THESIS

PREDICTION OF MEAT TENDERNESS USING HIGH RESOLUTION IMAGING

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ABSTRACT OF THESIS

PREDICTION OF MEAT TENDERNESS USING HIGH RESOLUTION IMAGING

Tenderness plays an important role in the sensory attributes of beef products. The objective of this study was to obtain the highest quality and resolution images of cross-sections of beef *Longissimus dorsi* surfaces that could likely be replicated in a commercial environment; and, to develop algorithms and regression equations that predict aged beef shear force. Fifty carcasses were identified at each of three commercial beef processing facilities in Colorado, Nebraska and Texas (total N = 150). A-maturity carcasses were selected to fill an equal distribution over the entire range of beef marbling scores; 1/3 of carcasses represented marbling scores from Practically Devoid^{00} to Slight^{40}, 1/3 from Slight^{50} to Small^{90} and 1/3 from Modest^{00} or higher. Carcasses derived from cattle supplemented with Zilpaterol hydrochloride (n = 25, based on harvest facility records) were identified as such. Samples were excised from the *Longissimus* muscle immediately posterior to the 12th/13th rib interface and imaged using the Tenera Technology High Resolution Imaging System; in addition, reflectance measurements (L*, a*, b*) were obtained. Samples were aged for either 7 or 14 days prior to freezing. Steaks were fabricated from frozen samples for Warner-Bratzler shear force (WBSF) determination. Images were analyzed using the custom developed Tenera Technology

ZARMT software program, generating 10 output variables (diaSml, propSml, diaLrg, propLrg, ratDia, ratProp, medDia, medProp, diaNormMax and propNormMax) thought to represent ultra-structural characteristics of muscle such as fiber diameter, proportion of large versus small fibers and predominant size of muscle fiber within a given sample, which have previously been associated with beef tenderness (Hiner et al., 1953; Tuma et al., 1962; Herring et al., 1965; Cooper et al., 1968).

In 14d aged steaks from harvest facility one, the use of high resolution variables explained an additional 11% of the variation in WBSF value over the use of marbling and color variables alone. Within harvest facility two and three, high resolution variables allowed for explanation of an additional 25% and 17% of the variation in 14d WBSF respectively. For samples aged 7d, high resolution variables allowed for explanation of an additional 8%, 14% and 34% of the variation in WBSF values of steaks from harvest facility one, two and three respectively. Fourteen days postmortem, inclusion of high resolution variables improved classification of "tender" steaks (WBSF $\leq$ 3.7, Platter et al., 2003a) 40%, -3% and 7% from harvest facility one, two and three respectively. Classification of "tough" steaks (WBSF > 3.7, Platter et al., 2003a) within steaks aged 14d was improved by -10%, 0% and 0% through use of high resolution variables. In classification of "tough" versus "tender" steaks 7d postmortem, equations containing high resolution variables correctly classified and additional 6%, 14.3% and 7.1% of "tender" steaks and 0%, -5.9% and 9.1% of "tough" steaks from harvest facility one, two and three respectively. Compared with the use of marbling and reflectance measurements alone, the use of high resolution variables improved the ability to explain WBSF at 7d and 14d, as well as in the designation of "tough" and "tender"

steaks/carcasses, suggesting this technology, or one measuring similar traits could improve the assurance of tender beef products at the consumer level.

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CHAPTER I

INTRODUCTION

Tenderness of beef products is essential both to eating quality (Miller et al., 2001; Platter et al., 2003a) and consumer purchasing decisions (Platter et al., 2005). Additionally, tenderness is the most important factor influencing the beef sensory experience. Tenderness has repeatedly been cited as being among the top five beef quality challenges to the industry (Smith et al., 1992, 1995a, 2000). The importance of beef tenderness on the global marketplace was demonstrated by Smith (1995b), where importers of US beef ranked "its exemplary tenderness and flavor" as the second most important reason for purchase. In determining the importance that tenderness has on beef demand, several studies have found that consumers are willing to pay more for cuts expressing superior tenderness (Platter et al., 2005), or for cuts that have been guaranteed as tender (Boleman et al., 1997, Miller et al., 2001, Shackelford et al., 2001).

Smith and Carpenter (1974) reported that tenderness can be related to three principal components: acto-myosin interactions, background effects, and bulk-density/lubrication effects. Acto-mysoin interactions represent the contribution of the contractile state of muscle to meat tenderness. Background effects reference the contribution of collagen to meat tenderness. Finally, the bulk-density or lubrication effect relates to the decrease in resistance during chewing that can be associated with

increasing levels of intramuscular fat. Relating these components to conventional evaluations of beef quality, only marbling is associated directly with the bulk-density/lubrication effect. Marbling may also impact background effects relating to connective tissue content, as increased levels of intramuscular fat have been hypothesized to thin the connective tissue layers of the epi- and perimysium (Smith and Carpenter, 1974). Unfortunately, marbling has been shown to have only a low to moderate association with tenderness (Smith and Carpenter, 1974). As a result, substantial variation in beef tenderness is still unaccounted for by traditional evaluations of beef quality. More specifically, within the current beef quality evaluation system used by USDA-AMS, over 75% of A-maturity cattle harvested fall within a narrow range of marbling scores, corresponding to the USDA Select and low Choice grades (Smith et al., 2006). Within this majority, use of marbling alone has shown limited ability to differentiate carcasses into groups indicative of differences in value at the consumer level, specifically regarding tenderness (Smith et al., 1995a, Wulf et al., 1997). Consequently, during the National Beef Instrument Assessment Planning Symposium's (NBIAP) held in 2002 and 2007, focus was directed toward the development of an objective means of evaluating carcass tenderness (NCBA, 2002, NCBA, 2007). Therefore, the present study was conducted to determine the ability of high resolution imaging to enhance the ability of current predictors of meat tenderness.

CHAPTER II

REVIEW OF LITERATURE

Measures of Beef Tenderness

Considerable research has been conducted to develop objective methods for measurement of beef tenderness. From this work, the most commonly accepted mechanical measures of tenderness are Warner-Bratzler shear force (WBSF) and slice shear force (SSF). Each system measures the amount of mechanical force required to shear through a sample of cooked beef using a blunt edged blade. The cooking step of each procedure is critical, given the well documented relationship between increased degree of doneness and increased shear force and/or decreased tenderness (Ritchey and Hostetler, 1964; Parrish et al., 1973; Cross et al., 1976; Warkup, 1994; Wheeler et al., 1994; Lorenzen et al., 2003). To objectively determine tenderness differences between samples, and in an attempt to allow comparisons between different experiments from various laboratories, in 1978 the American Meat Science Association (AMSA) published "Guidelines for Cookery and Sensory Evaluation of Meat." This document was amended in 1995, and dictates that steaks used for WBSF measurements (2.54 cm in thickness) should be cooked to an internal temperature of 71^{o} C (AMSA, 1995). Slice Shear Force deviates slightly from these guidelines such that a peak internal temperature of 70^{o} C is recommended (Shackelford, 1999a). Following cooking, the two shear force procedures

differ also in how steaks are handled and samples are removed. For the WBSF procedure, steaks are allowed to cool to room temperature before the removal of a series of circular core sample(s) 1.27 cm in diameter (AMSA, 1995). By comparison, in the SSF procedure, a warm 1 x 5 cm strip is removed immediately following cooking from the center of the steak (Shackelford, 1999a). In either system, to most accurately evaluate tenderness, samples must be removed parallel to the muscle fiber orientation. This is critical, as using the WBSF procedure many workers have found increases in shear force values of cores removed in a manner parallel to muscle fibers rather than perpendicular to the cut surface (Hostetler and Ritchey, 1964; Wheeler et al., 1994).

Use of objective, mechanical measures of tenderness are of little value to the industry if correlations are not made between shear force values and consumer acceptability of tenderness. Several studies have attempted to define "threshold" values for WBSF at which a majority of consumers would deem a product acceptable/unacceptable in terms of tenderness (Shackelford et al., 1991; Miller et al., 2001; Platter et al., 2003a), and ranges of WBSF values which would lead to the purchase of additional steaks (Boleman et al., 1997). Shackelford et al. (1991) determined that, at WBSF values less than 4.6 kg, steaks should have a 50% chance of being rated as "slightly tender" or higher by a trained sensory panel. In testing against data from the National Consumer Retail Beef Study (Savell et al., 1987), these workers showed that the 4.6 kg threshold accurately classified 88.6% of steaks that would be rated by consumers as less than "slightly tender." Similar findings were achieved by Miller et al. (2001) who showed that the transition in consumer differentiation of tough versus tender occurred within the range of WBSF values between 4.3 – 4.9 kg. Platter et al. (2003a) found that

consumer perceived tenderness changed little at WBSF measurements above 5.5 kg and below 3.0 kg, however a steep decline in sensory ratings for tenderness occurred as shear force increased between these two values. More specifically, Platter et al. (2003a) described that at a WBSF value of 4.4 kg, the probability of consumers rating a steak as acceptable in terms of tenderness would be .50. Boleman et al. (1997) classified steaks into three known ranges of WBSF values (2.27 to 3.58, 4.08 to 5.40, 5.90 to 7.91) and determined that after consumers prepared steaks from each of the three categories without knowledge of WBSF values, a greater (55.3% versus 12.6% versus 32.0%) percentage of consumers chose to repurchase steaks from the group corresponding to the lowest WBSF values (2.27 to 3.58 kg).

Several other forms of tenderness evaluation also exist, and are performed through the use of either a trained, laboratory consumer or household sensory panel (Smith et al., 2008). A beneficial attribute of sensory panel evaluation is the ability of panelists to discriminate between connective tissue and muscle fibers (Hostetler et al., 1973), as the contribution of connective tissue to tenderness is generally obscured by the use of the traditional mechanical measures of tenderness (Smith et al., 2008). Nonetheless, many workers have found moderately high correlations between sensory panel ratings for tenderness and mechanical measures of shear force (Bratzler and Smith, 1963; Breidenstein et al., 1968; Otremba et al., 1999; Reagan et al., 1976; Wheeler et al., 1996).

In comparing the ability of different tenderness measurement methods (WBSF versus trained sensory panel evaluation) to predict the likelihood of consumer acceptability of beef steaks from the *Longissimus dorsi* (LD), Platter et al. (2003a)

determined that sensory panel evaluation was more accurate (78.7%) compared to WBSF (70.7%) in determining whether two-thirds of consumers would rate a steak as acceptable in terms of tenderness. Shackelford et al. (1995) reported that 73% of differences in consumer tenderness ratings of the LD could be explained by WBSF as the sole predictor within a regression model; however Schakelford et al. also suggested that WBSF of the LD may not apply to prediction of tenderness ratings within other muscles of the carcass such as the *Gluteus medius* ($r^2 = 0.00$). Lorenzen et al. (2003) found low ($r = -0.26$) but significant correlations between WBSF and consumer sensory panel ratings for tenderness. These lower correlations may be due to the variance associated with the non-standardized preparation of steaks within the consumer panel portion of this work (Lorenzen et al., 2003). As cited by Platter et al. (2003a), Meilgaard et al. (1999) stated reasons for decreased abilities of WBSF (specifically in evaluation of the LD) in the prediction of consumer acceptability were due to the idea that "threshold" values were "ill-defined in theory" and "may not even exist."

Prediction of Beef Tenderness

Belk et al. (1997) described that an ideal system for prediction of beef tenderness would involve an objective, noninvasive, accurate technology. Attempts to adhere to these criteria have brought forth several potential technologies, including slice shear force (SSF), objective color measurement, and near-infrared reflectance (NIR) (Woerner et al., 2008). Although SSF is undoubtedly a rapid, objective and accurate way by which to determine tenderness (Shackelford et al., 1999a; 1999b;1997; Vote et al., 2003), this technology would result in the loss of one steak from each animal harvested (Shackelford

et al., 1999a), making it much too invasive for overwhelming acceptance within the industry (Woerner et al., 2008). Objective color measurement and NIR have both shown the capability to quickly and non-invasively predict tenderness (Hildrum et al., 1994; Park et al., 1998; Price et al., 2007; Vote et al., 2003; Wulf and Page, 2000; Wulf et al., 1997).

Still reflectance measurement technologies are unable to describe which specific factors influence color measurements that they obtain. This complication is most notable within published articles when discrepancies exist for values of color (L*, a* and b*) or NIR measures that differ in their relative association with postmortem muscle tenderness (Vote et al., 2003). Meat color has been attributed to four primary components: muscle fiber content, myoglobin content, physiological maturity and ultimate muscle pH (Purchas et al., 2002; Purchas et al., 1999; Seideman et al., 1984). All of these factors are interrelated to muscle color and meat tenderness as demonstrated by **Figure 2.1**.

Muscle Fiber Content. Muscle fiber profile may play a primary role in color based on the intrinsic factors associated with each fiber type. Bovine skeletal muscle is made up of a heterogeneous blend of several fiber types (Pette and Staron, 2001). The metabolic, structural and contractile properties, along with other intrinsic properties, differ greatly between muscle fiber types (Schiaffino and Reggiani, 1996; Bottinelli and Reggiani, 2000). Through only slight differences in amino acid sequence, assorted isoforms of major contractile proteins (specifically myosin) exist within muscle, dictating both the metabolic and structural properties of meat (Schiaffino and Reggiani, 1996). During the transition of muscle to meat, the properties of the different muscle fiber types influence postmortem metabolism in such a way that dramatic differences can be noted in

the characteristics (including color) of meat (Ryu and Kim, 2005; 2006). Differences in myosin isoforms also dictate the ATPase reaction, allowing for differentiation of fiber type under laboratory conditions (Bottinelli and Reggiani, 2000). However, utilization of different fiber typing methods can yield differing results, even for the same set of muscle fibers (Picard et al., 1998). Most recently, the use of immuno-histochemistry and mRNA analysis has given rise to four distinct fiber types, type I, IIA, IIX and IIB.

Muscle fibers characterized as type I, beta-red, or slow-oxidative (SLO) are higher in myoglobin content as their metabolism is primarily oxidative (Seideman et al., 1984; Choi and Kim, 2009). Coincidentally, muscles with a greater content of type I fibers will be darker and redder in appearance (Seideman et al., 1984). This difference is most notable in muscles of posture such as the *Psoas major* (Hunt and Hedrick, 1977). In sharp contrast to type I muscle fibers are type IIB, alpha-white, fast-glycolytic (FG) fibers. Type IIB fibers are much lower in myoglobin content and rely almost entirely on glycolytic metabolism . The result of these characteristics is that muscles possessing a greater content of type II fibers will be lighter in perceived color (Choi and Kim, 2009). Within a beef carcass, the *Semitendinosus* serves as an example of a lighter colored muscle resulting from higher content of type IIB fibers (Hunt and Hedrick, 1977; Kirchoffer et al., 2002). Intermediate, type IIA/IIX, alpha-red fibers are more similar to type IIB fibers in terms of metabolism being more glycolytic. Through utilization of myosin-ATPase staining procedures, these fibers display color intermediate to that of type I and type IIB fiber types.

Critical to cattle management practices is the capability of all muscle fibers to transition between the various fiber types based on numerous intrinsic and extrinsic

influences (Ashmore and Doerr, 1971; Ashmore, 1974). These transitions can be caused by such factors as growth, physiological maturity, disease (Ashmore, 1974), presence of thyroid or growth hormones (Pette and Staron, 1997; 2000), and/or the presence of beta-agonists (Oksbjerg et al., 1995). This constant state of transition between various muscle fiber types allows for an incredibly diverse make-up between carcasses and within cuts derived from the same carcass (Kirchoffer et al., 2002). This diversity yields immense differences in the contribution of muscle fiber type to beef quality and color of lean.

Myoglobin. The quantity and oxidation state of heme iron in myoglobin make substantial contributions to muscle color. Within animals of similar physiological maturity, elevated myoglobin content can be attributed to differences in muscle fiber profile (Seideman, 1984). However, increasing physiological maturity can also result in increasing myoglobin content within muscle tissue. In either instance, the result of increased myoglobin content within muscle is a darker perceived color, which is typically associated with decreased tenderness (Hodgson et al., 1992; Hilton et al., 1997). Increased physiological maturity is associated with connective tissue cross-linking which causes increased toughness, and therefore has an inverse relationship with beef tenderness (Berry et al., 1974; Boleman et al., 1996; Breidenstein et al., 1968; Cross et al., 1973). This relationship substantiates the claim that darker perceived or measured color is associated with decreased tenderness.

Within animals of similar physiological maturity, the simple relationship that darker beef is less tender can be contradicted by the impact that muscle fiber profile can have on color and tenderness. The *Psoas major* is an example of a cut that may be derived from a youthful beef carcass, yet may be darker as a result of increased

myoglobin content due to substantially greater presence of type I, red muscle fibers and not advanced maturity. Several workers have shown that this increase in red fiber content may ultimately be beneficial to beef palatability (Calkins et al., 1981; Ockerman et al., 1984). In contrast, increased percentages of type IIB, white muscle fibers may contribute to a more youthful lean color that is theoretically indicative of higher quality beef (Urbain, 1952 as cited by Seideman et al., 1984). However, increased percentages of type IIB fibers have been associated with decreased tenderness (Calkins et al., 1981; Ockerman et al., 1984). Hence, conflicting associations between the "cause" of postmortem muscle color and its indications of meat paltability results in concerns regarding the benefits of objective color measurement in the prediction of tenderness; one must know what factors might be influencing color measurements before the measurement can be meaningful in the prediction of tenderness.

pH. Postmortem metabolism of muscle glycogen under anaerobic conditions leads to accumulation of lactate. This causes a decrease in the ultimate pH of beef. In a summary of factors influencing meat color, Seideman et al. (1984) stated that both the rate of postmortem pH decline and the ultimate pH influence the intensity of color in fresh meat. The effect of pH on lean color is directly related to changes in the amount of free water within muscle, alterations in sarcoplasmic and myofibrillar proteins and variation in mitochondrial respiration.

