Assessing Outcomes of Selection Using Genetic Panels for Prediction of Carcass Quality in Beef Cattle

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Abstract

Marbling and tenderness are economically important traits that have been shown to be highly heritable in beef cattle. As a result, genetic panel technology has become available for beef producers to use as a selection tool and has grown in popularity in the beef industry since its validation. Therefore, the objectives of the outlined studies were to 1.) determine whether beef cattle genetically selected for tenderness generated a more tender product, and to 2.) evaluate the effectiveness of genetic panel marbling indexes [Igenity® (IT) and PredicGEN™ (PG)] to predict marbling of crossbred cattle. In the first study, Igenity® (IT) panel results were provided by a cattle producer for fifty-two steers. Carcasses from those steers were grouped based upon their IT tenderness index scores. Steaks from boneless strip loins collected from the carcasses were analyzed for Warner-Bratzler shear force (WBSF) and consumer sensory evaluations. In the second study, blood samples from twenty-three steers were submitted for genetic panel analysis, and their carcasses were grouped by IT and PG marbling index scores. Steaks from those carcasses were analyzed for WBSF and a consumer sensory panel. Based on results from the first experiment, the cattle that had been selected to be tender were very tender, suggesting a successful selection outcome. Although all the carcasses attained the USDA *Certified Tender* threshold (< 4.4kg WBSF), currently few processing plants participate in the USDA Tenderness premium program. This means producers will still benefit the most by separating their cattle based on Marbling Score (MS), which is what is used to determine USDA Quality Grade. Based on results from the second experiment, MS can be predicted using commercially available genetic panels. In conclusion, genetic panels could be a beneficial tool to producers for selection within their herd and for making decisions about retaining ownership at the feedlot.

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µM – micromolar

ADG – average daily gain

AMS – Agriculture Marketing Service

ASTM – American Society for Testing and Materials

ATP – adenosine triphosphate

BW – birth weight

C – Celsius

C – cytosine

Ca++ – Calcium ion

CAB – Certified Angus Beef

CAPN1 – micromolar calcium activated neutral protease gene, calpain-1, µ-calpain

CAST – calpastatin, calpain inhibitor gene

CC – homozygous ‘fat’ genotype

CED – calving ease direct

CEM – calving ease maternal

CP – creatine phosphate

CT – heterozygous leptin genotype

D-loop – displacement loop

DMI – dry matter intake

DNA – deoxyribonucleic acid

EPD – expected progeny difference

G6PDH – glucose-6-phosphate dehydrogenase

HCW – hot carcass weight

HP – heifer pregnancy

IACUC – Institutional Animal Care and Use Committee

IMF – intramuscular fat

IMFC – intramuscular fat content

IMPS – Institutional Meat Purchase Specifications

IRB – Institutional Review Board

IT- Igenity®

MBV – molecular breeding value

mM – millimolar

MS – marbling score

mtDNA – mitochondrial DNA

MW – mature weight

NADPH – nicotinamide adenine dinucleotide phosphate

PG – PredicGEN™

REA – ribeye area

RFI – residual feed intake

RORC – retinoid related orphan receptor C (gamma)

SAS – Statistical Analysis System

SEM – standard error of the mean

SNP – single nucleotide polymorphism

T – thymine

TFAM – mitochondrial transcription factor A

TG – thyroglobulin

TP – Taste panel

TT – homozygous ‘lean’ genotype

USDA – United States Department of Agriculture

WBSF – Warner-Bratzler shear force

WW – weaning weight

YG – USDA Yield Grade

YW – yearling weight

Chapter 1: Review of Literature

1.1 Introduction

From the time animals were domesticated, humans inevitably made predetermined mating decisions to select for specific traits. More recently, selection of livestock has evolved from visual appraisal to genetic testing with performance measurements, progeny testing, expected progeny differences (EPD), and genetic panels indexes falling in between. The progression of selection methods has moved from rapid, cheap, and less accurate (visual appraisal) techniques to more time consuming, expensive, and accurate (genetic testing) ones. Producers must decide which selection method or combination of methods meets their operation goals and budget. The goal of this thesis is to further understand the utilization of genetic testing on purebred and crossbred beef cattle to improve tenderness and quality grade.

