DISSERTATION

EFFECTS OF PRE-SLAUGHTER CATTLE MANAGEMENT ON POSTMORTEM TENDERIZATION OF BEEF

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY SANDRA LYNN GRUBER ENTITILED "EFFECTS OF PRE-SLAUGHER CATTLE MANAGEMENT ON POSTMORTEM TENDERIZATION OF BEEF" BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTAION

EFFECTS OF PRE-SLAUGHTER CATTLE MANAGEMENT ON POSTMORTEM TENDERIZATION OF BEEF

Two independent studies were used to investigate the effects of pre-slaughter cattle management on postmortem tenderization of beef. In experiment I, relationships between behavioral and physiological symptoms of pre-slaughter stress and beef LM tenderness were examined using calf-fed steers (n = 79) and heifers (n = 77). Pen, chute, and post-transportation behavior scores were assigned to each animal. Physiological indicators of stress included cortisol, epinephrine, creatine kinase, lactate, glucose, heart rate, respiration rate, and rectal temperature.

Positive correlations ($P < 0.05$) between behavior scores and physiological parameters indicated that cattle that exhibited behavioral symptoms of stress also responded physiologically. Mean Warner-Bratzler shear force (WBSF) was positively correlated ($P < 0.05$) with all 3 measures of stressful behavior ($r \geq 0.23$). Plasma lactate concentration at slaughter was associated ($P < 0.05$) with mean WBSF and response to postmortem aging.

In Experiment II, effects of genotype and implant program on LM and gluteus muscle (GM) postmortem tenderization were investigated using crossbred steer (n = 185) and heifer (n = 158) calves. The 3-marker GeneSTAR Tenderness panel was used to

determine each animal's genotype. Calves were assigned to 1 of 2 implant programs, conventional or delayed.

Steaks from conventionally implanted cattle had WBSF values that were approximately 0.2 kg higher ($P < 0.05$) than steaks from animals that received a single delayed implant, but only during the early postmortem period (3 to 7 d). For both muscles, a linear effect of genotype on WBSF was detected ($P < 0.05$). Steaks from cattle with most desirable genotype had WBSF values 0.33 kg lower than steaks from cattle with the least desirable genotype. Pre-slaughter factors (sex, implant program, and genotype) controlled in Experiment II each accounted for less than 10 % of the explained variation in tenderness of the experimental population

Results of Experiment I identified behavioral and physiological symptoms of acute pre-slaughter stress that were associated with pH-independent differences in tenderness. Findings from Experiment II suggest that pre-slaughter cattle management can impact beef tenderness, but the factors evaluated only accounted for a small proportion of variation in beef tenderness for the experimental population.

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CHAPTER I

OBJECTIVE OF DISSERTATION

The objective of this dissertation was to examine the effects of pre-slaughter cattle management on postmortem tenderization of beef. Two independent studies (funded by the beef check-off) were completed to investigate these effects; their individual objectives were as follows:

1) To gain further insight into relationships of behavioral and physiological pre-slaughter stress responses to meat quality characteristics and LM tenderness.

2) To examine the effects of genetic tenderness markers and implant strategy on longissimus and gluteus muscle tenderness of calf-fed steers and heifers.

CHAPTER II

REVIEW OF LITERATURE

Mechanisms of Stress

Stress is a broad term that refers to stimuli (external or internal) that disrupt an animal's homeostasis (von Borell, 2001). Stressors can be physical, psychological, and often contain components of both classifications (Grandin, 1997; von Borell, 2001). Common stressors encountered by livestock include weather extremes, handling, estrus, mixing or separation, and transportation (Minton, 1994; Ferguson et al., 2001). Although some stressors are unavoidable in food animal production (i.e., transportation and handling), livestock production systems are designed to minimize stress, firstly for the welfare of animals, but also because of the negative effects of stress on important production traits such animal health (Salak-Johnson and McGlone, 2007), live weight gain (Mitlöhner et al., 2001), and meat quality (Scanga et al., 1998).

Physiological Responses to Stress. In response to stress, the central nervous system induces physiological responses that result in the activation of the sympatho-adrenal (SA) axis (also referred to as the hypothalamic-adrenal medullary system) and the hypothalamo-pituitary-adrenocortical (HPA) axis (Minton, 1994; von Borrell, 2001). Commonly referred to as the fight-flight response, the SA axis is a short-acting stress-response that was first conceptualized by Walter Cannon in 1914 (Cannon, 1914; von Borrell, 2001). The SA axis involves the hypothalamus, pituitary gland, and the

sympathetic neural pathways to the adrenal medulla (von Borrell, 2001). Activation of the SA axis in response to acute stress results in the secretion of catecholamines from the adrenal medulla directly into the blood stream. Catecholamines are amino acid derivatives synthesized from tyrosine that influence adrenergic receptors throughout the body. In mammals, the most abundant catecholamines are epinephrine (adrenaline), norepinephrine (noradrenaline), and dopamine (Hossner, 2005). Epinephrine is considered the primary mediator in response to acute stress, and according to Hossner (2005), circulating concentrations of norepinephrine and dopamine result from "spillover" into the blood during sympathetic activation of the adrenal medulla.

In response to stress, epinephrine acts to mobilize energy by increasing glycogenolysis and gluconeogenesis in the liver and glycogenolysis in skeletal muscle. Epinephrine also stimulates the pancreas to reduce the release of insulin and increase the secretion of glucagon. These combined effects provide a high level of blood glucose to be used as an energy source during stressful events (Hossner, 2005). Additionally, release of epinephrine during the flight-fight response prepares the animal for an active coping response to stressors by increasing heart rate and blood pressure (von Borrell, 2001).

In contrast to the SA axis, the HPA axis, conceptualized by Hans Selye in 1936, is considered a longer-term, sustained response to stress (Selye, 1936; von Borell, 2001). In response to stress, small bodied neurons in the hypothalamus synthesize and secrete the amino-acid neuropeptides corticotrophin releasing hormone (CRH) and vasopressin (VP) (Boron and Boulpaep, 2005). Transported to the anterior pituitary by the hypopheseal portal blood system, the combination of CRH and VP increases production of

adrenocorticotropin hormone (ACTH) (von Borrell, 2001). In respect to the secretion of ACTH, pigs and cattle have greater secretory responses to ACTH than VP. On the other hand, in sheep, VP is a far more potent stimulator of ACTH secretion than CRH (Minton, 1994). Adrenocoticotropic hormone is the primary regulator of glucocorticoid hormone production, and with regard to stress response, cortisol is the prototypic glucocorticoid. Increased ACTH results in acute synthesis and secretion of cortisol from the adrenal cortex by: 1) stimulating the conversion of cholesterol to pregnenlone, which is the rate limiting step in cortisol formation, 2) increasing the synthesis of several proteins needed for cortisol synthesis (e.g., P-450 enzymes and LDL receptors), which occurs over a longer time frame (Boron and Boulpaep, 2005). Cortisol increases hepatic glucose and glycogen synthesis, as well as reducing protein synthesis and stimulating protein degradation to mobilize amino acids for gluconeogenesis (Boron and Boulpaep, 2005; Hossner, 2005).

Although the SA and HPA axes are often discussed separately, they work together to help animals cope with stress. Axelrod and Reisine (1984) provided the following summary of the physiological response to stress (Figure 2.1):

> "Stress stimulates several adaptive hormonal responses. Prominent among these responses are the secretion of catecholamines from the adrenal medulla, corticoids from the adrenal cortex, and adrenocorticotropin from the anterior pituitary. A number of complex interactions are involved in the regulation of these hormones. Glucocorticoids regulate catecholamine biosynthesis in the adrenal medulla and catecholamines stimulate adrenocorticotropin release from the anterior pituitary. In addition, other hormones, including corticotrophin-

releasing factor, vasoactive intestinal peptide, and arginine vasopressin stimulate while the corticosteroids and somatostatin inhibit adrenocorticotropin secretion. Together these agents appear to determine the complex physiologic responses to a variety of stressors" (Axelrod and Reisine, 1984).

Behavioral Responses to Stress. To determine whether animals are experiencing stress, researchers commonly document animals' behaviors or responses to certain events (e.g., handling). Likewise, an assessment of an animal's overall temperament is often made as an indicator of how that animal will react to future stressful situations (e.g., transportation). Common measures of cattle behavior and temperament are often made: 1) in an unrestrained environment (e.g. pen score); 2) during routine handling (e.g., chute score); and, 3) during or following transportation.

Measures of cattle behavior/temperament in unrestrained environments often involve reactions to human presence, and are frequently a measurement (subjective or objective) of an animal's flight zone (Fordyce et al., 1982; Grandin, 1993). The flight zone is essentially how close an animal will let a human come before it attempts to flee (Grandin, 2000). Fordyce et al. (1982) recorded the closest distance (paces) an observer could get to each animal (bull or heifer) as a measure of cattle temperament (referred to as a yard test). Vann and Randel (2003) described the following subjective pen temperament scoring system: 1 = non-aggressive, not excited by humans to 5 = aggressive, runs into fences and at humans if approached. Similar subjective, discrete, pen temperament scores also have been reported by others (Curley et al., 2003, Vann et al., 2008). In an extensive review of the human-animal relationship in farmed animal species, Waiblinger

et al. (2006) highlighted numerous studies that measured the behavior of cattle based on reactions to stationary or moving humans (also referred to as fear and avoidance tests).

Estimates of cattle temperament also are frequently obtained during routine handling. The 2 most common cattle behavior measurements obtained during handling are: 1) an animal's response to confinement in a chute/crush; and, 2) the speed that an animal exits the chute/crush. Chute scores or crush tests often are discrete scales that describe the animal's vigor of movement while contained (Fordyce et al. 1982, 1985, 1988; Grandin, 1993). For example, Grandin (1993) described in detail the following 5-point chute scoring system: 1 = calm, no movement; 2 = slightly restless; 3 = squirming, occasionally shaking the squeeze chute; 4 = continuous, very vigorous movement and shaking of the squeeze chute; 5 = rearing, twisting of the body and struggling violently. In addition to an animal's behavior in a chute, researchers often attempt to quantify cattle temperament based on the speed at which cattle exit a crush. Exit speed scores are subjectively assigned, such as those reported by Baszak et al. (2006) where 1 = walk, 2 = trot, and 3 = run or gallop. Or, flight speed/exit velocity measurements (m/s) may be obtained objectively using 2 sets of light-beam generators, reflectors, and a timer (Burrow et al., 1988; Burrow and Dillon, 1997; Petherick et al., 2002; Curley et al., 2006; King et al., 2006; Vann et al., 2008). Exit velocity (m/s) has gained popularity as an assessment of cattle temperament because speed is objectively measured as opposed to behavior scores, which are assigned subjectively by human handlers/scorers. In fact, studies that only include one measure of behavior often solely rely on exit velocity (m/s) to categorize cattle temperament (Burrow et al., 1999; Petherick et al., 2002).

Transportation (including loading and unloading) is considered one of the most severe stressors experienced by animals in food production systems (María et al., 2004). However, the ability to assess whether an animal is experiencing transportation-induced stress, based on its behavior, is relatively difficult. It is plausible to think that earlier measures of cattle temperament (i.e., pen, chute, or exit speed scores) may indicate how cattle will react to transportation (Vann et al., 2008), but few attempts have been made to develop or monitor behavioral changes during transport. The majority of studies that have investigated the transportation of cattle have made behavioral observations such as social interactions (e.g., butts and mounts), elimination (defecation and urination), frequency of standing and laying, and the orientation that cattle stand while being transported (Kent and Ewbank, 1983; Kenny and Tarrant, 1987; Tarrant, 1990; Swanson and Morrow-Tesch, 2001). María et al. (2004) developed a comprehensive objective scoring system for evaluating the stress to cattle of commercial loading and unloading. The authors' scoring system created weighted scores for loading and unloading based on time (min) and the frequency of events such as falls, balks, vocalizations, and reversals (María et al., 2004).

Numerous studies have included multiple measures of cattle behavior (Hearnshaw et al., 1979; Fordyce et al., 1982, 1985, 1988; Burrow and Corbet, 2000; Curley et al., 2003; Baker et al., 2003; Vann and Randel, 2003; Baszczak et al., 2006; Vann et al., 2008). Because measures of behavior/temperament can be obtained at various stages throughout production, and during what could be perceived as different levels of stress, attempts have been made to investigate the relationships among different scores. Low to moderate correlations have been reported between exit velocity (m/s) and crush/chute tests (Burrow

and Corbet, 2000; Baker et al., 2003; Curley et al. 2003; Van and Randel, 2003) and between exit velocity (m/s) and pen score (Curley et al. 2003; Van and Randel, 2003). Burrow and Corbet (2000) suggested that flight speed scores and crush scores measure different aspects of cattle behavior. Additionally, some researchers have attempted to create an overall temperament score for a bovine using multiple measures of behavior (Hearnshaw et al., 1979; Fordyce et al., 1985; Vann et al., 2008). For example, Fordyce et al. (1985) assumed that all measures of behavior observed in a crush test were additive and therefore summed them to create a temperament ranking (crush score + audible respirations + bellowing + kicking + going down). Vann et al. (2008) calculated an overall temperament score for each animal using pen score (PS) and exit velocity (EV) measures (PS + EV/2).

Measuring the Effects of Stress on Physiological and Behavioral Traits

To objectively determine if cattle are stressed, researchers often measure blood metabolites as indices of SA and/or HPA activation. Common blood constituents that are measured include cortisol (Crookshank et al., 1979; Warriss et al., 1995; María et al., 2004), catecholamines (Rulofson et al., 1988; Mitchell et al., 1988; Lay et al., 1992a), glucose (Crookshank et al., 1979; Kenny and Tarrant, 1987; María et al., 2004), lactate (Mitchell et al., 1988; Shaefer et al., 1988; María et al., 2004) and creatine kinase (Crookshank et al., 1979; Warriss et al., 1995; María et al., 2004). Frequent methods used to collect blood from cattle for analyses of physiological stress indicators include venipuncture (Knowles et al., 1999; King et al., 2006), indwelling catheters (Lay et al., 1992a; Apple et al., 2005), and sampling during exsanguination (Mitchell et al., 1988; María et al., 2004). However, in a review of pre-slaughter and slaughter treatments of

livestock by measurement of plasma constituents, Shaw and Tume (1992) concluded that electrical and mechanical stunning methods can cause increases in catecholamine and glucose levels, but cortisol levels were unaffected by stunning methods. Additionally, some concern has been expressed that restraining animals to collect blood may initiate or add to the physiological stress response, so to combat this issue some researchers have attempted to train or habituate animals to handling before initiation of treatments (McVeigh et al., 1982; Lay et al., 1992a), or have used dairy cows that are frequently handled or halter led as experimental units (Lay et al., 1992b). Additionally, measurements of cortisol in fecal samples (Palme et al., 2000) or catecholamine concentrations in urine samples have been examined (Lowe et al., 2004) as alternatives to blood collection.

Several studies have investigated the effects of loading/unloading (Kenny and Tarrant, 1987; María et al., 2004), transport duration (Knowles et al., 1999), stocking density (Tarrant, 1990), and lairage/recovery times (Crookshank et al., 1979) on the physiological stress response of cattle. For example, Kenny and Tarrant (1987) reported the response of 12 groups of 6 steers to 3 treatments: 1) loading/unloading; 2) confinement for 1 h on a stationary truck; and, 3) confinement for 1 h on a moving truck. The authors measured plasma creatine kinase, plasma glucose, plasma cortisol, heart rate, and rectal temperature as indicators of stress (Kenny and Tarrant, 1987). Results from the study showed that of the 5 physiological indicators measured, only heart rate increased with loading/unloading. Confinement on a stationary truck caused increased plasma creatine kinase activity (indicative of physical activity, and possibly bruising), glucose content, and rectal temperature, compared to resting measurements (Kenny and

Tarrant, 1987). Confinement on a moving truck caused a rise in plasma cortisol levels compared to resting values (Kenny and Tarrant, 1987).

Although physiological measurements are reliable objective indicators of stress (preferably, more than one indicator is measured), these measurements are often time-consuming and expensive to obtain. Therefore, measures of behavior are frequently used to classify an animal's temperament (e.g., Calm vs. Flighty). Some studies have documented both behavioral and physiological responses to stress. For instance, Curley et al. (2006) evaluated the relationship of temperament appraisals (pen score, chute score, and exit velocity) of Brahman bulls with serum cortisol concentrations. The authors reported that serum cortisol concentrations were positively correlated with pen scores and exit velocity measurements, and concluded that exit velocity could be used as a possible predictor of temperament and stress responsiveness to future cattle handling events (Curley et al., 2006). Similarly, María et al. (2004) reported that cattle with the most undesirable loading scores had higher concentrations of cortisol, creatine kinase, and lactate.

Effects of Stress and Behavior on Meat Quality

pH and Tenderness. As described previously, stress can result in increased glycogenolysis in both the liver and skeletal muscle. The extent of muscle glycogen breakdown before slaughter can have pronounced effects on beef quality. After exsanguination, muscle relies on the anaerobic metabolism of glycogen to maintain homeostasis. In the absence of a functional circulatory system, lactate remains in postmortem muscle and increases in concentration (pH decrease) until muscle glycogen

stores are depleted or the pH becomes too low for the enzymes involved in glycolysis to function (Aberle et al., 2001).

The most common association between beef quality and cattle temperament or stress is the dark cutting condition (Scanga et al., 1998). Dark-cutters experience pre-slaughter stress that depletes muscle glycogen stores, resulting in reduced postmortem lactate production and high ultimate muscle pH (> 6.2)(Figure 2.2). Varying degrees of pre-slaughter glycogen breakdown can produce a wide range in ultimate muscle pH (Immonen and Puolanne, 2000). The rate of postmortem pH decline and ultimate muscle pH can affect various meat quality attributes such as color and water holding capacity; however, this review is confined to the effects of ultimate pH on meat tenderness.

Watanabe et al. (1996) investigated the effects of the ultimate pH of meat on tenderness changes during aging. The researchers produced a range of ultimate LM pH (5.4 to 7.2) by injecting sheep with adrenaline or by adrenaline injection and subsequent exercise. A curvilinear relationship between ultimate pH and shear force was reported (Figure 2.3; Watanabe et al., 1996). Shear force of LM with high ultimate pH (> 6.3) changed rapidly over time, and shear force of LM with ultimate low pH (< 5.8) declined at a moderate pace (Figure 3; Watanabe et al., 1996). Longissimus muscle with intermediate ultimate pH (5.8 – 6.3) had the slowest rate of tenderization (Figure 3; Watanabe et al., 1996). Shear force values from all pH groups (low, intermediate, and high) were equivalent at 6 d postmortem and the authors concluded that ultimate tenderness was not influenced by ultimate pH (Watanabe et al., 1996). In contrast, Silva et al. (1999) reported that tenderness (WBSF) at 1, 6, and 13 d postmortem increased linearly with increasing pH (5.5 to 6.7).

Stress, Behavior, and Tenderness. Previous studies have reported that cattle with excitable temperaments or that endure pre-slaughter stress have reduced tenderness compared to calm or unstressed cattle (Lewis et al., 1962; Voisinet et al., 1997; Vann et al., 2004; King et al., 2006; Warner et al., 2007). Warner et al. (2007) investigated the effects of acute pre-slaughter stress (6 electric prods 15 min before slaughter) on beef quality traits. Cattle subjected to the pre-slaughter stress treatment had higher plasma lactate at slaughter and produced LM steaks that were consumer rated as less tender, juicy, and flavorful than control steaks (Warner et al., 2007). WBSF values did not differ between treatments (Warner et al., 2007). The acute stress treatment was associated with reduced water holding capacity, but pre-rigor muscle pH and temperature and ultimate muscle pH did not differ between carcasses from stressed and control cattle (Warner et al., 2007). Lewis et al. (1962) reported that pre-slaughter stress (3 electric prods every 20 min, 24 hr before slaughter) resulted in increased LM shear force values and reduced sensory panel scores for tenderness; these effects were independent of LM pH.

Voisinet et al. (1997) showed that cattle with excitable temperaments had higher WBSF values than cattle with calm temperaments; however, the increase in WBSF was associated with an increase in the incidence of carcasses that were downgraded by USDA graders due to dark colored lean ("borderline dark-cutters"). King et al. (2006) reported temperament influenced LM WBSF independently of ultimate LM pH and 72-hr calpastatin activity. At 0.5 hr postmortem, carcasses from calm cattle had slightly higher LM pH values than carcasses from excitable cattle (6.1 vs. 5.9; King et al., 2006).

The mechanism associated with stress and decreased tenderness is not clear. Sensky et al. (1996) reported results that suggested that variations in plasma epinephrine

concentrations perturb the calpain system. This hypothesis is in general agreement with the effects that synthetic β-agonists have on postmortem proteolysis and meat tenderness (Dunshea et al., 2005; Strydom et al., 2009). In contrast to the preceding results, several researchers have reported that there is no association between cattle temperament/stress and tenderness (Burrow et al., 1999; Petherick et al., 2002; Colditz et al., 2007).

