that cholesterol content is an inherent characteristic that is dependent on needs for cellular membrane functions. Biological cellular functions determine cholesterol content of muscle tissue, not environmental factors such as feed, diet or breed.

#### **IV. Mineral Content**

Mineral content of fresh meat is roughly 1% and can be divided into macrominerals and microminerals (trace minerals). Humans cannot synthesize minerals and must consume minerals within their daily diet. Those minerals required for biological functions are considered essential nutrients (Kinsman et al., 1994), including being a cofactor of enzyme systems (Kinsman et al., 1994), while other minerals are considered non-essential. Meat is a good source of all minerals with the exception of calcium (Hui et al., 2001). Kinsman et al. (1994) stated that the biologically important minerals for human nutrition are present in sufficient amounts in the flesh of animals due to muscles possessing similar mineral-dependent biological systems.

The mineral of greatest importance within muscle food is iron, with its bioavailability being an important factor of meat (Hui et al., 2001). Dietary iron is classified as either heme or non heme iron with heme iron being essential in transporting of oxygen within the blood and is attached to both myoglobin and hemoglobin molecules. Meats with a large concentration of these pigments are a good source of iron (Kinsman et al., 1994). A portion of the iron located in meats is heme iron which is present in animal

tissue, but heme iron is not available in any plant tissue (Hui et al., 2001; Kinsman et al., 1994). Heme iron absorption is much greater than non heme and its absorption is not affected by other dietary factors (Hui et al., 2001). Two factors can have an impact on the concentration of iron in meat, muscle type (Type I muscle fibers have a greater concentration of iron compared to Type IIB) and age of the animal at harvest (as animals age, the iron content will increase).

The mineral potassium is important for electrical and cellular functions within animals (including humans; Mateescu et al., 2012). Three trials conducted by Clark et al. (1972) evaluated the potassium distribution within beef carcasses dependent on different slaughter weights. It was reported within the trial comparing Hereford steers that lighter weight cattle had a greater percentage (23.7% vs. 20.8% vs. 19.3%) of potassium than intermediate and heavier cattle. These results were similar to a trial another trial conducted in the same study where Clark et al. (1972) also evaluated Hereford Angus crossbred steers. The lighter cattle again had a greater percentage (20.6% vs. 17.9%) of potassium when two weight groups were evaluated. This difference in potassium content was also observed in for all retail cuts evaluated; greater concentration of potassium were observed within the hindquarters of beef carcasses. Clark et al. (1972) concluded that the difference observed within potassium concentrations were related to the fat content of the samples; and indicator that fatter animals have decreased concentrations potassium when compared to leaner animals.

Mineral content varies more due to muscle effects than breed and sex differences (Doornebal and Murray., 1981). Mateescu et al. (2012) estimated the heritability of

mineral concentration within the *Longissimus* muscle of Angus beef cattle and concluded iron and sodium were highly and moderately heritable while other minerals were lowly heritable. An exception was calcium, where no genetic variation was observed.

Doornenbal and Murray (1981) reported that *Longissimus dorsi* samples from Chianina cattle possessed greater ( $P \leq 0.01$ ; 35.9 vs. 29.3, 29.3, and 29.0 ppm, respectively) concentrations of calcium when compared to Charolais, Simmental, and Limousin.

However, Charolais were had decreased calcium concentrations within the *Semimembranosus* ( $P \leq 0.01$ ; 24.6 vs. 31.2, 32.4, and 32.7 ppm) when compared to Simmental, Limousin, and Chianina cattle. Furthermore, sodium content was less ( $P \leq 0.01$ ) in the Limousin (624 vs. 609 and 624 ppm) than that of Charolais and Chianina cattle (Doornenbal and Murray, 1981). Similarly, Murray et al. (1981) concluded that mineral content and shear force may differ due to breed, gender, age, and muscle, however, no relationship between shear force value and mineral content were observed. This research suggests that concentrations of calcium, iron, sodium, and potassium in fresh meat are not useful for predicting shear force values of cooked beef.

## **V. Tenderness of Beef**

Palatability of beef is determined by tenderness, juiciness, and flavor; with tenderness being the most important economic factor (Belew et al., 2003). Factors that influence tenderness include postmortem proteolysis, marbling or intramuscular fat, connective tissue, and the contraction state of the muscle (Belew et al., 2003). The National Beef Quality Audit (2011) stated that the factors that contribute to eating satisfaction include flavor, tenderness, and juiciness. They found that eating satisfaction

ranked second only to food safety in importance to packers, retailers, and foodservice operators and that eating satisfaction is a key issue for not only consumers but also for the beef industry in general. Huffman et al. (1996) showed that 51% of consumers considered tenderness the attribute they want most in a steak for both the home and restaurant environment.

Warner-Bratzler shear force value is an objective way to determine beef tenderness. Belew et al. (2003) reported that Warner-Bratzler values varied among and within bovine muscles and that understanding these variations in tenderness may allow for appropriate use of various cuts of beef toward specific purposes matching the palatability of these cuts within the marketplace. The ability of consumers to determine differences in tenderness levels is important for establishing the values of beef tenderness (Miller et al., 2001). Consumers are able to differentiate between steaks of varying Warner-Bratzler shear force values, similar to the tenderness instruments, and are willing to pay a higher price or premium for more tender steaks with 78% of consumers willing to pay that premium (Miller et al., 2001).

Shackelford et al. (1991) reported Warner-Bratzler values of 4.6 kg and 3.9 kg for retail and food service respectively were considered acceptable for tenderness. Furthermore, Shackelford et al. (1991) indicated that a 4.6 kg value is 88.6% accurate in determining a steak to be “slightly tender” when evaluated by consumers. Huffman et al. (1996) reported similar Warner-Bratzler shear force values of 4.1 kg or less as a target value of steaks to ensure high levels (98%) of consumer acceptability in both the home and restaurant. A consumer preference value between 4.3 and 4.9 kg was determined by

Miller et al. (2001) to differentiate from tender to tough. Similarly, acceptable levels for consumers in both home and restaurant occurred between 4.4 and 5.2 kg in consumer's homes and a slightly higher range of 4.8 to 5.6 kg in the restaurant setting (Huffman et al., 1996).

The meat industry's ultimate goal is to produce a product that reaches the consumer's table that has a high degree of eating satisfaction, is repeatable, as well as being available to the consumers within a reasonable price range (Smith and Carpenter, 1976). It is important that the beef industry focus on what consumers want and attempt to meet the demands of the consumers for tenderness and possibly increase the consumption of beef (Miller et al., 1995). From 1990 to 1999, a 20% increase in tenderness was reported by the National Beef Tenderness Survey and from the 1999 to 2005/2006 survey. An additional 18% overall increase was reported in the 2010 National Beef Quality Audit (NCBA 2010). With tenderness playing a large role in the marketing of beef, management improvements and genetic selection of cattle is of importance to producers (Hosch Stevenson, 2012).

## **VI. Vein Steaks**

Toward the posterior end of the strip loin the *Longissimus dorsi* decreases in size and narrows while the *Glutues medius* increases in size. Strip steaks that contain the *Gluteus medius* also include a piece of connective tissue, half-moon in shape, lying directly beneath the surface fat of the strip loin that separates the *Gluteus medius* from the *Longissimus dorsi*. These steaks are known as vein steaks and are less tender and have a decreased value when compared to strip steaks without the *Gluteus medius* muscle. The



decrease in value is related to the amount of connective tissue present in the vein steaks. The amount of connective tissue that is present within a muscle or steak is determined by a number of things such as; animal age, muscle location, muscle use, and gender of the animal. Each of these factors can affect the concentration of connective tissue (Aberle et al., 2001). Connective tissue content is based on the functional demands placed upon that muscle during life. A tough web-like structure will be formed that may surround the muscles (epimysium) such as the connective tissue observed within vein steaks (Aberle et al., 2001). When meat is cooked the connective tissue will shrink and develop tension that leads to an increase in toughness within the meat, also known as background toughness (Du and McCorminck, 2006).

In addition to connective tissue creating toughness within the vein steaks, differences within tenderness of the *Longissimus dorsi* and *Gluteus medius* have been studied. The *Longissimus dorsi* represents a larger proportion of total carcass value than any other muscle (Wheeler et al. 2000). Slanger et al. (1985) evaluated the effects of crossbreeding beef cattle on meat tenderness and reported the *Longissimus* muscles to be more tender than the *Gluteus medius* muscle. These differences could be explained by the fact that the *Gluteus medius* muscle is utilized to generate force and aid the animal in movement, while the *Longissimus dorsi* is primarily a muscle of support, with little involvement in the generation of force for movement (Slanger et al., 1985). Similar results were observed when a consumer sensory panel was conducted by Wheeler et al. (2000) with 92% of the *Longissimus dorsi* muscles being rated as “slightly tender” compared to only 89% of the *Gluteus medius* muscles. Wheeler et al. (2001), using an

eight member trained sensory panel once again indicated the *Longissimus* to be more tender than the *Gluteus medius* (Wheeler et al., 2001). In a review by Sullivan and Calkins (2011), the major muscles of the carcass were based upon Warner-Bratzler shear force and sensory traits. Sullivan and Calkins (2011) noted that when Warner-Bratzler values were compared, *Longissimus* muscles were grouped within the tenderness category of intermediate indicating values of 3.9 to 4.6 kg, compared to *Gluteus medius* being placed within the tough category for values of greater than 4.6 kg.

## **VII. Conclusion**

With the enormous amount of genetic diversity within the United States beef cattle industry it is important to understand the attributes each of these breeds possesses. Producing a consistent, high quality, affordable product that is acceptable to consumers while also creating a desirable eating experience is a goal within the beef industry. There is also an increasing demand by consumers for healthier red meat options such as reducing fat content within their diet.

While numerous other breeds have been studied extensively, little research has been focused on the double muscled Piedmontese cattle and how the genetic diversity of this breed compares to cattle possessing the homozygous dominant allele of the myostatin gene. Such attributes considered within this study include tenderness and nutritional analysis differences (proximate, lipid, and mineral). From a production and economic standpoint strip loins were evaluated for number and percentage of vein steaks within the strip loin.

## MATERIALS AND METHODS

### *Production System*

Fifty-nine calf-fed steers and fifty-nine yearling heifers of Piedmontese influence were utilized for this trial. Both steers and heifers were divided into three distinct categories based upon which allele of the myostatin gene each animal possessed. The three alleles represented consisted of homozygous dominant (MM, active myostatin gene), heterozygous (Mm, partially recessive myostatin gene), and homozygous recessive (mm, mutated myostatin gene). To ensure possession of alleles, DNA testing was performed to confirmed cattle genotypes as MM, Mm, or mm for the myostatin gene. Steers and heifers were delivered to the Agricultural Research and Development Center Research Feedlot and fed a common finishing diet within an all-natural program for 232 (steers) and 191 (heifers) d. Common finishing diets consisted of 20% *Sweet Bran*®, 20% modified distiller grain plus soluble, 48% high-moisture: dry rolled corn blend, 8% grass hay, and 4% supplementation. Cattle received no implants and diet supplements contained no additives as to full fill the requirements of the all-natural feeding program.

### *Sample Collection*

Piedmontese cattle were harvested at Nebraska Prime (Hastings, NE) on August 12, 2011 (steers) and January 20, 2012 (heifers). Allele breakdown for steers was: 21 were carriers of the homozygous dominant allele, 22 were carriers of the heterozygous allele, and 16 were carriers of the homozygous recessive allele of the myostating gene. Allele breakdown for heifers was: 21 were carriers of the homozygous dominant allele,

22 were carriers of the heterozygous allele, and 16 were carriers of the homozygous recessive allele of the myostating gene.