During postmortem pH decline, muscle approaches its isoelectric point (5.1 to 5.2); as this occurs, the amount of free water at the surface of the muscle increases because the net charge on myofibrillar protein is reduced and less water is bound. Consequently, more light is reflected off the meat surface, leading to a lighter perceived

and measured color. The most extreme example of this is the PSE or Pale, Soft and Exudative condition first described by Briskey and Wismer-Pedersen (1961), and characterized extensively in porcine muscle with low ultimate pH. The term PSE is not specifically applied to beef; however the changes seen in beef of a low ultimate pH can resemble conditions found in PSE pork. Ultrastructrally, meat of a lower ultimate pH will display myofibrils that are more "open" in structure, causing more light to be scattered, thereby appearing lighter in color (Walters, 1975). Low ultimate pH also will allow myoglobin to be more readily oxidized to metmyoglobin which displays a brown hue (Walters, 1975). Finally, with decreasing pH, mitochondrial metabolic potential has been shown to decrease (Cheah and Cheah, 1971; Ashmore et al., 1971; Tang et al., 2005) theoretically producing a brighter, more desirable lean color as there is less competition for oxygen between myoglobin and mitochondria.

Abnormal postmortem pH decline also can negatively impact meat color through the development of dark colored beef that is associated with a high ultimate pH. At higher pH values, the amount of free water at the surface of the ribeye decreases, decreasing the ability of the meat surface to reflect light, creating darker perceived color. Walters (1975) reported that beef of high ultimate pH expressed muscle fibers that were more swollen and packed tightly together. Consequently, oxygen is less readily absorbed. High pH also causes increases in the level of mitochondrial respiration, resulting in reduced concentrations of the bright, cherry-red oxymyoglobin pigment. The result is a thinner layer oxymyoglobin at the exposed surface of the ribeye, resulting in a darker, more purple color, as the deoxymyoglobin layer below the ribeye surface becomes more evident. As cited by MacDougall (1982), Lawrie (1958) determined that

the activity of mitochondrial cytochrome oxidase was increased at pH levels above 6.0, thereby increasing oxygen consumption and the appearance of deoxymyoglobin. This was confirmed by Walters (1975) who described that the respiratory rate of meat with a high ultimate pH was greater, resulting in a thinner layer of oxymyoglobin pigment at the surface, allowing the underlying purple, deoxymyoglobin pigment to become more visible.

Objective Measurement of Beef Color

Fresh meat color is the most important factor driving consumer purchasing decisions at the retail level. This is a result of a consumer perception that meat displaying a light, bright-red color is indicative of a high quality product (Urbain, 1952 as cited by Seideman, 1984). However, color perception is a subjective and psychological experience (MacDougall, 1982). In order to objectively measure color, it must be realized that color exists as a value based on the three primary colors in a three-dimensional space (MacDougall, 1982). Following the development of the L*, a* and b* measurements by Hunter, Commission Internationale de l'Eclairage (CIE) adopted this system as a universal color measurement in 1931 (MacDougall, 1982). Development of technology to measure color on various grids corresponding to the psychological surface color solid was summarized by Hunter (1958). This author cited the work of Judd, Hunter, and Scofield that eventually led to the development of the CIE L*, a*, b* system which is utilized today in many objective color measurement systems.

In addition to CIE L*, a*, b* color space, spectroscopy is used as an objective measure of color. The use of near-infrared reflectance (NIR) spectroscopy serves as a

determination of the interaction between the different constituents (proteins, carbohydrates, lipids, minerals and vitamins) in meat products and as a predictor of muscle tenderness (Nollet and Toldra, 2006). Measurements obtained within the NIR spectra involve three processes, reflectance, transmittance, and transflection (Nollet and Toldra, 2006). Reflectance is most simply defined as the energy reflected from the surface of a dense sample. Transmission measurements are based on the passage of energy through a substance; this measurement is typically associated with liquid samples. Transmittance is a combination of these measures and is best utilized for heterogeneous samples such as meat (Nollet and Toldra, 2006). The application of NIR to beef in the prediction of tenderness is of interest, as the technology can expose changes in the state of water and hydrogen bonds, such as those that occur during meat aging (Hildrum et al., 1994).

Use of Color in Prediction of Beef Tenderness

In application, numerous studies have found contrasting results in the measures of color that correlate to the ultimate tenderness of a product. The most uniform results have come from subjective evaluation of lean maturity, typically performed by trained USDA graders. However, almost all of these experiments involved carcasses representing the entire range (A – E) of lean maturity scores. The work of Hodgson et al. (1992) and Hilton et al. (1997) demonstrated a correlation between lean color and cooked beef palatability. These studies, however, did not use objective instrument evaluation of color, and involved carcasses from a wide range of maturity, mainly corresponding to mature cows.

Within the CIE L*, a*, b* color space, Wulf et al. (1997) found a significant relationship between b* color measurement and Warner-Bratzler shear force (r = -.38), as well as trained sensory panel ratings for tenderness (r = .37). Additionally, the same workers found a significant correlation between all objective measures of color and 24h calpastatin activity, possibly explaining some of the observed tenderness differences. Wulf and Page (2000) also reported a "higher the better" trend between b* values and tenderness. In an attempt to segregate carcasses into a group certified as "tender", Vote et al. (2003) determined that a* and b* were effective, indicating that higher values were more closely associated with lower WBSF values. These workers were able to combine a* and b* measurements with other output variables from the CVS BeefCam system (RMS Inc., Fort Collins, CO) in the certification of 80% of carcasses as "tender". Wyle et al. (2003) found that all measurements of lean and fat color (CIE L*, a*, b*), generated from the CVS BeefCam system were significant ($P < 0.05$) for entry into regression models to predict WBSF. Based on the two regression models generated by Wyle, et al. (2003) the frequency of tough steaks within groups certified as tender was lower than within the total population. Vote et al. (2009) found significant correlations of all color measurement variables (L* = -0.12, a* = -0.11, b* = -0.11) generated by the CVS BeefCam system and WBSF. Nonetheless, in an evaluation of three objective systems to determine beef tenderness, Wheeler et al. (2002) concluded that objective color measurement may not be accurate enough in classification of "tough" versus "tender" carcasses to warrant use in a commercial setting.

Mitsumoto et al. (1991) first found a correlation between NIR measurements taken in approximately the 1080 nm range and WBSF values (r = 0.798). Park et al.

(1998) indicated that NIR might be capable of predicting WBSF values, specifically suggesting that absorption was higher in extremely tough steaks in the range of wavelengths between 1,100 to 1,350 nm. In the reflectance spectra of 750 – 1098 nm, Byrne et al. (1998) indicated a moderate correlation (r = 0.53) using NIR to predict 14d WBSF. At 7d postmortem, Rodbotten et al. (2000) showed low to moderate correlations (r = 0.47 to 0.55) of NIR variables with WBSF. Shackelford et al. (2005) reported that NIR may have the potential to identify those steaks of the LD that are exceptionally tender within the USDA Select grade, however the equation developed to predict tenderness was only able to explain 22% of the variance in SSF. More recently, Price et al. (2007) found that NIR was correctly able to classify 92.9% of tough carcasses, in addition to showing the highest consumer satisfaction with steaks from the "tender" and "intermediate" classifications. However, in analysis of the ability of NIR to predict tenderness when used with visible color variables, Bowling et al. (2009) found no additive effect of variables measured with the NIR spectra to those generated using the CIE L*, a*, b* color space.

Alternative Predictors of Beef Tenderness

The evaluations of factors influencing color also have been used in prediction of beef tenderness. Using Holstein bulls and steers, Purchas (1990) demonstrated that graphing shear force versus pH resulted in a curvilinear relationship such that shear force values increased to a maximum around a pH of 6.1, and then declined once pH began to exceed 6.1. These results were similar to the findings of Bouton et al. (1973) who determined that beef was toughest in the range of pH from 5.8 to 6.0. However, Purchas

and Aungsupakorn (1993) were only able to account for a maximum of 37% of the variation in shear force value utilizing pH as a predictor. Combining pH with subjective color evaluation in tenderness prediction of over 3,400 beef carcasses, Jeremiah et al. (1991) were only able to account for 22% of the variation in shear force values. Shackelford el al. (1994) found low and non-significant relationships between pH measurements at both 3h and 48h post-mortem with Warner-Bratzler shear force. Jones and Tatum (1994) found a low ($r = 0.16$) but significant relationship between pH at 3h post-mortem and 10d WBSF.

In an invesigation of the role that pH plays on tenderness, Bouton et al. (1972) utilized lamb meat to determine that pH influenced the mechanical properties and adhesion between adjacent muscle fibers. Yu and Lee (1986) found that, within beef of high ultimate pH, there was increased activity of neutral proteases which degraded Z-line proteins; while in beef of low ultimate pH (5.5 to 5.7), acidic proteases were more active and targeted M-line proteins as well as myosin heavy chains. These findings possibly account for decreased tenderness at an intermediate value of pH where neither of these enzyme systems is overly active. Alternative effects of pH on the calpains were documented by Koohmaraie (1992) who found that autolysis of mu-calpain increased with decreasing pH. Dutson (1983) found both high and low ultimate pH accelerated the degradation of myofibrillar proteins. Purchas (1990) and Purchas and Aungsupakorn (1993) suggested that increased toughness of beef within intermediate pH values may be due to differences in sarcomere length. Watanabe et al. (1996) found the relationship between pH and beef tenderness may be related to differences in the rate of aging at differing pH values.

Muscle Ultrastructure and Beef Tenderness

Muscle ultrastructure has long been associated with meat quality and tenderness. It has been shown repetitively that increased fiber diameter is associated with decreased tenderness and/or increased shear force values (Hiner et al., 1953; Tuma et al., 1962; Herring et al., 1965; Cooper et al., 1968). This work also substantiates the findings that muscle fiber profile may impact tenderness given the well documented relationship between fiber diameter and fiber type, with red fibers corresponding to smaller diameters, and white fibers to larger diameters. Most recently, Seideman et al. (1988) found a negative relationship between tenderness and muscle fiber size, proposing that the difference was due to decreased myofibrils per unit area. The authors postulated that an increased number of myofibrils per unit area led to increased tenderness.

Contradicting the association of increased WBSF with increasing fiber diameter, Tuma et al. (1962) noted an increase in fiber diameter with animal age, and when the effect of animal age was removed, fiber diameter served as a poor predictor of tenderness at 14d postmortem. As cited by Crouse et al. (1991), Berry et al. (1971) found similar results, reporting that there was only a small correlation between muscle fiber diameter and tenderness when the effect of age was removed. Lewis et al. (1977) using 7d aged steaks from the LD, showed a small ($r = -0.03$) and non-significant relationship between fiber diameter and trained sensory panel ratings for tenderness. Crouse et al. (1991) found that the influence of fiber diameter on tenderness was important early postmortem; however, after extended periods of aging or after the removal of the effect of aging, the relationship between fiber diameter and tenderness was non-significant. Clear examples

of differences in fiber diameter and tenderness exist between certain muscles, such as those between the *Psoas major* and the *Semitendinosus.* However, given the contradictions between the aforementioned experiments, uncertainty arises as to whether tenderness differences are due to fiber size, fiber type or potentially the increases in collagen cross-linking that occurs as animals age and fiber size increases.

Cassens (1977) summarized the effect of fiber type on muscle by stating that "the properties of a muscle, be they visual appearance, physiological parameters or biochemical characteristics, are a reflection of the proportion of the muscle fiber types present." Prior to this, Ashmore et al. (1972) hypothesized that the quality of a meat product is a direct reflection of the proportion of different fiber types present. The first investigation of these theories was performed by Melton et al. (1974; 1975) who examined the relationship between fiber type and sensory quality attributes in beef using twenty-one Hereford bulls. This experiment failed to find a significant relationship between the areas of any fiber type and WBSF or trained sensory panel ratings for tenderness. However, variability in WBSF value was minimal within this experiment (Mean = 3.32, SD = 0.47). These results were in line with those of May et al. (1977) who, using thirty-six crossbred steers, found non-significant relationships between fiber type and all palatability attributes. Contrary to earlier findings, Calkins et al. (1981) showed a significant relationship between fiber type and tenderness ratings. Using sixty-five A-maturity carcasses representing USDA marbling scores from Moderately Abundant to Practically Devoid, these workers found a negative correlation ($r = -0.40$) between percent of α-white muscle fibers and trained sensory panel tenderness ratings. Moreover, Calkins et al. (1981) found a positive correlation ($r = 0.44$) between percent of

α-red fiber area and trained sensory panel ratings for tenderness. In addition to percentages of different fiber types, Calkins et al. (1981) demonstrated that the ratio of α-white to α-red (i.e., white : intermediate) muscle fibers had a significant correlation with trained sensory panel tenderness ratings ($r = -.42$). This experiment may have been more successful than previous attempts to characterize palatability attributes based on muscle fiber type because it utilized larger sample sizes, included a broader range of marbling scores and aged samples for a more extended period (10 to 14d versus 4d). Crouse et al. (1991) showed a significant correlation of percentage white muscle fiber area with 1d shear force, and a negative correlation with 1d and 6d trained sensory panel ratings for tenderness. Ockerman et al. (1984) demonstrated a significant ($p < 0.05$) positive correlation between percentage of red muscle fibers and trained sensory panel ratings for tenderness, as well as a significant negative correlation between percent white muscle fibers and sensory tenderness ratings.

Contradicting these findings are those of Seideman and Theer (1986) who found that increases in percent and percent of area of white fibers were correlated with increased trained sensory panel tenderness ratings and decreased WBSF values. These workers also found that increases in the percent and percent area of intermediate fibers were correlated with decreased trained sensory panel tenderness ratings and increased WBSF values. No correlation was found to exist between either percent or percent of area of red fibers and trained sensory panel ratings for tenderness or WBSF values. It is important to note that the work of Seideman and Theer (1986) involved samples from both bulls and steers. Seideman and Theer (1986) showed an increase in the percent and percent area of red fibers in bulls. Steers also have been proven to have increases in the

percent and percent area of white fibers. Coincidentally, steers have also been shown to possess increased tenderness in comparison to bulls. These characteristics may confound studies involving both bulls and steers in the use of fiber typing to predict tenderness. Whipple et al. (1990) found no correlation existed between muscle fiber traits and 14d WBSF values; however, percentage of area of β-red muscle fibers did have a partial R^2 value of .16, and was significant at the 0.10 level for entry into regression models for prediction of trained sensory panel tenderness, indicating that it may be important in predicting tenderness ratings. The results of Wegner et al. (2000) and Vestergaard et al. (2000) coincided with those of Whipple et al. (1990) and suggested no relationship between muscle fiber characteristics and shear force value; however, both studies also utilized bull calves in their experiment.

Beyond differences in fiber diameter, additional reasoning to explain the impact of muscle fiber type on tenderness has been proposed by many authors. Ouali and Talmant (1990) stated that the rate of aging is faster in white muscle fibers than in red. These workers also showed that red muscle fibers have the highest calpain content, but also suggested that expression of these proteases was muscle dependant. Whipple et al. (1990) proposed that the activity of the inhibitor to the calcium dependent proteases (calpastatin) at 1d post-mortem may be related to the area of red fibers in addition to ultimate pH. Further work has shown that red muscle fibers have the highest content of calpastatin (Koohmaraie, 1996). In carcasses where electrical stimulation is applied, it has been noted that white-muscles respond more intensively at high voltages. Cornforth et al. (1980) demonstrated that red muscle fibers are more susceptible to cold shortening, potentially leading to tougher meat. Gann and Merkel (1978) showed that degradation of

the Z-line during aging was much lower in red muscle type I myofibrils than in type II at 1h, 48h and 216h post-mortem.

Summarizing the effects of muscle fiber type on beef tenderness, it is now commonly accepted that increased red muscle fiber content is more highly correlated with beef tenderness. Fiber profile differences exist between many muscles of the carcass, with muscles characterized as being "redder" (or having increased red muscle fiber content) being more tender (Kirchoffer et al., 2002). The work of Calkins et al. (1981) and similar studies, established that those carcasses expressing greater red muscle fiber typically yield more tender cuts. Prior to this, although not fiber typing studies, the work of Hiner et al. (1953), Tuma et al. (1962), Herring et al. (1965), and Cooper et al. (1968) established that larger muscle fiber diameters are associated with decreased tenderness. In proving the benefit of analysis of size and proportional differences of muscle fibers in prediction of muscle tenderness, these workers established the basis for the potential capabilities that high resolution imaging may have to augment current tenderness predictions.