Sex Effects on Tenderness

Tatum et al. (2007) conducted a thorough review of the literature investigating the difference in tenderness between beef from steers and heifers. The authors compared WBSF measurements of steers (n = 3054) and heifers (n = 1870) included in 10 experiments from 1985 through 2006 (Tatum et al., 2007). The data presented by Tatum et al. (2007) suggested that LM steaks from heifers were slightly tougher (0.25 kg) than LM steaks produced from steers (Table 2.1). Several studies also have reported that beef produced from heifers is more variable in tenderness than beef produced from steers (Maher et al., 2004); and, that *Bos indicus* (Voisinet et al., 1997) and *Bos taurus* (Jeremiah et al., 1991; Wulf et al., 1996; Maher et al., 2004) heifers produce a higher frequency of tough steaks than steers (Tatum et al., 2007). Although the majority of studies summarized by Tatum et al. (2007) indicated that heifers produce beef that is slightly less tender than beef produced by steers, several studies have reported no difference in tenderness between beef from steers and heifers (Gracia et al., 1970; Prost et al., 1975; Bouton et al., 1982), or that beef from heifers is more tender than beef from steers (Kropf and Graf, 1959). It should be noted that some of these studies were excluded by Tatum et al. (2007) do to small sample sizes (< 20 animals per sex) or because measures of variation were not reported.

Tatum et al. (2007) identified 3 factors that are believed to be associated with sex effects on tenderness: 1) calpastatin effects on early postmortem tenderization; 2) temperament and reaction to pre-slaughter stress; and, 3) hormonal effects (endogenous and exogenous). Tenderness of beef increases with increasing time of postmortem storage, and it is widely accepted that the calpain proteolytic system is responsible, at least in part, for postmortem tenderization. The components of the calpain proteolytic system believed to be involved in postmortem proteolysis of beef are the calcium activated enzyme μ-calpain and its inhibitor, calpastatin (for review see Gruber, 2006). Measures of calpastatin activity (24 hr postmortem) have been associated with beef tenderness (Koohmaraie et al., 1995; Koohmaraie et al., 1996); high calpastatin activity reflects reduced postmortem proteolysis and is thus associated with increased beef toughness. Several studies have reported that, compared to LM from steers, LM from heifers had a higher 24-hr calpastatin activity and correspondingly higher WBSF measurements (Wulf et al., 1996; O'Connor et al., 1997; Wulf et al, 1997). The difference in LM WBSF between steers and heifers is particularly evident during the early postmortem storage period (Figure 2.4; Choat et al., 2006; Woerner and Tatum, 2007).

Tatum et al. (2007) also suggested that the higher circulating estrogen levels of heifers may contribute to their tendency to produce beef that is less tender than beef produced by steers. The effect of endogenous estrogen levels on beef tenderness has been investigated by utilizing spayed heifers; spaying reduces serum estrogen concentrations and prevents estrus (Vestergaard et al., 1995). Choat et al. (2006) used non-implanted cattle to compare 7-d WBSF measurements of steers, intact heifers, and

spayed heifers. Intact heifers produced LM steaks with higher WBSF measurements than LM steaks from steers; however, LM WBSF measurements did not differ between steers and spayed heifers (Choat et al., 2006). Jeffery et al. (1997) reported lower LM WBSF values for spayed heifers compared to intact heifers. In contrast, others have reported that tenderness between intact and spayed heifers did not differ (Bouton et al., 1982; Vestergaard et al., 1995; Field et al., 1996).

Anabolic Implants

Anabolic steroids have been approved for use in cattle production since 1954 (oral application of diethylstilbestrol), and since that time, a number of anabolic growth promoting compounds have been approved by the FDA for use in the United States (Raun and Preston, 1997; Table 2.2). Currently, anabolic agents for growing cattle can be classified as estrogens, androgens, or progestins. Compounds with estrogenic activity include 17β-estradiol (E2), estradiol benzoate (EB, approximately 72.34% E2), and zeranol (Botts et al., 1997). Synthetic androgens approved for use in cattle are testosterone propionate and trenbolone acetate (TBA; Botts et al., 1997). Compounds classified as progestins include progesterone and melengestrol acetate (MGA; Botts et al., 1997). These active ingredients alone or in combination are currently marketed under numerous trade names, and are classified as estrogenic, androgenic, or combination implants (Table 2.3).

In general, cattle finished < 130 d receive a single implant during the finishing period, whereas cattle requiring > 130 d of finishing are administered 2 implants; re-implantation typically occurs 70 to 120 d after administration of the initial implant (Nichols et al., 2005). Galyean (1997) surveyed beef nutrition consultants who serviced

feedlots in all major cattle feeding states and reported that, in general, most consultants recommended a combination terminal implant (TBA + E) administered 80 to 140 d before slaughter for steers and heifers not receiving MGA. For heifers receiving MGA, a terminal implant (80 to 140 d before harvest) containing TBA alone was recommended (Gaylean, 1997). When steers and heifers received more than 1 implant, the initial implant was typically estrogenic (EB or zeranol; Galyean, 1997).

Mechanisms of Action. Dayton and White (2008) stated that "despite general agreement on the effectiveness of anabolic steroids, there has been no consensus as to the cellular mechanism(s) responsible for the anabolic effects of either estrogenic or androgenic steroids." Several hypotheses have been suggested as direct and indirect mechanisms of action for estrogen induced protein accretion. Estrogen receptors have been located on bovine skeletal muscle (Meyer and Rapp, 1985; Sauerwein and Meyer, 1989; Hancock et al., 1991), and although the concentration of estrogen receptors on skeletal muscle is 1000-fold lower than that of the uterus, nuclear binding coupled with increased RNA and protein synthesis has been suggested as a possible direct mechanism of action for anabolic estrogen effects (Meyer and Rapp, 1985; Hancock et al., 1991). One discrepancy identified with this is that estrogen receptors have been isolated from the skeletal muscle of rats, but rats do not exhibit an anabolic response to estrogen administration (Hancock et al., 1991; Hossner, 1995).

The classical hypothesis for an indirect estrogenic mechanism of action involves anabolic effects mediated through an increase in growth hormone (somatotropin) secretion. In support of this hypothesis, anterior pituitary weight, cell size and number, and secretion and circulating concentration of growth hormone (GH) have been reported

to increase following administration of estrogen to ruminants (Hancock et al., 1991). Moreover, GH and estrogen have similar effects on nitrogen metabolism (Hossner, 1995). Lack of support for the classical hypothesis have been detailed as: 1) when administered together, GH and estrogen have an additive anabolic response, suggesting that each hormone has an independent pathway and, 2) estrogen administration also increases GH secretion in rats, but rats do not exhibit an anabolic response to estrogen (Hancock et al, 1991; Hossner, 2005).

Alternative indirect hypotheses for the mechanism of estrogenic steroidal action include responses mediated by insulin like growth factor-I (IGF-I) and catechol estrogen formation (Hancock et al., 1991; Hossner, 2005). Catechol estrogens (CE) are estrogen metabolites formed by hydroxylation. The CE inhibit catecholamine formation; catecholamines mobilize energy glycogenolysis, and therefore can have catabolic effects on skeletal muscle (Hancock et al., 1991). Administration of estrogen to ruminants also has been reported to increase the number of hepatic GH receptors by 250% (Hancock et al., 1991; Hossner, 2005). Growth hormone receptors mediate IGF-I secretion by the liver; IGF-I stimulates amino acid uptake and protein synthesis in skeletal muscle, and may be an integral portion of an indirect mechanism of action for anabolic estrogen effects (Hancock et al., 1991; Hossner, 2005).

Natural and synthetic androgens have different mechanisms of action (Hancock et al., 1991). Testosterone and dihydrotestosterone (DHT, an active metabolite of testosterone) primarily cause protein accretion through an increase in protein synthesis. Conversely, trenbolone acetate (TBA, a structural analog of testosterone) reduces both protein synthesis and degradation; TBA reduces degradation to a greater extent than the

reduction in synthesis thereby resulting in net protein accretion (Sinnett-Smith et al., 1987; Hancock et al., 1991). It has been suggested that the anabolic response to androgens may be mediated by a direct response similar to that for estrogens (Hancock et al., 1991; Hossner, 2005). In support of a direct mechanism of action, Sinnett-Smith et al. (1987) reported that when sheep where implanted with TBA, the number of androgen receptors in skeletal muscle increased. When administered in combination, TBA and estrogen produced an additive growth response in steers, without increasing GH concentrations. Hossner (2005) suggested that these findings indicate that the combination treatment may have a direct mechanism of action with no involvement of the endocrine-somatotropic axis.

Several indirect mechanisms of action also have been suggested for androgens. Catabolic effects on skeletal muscle occur in response to corticosteroids. Androgens displace corticoid steroids from receptors by competitive binding, and are also believed to down-regulate corticosteroid receptors (Hancock et al., 1991). Androgens also have been reported to decrease plasma thyroxine levels and this has been suggested as another possible indirect mechanism of anabolic androgen action (Hancock et al, 1991). Additional indirect mechanisms of action for testosterone (not TBA) include increased plasma insulin concentrations and an anabolic response mediated through estrogen. TBA has not been shown to increase insulin concentrations. Furthermore, only testosterone and DHT can be converted to estrogen via aromatization; TBA cannot be metabolized to estrogen *in vivo* (Sillence, 2004).

Recent research involving both estradiol and TBA suggests that the mechanism of anabolic action for both steroid hormones may be mediated through IGF-I and its affects

on satellite cells (Chung and Johnson, 2008; Dayton and White, 2008). In meat animals, skeletal muscle fibers are postmiotic. In other words, the muscle fibers and the nuclei within each fiber cannot divide. Therefore, if postnatal muscle hypertrophy is to occur, muscle fibers need an external source of DNA (Chung and Johnson, 2008). According to Allen et al. (1979), 60 to 90 % of DNA within mature skeletal muscle is accumulated postnatal (Chung and Johnson, 2008). Mononucleated satellite cells located between the basal lamina and the sarcolemma of the muscle fiber are recognized as the source of external DNA needed for postnatal muscle growth (Mauro, 1961). Chung and Johnson (2008) suggested that activation, increased proliferation, and fusion of satellite cells may be an important mechanism by which anabolic steroids enhance muscle hypertrophy.

As discussed previously, muscle from implanted cattle (TBA + E2) may produce more IGF-I than muscle from non-implanted cattle (Johnson et al., 1996a, 1998; White, 2003). According to Chung and Johnson (2008), IGF-I is a progression factor that aids cells through the cell cycle; it is a potent stimulator of satellite cell proliferation and promotes muscle cell differentiation. Kamanga-Sollo et al. (2004) showed *in vitro* that TBA and E2 can increase the rate of proliferation of cultured satellite cells from bovine skeletal muscle (Chung and Johnson, 2008). Johnson et al. (1998) reported that semimembranosus muscle from steers implanted with TBA and estradiol-17β contained a greater number of satellite cells than did corresponding muscles from non-implanted cattle (Dayton and White, 2008). The increase in IGF-I in bovine skeletal muscle from implanted cattle coupled with increased satellite cell proliferation may support a mechanism of steroidal action (Hossner, 2005; Chung and Johnson, 2008; Dayton and White, 2008).

Implant Effects on Beef Quality. Approximately 97% of U.S. feedlot cattle receive 1 or more implants during the finishing period (USDA, 2000). Administration of anabolic implants to steers and heifers can increase growth rate by 10 to 20 % (Dikeman, 2007) and feed efficiency by 8 % (Duckett, 1996). Use of growth promoting implants also consistently increases hot carcass weight and LM area of fed cattle (Duckett et al., 1997; Roeber et al., 2000; Duckett and Andrae, 2001; Schneider et al., 2007). Although steroidal implants provide economic rewards by enhancing growth and performance, negative effects on carcass quality attributes have been reported (Morgan, 1997).

In general, it is recognized that application of finishing implants to steers and heifers can reduce marbling score, decrease the percentage of carcasses grading choice, and increase skeletal maturity (Dikeman, 2007). Morgan (1997) used the Oklahoma State University (OSU) Implant Data Base to compare the effects of numerous implant strategies on marbling score and grade distribution of steers and heifers (Table 2.4); implant strategies varied by the number of implants administered, classification (androgen, estrogen, and combination), and potency. Compared to non-implanted controls, the impact of implants on marbling score ranged from - 9 to - 47 degrees (Table 2.4; Morgan, 1997). Implanting steers and heifers numerically reduced the percentage of cattle grading Choice by 2.1 to 26.0 % (Table 2.4; Morgan, 1997). However, several studies also have reported no difference in marbling scores between non-implanted and implanted cattle (Gerken et al., 1995; Johnson et al., 1996b; Smith et al., 2007).

Duckett et al. (1999) investigated the effects of anabolic implants on beef intramuscular lipid content. In that study, non-implanted steers were compared to steers administered: 1) a strong combination implant at d 0; 2) a strong combination implant at

d 0 and 61; or 3) a moderate estrogenic implant on d 0 and a strong combination implant on d 61 (Duckett et al., 1999). Implanting reduced ($P < 0.05$) marbling score by approximately 50 degrees ($Small^{00}$ vs. $Small^{50}$); marbling scores did not differ ($P > 0.05$) among implanted cattle (Duckett et al., 1999). Compared to control steers, implanting increased LM area by 7 % (Duckett et al., 1999). Total fatty acid percentage (LM) was less ($P < 0.05$) for implanted steers than for control steers; however, when the increases in LM area were accounted for, implanting did not change amount of total fatty acids (Duckett et al., 1999). Duckett et al. (1999) concluded that implanting alters intramuscular lipid amount through a dilution effect with increased LM area. Smith et al. (2007) reported that implanting did not alter mRNA expression of acetyl CoA carboxylase, stearoyl CoA desaturase, or lipoprotein lipase (enzymes involved in the uptake and biosynthesis of fatty acids) in intramuscular adipose tissue. Additionally, the number of intramuscular adipocytes per g of LM tissue was greater for implanted cattle than for non-implanted controls (Smith et al., 2007). Similarly to Duckett et al. (1999), Smith et al. (2007) concluded that anabolic implants do not have a direct effect on intramuscular fat deposition (cellularity or enzyme expression). It should be noted that administration of finishing implants by Smith et al. (2007) resulted in increased LM area, but did not reduce marbling score.

The traditional belief is that intramuscular fat (marbling) is a late developing tissue; however, contemporary research suggests that intramuscular fat develops at a constant rate throughout cattle growth (Bruns et al., 2004). Recently, it has been suggested that the use of "delayed" implant strategies may be effective at reducing or eliminating the impacts of anabolic implants on marbling scores. To investigate the effects of implant

timing on marbling score, Bruns et al. (2005) applied 3 implant strategies to crossbred steers:1) no implant, control; 2) early implant - a moderate combination implant administered on d 0; and, 3) delayed implant - a moderate combination implant administered on d 57. Marbling scores of cattle administered a delayed implant did not differ ($P > 0.05$) from those of control or early implanted cattle; however, cattle in the early implant treatment group produced carcasses with lower ($P < 0.05$) marbling scores than control cattle (Bruns et al., 2005). Furthermore, compared to early implanted cattle, control and delayed implant cattle produced an average of 15 % more carcasses that graded Modest^{00} or higher (premium choice). Other reports on the effects of delayed implanting on marbling score have produced variable results. Griffin et al. (2009) conducted a 2 year study that compared traditional (initial implant d 0/ re-implant d 115) and delayed (initial implant d 30/re-implant 115) strategies for calf-fed steers (initial implant = Synovex-S, re-implant = Synovex choice). During year 1 of the study, cattle in the delayed treatment group produced 22% more choice carcasses than traditionally implanted cattle (Griffin et al., 2009). However, in year 2, traditionally implanted cattle produced 15 % more Choice carcasses than cattle that received a delayed implant (Griffin et al. 2008). Several additional studies have investigated the impact of initial implant timing on marbling scores; unfortunately, these studies often confound implant timing with the number of implants administered or dosage of steroids used (Mader, 1994; Scheffler et al., 2003; Tatum et al., 2008).

In addition to affects on marbling, it is generally recognized that implants tend to decrease tenderness (Dikeman, 2007). Morgan (1997) also summarized the effects of various implant strategies on WBSF (Table 2.5). On average, WBSF values for steaks

from implanted cattle were approximately 0.50 kg greater than control steaks (Morgan, 1997). Summarizing WBSF results from the OSU Implant Database should be done with caution however, as postmortem aging times vary by study (Morgan, 1997). Barham et al. (2003) used Bos indicus steers to investigate the effects of implanting (control vs. moderate estrogenic/moderate combination vs. moderate combination/moderate combination) and postmortem aging on WBSF. Compared to LM steaks from implanted cattle, steaks from non-implanted cattle had lower ($P < 0.05$) WBSF values after 3, 7, and 14 d of postmortem storage (Barham et al., 2003). No implant treatment differences ($P > 0.05$) were detected following 21 d of storage (Barham et al., 2003). Platter et al. (2003) reported the effects of 10 lifetime implant strategies on consumer ratings of beef palatability; LM steaks (aged 14 d postmortem) from control steers had lower ($P < 0.05$) WBSF values and were rated by consumers as more desirable for tenderness like/dislike than LM steaks from steers in all other groups (Platter et al., 2003). In contrast, Nichols et al. (2005) summarized the effects of various implant strategies on WBSF (Table 2.6) and concluded that steroidal implants have limited, if any, effects on WBSF.

A limited amount of information is available regarding the effects of steroidal implants on the tenderness of beef from heifers; however, recent data presented by Schneider et al. (2007) suggests that the number and potency of hormonal implants administered to heifers during the finishing period can affect beef tenderness. Findings of Schneider et al. (2007) showed that administering a single finishing implant to heifers did not impact 14-d LM WBSF; however, 14-d LM steaks from heifers receiving 2 successive finishing implants was significantly higher than 14-d LM steaks from non-implanted and single implant heifers. Results summarized in Figure 2.2 also suggest that

not all 2-implant programs negatively impacted tenderness, only programs that utilized more potent combination (androgenic and estrogenic compounds) implants significantly affected 14-d WBSF (Schneider et al., 2007). Moreover, heifers that received implants containing both E2 and TBA had higher WBSF values at 3, 7, 14, and 21 d postmortem than did heifers that were administered implants containing TBA alone (Schneider et al., 2007). Data presented by Schneider et al. (2007), as well as others, emphasize the fact that the effects of steroidal implants on beef tenderness can be influenced by sex, animal age, dosage, classification of implant (estrogen, androgen, or combination) number of implants administered throughout an animals lifetime, and postmortem aging time (Prichard and Rust, 1997; Roeber et al., 2000; Platter et al., 2003; Barham et al., 2003).

Genetic Effects on Tenderness

Research suggests that genetic differences in beef tenderness are associated with genetic variation in activities of μ-calpain and calpastatin during the early postmortem period (Shackelford et al., 1994; Wulf et al., 1996; O'Connor et al., 1997). Gene mapping studies have identified CAPN1 and CAST as the genes that encode for μ-calpain and calpastatin, respectively. Table 2.7 displays a summary of single nucleotide polymorphisms (SNP) that have been investigated as possible genetic markers for beef tenderness. Most of these genetic markers/SNPs have been applied to Bos taurus and Bos indicus populations, but it should be noted that not all markers segregate in both biological types of cattle (Casas et al., 2005). To date, several genetic markers have been made commercially available to facilitate marker-assisted selection for beef tenderness. Of specific interest for the current research is the 3-marker panel offered by Pfizer Animal Genetics (Harahan, LA). Marketed under the trade name GeneSTAR, it consists

of the CAST gene marker for calpastatin and μ-calpain markers 316 and 4751 (Table 2.7). Van Eenennaam et al. (2007) validated the GeneSTAR Tenderness panel in over 1,000 Bos taurus and Bos indicus cattle (372 Charolais × Angus, 260 Hereford, and 670 Brahman). Their findings showed approximately a 1 kg difference in Warner-Bratzler shear force between the most and least tender genotypes (Table 2.8). Results presented by Van Eenennaam et al. (2007) showed that there was a significant association between the combination of all three markers and WBSF, as well as an association between CAST and the μ-calpain haplotype with WBSF. For each favorable CAST allele (T), a decrease of 0.15 kg in WBSF was observed; substituting the calpain C-C haplotype for the G-T haplotype was estimated to decrease WBSF by 0.34 kg (Van Eenennaam et al., 2007).

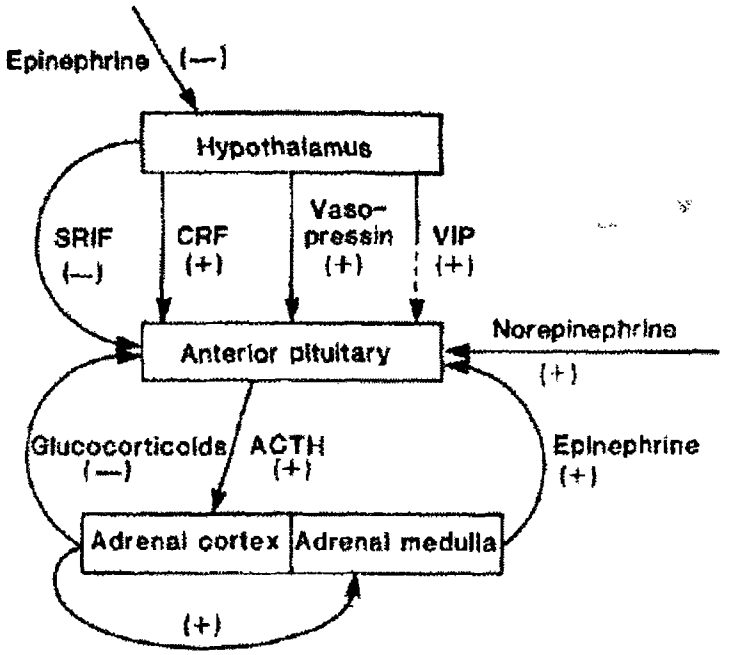

Figure 2.1. Multihormonal response to stress (Axelrod and Reisine, 1984). SRIF = somatostatin-releasing inhibitor factor; CRF = corticotrophin releasing factor/corticotrophin releasing hormone; VIP = vasodilatory intestinal peptide; ACTH = adrenocorticotropin hormone.