1.2 Beef quality

Traits that fall into the category of beef quality are considered as such because they are indicators of palatability, or the state of being acceptable to the palate or taste. Traits which are considered when evaluating overall beef palatability include tenderness, juiciness, and flavor. Palatability is defined as the complex of sensations resulting from the odor, taste, feel, and ease of chewing (Blumer, 1963). There are many factors that play a role in overall beef palatability, with the amount of fat and connective tissue being main factors that impact overall eating quality of beef (Ramsbottom et al., 1945; Huff and Parrish, 1993; Magolski et al., 2013). Furthermore, events that take place during the conversion of muscle to meat affect traits like color stability, postmortem enzymatic activity, and water holding capacity, which affect

juiciness, flavor, texture, and tenderness (Koohmaraie, 1992; Morgan et al., 1993; Aberle et al., 2001). Consumers have been shown to rank palatability traits, including marbling and tenderness, at the top of their list for importance in driving future purchasing decisions (Henchion et al., 2014; Henchion et al., 2017). Additionally, Banovic et al. (2009) determined that consumer purchasing intentions are heavily influenced by their eating experience. Therefore, it can be inferred that consumer preferences and the attributes that affect purchasing decisions drive the beef industry's focus on United States Department of Agriculture (USDA) quality grade. For this reason, it is imperative that the beef industry focus on improvements in carcass quality when considering management choices.

1.2.1 Factors affecting beef quality

1.2.1.1 Tenderness

Picard et al. (2014) identify variability in tenderness to be a big issue facing the beef industry. Koohmaraie et al. (1995) performed a consumer survey and saw that consumers consider tenderness to be the most important palatability trait involved in meat quality. This supports the work of Savell and Shackelford (1992), who observed a positive relationship between the price of a cut and its relative tenderness. This is further supported by Platter et al. (2005), who observed that consumers were more likely to bid on a steak during an experimental auction if it had a high marbling score or low WBSF value. It is for this reason that many beef producers consider improvement of tenderness in their overall production goals (Schroeder et al., 2013). Additionally, the USDA and the American Society for Testing and Materials (ASTM) have implemented tenderness as an additional carcass quality standard (ASTM, 2011). Under these

guidelines, producers can receive a premium for beef that is certified as inherently tender, yet there is not currently a premium associated with using selection tools to predict that value (Smith, 2020).

Tenderness of the muscle is influenced by several factors including sarcomere length, or degree of muscle fiber shortening, and chilling rate (Locker and Hagyard, 1963). Furthermore, muscle fiber diameter, connective tissue content, degree of doneness, and muscle fiber type can affect tenderness of meat (Parrish et al., 1973; Calkins et al., 1981; Klont et al., 1998). Different muscles contain different degrees of connective tissue content in general, which leads to differing degrees of tenderness within and between muscles (Ramsbottom et al., 1945). Furthermore, tenderness is influenced by the age of the animal, and therefore the amount of cross-linking between connective tissue fibers (Huff and Parrish, 1993). Additionally, ultimate pH, temperature, and postmortem proteolysis play a large role in meat tenderness (Locker and Hagyard, 1963; Yu and Lee, 1986; Koohmaraie, 1992; Huff and Parrish, 1993; Rios-Mera et al., 2017). Amount of intramuscular fat has also been shown to influence tenderness through the decrease in bulk density of each bite (Miller, 1994) as well as the disruption in the connective tissue matrix (Li et al., 2006). Alongside inherent tenderness, postmortem proteolysis is thought to have the largest influence on ultimate meat tenderness (Koohmaraie, 1992; Huff and Parrish, 1993).

During the conversion of muscle to meat, several different events take place. Once the animal is exsanguinated, or rid of the blood, body tissues become depleted of oxygen, which disrupts normal homeostatic processes (Matarneh et al., 2017). Skeletal muscles, however, continue to synthesize and utilize adenosine triphosphate (ATP) in an effort to regain cellular homeostasis.

Once oxygen is depleted, glycogen stored in the muscle and creatine phosphate (CP) are metabolized anaerobically for ATP production (Matarneh et al., 2017). Rigor mortis onset occurs when ATP production falls behind ATP hydrolysis, which leads to muscles remaining in the contracted state (Aberle et al., 2001). This is because of the lack of ATP in the muscle tissue, which normally causes the conformational shift in the protein myosin to allow relaxation of the muscle. The irreversible binding of actin and myosin within the sarcomere is the cause of the stiffness observed during rigor mortis (Matarneh et al., 2017). With the loss of the circulatory system, the body is no longer able to regulate heat or to remove waste products generated during anaerobic respiration, thus causing the pH decline in normal meat (Paredi et al., 2012). With depleted ATP stores, the Sarco-endoplasmic Reticulum Calcium pump responsible for Calcium (Ca^{++}) re-sequestration into the sarcoplasmic reticulum is no longer able to pump Ca^{++} against its concentration gradient, leaving the Ca^{++} concentration in the cytosol of the muscle cells much higher than in the living tissue (Paredi et al., 2012). This allows for activation of the calpain system, which degrades the proteins titin, desmin, and viniculin, which all reside near the z-disc of the sarcomere of the muscle cell (Taylor et al., 1995). This causes disruption of the cellular integrity. Calpains are responsible for the resolution of rigor mortis and therefore the post-mortem tenderization process (Koohmaraie, 1992; Aberle et al., 2001). This proteolysis, which Koohmararie et al. (1992) implicated as being responsible for the majority of meat tenderness during postmortem aging, has been shown to be highly influenced by genetics (Page et al., 2002; Casas et al., 2006; Barendse et al., 2007a).