Contributions of Collagen to Beef Tenderness

Approximately 1 to 4% of any meat product consists of connective tissues comprised primarily of the protein collagen. Excluding tendons, the connective tissues of a muscle can be subdivided into three distinct layers from the outermost epimysium (surrounding the entire muscle), leading next to the perimysium (surrounding muscle bundles) to the inner most endomysium (surrounding muscle fibers). Bailey (1985) stated that it is the perimysial connective tissue that provides the greatest contribution to

beef tenderness. Purslow (2005) stated that it is the perimysial component of connective tissue that varies the most between muscles. Consequently, measurement of perimysial attributes may ultimately augment predictions of beef tenderness. Nishimura et al. (1999) demonstrated that both the peri- and endomysium can stretch and become more disorganized with increased deposition of intra-muscular lipid, resulting in increased tenderness. Ultrastructually, individual collagen fibrils are far too small (45 to 65 nm) to be measured using existing technologies for tenderness prediction. The aforementioned studies focused on the quantity of connective tissue present, however Bailey (1985) stated that it is the "quality" of collagen, referring to the degree of cross-linking, that contributes much more readily to the texture of meat. Lepetit (2007) stated that no one individual characteristic of intramuscular connective tissue may be critically linked to beef tenderness.

The first correlation between muscle fiber type and connective tissue (collagen) content was made by Beatty et al. (1967) who used fetal, neonatal and infant monkeys to demonstrate that red muscles (muscles high in red fiber content) possessed less collagen than white muscles. These results were similar to those obtained by Valin et al. (1982) who used lamb meat to show decreased levels of collagen as muscles became "redder" and more oxidative. More recently, Nishimura et al. (1999) found a much greater amount of collagen (approximately 4% versus 2%) between the *Semitendinosus* and LD of fattened Japanese Black cattle.

McCormick (1998) cited unpublished data comparing the collagen content and amount of cross-linking between five muscles of the bovine carcass. In the McCormick (1998) experiment, the investigator found that the LD contained substaintially less

collagen and cross linking than both the *Biceps femoris* and *Semimembranosus* (both muscles that are characterized as "white" due to white muscle fiber content greater than 40%; Kirchoffer et al., 2002). Interestingly, McCormick (1998) also showed in a comparison between the *Gluteus medius* and *Psoas major* (a predominantly red muscle), the *Gluteus medius* to have a higher level of collagen, but lower levels of cross linking. Tatum et al. (1990) utilized steers sired by Piedmontese, Gelbvieh and Red Angus bulls in a comprehensive study of beef quality attributes including collagen content. Within this experiment, calves sired from Piedmontese bulls exhibited the highest percentage of white muscle fibers and the smallest area of red muscle fibers. However, no difference was found in the collagen content of steaks derived from the *Longissimus dorsi* of Piedmontese-sired steers. Workers have attributed the superior tenderness of meat from double muscled animals to decreased amounts of collagen. However, it is known that double muscled animals have greater percentages of white muscle fibers, and reduced areas of red fibers, contradicting findings of previous research which correlated increased collagen content with white muscle fibers.

Influence of Maturity on Fiber Type

Animal age and maturity have been linked to effects on muscle fibers and tenderness. Seideman et al. (1986) found that the percent, as well as the percent of total muscle area, of white muscle fibers increased with animal age. Ashmore et al. (1972) demonstrated that as animals age, there is a transformation from intermediate type muscle fibers to large, white type IIB fibers. The transition of muscle fibers from either type I or type IIA/X to the larger type IIB variety produces muscle with larger average fiber

diameter as animals mature. Johnston et al., 1975 found a significant effect of days on feed on the size of β-red muscle fibers, and of breed (Charolais versus Angus) on the area of white muscle fibers present and the size of muscle fibers.

Effect of Selection and Management on Tenderness

Substantial evidence has been presented linking the effect of selection pressure for increased meat production to muscle fiber profiles. Ashmore et al. (1972) theorized that, in the selection of domesticated animals for increased meat yield, a transformation to a population with a greater percentage of large, white muscle fibers will occur. Additionally, this author stated that this selection may inadvertently promote alterations in the quality of meat products derived from domesticated animals. These impacts include decreases in intramuscular lipid content, negative impacts on postmortem color, and potentially decreased tenderness.

In the production of beef cattle, there is increasing use of growth enhancing management practices that can influence meat quality characteristics such as color and tenderness. The use of growth promoting implants containing steroid hormones, as well as the feeding of beta-adrenergic agonists, have become common practices in today's beef industry as a means to improve efficiency and retail yield. Tatum (2006) cited a survey of feedlot management practices (NAHMS, 2000) which indicated that over 97% of feedlot cattle receive one or more hormone-based, growth promoting implant during finishing. The use of beta-adrenergic agonists also has increased rapidly in the last decade, with approximately 40% of cattle fed in the United States receiving some form of beta-adrenergic agonist in their ration (Tatum, 2006). Though these practices produce

substantial increases in efficiency, this gain in productivity also can have adverse effects on beef quality due to the impact of hormone-based implants and beta-adrenergic agonists on the intrinsic properties of muscle.

Multiple studies have cited an increase in WBSF values in beef derived from cattle known to be implanted with growth promoting steroid implants (Samber et al., 1996; Foutz et al., 1997; Platter et al., 2003b; Schneider et al., 2007), as well as a decrease in consumer acceptability (Roeber et al., 2000; Platter et al., 2003b). Prior to this work, Ashmore (1974) stated that hormones may play a crucial role in the transition of intermediate-type muscle fibers to large white fibers, citing the work of Bass (1971) who demonstrated a transition of intermediate type muscle fibers to large white fibers in the presence of testosterone in the male guinea pig. Using Holstein heifers, Crouse et al. (1987) found an increase in fiber diameter and the predominance of type IIA fibers in animals administered trenbolone acetate. Baxa et al. (2010) found an increase in the expression of myosin heavy-chain type IIA mRNA following implantation with a combination trenbolone aceate/estradiol implant. Consequently, growth enhancement technologies such as hormone-based implants must be administered judiciously to minimize adverse effects on meat quality and tenderness (Tatum, 2006).

The beta-adrenergic agonists commercially available to cattle producers today (Ractopamine hydrochloride and Zilpaterol hydrochloride) are synthetic peptides that mimic the actions of the catecholamines epinephrine and norepinephrine, which are produced in the body (Mersmann, 1998). As cited in Mersmann (1998), Cunningham (1965) first presented data indicating that the use of compounds that may alter the function of cyclic adenosine monophosphate (CAMP) may influence animal growth.

Epinephrine was explicitly cited in this work, which was the first that suggested beta-adrenergic agonists may have some effect on animal growth. Beta-adrenergic agonists bind to G – protein – coupled beta receptors found throughout the body (Mersmann, 1998). Binding of the beta-receptors initiates a cascade of events: CAMP binds to protein kinase A, releasing a subunit responsible for phosphorylation of numerous intracellular components. The CAMP response element binding protein (CREB) is phosphorylated by protein kinase A, after which transcription of numerous genes in the cell occurs. Ultimately, protein accretion is increased while degredation is decreased (Beermann, 2002).

Ractopamine hydrocholoride and Zilpaterol hydrochloride have been used in beef cattle to create dramatic improvements in efficiency and muscle expression. Dikeman (2007) stated that beta-adrenergic agonists must be used responsibly to avoid negative impacts on marbling and tenderness of meat products. The effects of ractopamine hydrochloride supplementation in swine have been well documented, with most work showing an increase in the glycolytic fiber content of muscle, a subsequent decrease in lipid content and accelerated postmortem pH decline that may produce pork which is more prone to eating quality issues. Gonzalez et al. (2007) found that ractopamine hydrochloride supplementation in conjunction with trenbolone acetate implantation in cattle increased the percentage of type II muscle fibers in culled crossbred beef cattle. Gonzalez et al. (2008) confirmed these findings by the feeding of ractopamine hydrochloride at four different levels without any growth promoting implants. Finally, Gonzalez et al. (2009) attained similar results in the feeding of ractopamine hydrochloride to steers during the finishing phase, showing an increase in type II fiber

content in five of six muscles examined from cattle supplemented with Ractopamine hydrochloride. Gruber et al. (2008) studied the effects of ractopamine hydrochloride on beef sensory characteristics, and reported steers supplemented with the beta-adrenergic agonist produced steaks with greater WBSF values and decreased sensory panel ratings for tenderness. Avendano-Reyes et al. (2006) attained similar findings, showing increased shear force values in steaks from carcasses of steers supplemented with ractopamine hydrochloride.

The most recent beta-adrenergic agonist approved for use in cattle is Zilpaterol hydrochloride. Dikeman (2007) cited the findings of Dikeman (2003) who found that Zilpaterol hydrochloride supplementation dramatically improves growth, dressing percentage and muscling in cattle. A limited number of publications exist describing the effects of Zilpaterol hydrochloride on muscle fiber type. However, the work of Baxa et al. (2010) indicated that Zilpaterol hydrochloride supplementation increased the expression of myosin heavy chain type IIX mRNA. Substantiating these findings, Rathmann et al. (2009) showed an increase in the expression of myosin heavy chain type IIX mRNA and a decrease in myosin heavy chain type IIA mRNA in cattle supplemented with Zilpaterol hydrochloride. These findings indicate a potential transformation of small red muscle fibers to "intermediate" type muscle fibers, which may adversely affect meat tenderness (Calkins et al., 1981).

The impact of Zilpaterol hydrochloride on beef quality attributes such as marbling, color and tenderness have been well documented. Montgomery et al. (2008) found decreased marbling scores in steers supplemented with Zilpaterol hydrochloride. Hilton et al. (2009) also found that supplementation of Zilpaterol hydrochloride in the

diet of beef steers decreased quality grade. Similarly, Montgomery et al. (2009) found reduced marbling scores and quality grades in steers and heifers supplemented with Zilpaterol hydrochloride. Rousel and Nel (1996), as cited by Kellermeier et al. (2008) found no differences in L* values, but higher a* and b* values in carcasses of cattle supplemented with Zilpaterol hydrochloride. Hilton et al. (2009) were unable to find differences in lean maturity, color score or CIE L* values between cattle supplemented with Zilpaterol hydrochloride and control groups, but did find increased CIE a* and b* values and increased color scores through a 5d display period within steaks of the *Longissimus dorsi* from cattle treated with Zilpaterol hydrochloride. These results indicate that Zilpaterol hydrochloride supplementation may cause beef to appear redder (a*) and yellower (b*) both indicators that have been associated with increased tenderness through objective color measurement (Wulf et al., 1997; Wulf and Page, 2000). However, Zilpaterol hydrochloride has been documented to increase beef shear force (Avendano-Reyes et al., 2006; Kellermeier et al., 2009; Hilton et al., 2009; Shook et al., 2009; Brooks et al., 2009) and decrease sensory panel ratings for tenderness (Hilton et al., 2009).

Several hypotheses have been advanced to explain reduced tenderness of steaks derived from cattle supplemented with beta-adrenergic agonists. Several workers (Koohmaraie et al., 1991; Wheeler and Koohmaraie, 1992; Geesink et al., 1993) suggested that an increase in calpastatin activity postmortem is responsible for decreased tenderness of meat products produced from animals fed beta-adrenergic agonists. However, recently, Hilton et al. (2009) failed to find an effect of Zilpaterol hydrochloride supplementation on calpastatin or calpain activity. Regardless of the reason, the

documented effect of Zilpaterol supplementation on color (increased a* and b* values) as well as tenderness (increased WBSF values) poses issues with objective color measurement technologies, as theoretically, given higher a* and b* values, cattle supplemented with Zilpaterol hydrochloride would, based on objective color measurement, be expected to be more tender (Wulf et al., 1997; Wulf and Page, 2000). This paradigm demonstrates need for technologies that can differentiate muscle ultrastucture and potentially explain which variables are influencing muscle color.

Summary

Objective color measurement is a relatively accurate and non-invasive way in which to predict beef tenderness (Wulf et al., 1997; Wulf and Page, 2000; Vote et al., 2003; Wyle, et al., 2003; Mistumoto et al., 1991; Park et al., 1998; Byrne et al., 1998; Rodbotten et al., 2000; Shackelford et al., 2005). The use of CIE L*, a*, b* and NIR have proven to adhere to the requirements established by Belk et al. (1997) in being a rapid, non-invasive technology. However, the accuracy of these technologies still remains unacceptable for overwhelming industry acceptance (Wheeler, 2002), particularly given the contradicting reports that exist regarding which values of these measurements best correlate to beef tenderness (Vote et al., 2003).

Muscle ultrastructure and particularly fiber type are central components to the outward appearance of a muscle (Seideman et al., 1984; Hunt and Hedrick, 1977; Ryu and Kim, 2005, 2006; Choi and Kim, 2008). The intrinsic properties of muscle have dramatic impacts on postmortem metabolism, pH and myoglobin content, all of which dictate the perceived or measured reflectance of the lean surface (Schiaffino and

Reggiani, 1996). Ultimately, a muscle may be "redder" due to increased content of red muscle fibers, which are perhaps associated with increased tenderness (Calkins et al., 1981); or, due to increased physiological age, which is thought to be associated with reduced tenderness (Hodgson et al., 1992; Hilton et al., 1998); or, due to increased pH which is associated with reduced or improved tenderness depending on the value (Purchas, 1990). Additionally, given the advancement of growth enhancing technologies, beef reflectance may be increased (Hilton et al., 2009) due to conversion of small, oxidative, red muscle fibers into large, white, glycolytic fibers (Rathmann et al., 2009; Baxa et al., 2010), which may be detrimental to beef tenderness (Calkins et al., 1981). The inability of traditional color measurements to explain differences in the factors influencing muscle color demonstrates the precise need for a technology that can measure ultrastructural traits in order to enhance the accuracy of tenderness prediction when coupled with objective color measurement.

CHAPTER III

PREDICTION OF MEAT TENDERNESS USING HIGH RESOLUTION IMAGING

Materials and Methods

Tenera Technology-High Resolution Beef Imaging System

For this research, a lens system with a nominal resolution of 1.25 to 1.3 microns was employed. With diffraction effects, as well as limitations of the camera optics and electronics, the true resolution, roughly speaking, was around 4 to 6 microns. This resolution is near the theoretical limits of resolution, and is likely the best that can be expected for a commercial system given current available digital imaging technology. The camera utilized within the system was a Canon 5D camera with about a 15 megapixel imager (4770 x 3177 pixels) -- by comparison, a television screen is approximately 300 x 500 pixels. A macro lens with an enlarger element was employed to give the desired resolution.

The camera and lens were mounted to a ZenBot 1216 CNC router (Zenbot CNC, Tulare, CA) (**Figures 2.2 and 2.3**). The router element was replaced by the camera and lens, and the router base was changed by replacing the wooden platform with a plastic platform on which jigs could be attached for sample placement. The Zenbot had the ability to move on three axes via control from custom developed ZARMT software

(Tenera Technology, Boulder, CO). The ZARMT software automated focus of the imaging system by z-axis movement and bracketing of the distances of highest focus. Because the magnification was constant, this provided a fixed field of view and, therefore, the same resolution per linear distance on each sample. Illumination of the sample was accomplished via a small battery powered LED system mounted at approximately a 45^{o} angle to the lens and sample surface and was applied in an open lighting environment similar to any found within a commercial carcass cooler. Matched polarizing lenses on the camera and the illuminator were used to reduce the amount of glare in images.

Sample Collection

Fifty carcasses were identified at each of three commercial beef processing facilities located in Colorado, Nebraska and Texas (total N = 150). Carcass selection criteria were based on official USDA quality grade, with all carcasses coming from the A-maturity class. One-third (n = 50) of carcasses were from USDA Standard through low Select (marbling score $\leq$ Slight^{40}), approximately one third (n = 49) were from high Select (marbling score $\geq$ Slight^{50}) through USDA low Choice, and approximately one third (n = 51) were from upper 2/3 USDA Choice and higher (marbling score $\geq$ Modest^{00}). Carcasses selected from harvest facility two consisted of half (n = 25) from cattle of unknown pre-harvest diet and half (n = 25) from cattle known (through plant records) to have been supplemented with Zilpaterol hydrochloride antemortem. Carcasses derived from cattle supplemented with Zilpaterol hydrochloride were noted within the group.

A boneless portion (7.62 cm in width) of strip loin immediately posterior to the $12^{th}/13^{th}$ rib interface was dissected from the right side of carcasses in two of three facilities. Management at the third facility dictated an equal sample size (3.8 cm in width) be dissected from both the right and left side of carcasses. In all facilities, the portions removed from the right half of the carcass were used for imaging under the Tenera Technology High Resolution Imaging System such that the surface reflecting the exposed ribeye at the $12^{th}/13^{th}$ rib interface was evaluated.

The Tenera Technology High Resolution Imaging System captured images at four locations (A, B, C, D) on each sample (**Figure 2.4**). Calibration was achieved before each sample was imaged via photographs taken of the calibration tool at the bottom left corner of the imaging tray. Samples were placed in the imaging tray (**Figure 2.4**) and the coordinates of position A (**Figure 2.4**) were manually entered into the ZARMT program. Based on the coordinates of position A, the ZARMT software automatically controlled movement to locations B, C and D. Images were captured at International Organization for Standardization (ISO) of 100, 200, and 400 (corresponding to the measure that would be film speed or the sensitivity of the image processor to light) as well as apertures of 2.8 and 5.6 for a total of at least 5 images at each location. Imaging within the medial portion of samples was avoided as the pennate orientation of muscle fibers within this region made image collection extremely difficult.