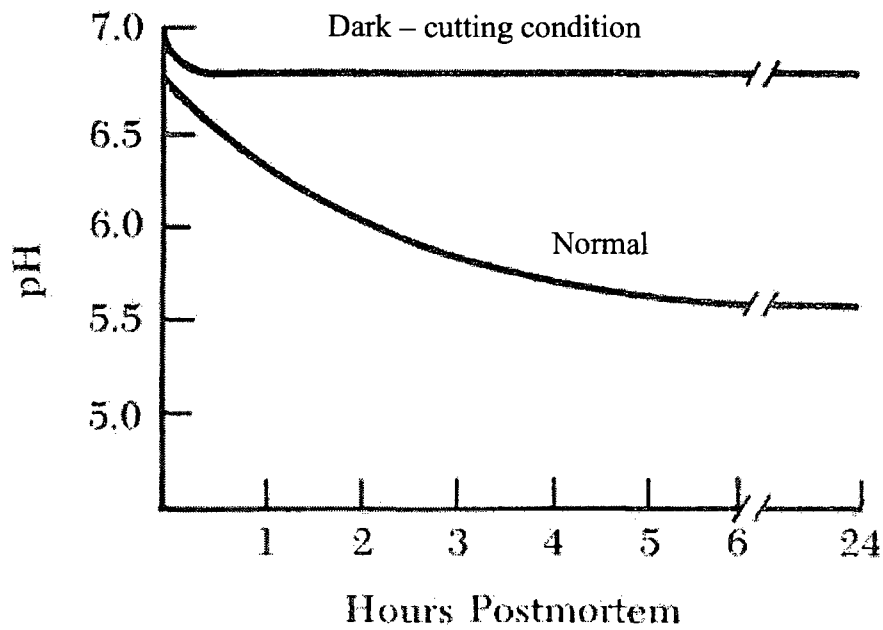

Figure 2.2. Postmortem pH decline. Adapted from Aberle et al. (2001).

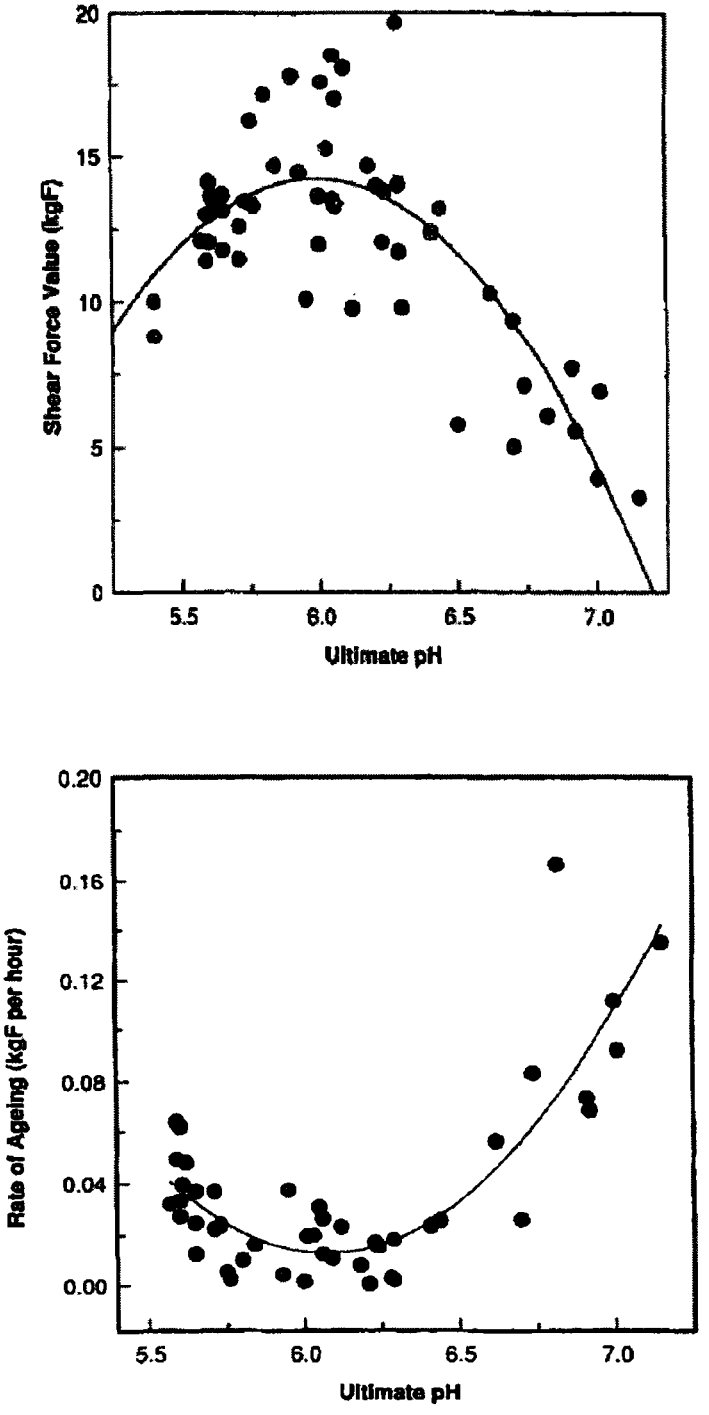

Figure 2.3. Shear force values (1 d postmortem) for sheep loin muscle at pH 5.4 to 7.2 (top). Rate of change in shear force (d 1 through 6 postmortem) for sheep loin muscle at pH 5.4 to 7.2 (bottom). Adapted from Watanabe et al. (1996).

Table 2.1. Summary of studies comparing mean values for Warner-Bratzler shear force of LM samples from steers vs. heifers[1]

		Heifers		Steers		
Study	Age, d	No. of animals	WBSF, kg	No. of animals	WBSF, kg	Mean difference[2], kg
Greathouse (1985)	11	42	4.00	42	4.50	-0.50
Jeremiah et al. (1991)[3]	6	978	6.21	1985	5.68	0.53*
Huffhines et al. (1993)	18	198	2.95	200	2.74	0.21*
Wulf et al. (1996)	14	170	3.14	222	2.91	0.23*
O'Connor et al. (1997)	14	125	3.00	138	2.78	0.22*
Busby et al. (2001)	14	88	6.95	151	6.63	0.32*
Maher et al. (2004)	14	81	5.38	81	4.54	0.84*
Choat et al. (2006) – Exp I	14	51	3.62	96	3.31	0.31*
Choat et al. (2006) – Exp II	14	60	3.36	60	3.11	0.25*
Gruber et al. (2006b)	14	77	3.50	79	3.56	-0.06
Standardized mean sex effect[4]						0.25

[1]Source: Tatum et al. (2007).

[2]Mean WBSF difference = (Mean $WBSF_{heifer}$ – Mean $WBSF_{steer}$).

[3]WBSF obtained using 2.5 cm cores. All other studies used 1.3 cm cores.

[4]Calculated using methodology described by Lipsey and Wilson (2001).

*WBSF of steers differed from heifers ($P < 0.05$).

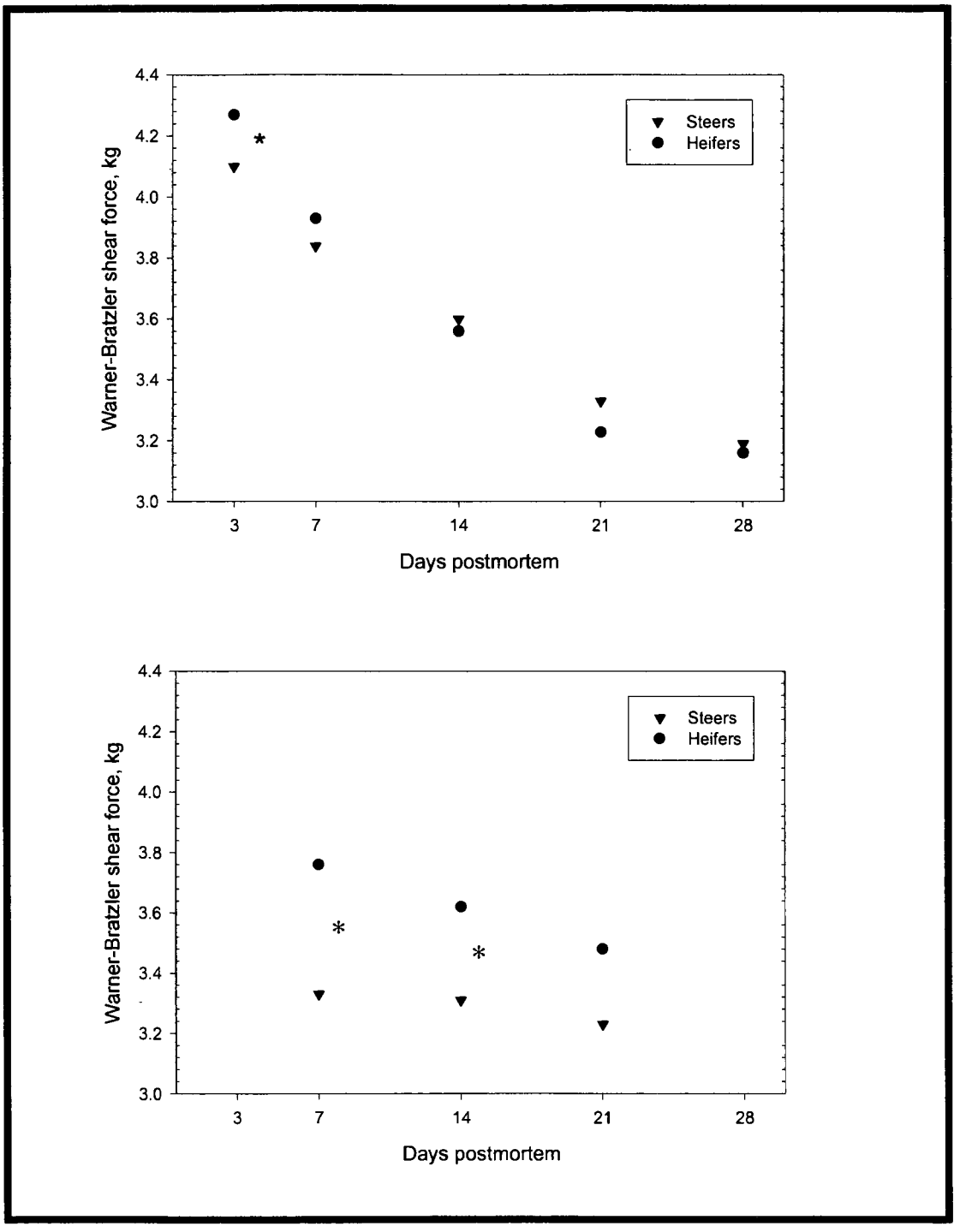

Figure 2.4. Postmortem changes in LM Warner-Bratzler shear force (WBSF) for steers and heifers (top: Woerner and Tatum, 2007; bottom: Choat et al., 2007). * Mean WBSF differed between steers and heifers ($P < 0.05$).

Table 2.2. Chronology of cattle anabolic agents in the U.S.[1]

Year	Event
1954	Oral DES approved for cattle
1956	Estradiol benzoate/progesterone implants approved for steer
1957	DES implants approved for cattle
1958	Estradiol benzoate/testosterone propionate implants approved for heifers
1968	Oral MGA approved for heifers
1969	Zeranol implants (36 mg) approved for cattle
1979	FDA bans all use of DES in cattle production
1982	Silastic estradiol implant approved for cattle
1984	Estradiol benzoate/progesterone implants approved for calves
1987	Trenbolone acetate (TBA) implants approved for cattle
1991	Estradiol/TBA implants approved for steers
1993	BST approved for lactating dairy cows
1994	Estradiol/TBA implants approved for heifers
1995	72 mg Zeranol implants approved for cattle
1996	Estradiol/TBA implants approved for stocker cattle
2006	The suspension implant Duralease (EB) is approved for use in steers/heifers
2007	Slow-release delivery system (Revalor-XS, TBA/Estradiol) is approved for steers

[1]Source: Raun and Preston (1997).

Table 2.3. Summary of commercially available anabolic implants categorized by type (estrogenic, androgenic, or combination) and potency (low, medium, or high)[1]

				Active Ingredients (mg)				Approved Uses					
								Calves		Stockers		Feedlot	
		Implant	Company[2]	Estrogen[3]	Progesterone	Testosterone Propionate	Trenbolone Acetate	Effective Period[4]	Steers	Heifers	Steers	Heifers	Steers	Heifers
Estrogenic	Low	Ralgro	IS-P	11–13[5]				70	×	×	×	×	×	×
		Synovex-C	FD	7.2[6]	100			120	×	×			×	
		Component E-C	VL	7.2[6]	100			120	×	×			×	
		Ralgro Magnum	IS-P	72 [5]				90					×	
		Compudose	VL	25.7				175	×		×		×	×
	Medium	Encore	VL	43.9				336	×		×		×	×
		Synovex-S	FD	14.5[6]	200			120			×		×	
		Component E-S	VL	14.5[6]	200			120			×		×	
		Synovex-H	FD	14.5[6]		200		120				×		×
		Component E-H	VL	14.5[6]		200		120				×		×
Androgenic		Finaplix-S[7]	IS-P				140	105					×	
		Component T-S[7]	VL				140	105					×	
		Finaplix-H	IS-P				200	105						×
		Component T-H	VL				200	105						×
Combination	Low	Revalor-G	IS-P	8			40	120			×	×		
		Component-TEG	VL	8			40	120			×	×		
		Synovex T40	FD	8			40	120			×	×		
		Revalor-IS	IS-P	16			80	120					×	
		Component TE-IS	VL	16			80	120					×	
		Synovex T80	FD	16			80	120					×	
		Revalor-IH	IS-P	8			80	120						×
		Component TE-IH	VL	8			80	120						×
		Synovex - Choice	FD	10			100	120					×	

Medium	Revalor-S	IS-P	24	120	120	×	
	Component TES	VL	24	120	120	×	
	Synovex – T120	FD	24	120	120	×	
	Revalor-H	IS-P	14	140	120		×
	Component TEH	VL	14	140	120		×
High	Revalor-200	IS-P	20	200	120	×	×
	Synovex-Plus	FD	20	200	120	×	×
	Component TE 200	VL	20	200	120	×	×
	Revalor-XS	IS-P	40	200	200	×	

[1]Adapted from various publication (scientific and trade) including, but not limited to: McCollum (1998); Duckett and Andrae (2001); Platter (2003); Campbell (2007); FDA (2008).

[2]IS-P = Intervet Schering-Plough, Millsboro, DE. FD = Fort Dodge Animal Health, Ft. Dodge, IA. VetLife, Ivy Laboratories, Overland Park, KS.

[3]Estrogenic activity.

[4]Refer to manufacture's label for most accurate claims.

[5]Zeronal is 31 to 36% active estrogen.

[6]Estradial benzoate contains 72.34% Estradiol 17β.

[7]Implants are approved by the FDA, but are no longer manufactured.

Table 2.4. Effects of implant strategy on marbling score and percentage choice[1]

First implant	Second implant	Third implant	Marbling score	Choice, %
-	-	-	436[2]	78.5
ME[3]	-	-	-12[4]	-4.9[4]
ME	ME	-	-16	-5.7
ME	ME	ME	-12	-3.5
A	-	-	-9	-4.2
A	A	-	NA[5]	-2.1
ME/A	ME/A	-	-12	-9.3
SE	-	-	-24	-14.3
SE	SE	-	-47	-24.0
SE/A	-	-	-19	-6.2
SE/A	SE/A	-	-24	-24.0
MC	-	-	-12	-23.0
MC	MC	-	-26	-24.0
SE	MC	-	-21	-23.0
SC	-	-	-29	-20.0
SC	SC	-	-20	-26.0

[1]Source: Morgan (1997).
[2]Marbling score: 300 to 399 = select; 400 to 499 = small.
[3]Implant classification: ME = mild estrogen, SE = strong estrogen, A = androgen, MC = mild combination, SC = strong combination.
[4]Change in marbling score and percentage choice compared to non-implanted controls.
[5]NA = not available.

Table 2.5. Effects of implant strategy on Warner-Bratzler shear force (WBSF)[1]

First implant	Second implant	Third implant	WBSF, kg[2]
-	-	-	3.63
ME[3]	-	-	+ 0.05
ME	ME	ME	+ 0.42
A	-	-	+ 0.59
ME/A	ME/A	-	+ 0.71
SE	-	-	+ 0.43
SE	SE	-	+ 0.44
SE/A	-	-	+ 0.49
SE/A	SE/A	-	+ 0.64
MC	-	-	+ 0.11
MC	MC	-	+ 0.77
SC	-	-	+ 0.77
SC	SC	-	+ 0.59

[1]Source: Morgan (1997).
[2]Change in WBSF compared to non-implanted controls.
[3]Implant classification: ME = mild estrogen, SE = strong estrogen, A = androgen, MC = mild combination, SC = strong combination.

Table 2.6. Summary of published experiments showing the effects of implants on Warner-Bratzler shear force (WBSF,kg)[1]

Reference	Year	N	Sex[2]	Control	E_2[3]	0/TBA[4]	E2/TBA[5]	TBA/0[6]	TBA/TBA[7]	TBA × 342
Crouse et al.	1987	77	h	5.45	-	-	-	5.37	-	-
Trenkle	1990	150	s	2.86	3.22	-	3.67	3.31	3.40	-
Apple et al.	1991	72	s	4.01	3.97	-	-	4.23	-	-
Huck et al.	1991	80	s	3.68	3.90	-	3.90	-	3.71	-
Huffman et al.	1991	46	s	4.82	5.50	-	-	5.98	-	-
Hunt et al.	1991	30	s	3.40	-	-	-	3.25	-	-
Gerken et al.[8]	1995	24	s	4.16	4.56	-	-	4.29	-	-
Cranwell et al.	1996	60	cc	5.10[a]	4.90[b]	-	-	4.85[b]	-	-
Kerth et al.	1996	12	s	2.07	-	-	-	2.35	-	-
Milton et al.	1996	480	s	3.64	-	3.79	3.81	-	3.81	-
Milton et al.	1996	216	s	3.85	3.89	-	3.76	3.82	3.87	-
Nichols et al.	1996	600	h	3.71[a]	-	-	-	3.91[b]	-	-
Samber et al.	1996	560	s	2.58[a]	-	-	2.69[ab]	-	3.01[c]	2.89[bc]
Foutz et al.[9]	1997	140	s	4.00	4.43	-	-	4.20	-	-
Beirman et al.	1999	480	s	5.41	-	5.36	-	5.76	5.37	-
Pruneda et al.	1999	100	s	3.38	-	-	-	3.56	3.55	-
Pritchard	2000	310	s	4.02[b]	3.84[a]	-	4.06[ab]	-	4.15[b]	-
Roeber et al.	2000	298	s	2.97[a]	3.19[ab]	3.42[ab]	3.35[ab]	3.51[b]	3.27[ab]	-
Kerth et al.	2002	320	h	3.49[a]	-	3.53[a]	3.39[ab]	2.92[b]	3.18[b]	-

[1]Source: Nichols et al. (2002).
[2]H = heifer; s = steer; cc = cull cows.
[3]Estradiol (E_2) only implant.
[4]No initial implant, and an implant containing TBA at re-implant.
[5]Estradiol (E_2) initial implant, and an implant containing TBA at re-implant.
[6]TBA initial implant, and no re-implant.
[7]Initial and re-implant both contained TBA.
[8]WBSF values averaged for striploin, top sirloin, and top round steaks.
[9]Control vs. all implants ($P < 0.05$); control vs. TBA ($P < 0.10$).

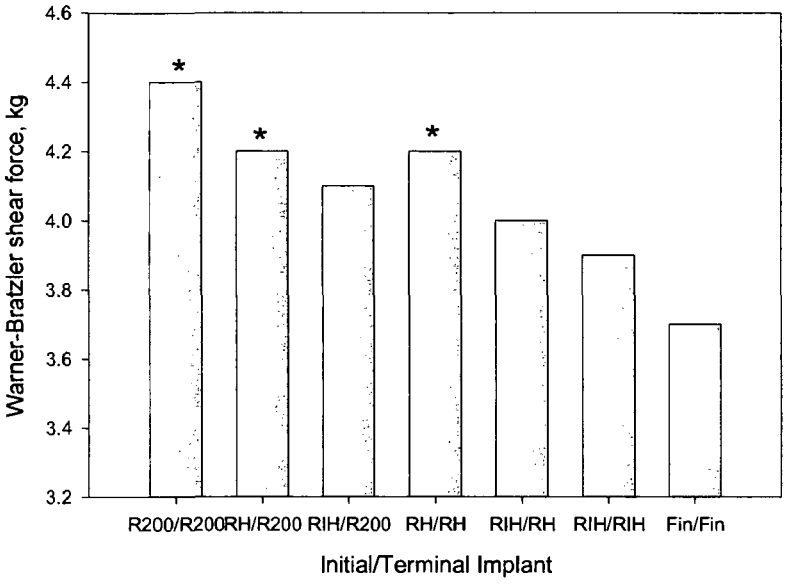

Figure 2.5. Effects of heifer finishing implants on 14-d LM Warner-Bratzler shear force (Tatum et al, 2007; Schneider et al., 2007). *Differs ($P < 0.05$) from non-implanted control.