For both steers and heifers at 3 d post-mortem the eye of round (IMPS # 171C, NAMP, 2007) and strip loins (IMPS # 180, NAMP, 2007) were collected from each carcass. To ensure identity of the eye of round and strip loin during fabrication, prior to fabrication, eye of rounds and strip loins from the left side of each carcass were numbered using push-pins and carcass ink. These sides were then followed through fabrication and samples removed from the production line. Individual identity of each cut was maintained throughout the fabrication process. The collected eyes of round and strip loin samples were collected, identified with additional tags, vacuum-packaged, boxed, and shipped back to the Loeffel Meat Laboratory at the University of Nebraska-Lincoln. Samples were placed in the carcass cooler at 2°C until fabricated. During fabrication of steer carcasses, one strip loin from a homozygous dominant steer was lost.

### ***Eye of Round Fabrication***

Upon arrival at the Loeffel Meat Laboratory, three steaks were cut from the middle of each eye of round. The first steak removed was a 1.3 cm thick steak utilized for nutrient analysis, the second steak removed was a 2.5 cm thick steak for Warner-Bratzler shear force analysis (WBSF), and third and final steak was a 1.3 cm thick steak utilized as a backup. Each steak was then individually vacuum-packed (nylon-polyethylene vacuum pouches; 3 mil STD barrier, Prime Source; St. Louis, MO) using a Multivac Packaging machine (MULTIVAC C500, Multivac Inc. Kansas City, MO). Steaks were then stored at 3°C for 14 d (post-harvest aging). Backup steaks and steaks

identified for nutrient analysis were frozen after 14 days of aging in a -20°C freezer.

Nutrient steaks were then shipped to Midwest Laboratories, Inc. (Omaha, NE) for proximate, lipid, and mineral analysis.

### ***Strip Loin Fabrication***

Upon arrival at the Loeffel Meat Laboratory prior to fabrication, each strip loin was measured for loin weight, loin length, sirloin face width, rib face width, sirloin tail length, rib tail length and fat thickness at the rib face. A photograph was then taken of each strip loin at the vein steak face. Loins were then cut from anterior to posterior. The first three steaks were cut as follows: one 2.5 cm thick steak for WBSF, one 1.3 cm thick steak for nutrient analysis, and one 1.3 cm thick steak to serve as a backup. The remainder of the strip loin was then cut into 2.5 cm steaks where the following information was gathered for each individual strip loin: total number of steaks, total number of vein steaks, total number of non-vein steaks. Weight of each individual steak was noted. The two 1.3 cm thick steaks collected for nutrient analysis and backup were considered as one 2.5 cm thick steak for the above information gathered (e.g., steak number). Tracings were also completed for all vein steak using acetate paper. A second photograph was then taken of each strip loin with all steaks laid out with the posterior side of each steak facing upward.

Steaks for nutrient analysis were trimmed to 0.3 cm subcutaneous fat. The first three steaks collected for shear force, nutrient analysis, and to use as backup were individually vacuum-packaged in a nylon-polyethylene vacuum pouches (3 mil STD barrier, Prime Source; St. Louis, MO) using a Multivac Packaging machine

(MULTIVAC C500, Multivac Inc. Kansas City, MO). Steaks were then stored at 3°C for 14 d (post-harvest aging). Steaks identified for Warner-Bratzler were cooked after 14 d postmortem and were fresh - never frozen. Nutrient analysis and backup steaks were placed in -20°C and frozen. Nutrient analysis steaks were then shipped to Midwest Laboratories, Inc. (Omaha, NE) for proximate, lipid and mineral analysis.

### ***Warner-Bratzler Shear Force***

At the conclusion of the 14 d aging period, steaks identified for WBSF were cooked fresh never frozen on a Hamilton Beach Indoor-Outdoor Grill (Hamilton Beach/Proctor Silex, Inc., Catalog No. HB9, Model 31605A, Series type G16 Grill, 120 v ~60 Hz, 1200 W) to an internal temperature of 71°C. Internal temperature of steaks was monitored using an OMEGA thermometer (Model 450A, OMEGA Engineering Inc., Stamford, CT) with a type T thermocouple (Model L-0044T Fine Wire Thermocouple, OMEGA Engineering Inc., Stamford, CT) inserted in to the geometric center of the steak using a pointed metal needle for guidance. Initial weights prior to thermocouples being inserted and final weights with thermocouples removed were measured immediately after removal from grill to calculate cooking loss of each steak; initial and final temperatures were recorded. Steaks were cooked on one side to an internal temperature of 35°C and were then cooked on the other side to an internal temperature of 71°C. Cooked steaks were placed on plastic trays, covered with oxygen-permeable film, and placed in a 3°C cooler overnight.

The following morning steaks were removed from the cooler and six 1.3 cm-diameter cores were prepared parallel to the muscle fibers using a Delta 20.3 cm Drill

Press (Mfg. Ser. No. W9609, Model 11-950, Delta International Machinery Corp., Pittsburgh, PA). The cores were then sheared using a tabletop Warner-Bratzler Shear Force Machine (Saltner Brecknell, Model 235 6X: Motor for Shearer: Bodine Electric Company, Small Motor S/N 0291KUIL 0009 Chicago, IL) and results were recorded for each core and the average of the six cores was determined for each steak.

### ***Nutrient Analysis***

One 1.3 cm-thick steak per loin trimmed to 0.3 cm subcutaneous fat and one 1.3 cm-thick steak per eye of round were shipped to Midwest Laboratories, Inc. (Omaha, NE) for further analysis. Midwest Laboratories, Inc. followed protocols listed in the AOAC. The following methods were used; to conduct moisture (AOAC 950.46), protein (AOAC 990.03), fat (AOAC 991.36), ash (900.02a), total dietary fiber (AOAC 991.43), cholesterol (AOAC 976.26), fatty acid profile (AOAC 996.06), and minerals (AOAC985.01 mod.) Proximate, lipid, and mineral analysis results were reported for a 113.40 gram serving size.

### ***Statistical Analysis***

Data were analyzed in a completely randomized design using ANOVA in PROC GLM in SAS (Version 9.2. Cary, N.C.) Fixed and random effects were the different inactive myostatin mutation and animal used, respectively. Separation of means was determined using LS MEANS and DIFF LINES options of SAS, with significance determined at  $P \leq 0.05$ .

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**MANUSCRIPT 1**

**Nutrient and Tenderness Differences in Beef from Cattle due to Mutation of the  
Myostatin Gene<sup>1,2</sup>**

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## ABSTRACT

The objectives of this study were to determine tenderness, nutrient, and composition differences of meat from cattle with different alleles of the myostatin gene. Fifty-nine steers and 59 heifers were genotyped for the myostatin gene; 21 steers and 19 heifers were identified as carriers for the homozygous dominant allele (MM), 22 steers and 20 heifers were carriers of the heterozygous allele (Mm), and 16 steers and 20 heifers were carriers of the homozygous recessive allele (mm) of the myostatin gene were harvested. Three d post mortem *Longissimus dorsi* (LD) and *Semitendinosus* (ST) were collected each carcass. From both the LD and ST, one 1.3 cm steaks were analyzed for nutrient content and one 2.5 cm steaks removed for Warner-Bratzler shear force (WBS) analysis (14 d post-mortem). The LD and ST steaks collected from steers and heifers were analyzed separately. Steaks from ST of MM steers had greater ( $P < 0.001$ ) for WBS values (3.6 vs. 3.1 kg) than steaks mm steers. There were no differences ( $P = 0.29$ ) in WBS values among genotypes for the ST of heifers and there was also no difference in the LD of both steers ( $P = 0.13$ ) and heifers ( $P = 0.16$ ). Fat content from mm steaks decreased ( $P < 0.001$ ) while moisture ( $P < 0.001$ ) and protein increased ( $P < 0.001$ ) compared to MM and Mm. Calories decreased with increasing copies of recessive gene ( $P < 0.001$ ). Steaks from mm had greater concentrations of cholesterol ( $P < 0.001$ ), decreased levels of saturated fatty acids ( $P < 0.001$ ), monounsaturated fatty acids ( $P < 0.001$ ), polyunsaturated fatty acids ( $P < 0.001$ ) and trans fatty acids ( $P < 0.001$ ) than MM. Fat thickness decreased ( $P < 0.001$ ) for heterozygous and homozygous recessive cattle compared to homozygous dominant cattle. In summary, steaks from cattle

homozygous recessive had decreased fat content, greater cholesterol content, decreased levels of saturated, monounsaturated, polyunsaturated, and trans fatty acids, and greater protein concentration when compared to cattle homozygous dominant for the myostatin gene.

**Key Words:** Piedmontese, beef, myostatin, nutrient analysis



## Introduction

The Piedmontese breed of cattle is a breed that possesses a genetic mutation of the myostatin gene. Commonly referred to as double muscling, the mutation of the myostatin gene in Piedmontese cattle results in a dramatic increase in overall muscle mass due to the inability of myostatin to control/regulate myogenesis.. The increase in muscle mass is due to pre-natal muscle hyperplasia, which in turn results in Piedmontese cattle producing carcasses that are heavier muscled and leaner compared to cattle that do not possess a myostatin mutation (Cases et al., 1998).

A concern of the beef industry is the impact of the myostatin mutation on beef tenderness due to the increase muscle mass and decrease overall fat composition . However, researchers have reported that steaks from some double muscle cattle are more tender than steaks from cattle without the myostatin mutation. Factors that influence tenderness include postmortem proteolysis, marbling or intramuscular fat, connective tissue, and the contraction state of the muscle (Belew et al., 2003). Double muscled cattle have improved meat tenderness compared to homozygous normal cattle that carry zero copies of the mutated myostatin gene. Previous research conducted by Wheeler et al. (2001) showed that the percent composition of the Piedmontese breed had no effect on tenderness and that improved tenderness was dependent on the myostatin genotype. Further research by Wheeler et al. (1996) with the completion of a sensory panel showed meat from Piedmontese to receive the highest ratings indicating superior tenderness.

Environmental and genetic factors can impact fatty acid composition of meat (De Smet et al., 2004). A genetic factor for example is seen with double muscled animals that

tend to have an increased percent of polyunsaturated fatty acid content of the phospholipid fraction (De Smet et al., 2004; Wood et al., 2008). Fatty acid composition is also altered by overall fat and muscle content of the animal. As an animal fattens the triacylglycerols dilute the phospholipids concentration and thus decrease the polyunsaturated to saturated fatty acid ratio (De Smet et al., 2004). Cholesterol is also a component of meat lipids that help stabilize the membrane of cells (Du et al., 2009) and an increase in cholesterol is correlated to an increase in polyunsaturated fatty acids as phospholipids are rich in polyunsaturated fatty acids (Rule et al., 1997). Cholesterol content is thus determined based upon biological cellular functions and not environmental factors such as feed, diet, or breed.

It was hypothesized that meat from homozygous recessive steers and heifers would be equivalent in tenderness to homozygous dominant and heterozygous recessive cattle even though the product is leaner. Therefore, this study was conducted to compare tenderness, nutritional, and compositional differences of cattle due to mutations of the myostatin gene.

### **Materials & Methods**

Fifty-nine steers and fifty-nine heifers of Piedmontese influence were utilized for this trial. Steers and heifers were divided into three categories based upon which allele of the myostatin gene each animal possessed. The three alleles represented consisted of homozygous dominant (MM, active myostatin gene), heterozygous (Mm, partially recessive myostatin gene), and homozygous recessive (mm, mutated myostatin gene). To ensure possession of alleles, DNA testing was performed to confirmed cattle genotypes

as either MM, Mm, or mm for the myostatin gene. Steers and heifers were delivered to the Agricultural Research and Development Center Research Feedlot and fed a common finishing diet within an all-natural program for 232 (steers) and 191 (heifers) d. Common finishing diets consisted of 20% *Sweet Bran*®, 20% modified distiller grain plus soluble, 48% high-moisture: dry rolled corn blend, 8% grass hay, and 4% supplementation. Cattle received no implants and diet supplements contained no additives as to full fill the requirements of the all-natural feeding program.