Lean color measurements (L*, a* and b*) were obtained from the imaged surface following a bloom time of approximately one hour; this time was used to assure stable lean color as documented by Wulf and Wise (1999). Color measurements were obtained using a portable spectrophotometer with a port size of 1.27 cm and a D-65 illuminant,

calibrated using a black and white tile (Miniscan XE Model 45/0-L, Hunter Laboratories, Reston, VA). Final color values were means calculated from three measurements obtained at three different locations (free of intramuscular fat) on the steak lean surface.

Samples were transported in vacuum package bags on ice packs within coolers to the Colorado State University Meat Laboratory where they were fabricated into equal portions, approximately 3.8 cm in thickness. Samples obtained from the third facility required no further fabrication as they were already approximately 3.8 cm in thickness. Samples were vacuum packaged and assigned to a 7d or 14d aging period at 2^{o}C. All 14d samples were removed from the surface that was imaged using the Tenera Technology system. Following aging, samples subsequently were frozen (-20^{o}C). Frozen samples were fabricated into steaks (2.54-cm thick) and trimmed to approximately 0.32 cm external fat using a band saw (Model 400, AEW-Thurne, AEW Engineering Co. Ltd, Norwich, England).

Warner-Bratzler Shear Force (WBSF) Determination

Steaks for WBSF were thawed at 2^{o}C for approximately 36h immediately following fabrication. This produced a raw internal temperature between 0 and 4^{o} C before cooking. Steaks were cooked on a belt grill (model TBG-60, MagiGrill, MagiKitch'n Inc., Quakertown, PA) for 6m, 28s at a setting of 163^{o}C for the top and bottom heating plate, to a target peak internal temperature of 71^{o}C. Peak internal temperature was measured at the approximate geometric center of each steak utilizing a Type K thermocouple (model 340, Cooper-Atkins Corporation, Middlefield, CT). Following cooking, steaks were permitted to equilibrate to room temperature (22^{o}C).

Cores (1 or 2, 1.27 cm in diameter) were removed from 6 locations on each steak in a manner such that each core was parallel to the orientation of muscle fibers. These locations were approximately the same regions from which images were obtained during sample collection. Each core was identified by its location of origin and sheared perpendicular to the muscle fiber orientation using a universal testing machine (model 4443, Instron Corp., Canton, MA) equipped with a Warner-Bratzler shear attachment. Crosshead speed was set at 200 mm/m. Peak shear force of each core was recorded.

Image Analysis

The analysis of raw images from the ZARMT program was conducted as a multi-step procedure that ultimately converted the two dimensional images captured within the processing facility to a one dimensional waveform from which variables could be extracted. This conversion was commercially convenient and possibly required with conventional computer software and hardware for a number of reasons, including:

1. The amount of processing that would be required is 10 to 100 times larger with a two-dimensional array of pixels compared with a one-dimensional array of pixels.

2. One-dimensional features can be collected by a variety of means, including non-imaging methods such as laser scanners, which would lead to a much less costly and complicated data acquisition methodology.

3. Standardization across a broad range of biological variability in fiber orientation, topographical variability of the steak surface, and variation in

non-muscle components within images (i.e., fat) was more easily facilitated by one- rather than two-dimensional data.

A flow chart depicting image analysis is presented in **Figure 2.5** and explained below.

Separate into RGB values – The original images (**Figure 2.6**) were broken into their RGB information, and it was determined that the red information accounted for the majority of information in the images obtained (this was done using Matlab by Mathworks, Natick, MA). Part of the reason for this is that the images were purposefully underexposed so as not to saturate the red values, leading to very low values in the blue and green planes (the steak surface is almost exactly at the red hue).

Take red plane – The information from the red plane was converted into a separate image, which was essentially then a grayscale image.

Median filter – The camera system employed had a large electronic noise, due both to the intrinsic nature of the electronics, as well as the low level of illumination. This has the appearance in the image of a salt-and-pepper effect. A median filter with a dimension of 15 pixels was employed, using Paint Shop Pro (Corel, Ottawa, Canada). This chooses the median value, within a box 15 pixels on a side, that is preferable to a blur or mean filter (or a Gaussian filter) in that it far better reduces extreme values, and is often employed for this purpose. The size of a median filter, it should be noted, is frequently larger than that of a mean filter, and behaves better over larger distances.

Manual/automated estimate of fat – This took place in two steps. In the first step, large portions of intramuscular fat pixels were removed from the image by hand in Paint Shop Pro (**Figure 2.7**), and then for the remaining primarily lean meat portions, the median value was calculated and a series of images were created that had removed pixels that were more than a specified percentage from the median value (8, 12, 16, 20, and 24%). In addition, to ensure that regions adjacent to the fat also were removed, an erosion of 21 pixels from the black pixels was performed. It should be noted that this methodology also removes two other artifacts of the images: areas of glare and unusual intensity, as well as deep shadows. Because the erosion gives significant artifacts in the lean meat portions, this is unsuitable for image analysis, but serves well to eliminate regions of fat, glare and shadow.

Lean meat image – The final lean meat image (**Figure 2.8**) was obtained by taking only those pixels from the lean meat image obtained at the conclusion of the median filter that were not determined to be fat, glare or shadow in the manual/automated estimate of fat of the previous step.

Convert into 1D waveform – The images were converted into 1-D waveforms (**Figure 2.8**) by taking every 6^{th} row in the image of the previous step. It should be noted that the analysis as described below was also performed on every 6^{th} column, but gave information that was not apparently different. The waveform is presented as RGB value plotted against the number of pixels or samples in a given row.

Extract features – A large number of different features were extracted from the waveform, and generally were of two types. The first type of analysis was a discrete analysis of peaks and troughs, with particular attention to the distances between these. A second type of analysis was a Fourier transform analysis, which transforms the data from amplitude/distance to amplitude/frequency. Examples of a number of the discrete analyses include:

- diaSml - The diameter fraction of the data that was in the range of 40 to 60 microns.
- propSml -The proportion of the area fraction of the data (diameters giving rise to area) that was in the range of 40-60 microns.
    - The previous two variables refer to measures of the "small" muscle fibers, quite likely indicative of red fibers.
- diaLrg - The diameter fraction of the data that was in the range of 65 to 90 microns.
- propLrg -The proportion of the area fraction of the data (diameters giving rise to area) that was in the range of 65-90 microns.
    - The previous two variables refer to the measures of the "large" muscle fibers, quite likely indicative of white or intermediate fibers.
- ratDia - the ratio of diaSml to diaLrg.
- ratProp - the ratio of propSml to propLrg.

- medDia - the median length in the range of 40 to 90 microns.
- medProp - the median area in the range of diameters 40 to 90 microns.
- diaNormMax/propNormMax - the 10th percentile at each diameter histogram position was obtained, resulting in a baseline curve. This curve was subtracted from the histogram to give the excursions from the baseline. It was expected that this would highlight regions of the histogram that were more divergent. The position of the maximum excursion was recorded, and was adjusted in the case of areasNormMax for the area versus the linear dimension.
    - Simply put, the previous two variables were outputted at whole numbers which corresponded to given ranges in muscle fiber diameters and areas. Ultimately, these variables identify the predominant range of muscle fiber diameter and area. Lower numeric values of these variables are indicative of more predominant number of fibers falling into smaller classifications of fiber diameter and area, potentially being beneficial to beef tenderness.

Statistical analysis

Statistical analyses were performed using SAS (SAS Institute Inc., Cary, NC). Data sets were analyzed by individual harvest facility to avoid confounding issues of equipment malfunction between collection locations. Summary statistics (mean, standard

deviation and range) were computed using the MEANS procedure. PROC T-TEST was used to compare the effect of processing facilities and Zilpaterol supplementation on all variables. Alpha was set at 0.05. Correlation coefficients were determined utilizing the PROC CORR procedure. Outliers (n = 6) were removed from the data set as determined by Cook's D-value (D > 0.5). This statistic determines the effect of single points on the regression line, with larger values indicating greater effects. For exploratory purposes forward, backward, stepwise, lowest C_p, and highest adjusted R^2 model selections were performed using PROC REG. Significance level for entry into, or exit from, the model was set at $\alpha = 0.10$. Ultimately, lowest C_p was used for model selection, with some models including variables that were indicated through alternative selection methods as having strong predictive capabilities. Homogeneity of error variance for WBSF models was checked by plotting residuals versus actual WBSF value. Interaction variables were included in the model to allow the effect of some predictors (marbling, a*, b* diaSml, propSml) to depend on whether or not Zilpaterol hydrochloride was supplemented. The DISCRIM procedure was used to estimate the percentage of samples that could be correctly classified by the models as "tough" versus "tender." Cross-validation of error estimates were used to insure points were not used to predict themselves.

Results and Discussion

Means and standard deviations (**SD**) for marbling score (**MS**), CIE L* (higher the value, lighter the color), CIE a* (higher the value, redder the color), CIE b* (higher the value, more yellow the color), diaSml, propSml, diaLrg, propLrg, ratDia, ratProp, medDia, medProp, diaNormMax, propNormMax, average 14d WBSF and average 7d

WBSF by harvest facility are presented in **Tables 3.1 to 3.3**. Variation in marbling score was uniform as a result of the selection criterion that was utilized during sample collection. L*, a* and b* measurements were highly variable throughout the experiment, which may be a result of differences in pre- and post-harvest management strategies employed at each facility from which samples were collected, or as a result of unavoidable equipment malfunction during data collection. Mean values for L* differed ($P < 0.05$) by plant (facility one = 7.2, facility two = 41.6, facility three = 36.5, root mean square error (RMSE) = 2.14). The abnormally low values at facility three may have been due to equipment malfunction that was unavoidable and irreparable during data collection. Data was analyzed separately by harvest facility to insure equipment malfunction did not skew algorithm development by artificially creating significant differences in color measurements between locations.

diaSml, propSml, diaLrg, propLrg, ratDia, ratProp, medDia, medProp, diaNormMax, propNormMax were all generated using the image analysis software from Tenera Technology. These traits were low in variability, which was not ideal for algorithm development. However, this may be indicative of a very uniform sample population in terms of WBSF as approximately 10% of samples collected could be categorized as "tough" (WBSF > 4.4 kg; Platter, et al., 2003) at the 14d aging period. For purposes of algorithm development, additional steaks with WBSF values over 3.7 kg were also classified as "tough". According to Platter et al. (2003), a WBSF value of 3.7 kg represents the mark at which approximately two-thirds of consumer will deem a steak acceptable in terms of tenderness.

Simple correlation coefficients (R^2) between all variables and 14d and 7d WBSF by harvest facility are presented in **Table 3.4**. Half of the samples (n = 25) from harvest facility two were obtained from the carcasses of cattle that were known (through plant records) to have been supplemented with Zilpaterol hydrochloride prior to harvest. Zilpaterol hydrochloride supplementation had a significant ($P < 0.05$) effect on marbling, a*, b*, diaSml and propSml within those carcasses sampled from facility two. Consequently, interactions terms for these five traits were allowed to enter equations during model selection.

The correlation coefficient between marbling and 7d and 14d WBSF across all facilities was negative ($P < 0.05$). Other workers also have found that increased marbling is associated with decreased WBSF (Vote et al., 2003; Smith et al., 1984). Correlation coefficients between all color measurements and 14d WBSF were negative and significant ($P < 0.05$) within harvest facility 1. Within harvest facility two, a* and b* showed a significant negative relationship with 7d and 14d WBSF values. These findings are in agreement with those of Wulf et al. (1997) and Vote et al. (2003). All color measurements within harvest facility three were negative and non-significantly associated with 7d and 14d WBSF values.

Contrasting the findings of Wulf et al. (1997) and Vote et al. (2003), color measurements for L* within harvest facility two showed a positive, but non-significant correlation with 7d and 14d WBSF. This may be due to the presence of samples obtained from carcasses of cattle supplemented with Zilpaterol hydrochloride. If Zilpaterol hydrochloride causes an increase in white muscle fiber content, lean color may appear "brighter" (higher L*), however given the positive relationship of white muscle fiber

content with WBSF (Calkins et al., 1981) this brighter color may not be indicative of increased tenderness as in previous studies (Wulf et al., 1997; Wulf and Page, 2000).

Simple correlation coefficients between variables generated by the Tenera Technology software (diaSml, propSml, diaLrg, propLrg, ratDia, ratProp, medDia, medProp, diaNormMax, propNormMax) and visible color measurements (L*, a*, b*) are displayed in **Table 3.3**. The general lack of association between most Tenera Technology variables with L*, a* and b* measurements ($P > 0.05$) indicated that the traits generated by the software program were indeed unique attributes that have not been associated (through color measurement) with WBSF previously. This is essential to the merit of the technology as it indicates potential for an enhanced tenderness prediction model utilizing color measurement and variables such as those measured by the Tenera Technology High Resolution Imaging System.

Model selection was performed on data from each plant individually utilizing forward, backward, stepwise and maximum R^2 approaches for exploratory purposes. The lowest C_p method was used for final model selection. Model R^2, C_p, root mean squared-error (RMSE), partial R^2, and beta-coefficients for variables entered into the prediction model are displayed in **Table 3.4**. Variables are listed in order of significance for entry into the model. In all instances, addition of high resolution variables resulted in some improvement in prediction of WBSF, while decreasing the RMSE of the model.

Figures 3.1 to 3.6 display actual versus predicted WBSF values for 14d and 7d aged steaks obtained from each harvest facility. In steaks aged 14d from harvest facility one, the use of high resolution variables in conjunction with marbling and objective color measurements explained an additional 11% of variation in WBSF ($R^2 = 0.42$ versus 0.31)

over use of marbling an color variables alone. In predicting WBSF of 7d aged steaks from harvest facility one, the prediction equation enhanced with high resolution variables explained an additional 8% of the variation in WBSF (R^2 = 0.36 versus 0.28). Similarly, high resolution variables in conjunction with marbling and color measurements were able to explain an additional 25% of the variation in WBSF (R^2 = 0.66 versus 0.42) in 14d aged steaks from harvest facility two. In prediction of WBSF in steaks aged 7d from harvest facility two, high resolution variables allowed for the additional explanation of 14% of the variation in WBSF (R^2 = 0.55 versus 0.41). In steaks obtained from harvest facility three, marbling and color variables were only able to explain 9% of the variation in WBSF at both 14d and 7d postmortem. Use of high resolution variables allowed for explanation of an additional 17% of the variation in WBSF (R^2 = 0.26 versus 0.09) 14d postmortem and 34% of the variation in WBSF (R^2 = 0.43 versus 0.09) 7d postmortem.

All models containing marbling as a predictor of WBSF, showed a negative β-coefficients for marbling score. β-coefficients for all measures of color were negative, except within facility two, which included samples obtained from carcasses of cattle supplemented with Zilpaterol hydrochloride. A significant interaction between Zilpaterol supplementation and a* measurements yielded a negative β-coefficient. These findings may be indicative that samples with a greater proportion of red muscle fibers (as indicated by higher a* values) may be more tender, particularly in cattle supplemented with Zilpaterol hydrochloride. The interaction between b* and Zilpaterol hydrochloride supplementation was significantly positive, accounting for 15% of the variation in WBSF within 14d aged samples obtained from facility two. These findings contradict those of Wulf et al. (1997) and Vote et al. (2003), however may indicate that as muscle fibers are

transformed from the small, red oxidative fibers to large, white, glycolytic fiber types through the use of beta-adrenergic agonists, color measurements may be effected, specifically, by increasing a* and b* values. If increased white fiber content is the cause of higher b* values, this may not be associated with increased tenderness as has been reported in previous work (Wulf et al., 1997; Wulf and Page, 2000; Vote et al., 2003).

High resolution variables that were able to explain the greatest proportion of WBSF within regression equations differed by harvest facility. In samples obtained from harvest facility one, the ratDia and ratProp variables explained 8% and 7% of WBSF at 14d and 7d respectively. Both variables had a non-significant negative correlation with 14d and 7d WBSF. This is reasonable to assume given the association of increasing fiber diameter with decreasing tenderness. Moreover, if a greater proportion of smaller diameter fibers were present within a muscle, based on the findings of multiple workers (Hiner et al., 1953; Tuma et al., 1962; Herring et al., 1965; Cooper et al., 1968; Seideman et al., 1988) WBSF values could be expected to decrease. The propLrg and diaSml variables also entered into prediction equations for 7d and 14d WBSF within harvest facility one, however both variables explain 3% or less of the variation in WBSF within the sample population. diaSml showed a negative correlation with 7d and 14d WBSF values, which is expected given the documented relationship between decreased fiber size and decreased WBSF values (Hiner et al., 1953; Tuma et al., 1962; Herring et al., 1965; Cooper et al., 1968; Seideman et al., 1988). However, propLrg also showed a negative relationship with 7d and 14d WBSF which contrasts the findings of previous authors who associated increased proportions of large muscle fibers with decreasing tenderness.