Table 2.7. Summary of genetic markers that have been investigated for genetic assisted selection of beef tenderness

Gene marker	SNP[1]	Gene[2]	Citations
UoGCAST1	G/C	calpastatin	Schenkel et al. (2006) Van Eenennaam et al. (2007)
CAST	G/A	calpastatin	Barendese (2002) Casas et al. (2006) Van Eenennaam et al. (2007)
316	G/C	μ-calpain	Page et al. (2002) Page et al. (2004) Casas et al. (2005) White et al. (2005) Van Eenennaam et al. (2007)
530	G/C	μ-calpain	Page et al. (2002) Page et al. (2004) Casas et al. (2005) White et al. (2005)
4751	C/T	μ-calpain	White et al. (2005) Casas et al. (2006) Van Eenennaam et al. (2007)
4753	A/C	μ-calpain	Casas et al. (2005) White et al. (2005)
5331	A/T	μ-calpain	Casas et al. (2005) White et al. (2005)

[1]SNP = single nucleotide polymorphism.
[2]Calpastatin and μ-calpain genes are commonly referred to as CAST and CAPN1 in the literature, respectively.

Table 2.8. Genotypic effects of the GeneSTAR tenderness panel on Warner-Bratzler shear force (Van Eenennaam et al., 2007)

Genotype						
CAST (T1)	CAPN1 316 (T2)	CAPN1 4751 (T3)	Estimate, kg	SE	No.	%
2 = CC	2 = CC	2 = CC	-1.0	0.2	11	0.8
		1 = CT	-0.8	0.2	9	0.7
		0 = TT	-0.6	0.4	0	0.0
	1 = CG	2 = CC	-0.8	0.2	71	5.5
		1 = CT	-0.6	0.1	80	6.1
		0 = TT	-0.5	0.2	13	1.0
	0 = GG	2 = CC	-0.7	0.2	54	4.2
		1 = CT	-0.5	0.1	143	11.0
		0 = TT	-0.3	0.1	321	24.7
1 = CG	2 = CG	2 = CC	-0.8	0.2	5	0.4
		1 = CT	-0.7	0.2	3	0.2
		0 = TT	-0.5	0.4	0	0.0
	1 = CG	2 = CC	-0.7	0.1	38	2.9
		1 = CT	-0.5	0.1	25	1.9
		0 = TT	-0.3	0.2	7	0.5
	0 = GG	2 = CC	-0.5	0.1	62	4.8
		1 = CT	-0.3	0.1	53	4.1
		0 = TT	-0.2	0.0	285	21.9
0 = GG	2 = CC	2 = CC	-0.7	0.2	0	0.0
		1 = CT	-0.5	0.2	1	0.1
		0 = TT	-0.3	0.3	0	0.0
	1 = CG	2 = CC	-0.5	0.1	9	0.7
		1 = CT	-0.3	0.1	7	0.5
		0 = TT	-0.2	0.2	6	0.5
	0 = GG	2 = CC	-0.4	0.1	17	1.3
		1 = CT	-0.2	0.1	15	1.2
		0 = TT	0.0	0.0	67	5.2

CHAPTER III

RELATIONSHIPS OF BEHAVIORAL AND PHYSIOLOGICAL SYMPTOMS OF PRE-SLAUGHTER STRESS TO BEEF LM TENDERNESS

ABSTRACT

The relationships between behavioral and physiological symptoms of pre-slaughter stress and LM tenderness were investigated using bos taurus steers (n = 79) and heifers (n = 77). All behavioral reactions to pre-slaughter stress (pen, chute, and post-transportation score) were positively correlated ($P < 0.05$) with each other. Increased chute scores (more agitated) were associated ($P < 0.05$) with increased heart rate (r = 0.45), rectal temperature (r = 0.37), serum cortisol concentration (r = 0.29), and plasma epinephrine concentration (r = 0.42). Higher post-transportation behavior scores were associated ($P < 0.05$) with increased concentrations of plasma lactate (r = 0.33) and serum creatine kinase (r = 0.28) at slaughter. Positive correlations between behavior scores and physiological traits indicated that animals that showed behavioral responses to handling and transport also responded with physiological symptoms of stress. Epinephrine concentration, measured as an indicator of acute stress during handling, was positively correlated (r = 0.22 to 0.42; $P < 0.05$) with heart rate and rectal temperature during restraint, plasma lactate and serum creatine kinase concentration at slaughter, and LM Warner-Bratzler

shear force (WBSF). Plasma lactate concentration at slaughter, used to reflect an adrenergic stress response to transportation, was associated ($P < 0.05$) with lower LM pH (r = -0.30) and higher WBSF (r = 0.26). Categorical analyses of behavior scores (calm vs. restless vs. nervous) indicated that cattle that had adverse behavioral reactions to handling had increased ($P < 0.05$) plasma epinephrine concentration, heart rate, and rectal temperatures during chute restraint, elevated ($P < 0.05$) plasma lactate concentrations at slaughter, and increased ($P < 0.05$) WBSF. Cattle that were agitated in lairage had higher ($P < 0.05$) plasma glucose and lactate concentrations at slaughter and produced LM steaks that were 0.34 kg tougher ($P < 0.05$) than calm cattle. No carcasses were identified as dark cutters, and LM pH did not differ ($P > 0.05$) by behavior category. Plasma lactate concentration at slaughter was associated ($P < 0.05$) with response to postmortem aging. Steaks from cattle with the highest plasma lactate concentrations at slaughter (91st to 100th percentile) had a delayed response to aging that persisted until 14 d postmortem. Results of this study identified behavioral and physiological symptoms of acute pre-slaughter stress that were associated with pH-independent differences in LM tenderness.

INTRODUCTION

Handling and transport of cattle immediately before slaughter elicit a broad range of responses among individual animals (Grandin, 1997; von Borell, 2001). While some cattle appear psychologically and physically unaffected by routine pre-slaughter handling and transport practices, others exhibit various combinations of stereotypical behaviors (e.g., nervousness, balking, excitement, fear, avoidance/flight, vocalization, aggression)

and physiological reactions that are symptomatic of stress (von Borell, 2001; Broom, 2007; Knowles and Warriss, 2007).

Acute exposure of cattle to adverse stimuli (stressors) causes release of catecholamines (epinephrine and norepinephrine), resulting in a cascade of effects including tachycardia, increased metabolic rate, and higher core body temperature. Catecholamines also stimulate glycogenolysis, leading to mobilization of hepatic and muscle glycogen stores, together with elevated blood glucose and lactate concentrations (Knowles and Warriss, 2007). Effects of this acute autonomic stress response on postmortem muscle pH and meat quality (i.e., elevated muscle pH accompanied by dark, firm, dry muscle characteristics) are well documented (Knowles, 1999).

A growing body of evidence suggests that acute pre-slaughter stress not only affects muscle color, firmness, and water holding capacity, but also reduces meat tenderness (Wulf et al., 2002). Watanabe et al. (1996) demonstrated that postmortem tenderization rate was influenced by epinephrine-induced differences in final muscle pH resulting in early postmortem differences in sheep meat tenderness. Recent evidence, however, suggests that stress-induced differences in meat tenderness are not always associated with differences in muscle pH (Ferguson and Warner, 2008). This study was conducted to gain further insight into relationships of behavioral and physiological pre-slaughter stress responses to meat quality characteristics and LM tenderness.

MATERIALS AND METHODS

Animals and Management

Care, handling, and sampling of animals described herein were approved by the Colorado State University Animal Care and Use Committee. Crossbred (50 % British × 50 % Continental European) steer (n = 79) and heifer (n = 77) calves, contemporaries from a single herd produced by mating 4 Charolais bulls to British crossbred cows, were used in this study. Following immunization and weaning (on the ranch at approximately 6 mo of age), calves were transported to the Colorado State University Beef Research Feedlot (Fort Collins, CO) for growing and finishing. Upon arrival, steers and heifers were penned separately. Following a 25-d acclimation period, calves were weighed individually (initial BW) and initial implants were administered. Steers received initial implants containing 80 mg trenbolone acetate and 16 mg 17- β estradiol (Revalor-IS, Intervet Schering-Plough, Millsboro, DE); heifers received initial implants containing 80 mg trenbolone acetate and 8 mg 17-β estradiol (Revalor-IH, Intervet Schering-Plough, Millsboro, DE).

Calves were sorted by sex and sire, stratified by initial BW, and randomly allocated to 16 pens (9 to 11 animals per pen) for finishing. A steam flaked corn-based finishing diet (2.45 Mcal/lb NEm, 1.48 Mcal/lb NEg, and 12.4 % CP, DM basis) was provided once daily and consumed ad libitum. The finishing diet for heifers included melengestrol acetate (MGA, Pfizer Animal Health, Kalamazoo, MI) fed at a daily rate of 0.50 mg/heifer. Sixty-three d after the beginning of the finishing period, cattle were re-implanted with the same implant products that were administered at the beginning of the finishing period.

Behavioral Responses

Behavioral reactions to the following 3 events were observed: 1) human presence in a confined area – pen score, 2) confinement in a squeeze chute – chute score, and 3) transportation from feedlot to packing facility – post-transportation score. For each event, reactions of individual animals were scored using a 15-cm, semi-structured continuous line scale adapted from guidelines published by the Beef Improvement Federation (BIF, 2002). The line scale was equally divided into 5 sections that represented the following behaviors: 1) calm (0 to 2.9 cm) - cattle that were docile, undisturbed, calm, and that had a small flight zone; 2) restless (3.0 to 5.9 cm) - cattle that were quiet, not easily disturbed, but were slightly restless; 3) nervous (6.0 to 8.9 cm) - cattle that were nervous and easily disturbed; 4) flighty (9.0 to 11.9 cm) - cattle that were very fearful, easily excited or agitated, and that had a large flight zone; and 5) aggressive (12.0 to 15.0 cm) - cattle that were very fearful, easily excited or agitated, and that exhibited aggressive behavior.

Pre-Slaughter Procedures and Measurements

Pen Observation. Within 1 mo of slaughter, 2 evaluators assigned behavior scores (described previously) by briefly walking through each pen (40 m × 6.1 m) of cattle and independently scoring each animal's reaction to human presence in the pen. Evaluators assigned pen scores on different days; on both days, all animals were scored within a 3-h time period. The 2 evaluators' scores were averaged to obtain a single pen behavior score for each animal.

Chute Measurements and Observations. The cattle were scheduled for slaughter on 4 separate dates (90 to 111 d after final implant; Table 3.1). On the day before each

designated slaughter date, 4 to 5 animals representing each sex and sire were moved from their respective pens to a working facility where they were individually confined in a hydraulic squeeze chute (Moly Manufacturing, Inc., Lorraine, KS) and weighed (final BW). During weighing, a chute behavior score (described previously) was assigned to each animal by a single evaluator who assessed behavior immediately following application of light pressure to the animal's sides using the "squeeze" feature of the chute. Once the chute behavior score had been recorded, blood samples were collected from each animal via jugular venipuncture. Blood was collected into 10 ml non-heparinized tubes for serum cortisol analysis, and an additional 10 ml sample was collected into tubes containing K_2EDTA for plasma catecholamine determination. Blood samples collected for cortisol determination were allowed to coagulate at room temperature (22 °C) for 4 to 6 h and, then, were centrifuged at 1,200 × g for 25 min, after which serum was harvested. Blood samples collected in the K_2EDTA blood tubes were immediately placed on ice and, within approximately 30 min, were placed in the centrifuge (1,200 × g for 25 min, 4°C) for plasma collection. All serum and plasma samples were immediately frozen and stored at - 80°C. Following blood sample collection, each animal's heart rate (measured using a stethoscope), respiration rate (visual determination), and rectal temperature were recorded.

Transportation and Lairage. On the morning of each slaughter date, groups of 4 or 5 cattle representing each sex and sire were moved from their pens and loaded onto a semi-trailer. After loading was completed, the cattle were transported approximately 64 km to a commercial packing facility for slaughter.

Upon arrival at the packing facility, cattle (39 per slaughter date) were unloaded and placed into lairage pens. Within 10 min after unloading, 2 evaluators independently assigned a post-transportation behavior score (described previously) to each animal. Scores recorded by the 2 evaluators were averaged to provide a single post-transportation score for each animal. Following post-transportation scoring, the cattle were slaughtered using conventional, humane procedures.

Post-Slaughter Measurements

Collection of Blood Samples. During exsanguination, blood samples were collected from each animal and placed on ice. Samples were collected into non-heparinized 10 ml tubes for quantification of cortisol and creatine kinase and into 10 ml tubes containing potassium oxalate and sodium fluoride for subsequent measurements of glucose and lactate. Blood samples were transported to the laboratory at Colorado State University, and approximately 6 to 8 h later, serum and plasma were harvested following centrifugation at 2,500 × g for 25 min.

Postmortem Procedures and Carcass Data Collection. Prior to chilling, pre-rigor carcass sides traveled through 4 zones of electrical stimulation: 1) 16 V, 60 Hz, 15 s (1 s on, 1 s off); 2) 20 V, 60 Hz, 15 s (1 s on, 1 s off); 3) 24 V, 60 Hz, 20 s (1 s on; 1 s off); 4) 28 V, 60 Hz, 13 s (2 s on, 1 s off) and, then, were transferred to a cooler with an air temperature of 2°C. For the first 8 h of the 36-h chill period, sides were sprayed intermittently (2 min on, 8 min off) with a fine mist of 2°C water. After carcass chilling, ribbing, and blooming, a panel of 2 experienced evaluators (Colorado State University personnel) independently evaluated each carcass and assigned a marbling score.

Marbling assessments for each carcass were averaged, resulting in a single marbling score for each carcass.

Approximately 1 h following carcass ribbing, L*, a*, and b* values were measured (Hunter Lab Miniscan, Model 45/O-S, Reston, VI) in triplicate on the right and left LM of each carcass, and then averaged to obtain a single L*, a*, and b* value for each carcass.

Striploins (IMPS 180; USDA, 1996) were removed from the right side of each carcass and transported immediately (under refrigeration) to the Colorado State University Meat Laboratory. At the Meat Laboratory, each striploin was assigned to a sampling scheme that randomly identified sequential (anterior to posterior) 5.1-cm sections of the LM that would subsequently be assigned to each of 5 postmortem aging periods (3, 7, 14, 21, and 28 d). Each striploin was "faced" and appropriately sized LM sections were sequentially removed in the order that had been specified using the sampling scheme described above. Each section then was packaged in a vacuum-sealed bag and stored at 2°C. Following completion of the appropriate aging time, LM sections were frozen and stored at -20°C. Frozen LM sections were fabricated into 2.5-cm thick steaks with a band saw (model 400, AEW, Norwich, UK), repackaged in vacuum-sealed bags, and stored (-20°C) for subsequent Warner-Bratzler shear force (**WBSF**) determination.

Warner-Bratzler Shear Force Measurements. Frozen LM steaks were tempered for 36 to 40 h at 2°C (precooking internal steak temperatures were monitored to ensure that steak temperatures were between 1 and 5°C) and cooked on an electric conveyor grill (model TBG-60 MagiGrill, MagiKitch'n, Inc., Quakertown, PA) for a constant time of 6

min, 5 s at a setting of 163°C for the top and bottom heating platens to achieve a peak internal temperature target of 71°C. Peak internal temperature of each steak was measured by inserting a Type K thermocouple (model 39658-K, Atkins Technical, Gainesville, FL) into the geometric center of each steak.

After cooking, steaks were allowed to equilibrate to room temperature (22°C) and 6 to 10 cores (1.3 cm in diameter) were removed from each steak parallel to the muscle fiber orientation. Each core was sheared once, perpendicular to the muscle fiber orientation, using an universal testing machine (Instron Corp., Canton, MA) fitted with a WBSF head (cross head speed: 200 mm/min). Peak shear force measurements of cores from each steak were recorded and averaged to obtain a single WBSF value for each steak.

Analytical Procedures

Pre- and post-slaughter serum samples were shipped to the Department of Animal Sciences at New Mexico State University for determination of cortisol concentration (Kiyma et al, 2004) using a commercial RIA kit (Diagnostic Products Corp., Los Angeles, CA). Pre-slaughter plasma samples were transported to a commercial laboratory that used an enzyme immunoassay to quantify epinephrine concentration (American Laboratory Products Co., Windham, NH). Serum creatine kinase, plasma glucose (Stanbio Laboratory, Boerne, TX), and plasma lactate concentrations (Trinity Biotech, Wicklow, Ireland) were analyzed using commercial assay kits following manufacturers' directions.

Longissimus muscle samples removed from the anterior end of each striploin by "facing" were used for pH determination. Three d postmortem, 3 g of each tissue sample

was added to 30 ml of deionized water, homogenized thoroughly, and used to determine ultimate LM pH (Model 401A, Orion Research, Boston, MA).

Statistical Methods

Analyses of behavior scores, physiological parameters, and muscle quality traits (excluding WBSF) were conducted using a restricted maximum likelihood-based, mixed-effects model of SAS (PROC MIXED: SAS Inst. Inc., Cary, NC). The statistical model included sex and sire as fixed independent effects and slaughter group was included as a random effect. The sex × sire interaction was included and subsequently removed from the model if not significant ($P > 0.05$). Analyses that examined the effects of behavior classification (calm, restless, or nervous) on physiological parameters used procedures identical to those detailed above, with the addition of behavior category as a fixed, independent effect. Due to unequal distributions of behavior scores, the behavior category × sex and behavior category × sire interactions were not tested.

Data for WBSF were analyzed using a restricted maximum likelihood-based, mixed-effects model, repeated measures analysis (PROC MIXED; SAS Inst. Inc., Cary, NC). The statistical model included sex, sire, and postmortem aging period (AGE) as independent fixed effects, along with random effects of slaughter group and individual animal. All relevant three- and two-way interactions of fixed effects were included and subsequently removed from the model if not significant ($P > 0.05$). The final ANOVA model for WBSF included sex, sire, age, and sire × age as fixed effects. Analyses that examined the effects of behavior or lactate category on WBSF were conducted using procedures identical to those detailed above, with the addition of behavior (or lactate) category as a fixed independent effect. The behavior (or lactate) category × age

interaction was the only interaction between behavior (or lactate) category and other main effects that was included. For all WBSF analyses, AGE was treated as a repeated measurement and a spatial power covariance structure was used.

For all analyses, individual animal served as the experimental unit, the Kenward-Roger approximation was used to calculate denominator degrees of freedom, and means were separated using the PDIFF option at a significance level of $P < 0.05$. Simple correlations among continuous traits were calculated using PROC CORR (SAS Inst. Inc., Cary, NC). For correlation analyses, pen, chute, and post-transportation scores were treated as continuous variables.

RESULTS AND DISCUSSION

Experimental Conditions

Environmental temperatures coinciding with handling and transportation, together with time intervals for loading, transportation, and lairage of animals comprising each of the 4 slaughter groups are provided in Table 3.1. Handling and transportation of all groups occurred in mild, dry weather conditions at mean temperatures ranging from 7 to 26°C. Loading of trucks for transportation of cattle to the packing facility required between 4 and 6 min for each of the 4 slaughter groups. All animals were loaded without difficulty and required minimal coaxing or prodding. Mean travel times between the feedlot and packing facility ranged from 64 to 90 min and duration of lairage at the packing facility for the 4 groups averaged from 121 to 135 min (Table 3.1).

Behavioral and Physiological Reactions to Handling and Transportation

Cattle comprising the experimental sample showed considerable variation in both behavioral and physiological reactions to pre-slaughter handling and transportation (Table 3.2). Scores (pen, chute, and post-transportation) used to quantify stressful behavior of individual cattle ranged from calm to flighty; none of the animals exhibited aggressive behavior during the 3 scoring events (Table 3.2). Pen scoring (a non-restrained event that does not involve physical contact with animals) showed that approximately 39% of the cattle remained calm when confronted with human presence in the pen, whereas 61% showed some aversion to non-physical human interaction (Figure 1). When cattle were subjected to physical handling (movement through a chute system, coupled with restraint in a squeeze chute) or transported (loading, hauling, and unloading), approximately 30% of the cattle remained calm, whereas 70% showed some visible evidence of stressful behavior (Figure 1). Approximately 20%, 21%, and 8% of the animals reacted adversely to non-physical human interaction, physical handling with chute restraint, and transportation, respectively, with behaviors that were characterized as nervous or flighty (Figure 1). Scores for pen, chute, and post-transportation behaviors were positively correlated ($P < 0.05$) with one another (Table 3.3), suggesting that individual cattle exhibited somewhat consistent behaviors during the 3 different scoring events.

Measurements of heart rate, respiration rate, rectal temperature, and concentrations of serum cortisol (CH cortisol) and plasma epinephrine were used as physiological indicators of stress associated with physical handling and chute restraint. Chute behavior score was positively correlated ($P < 0.05$) with heart rate ($r = 0.45$), rectal temperature (r

= 0.37), CH cortisol concentration (r = 0.29), and plasma epinephrine (r = 0.42) concentration (Table 3.3), indicating that cattle exhibiting behavioral symptoms of stress during confinement in the chute also responded physiologically with increased circulating levels of cortisol and epinephrine, together with accelerated heart rates and elevated body temperatures.

Concentrations of serum cortisol (PT cortisol), plasma glucose, plasma lactate, and serum creatine kinase, quantified using blood samples obtained at exsanguination, were used to reflect physiological reactions of animals to transportation stress. Post-transportation behavior score was not correlated ($P > 0.05$) with concentrations of PT cortisol (r = -0.06) or glucose (r = 0.09); however, higher post-transportation scores (indicative of more stressful behavior immediately after delivery to the packing facility), were associated ($P < 0.05$) with increased plasma lactate (r = 0.33) and serum creatine kinase (r = 0.28) concentrations at slaughter (Table 3.3). Three animals had serum concentrations of creatine kinase that were more than 4 times higher than the mean concentration, resulting in an extremely high coefficient of variation (93%) for that trait (Table 3.2). Creatine kinase is released into the blood when there is muscle damage, as occurs with physical exertion or bruising (Broom et al., 2002). In the present study, the 5 blood samples with the highest serum creatine kinase concentrations all were collected from cattle with carcass bruises that involved damage to muscle tissue. The 3 observations that were 4 times higher than the mean creatine kinase concentration were considered outliers and were removed from all analyses.