Piedmontese cattle were harvested at Nebraska Prime (Hastings, NE). At 3 d postmortem samples were taken from the left side of the carcass where from the steers fifty-nine eye of rounds (IMPS # 171C, NAMP, 2007) and fifty-eight strip loins (IMPS # 180, NAMP, 2007) gathered. One strip loin identified from a steers genotyped as heterozygous recessive was lost during fabrication. Heifers (n = 19, 20, and 20) were also harvested and fifty-nine eye of rounds (IMPS # 171C, NAMP, 2007) and fifty-nine strip loins (IMPS # 180, NAMP, 2007) were also gathered. Samples were vacuum packaged, boxed, and shipped back to Loeffel Meat Laboratory at the University of Nebraska-Lincoln and placed in the carcass cooler at 2°C until fabricated.

Three steaks were taken from the center of each eye of round, with one 1.3 cm steak cut for nutrient analysis, one 2.5 cm steak for Warner-Bratzler shear force testing, and one 1.3 cm steak was cut and saved as a backup. All steaks were individual vacuum-packaged and placed in a 3°C cooler and allowed to age 14 days post-harvest. Warner-Bratzler steaks were cooked fresh and never frozen after aging for 14 days. Steaks designated for nutrient analysis and backups were frozen after 14 days in a -20°C freezer.

Steaks for nutrient analysis were shipped to Midwest Laboratories, Inc. (Omaha, NE) for proximate, lipid, and mineral analysis.

For strip loin fabrication three steaks were cut as followed from the most anterior end: one 2.5 cm steak for Warner-Bratzler shear force, one 1.3 cm steak for nutrient analysis, and one 1.3 cm steak to be saved as a backup. Steaks identified for nutrient analysis for trimmed to 0.3 cm subcutaneous fat. The three most anterior steaks taken for shear force, nutrient analysis, and to use as backup were individually vacuum packaged and placed in a 3°C cooler and allowed to age for a total of 14 days postmortem. After 14 days of aging, Warner-Bratzler steaks were cooked fresh never frozen, while nutrient analysis and Warner-Bratzler steaks were placed in -20°C and frozen. Nutrient analysis steaks were then shipped to Midwest Laboratories, Inc. (Omaha, NE) for proximate, lipid and mineral analysis. Midwest Laboratories, Inc then followed AOAC protocols to conduct moisture, protein, fat, ash, total dietary fiber, cholesterol, fatty acid composition, and mineral analysis on both eye of rounds and strip loin samples.

Warner-Bratzler steaks were cooked on a Hamilton Beach Indoor-Outdoor Grill (Hamilton Beach/Proctor Silex, Inc., Catalog No. HB9, Model 31605A, Series type G16 Grill, 120 v ~60 Hz, 1200 W) after 14 days of aging. A thermocouple (Model L-0044T Fine Wire Thermocouple, OMEGA Engineering Inc., Stamford, CT) was inserted into the geometric center of each steak and temperature was monitored using a OMEGA thermometer (Model 450A, OMEGA Engineering Inc., Stamford, CT). Initial weight of raw steaks prior to insertion of thermocouples and cooked steaks after the removal of thermocouples were measured to calculate drip loss of each steak. Initial and final

temperatures were also noted. Steaks were allowed to cook on one side until they reached an internal temperature reached 35°C and were then turned over to continue cooking until they reached 71°C (AMSA 1995). When steaks reached 71°C they were placed on a plastic tray and covered with oxygen-permeable film and placed into a 3°C cooler overnight.

The next morning steaks were removed from the cooler and six 1.3 cm cores were taken parallel to the muscle fibers using a Delta Drill Press (Delta 20.3 cm Drill Press, Mfg. Ser. No. W9609, Model 11-950, Delta International Machinery Corp., Pittsburgh, PA). Cores were then sheared using a tabletop Warner-Bratzler Shear Force Machine (Saltner Brecknell, Model 235 6X: Motor for Shearer: Bodine Electric Company, Small Motor S/N 0291KUIL 0009 Chicago, IL) and results were recorded for each core that was sheared and the average of the six cores was determined for each steak

Data was analyzed in a completely randomized design using ANOVA in PROC GLM in SAS (Version 9.2. Cary, N.C.) Fixed effects were the inactive myostatin mutation and random effects were the animals used. Separation of means was determined using LS MEANS and DIFF LINES options of SAS, with significance determined at  $P \leq 0.05$ .

## **Results & Discussion**

### *Carcass Traits*

Carcass traits for steers and heifers with differences due to the mutation of the myostatin gene are found in Tables 1 and 2. For both steers and heifers with increasing

copies of the recessive myostatin gene loin muscle area increased ( $P < 0.001$ ), while marbling score decreased ( $P < 0.001$ ), and calculated yield grade also decreased ( $P < 0.001$ ). These findings agree with Cases et al. (1998) who suggest that double muscled cattle such as Piedmontese have an increase in skeletal mass but also produce carcasses that are leaner. Moreover, Kambadur et al. (1997) states that myostatin is “partially recessive” because as there was some noticeable differences in cattle that are heterozygous recessive compared to homozygous dominant cattle. Hot carcass weights of homozygous recessive steers was lower ( $P < 0.001$ ) when compared to homozygous dominant and heterozygous recessive carcasses. There was no difference ( $P = 0.16$ ) in the hot carcass weight of heifers.

#### *Warner-Bratzler Shear Force*

Eye of round steaks from steers possessing either the homozygous recessive or heterozygous allele for the myostatin gene had WBSF values that were less ( $P \leq 0.001$ ) when compared to steaks from steer with the homozygous dominant (2.99 and 3.10 vs. 3.60 kg; Table 3). These findings agree with Wheeler et al. (1996) whose results suggest that Piedmontese heterozygous recessive cattle with one copy have improved tenderness of lower-valued cuts, such as the eyes of rounds, which could result in increased carcass value. In the current study, steaks from the eye of rounds of heifers showed no difference in tenderness ( $P = 0.29$ ).

When strip loins of steers and heifers were analyzed there was also no difference in Warner-Bratzler shear force values detected for beef from steers ( $P = 0.13$ ) or beef from heifers ( $P = 0.16$ ). These results are in contrast to the results reported by Wheeler et

al. (1996) who reported lower shear force values and improved sensory panel tenderness ratings of *Longissimus* from Piedmontese than other breeds, such as Angus. Thus, beef from homozygous recessive cattle is equivalent in tenderness to homozygous dominant and heterozygous recessive cattle.

### *Proximate Analysis*

As expected with increasing copies of the recessive gene for myostatin overall fat percent decreased ( $P < 0.001$ ) and percent protein increased ( $P < 0.001$ ) for strip loins and eye of rounds for both steers and heifers. These data are consistent with Casas et al. (1998) who noted that double muscled cattle have an increase in skeletal muscle mass as well as yielding leaner carcasses than those cattle that do not possess a myostatin mutation. Furthermore, Wheeler et al. (1996) reported that percent protein was greatest and percent lipid was least in raw *Longissimus* steaks of Piedmontese-sired steers compared to Hereford, Angus, Charlois, Gelbvieh, Pinzgauer, Shorthorn, Galloway, Longhorn, Nellore, Piedmontese, or Saler sires.

Percent moisture was also consistent for beef from steers and heifers, as copies of the recessive myostatin gene increased percentage of moisture also increased ( $P < 0.001$ ). These results were also expected as greater fat content within a carcass results in decreased water content due to fat containing limited amounts of water (Romans et al., 1994). Calorie content in strip loins and eye of rounds also decreased ( $P < 0.001$ ) with increasing copies of the recessive gene. Ash content was greater ( $P \leq 0.001$ ) in steaks from homozygous recessive cattle with compared to homozygous dominant except for eye of round of steers where homozygous recessive was greater ( $P =$

0.02) than heterozygous recessive. There were no differences ( $P \leq 0.69$ ) in percent of carbohydrates between genotypes. Carbohydrates are stored in the animal's body as glycogen and are converted to lactic acid postmortem (Romans et al., 1994). Thus few carbohydrates remain in the meat once animals are harvested.

### *Lipid Analysis*

Lipid analysis results are summarized in Tables 9 through 12. Steaks from homozygous recessive cattle had greater concentration of cholesterol ( $P \leq 0.001$ ) than homozygous dominant steaks. As well as being a component of meat lipids cholesterol helps stabilize the cell membrane (Du et al., 2009). With Piedmontese cattle having increase muscle mass due to hyperplasia (increased fiber number) it is expected for these cattle to have increased cholesterol concentration to stabilize the cell membrane from the increased number of muscle cells. This is supported by Wheeler et al. (1987) who noted that cholesterol content is dependent on need for cellular membrane function and not environmental factors such as feed, diet, or breed.

Saturated fatty acids and monounsaturated fatty acids decreased ( $P < 0.001$ ) with increasing copies of the recessive gene for myostatin. As stated above steaks from homozygous dominant cattle had an increase in overall fat content compared to homozygous recessive. As cattle such as homozygous recessive cattle fatten, monounsaturated fatty acid increased (De Smet et al., 2004) and the results within the current study agree with this. Trans fatty acids also followed the same patterns with the exception of the strip loins of heifers where homozygous recessive steaks had a lower concentration ( $P < 0.001$ ) compared to heterozygous recessive and homozygous



dominant. Within the strip loins of both steers and eye of round of heifers polyunsaturated fatty acid concentration decreased ( $P < 0.001$ ) with increasing copies of the recessive myostatin gene while strip loins from homozygous dominant heifers had a greater concentration ( $P < 0.001$ ) of polyunsaturated fatty acids and eye of round steaks from homozygous recessive steers was greater ( $P < 0.001$ ) when compared to homozygous dominant and heterozygous recessive.

### *Mineral Analysis*

Mineral analysis revealed less consistent patterns between heifers and steers and between eye of rounds and strip loins compared to proximate and lipid analysis. Sodium concentration increased ( $P = 0.04$ ) within the strip loin of homozygous recessive steers compared to heterozygous recessive (Table 13). Potassium analysis indicated an increase ( $P < 0.001$ ) in concentration with increased copies of the recessive myostatin gene. These results agree with the findings of Clark et al. (1972) where they concluded that differences in potassium concentration was attributed to fat content and that fatter animals have decreased concentrations of potassium than leaner animals. Calcium was greater ( $P = 0.001$ ) in heterozygous recessive and homozygous recessive steer samples compared to homozygous dominant. In the strip loins of heifers potassium also increased ( $P < 0.001$ ) with increasing copies of the recessive gene for myostatin, as did calcium ( $P < 0.001$ ). Sodium was greater ( $P = 0.02$ ) in heterozygous recessive and homozygous recessive heifers steaks than homozygous dominant (Table 16). No difference was observed in iron concentration of strip loins for both heifers ( $P = 0.12$ ) and steers ( $P = 0.94$ ).

Within the eye of round steaks of both homozygous recessive steers and heifers samples had a decreased concentration ( $P < .001$ ) of iron compared to homozygous dominant and heterozygous recessive samples. Iron is found in both myoglobin and hemoglobin molecules and muscle foods with a large concentration of these pigments, such as red muscle fibers, are a good source of iron (Kinsman et al., 1994). With double muscled cattle have a greater proportion of white muscle fibers and decreased proportion of red fibers (Aberle et al., 2001; Wegner et al., 2000) it is not surprising to see steaks from homozygous recessive cattle having a decreased concentration of iron than homozygous dominant cattle. Potassium analysis from eye of round steaks indicated that steaks from homozygous recessive cattle had a higher concentration ( $P < 0.001$ ) than homozygous dominant. Mineral analysis for sodium and calcium were inconsistent between steer and heifer steak samples. Heterozygous recessive steer steaks had a decreased sodium concentration ( $P = 0.04$ ) than homozygous dominant while no difference in sodium levels of heifers were noted ( $P = 0.77$ ). Meat from homozygous recessive heifers possessed a higher ( $P = 0.007$ ) concentration of calcium than homozygous dominant and heterozygous recessive heifers and no difference ( $P = 0.39$ ) in calcium concentration was found for steers within the eye of round steaks.