The equation developed to predict 14d WBSF of steaks from harvest facility two included interaction terms between Zilpaterol hydrochloride and diaSml as well as b*, which explained 28% and 8% of the variation in WBSF respectively. diaSml also entered the prediction equation without interaction, however explained much less (3%) of the variation in WBSF values. Interestingly, across all facilities, diaSml and propSml showed non-significant positive correlations with 7d and 14d WBSF. This is contradictory to the theory that increased numbers of small fibers could produce increased tenderness and decreased WBSF values (Hiner et al., 1953; Tuma et al., 1962; Herring et al., 1965; Cooper et al., 1968; Seideman et al., 1988). Explanation for this may include the range established for "small" muscle fiber diameters in the present study was such that measurements may have encompassed some portion of the white and intermediate muscle fiber population (Cooper et al.,1966). Although the aforementioned study did not directly involve the measurement of diameter specific to fiber types, these workers did find an increase in the range of muscle fiber diameter of only approximately 5 microns between cattle of A and C maturity. This increase showed cattle of C maturity to have an approximate average fiber diameter of 55 microns. Assuming that this increase in maturity also corresponded with increases in white fiber content, as documented previously by the work of Ashmore et al. (1972), the hypothesis may be made that the range for "small" fibers of 40-60 microns used in the current study, was not fully sufficient to differentiate all white and intermediate fibers from red. Without full differentiation of diameters indicative of the different muscle fiber types present, the capability of this technology to most effectively predict tenderness differences may have been impaired.

The equation developed to predict 7d WBSF of steaks from harvest facility two used the marbling variable to explain the vast majority (36%) of the variation in WBSF. At both facility two and three, marbling was able to explain a much greater proportion of the variation in WBSF at 7d than at 14d. However, through the use of high resolution variables (specifically medDia, medProp and diaNormMax) an additional 14% of the variation in 7d WBSF in steaks from harvest facility three was accounted for. medDia and medProp both showed a non-significant, negative correlation with WBSF at 7d; this is logical based on the theory that as median fiber diameter decreases, WBSF should also decrease. Additionally, with decreases in the proportion of large fibers, previous workers have also shown decreases in WBSF values (Seideman et al., 1988).

Samples from harvest facility three aged 14d postmortem were unique in expressing the only prediction equation not using marbling as a predictor for WBSF. This may be due to an incredibly narrow range in WBSF values (2.3 to 4.8 kg), with only 2 of 50 steaks shearing over 4.4 kg, and an additional 5 steaks shearing over 3.7 kg. Consequently, with limited variation in WBSF value, algorithm development was very difficult. The medProp variable explained the greatest portion (19%) of variation in WBSF, with L*, a* and ratDia accounting for another 3%, 3% and 1% respectively. Of the six equations developed in the current study, the 14d prediction equation for WBSF of steaks from facility three expressed the largest C_p value, indicating the greatest bias as a result of omitting terms in the prediction of WBSF.

The majority of variation in WBSF within 7d aged samples from facility three was explained by the medProp and propNormMax variables, both accounting for 15% (30% combined) of variation in WBSF values. propNormMax and diaNormMax

expressed the most variation (**SD** = 1.6 to 2.1) of any of the high resolution variables generated by the Tenera Technology system. Both variables were also the most highly correlated with WBSF at both 7d and 14d postmortem. However, this relationship was negative in the case of harvest facility one, strongly positive in the case of harvest facility two, and mixed (positive and negative) in the case of harvest facility three. Theoretically, lower values of these two variables indicate an abundance of smaller fibers within the lean surface, which should theoretically result in lower WBSF values. This concept validates entry of propNormMax into the 7d prediction equation with a positive correlation and positive β-coefficient. Conflicts in the relationship elsewhere in the dataset may be due to more uniform distribution of fiber diameters within other samples as the variables (diaNormMax and propNormMax) are based on protrusions in a histogram.

Determining the predictive capability of all equations generated for WBSF prediction, a shear force value of 3.7 kg was used to define "tough" as this maximized the number of "tough" samples within the dataset, and has been shown to be the critical level at which two-thirds of consumers will deem a steak acceptable in terms of tenderness (Platter et al., 2003a). The 14d equation generated for harvest facility one correctly classified 92.5% of "tender" steaks and 44.4% of "tough" steaks. Compared to the use of marbling and color variable alone, this represents an increase of 40% (92.5% versus 52.5%) in the correct classification of "tender" steaks, however this equation also was 10% less effective in classification of "tough" steaks (44.4% versus 54.4%). Within the 7d aged sample set from facility one, the use of high resolution variables correctly classified an additional 6% of "tender" steaks (62.5%) while not sacrificing any

classification capability of "tough" steaks (76.5%). Within 14d aged steaks from harvest facility two, the equation generated by this work correctly classified 55.2% of "tender" steaks and 68.4% of "tough" steaks. This represents a decrease of 3.4% in classification capabilities of tender steaks when compared to the use of marbling and color variables alone. However, within 7d aged steaks from harvest facility two, use of high resolution variables in conjunction with marbling and color measurements increased the classification capabilities for "tender" steaks by 14.3%. Marbling and color variables alone were able to correctly classify an additional 5.9% of "tough" steaks however. Use of high resolution variables in conjunction with objective color measurement improved classification of "tender" steaks by 7% (60.4% versus 67.4%) within 14d aged steaks from facility three. No advantage in classification of "tough" steaks was observed in the use of high resolution variables. Within steaks aged 7d from harvest facility three, use of high resolution variables improved classification of "tender" steaks by 7.1% (60.7% versus 53.6%) and classification of "tough" steaks by 9.1% (50% versus 59.1%).

Utilizing high resolution variables generated by the Tenera Technology software program, a small and additive increase in WBSF predictive capability resulted in most instances. The differences in the predictive ability of these variables within different harvest facilities from which samples were obtained may most simply have been due to variation, or lack thereof, in the range of WBSF values within samples from each harvest facility. Additionally, differences in muscle ultra-structure as a result of feeding Zilpaterol hydrochloride antemortem, as well as the effect this supplementation may have had on the postmortem proteolytic degradation of muscle proteins may have enhanced the ability of the technology to differentiate WBSF and postmortem tenderness following

aging. Steaks obtained from carcasses of cattle supplemented with Zilpaterol hydrochloride did prove exceptionally useful in the development of prediction equations in that these samples were substantially tougher ($P < 0.05$) and provided much greater variation in variables generated by the Tenera Technology system. This could be indicative that in a total population with a greater range in tenderness and ultra-structural characteristics, the Tenera Technology High Resolution Imaging System could be better proven.

Typical to any prototype bench-top device, there were numerous technical issues surrounding the initial use of the Tenera Technology High Resolution Imaging System. Most importantly, WBSF data for the sample population were such that there were few "tough" samples to allow for an ideal algorithm development to predict WBSF. Mechanical issues with illumination and image analysis resulted in less than ideal output from the image analysis software. Sample collection problems with regard to effects of pre- and post-harvest variables between locations could not be fully controlled. Finally, the limitations of collecting samples in a commercial setting utilizing a bench top device were less than ideal to the development of the equipment while in the field. Ultimately, the image produced attained from the Tenera Technology High Resolution Imaging System was not of high enough quality to allow for extraction of all variables which may be beneficial to enhanced prediction of beef tenderness. It is our conclusion that the technology does not exist at this time to use digital imaging within a commercial setting to measure all traits necessary to optimize tenderness prediction. However, the small additive gains that were demonstrated by this technology represent the need to fully

explore alternative methods that may be able to more effectively extract features on the ultra-structural level that are necessary for enhancing predictions of beef tenderness.

Implications

The Tenera Technology High Resolution Imaging System was able to produce variables that had an additive effect in prediction of beef tenderness when coupled with marbling and objective color measurements. These results indicate that those variables produced are measuring muscle qualities that have not previously been assessed by traditional measures of muscle color. With increased development of alternative technologies that may be able to more effectively measure these traits (and others) in a commercial setting, the usefulness of variables generated from ultra-structural measurements should be greatly improved. The discrepancies from previous research with regard to color measurements obtained in this experiment demonstrate the specific need for the Tenera Technology High Resolution Imaging System, and other systems that may measure equivalent variables. Without the ability to explain color variables through ultra-structural evaluation of muscle, current tenderness predictors will be substantially less effective.

Figure 2.1. The interrelationship between factors influencing muscle color and tenderness.

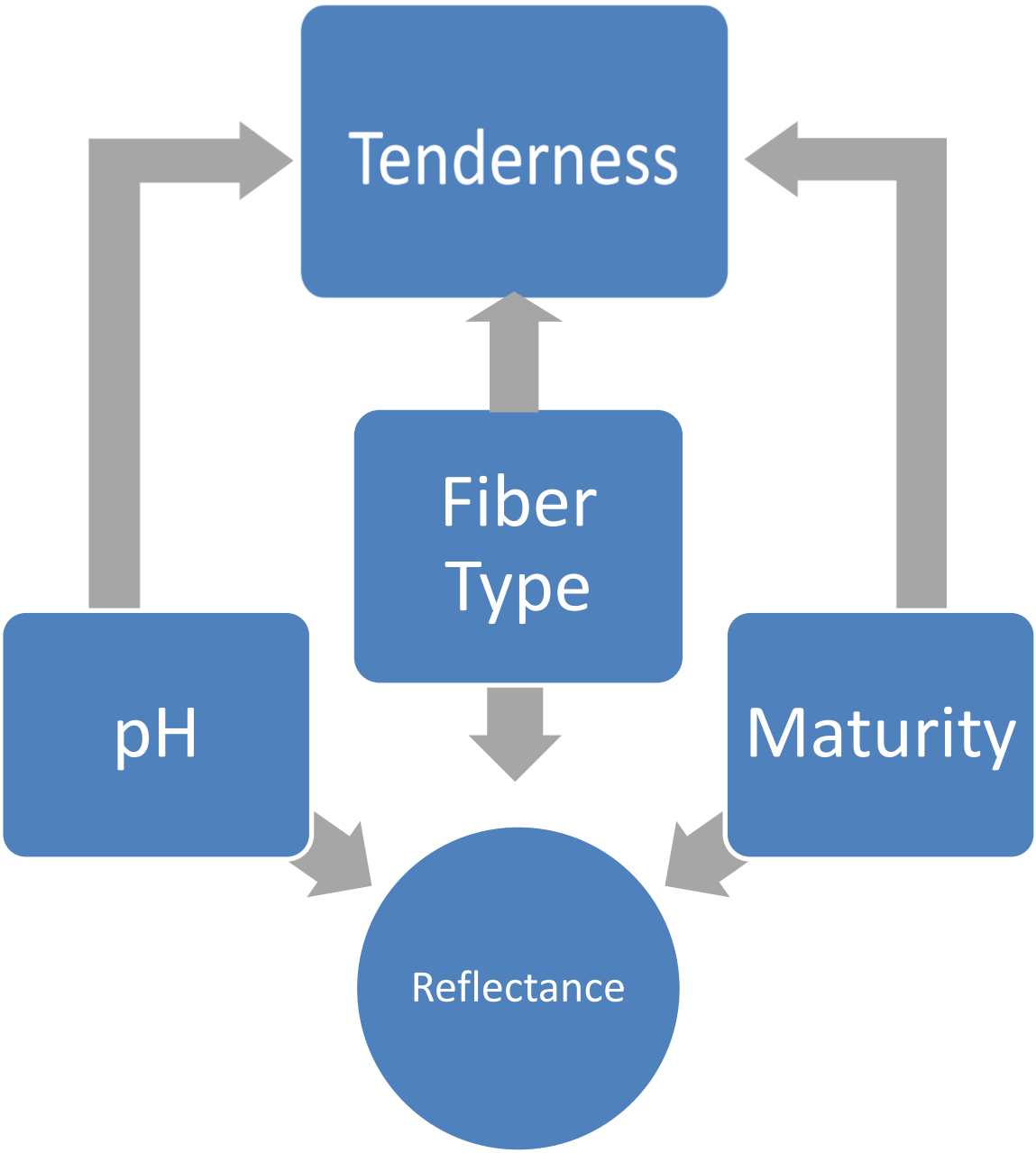

Figure 2.2. The ZenBot and camera apparatus.

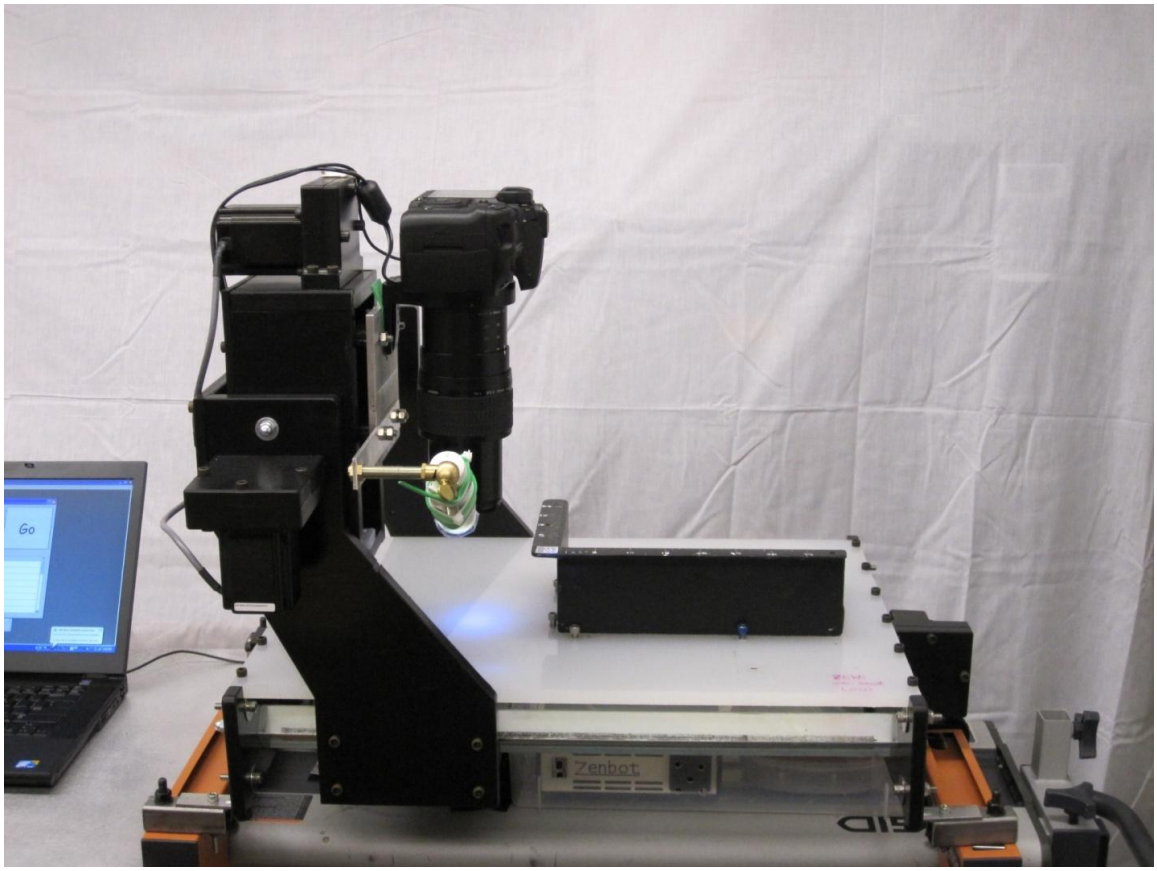

Figure 2.3. The ZenBot and camera apparatus.

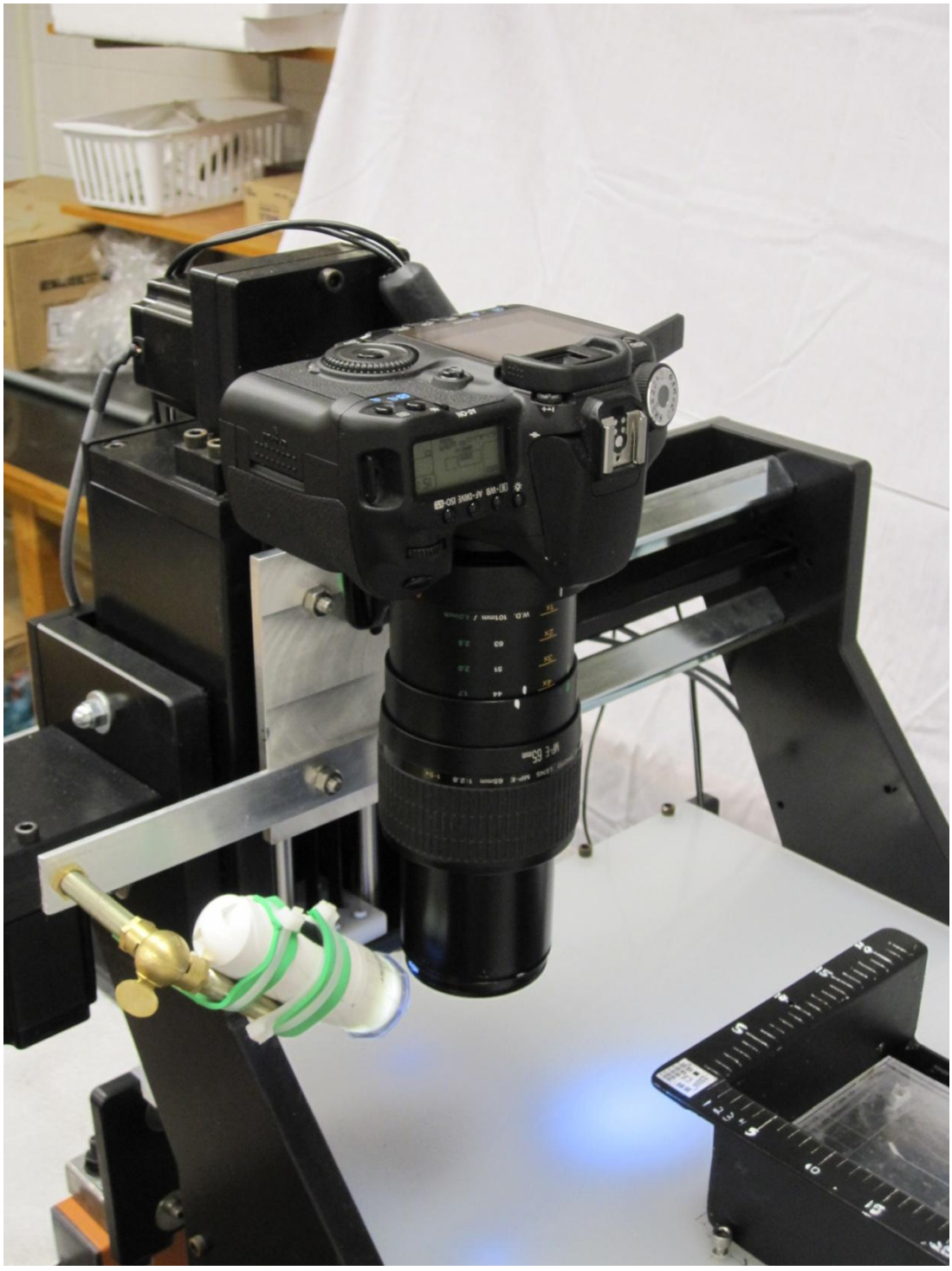

Figure 2.4. The imaging tray with sample, letters correspond to imaging locations.