No physiological measurements were recorded at the time pen behavior scores were assigned; however, results of correlation analyses (Table 3.3) suggested that pen behavior

was indicative of physiological reactions that occurred during later events when cattle were physically handled or transported. Higher pen behavior scores, indicative of greater aversion of animals to human presence in the pen, were associated with accelerated heart rate (r = 0.32), elevated rectal temperature (r = 0.33), and increased concentrations of CH cortisol (r = 0.20) and epinephrine (r = 0.35) during chute restraint, as well as elevated concentrations of serum creatine kinase (r = 0.30) and plasma lactate (r = 0.34) following transportation. These results suggest that assessment of pen behavior may be effective for identifying reactive cattle that respond adversely to stressful stimuli during future events involving physical handling or transportation.

Previous research involving both steers and heifers suggests that heifers tend to be more reactive to handling stress (Voisinet et al., 1997; Wulf et al., 1997; Vann and Randel, 2003). In the current study, heifers were more excitable ($P = 0.001$) than steers during pen behavior scoring and seemed to be slightly more reactive ($P = 0.084$) when confined in a chute (Table 3.4). Blood samples collected from steers and heifers during chute restraint had similar concentrations of CH cortisol and epinephrine; however heifers had more rapid ($P < 0.05$) respiration and heart rates. Post-transportation behavior of steers and heifers did not differ ($P = 0.814$), but analysis of blood samples collected at exsanguination showed that heifers had higher ($P < 0.05$) serum concentrations of PT cortisol and creatine kinase (Table 3.4).

Several previous reports have documented within-breed, genetic differences in cattle behavior (Le Neindre, et al., 1995; Burrow and Corbet, 2000; Halloway and Johnston, 2003). In the present study, sire was a significant source of variation in pen and chute behavior, CH cortisol, respiration rate, rectal temperature, heart rate, and post-

transportation plasma lactate concentration (Table 3.4). Collectively, behavioral and physiological responses summarized in Table 3.4 identified progeny of Sire 1 as being most reactive to pre-slaughter handling and transportation. The interaction between sex and sire was statistically significant for chute behavior score, heart rate, and PT cortisol; however, tests of interaction means for these traits (data not presented) did not reveal any biologically meaningful differences. Although sire and sex were significant sources of variation in pre-slaughter stress responses, the relationships among behavioral and physiological symptoms of stress represent effects over and above what can be explained by sire or sex.

Pre-Slaughter Stress and Beef Tenderness

Exposure of cattle to various physical or psychological challenges (stressors) activates 2 integrated neuroendocrine axes – the hypothalamic-pituitary-adrenal (**HPA**) axis and the sypatho-adrenal (**SA**) axis (Axelrod and Reisine, 1984; von Borell, 2001). Activation of the HPA axis, typically regarded as a long-term, sustained response to stress (von Borell, 2001), stimulates secretion of ACTH from the anterior pituitary which, in turn, causes production of cortisol by the adrenal cortex (Axelrod and Reisine, 1984). Release of cortisol into the circulatory system elevates plasma glucose concentration (due to hepatic glycogenolysis and gluconeogensis) and promotes protein catabolism (due to reduced protein synthesis and increased protein degradation) in skeletal muscle (Shaw and Tume, 1992; Gerrard and Grant, 2003; Boron and Boulpaep, 2005). Purchas et al. (1971) reported data suggesting that elevated blood cortisol at slaughter may be associated with less tender meat; however, further experimentation failed to establish consistent relationships between HPA activation and meat quality characteristics

(Purchas et al., 1973; Purchas et al., 1980; Shaw and Tume, 1992). In the current study, serum cortisol concentration, measured either during chute restraint (CH cortisol) or at slaughter (PT cortisol), was not ($P > 0.05$) correlated with measurements of LM pH, color (L*, a*, b*), or tenderness (Table 3.3).

The SA axis is activated by acute stress, resulting in the release of catecholamines (epinephrine and norepinephrine) from the adrenal medulla (Axelrod and Reisine, 1984). Epinephrine, acting via β-adrenergic receptors, mobilizes hepatic and muscle glycogen, elevates plasma glucose and lactate concentrations, and increases heart rate, body temperature, and respiration rate (Apple et al., 1995; Gerrard and Grant, 2003; Knowles and Warriss, 2007). Epinephrine-induced depletion of muscle glycogen has long been recognized as the root cause of high-pH, DFD meat (Apple et al., 2005). Moreover, experimental evidence suggests that pre-slaughter SA activation reduces meat tenderness (Ferguson and Warner, 2008). In the current study, plasma epinephrine concentration, measured as an indicator of acute stress during chute restraint, was positively correlated ($P < 0.05$, Table 3.3) with heart rate (r = 0.42), rectal temperature (r = 0.33), CH cortisol (r = 0.33), plasma lactate concentration at slaughter (r = 0.22), serum creatine kinase concentration at slaughter (r = 0.28), LM a* (r = 0.21), and LM WBSF (r = 0.22). Moreover, elevated plasma lactate concentration at slaughter, used to reflect SA activation during transportation and lairage, was associated ($P < 0.05$, Table 3.3) with lower LM pH (r = -0.30), higher values for all measures of LM color (r = 0.16, 0.21, and 0.26 for L*, a*, and b*, respectively), and greater LM WBSF (r = 0.26).

Data presented in Table 3.3 revealed several potentially important correlations among cattle behavior scores, physiological symptoms of acute stress, and LM WBSF that

warranted further examination. Additional least squares analyses were conducted, using chute behavior and post-transport behavior as independent, categorical variables, to examine these relationships (Table 3.5). Due to the few number of animals scored as flighty (9.0 to 11.9 cm; Figure 1), cattle that received behavioral scores $\geq$ 6.0 cm were classified as nervous for all categorical analyses. Cattle showing adverse behavioral reactions to physical handling and chute restraint (i.e., those with chute behaviors categorized as nervous) exhibited a pronounced acute stress response, characterized by elevated ($P < 0.05$) values for plasma epinephrine, heart rate, and rectal temperatures during confinement in the chute (Table 3.5). The same cattle had higher ($P < 0.05$) plasma lactate concentrations at slaughter and subsequently produced tougher ($P < 0.05$) LM steaks compared with calmer cattle (3.72 vs. 3.49 $\pm$ 0.11 kg; Table 3.5). In addition, cattle that showed behavioral symptoms of agitation, when observed following transportation to the packing facility (i.e., those with post-transportation behaviors categorized as restless or nervous), had higher ($P < 0.05$) plasma glucose and lactate concentrations at slaughter and produced LM steaks that were approximately 0.34 kg tougher ($P < 0.05$), compared with cattle exhibiting calm behavior.

A noteworthy aspect of results presented in Table 3.5 is that final LM pH did not differ ($P > 0.05$) among behavior categories, despite significant among-group differences in several other stress indicators. Values for final LM pH observed in the present study ranged from 5.2 to 5.7, with a mean of 5.4 (Table 3.2) and, even though handling and transport events in the current study produced measurable behavioral and physiological stress responses commonly associated with acute pre-slaughter stress (Table 3.2), no carcasses were classified as DFD, either visually or on the basis of LM pH

measurements. According to Warner et al. (2007), DFD muscle characteristics are produced by chronic stress and often do not coincide with symptoms of acute stress.

Page et al. (2001) published results of an industry wide survey of beef LM pH and reported a range of 5.2 to 6.9, with a mean of 5.5. In their study, more than 80% of the carcasses measured had LM pH values between 5.4 and 5.6, whereas most of the carcasses that were classified as DFD in the survey had LM pH values of 5.87 or greater (Page et al., 2001). A number of different studies have shown that the relationship between muscle pH and meat tenderness is curvilinear and that meat toughness tends to be greatest when final muscle pH is somewhat elevated, between 5.8 and 6.2 (Purchas et al., 1990; Watanabe et al 1996; Wulf et al., 2002). Correspondingly, stress-effects on meat tenderness, when observed, typically have been attributed to higher-than-normal muscle pH values (5.8 to 6.2) caused by stress (Watanabe et al., 1996; Wulf et al., 2002). Recently reported evidence, however, suggests that acute pre-slaughter stress reduces tenderness, even when muscle pH is unaffected (Warner et al., 2007). Our results are similar to those reported by Warner et al. (2007). In the current study, behavioral and physiological indices of acute pre-slaughter stress were associated with reduced ($P <$ 0.05) LM tenderness despite the fact that all values for final LM pH were less than 5.8 (Table 3.5).

Of particular interest in Table 3.5 was an apparent connection between post-transportation plasma lactate concentration and stress-induced differences in beef tenderness. Further analyses revealed that grouping animals according to differences in plasma lactate concentration at slaughter essentially categorized them according to mean differences in LM WBSF (Figure 2). Previous research has shown that elevated blood

lactate concentration at slaughter reflects an acute pre-slaughter SA response involving release of epinephrine, stimulation of β-adrenergic receptors, and concomitant changes in glucose metabolism (Shaw and Tume, 1992; Warner et al., 2007). Correspondingly, it is possible that the toughening effect of acute pre-slaughter stress observed in the present study was associated with adrenergic stimulation (Ferguson and Warner, 2008). Sensky et al. (1996) used intravenous infusion of epinephrine to simulate stress-induced adrenergic stimulation in swine and found that LM calpastatin activity at slaughter was increased by nearly two-fold, leading the researchers to conclude that epinephrine release in response to pre-slaughter stress could influence postmortem tenderization rate. In addition, several studies have demonstrated that adrenergic stimulation using synthetic β-agonists increases calpastatin activity, reduces postmortem tenderization, and increases meat toughness (Kretchmar et al., 1990; Koohmaraie, et al., 1991; Strydom et al., 2009), often without affecting final muscle pH (Hilton et al., 2009; Strydom et al., 2009). Calpastatin activity was not measured in the current study; however, increased plasma lactate concentration at slaughter (indicative of adrenergic stimulation during the immediate pre-slaughter period) was associated with a delayed aging response in LM samples between 3 and 7 d postmortem, resulting in significant among-group differences in 7-d LM WBSF (Table 3.6). For cattle with the highest plasma lactate concentrations (91^{st} to 100^{th}percentile), the delayed aging response and associated toughening effect persisted until the 14^{th} d of postmortem aging (Table 3.6). Strydom et al. (2009) reported a similar delay in the postmortem aging response for LM samples from cattle treated with either of 2 synthetic β-agonists, zilpaterol hydrochloride or clenbuterol. Though not

conclusive, these results suggest a possible mode of action for stress-induced differences in meat tenderness that merits further study.

Studies that have compared tenderness of beef produced by heifers and steers suggest that heifers often produce tougher beef (Tatum et al., 2007). Furthermore, there is evidence suggesting that the difference in tenderness between heifers and steers, when present, is related to heifers' greater reactivity to pre-slaughter stress (Voisinet et al., 1997; Wulf et al., 1997). In the current study, heifers showed a more pronounced stress response than did steers; however, sex class did not affect ($P > 0.05$) LM WBSF (Table 3.4).

Exposure of cattle to some degree of stress during pre-slaughter shipment is inevitable (Ferguson and Warner, 2008). Results of this study identified behavioral and physiological symptoms of acute pre-slaughter stress that were associated with differences in LM tenderness and underscore the importance of stress avoidance in the application of best management practices for ensuring beef tenderness.

Table 3.1. Simple means for duration and temperature of events stratified by slaughter group

	Slaughter group			
Trait	1	2	3	4
Number of cattle	39	39	39	39
Days on feed	153	160	167	174
Temperature during chute processing, °C	12.0	19.4	26.4	6.9
Duration of loading, min[1]	4.6	4.7	5.6	5.2
Duration of transport, min	67.7	64.2	89.7	73.8
Temperature during transport, °C	14.1	14.9	17.2	10.1
Time in lairage, min	135.4	122.7	121.1	132.6

[1]Cattle were loaded in groups of 4 to 5 animals. Loading duration represents an average time required to load each small group.

Table 3.2. Simple statistics for behavior scores, physiological parameters, and meat quality traits

Trait	Mean	Minimum	Maximum	CV, %
Behavioral reaction[1]				
Pen behavior score	4.16	0.25	10.45	58.3
Chute score	4.46	0.60	10.00	44.8
Post-transportation score	4.06	2.20	11.45	34.2
Physiological reaction[2]				
CH cortisol, ng/mL	44.3	4.9	111.0	40.5
CH epinephrine, pg/ml	183.6	37.0	850.0	77.8
Heart rate, beats/min	117.2	60	200	18.8
Respiration, breaths/min	42.7	28	80	18.9
Rectal temperature, °C	39.6	38.3	40.8	1.0
PT cortisol, ng/mL	47.9	7.9	142.3	47.5
PT glucose, mg/dL	225.6	90.8	718.5	45.1
PT lactate, mg/dL	107.8	51.4	182.5	22.9
PT creatine kinase, U/L	571.4	169.9	3876.1	93.0
Meat Quality				
Warner-Bratzler shear force, kg[3]	3.50	2.16	5.11	14.6
Marbling score[4]	415.1	265	650	16.8
LM pH[5]	5.39	5.23	5.74	1.9
L^*[6]	31.3	26.3	37.0	5.8
a^*[7]	7.39	5.29	10.21	11.6
b^*[8]	7.10	5.49	9.49	8.8

[1]Behavior scores assessed using a 15 cm semi-structured continuous line scale: Calm = 0 to 2.9cm, Restless = 3 to 5.9 cm, Nervous 6 to 8.9 cm, Flighty = 9.0 to 11.9 cm, and Aggressive ≥ 12 cm.
[2]Serum or plasma concentrations determined from blood samples taken during routine processing through the chute (CH) or on the slaughter floor, post-transportation (PT).
[3]Warner Bratzler shear force averaged across aging period (3, 7, 14, 21, and 28 d).
[4]200 = Traces, 300 = Slight, 400 = Small, 500 = Modest, and 600 = Moderate.
[5]Muscle pH at 72 h postmortem.
[6]L^*: 0 = black; 100 = white.
[7]a^*: Negative number = green; Positive numbers = red.
[8]b^*: Negative number = blue; Positive numbers = yellow.

Table 3.3. Simple correlations among behavior responses, physiological responses, and LM quality traits[1]

Trait	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.
1. Pen score[2]		0.31	0.29	0.20	0.35	0.13	0.11	0.34	0.30	0.32	0.03	0.33	-0.31	0.14	-0.13	-0.05	0.17	0.06	0.23
2. Chute score[2]	0.31		0.19	0.29	0.42	0.10	0.11	0.24	0.17	0.45	0.17	0.37	-0.02	0.12	0.02	-0.02	0.41	0.23	0.24
3. Post-transportation score[2]	0.29	0.19		0.02	0.12	-0.06	0.09	0.33	0.28	0.11	-0.08	0.17	-0.15	0.04	-0.06	-0.03	0.12	0.07	0.34
4. CH cortisol[3]	0.20	0.29	0.02		0.33	0.19	0.20	0.10	0.19	0.23	0.11	0.33	0.02	0.05	-0.04	0.03	0.15	0.12	0.10
5. CH epinephrine[3]	0.35	0.42	0.12	0.33		0.09	0.01	0.22	0.28	0.42	0.11	0.34	-0.11	0.14	0.00	-0.05	0.21	0.13	0.22
6. PT cortisol[3]	0.13	0.10	-0.06	0.19	0.09		0.12	0.21	-0.01	0.00	0.20	0.03	-0.17	0.03	-0.13	0.04	0.08	0.09	-0.03
7. PT glucose[3]	0.11	0.11	0.09	0.20	0.01	0.12		0.16	-0.09	-0.12	0.20	-0.02	0.00	0.13	-0.21	0.01	-0.14	-0.09	0.01
8. PT lactate[3]	0.34	0.24	0.33	0.10	0.22	0.21	0.16		0.21	0.18	0.07	0.14	-0.20	0.11	-0.30	0.16	0.21	0.26	0.26
9. PT creatine kinase[3]	0.30	0.17	0.28	0.19	0.28	-0.01	-0.09	0.21		0.39	0.07	0.32	-0.29	-0.01	-0.02	-0.02	0.14	0.07	0.08
10. Heart rate	0.32	0.45	0.11	0.23	0.42	0.00	-0.12	0.18	0.39		0.11	0.44	-0.11	0.07	-0.07	-0.13	0.31	0.04	0.19
11. Respiration	0.03	0.17	-0.08	0.11	0.11	0.20	0.20	0.07	0.07	0.11		0.13	-0.16	-0.05	0.04	-0.04	0.23	0.13	0.07
12. Rectal temperature	0.33	0.37	0.17	0.33	0.34	0.03	-0.02	0.14	0.32	0.44	0.13		-0.16	0.17	-0.02	-0.03	0.26	0.10	0.22
13. ADG	-0.31	-0.02	-0.15	0.02	-0.11	-0.17	0.00	-0.20	-0.29	-0.11	-0.16	-0.16		-0.07	0.06	0.29	-0.16	0.16	-0.01
14. Marbling score	0.14	0.12	0.04	0.05	0.14	0.03	0.13	0.11	-0.01	0.07	-0.05	0.17	-0.07		-0.06	0.02	0.16	0.02	-0.15
15. pH[4]	-0.13	0.02	-0.06	-0.04	0.00	-0.13	-0.21	-0.30	-0.02	-0.07	0.04	-0.02	0.06	-0.06		0.21	0.03	0.24	0.10
16. L*[5]	-0.05	-0.02	-0.03	0.03	-0.05	0.04	0.01	0.16	-0.02	-0.13	-0.04	-0.03	0.29	0.02	0.21		-0.11	0.75	0.02
17. a*[6]	0.17	0.41	0.12	0.15	0.21	0.08	-0.14	0.21	0.14	0.31	0.23	0.26	-0.16	0.16	0.03	-0.11		0.45	0.10
18. b*[7]	0.06	0.23	0.07	0.12	0.13	0.09	-0.09	0.26	0.07	0.04	0.13	0.10	0.16	0.02	0.24	0.75	0.45		0.14
19. WBSF[8]	0.23	0.24	0.34	0.10	0.22	-0.03	0.01	0.26	0.08	0.19	0.07	0.22	-0.01	-0.15	0.10	0.02	0.10	0.14	

[1] $|r| \geq 0.16$ ($P < 0.05$).

[2] Semi-structured continuous line scale: 0 = calm; ≥ 6 = nervous.

[3] Serum or plasma concentrations determined from blood samples taken during routine processing through the chute (CH) or on the slaughter floor, post-transportation (PT).

[4] Muscle pH at 72 h postmortem.

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

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

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

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

Table 3.4. Least squares means for behavior scores and physiological parameters corresponding to sex and sire effects

			Sex				Sire			
Trait	P_{Sex}	SEM	Heifer	Steer	P_{Sire}	SEM	1	2	3	4
Number of animals	-	-	77	79	-	-	37	40	39	40
Pen behavior score[1]	0.001	0.27	4.95	3.43	0.005	0.37	5.32^{a}	3.82^{b}	3.92^{b}	3.71^{b}
Chute score[1,3]	0.084	0.26	4.74	4.22	0.001	0.34	5.30^{a}	4.18^{bc}	4.78^{ab}	3.65^{c}
Post-transportation score[1]	0.814	0.32	4.09	4.04	0.083	0.36	4.42	3.83	4.23	3.78
CH Cortisol, ng/mL[2]	0.584	2.7	43.6	45.1	0.007	3.4	51.4^{a}	45.2^{ab}	43.3^{b}	37.7^{b}
CH Epinephrine, pg/ml[2]	0.364	16.2	194.3	173.6	0.080	23.2	217.6	142.5	207.1	168.7
PT Cortisol, ng/mL[2,3]	0.025	4.85	51.9	44.2	0.525	5.5	49.4	46.2	45.2	51.4
PT Glucose, mg/dL[2]	0.438	24.8	232.2	220.4	0.078	26.8	243.4	210.3	203.0	248.6
PT Lactate, mg/dL[2]	0.146	2.9	111.1	105.4	0.029	4.2	117.3^{a}	102.7^{b}	102.5^{b}	110.5^{ab}
PT Creatine kinase, U/L[2]	0.001	71.7	714.6	440.0	0.297	94.4	707.3	517.5	579.7	504.6
Respiration, breaths/min	0.001	2.26	45.4	40.1	0.021	2.39	44.0^{ab}	41.5^{bc}	44.7^{a}	40.7^{c}
Rectal temperature, °C	0.370	0.07	39.6	39.6	0.001	0.08	39.8^{a}	39.5^{b}	39.7^{a}	39.4^{b}
Heart Rate, beats/min[3]	0.048	5.0	120.5	114.5	0.001	5.5	128.6^{a}	114.0^{bc}	120.2^{ab}	107.2^{c}
Marbling score[4]	0.001	8.0	439.3	392.3	0.002	10.9	444.3^{a}	416.3^{b}	387.6^{b}	415.0^{b}
pH[5]	0.637	0.04	5.39	5.39	0.096	0.04	5.37	5.39	5.41	5.40
L*[6]	0.148	0.55	31.1	31.5	0.003	0.57	30.9^{b}	31.5^{ab}	30.8^{b}	32.0^{a}
a*[7]	0.001	0.13	7.6	7.1	0.001	0.15	7.8^{a}	7.2^{b}	7.4^{ab}	7.1^{b}
b*[8]	0.226	0.22	7.2	7.1	0.294	0.23	7.1	7.1	7.0	7.2
Warner-Bratzler shear force, kg[9]	0.237	0.10	3.47	3.55	0.002	0.11	3.72^{a}	3.38^{b}	3.59^{a}	3.35^{b}

[1]Behavior scores assessed using a semi-structured continuous line scale 0 = calm; ≥ 6 = nervous.