In summary, steaks from homozygous recessive cattle had a decreased fat content, greater cholesterol, and decreased concentration of saturated, monounsaturated, polyunsaturated, and trans fatty acid, and greater protein levels when compared to homozygous normal cattle. Furthermore, as hypothesized beef from homozygous

recessive cattle is equivalent for tenderness compared to homozygous dominant and heterozygous recessive cattle even though the product is leaner.

**Table 1: Steers carcass traits**

Trait	Myostatin Genotype <sup>1</sup>			SEM	P-Value
	MM	Mm	mm		
Hot Carcass Weight (kg)	1936.62 <sup>a</sup>	1864.49 <sup>a</sup>	17586.62 <sup>b</sup>	45.354	0.001
Marbling <sup>2</sup>	721.00 <sup>a</sup>	502.27 <sup>b</sup>	343.12 <sup>c</sup>	24.132	< 0.001
Loin Muscle area, cm <sup>2</sup>	85.10 <sup>c</sup>	103.16 <sup>b</sup>	109.42 <sup>a</sup>	1.111	< 0.001
CYG <sup>3</sup>	3.09 <sup>a</sup>	1.38 <sup>b</sup>	0.26 <sup>c</sup>	0.159	< 0.001

<sup>abc</sup> Means with different superscripts within the same row are considered different  $P \leq 0.05$ .

<sup>1</sup>Myostatin: homozygous active (MM), heterozygous partially recessive (Mm), and homozygous recessive inactive (mm).

<sup>2</sup>Marbling: 700 = MD, 600 = MT, 500 = SM, 400 = SL, 300 = TR, 200 = PD

<sup>3</sup>Calculated Yield Grade =  $2.5 + (2.5 * 12^{\text{th}} \text{ rib fat, in.}) + (0.0038 * \text{HCW, lb.}) - (0.32 * \text{LM area, in}^2) + (0.2 * \text{estimated KPH, \%})$

**Table 2: Heifer carcass traits**

<b>Trait</b>	<b>Myostatin Genotype<sup>1</sup></b>			<b>SEM</b>	<b>P-Value</b>
	<b>MM</b>	<b>Mm</b>	<b>mm</b>		
Hot Carcass Weight (kg)	2002.33	1933.07	1884.30	60.370	0.16
Marbling <sup>2</sup>	556.32 <sup>a</sup>	414.00 <sup>b</sup>	316.50 <sup>c</sup>	19.070	< 0.001
Loin Muscle area, cm <sup>2</sup>	90.06 <sup>c</sup>	105.16 <sup>b</sup>	124.32 <sup>a</sup>	4.103	< 0.001
CYG <sup>3</sup>	3.30 <sup>a</sup>	1.40 <sup>b</sup>	0.04 <sup>c</sup>	0.216	< 0.001

<sup>abc</sup> Means with different superscripts within the same row are considered different  $P \leq 0.05$ .

<sup>1</sup>Myostatin: homozygous active (MM), heterozygous partially recessive (Mm), and homozygous recessive inactive (mm).

<sup>2</sup>Marbling: 700 = MD, 600 = MT, 500 = SM, 400 = SL, 300 = TR, 200 = PD

<sup>3</sup>Calculated Yield Grade =  $2.5 + (2.5 * 12^{\text{th}} \text{ rib fat, in.}) + (0.0038 * \text{HCW, lb.}) - (0.32 * \text{LM area, in}^2) + (0.2 * \text{estimated KPH, \%})$

**Table 3: Steers cooking loss and shear force**

<b>Trait</b>	<b>Myostatin Genotype<sup>1</sup></b>			<b>SEM</b>	<b>P-Value</b>
	<b>MM</b>	<b>Mm</b>	<b>mm</b>		
% Strip Steak Cooking Loss	16.34	17.61	15.96	1.574	0.69
Strip Steak Shear Force (kg)	2.62	2.79	2.87	0.095	0.13
% Eye of Round Cooking Loss	12.21	14.05	15.34	1.680	0.38
Eye of Round Shear Force (kg)	3.6 <sup>a</sup>	2.99 <sup>b</sup>	3.10 <sup>b</sup>	0.052	<0.001

<sup>abc</sup> Means with different superscripts within the same row are considered different  $P \leq 0.05$ .

<sup>1</sup>Myostatin: homozygous active (MM), heterozygous partially recessive (Mm), and homozygous recessive inactive (mm).

**Table 4: Heifer cooking loss and shear force**

<b>Trait</b>	<b>Myostatin Genotype<sup>1</sup></b>			<b>SEM</b>	<b>P-Value</b>
	<b>MM</b>	<b>Mm</b>	<b>mm</b>		
% Strip Steak Cooking Loss	14.35 <sup>b</sup>	21.19 <sup>a</sup>	15.96 <sup>b</sup>	1.254	< 0.001
Strip Steak Shear Force (kg)	2.62	3.08	2.82	0.097	0.16
% Eye of Round Cooking Loss	23.16	26.14	27.00	1.551	0.19
Eye of Round Shear Force (kg)	3.60	3.70	3.45	0.117	0.29

<sup>abc</sup> Means with different superscripts within the same row are considered different  $P \leq 0.05$ .

<sup>1</sup>Myostatin: homozygous active (MM), heterozygous partially recessive (Mm), and homozygous recessive inactive (mm).

**Table 5: Steers proximate analysis strip loin**

Proximate	Unit	Myostatin Genotype <sup>1</sup>			P-Value	
		MM	Mm	mm		
Number of Strips Loins Analyzed		20	22	16		
Moisture	%	58.92 <sup>c</sup>	65.23 <sup>b</sup>	70.45 <sup>a</sup>	0.509	< 0.001
Protein	%	19.39 <sup>c</sup>	20.82 <sup>b</sup>	24.22 <sup>a</sup>	0.291	< 0.001
Fat	%	20.72 <sup>a</sup>	13.15 <sup>b</sup>	3.49 <sup>c</sup>	0.728	< 0.001
Ash	%	0.635 <sup>c</sup>	0.828 <sup>b</sup>	0.954 <sup>a</sup>	0.042	< 0.001
Carbohydrates	%	1.028	0.78	1.34	0.354	0.50
Calories	CALORIES	298.40 <sup>a</sup>	227.41 <sup>b</sup>	148.37 <sup>c</sup>	6.115	< 0.001

<sup>abc</sup> Means with different superscripts within the same row are considered different  $P \leq 0.05$ .

<sup>1</sup>Myostatin: homozygous active (MM), heterozygous partially recessive (Mm), and homozygous recessive inactive (mm).



**Table 6: Heifers proximate analysis strip loin**

Proximate	Unit	Myostatin Genotype <sup>1</sup>			SEM	P-Value
		MM	Mm	mm		
Number of Strips Loins Analyzed		19	20	20		
Moisture	%	57.00 <sup>c</sup>	62.29 <sup>b</sup>	67.27 <sup>a</sup>	0.603	< 0.001
Protein	%	19.65 <sup>c</sup>	20.88 <sup>b</sup>	22.32 <sup>a</sup>	0.257	< 0.001
Fat	%	21.48 <sup>a</sup>	15.96 <sup>b</sup>	9.46 <sup>c</sup>	0.728	< 0.001
Ash	%	0.50 <sup>b</sup>	0.74 <sup>a</sup>	0.81 <sup>a</sup>	0.046	< 0.001
Carbohydrates	%	0.66	0.48	0.59	0.135	0.69
Calories	CALORIES	306.58 <sup>a</sup>	255.90 <sup>b</sup>	197.30 <sup>c</sup>	6.627	< 0.001

<sup>abc</sup> Means with different superscripts within the same row are considered different  $P \leq 0.05$ .

<sup>1</sup>Myostatin: homozygous active (MM), heterozygous partially recessive (Mm), and homozygous recessive inactive (mm).

**Table 7: Steers proximate analysis eye of round**

Proximate	Unit	Myostatin Genotype <sup>1</sup>			SEM	P-Value
		MM	Mm	mm		
Number of Eyes Analyzed		21	22	16		
Moisture	%	69.36 <sup>c</sup>	72.67 <sup>b</sup>	73.89 <sup>a</sup>	0.306	< 0.001
Protein	%	21.51 <sup>c</sup>	23.44 <sup>b</sup>	24.25 <sup>a</sup>	0.185	< 0.001
Fat	%	7.88 <sup>a</sup>	3.51 <sup>b</sup>	0.78 <sup>c</sup>	0.418	< 0.001
Ash	%	0.92 <sup>b</sup>	1.05 <sup>a</sup>	0.93 <sup>b</sup>	0.040	0.02
Carbohydrates	%	1.08	0.31	0.478	0.339	0.11
Calories	CALORIES	178.71 <sup>a</sup>	140.77 <sup>b</sup>	117.63 <sup>c</sup>	3.654	< 0.001

<sup>abc</sup> Means with different superscripts within the same row are considered different  $P \leq 0.05$ .

<sup>1</sup>Myostatin: homozygous active (MM), heterozygous partially recessive (Mm), and homozygous recessive inactive (mm).

**Table 8: Heifers proximate analysis eye of round**

Proximate	Unit	Myostatin Genotype <sup>1</sup>			SEM	P-Value
		MM	Mm	mm		
Number of Eyes Analyzed		19	20	20		
Moisture	%	65.21 <sup>c</sup>	69.38 <sup>b</sup>	72.78 <sup>a</sup>	0.449	< 0.001
Protein	%	20.79 <sup>c</sup>	22.73 <sup>b</sup>	23.68 <sup>a</sup>	0.218	< 0.001
Fat	%	12.85 <sup>a</sup>	6.91 <sup>b</sup>	2.08 <sup>c</sup>	0.590	< 0.001
Ash	%	0.91 <sup>b</sup>	0.67 <sup>c</sup>	1.04 <sup>a</sup>	0.044	< 0.0001
Carbohydrates	%	0.45	0.57	0.80	0.144	0.22
Calories	CALORIES	224.16 <sup>a</sup>	173.30 <sup>b</sup>	129.20 <sup>c</sup>	5.258	< 0.001

<sup>abc</sup> Means with different superscripts within the same row are considered different  $P \leq 0.05$ .

<sup>1</sup>Myostatin: homozygous active (MM), heterozygous partially recessive (Mm), and homozygous recessive inactive (mm).

**Table 9: Steers lipid analysis strip loin**

<b>Lipid</b>	<b>Unit</b>	<b>Myostatin Genotype<sup>1</sup></b>			<b>SEM</b>	<b>P-Value</b>
		<b>MM</b>	<b>Mm</b>	<b>mm</b>		
Number of Strips Loins Analyzed		20	22	16		
Cholesterol	mg/100g	43.35 <sup>b</sup>	43.18 <sup>b</sup>	48.31 <sup>a</sup>	1.003	< 0.001
Saturated Fatty Acids	mg/100g	9.65 <sup>a</sup>	6.14 <sup>b</sup>	1.73 <sup>c</sup>	0.450	< 0.001
Monounsaturated Fatty Acids	mg/100g	10.16 <sup>a</sup>	6.28 <sup>b</sup>	1.43 <sup>c</sup>	0.519	< 0.001
Polyunsaturated Fatty Acids	mg/100g	0.70 <sup>a</sup>	0.56 <sup>b</sup>	0.27 <sup>c</sup>	0.039	< 0.001
Trans Fatty Acids	mg/100g	0.20 <sup>a</sup>	0.16 <sup>b</sup>	0.05 <sup>c</sup>	0.015	< 0.001

<sup>abc</sup> Means with different superscripts within the same row are considered different  $P \leq 0.05$ .

<sup>1</sup>Myostatin: homozygous active (MM), heterozygous partially recessive (Mm), and homozygous recessive inactive (mm).