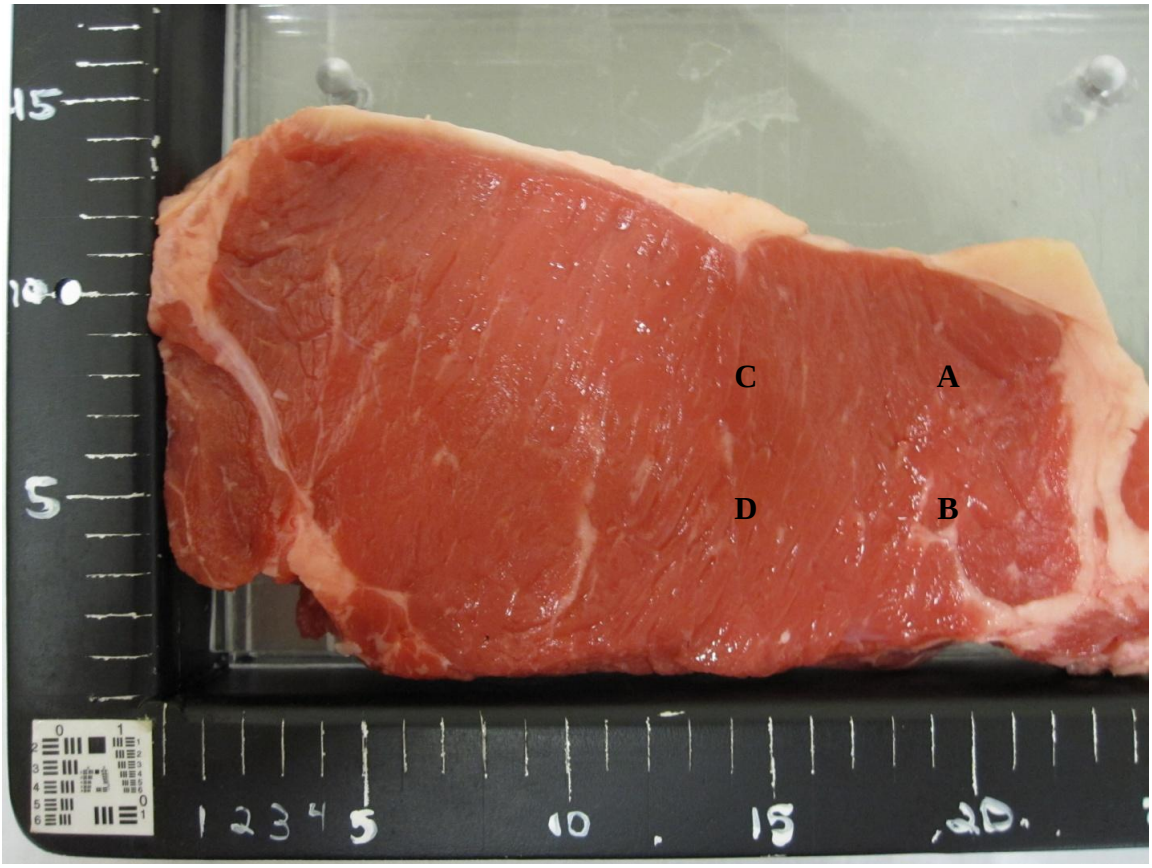

Figure 2.5. The flow chart for image analysis.

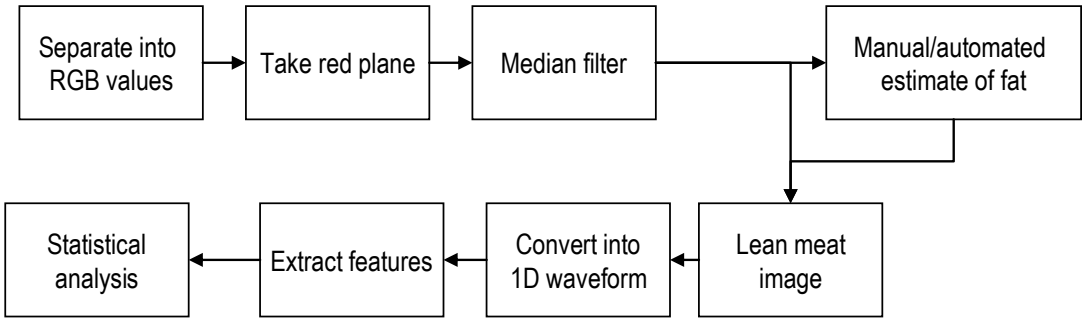

Figure 2.6. Original image obtained using Tenera Technology High Resolution Imaging System (Note the appearance of muscle fibers as “clouds” within bundles).

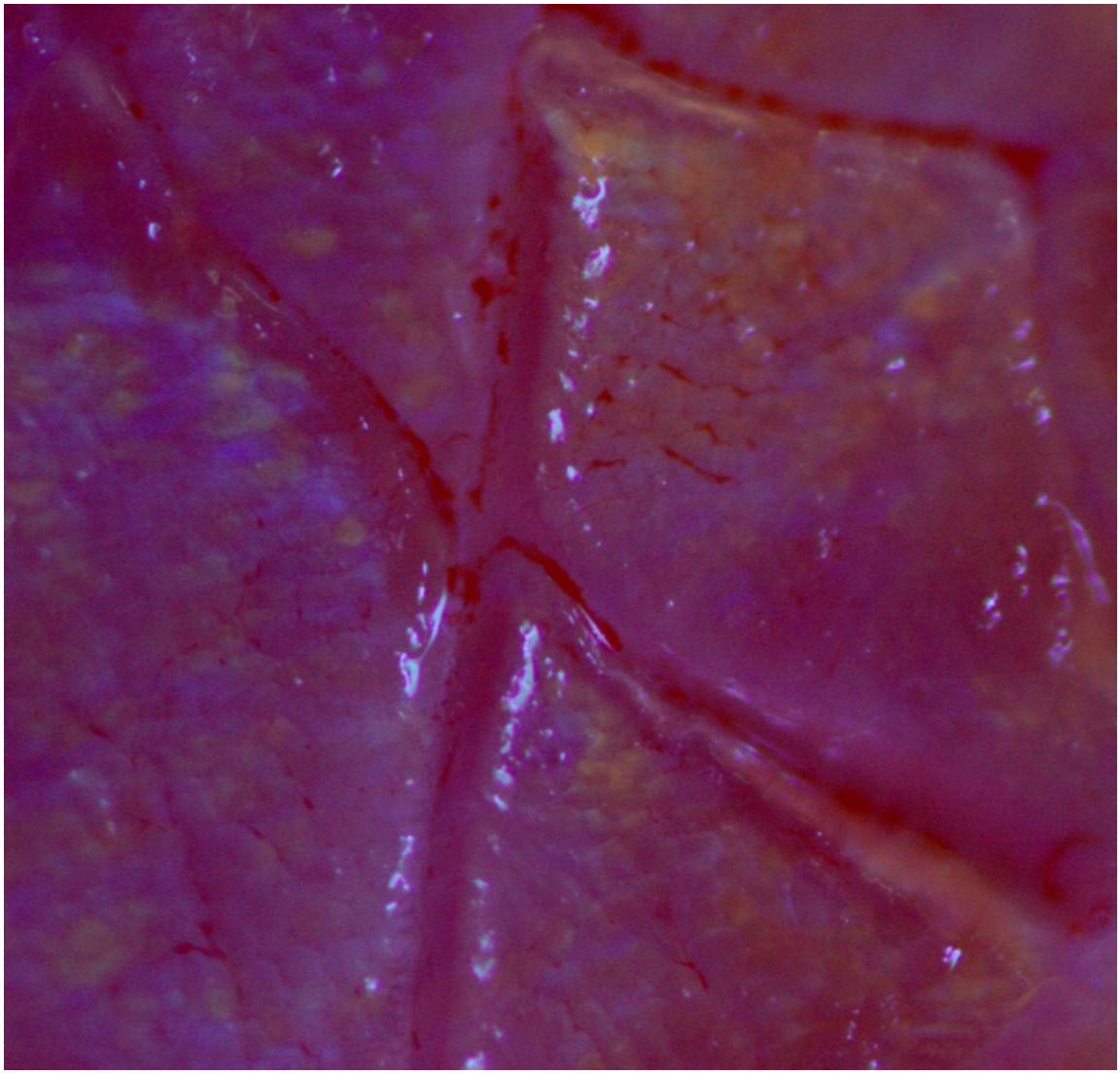

Figure 2.7. Image after manual fat removal, median filter and erosion.

Figure 2.8. Final image (merger of those pixels eliminated by removal of fat/median filter and the original image of lean meat).

Figure 2.9. 1-dimesional waveform.

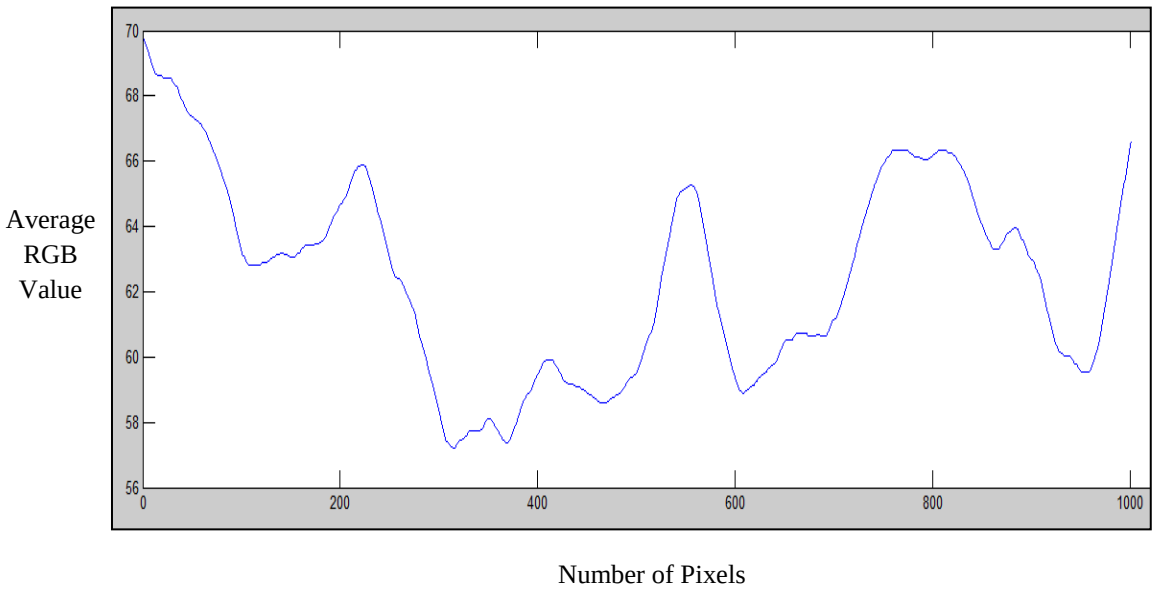

Figure 3.1. Actual versus predicted 14-day Warner-Bratzler shear force (WBSF) for harvest facility one.

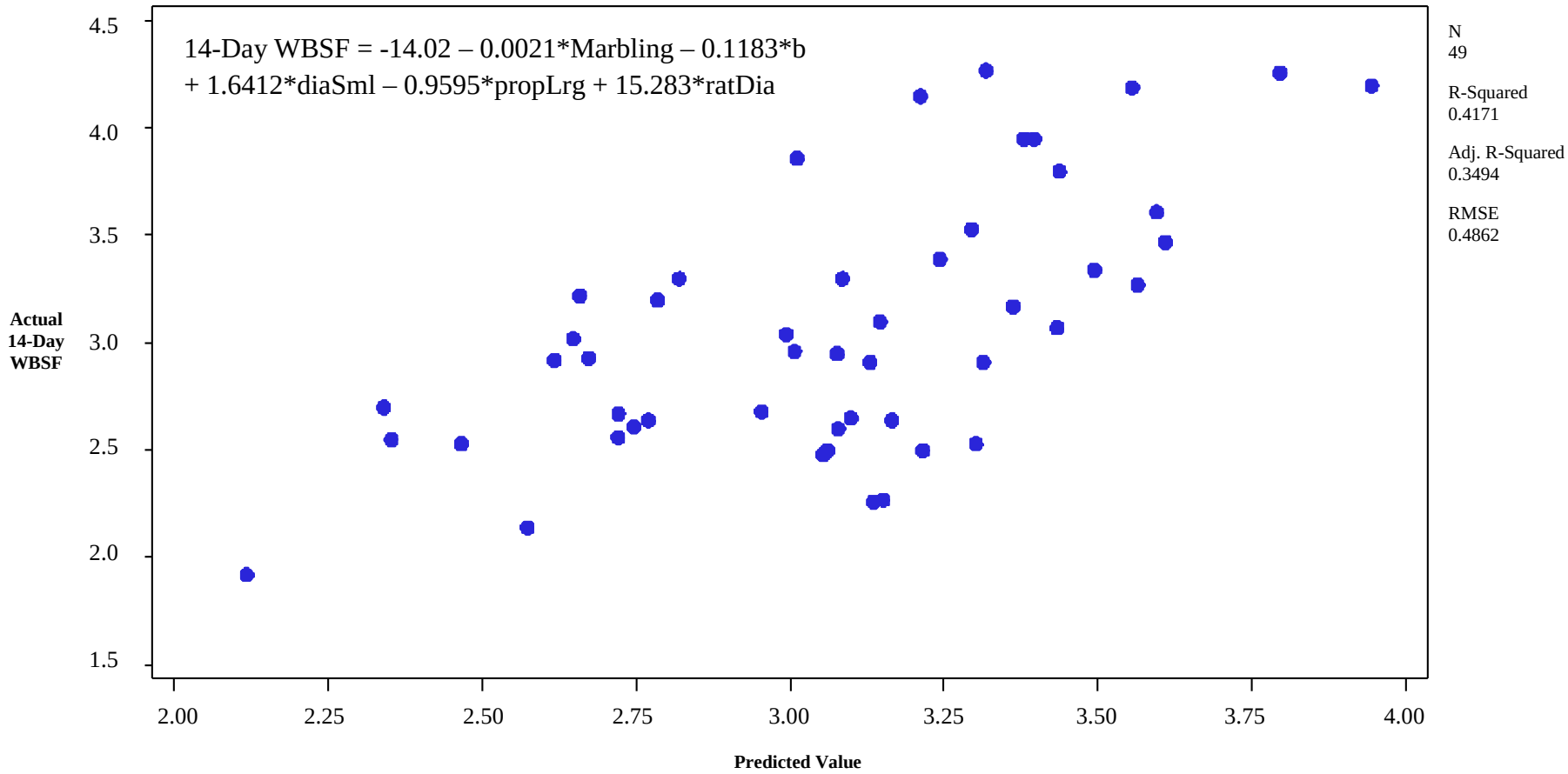

Figure 3.2. Actual versus predicted 14-day Warner-Bratzler shear force (WBSF) for harvest facility two.