[2]Serum or plasma concentrations determined from blood samples taken during routine processing through the chute (CH) or on the slaughter floor, post-transportation (PT).

[3]Sex × sire interaction ($P < 0.05$).

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

[5]Muscle pH at 72 h postmortem.

[6]L^*: 0 = black; 100 = white.
[7]a^*: Negative number = green; Positive numbers = red.
[8]b^*: Negative number = blue; Positive numbers = yellow.
[9]Sire × AGE interaction ($P = 0.019$).
[a-c]Means without a common superscript differ $P < 0.05$.

Table 3.5. Least squares means showing the effects of chute and post-transportation behaviors on acute stress indicators, LM pH, and LM Warner-Bratzler shear force

	Chute behavior[1]				Post-transportation behavior[2]			
Trait	*P*-value	Calm	Restless	Nervous	*P*-value	Calm	Restless	Nervous
CH epinephrine, pg/ml	0.001	156.5 ± 20.2^{b}	156.6 ± 15.0^{b}	290.8 ± 24.3^{a}	0.575	173.0 ± 20.9	184.7 ± 14.5	224.0 ± 43.5
Heart rate, beats/min	0.001	110.1 ± 5.3^{b}	116.5 ± 5.0^{b}	130.1 ± 5.6^{a}	0.908	117.2 ± 5.6	117.4 ± 5.1	120.0 ± 7.5
Rectal temp, °C	0.006	39.5 ± 0.08^{b}	39.6 ± 0.07^{a}	39.8 ± 0.09^{a}	0.342	39.5 ± 0.08	39.6 ± 0.06	39.6 ± 0.12
PT Glucose, mg/dL	0.137	203.7 ± 26.7	230.3 ± 25.0	248.0 ± 28.4	0.003	182.3 ± 29.7^{b}	243.2 ± 27.5^{a}	256.5 ± 37.7^{a}
PT Lactate, mg/dL	0.006	106.9 ± 4.1^{b}	103.6 ± 3.4^{b}	120.6 ± 4.8^{a}	0.001	97.1 ± 4.1^{b}	111.3 ± 3.0^{a}	124.7 ± 7.3^{a}
LM pH	0.065	5.38 ± 0.04	5.41 ± 0.04	5.37 ± 0.04	0.343	5.39 ± 0.04	5.40 ± 0.04	5.36 ± 0.05
LM WBSF, kg	0.023	3.49 ± 0.10^{b}	3.43 ± 0.09^{b}	3.72 ± 0.11^{a}	0.005	3.30 ± 0.10^{b}	3.59 ± 0.08^{a}	3.68 ± 0.15^{a}

[1]Categories established from the 15 cm semi-structured continuous line scale: 0 to 2.9 cm = calm; 3 to 5.9 = restless; ≥ 6 = nervous. Animals per subclass: Calm = 46, Restless = 78, and Nervous = 32.

[2] Categories established from the 15 cm semi-structured continuous line scale: 0 to 2.9 cm = calm; 3 to 5.9 = restless; ≥ 6 = nervous. Animals per subclass: Calm = 47, Restless = 97, and Nervous = 12.

[a-c]Means without a common superscript differ ($P < 0.05$).

Table 3.6. Least squares means showing the effect of the PT lactate × AGE interaction ($P = 0.029$) on Warner-Bratzler shear force (kg)

			Postmortem aging period, d				
PT lactate, percentile	n	SEM	3	7	14	21	28
1 to 10	15	0.19	3.82^{bcd}	3.22^{fghi}	3.27^{fghi}	2.94^{ij}	2.76^{ij}
11 to 30	30	0.15	4.01^{abc}	3.49^{def}	3.37^{fgh}	3.15^{ghij}	2.99^{ij}
31 to 70	60	0.13	4.19^{ab}	3.69^{cde}	3.43^{efg}	3.10^{hij}	3.01^{ij}
71 to 90	30	0.15	4.30^{a}	3.92^{bc}	3.43^{defg}	3.30^{fgh}	3.25^{fghi}
91 to 100	15	0.19	4.09^{ab}	4.15^{ab}	4.03^{abc}	3.41^{efgh}	3.21^{fghij}

[a-j]Means without a common superscript differ $P < 0.05$.

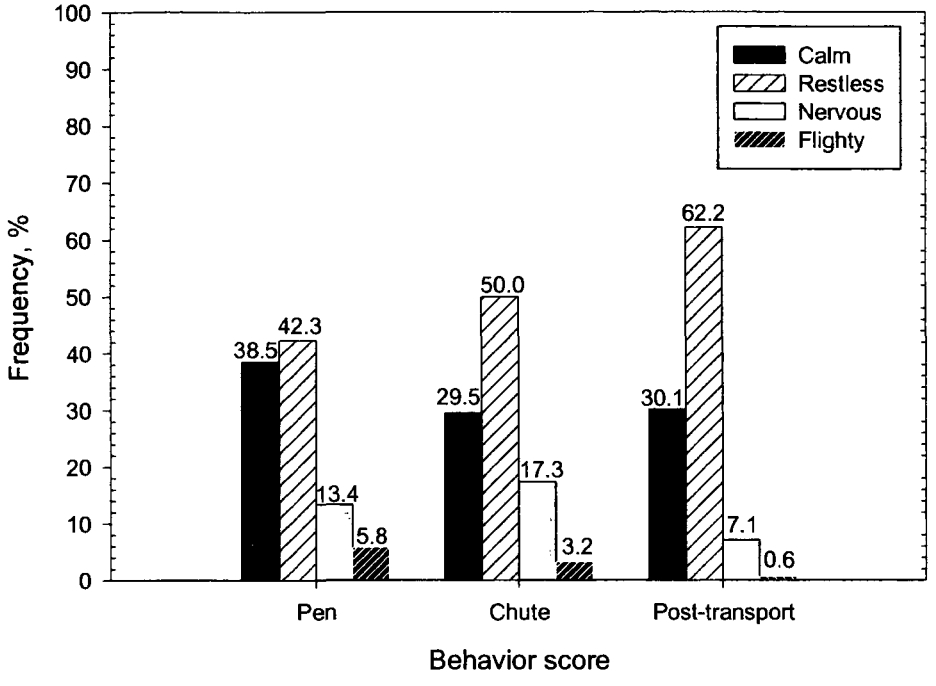

Figure 3.1. Distribution of behaviors observed at each scoring event

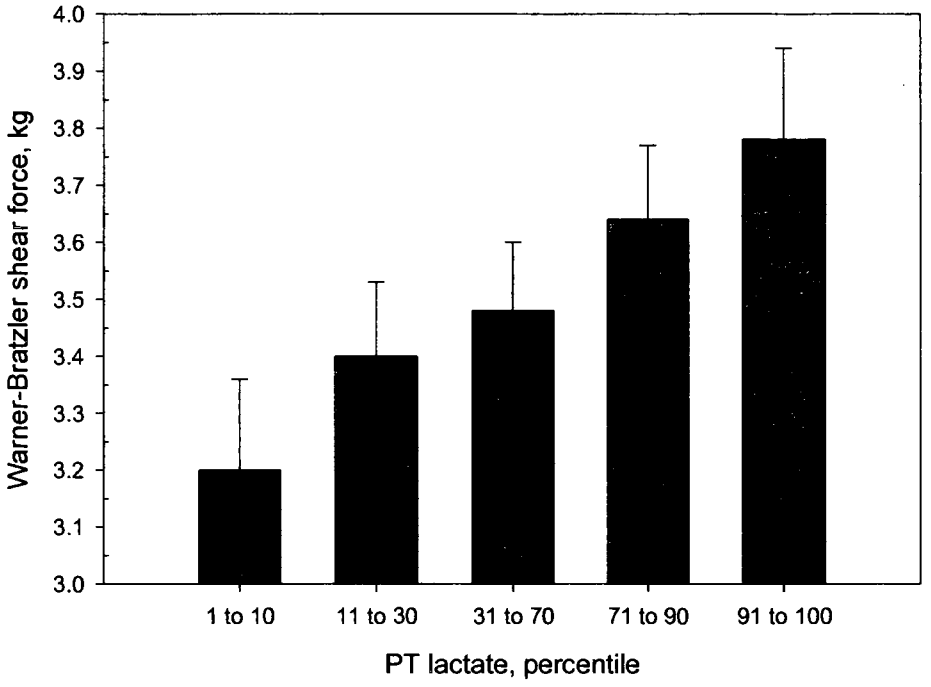

Figure 3.2. Effect of PT lactate concentration on LM Warner-Bratzler shear force. Linear contrast $P = 0.003$

CHAPTER IV

EFFECTS OF GENETIC MARKERS AND IMPLANT STRATEGY ON LONGISSIMUS AND GLUTEUS MUSCLE TENDERNESS OF CALF-FED STEERS AND HEIFERS

ABSTRACT

Effects of genotype (GEN) and implant program (IMP) on LM and gluteus muscle (GM) postmortem tenderization were investigated using crossbred steer (n = 185) and heifer (n = 158) calves. The 3-marker GeneSTAR Tenderness panel (CAST, CAPN1 316, and CAPN1 4751) was used to determine each animal's GEN (reported as total number of favorable alleles, 0 through 6). Calves were randomly assigned to 1 of 2 implant programs, conventional (CNV) or delayed (DEL). Cattle in the CNV group were implanted at the beginning of the finishing period with Revalor-IS or -IH, and then re-implanted 59 d later with Revalor-S or -H. Calves in the DEL group received a single, terminal implant (Revalor-S or -H) administered 45 d after initiation of the finishing period. Warner-Bratzler shear force (WBSF) was measured on LM and GM steaks at 3, 7, 14, 21, and 28 d postmortem. No interactions among the main effects of sex, IMP, or GEN were detected ($P > 0.05$). An IMP × postmortem aging (AGE) interaction was detected ($P < 0.05$) for LM and GM WBSF. For both muscles, steaks from CNV cattle

had WBSF values that were approximately 0.2 kg higher ($P < 0.05$) than steaks from DEL animals, but only during the early postmortem period (3 to 7 d). A linear effect of GEN on WBSF was detected ($P < 0.05$) for LM and GM steaks. Within each muscle, steaks from cattle with 6 favorable alleles had WBSF values 0.33 kg lower than steaks from cattle with 1 favorable allele. The GEN × AGE interaction was not significant for either muscle, but there was a numerical trend for the effect of GEN on WBSF to diminish as AGE increased. To investigate how genetic markers could be interfaced with current beef carcass quality grading (QG), cattle were sorted into 2 gene marker groups (**GMG**), ≤ 3 vs. ≥ 4 favorable alleles. For both muscles, GMG was only effective at identifying tenderness differences within the Select grade. When aged ≤ 14 d, Select LM steaks from cattle with ≥ 4 alleles had lower ($P < 0.05$) WBSF values than LM steaks from animals with ≤ 3 alleles. Pre-slaughter factors (Sex, IMP, and GMG) controlled in the present study each accounted for less than 7 % of the explained variation in tenderness of the test population. Results from this study suggest that the 3 GeneSTAR tenderness markers were associated with small differences (0.33 kg) in WBSF, and may be useful for increasing consistency of Select beef, but these specific markers only accounted for a minor amount of variation in beef tenderness.

INTRODUCTION

For the majority of beef consumers, tenderness is the sensory attribute that has the greatest influence on eating satisfaction (Huffman et al., 1996), and market research has shown that improving beef tenderness increases both the likelihood that consumers will purchase beef and the price they are willing to pay (Boleman et al., 1997; Platter et al.,

2003). Systems that facilitate production of consistently tender beef by controlling pre- and post-slaughter processes known to impact tenderness would assist the industry in attaining goals of building beef demand and adding value to cattle (Tatum et al., 2000; NCBA, 2006). Tatum et al. (1999) identified postmortem aging as a critical control point in management systems designed to reduce the incidence of beef tenderness problems; however, research suggests that postmortem tenderization can be influenced by numerous factors such as USDA quality grade, muscle within a carcass (Gruber et al., 2006a), genetics (Wulf et al., 1996), and use of hormonal implants (Schneider et al., 2007).

The association between commercial genetic markers and beef LM tenderness has been validated (Van Eenennaam et al., 2007), but little is known regarding the relationships among genetic markers, pre-slaughter management factors, and postmortem tenderization of different beef muscles. Moreover, delayed implanting recently has been recommended for enhancing marbling deposition in feedlot cattle (Corah and McCulley, 2006), but the effects of delayed implanting on beef tenderization are not well documented.

While the effects of various pre-slaughter factors on beef tenderness have been studied individually, it is not known how these factors interact within the beef chain to affect postmortem tenderization of beef. Therefore, the current study was designed to characterize interactions among delayed implanting, genetic markers, and postmortem tenderization of 2 beef muscles from calf-fed steers and heifers.

MATERIALS AND METHODS

Animals and Management

Care, handling, and sampling of animals described herein were approved by the Colorado State University Animal Care and Use Committee. Male and female contemporaries (born 9-Jan to 25-May, 2006) from 2 different *Bos taurus* crossbred cowherds were identified for use in this study. Crossbred steer (n = 185) and heifer (n = 158) calves (289 calves sired by Charolais bulls and 54 calves sired by Angus bulls) were weaned at conventional ages, pre-conditioned, and placed in the feedlot at the Colorado State University Eastern Colorado Research Center (Akron, CO) for finishing.

Within each source, calves were sorted by sex, stratified by weaning weight, and randomly assigned to 1 of 2 implant programs, conventional (**CNV**) or delayed (**DEL**). Calves in the CNV group were implanted at the beginning of the finishing period (11-Dec-06; approximately 140 to 169 d before slaughter); steers were administered implants containing 16 mg 17-β estradiol and 80 mg trenbolone acetate (Revalor-IS, Intervet, Inc., Millsboro, DE) and heifers received initial implants containing 8 mg 17-β estradiol and 80 mg trenbolone acetate (Revalor IH, Intervet, Inc., Millsboro, DE). Cattle in the CNV treatment group were re-implanted 59 d later; steers received terminal implants containing 24 mg 17-β estradiol and 120 mg trenbolone acetate (Revalor-S, Intervet, Inc., Millsboro, DE) and heifers were administered terminal implants containing 14 mg 17-β estradiol and 140 mg trenbolone acetate (Revalor H, Intervet, Inc., Millsboro, DE). Calves in the DEL group received a single, terminal implant (steers received Revalor-S, heifers received Revalor-H) administered 45 d after initiation of the finishing period.

The finishing diet, consisting of 81.3 % dry-rolled corn, 5.0 % wheat straw, 5.0 % sunflower meal, 4.5 % finisher pellet, and 4.2 % alfalfa hay (DM basis), was formulated to contain 12.4 % CP and to meet or exceed NRC nutrient requirements for growing and finishing cattle (NRC, 1996). All cattle were fed Rumensin and Tylan (Elanco Animal Health, Greenfield, IN) and heifers were supplemented with melengestrol acetate (MGA; Pfizer Animal Health, Kalamazoo, MI). Diets were dispensed once daily and provided cattle with ad libitum access to feed. Individual BW were recorded on 11-Dec-2006 (initial BW, all cattle), 25-Jan-2007 (terminal implant BW, DEL cattle), 8-Feb-2007 (terminal implant BW, CNV cattle), and 24 to 48 h before slaughter (final BW, all cattle).

Genotyping

Hair samples for genotyping were obtained from each animal at the time final BW was recorded. Using commercially available collectors, a tail hair sample was obtained from each animal and submitted to Pfizer Animal Genetics (Harahan, LA) to obtain GeneSTAR Tenderness results. The GeneSTAR Tenderness panel consisted of 3 markers for 2 different genes: 1) T1 – calpastatin, CAST (Barendese, 2002), 2) T2 – μ-calpain, CAPN1 316 (Page et al., 2002), and 3) T3 - μ-calpain, CAPN1 4751 (White et al., 2005).

Morbidity and Behavior

Additional pre-slaughter factors that have been found to affect postmortem tenderization were monitored throughout the growing/finishing period (Wulf et al., 1997; Gardner et al., 1999; King et al., 2006). Cattle behavior was scored in the pen (pen score) using procedures developed by Gruber et al. (2006b). Briefly, behavior scores were assigned to cattle using a 15-cm semi-structured line scale that was equally divided into 5

sections to represent the following behaviors: 1) calm: 0 to 2.9 cm, 2) restless: 3.0 to 5.9 cm, 3) nervous: 6.0 to 8.9 cm, 4) flighty: 9.0 to 11.9 cm, and 5) aggressive: 12.0 to 15.0 cm. Morbidity was quantified by the number of diagnosed cases during growing/finishing in the feedlot and using a post-slaughter lung scoring system that identified either the presence or absence of any lesions (dark depressed purple areas primarily in the right, anterior ventral lobe).

Slaughter and Carcass Data Collection

Cattle were slaughtered using humane procedures at a commercial beef processing plant (approximately 145 km from the feedlot) on 3 dates (Table 4.1). Following slaughter, pre-rigor carcasses traveled through 4 zones of electrical stimulation: 1) 16 V, 60 Hz, 15 s (1 s on, 1 s off); 2) 20 V, 60 Hz, 15 s (1 s on, 1 s off); 3) 24 V, 60 Hz, 20 s (1 s on; 1 s off); 4) 28 V, 60 Hz, 13 s (2 s on, 1 s off) and, then, were chilled (air temperature 2°C) for 48 h. After the carcass-chilling period, a USDA grader assigned scores to each carcass for marbling and lean maturity. In addition, a panel of 2 experienced evaluators (Colorado State University personnel) independently evaluated each carcass and recorded measurements/assessments of fat thickness, adjusted fat thickness, KPH, and skeletal maturity. Values for each trait recorded by the 2 evaluators were averaged, resulting in a single value for each grade factor for each carcass. Measurements of LM area were obtained for each carcass using a video image analysis system (e + v Technology GmbH, Model VBG 2000, Oranienburg, Germany) maintained by the beef processing facility. Approximately 1 h after carcass ribbing, L*, a*, and b* values were measured (Hunter Lab Miniscan, Model 45/O-S, Reston, VI) in triplicate on the right LM of each carcass.

Muscle Samples

At 48 h postmortem, striploins (IMPS 180; USDA, 1996) and top sirloin butts (IMPS 184, USDA, 1996) were removed from the right side of each carcass and transported immediately (under refrigeration) to the Colorado State University Meat Laboratory. At the Meat Laboratory, the *gluteus medius* muscle (**GM**) was separated from each top sirloin butt and samples of each GM and LM were obtained for pH determination.

Each GM and striploin was fabricated into 5 sections that were then randomly assigned to each of 5 postmortem aging periods (3, 7, 14, 21, and 28 d). Muscle sections were placed into vacuum-sealed bags and stored at 2°C. Following completion of the appropriate aging period, muscle sections were frozen and stored at -20°C. Frozen muscle samples were fabricated into 2.54-cm-thick steaks using a band saw (model 400, AEW, Norwich, UK).

Warner-Bratzler Shear Force Measurement

Frozen LM and GM steaks were tempered for 36 to 40 h at 2°C (precooking internal steak temperatures were monitored to ensure that steak temperatures were between 1 and 5°C) and cooked on an electric conveyor grill (model TBG-60 MagiGrill, MagiKitch'n, Inc., Quakertown, PA) for a constant time of 6 min, 5 s at a setting of 163°C for the top and bottom heating platens to achieve a peak internal temperature target of 71°C. Peak internal temperature of each steak was measured by inserting a Type K thermocouple (model 39658-K, Atkins Technical, Gainesville, FL) in the geometric center of each steak.

After cooking, steaks were allowed to equilibrate to room temperature (22°C) and 6 to 10 cores (1.3 cm in diameter) were removed from each steak parallel to the muscle

fiber orientation. Each core was sheared once, perpendicular to the muscle fiber orientation, using an universal testing machine (Instron Corp., Canton, MA) fitted with a WBSF head (cross head speed: 200 mm/min). Peak shear force measurements of cores from each steak were recorded and averaged to obtain a single WBSF value for each steak.

Statistical Analysis

Analyses of WBSF were conducted within muscle using a restricted maximum likelihood-based, mixed-effects model for repeated measures (PROC MIXED; SAS Inst. Inc., Cary, NC). The statistical model included sex, implant program (**IMP**), genotype (**GEN**), and aging period (**AGE**) as independent fixed effects. All relevant 3- and 2-way interactions of fixed effects were included and subsequently removed from the model and pooled with the residual if not significant ($P > 0.05$). Animal was included as a random effect, a spatial power covariance structure was used, and the Kenward-Roger approximation was used to calculate denominator df. Shear force measurements for the GM were adjusted to a common peak internal temperature using analysis of covariance.

To investigate the effects of behavior and health on WBSF, analyses were conducted using behavior or morbidity as a fixed independent categorical variable along with sex, IMP, and AGE. Animal was included as a random effect. For all shear force analyses, animal served as the experimental unit, AGE was treated as a repeated measurement, and means were separated using the PDIFF option at a significance level of $P < 0.05$.

Statistical analyses of muscle quality (excluding WBSF), carcass, and growth traits also were conducted using the mixed models procedure of SAS. The statistical models included sex, IMP, and GEN as independent fixed effects. All relevant 3- and 2-way

interactions of fixed effects were included and subsequently removed from the model if not significant ($P > 0.05$). Kill group was included as a random effect, and the Kenward-Roger approximation was used to calculate denominator df.