**Table 10: Heifers lipid analysis strip loin**

<b>Lipid</b>	<b>Unit</b>	<b>Myostatin Genotype<sup>1</sup></b>			<b>SEM</b>	<b>P-Value</b>
		<b>MM</b>	<b>Mm</b>	<b>mm</b>		
Number of Strips Loins Analyzed		19	20	20		
Cholesterol	mg/100g	42.26 <sup>c</sup>	46.65 <sup>b</sup>	49.70 <sup>a</sup>	1.100	< 0.001
Saturated Fatty Acids	mg/100g	9.64 <sup>a</sup>	7.19 <sup>b</sup>	4.37 <sup>c</sup>	0.458	< 0.001
Monounsaturated Fatty Acids	mg/100g	10.85 <sup>a</sup>	7.92 <sup>b</sup>	4.36 <sup>c</sup>	0.539	< 0.001
Polyunsaturated Fatty Acids	mg/100g	0.75 <sup>a</sup>	0.64 <sup>b</sup>	0.59 <sup>b</sup>	0.038	< 0.001
Trans Fatty Acids	mg/100g	0.23 <sup>a</sup>	0.21 <sup>a</sup>	0.14 <sup>b</sup>	0.018	< 0.001

<sup>abc</sup> Means with different superscripts within the same row are considered different  $P \leq 0.05$ .

<sup>1</sup>Myostatin: homozygous active (MM), heterozygous partially recessive (Mm), and homozygous recessive inactive (mm).

**Table 11: Steers Lipid analysis eye of round**

<b>Lipid</b>	<b>Unit</b>	<b>Myostatin Genotype<sup>1</sup></b>			<b>SEM</b>	<b>P-Value</b>
		<b>MM</b>	<b>Mm</b>	<b>mm</b>		
Number of Eyes Analyzed		21	22	16		
Cholesterol	mg/100g	47.29 <sup>b</sup>	48.86 <sup>b</sup>	53.75 <sup>a</sup>	0.741	< 0.001
Saturated Fatty Acids	mg/100g	9.36 <sup>a</sup>	5.91 <sup>b</sup>	1.50 <sup>c</sup>	0.423	< 0.001
Monounsaturated Fatty Acids	mg/100g	10.29 <sup>a</sup>	6.08 <sup>b</sup>	1.32 <sup>c</sup>	0.513	< 0.001
Polyunsaturated Fatty Acids	mg/100g	1.03 <sup>a</sup>	0.89 <sup>a</sup>	0.64 <sup>b</sup>	0.090	< 0.001
Trans Fatty Acids	mg/100g	0.18 <sup>a</sup>	0.13 <sup>b</sup>	0.04 <sup>c</sup>	0.014	< 0.001

<sup>abc</sup> Means with different superscripts within the same row are considered different  $P \leq 0.05$ .

<sup>1</sup>Myostatin: homozygous active (MM), heterozygous partially recessive (Mm), and homozygous recessive inactive (mm).

**Table 12: Heifers lipid analysis eye of round**

<b>Lipid</b>	<b>Unit</b>	<b>Myostatin Genotype<sup>1</sup></b>			<b>SEM</b>	<b>P-Value</b>
		<b>MM</b>	<b>Mm</b>	<b>Mm</b>		
Number of Eyes Analyzed		19	20	20		
Cholesterol	mg/100g	41.47 <sup>c</sup>	43.70 <sup>b</sup>	48.55 <sup>a</sup>	0.724	< 0.001
Saturated Fatty Acids	mg/100g	5.52 <sup>a</sup>	2.994 <sup>b</sup>	0.88 <sup>c</sup>	0.362	< 0.001
Monounsaturated Fatty Acids	mg/100g	6.71 <sup>a</sup>	3.44 <sup>b</sup>	0.94 <sup>c</sup>	0.435	< 0.001
Polyunsaturated Fatty Acids	mg/100g	0.49 <sup>a</sup>	0.40 <sup>b</sup>	0.23 <sup>c</sup>	0.034	< 0.001
Trans Fatty Acids	mg/100g	0.13 <sup>a</sup>	0.08 <sup>b</sup>	0.03 <sup>c</sup>	0.01013	< 0.001

<sup>abc</sup> Means with different superscripts within the same row are considered different  $P \leq 0.05$ .

<sup>1</sup>Myostatin: homozygous active (MM), heterozygous partially recessive (Mm), and homozygous recessive inactive (mm).

**Table 13: Steers mineral analysis strip loin**

Mineral	Unit	Myostatin Genotype <sup>1</sup>			SEM	P-Value
		MM	Mm	mm		
Number of Strips Loins Analyzed		20	22	16		
Sodium	mg/kg	437.70 <sup>ab</sup>	419.80 <sup>b</sup>	443.52 <sup>a</sup>	7.401	0.04
Potassium	mg/kg	2800.85 <sup>c</sup>	3054.68 <sup>b</sup>	3305.19 <sup>a</sup>	47.470	< 0.001
Calcium	mg/kg	59.70 <sup>b</sup>	75.53 <sup>a</sup>	81.53 <sup>a</sup>	3.927	< 0.001
Iron	mg/kg	13.07	13.99	12.51	0.519	0.94

<sup>abc</sup> Means with different superscripts within the same row are considered different  $P \leq 0.05$ .

<sup>1</sup>Myostatin: homozygous active (MM), heterozygous partially recessive (Mm), and homozygous recessive inactive (mm).



**Table 14: Heifers mineral analysis strip loin**

Mineral	Unit	Myostatin Genotype <sup>1</sup>			SEM	P-Value
		MM	Mm	mm		
Number of Strips Loins Analyzed		19	20	20		
Sodium	mg/kg	381.82 <sup>b</sup>	401.68 <sup>a</sup>	404.39 <sup>a</sup>	5.915	0.02
Potassium	mg/kg	2597.21 <sup>c</sup>	2939.90 <sup>b</sup>	3134.65 <sup>a</sup>	37.978	< 0.001
Calcium	mg/kg	69.77 <sup>c</sup>	85.96 <sup>b</sup>	94.23 <sup>a</sup>	2.714	< 0.001
Iron	mg/kg	13.26	14.76	14.1	0.511	0.125

<sup>abc</sup> Means with different superscripts within the same row are considered different  $P \leq 0.05$ .

<sup>1</sup>Myostatin: homozygous active (MM), heterozygous partially recessive (Mm), and homozygous recessive inactive (mm).

**Table 15: Steers mineral analysis eye of round**

Mineral	Unit	Myostatin Genotype <sup>1</sup>			SEM	P-Value
		MM	Mm	mm		
Number of Eyes Analyzed		21	22	16		
Sodium	mg/kg	418.77 <sup>a</sup>	399.87 <sup>b</sup>	408.11 <sup>ab</sup>	5.985	0.04
Potassium	mg/kg	3483.76 <sup>b</sup>	3684.41 <sup>a</sup>	3754.31 <sup>a</sup>	36.109	< 0.001
Calcium	mg/kg	42.63	43.03	45.31	1.532	0.38
Iron	mg/kg	14.06 <sup>a</sup>	13.10 <sup>a</sup>	10.94 <sup>b</sup>	0.413	< 0.001

<sup>abc</sup> Means with different superscripts within the same row are considered different  $P \leq 0.05$ .

<sup>1</sup>Myostatin: homozygous active (MM), heterozygous partially recessive (Mm), and homozygous recessive inactive (mm).

**Table 16: Heifers mineral analysis eye of round**

<b>Mineral</b>	<b>Unit</b>	<b>Myostatin Genotype<sup>1</sup></b>			<b>SEM</b>	<b>P-Value</b>
		<b>MM</b>	<b>Mm</b>	<b>Mm</b>		
Number of Eyes Analyzed		19	20	20		
Sodium	mg/kg	368.94	373.89	373.31	5.280	0.77
Potassium	mg/kg	3091.16 <sup>c</sup>	3398.40 <sup>b</sup>	3529.20 <sup>a</sup>	35.151	< 0.001
Calcium	mg/kg	61.80 <sup>b</sup>	61.48 <sup>b</sup>	67.01 <sup>a</sup>	1.912	0.007
Iron	mg/kg	15.35 <sup>a</sup>	14.60 <sup>a</sup>	12.49 <sup>b</sup>	0.315	<0.001

<sup>abc</sup> Means with different superscripts within the same row are considered different  $P \leq 0.05$ .

<sup>1</sup>Myostatin: homozygous active (MM), heterozygous partially recessive (Mm), and homozygous recessive inactive (mm).

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**MANUSCRIPT 2****Vein Steak Differences in Strip Loins Due to Mutation of the Myostatin Gene<sup>1,2</sup>**

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## ABSTRACT

The objective of this study was to determine the musculature differences of strip loins of cattle with variations of the myostatin gene. Fifty-nine steers and 59 heifers were genotyped for the myostatin gene; 21 steers and 19 heifers were identified as carriers for the homozygous dominant allele (MM), 22 steers and 20 heifers were carriers of the heterozygous allele (Mm), and 16 steers and 20 heifers were carriers of the homozygous recessive allele (mm) of the myostatin gene were harvested. Strip loins were measured for fat thickness, loin weight, loin length, sirloin face width, rib face width, sirloin tail length, and rib tail length. Strip loins were then cut into 2.5 cm-thick steaks and the following noted: total number of steaks, total number of vein steaks, total number of non-vein steaks, and weight of each individual steak. Vein steaks were defined as steaks that contained the *Gluteus medius*. Strip loins from steers and heifers were analyzed separately. With increasing copies of the recessive gene for myostatin fat thickness decreased ( $P < 0.01$ ). For steers the average steak weight for Mm and mm samples was greater ( $P < 0.01$ ) than MM. The mm samples had a fewer number of total steaks ( $P < 0.01$ ) and fewer number of vein steaks ( $P < 0.01$ ) when compared to MM and Mm samples. Number of non-vein steaks was greater ( $P < 0.01$ ) for MM strip loins than Mm and mm. Strip loins of mm cattle were shorter ( $P < 0.01$ ) than MM and Mm. A wider sirloin face ( $P < 0.01$ ) and rib face ( $P < 0.01$ ) were observed for the Mm and mm samples compared to MM strip loins. Total weight of veins steaks and weight percentage of vein steaks did not differ between genotypes. When strip loins from heifers were analyzed; mm heifers were shorter ( $P < 0.01$ ) and had a wider rib face ( $P < 0.01$ ) when compared to

MM and Mm loins. The Mm strip loins had a greater number of non-vein steaks ( $P < 0.01$ ), and a decreased percentage of vein steaks ( $P = 0.01$ ) compared to MM and mm samples. As observed with steers, with increasing copies of the recessive gene for myostatin fat thickness also decreased ( $P < 0.01$ ). Mean steak weight, total weight of vein steaks, average steak weight, and percent weight of vein steaks within the strip loin did not differ among genotypes. In conclusion, Mm and mm steer samples yielded a heavier wider strip loin and higher average steak weight than MM while no difference in proportion of weight and percentage of vein steaks were noted.

**Key Words:** beef, myostatin, vein steaks



## Introduction

The recessive myostatin gene in Piedmontese cattle is a negative regulator of myogenesis that leads to an increase in muscle fiber number (hyperplasia). This result in approximately 20% increase in muscle mass of double muscled cattle (Kambadur et al., 1997). Cattle homozygous for the recessive myostatin gene commonly possess approximately twice the number of muscle fibers compared to conventional cattle (Swatland and Kieffer, 1974). Typically for double muscling to be observed phenotypically, cattle must be homozygous recessive for the myostatin gene. However, Kambadur et al. (1997) defined the allele as “partially recessive” due to some noticeable differences in muscularity of animals who are heterozygous for the recessive myostatin gene. Cattle whom are heterozygous or homozygous for the myostatin mutation will yield a greater amount of closely trimmed retail cuts with a greater proportion of the most desirable cuts such as the strip loin and rib roll (Casas et al., 1998; Kambadur et al 1997).