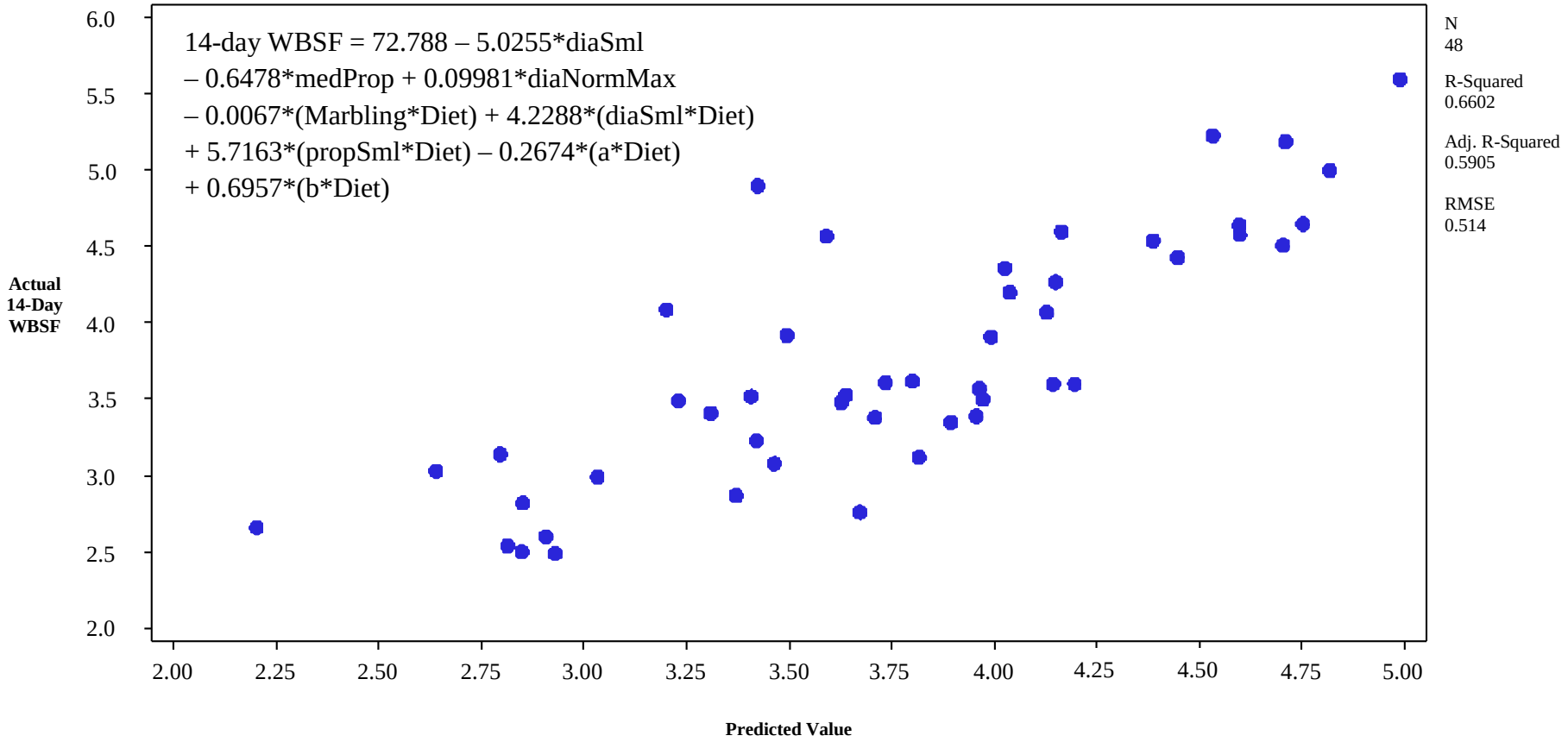

Figure 3.3. Actual versus predicted 14-day Warner-Bratzler shear force (WBSF) for harvest facility three.

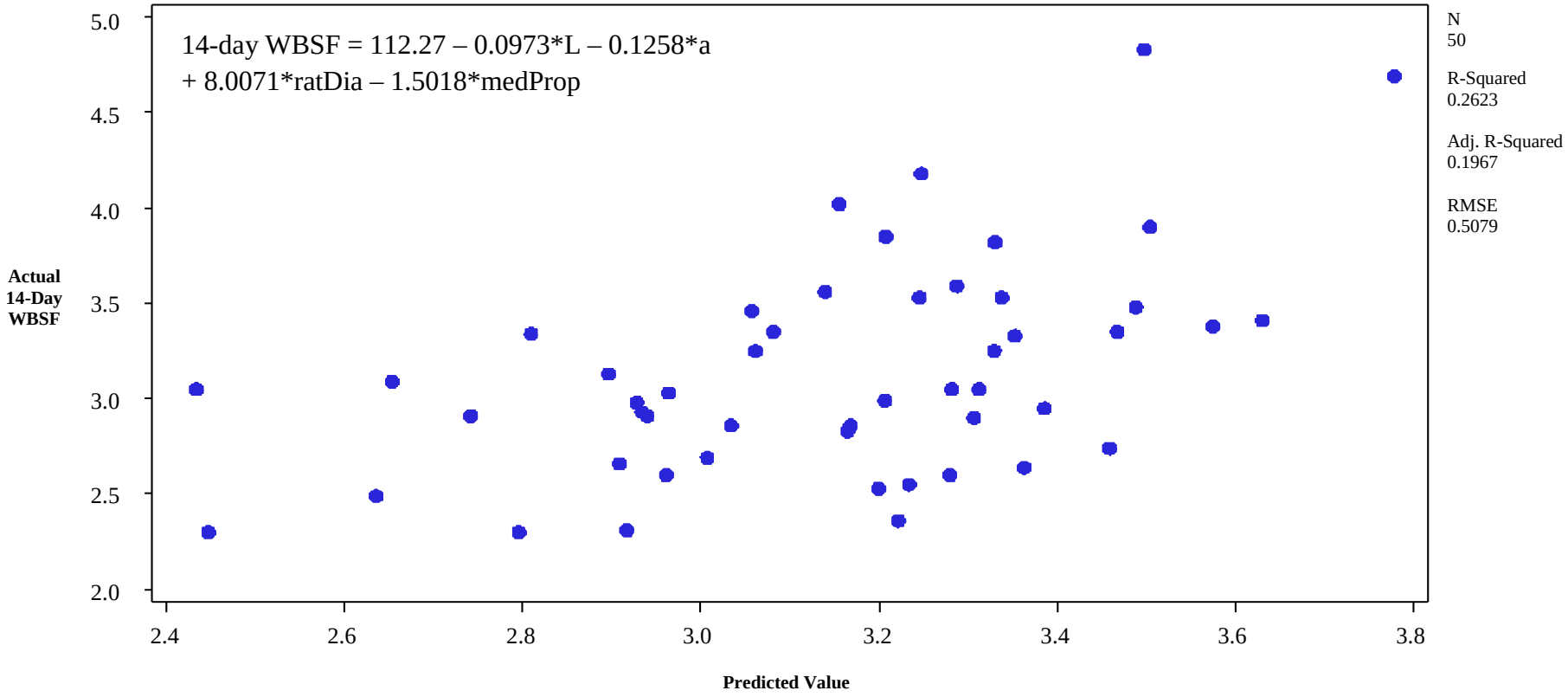

Figure 3.4. Actual versus predicted 7-day Warner-Bratzler shear force (WBSF) for harvest facility one.

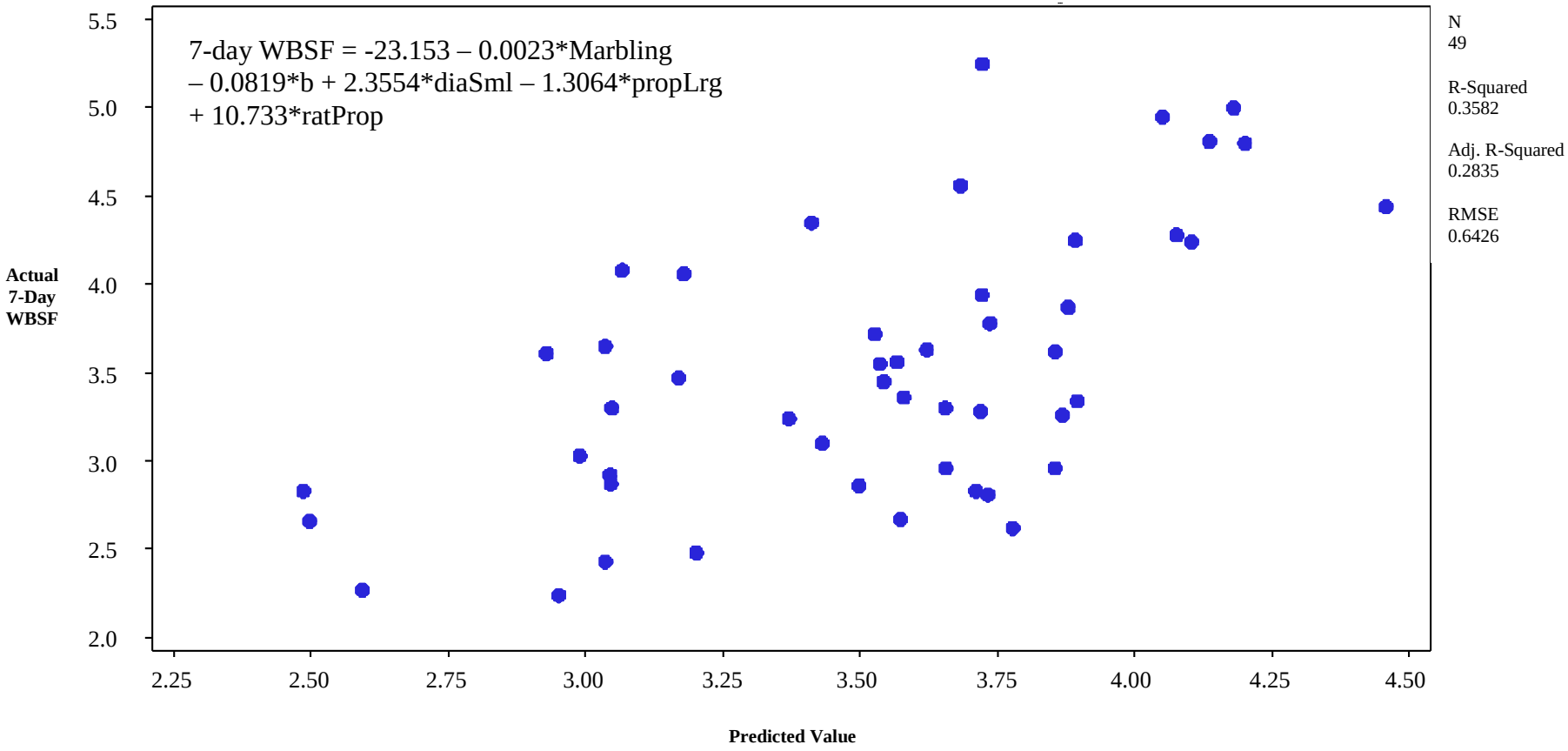

Figure 3.5. Actual versus predicted 14-day Warner-Bratzler shear force (WBSF) for harvest facility two.

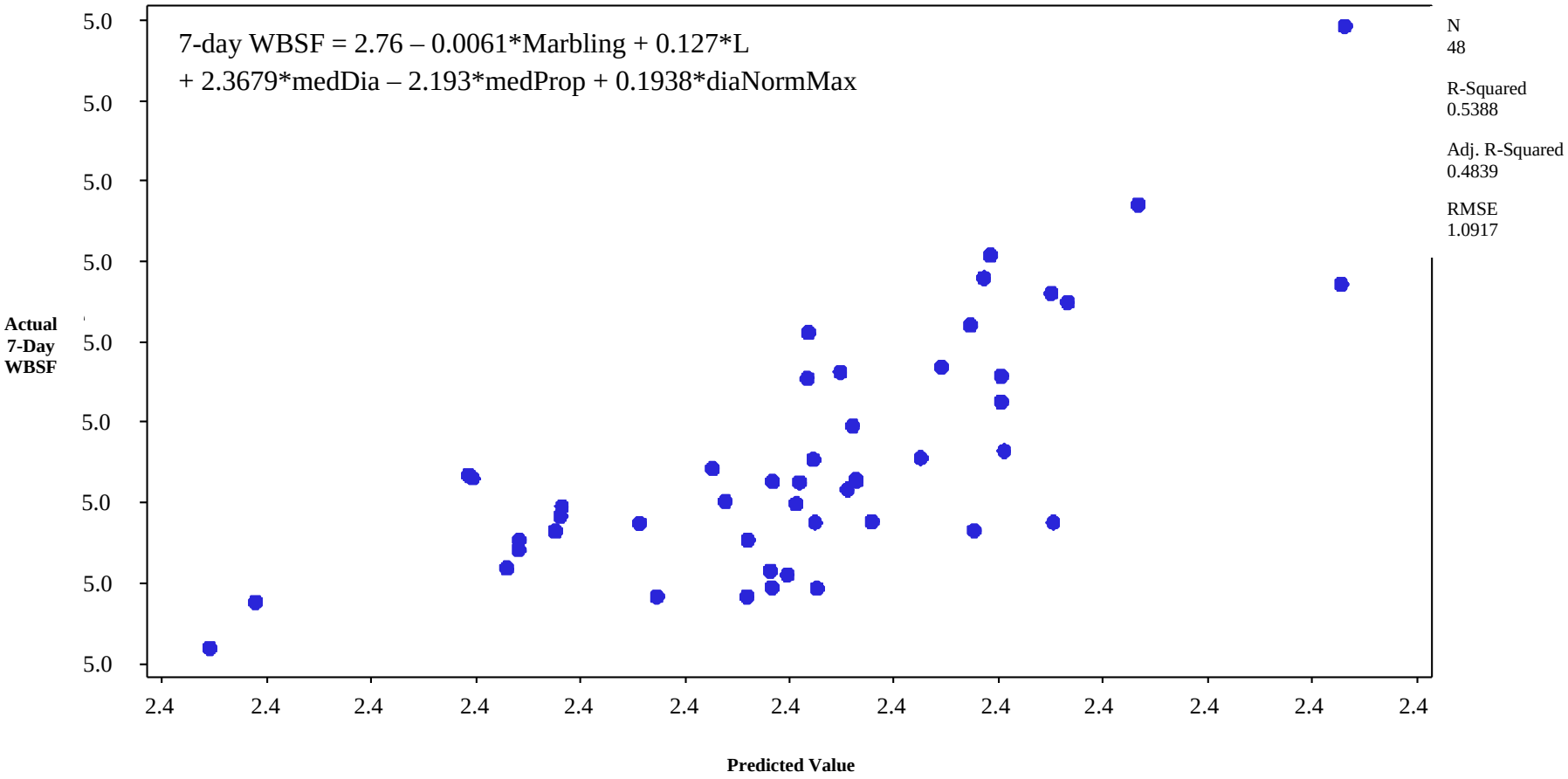

Figure 3.6. Actual versus predicted 7-day Warner-Bratzler shear force (WBSF) for harvest facility three.

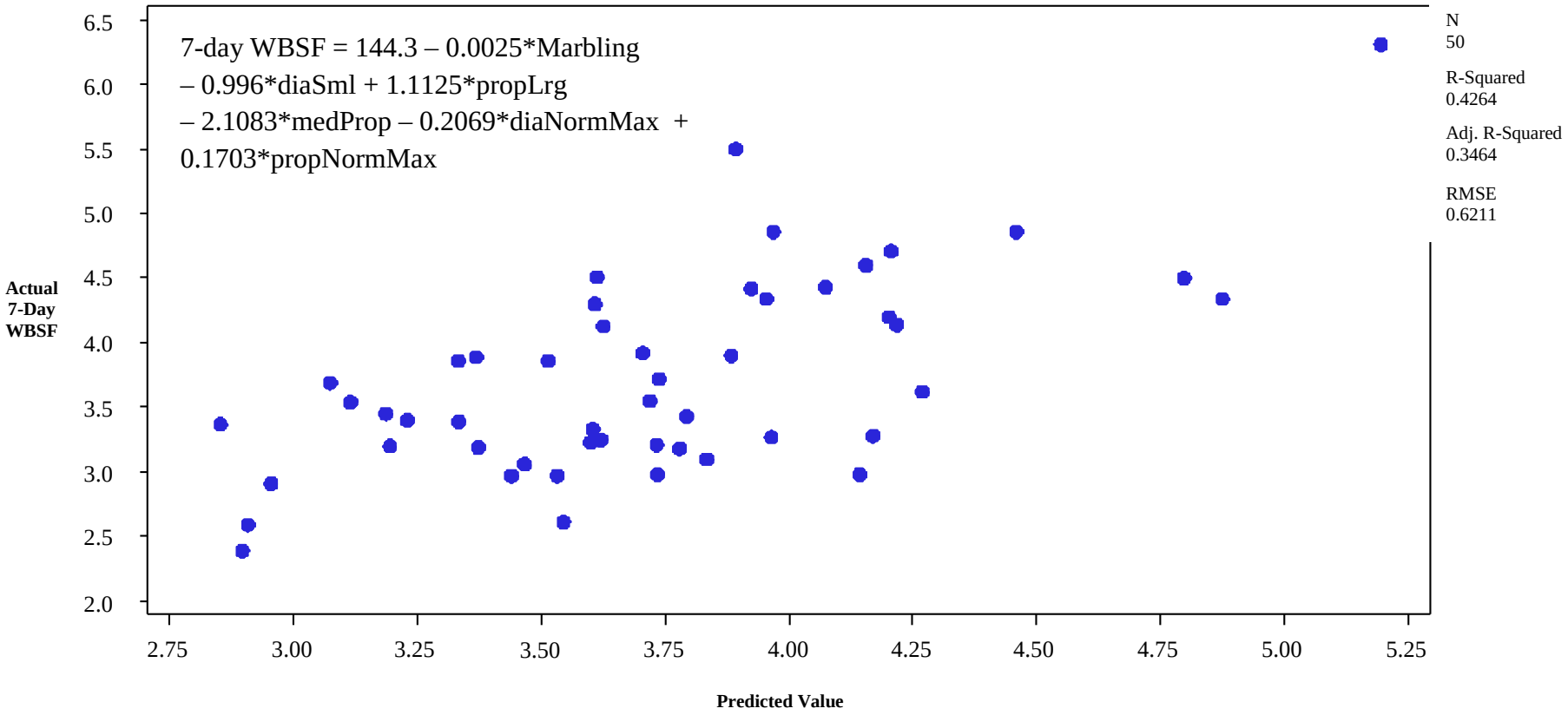

Table 3.1. Summary statistics of Plant One for marbling score, color measurements, output variables from the Tenera Technology-High Resolution Beef Imaging System and Warner-Bratzler shear force (WBSF) measurement