The proportion of variation in WBSF explained by pre- and post-slaughter variables was estimated using the Type III sums of squares approximation in PROC MIXED. For Type III analyses, individual animal (sex × IMP × genetic marker group) was included as a random effect. Proportions of explained variation (EV) were calculated as the ratio of SS for a variable to EV.

RESULTS AND DISCUSSION

The test population in this study was constrained to *Bos taurus* cattle and consisted of both steers (54% of sample) and heifers (46% of sample). The calves were weaned at 4 to 7 mo of age, backgrounded (in drylot) for approximately 90 d, finished on a high-concentrate corn-based diet for 137 to 166 d, and slaughtered at ages ranging from 12 to 16 mo. Cattle feeding and management practices were consistent with recommended pre-slaughter management practices for producing tender beef (Tatum, 2006). Descriptive statistics for growth, carcass, muscle quality, and behavior traits of the experimental sample are displayed in Table 4.2.

The primary objective of this study was to examine the combined effects of several pre-slaughter factors and their interactions with postmortem tenderization of the LM and GM. Results from analysis of variance summarizing effects of sex-class, IMP, GEN, and AGE on WBSF of LM and GM steaks are presented in Table 4.3. No interactions among sex-class, IMP, and GEN were detected ($P > 0.05$), indicating that effects of these factors

on LM and GM WBSF were independent. Correspondingly, only main effects and their interactions with AGE will be presented and discussed.

Sex-Class Effects

Previous studies comparing tenderness of beef from steers and heifers suggest that, on average, heifers produce beef that is slightly tougher than beef produced by steers (Tatum et al., 2007). In addition, results of some studies indicate that heifer beef may require a longer postmortem aging period to be comparable in tenderness to beef produced by steers (Wulf et al., 1996; O'Connor et al., 1997; Choat et al., 2006). In the current study, sex-class did not affect ($P > 0.05$) WBSF values of steaks from either muscle (Table 4.4). Furthermore, the interaction between sex-class and AGE (Table 4.3) was not significant for the LM ($P = 0.22$) or GM ($P = 0.29$), indicating that beef cuts from steers and heifers responded similarly to postmortem aging and would not require different aging periods to produce steaks with similar levels of tenderness.

Wulf et al. (1997) found that heifers were more excitable than steers and that muscles from heifers had higher ultimate pH values, lower a* and b* values, and a higher 24-h calpastatin activity, all of which were associated with higher WBSF values and lower sensory panel ratings for tenderness. In the current study, pen behavior scores showed that heifers were more excitable than steers (Table 4.4). However, LM and GM pH values (Table 4.4) were identical for steers and heifers and, in contrast to results reported by Wulf et al. (1997), heifers in the current study had higher LM a* and b* values than did steers (Table 4.4).

Implant Effects

A growing body of scientific evidence suggests that aggressive use of hormonal implants during finishing adversely affects tenderness and consumer acceptability of beef (Morgan, 1997; Roeber et al., 2000; Platter et al., 2003). Recent research suggests, however, that the detrimental effects on tenderness of all but the most aggressive implant programs are mitigated by postmortem aging periods of 14 to 28 d (Schneider et al., 2007).

Implant programs compared in the current study included: 1) a CNV 2-implant program in which cattle received a relatively mild initial implant (Revalor-IS or -IH) and were re-implanted with a moderate-dose terminal implant (Revalor-S or -H) approximately 80 to 110 d prior to slaughter, and 2) a DEL-implant program in which cattle were not given an initial implant, but received a moderate-dose terminal implant (Revalor-S or -H) administered after 45 d of finishing (approximately 95 to 125 d prior to slaughter). Neither of the 2 implant programs compared in this study would be considered overly aggressive (Morgan, 1997; Montgomery et al., 2001).

In the current study, effects of implant program on LM and GM WBSF depended on length of the postmortem aging period as indicated by significant IMP × AGE interactions for both muscles (Table 4.3). Compared with the CNV group, mean WBSF values for LM and GM steaks were reduced ($P < 0.05$) by delayed implanting, but only during the early stages of postmortem aging (Figures 4.1 and 4.2). Essentially, all differences in LM and GM WBSF between the 2 implant programs were eliminated once striploins and top sirloins had been aged for 14 d or longer. The only exception was a slight difference (0.14 kg) in GM WBSF between the 2 implant groups at 21 d

postmortem (Figure 4.2). These results suggest that if LM and GM steaks are aged for a minimum of 14 d, the effects of a CNV implant program on beef tenderness would be similar to that of a DEL implant strategy.

Delayed implanting has been recommended as a management strategy for enhancing marbling deposition in feedlot cattle without substantially reducing growth performance (Bruns et al., 2005; Corah and McCulley, 2006). The effects of IMP on growth and carcass characteristics are displayed in Table 4.5. Implant program did not affect ($P >$ 0.05) final BW, ADG, hot carcass weight, LM area, adjusted fat thickness, kidney, pelvic and heart fat percentage, or yield grade (Table 4.5). Conventional carcasses had slightly higher ($P < 0.05$) skeletal and overall maturity scores than DEL carcasses (Table 4.5). Compared to carcasses from CNV cattle, DEL cattle tended to produce a greater ($P =$ 0.09) percentage of carcasses grading USDA Choice or higher (71.5 vs. 62.0 ± 4.9 %) with higher ($P = 0.07$) marbling scores (444.6 vs. 428.2 ± 6.9; Small = 400). Similar to the present study, Woerner and Tatum (2007) applied CNV and DEL implant programs to a test population of cattle that were comparable to the current experimental sample (steer and heifer contemporaries from the same 2 crossbred cowherds). In that study, conventionally implanted cattle had greater hot carcass weights and LM areas than cattle that received a single delayed implant (Woerner and Tatum, 2007). Although not statistically significant, Woerner and Tatum (2007) reported that there was a trend for cattle that received conventional initial and terminal implants to produce carcasses with lower mean marbling scores (20 points) and a reduced quality grade performance(14% fewer Choice or higher) than cattle that received a single delayed terminal implant.

Genotype Effects

Cattle genotypes determined using the GeneSTAR Tenderness 3-marker panel and allelic frequencies for each tenderness marker (T1, T2, and T3) are presented in Table 4.6. Frequency of the favorable T1 allele was very high (0.88) among cattle comprising the experimental sample. Moreover, all cattle in the current study had at least 1 favorable T1 allele (Table 4.6). Frequencies of favorable T2 and T3 alleles among cattle in the test population were 0.25 and 0.60, respectively (Table 4.6). Tenderness genotypes and allelic frequencies for cattle in the current study were very similar to those reported by Van Eenennaam et al. (2007) for Charolais × Angus cattle. In the latter study (Van Eenennaam et al., 2007), frequencies of favorable T1, T2, and T3 alleles reported for Charolais × Angus cattle were 0.94, 0.23, and 0.46, respectively.

Information showing how T1, T2, and T3 alleles segregated in the experimental sample is provided in Table 4.7. In a recent validation study, Van Eenennaam et al. (2007) reported that the marker effect for the GeneSTAR tenderness panel was additive, but not equal. The CAPN1 316 (T2) and 4751 (T3) markers were linked, and therefore the association of the CAST (T1) marker and the μ-calpain haplotype with WBSF was reported (Van Eenennaam et al., 2007). The distribution of the genotypes in the current experimental sample (Table 4.7) was not suitable for haplotype analysis, and therefore an animal's genotype was expressed as the total number of favorable alleles for tenderness. Based on GeneSTAR Tenderness, an animal could possess from 0 to 6 favorable tenderness alleles (0, 1, or 2 favorable alleles for each of 3 markers). The distribution of genotypes observed in the experimental sample, expressed as total number of favorable

tenderness alleles, is displayed in Table 4.8. Genotypes were normally distributed, with more than 90% of the animals having from 2 to 5 favorable alleles (Table 4.8).

All animals with a single, favorable allele for tenderness had 1 favorable T1 allele. Of cattle that had 2 favorable alleles for tenderness, 60% had 2 favorable T1 alleles, whereas 40% had 1 favorable T1 allele and 1 favorable T3 allele. Most of the 3-allele and 4-allele cattle had 2 favorable T1 alleles, together with either 1 or 2 favorable T3 alleles. Of the 5-allele cattle, approximately 93% possessed 2 favorable alleles for T1 and T3, and 1 favorable T2 allele. In this experimental population of cattle, it was rare for an animal to have a favorable T2 allele, without also having either 1 or 2 favorable T3 alleles. This is consistent with findings of Van Eenennaam et al. (2007) based on genotypic information from several different *Bos taurus* and *Bos indicus* populations.

It is noteworthy that in the validation study, Van Eenennaam et al. (2007) reported a significant association between genotype expressed as total number of favorable tenderness alleles and WBSF. Furthermore, results from that study suggest that the difference between defining genotype as additive and equal (as in the current study) and defining genotype as additive but allowing markers to have effects of different magnitudes (CAST and μ-calpain haplotype) was very small. According to estimates reported by Van Eenennaam et al. (2007), within additive and equal genotypes (i.e., the total number of favorable alleles) the range of expected change in WBSF among the various combinations of markers was only 0.1 kg.

In the current study, there was a linear relationship ($P > 0.05$) between the number of favorable alleles for tenderness and WBSF of LM and GM steaks (Table 4.9). Cattle with 6 favorable alleles produced LM and GM steaks with mean WBSF values that were

0.33 kg lower than mean WBSF values for LM and GM steaks produced by cattle with only 1 favorable allele (Table 4.9). In the validation study, Van Eenennaam et al. (2007) reported that cattle with 6 favorable alleles for tenderness produced 14-d aged LM steaks with WBSF values that were approximately 0.8 kg lower than that from cattle with 1 favorable allele. For both muscles in the current study, the GEN × AGE interaction was not significant ($P > 0.05$). However, there was a tendency ($P < 0.20$) for effects of GEN on LM and GM WBSF to diminish as postmortem aging time increased. The mean difference in WBSF between LM steaks from 1 allele and 6 allele cattle was approximately 0.5 kg at 3 d postmortem and 0.2 kg at 28 d postmortem (data not presented). For GM steaks, mean difference in WBSF between the most and least desirable genotypes was approximately 0.5 kg at 3 d postmortem and 0 kg following 28 d of storage (data not presented). To date, the majority of studies investigating the effects of genetic markers on WBSF have involved only 14 d aged LM steaks. Schenkel et al. (2006) examined the effects of a CAST SNP (UoGCAST1 G to C substitution) on WBSF of 2, 7, 14, and 21 d aged LM steaks in addition to 7 d-aged semitendinosus steaks, however, this CAST SNP differed from the one utilized in the present study.

Additional Factors and Their Effects

Cattle Behavior. No cattle in the current study exhibited aggressive behavior, and only 2 animals were classified as "flighty". Animals scored as "flighty" were included in the "nervous" category for all analyses. Pen behavior scores classified approximately 60 % of all cattle as calm, 36 % as restless, and 4 % as nervous (data not presented). Pen behavior influenced WBSF values for both LM ($P = 0.013$) and GM ($P = 0.001$) steaks, but had a greater affect on tenderness of the GM. Cattle exhibiting calm pen behavior

produced LM steaks with mean WBSF values that were 0.22 kg lower than those for cattle characterized as nervous (data not presented). Perhaps most noteworthy was the fact that GM steaks produced by cattle with nervous pen behavior showed much less tenderness improvement in response to postmortem aging than did GM steaks from calmer cattle (behavior × AGE, $P = 0.019$). From 3 to 28 d postmortem, WBSF values for GM steaks from calm and restless cattle decreased 0.81 and 0.94 kg respectively, whereas WBSF values for GM steaks produced by nervous cattle were reduced by only 0.59 kg (Figure 4.3). Following 28 d of postmortem storage, top sirloin steaks from nervous cattle had WBSF values approximately 0.60 kg higher ($P < 0.05$) than top sirloin steaks from calm or nervous cattle (Figure 4.3).

Morbidity. The number of animals treated for illness 1 or more times during the experiment was approximately 1 in 5, whereas the incidence of cattle with 1 or more detectable lung lesions at slaughter was about 1 in 20 (data not presented). Gardner et al. (1999) reported that cattle with detectable lung lesions at slaughter produced tougher LM steaks (aged for 7 d) compared with steers that did not have lung lesions. In the current study, treating cattle for illness did not affect ($P > 0.05$) WBSF values for LM or GM steaks. In addition, the presence of detectable lung lesions had no effect ($P > 0.05$) on LM or GM tenderness.

Application of Genotyping for Tenderness Management

Of the pre-slaughter factors evaluated in the current study, genotype had the largest affect on beef tenderness. However, few, if any attempts have been made to use genetic markers to manage end-product tenderness. Application of genetic marker technology by

consumer-driven beef marketing programs could enhance their effectiveness for assuring consistent product tenderness and consumer satisfaction.

The U.S. beef industry currently relies on USDA quality grades (**QG**) to categorize carcasses and beef cuts according to expected differences in eating quality. Moreover, marbling score is a key carcass specification for certified beef programs; therefore, additional analyses were conducted to examine integration of genetic markers with existing beef carcass grading/classification systems.

Further analyses of the test population revealed that dividing the array of genotypes into 2 gene marker groups (**GMG**) – those with 3 or fewer favorable alleles vs. those with 4 or more favorable alleles – effectively stratified cattle comprising the experimental sample according to genotypic differences in mean LM WBSF (data not presented). Most of the cattle (> 88%) with 4 or more favorable alleles had 2 favorable T1 (CAST) alleles and at least 2 favorable T2/ T3 (CAPN1) alleles; however, a small subset (approximately 11.5%) of cattle within this group had a single favorable allele for T1 (CAST) together with 3 or more favorable alleles for T2/ T3 (CAPN1).

The effects of GMG, QG (Select, low Choice, and upper ⅔ Choice or higher), and AGE on LM and GM WBSF are summarized in Table 4.10. For top sirloin steaks, GMG was only effective at identifying tenderness differences within the Select (**SE**) grade (GMG × QG, $P = 0.027$). Within the SE grade, GM steaks from animals with ≥ 4 favorable alleles had WBSF values approximately 0.24 kg lower ($P < 0.05$) than GM steaks from cattle with ≤ 3 favorable alleles (Figure 4.4). The GMG × AGE interaction ($P = 0.025$) indicated that GM steaks from the more desirable genotype only had lower WBSF values at 3 d postmortem (Figure 4.5).

A significant GMG × QG × AGE interaction was detected for LM WBSF (Table 4.10). Least squares means for the 3-way interaction suggest that GMG was most effective at identifying LM tenderness differences within Select carcasses (Table 4.11). Regardless of postmortem aging period, GMG did not separate upper ⅔ Choice or higher **(UCH)** striploin steaks according to WBSF (Table 4.11). Low Choice **(LCH)** LM steaks from cattle with ≥ 4 favorable alleles for tenderness had lower ($P < 0.05$) WBSF values than LCH LM steaks from cattle with ≤ 3 favorable alleles, but only during the early postmortem period (3 d). At 3, 7, and 14 d postmortem, SE striplon steaks fabricated from cattle with ≤ 3 favorable alleles had higher ($P < 0.05$) shear force values than SE striploin steaks from animals with ≥ 4 favorable alleles (Table 4.11).

To estimate the potential merit that genetic marker technology may have for branded beef programs that use SE carcasses, non-linear regression was used to fit "aging curves" to the GMG × QG × AGE least squares means for striploin steaks (Gruber et al., 2006a). These exponential decay models were then used to calculate the number of days of postmortem aging required for LM samples in each subclass to achieve a specified WBSF value (3.70 kg, Platter et al., 2003). Regardless of GMG, UCH and LCH LM steaks required approximately 7 and 11 d of postmortem aging, respectively, to achieve the targeted WBSF value (Table 4.11). Select striploin steaks from cattle with ≥ 4 favorable alleles required 10 d of postmortem aging to reach 3.7 kg, whereas LM steaks from animals with ≤ 3 favorable alleles required 18 d of aging to achieve the targeted WBSF value (Table 4.11). Information presented in Table 4.11 may assist consumer-driven beef programs increase the consistency of their Select brands by identifying ways to manage genotype and postmortem aging.

Relative Importance of Pre- and Post-Slaughter Management Factors

Analyses in the previous sections estimated the magnitude of change in WBSF associated with various pre-slaughter management factors. Information pertaining to experimental sample variance, and the proportion of variation that can be attributed to different pre-and post-slaughter variables, could provide additional information about the contribution of each management factor to total tenderness variation. Type III sums of squares analyses were conducted for the LM and GM using sex, IMP, GMG, and AGE, as the factors of interest (Table 4.12). The repeated measures design of the present study also allowed for an estimate of between animal variation in beef tenderness. The experimental sample variance for WBSF was approximately 0.20 kg^2 for LM steaks and 0.22 kg^2 for GM steaks. Approximately 95 % of all LM WBSF observations for striploin steaks were between 2.78 and 4.58 kg; 95% of GM WBSF observations were between 3.36 and 5.24 kg.

Accounting for sex, IMP, GMG, AGE, and individual animal explained approximately 72.0 % of total variation in LM WBSF (i.e., explained variation) and 61.0 % of total variation in GM WBSF (Table 4.12). Postmortem aging accounted for more than 30 % of the explained variation in LM and GM WBSF. Sex and IMP each accounted for < 1 % of the explained variation in shear force of striploin and top sirloin steaks. Gene marker group and its interaction with AGE accounted for 1.5 and 0.8 % of the explained variation in LM and GM tenderness, respectively (Table 4.12). Variation in WBSF among animals within a sex × IMP × AGE subclass (between animal variation) accounted for 51 % of the explained variation in LM shear force (Table 4.12). Animal-to-animal variance accounted for 63.1% of the explained variation in GM WBSF.

Individual animal variation in WBSF was likely due to both environmental and genetic factors not accounted for in the present study. If a portion of animal-to-animal variation observed in the present study represents genetic differences in tenderness, then it is associated with genetic effects not explained by the calpastatin and μ-calpain SNPs quantified in the test population.

Results from the previous section suggested that tenderness markers may be most beneficial when applied within the Select quality grade. Type III sums of squares analyses conducted within the Select grade showed that GMG and its interaction with AGE accounted for 5.5 and 6.8 % of the explained variation in Select LM and GM steaks, respectively. It should be noted that analyses that included genotype expressed as 0 through 6 favorable alleles still only accounted for < 7 % of the explained variation in beef tenderness.

Results from this study suggest that the GeneSTAR 3 marker tenderness panel effectively identified differences ($\leq$ 0.33 kg) in LM and GM shear force. Moreover, the present study suggests that genetic marker technology could be used to increase the tenderness and consistency of Select striploins. However, the amount of variability in tenderness explained by the calpastatin and μ-calpain SNP is minimal. In the current study, sex, implant program, and genotype each accounted for < 7% of the explained experimental population variance.

Table 4.1. Number of animals in the experimental sample stratified by slaughter date, source, sex, and treatment

		Slaughter date					
		30-Apr-07		15-May-07		29-May-07	
Source	Treatment	Steers	Heifers	Steers	Heifers	Steers	Heifers
Ranch A	Conventional	18	19	18	14	15	12
	Delayed	16	19	18	14	15	15
Ranch B	Conventional	11	10	15	11	14	9
	Delayed	11	9	17	12	17	14

Table 4.2. Simple statistics for growth, carcass, muscle quality, and behavior traits

Trait	Mean	Minimum	Maximum	CV, %
Growth				
Age at slaughter, d	430.5	366	491	5.4
Initial BW, kg	355.7	259.9	479.5	11.4
Final BW, kg	601.1	474.5	747.5	8.5
Days on feed	152.3	137	166	7.7
Average daily gain, kg	1.61	0.96	2.25	13.8
Carcass				
Hot carcass weight, kg	369.1	287.6	452.2	8.6
Adjusted fat thickness, cm	1.40	0.56	2.49	26.5
LM area, cm^2	90.3	68.4	120.6	10.3
Kidney, pelvic, and heart fat, %	2.29	1.0	4.0	19.0
Yield grade	2.95	1.26	4.60	21.9
Marbling score[1]	431.2	300.0	890.0	20.5
Skeletal maturity[2]	42.3	20.0	80.0	24.4
Lean maturity[2]	36.5	10.0	120.0	29.4
Overall maturity[2]	40.7	20.0	80.0	21.8
Meat quality				
L*[3]	38.5	26.3	46.5	7.9
a*[4]	13.7	6.7	34.2	42.4
b*[5]	14.2	9.1	27.6	25.7
LM pH[6]	5.43	5.26	6.15	1.4
Gluteus muscle (GM) pH[6]	5.41	5.24	5.60	1.2
LM Warner-Bratzler shear force, kg[7]	3.68	2.04	6.79	20.7
GM Warner-Bratzler shear force, kg[7]	4.30	2.48	8.38	16.0
Behavior[8]				
Pen behavior score	2.88	0.25	10.9	55.6

[1]300 = Slight[0]; 400 = Small[0]; 500 = Modest[0].
[2]A-maturity = 0 to 99; B-maturity = 100 to 199.
[3]L^*: 0 = black; 100 = white.
[4]a^*: Negative number = green; Positive numbers = red.
[5]b^*: Negative number = blue; Positive numbers = yellow.
[6]Muscle pH taken 72 to 96 h postmortem.
[7]Shear force measurements were computed across postmortem aging period (3, 7, 14, 21, and 28 d).
[8] Behavior assessed using a 15 cm semi-structured continuous line scale: Calm = 0 to 2.9cm, Restless = 3 to 5.9 cm, Nervous 6 to 8.9 cm, Flighty = 9.0 to 11.9 cm, and Aggressive ≥ 12 cm (Gruber et al., 2006b).