Toward the posterior end of the strip loin the *Gluteus medius* increases in size while the *Longissimus dorsi* decreases in size and narrows. The *Longissimus dorsi* represents a larger proportion of total carcass value than any other muscle within the beef carcass (Wheeler et al. 2000). Steaks that include the *Gluteus medius* possess a piece of connective tissue separating it from the *Longissimus dorsi*. This connective tissue forms a web like structure and when cooked will shrink and thus develop tension resulting in decreased tenderness (Du and McCorminck, 2006). Tenderness differences can also be observed between the *Longissimus dorsi* and *Gluteus medius* muscles. When crossbreeding of cattle and its effects on tenderness were studied Slinger et al. (1985)

results indicate the longissimus muscle to be more tender than the *Gluteus medius*. Wheeler et al. (2000) observed similar results with the completion of a consumer taste panel where 92% of the *Longissimus dorsi* muscles were rated as “slightly tender”; in comparison to only 89% of the *Gluteus medius* muscles. Steaks containing the *Gluteus medius* are known as vein steaks (Figure 1) and are less tender and thus lower in value than strip steaks without the *Glutues medius*.

Therefore, this study was conducted to compare the amount and musculature differences of strip loin of cattle due to the inactive myostatin mutation and determine if homozygous recessive cattle would produce a greater numbers of vein steaks from the strip loin containing the *Gluteus medius* when compared to homozygous dominant and heterozygous recessive cattle for the mutation of the myostatin gene.

### **Materials & Methods**

Fifty-nine calf-fed steers and fifty-nine yearling heifers of Piedmontese influence were utilized for this trial. Both steers and heifers were divided into three distinct categories based upon which allele of the myostatin gene each animal possessed. The three alleles represented consisted of homozygous dominant (MM, active myostatin gene), heterozygous (Mm, partially recessive myostatin gene), and homozygous recessive (mm, mutated myostatin gene). To ensure possession of alleles, DNA testing was performed to confirmed cattle genotypes as MM, Mm, or mm for the myostatin gene. Steers and heifers were delivered to the Agricultural Research and Development Center Research Feedlot and fed a common finishing diet within an all-natural program for 232 (steers) and 191 (heifers) d. Common finishing diets consisted of 20% *Sweet Bran*®,

20% modified distiller grain plus soluble, 48% high-moisture: dry rolled corn blend, 8% grass hay, and 4% supplementation. Cattle received no implants and diet supplements contained no additives as to full fill the requirements of the all-natural feeding program.

Piedmontese influenced cattle were harvested at Nebraska Prime (Hastings, NE). At 3 d postmortem samples were collected from the left side of the carcass where fifty-eight strip loins (IMPS # 180, NAMP, 2007) were gathered from the steers and fifty-nine strip loins collected from the heifer carcasses. One strip loin was lost during fabrication from a steer genotyped for homozygous dominant for the recessive myostatin gene. The collected samples were individually vacuum packaged, boxed, and shipped to Loeffel Meat Laboratory at the University of Nebraska-Lincoln and placed in a cooler at 2°C until fabricated.

Strip loins were measured for loin weight, loin length, sirloin face width, rib face width, sirloin tail length, rib tail length and fat thickness at the rib face prior to fabrication. Loins were cut from anterior to posterior. Three steaks were cut as followed from the most anterior end: one 2.5 cm-thick steak for Warner-Bratzler shear force, one 1.3 cm-thick steak for nutrient analysis, and one 1.3 cm-thick steak to be saved as a backup. The remainder of the strip loin was cut into 2.5 cm-thick steaks where total number of steaks, total number of vein steaks, total number of non-vein steaks, and weight of each individual steak was noted. The two 1.3 cm steaks collected for nutrient analysis and backup were considered one 2.5 cm steak for the above gathered information (e.g., steak number).

Data were analyzed in a completely randomized design using ANOVA in PROC GLM in SAS (Version 9.2. Cary, N.C.) Fixed and random effects were the different inactive myostatin mutation and animal used, respectively. Separation of means was determined using LS MEANS and DIFF LINES options of SAS, with significance determined at  $P \leq 0.05$ .

## **Results & Discussion**

### *Steers*

Strip loin dimensional measurements from steers are found in Table 1. Fat thickness decreased as copies for the recessive myostatin gene increased ( $P < 0.001$ ). These results agree with Casas et al. (1998) who showed that double muscled cattle yield leaner carcasses compared to cattle that do not possess a myostatin mutation. Homozygous recessive cattle yielded loins that were shorter in length ( $P < 0.001$ ) compared to strip loins from homozygous dominant and heterozygous recessive. While having an increase in strip loin weight ( $P = 0.03$ ) homozygous recessive and heterozygous recessive cattle had strip loins that were also wider for both the sirloin face width ( $P = 0.03$ ) and rib face width ( $P < 0.001$ ) compared to homozygous dominant. Sirloin tail length ( $P = 0.006$ ) and rib tail length ( $P = 0.02$ ) were shorter for homozygous recessive cattle compared to heterozygous recessive.

Number, weight, and proportion of vein steaks of strip loins from steers are found in Table 2. As expected with homozygous recessive cattle possessing shorter strip loins they also yielded fewer total steaks ( $P < 0.001$ ) and fewer vein steaks ( $P < 0.001$ ) than

loins from homozygous dominant cattle. This was reflected in combined steak weight ( $P = 0.05$ ) being greater for heterozygous recessive and homozygous recessive than homozygous dominant. When average steak weight was analyzed heterozygous recessive and homozygous recessive were heavier ( $P < 0.001$ ) than homozygous dominant which was expected as heterozygous recessive and homozygous recessive strip loins were also heavier. When comparing number of non-vein steaks, homozygous recessive and heterozygous recessive had fewer steaks ( $P < 0.001$ ) compared to homozygous dominant. The weight and numeric percentage of vein steaks within the strip loin were not altered due to the different genotypes. With vein steaks being of lower value compared to strip loin steaks without the *Gluteus medius* the above results indicate no differences for the overall percentages of vein steak weight and percentages of vein steaks within the strip loin are desirable from an economic standpoint. Thus, within the Piedmontese steers, increasing copies of the recessive gene for myostatin were not detrimental to the percentage of vein steaks derived from the strip loin. Strip loins from homozygous recessive cattle yielded fewer total steaks that were wider and heavier. This would result in possible unattractiveness to consumers due to a larger portion size if cut to equal thickness or a thinner steak if cut to a constant weight. Thinner steaks would be more difficult for consumers to reach a desirable internal temperature while cooking. A consumer survey conducted by Leick et al. (2011) explored consumer's willingness to select thinner steaks in a retail setting. Their results indicate that consumers preferred the thinner steaks that would likely correspond to steaks having the largest surface area. Selection of steaks was not based on the thinner steaks being least expensive.

*Heifer*

As expected, increasing copies of the recessive gene for myostatin decreased fat thickness ( $P < 0.001$ ) from the strip loins of heifers similar to results seen from strip loins of steers. Loin length ( $P < 0.001$ ) of heifers was consistent with those observed within the steers as homozygous recessive cattle yielded shorter loins compared to homozygous dominant and heterozygous recessive and possessed a wider rib face ( $P < 0.001$ ). However, there were no differences for overall loin weight, sirloin face width, sirloin tail length, and rib tail length.

When comparing strip loins from homozygous recessive cattle to heterozygous recessive, homozygous recessive strip loins had a lower total number of steaks ( $P = 0.0290$ ) which is as expected due to homozygous recessive strip loins being shorter in length. The strip loins from heterozygous recessive heifers had a greater number of non-vein steaks ( $P = 0.002$ ), and a lower percentage of vein steaks ( $P = 0.01$ ) compared to homozygous dominant and homozygous recessive. These differences in percent vein steaks were inconsistent and showed no meaningful pattern. Overall mean steak weight, total weight of vein steaks, average steak weight, and percent weight of vein steaks within the strip loin did not differ among genotypes. These results suggest musculature differences are not only observed within the cattle from different genotypes, but also differences are apparent due to sex as patterns observed within the steers were not consistent when data collected from heifers were analyzed.

In summary, homozygous dominant steers yielded a heavier wider strip loin resulting in higher average steak weight than homozygous dominant cattle. Furthermore,

there was no difference in proportion of weight and percentage of vein steaks. Similar patterns were not seen within the strip loins of the heifers as there was no difference in loin weight and average steak weight and strip loins from heterozygous recessive heifers possessed a lower percentage of vein steaks compared to homozygous dominant and homozygous recessive heifers.

**Table 1: Steer dimensional measurements of strip loins from cattle with MM, Mm, or mm genotype of the myostatin gene.**

Measurements	Myostatin Genotype <sup>1</sup>			SEM	P-Value
	MM	Mm	mm		
Fat Thickness (cm)	1.42 <sup>a</sup>	0.69 <sup>b</sup>	0.33 <sup>c</sup>	0.079	< 0.001
Loin Weight (kg)	4.99 <sup>b</sup>	5.48 <sup>a</sup>	5.10 <sup>a</sup>	0.149	0.03
Loin Length (cm)	38.95 <sup>a</sup>	37.65 <sup>a</sup>	34.62 <sup>b</sup>	0.588	< 0.001
Sirloin Face Width (cm)	22.64 <sup>b</sup>	24.47 <sup>a</sup>	24.24 <sup>a</sup>	0.356	< 0.001
Rib Face Width (cm)	19.00 <sup>b</sup>	21.34 <sup>a</sup>	22.03 <sup>a</sup>	0.343	< 0.001
Sirloin Tail Length (cm)	6.54 <sup>b</sup>	7.63 <sup>a</sup>	6.18 <sup>b</sup>	0.348	0.006
Rib Tail Length (cm)	2.98 <sup>ab</sup>	3.18 <sup>a</sup>	2.62 <sup>b</sup>	0.150	0.02

<sup>abc</sup> Means with different superscripts within the same row are considered different  $P \leq 0.05$ .

<sup>1</sup>Myostatin: homozygous active (MM), heterozygous partially recessive (Mm), and homozygous recessive inactive (mm).



**Table 2: Steers number, weight, and proportion of vein steaks from strip loins of cattle with MM, Mm, or mm genotype of the myostatin gene.**

Steak Trait	Myostatin Genotype <sup>1</sup>			SEM	P-Value
	MM	Mm	mm		
Number of Loins Analyzed	20	22	16		
Total Steaks	14.7 <sup>a</sup>	13.77 <sup>b</sup>	12.38 <sup>c</sup>	0.233	< 0.001
Number Vein Steaks	4.60 <sup>a</sup>	4.18 <sup>b</sup>	3.56 <sup>c</sup>	0.151	< 0.001
Non-Vein Steaks	10.15 <sup>a</sup>	9.50 <sup>b</sup>	8.87 <sup>b</sup>	0.238	< 0.001
Average Steak Weight (g)	330.98 <sup>b</sup>	385.16 <sup>a</sup>	386.35 <sup>a</sup>	8.860	< 0.001
% of Vein Steaks in loin	31.37	30.39	28.82	1.068	0.21
Combined Weight of Steaks (g)	4993.37 <sup>b</sup>	5446.37 <sup>a</sup>	5093.29 <sup>ab</sup>	137.653	0.05
Total Weight of Vein Steaks (g)	1569.11	1704.30	1505.81	68.119	0.12
% Weight of Vein Steaks	31.42	31.35	29.35	1.109	0.30

<sup>abc</sup> Means with different superscripts within the same row are considered different  $P \leq 0.05$ .

<sup>1</sup>Myostatin: homozygous active (MM), heterozygous partially recessive (Mm), and homozygous recessive inactive (mm).

**Table 3: Heifer dimensional measurements of strip loin from cattle MM, Mm, or mm genotype of the myostatin gene.**

Measurements	Myostatin Genotype <sup>1</sup>			SEM	P-Value
	MM	Mm	mm		
Fat Thickness (cm)	1.40 <sup>a</sup>	0.81 <sup>b</sup>	0.48 <sup>c</sup>	0.084	< 0.001
Loin Weight (kg)	6.62	6.77	6.59	0.188	0.77
Loin Length (cm)	40.22 <sup>a</sup>	40.20 <sup>a</sup>	37.50 <sup>b</sup>	0.491	< 0.001
Sirloin Face Width (cm)	25.36	24.63	25.34	0.295	0.13
Rib Face Width (cm)	18.98 <sup>b</sup>	19.72 <sup>b</sup>	21.40 <sup>a</sup>	0.313	< 0.001
Sirloin Tail Length (cm)	7.50	7.84	4.73	1.018	0.06
Rib Tail Length (cm)	3.00	3.18	2.98	0.117	0.39

<sup>abc</sup> Means with different superscripts within the same row are considered different  $P \leq 0.05$ .