Calpains (calcium-activated cysteine proteases) play a key role in the regulation of protein turnover in living skeletal muscle. Health scientists are most interested in the calpain system

for use in enhancement of muscle growth (Huang and Forsberg, 1998) and wound healing (Wing et al., 2011; Nassar et al., 2012; Bennato et al., 2013). The calpain system has been shown to play an important role in the regulation of levels of individual proteins, overall growth of tissues, and atrophy of tissue cells through the degradation of intracellular proteins, but it has been implicated in pathologies that exhibit muscle wasting as a symptom (Huang and Forsberg, 1998). Regarding the study of meat tenderness, the calpain/calpastatin system is widely accepted to be the key cause for meat tenderness post-mortem (Koohmaraie, 1992; 1994; 1996; Koohmaraie et al., 1995; Geesink et al., 2006; Warner et al., 2010). Calpain-1, or μ-calpain, requires micromolar (μM) concentrations of Ca^{++} for activation, and calpain-2, or m-calpain, requires millimolar (mM) concentrations of Ca^{++} for activation (Croall and DeMartino, 1991). Calpastatin is a potent competitive inhibitor of calpain-1 (Koohmaraie et al., 1995; Goll et al., 2003; Kemp et al., 2009). Murachi et al. (1981) reported that calpastatin inhibits both calpain-1 and calpain-2, and is activated by a similar concentration of Ca++ than what is required to activate calpain-1. The authors report that calpastatin is also susceptible to proteolysis, but its fragments remain inhibitory to calpains nonetheless.

It is well characterized that variations in deoxyribonucleic acid (DNA) at genes for both bovine micromolar calcium-activated neutral protease, or calpain-1 (CAPN1; bovine chromosome 29), and its inhibitor, calpastatin (CAST; bovine chromosome 7) affect meat tenderness (Page et al., 2002; White et al., 2005; Casas et al., 2006; Drinkwater et al., 2006; Morris et al., 2006; Van Eenennaam et al., 2007). Page et al. (2002) contributed variation in the CAPN1 gene to variation in meat tenderness postmortem. The authors suggest that prediction of meat tenderness phenotypes using genotypic information in large, unrelated populations would be a

valuable tool for cattle breeders to improve meat tenderness. Barendse et al. (2007a) observed an epistatic relationship between SNP on the CAPN1 gene and SNP on the CAST gene, which means that expression of one gene suppresses the effects of another gene. They observed an additive x dominance interaction, primarily, meaning that the dominant deviation at one locus is altered or rescaled by the additive deviation at the other locus (Hansen and Wagner, 2001). The authors also suggest that animal breed appears to alter the effect of its genotype. Additionally, Morris et al. (2006) suggest that, based on the small breed difference between *Bos taurus* cattle in their study in terms of Warner-Bratzler shear force (WBSF) accompanied by the large difference in allele frequency of the CAPN1 gene and small difference in allele frequency of the CAST gene, there are likely other genes which affect tenderness but have yet to be identified.

Though there is a tenderness premium available to beef producers (ASTM, 2011; Yates et al., 2013), there are currently few animal processing plants which participate the program (Morris, 2017). Therefore, genetic selection goals still need to be centered around marbling to help producers capture USDA carcass quality premiums, or avoid discounts for carcass quality, which focus primarily on marbling.

1.2.1.2 Marbling

Marbling is defined as intramuscular fat (IMF), or the white flecks/streaks of fat between the muscle fascicles (Ferguson, 2004). Many factors affect beef palatability in general, but amount of marbling has been found to have a large impact on variation in tenderness and consumer acceptability when compared to other beef palatability traits (Magolski et al., 2013). Greater

amounts of marbling have been associated with improvements in tenderness, juiciness, and flavor (Smith et al., 1987; Jones et al., 1991; Neely et al., 1999; Hunt et al., 2014, Corbin et al., 2015; Lucherk et al., 2016). Marbling is thought to promote structural disorganization of intramuscular connective tissue, leading to these improvements in tenderness directly (Li et al., 2006; Corbin et al., 2015). Furthermore, increased marbling is thought to decrease the bulk density of each bite as well as provide lubrication, which provides an indirect improvement to tenderness (Millar, 1994). Greater marbling has been found consistently to improve overall beef palatability (McBee and Wiles, 1967; Jones et al., 1991; Luchak et al., 1998; Li et al., 2006; Hunt et al., 2014). Corbin et al. (2015) concluded that intramuscular fat level was the primary driver of beef flavor acceptability. Crouse and Smith (1978) observed that marbling accounted for 2-3% of variation in sensory panelist perception of tenderness and acceptability, which is supported by Emerson et al. (2013).