Table 4.3. Summary of results from least squares analysis of variance for LM and Gluteus Warner-Bratzler shear force

		Muscle	
Source of variation	df	Longissimus	Gluteus[1]
Sex	1	0.3928	0.3672
Implant program (IMP)	1	0.0220	0.0436
Genotype (GEN)	5	0.0211	0.2303
Postmortem aging period (AGE)	4	< 0.0001	< 0.0001
Sex × IMP × GEN	5	0.7631	0.9086
Sex × IMP × AGE	4	0.2577	0.7920
Sex × GEN × AGE	20	0.5096	0.4925
IMP × GEN × AGE	20	0.6892	0.0829
Sex × IMP	1	0.3424	0.8244
Sex × GEN	5	0.5934	0.6195
Sex × Age	4	0.2188	0.2930
IMP × GEN	5	0.8062	0.3260
IMP × AGE	4	0.0440	0.0274
GEN × AGE	20	0.1378	0.1989

[1]Peak internal steak temperature ($P < 0.05$) was used as covariate for all analyses of GM WBSF.

Table 4.4. Least squares means showing the effect of sex-class on muscle quality traits and cattle behavior scores

			Sex	
Trait	*P*-value	SEM	Steer	Heifer
No. of animals	-	-	185	158
LM WBSF, kg	0.393	0.04	3.68	3.63
GM WBSF, kg	0.422	0.04	4.32	4.28
L*[1]	0.172	0.80	38.8	38.3
a*[2]	0.001	2.7	13.3	14.9
b*[3]	0.022	1.5	14.0	14.8
LM pH[4]	0.944	0.02	5.43	5.43
GM pH[4]	0.805	0.03	5.40	5.40
Pen behavior score[5]	0.0435	0.29	2.70	3.04
Chute behavior score[5]	< 0.0001	0.15	3.02	3.91
Post-transport behavior score[5]	0.437	0.08	1.79	1.72

[1]L*: 0 = black; 100 = white.
[2]a*: Negative number = green; Positive numbers = red.
[3]b*: Negative number = blue; Positive numbers = yellow.
[4]Muscle pH taken 72 to 96h postmortem.
[5]Behavior categories established from behavior scores (15 cm semi-structured continuous line scale): 0 to 2.9 cm = calm; 3.0 to 5.9 cm = restless; 6 to 8.9cm = nervous; ≥ 9 cm= flighty (Gruber et al., 2006b).

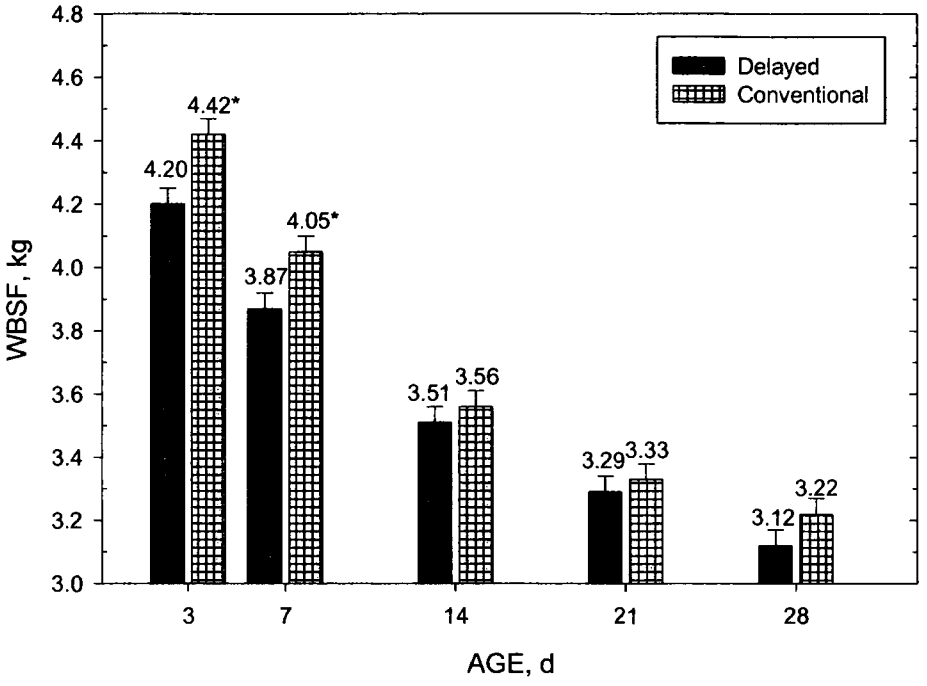

Figure 4.1. Effect of the implant program × postmortem aging period (AGE) interaction ($P = 0.044$) on LM Warner-Bratzler shear force (WBSF). *Within an aging period, least squares means differ between implant programs ($P < 0.05$).

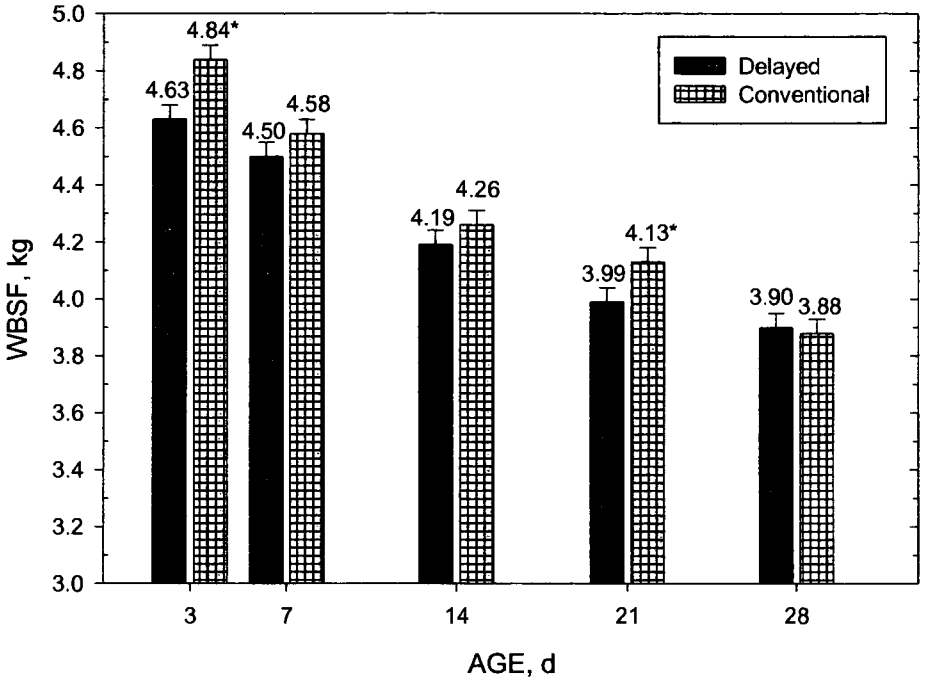

Figure 4.2. Effect of the implant program × postmortem aging period (AGE) interaction ($P = 0.027$) on gluteus muscle Warner-Bratzler shear force (WBSF). *Within an aging period, least squares means differ between implant programs ($P < 0.05$).

Table 4.5. Least squares means showing the effect of implant treatment on growth and carcass traits

			Implant treatment	
Trait	$P_{Implant}$	SEM	Delayed	Conventional
Animals	-	-	166	177
Initial BW, kg	0.511	16.2	355.0	357.3
Final BW, kg	0.704	10.5	598.6	600.4
Average daily gain, kg	0.726	0.05	1.61	1.60
Hot carcass weight, kg	0.241	5.9	366.6	370.1
Adjusted fat thickness, cm	0.447	0.05	1.37	1.42
LM area, cm^2	0.161	2.0	89.0	90.3
Kidney, pelvic, and heart fat, %	0.794	0.06	2.29	2.28
Yield grade	0.893	0.11	2.98	2.97
Skeletal maturity[1]	0.001	1.0	41.4	44.5
Lean maturity[1]	0.579	2.9	36.5	37.1
Overall maturity[1]	0.024	1.0	40.1	42.1
Marbling score[2]	0.066	6.9	444.6	428.2
USDA Choice or higher, %	0.094	4.9	71.5	62.0

[1]A-maturity = 0 to 99; B-maturity = 100 to 199.
[2]300 = Slight^0; 400 = Small^0; 500 = Modest^0.

Table 4.6. Genotypic and allelic frequencies for GeneSTAR Tenderness gene markers

		Genotype[1], %			Frequency	
GeneSTAR marker	Gene	0	1	2	Unfavorable allele	Favorable allele
T1 - CAST	Calpastatin	-	24	76	0.12	0.88
T2 - CAPN1 316	μ-Calpain	57	37	6	0.75	0.25
T3 - CAPN1 4751	μ-Calpain	18	44	38	0.40	0.60

[1]Number of favorable alleles.

Table 4.7. Distribution of favorable alleles for T1, T2, and T3 gene markers[1]

Genotype				
T1 – CAST[2]	T2 - CAPN1 316	T3 - CAPN1 4751	No. animals	% of test population
1	0	0	15	4.4
		1	25	7.3
		2	12	3.5
	1	0	-	-
		1	10	2.9
		2	13	3.8
	2	0	-	-
		1	2	0.6
		2	4	1.2
2	0	0	38	11.1
		1	71	20.7
		2	34	9.9
	1	0	8	2.3
		1	44	12.8
		2	52	15.1
	2	0	-	-
		1	-	-
		2	15	4.4

[1]Table format adapted from Van Eenennaam et al. (2007).
[2]The experimental population did not contain any animals with 0 favorable alleles for the T1 marker.

Table 4.8. Distribution of genotypes stratified by implant strategy and sex-class

			Conventional		Delayed	
Genotype[1]	No. animals	% of test population	Steer	Heifer	Steer	Heifer
1	15	4.4	8	4	1	2
2	63	18.4	16	8	23	16
3	101	29.4	19	28	27	27
4	93	27.1	30	18	27	18
5	56	16.3	13	16	11	16
6	15	4.4	5	1	5	4

[1]Genotype is expressed as the total number of favorable tenderness alleles for 3 different markers (CAST, CAPN1 316, and CAPN1 4751).

Table 4.9. Least squares means showing the effect of genotype on LM and gluteus Warner-Bratzler shear force (kg)

		Muscle[1]			
		Longissimus		Gluteus	
Favorable alleles	n	SEM	WBSF	SEM	WBSF
1	15	0.12	3.77	0.11	4.44
2	63	0.06	3.81	0.05	4.35
3	101	0.05	3.71	0.04	4.33
4	93	0.05	3.63	0.05	4.28
5	56	0.06	3.58	0.06	4.25
6	15	0.12	3.44	0.11	4.11
P-value linear contrast	-	-	0.008	-	0.015

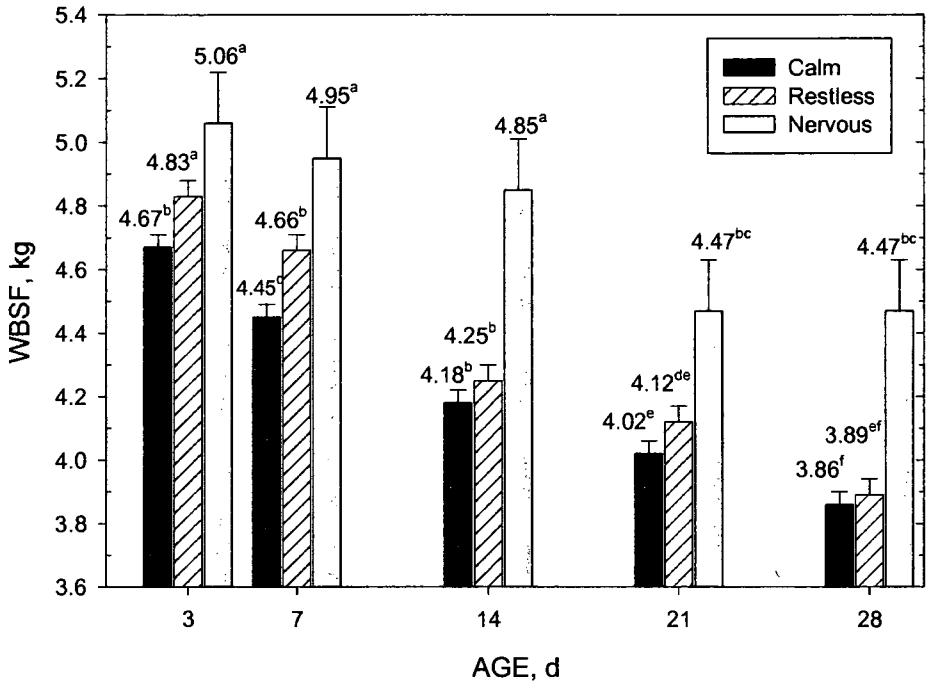

Figure 4.3. Effect of the pen behavior × postmortem aging period (AGE) interaction (P = 0.019) on gluteus muscle Warner-Bratzler shear force (WBSF). [a-f]Least squares means without a common superscript letter differ ($P < 0.05$).

Table 4.10. Summary of results from least squares analysis of variance for LM and Gluteus Warner-Bratzler shear force

Source of variation[1]	df	Muscle	
		Longissimus	Gluteus[2]
Sex	1	0.9435	0.6170
Implant program (IMP)	1	0.0749	0.1262
Genetic marker group (GMG)	1	0.0374	0.1714
Postmortem aging period (AGE)	4	0.0001	0.0001
Quality grade (QG)	2	0.0001	0.0167
IMP × AGE	4	0.0910	0.0456
QG × GMG × AGE	8	0.0136	0.4450
QG × GMG	2	0.0291	0.0282
QG × AGE	8	0.0787	0.6211
GMG × AGE	4	0.0107	0.0248

[1]Only significant interactions from previous ANOVA (Table 4.3) were included in the present analysis.

[2]Peak internal steak temperature ($P < 0.05$) was used as covariate for all analyses of GM WBSF.

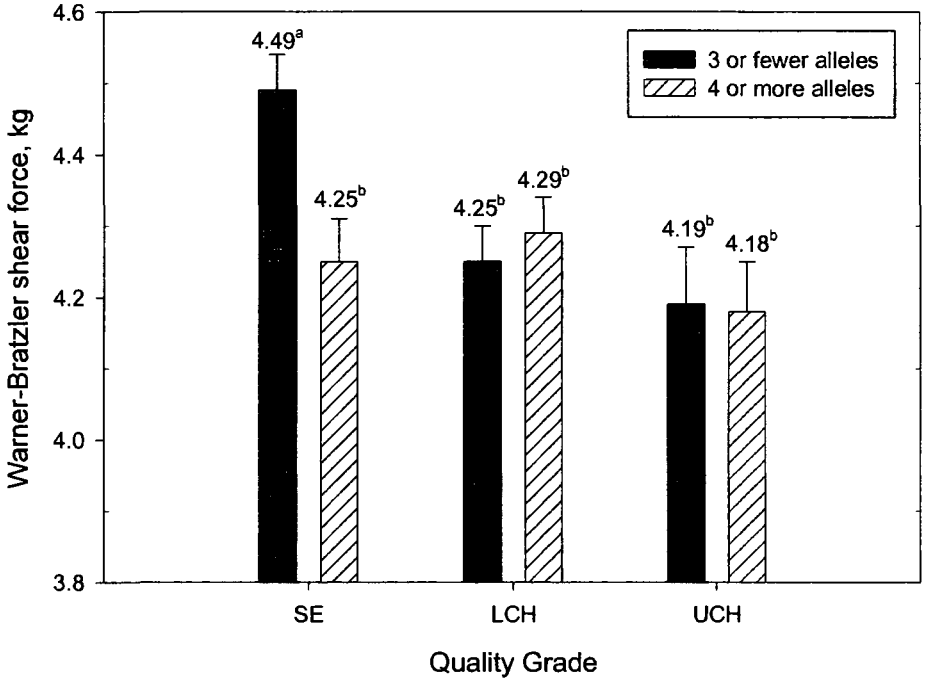

Figure 4.4. Effect of the quality grade × gene marker group interaction ($P = 0.028$) on gluteus muscle Warner-Bratzler shear force (WBSF). [a,b]Least squares means without a common superscript letter differ ($P < 0.05$).

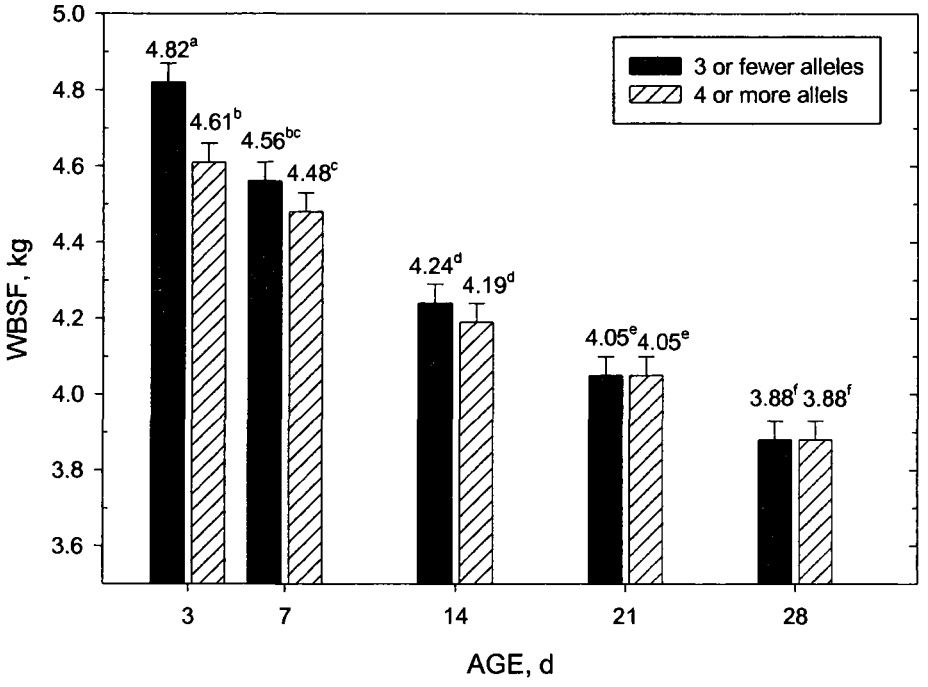

Figure 4.5. Effect of the gene marker group × postmortem aging period (AGE) interaction ($P = 0.025$) on gluteus muscle Warner-Bratzler shear force (WBSF). $^{a\text{-}e}$Least squares means without a common superscript letter differ ($P < 0.05$).

Table 4.11. Least squares means showing the QG × GMG × AGE interaction ($P = 0.011$) on WBSF of LM steaks

Quality grade[1]	GMG[2]	n	SEM	Postmortem aging period, d					d to 3.7 kg[3]
				3	7	14	21	28	
SE	≤ 3	78	0.07	4.72^{a}	4.43^{b}	3.88^{de}	3.49^{fghi}	3.39^{ghij}	18
	≥ 4	50	0.09	4.41^{b}	3.88^{de}	3.50^{fghi}	3.39^{ghij}	3.33^{hij}	10
LCH	≤ 3	72	0.07	4.37^{b}	3.95^{cd}	3.49^{fghi}	3.27^{jk}	3.07^{l}	11
	≥ 4	72	0.07	4.14^{c}	3.92^{d}	3.56^{fgh}	3.38^{hij}	3.00^{l}	12
UCH	≤ 3	29	0.11	4.07^{cd}	3.66^{ef}	3.32^{hij}	3.08^{kl}	3.08^{kl}	7
	≥ 4	42	0.09	3.97^{cd}	3.66^{ef}	3.30^{ij}	3.12^{kl}	3.14^{kl}	7

[1]Abbreviations used: SE = Select; LCH = low Choice; UCH = upper ⅔ Choice or higher (includes 6 Prime carcasses).
[2]GMG = Gene marker group, ≤ 3 or ≥ 4 favorable alleles for tenderness.
[3]d to 3.7 kg (Platter et al. 2003) predicted using non-linear regression models ($P < 0.05$) fit to least squares means for each QG × GMG × AGE subclass (Gruber et al., 2006a).
[a-l]Means without a common superscript letter differ ($P < 0.05$).

Table 4.12. Type III sums of squares analyses for LM and Gluteus muscle Warner-Bratzler shear force across all quality grades (pre-slaughter) and within the Select quality grade[1]

	Pre-slaughter				Within Select grade			
	Longissimus		Gluteus		Longissimus		Gluteus	
Source of variation	SS	% of EV	SS	% of EV	SS	% of EV	SS	% of EV
Sex	1.11	0.2	0.81	0.2	0.5	0.2	2.53	1.4
Implant program (IMP)	5.97	0.8	3.21	0.6	6.72	2.5	4.24	2.4
Genetic marker group (GMG)[2]	10.92	1.5	3.76	0.8	10.24	3.7	9.52	5.4
Postmortem aging period (AGE)	302.92	42.6	155.57	31.4	120.58	44.0	51.81	29.3
IMP × AGE	2.04	0.3	2.19	0.4	2.86	1.0	1.06	0.6
GMG × AGE	4.99	0.7	2.44	0.5	4.98	1.8	2.45	1.4
Individual animal	382.44	53.8	324.68	65.5	127.98	46.7	105.00	59.4
Internal steak temperature	-	-	2.80	0.6	-	-	0.13	0.1
Residual	271.39	-	301.30	-	107.84	-	126.77	-
Corrected total[3]	988.85	-	807.17	-	404.13	-	316.23	-

[1]SS = sums of squares; EV = explained variation.

[2]Analyses that included genotype as 1 through 6 favorable alleles accounted for < 7% of the explained variation.

[3]Sums of squares do not add to corrected total because individual Type III SS are adjusted for all other factors.

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