<sup>1</sup>Myostatin: homozygous active (MM), heterozygous partially recessive (Mm), and homozygous recessive inactive (mm).

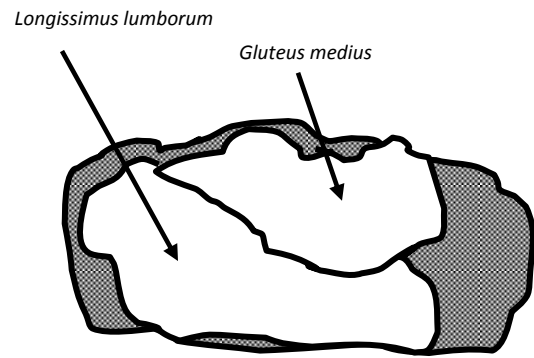
**Table 4: Heifer number, weight, and proportion of vein steaks from strip loins of cattle MM, Mm, or mm genotype of the myostatin gene.**

Steak Trait	Myostatin Genotype <sup>1</sup>			SEM	P-Value
	MM	Mm	mm		
Number of Loins Analyzed	19	20	20		
Total Steaks	12.63 <sup>ab</sup>	13.05 <sup>a</sup>	12.4 <sup>b</sup>	0.174	0.03
Number Vein Steaks	4.10	3.70	3.85	0.132	0.09
Non-Vein Steaks	8.53 <sup>b</sup>	9.35 <sup>a</sup>	8.55 <sup>b</sup>	0.179	0.002
Average Steak Weight (g)	514.91	497.77	506.09	11.233	0.55
% of Vein Steaks in loin	32.47 <sup>a</sup>	28.32 <sup>b</sup>	31.17 <sup>a</sup>	1.001	0.01
Combined Weight of Steaks (g)	6490.16	6513.00	6284.40	157.350	0.52
Total Weight of Vein Steaks (g)	2123.68	1996.00	1888.80	79.789	0.12
% Weight of Vein Steaks	32.55	30.73	30.32	1.089	0.31

<sup>abc</sup> Means with different superscripts within the same row are considered different  $P \leq 0.05$ .

<sup>1</sup>Myostatin: homozygous active (MM), heterozygous partially recessive (Mm), and homozygous recessive inactive (mm).

**Figure 1. Illustration showing vein steak from posterior end of strip loin**



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**MANUSCRIPT 3****Effectiveness of Three Antimicrobial Sprays and Their Effect on Color and Sensory  
Properties of Beef Steaks <sup>1,2</sup>**

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## ABSTRACT

The objectives of this study were to evaluate the effectiveness of three different antimicrobial sprays and their effects on color and sensory properties of beef steaks (bromine, BeefXide [lactic acid 45-60%, acetic acid 23-30% and potassium hydroxide >1%; Birko, Henderson, CO], lactic acid, and control). Treatments were applied to 60 *Psoas major* (PM) steaks and 60 *Gluteus medius* (GM) for six replications for total of 720 steaks. Three replications (180 steaks each) were used for sensory evaluation. The remaining replications used for microbial analysis were inoculated prior to antimicrobial treatment with approximately 3 log of generic e-coli and swabs for CFU's were taken. Treatments were applied via direct spray on average at 560 ppm bromine, 2.48 % BeefXide, and 4.17% lactic acid at 130°F. Following treatment, steaks were swabbed once more for CFU's. The non-inoculated steaks were frozen after treatment and thawed prior to consumer sensory evaluation and color measurements. Objective color was measured using a Minolta Chromameter. Values for, L\*, a\* and b\* were recorded. Percent discoloration was evaluated on raw steaks while in vacuum packages after thawing. Consumer evaluation steaks were cooked to 71°C and served. Panels were completed over 2 d with PM steaks prepared for day one and GM steaks for day two. Consumers (n = 204) used an 8 point hedonic scale to evaluate samples for juiciness and flavor (1 = extremely undesirable, 8 = extremely desirable) and off-flavor intensity (1 = extremely mild, 8 = extremely intense). Lactic acid and commercial blend treatments were the most effective antimicrobials ( $P < 0.01$ ) for both PM and GM steaks. Percent discoloration showed lactic acid-treated PM steaks had the most discoloration ( $P < 0.01$ ) compared to GM steaks where bromine-treated steaks had the most discoloration ( $P <$



0.01). Consumer evaluation of PM steaks showed lactic acid samples were more desirable ( $P = 0.05$ ) for juiciness and flavor ( $P = 0.01$ ) when compared to control, bromine, and commercial blend samples. There were no difference in off-flavor intensity among treatments for PM steaks and no preferences for juiciness, flavor, and off-flavor for GM steaks. In conclusion, lactic acid was the most effective for microbial treatment, but also showed the lightest color with the lowest  $L^*$  value while commercial blend-treated samples had less overall discoloration and more redness ( $a^*$ ).

**Key words:** beef flavor, antimicrobial, lactic acid, bromine

## Introduction

Antimicrobial inhibitors are important within the fresh meat industry to inhibit pathogens and extend shelf life by controlling growth of food spoilage microorganisms (Ray et al., 2008). Preferred antimicrobials will not change appearance characteristics such as color of raw meat and palatability properties such as juiciness, flavor, and off-flavor of cooked meat product (Diner et al., 2004).

Contamination of beef carcasses is an unavoidable result of processing and is generally considered to begin during processing on the slaughter line (Dickson et al., 1992). Most contamination occurs on the surface of the carcass while muscles within the carcass are basically sterile (Dickson 1992). However, the bacteria on the surface can be transferred to cuts during processing and fabrication by employees and equipment. Thus, control measures should be applied to help ensure meat products are safe for consumption.

One bacteria of major concern is *Escherichia coli*, which is commonly found in the intestinal tract of human and animal hosts such as cattle. *E-coli* are a Gram-negative, rod shaped, facultative anaerobic bacterium. It is normally present at very high levels in the content of the large intestine (Ray et al., 2008).

Organic acids such as lactic acid and citric acid are commonly used on beef carcasses to reduce bacterial load. Lactic acid is a weak organic acid that inhibits bacterium by neutralizing the membrane proton gradient, and reducing the water activity (Ray et al, 2008). Pipek et al. (2005) showed that steam treatment and spraying with a 2% solution of lactic acid reduced microbial counts on the surface of beef carcasses that then led to retarded microbial growth during storage and extension of shelf life. Citric acid has

also been shown to have some antimicrobial properties and is effective through chelating divalent cations (Ray et al., 2008).

More recently a study was completed by Laury et al. (2009) to validate the use of BeefXide, a commercial blend of lactic acid and citric acid, as an antimicrobial. Beef tips were inoculated with a cocktail composed of four *E. coli* strains then sprayed with Beefxide at a concentration of 2.5%. After treatment *E. coli* was reduced 1.4 log CFU/100 cm<sup>2</sup>. These results were similar to the effectiveness gathered for lactic acid spray within the same study and suggested that Beefxide is an effective antimicrobial option.

The objectives of this study were to determine the effectiveness of three different antimicrobials and their effects on color and sensory properties of beef steaks using a consumer taste panel.

### **Materials and Methods**

Three antimicrobials were used: bromine, Beefxide [a commercial blend (lactic acid 45-60%, acetic acid 23-30% and potassium hydroxide > 1%)], lactic acid, and an unsprayed control. Treatments were individually applied to 60 *Psoas major* (PM) steaks and 60 *Gluteus medius* (GM) for each of six replications for a total of 720 steaks. Three replications (180 steaks each) were used for sensory evaluation samples. The remaining three replications used for microbial analysis were inoculated prior to treatment with approximately 3 log of generic *E. coli* and swabs for CFU's were taken. Both side of *Psoas major* steaks (PM; n = 180) and *Gluteus medius* steaks (GM; n = 180) were inoculated e-coli via spray. One side of each steak (1 square inch) was swabbed with a BBL Culture Swab (Becton, Dickson and Company, Sparks, MD) and swabs placed into

a sterile test tube with 10 mL Butterfield's phosphate solution. Tubes were then vortexed and 1 mL of solution was placed on 3M petrifilm E-coli/coliform (3M Microbiology, St Paul, MN) and incubated for forty-eight hours at 35°C. After incubation initial CFU's were measured. Antimicrobial treatments were applied via direct spray using a spray cabinet supplied by Chad Equipment LLC (Birko, Olathe, KS) at 130°F on average 560 ppm bromine, 2.48% BeefXide, and 4.17% for lactic acid. Following antimicrobial treatment steaks used for microbial analysis were swabbed once more for CFU's. The following day steaks were sampled following the above listed protocol for enumeration of CFU's after treatment.

#### ***Objective and Subjective Color***

Vacuum packaged steaks were thawed overnight in a 3°C cooler prior to obtaining color measurements. Objective color measurements were made with a Minolta Chromameter CR-400 (Minolta Camera Company, Osaka, Japan). An 8 MM diameter area, illuminant D65 and a 2° standard observer were used. Measurements recorded included L\* (psychometric lightness), a\* (green to red) and b\* (blue to yellow). The Minolta was calibrated by normal standards with a white calibration plate that came with the machine from the manufacture. Three readings per steak within vacuum package were taken and the mean of the readings were used for statistical analysis.

Subjective percent surface discoloration were estimated by a five member trained panel of graduate students from the University of Nebraska-Lincoln Animal Science Department on raw steaks within vacuum packages. Percent discoloration was estimated using a range of 0 to 100%. Steaks were evaluated within vacuum packages to mimic what consumers would see when making purchasing decisions.

### ***Consumer Taste Panel***

A consumer taste panel consisting of 204 individuals was completed at the Capital City Christian Church, Lincoln, Nebraska over a period of two days. Prior to evaluation sessions panelists were informed of the experimental procedure verbally by a moderator and received a written consent form with further explanation. The moderator also informed panelists of the consumer evaluation sheet to be completed.

Steaks were thawed 24 hours prior in a 3°C cooler and then placed on a Hamilton Beach Indoor-Outdoor Grill (Hamilton Beach/Proctor Silex, Inc., Catalog No. HB9, Model 31605A, Series type G16 Grill, 120 v ~60 Hz, 1200 W), and cooked on one side until the steaks reached an internal temperature of 35° C. They were then turned over until they reached at internal temperature of 71°C (AMSA, 1995). Internal temperature of steaks was monitored using an OMEGA thermometer (Model 450A, OMEGA Engineering Inc., Stamford, CT) with a Type T copper-constantan probe (Omega Engineering Inc., Stamford, CT) inserted into the geometric center of each steak.

Steaks were then cut into 1-cm cubes, wrapped in aluminum foil and placed in a metal pan located in a warm water bath to maintain temperature. Taste panel sessions were initiated within 10 min of cutting. Individual steak identity was maintained throughout cooking and serving. A single sample was placed on a plate and served to consumers with each panelist evaluating eight samples. Each individual steak was evaluated by 5 consumers. Distilled water and crackers were available for pallet cleansing. Consumer taste panels were completed over two days with 176 *Psoas major* steaks prepared for day one and 176 *Gluteus medius steaks* prepared for day two.

The 204 consumers panelists (n = 101 for day one and n = 103 for day two) were asked to evaluate steaks on an 8-point hedonic scale for juiciness and flavor (1 = extremely undesirable, 8 = extremely desirable) and off-flavor intensity (1 = extremely mild, 8 = extremely intense). When a panelist detected an off flavor they were asked to describe it.

### ***Statistical Analysis***

Each steak was analyzed independently. Statistical analysis was conducted using SAS (Version 9.2, Cary, NC, 2002 – 2008) in a completely randomized design with main effects being the different microbial treatments and random effects of panelist was used (in consumer taste panel only). Analysis of Variance (ANOVA) was performed using the GLIMMIX procedure with mean separation determined using LS MEANS and DIFF LINES option within SAS. Significance was determined at  $P \leq 0.05$ .