Trait[a]	n	Mean	SD	Minimum	Maximum
Marbling score[b]	50	441.8	144.9	270	800
L*	50	7.2	0.6	6.4	8.6
a*	50	10.6	1.9	6.6	14.3
b*	50	10.5	1.1	8.7	12.8
diaSml	50	19.9	0.9	18.1	21.4
propSml	50	12.4	0.8	10.9	13.8
diaLrg	50	34.9	1.1	32.8	37.1
propLrg	50	42.0	0.6	40.8	43.6
ratDia	50	1.8	0.1	1.5	2.0
ratProp	50	3.4	0.3	3.0	4.0
medDia	50	72.1	0.9	70.5	73.9
medProp	50	78.9	0.8	77.5	80.4
diaNormMax	50	9.0	2.1	5.0	12.0
propNormMax	50	10.5	1.7	5.0	12.0
14-WBSF, kg	50	3.1	0.6	1.9	4.3
7-WBSF, kg	50	4.5	0.8	2.2	5.3

[a]Marbling score = as determined by official USDA grader on A-maturity carcasses; CIE L* = 0 = black, 100 = white; CIE a* = -60 = green, 60 = red; CIE b* = -60 = blue, 60 = yellow; diaSml = diamter fraction of the data that was in the range of 40 to 60 microns; propSml = proportion fraction of the data (i.e., treating the lengths as diameters giving rise to area) that was in the range of 40-60 microns; diaLrg = diameter fraction of the data that was in the range of 65 to 90 microns; propLrg = proportion fraction of the data (i.e., treating the lengths as diameters giving rise to area) that was in the range of 65-90 microns; ratDia = the ratio of diaSml to diaLrg; ratProp = the ratio of propSml to propLrg; medDia = the median diameter in the range of 40-90 microns; medProp = the median proportion in the range of diameters 40-90 microns; diaNormMax/propNormMax = 10^{th} percentile at each diameter histogram position was obtained, resulting in a baseline curve. This curve was subtracted from the histogram to give the excursions from the baseline. The position of the maximum excursion was recorded, and was adjusted in the case of propNormMax for the area versus the linear dimension

[a]Marbling: 200=Traces^{00}, 300=Slight^{00}, 400=Small^{00}, 500=Modest^{00}, 600=Moderate^{00}, 700=Slightly Abundant^{00}.

Table 3.2. Summary statistics of Plant Two for marbling score, color measurements, output variables from the Tenera Technology-High Resolution Beef Imaging System and Warner-Bratzler shear force (WBSF) measurement

Trait[a]	n	Mean	SD	Minimum	Maximum
Marbling score[b]	50	467.9	157.0	280	850
L*	50	41.6	2.4	35.2	47.6
a*	50	7.5	2.6	2.8	14.8
b*	50	17.1	1.3	15.0	20.4
diaSml	50	20.2	0.8	17.8	21.7
propSml	50	12.6	0.7	10.7	13.9
diaLrg	50	34.7	0.9	33.2	37.3
propLrg	50	42.0	0.5	40.8	43.1
ratDia	50	1.7	0.1	1.5	2.1
ratProp	50	3.3	0.2	3.0	4.0
medDia	50	71.8	0.8	70.6	73.9
medProp	50	78.6	0.7	77.5	80.3
diaNormMax	50	8.8	1.6	5.0	12.0
propNormMax	50	9.8	1.6	5.0	12.0
14-WBSF, kg	50	3.8	0.8	1.9	4.3
7-WBSF, kg	50	4.6	1.5	2.2	5.3

[a]Marbling score = as determined by official USDA grader on A-maturity carcasses; CIE L* = 0 = black, 100 = white; CIE a* = -60 = green, 60 = red; CIE b* = -60 = blue, 60 = yellow; diaSml = diamter fraction of the data that was in the range of 40 to 60 microns; propSml = proportion fraction of the data (i.e., treating the lengths as diameters giving rise to area) that was in the range of 40-60 microns; diaLrg = diameter fraction of the data that was in the range of 65 to 90 microns; propLrg = proportion fraction of the data (i.e., treating the lengths as diameters giving rise to area) that was in the range of 65-90 microns; ratDia = the ratio of diaSml to diaLrg; ratProp = the ratio of propSml to propLrg; medDia = the median diameter in the range of 40-90 microns; medProp = the median proportion in the range of diameters 40-90 microns; diaNormMax/propNormMax = 10^{th} percentile at each diameter histogram position was obtained, resulting in a baseline curve. This curve was subtracted from the histogram to give the excursions from the baseline. The position of the maximum excursion was recorded, and was adjusted in the case of propNormMax for the area versus the linear dimension

[a]Marbling: 200=Traces[00], 300=Slight[00], 400=Small[00], 500=Modest[00], 600=Moderate[00], 700=Slightly Abundant[00].

Table 3.3. Summary statistics of Plant Three for marbling score, color measurements, output variables from the Tenera Technology-High Resolution Beef Imaging System and Warner-Bratzler shear force (WBSF) measurement

Trait[a]	n	Mean	SD	Minimum	Maximum
Marbling score[b]	50	433.8	125.7	270	820
L*	50	36.5	2.8	30.3	45.2
a*	50	10.4	1.9	5.2	14.9
b*	50	11.1	0.8	9.7	12.8
diaSml	50	20.3	1.1	17.9	23.2
propSml	50	12.8	0.9	10.6	15.4
diaLrg	50	34.5	1.1	31.9	36.8
propLrg	50	41.8	0.6	40.8	43.6
ratDia	50	1.7	0.1	1.4	2.1
ratProp	50	3.3	0.3	2.7	4.0
medDia	50	71.7	1.0	69.9	74.0
medProp	50	78.5	0.8	77.0	80.4
diaNormMax	50	9.0	1.9	5.0	12.0
propNormMax	50	9.7	2.0	5.0	12.0
14-WBSF, kg	50	3.1	0.6	2.3	4.8
7-WBSF, kg	50	3.7	0.8	2.4	6.3

[a]Marbling score = as determined by official USDA grader on A-maturity carcasses; CIE L* = 0 = black, 100 = white; CIE a* = -60 = green, 60 = red; CIE b* = -60 = blue, 60 = yellow; diaSml = diamter fraction of the data that was in the range of 40 to 60 microns; propSml = proportion fraction of the data (i.e., treating the lengths as diameters giving rise to area) that was in the range of 40-60 microns; diaLrg = diameter fraction of the data that was in the range of 65 to 90 microns; propLrg = proportion fraction of the data (i.e., treating the lengths as diameters giving rise to area) that was in the range of 65-90 microns; ratDia = the ratio of diaSml to diaLrg; ratProp = the ratio of propSml to propLrg; medDia = the median diameter in the range of 40-90 microns; medProp = the median proportion in the range of diameters 40-90 microns; diaNormMax/propNormMax = 10^{th} percentile at each diameter histogram position was obtained, resulting in a baseline curve. This curve was subtracted from the histogram to give the excursions from the baseline. The position of the maximum excursion was recorded, and was adjusted in the case of propNormMax for the area versus the linear dimension

[a]Marbling: 200=Traces^{00}, 300=Slight^{00}, 400=Small^{00}, 500=Modest^{00}, 600=Moderate^{00}, 700=Slightly Abundant^{00}.

Table 3.4. Correlation coefficients of traits with 14-day and 7-day Warner-Bratzler shear force (WBSF, kg).

	Plant 1[b]		Plant 2[c]		Plant 3[d]	
Trait[a]	14-day WBSF	7-day WBSF	14-day WBSF	7-day WBSF	14-day WBSF	7-day WBSF
Marbling score	-0.53	-0.50	-0.54	-0.60	-0.20	-0.27
L*	-0.32	-0.21	0.25	0.21	-0.17	-0.14
a*	-0.38	-0.23	-0.39	-0.33	-0.04	-0.03
b*	-0.33	-0.23	-0.33	-0.33	-0.05	-0.01
diaSml	0.02	0.11	0.18	0.08	0.11	0.06
propSml	0.01	0.10	0.18	0.07	0.11	0.06
diaLrg	-0.03	-0.13	-0.13	-0.02	-0.13	-0.03
propLrg	-0.09	-0.19	-0.03	0.03	-0.15	-0.001
ratDia	-0.01	-0.11	-0.17	-0.05	-0.12	-0.05
ratProp	-0.01	-0.11	-0.16	-0.05	-0.13	-0.06
medDia	-0.02	-0.10	-0.12	-0.01	-0.16	-0.07
medProp	-0.001	-0.08	-0.17	-0.06	-0.19	-0.11
diaNormMax	-0.26	-0.18	0.20	0.35	0.05	-0.11
propNormMax	-0.20	-0.21	0.25	0.12	0.28	0.23

[a]Marbling score = as determined by official USDA grader on A-maturity carcasses; CIE L* = 0 = black, 100 = white; CIE a* = -60 = green, 60 = red; CIE b* = -60 = blue, 60 = yellow; diaSml = diamter fraction of the data that was in the range of 40 to 60 microns; propSml = proportion fraction of the data (i.e., treating the lengths as diameters giving rise to area) that was in the range of 40-60 microns; diaLrg = diameter fraction of the data that was in the range of 65 to 90 microns; propLrg = proportion fraction of the data (i.e., treating the lengths as diameters giving rise to area) that was in the range of 65-90 microns; ratDia = the ratio of diaSml to diaLrg; ratProp = the ratio of propSml to propLrg; medDia = the median diameter in the range of 40-90 microns; medProp = the median proportion in the range of diameters 40-90 microns; diaNormMax/propNormMax = 10^{th} percentile at each diameter histogram position was obtained, resulting in a baseline curve. This curve was subtracted from the histogram to give the excursions from the baseline. The position of the maximum excursion was recorded, and was adjusted in the case of propNormMax for the area versus the linear dimension

[b]$|r| > 0.27$ ($P < 0.05$) (n = 50)

[c]$|r| > 0.27$ ($P < 0.05$) (n = 50)

[d]$|r| > 0.27$ ($P < 0.05$) (n = 50)

Table 3.5. Correlations of variables generated by the Tenera Technology High Resolution Imaging System and Hunter Lab Miniscan L*, a* and b*.[a]

	Plant 1[b]			Plant 2[c]			Plant 3[d]		
Traits[a]	L*	a*	b*	L*	a*	b*	L*	a*	b*
diaSml	-0.06	-0.11	-0.10	0.18	-0.23	-0.22	-0.20	0.04	-0.10
propSml	-0.07	-0.12	-0.11	0.19	-0.21	-0.22	-0.20	0.04	-0.09
diaLrg	0.02	0.04	0.06	-0.13	0.22	0.29	0.14	-0.01	0.08
propLrg	-0.06	-0.05	-0.02	0.003	0.23	0.30	0.05	0.04	0.05
ratDia	0.04	0.08	0.09	-0.17	0.22	0.25	0.17	-0.02	0.09
ratProp	0.04	0.08	0.08	-0.17	0.22	0.25	0.17	-0.03	0.09
medDia	0.03	0.09	0.07	-0.13	0.20	0.23	0.16	-0.03	0.06
medProp	0.01	0.07	0.04	-0.15	0.21	0.22	0.14	-0.06	0.01
diaNormMax	0.30	0.38	0.27	-0.08	-0.01	-0.11	0.07	0.13	0.20
propNormMax	0.31	0.34	0.33	0.10	-0.04	-0.04	0.09	-0.02	0.06

[a]Marbling score = as determined by official USDA grader on A-maturity carcasses; CIE L* = 0 = black, 100 = white; CIE a* = -60 = green, 60 = red; CIE b* = -60 = blue, 60 = yellow; diaSml = diamter fraction of the data that was in the range of 40 to 60 microns; propSml = proportion fraction of the data (i.e., treating the lengths as diameters giving rise to area) that was in the range of 40-60 microns; diaLrg = diameter fraction of the data that was in the range of 65 to 90 microns; propLrg = proportion fraction of the data (i.e., treating the lengths as diameters giving rise to area) that was in the range of 65-90 microns; ratDia = the ratio of diaSml to diaLrg; ratProp = the ratio of propSml to propLrg; medDia = the median diameter in the range of 40-90 microns; medProp = the median proportion in the range of diameters 40-90 microns; diaNormMax/propNormMax = 10^{th} percentile at each diameter histogram position was obtained, resulting in a baseline curve. This curve was subtracted from the histogram to give the excursions from the baseline. The position of the maximum excursion was recorded, and was adjusted in the case of propNormMax for the area versus the linear dimension

[b]$|r| > 0.27$ ($P < 0.05$) (n = 50)

[c]$|r| > 0.27$ ($P < 0.05$) (n = 50)

[d]$|r| > 0.27$ ($P < 0.05$) (n = 50)

Table 3.6. Independent variables, R^2, C_p, root mean square error (RMSE), partial R^2 and β-coefficients for regression equations developed to predict 14-day and 7-day Warner-Bratzler shear force (WBSF, kg) within each harvest facility..

Plant 1							***Plant 1***						
Dependant Variable	Model R^2	C_p	RMSE	Variables in Model	Partial R^2	β-coefficient	Dependant Variable	Model R^2	C_p	RMSE	Variables in Model	Partial R^2	β-coefficient
14-day WBSF	0.42	-2.37	0.49	Marbling score	0.29	-0.00205	7-day WBSF	0.36	-0.02	0.64	Marbling score	0.26	-0.00232
				b	0.02	-0.11834					b	0.001	-0.08186
				diaSml	0.01	1.64123					diaSml	0.001	2.35541
				propLrg	0.02	-0.95952					propLrg	0.03	-1.30639
				ratDia	0.08	15.28285					ratProp	0.07	10.73310
Plant 2							***Plant 2***						
Dependant Variable	Model R^2	C_p	RMSE	Variables in Model	Partial R^2	β-coefficient	Dependant Variable	Model R^2	C_p	RMSE	Variables in Model	Partial R^2	β-coefficient
14-day WBSF	0.66	1.85	0.51	diaSml	0.03	-5.02548	7-day WBSF	0.55	4.22	1.09	Marbling score	0.36	-0.0061
				medProp	0.01	-0.64785					L	0.04	0.0127
				diaNormMax	0.04	0.09978					medDia	0.04	2.3679
				Marbling score x Diet[b]	0.08	-0.00666					medProp	0.06	-2.193
				diaSml x Diet[b]	0.28	4.22885					diaNormMax	0.04	0.1938
				propSml x Diet[b]	0.06	5.71627							
				a x Diet[b]	0.01	-0.26740							
				b x Diet[b]	0.15	0.69572							
Plant 3							***Plant 3***						
Dependant Variable	Model R^2	C_p	RMSE	Variables in Model	Partial R^2	β-coefficient	Dependant Variable	Model R^2	C_p	RMSE	Variables in Model	Partial R^2	β-coefficient
14-day WBSF	0.26	6.35	0.51	L	0.03	-0.09728	7-day WBSF	0.43	3.08	0.62	Marbling score	0.07	-0.00249
				a	0.03	-0.12577					diaSml	0.01	-0.99600
				ratDia	0.01	8.00715					propLrg	.002	1.11249
				medProp	0.19	-1.50185					medProp	0.15	-2.10828
											diaNormMax	0.06	-0.20688
											propNormMax	0.15	0.17031

[a]Marbling score = as determined by official USDA grader on A-maturity carcasses; CIE L* = 0 = black, 100 = white; CIE a* = -60 = green, 60 = red; CIE b* = -60 = blue, 60 = yellow; diaSml = diamter fraction of the data that was in the range of 40 to 60 microns; propSml = proportion fraction of the data (i.e., treating the lengths as diameters giving rise to area) that was in the range of 40-60 microns; diaLrg = diameter fraction of the data that was in the range of 65 to 90 microns; propLrg = proportion fraction of the data (i.e., treating the lengths as diameters giving rise to area) that was in the range of 65-90 microns; ratDia = the ratio of diaSml to diaLrg; ratProp = the ratio of propSml to propLrg; medDia = the median diameter in the range of 40-90 microns; medProp = the median proportion in the range of diameters 40-90 microns; diaNormMax/propNormMax = 10^{th} percentile at each diameter histogram position was obtained, resulting in a baseline curve. This curve was subtracted from the histogram to give the excursions from the baseline. The position of the maximum excursion was recorded, and was adjusted in the case of propNormMax for the area versus the linear dimension.

[b]Variable x diet = interaction between variable and Zilpaterol hydrochloride supplementation.

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