## **Results and Discussion**

Lactic acid and Beefxide were the most effective antimicrobial treatments ( $P < 0.01$ ) and were statistically equivalent (Tables 1 and 2) for both steak types. These results agree with the findings of Pipek et al. (2005) who noted that spraying a lactic acid solution at 2% reduced microbial counts on beef carcasses leading to reduced microbial growth during storage and thus increasing shelf life. Additionally, Laury et al. (2009) conducted research to validate BeefXide as an antimicrobial treatment for *E. coli*. and compared it to the effectiveness of lactic acid spray within the same study. Laury et al (2009) results were similar to those found within the current study as the effectiveness of BeefXide and lactic acid spray showed similar results.

*Psoas major* steaks treated with lactic acid had the largest percent discoloration ( $P < 0.001$ ) and the largest percent discoloration ( $P < 0.001$ ) for *Gluteus medius* steaks were bromine-treated (Tables 3 and 4). The objective L\*(lightness) values were lowest for both *Psoas major* and *Gluteus medius* steaks treated with lactic acid (Table 3 and 4,  $P = 0.06$  and  $P < 0.001$ , respectively). *Psoas major* steaks treated with lactic acid possessed the lowest a\* (redness) value ( $P < 0.001$ ), while bromine treated *Gluteus medius* steaks showed the lowest a\* value ( $P < 0.001$ ). There were no significant differences for b\* (blueness) values among *Psoas major* steaks compared to *Gluteus medius* steaks where control samples were lower ( $P < 0.001$ ) than the other treatments.

Within the consumer taste panel evaluation *Psoas major* steaks treated with lactic acid were more preferred for juiciness and flavor (Table 3,  $P = 0.05$  and  $P = 0.01$ , respectively). There were no significant differences for off-flavor intensity for *Psoas major* steaks among treatments. *Gluteus medius* steaks showed no significant preferences for juiciness, flavor, and off-flavor intensity as determined by the consumer taste panel.

These results suggest that lactic acid and Beefxide were the most effective antimicrobial treatment, while lactic acid samples revealed the lowest L\* value indicating the darkest color. Antimicrobials will continue to be needed to provide the food supply that will be demanded in the future (Branen et al., 1983) and after completion of this study lactic acid and Beefxide are viable antimicrobial treatments.

**Table 1: Mean antimicrobial treatment effects on *Psoas major* steaks**

	Treatment				SEM	P-Value
	Control	Bromine	BeefXide	Lactic Acid		
Log before	3.00	3.07	3.13	2.99		
Log after	2.49	2.54	2.17	1.89		
Log reduction	0.51 <sup>b</sup>	0.50 <sup>b</sup>	0.97 <sup>a</sup>	0.98 <sup>a</sup>	0.098	<.01

<sup>abc</sup> Means with different superscripts within the same row are considered different  $P \leq 0.05$



**Table 2: Mean antimicrobial treatment effects on *Gluteus medius* steaks**

	Treatment				SEM	P-Value
	Control	Bromine	BeefXide	Lactic Acid		
Log before	2.73	2.78	2.68	2.59		
Log after	2.20	2.14	1.35	1.02		
Log reduction	0.54 <sup>b</sup>	0.64 <sup>b</sup>	1.33 <sup>a</sup>	1.57 <sup>a</sup>	0.126	<.01

<sup>abc</sup> Means with different superscripts within the same row are considered different  $P \leq 0.05$

**Table 3: *Psoas major* color and sensory properties after treatment with antimicrobials**

	Treatment				SEM	P-Value
	Control	Bromine	BeefXide	Lactic Acid		
Juiciness	4.75 <sup>ab</sup>	4.68 <sup>b</sup>	4.69 <sup>b</sup>	4.99 <sup>a</sup>	0.125	0.05
Flavor	5.39 <sup>ab</sup>	5.40 <sup>a</sup>	5.19 <sup>b</sup>	5.57 <sup>a</sup>	0.113	0.007
Off-Flavor Intensity	2.49	2.41	2.56	2.65	0.151	0.16
Discoloration (%)	48.14 <sup>b</sup>	51.00 <sup>b</sup>	37.72 <sup>c</sup>	67.85 <sup>a</sup>	2.670	< 0.001
L* (%)	42.65 <sup>a</sup>	42.10 <sup>ab</sup>	42.23 <sup>ab</sup>	41.00 <sup>a</sup>	0.374	0.06
A* (%)	14.73 <sup>a</sup>	14.87 <sup>a</sup>	15.37 <sup>a</sup>	13.72 <sup>b</sup>	0.213	<.001
B* (%)	9.06	9.12	9.59	9.45	0.185	0.22

<sup>abc</sup> Means with different superscripts within the same row are considered different  $P \leq 0.05$

**Table 4: *Gluteus medius* color and sensory properties after treatment with antimicrobials**

	Treatment				SEM	P-Value
	Control	Bromine	BeefXide	Lactic Acid		
Juiciness	4.52	4.31	4.48	4.51	0.129	0.33
Flavor	5.18	5.21	5.18	5.17	0.107	0.99
Off-Flavor Intensity	2.75	2.65	2.74	2.83	0.155	0.51
Discoloration (%)	43.60 <sup>b</sup>	57.88 <sup>a</sup>	31.82 <sup>c</sup>	48.81 <sup>b</sup>	2.568	< 0.001
L* (%)	40.11 <sup>b</sup>	42.36 <sup>a</sup>	40.36 <sup>a</sup>	39.48 <sup>b</sup>	0.344	< 0.001
A* (%)	16.19 <sup>b</sup>	15.26 <sup>c</sup>	17.45 <sup>a</sup>	16.08 <sup>bc</sup>	0.274	< 0.001
B* (%)	10.24 <sup>b</sup>	10.58 <sup>b</sup>	11.19 <sup>a</sup>	11.09 <sup>a</sup>	0.148	< 0.001

<sup>abc</sup> Means with different superscripts within the same row are considered different  $P \leq 0.05$

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## APPENDICES

## Appendix 1: Taste Panel Consent Form

### TASTE PANEL CONSENT FORM

#### **IDENTIFICATION OF PROJECT**

Evaluation of Beef Steaks

#### **PURPOSE OF THE STUDY**

Participants will volunteer to taste beef fillet and beef sirloin steaks for flavor characteristics. Steaks will have been treated with USDA approved antimicrobial substances.

#### **EXPLANATION OF PROCEDURES**

Samples will be provided for you to place in your mouth for evaluation of beef flavor characteristics. These samples will be beef fillet and beef sirloin steaks which have been cooked and cut into bite-sized pieces for your evaluation. Participants will evaluate eight steak samples and evaluate them using an eight point scale, with eight being most desirable and one undesirable.

#### **POTENTIAL RISKS AND DISCOMFORTS**

There is no risk to participants with this group beyond the normal activity of eating. The ingredients will be identified on the consent form to avoid known food allergens.

#### **POTENTIAL BENEFITS**

Volunteering participants will help the Capitol City Christian Church earn money for activities. Society in general benefits from the production of meat products with improved consumer acceptance.

#### **ASSURANCE OF CONFIDENTIALITY**

Any information obtained in connection with this project and which could be identified with you will be kept confidential. Summary results and statistical data will not include individual observations, only group means will be reported.

#### **WITHDRAWAL FROM THE STUDY**

Participation in this study is voluntary. Your decision whether or not to participate will not affect your present or future relationship with the investigator or the University of Nebraska. If you decide to participate, you are free to withdraw your consent and to discontinue participation at any time without penalty.

#### **COMPENSATION FOR PARTICIPATION**

The Capitol City Christian Church will be paid for providing access to volunteers.

#### **OFFER TO ANSWER QUESTIONS**

If you have any questions, please do not hesitate to ask. If you think of questions later, please feel free to contact Chris Calkins, Ph.D. (402)-472-6314. If you have any additional questions concerning the rights of research subjects, you may contact the University of Nebraska-Lincoln Institutional Review Board (IRB), telephone 402-472-6965.

YOU ARE VOLUNTARILY MAKING A DECISION WHETHER OR NOT TO PARTICIPATE IN THIS RESEARCH TODAY. YOUR SIGNATURE CERTIFIES THAT YOU HAVE DECIDED TO PARTICIPATE HAVING READ THE INFORMATION PRESENTED. YOUR SIGNATURE ALSO CERTIFIES THAT YOU HAVE HAD AN ADEQUATE OPPORTUNITY TO DISCUSS THIS STUDY WITH THE INVESTIGATOR AND YOU HAVE HAD ALL YOUR QUESTIONS ANSWERED TO YOUR SATISFACTION. YOU WILL BE GIVEN A COPY OF THIS CONSENT FORM TO KEEP.

\_\_\_\_\_  
SIGNATURE OF SUBJECT

\_\_\_\_\_  
DATE

IN MY JUDGEMENT THE SUBJECT IS VOLUNTARILY AND KNOWINGLY GIVING INFORMED CONSENT AND POSSESSES THE LEGAL CAPACITY TO GIVE INFORMED CONSENT TO PARTICIPATE IN THIS RESEARCH STUDY.

\_\_\_\_\_  
SIGNATURE OF INVESTIGATOR

\_\_\_\_\_  
DATE

Chris R. Calkins, Ph.D.  
402-472-6314

## Appendix 2: Consumer Sensory Panel Evaluation Form

### Consumer Evaluation of Beef Steaks

Please evaluate each sensory attribute of the sample by using the rating scale (1-8) and then provide a description of off flavors identified.

Rating scales:

#### Juiciness

8 Extremely Juicy  
7 Very Juicy  
6 Moderately Juicy  
5 Slightly Juicy  
4 Slightly Dry  
3 Moderately Dry  
2 Very Dry  
1 Extremely Dry

#### Flavor

8 Extremely Desirable  
7 Very Desirable  
6 Moderately Desirable  
5 Slightly Desirable  
4 Slightly Undesirable  
3 Moderately Undesirable  
2 Very Undesirable  
1 Extremely Undesirable

#### Off Flavor Intensity

8 Extremely Intense  
7 Very Intense  
6 Moderately Intense  
5 Slightly Intense  
4 Slightly Mild  
3 Moderately Mild  
2 Very Mild  
1 Extremely Mild

Sample ID	Juiciness	Flavor	Off-Flavor Intensity	Description of off Flavors
986				
931				
260				
903				
168				
679				
578				
466				



## **RECOMMENDATIONS FOR FUTURE RESEARCH**

After completion of this research, it was determined that beef from homozygous recessive cattle is equal in tenderness to meat from homozygous dominant cattle. As well, after completion of nutrient analysis meat from homozygous recessive cattle had a lower fat content, higher cholesterol, and higher protein levels when compared to homozygous dominant cattle.

Completion of a trained sensory panel to evaluate flavor as well as tenderness is recommended. This would allow for comparison of shear force values to that noted of panelist. Furthermore, panelist would evaluate juiciness, connective tissue, and off-flavor where juiciness could be correlated with overall moisture percentage within sample.

Piedmontese cattle have a larger proportion of Type II fibers (white fibers), which use anaerobic metabolism. These fibers have the ability to work at a wider pH range and thus are more prone to oxidation. It is recommended that a retail display be completed on both eye of round steaks and strip loin steaks to determine the extent of oxidation that may occur due to increased white fiber concentration. As well, objective color would be evaluated using a Minolta Chromameter for L\*, a\*, and b\* values and subjective percent discoloration estimated by a trained panel.

Lastly, within this current study it was concluded that strip loins of steers showed no difference in proportion of weight and percentage of vein steaks while heterozygous recessive heifers possessed a lower percentage of vein steaks compared to strip loins from homozygous dominant and homozygous recessive heifers. To build on this study an economic analysis conducted to evaluate the overall profit/loss of producing homozygous

dominant and heterozygous recessive cattle is recommended. This analysis would determine if the increase muscle mass noted within Piedmontese cattle as well as their improved feed conversion offsets production cost.