Most IMF is deposited between muscle fiber bundles in the perimysial connective tissue in beef (Moody and Cassens, 1968). Fat cell growth occurs both due to hyperplasia, or increase in cell number, as well as hypertrophy, or increase in cell size (Park et al., 2018). Du et al. (2013) proposed that mesenchymal stem cells are first committed to myogenic or adipogenic cell lineages during fetal muscle development, and that adipocyte formation occurs between late gestation and 250 days after birth. Du et al. (2010) suggests that events that take place during this stage of fetal development have the potential to affect IMF deposition later in the animal's life. Furthermore, the nutritional and physiological condition of the fetus and the early postnatal stage affects the number of adipocytes and adipose tissue of animals (Du et al., 2013). Like tenderness, marbling degree has been shown to differ between and within individual muscles

(Hocquette et al., 2010). Amount of marbling that is deposited post-partum is heavily influenced by nutrition (Pethick et al., 2004), management (Meyer et al., 2005; Park et al., 2018), and genetics (Utrera and Van Vleck, 2004; Albrecht et al., 2011). Genetics can be manipulated through breed characteristics (Black et al., 2015; Greenwood et al., 2015) and general animal selection practices (Bonnet et al., 2007).

The leptin gene has been linked to intramuscular adipose tissue deposition (Bonnet et al., 2007). Leptin is a hormone that is secreted by white adipocytes to regulate appetite and energy metabolism in humans and mice (Houseknecht et al., 1998). Plasma leptin levels have been shown to increase linearly with increases in body mass and energy balance in sheep and steers (Blache et al., 2000; Yamada et al., 2003). Polymorphisms, or mutations, in the leptin gene have been shown to influence fat deposition in fed beef cattle (Buchanan et al., 2002; Geary et al., 2003; Yamada et al., 2003). The polymorphism observed in the leptin gene which affects fat deposition is a substitution of thymine (*T*) from cytosine (*C*), which causes an amino acid change in the circulating leptin (Buchanan et al., 2002). The three genotype possibilities an animal can exhibit are *CC* (homozygous 'lean'), *CT* (heterozygous), and *TT* (homozygous 'fat'). The *TT* genotype has been shown to result in animals that deposit 12th rib fat earlier in the finishing period and at lighter weights (Buchanan et al., 2002). Leptin genotype has been shown to affect subcutaneous fat deposition, and therefore can be anticipated to affect yield and quality grades in beef cattle, which would thus affect instances of discounts and premiums a producer could expect to receive when selling their cattle on a carcass value based pricing system. DeVuyst et al. (2007), however, observed that the *TT* cattle were more valuable overall than *CC* or *CT* cattle due to their increased overall adiposity. Additionally, increased levels of

circulating leptin, which is characteristic with the *CC* genotype, has been shown to decrease reproductive characteristics in cows and heifers (Bhowmik et al., 2019), which suggests selecting cattle for the *TT* genotype would show more improvements than simply with regard to marbling.

The Glucose-6-phosphate dehydrogenase (G6PDH) gene has also been implicated in determining the amount of marbling deposited (Bonnet et al., 2007). G6PDH is the rate-limiting enzyme involved in the pentose-phosphate pathway, and, when increased in quantity, has been shown to increase cell proliferation of all cells tested due to its role in providing NADPH for redox regulation (Tian et al., 1998). Therefore, the gene responsible for expression of G6PDH has been hypothesized to be involved in the deposition of intramuscular fat via adipocyte proliferation (Bonnet et al., 2007).

A supplemental gene implicated in amount of marbling fat observed in beef animals is the gene encoding thyroglobulin (TG), with the *TT* genotype showing the most marbling (Barendse, 1997; Shin and Chung, 2007). TG is the precursor to thyroid hormones T3 and T4, which each affect fat cell growth and differentiation (Santisteban et al., 1987). The effects of this gene, however, have been observed to be recessive (Thaller et al., 2003). Including this gene in marker panels, however, needs to be conducted with caution, as the *TT* genotype for TG has been associated with late-onset Graves' disease (an autoimmune disease characterized by clinical hyperthyroidism) in humans (Hsiao et al., 2008). No research to date, however, has been published regarding potential deleterious effects of selecting for the *TT* genotype in cattle.

Barendse (2003) identified the gene encoding the retinoid related orphan receptor C (gamma) (RORC) as being associated with fat deposition in muscle tissue. RORC has been identified as a candidate gene because it is a member of the steroid and thyroid hormone receptor superfamily (Petkovich et al., 1987; Evans, 1988), and because it binds retinoic acid and thyroid hormone (Evans, 1988), making it potentially responsible for regulation of adipocytes and the body's ability to process glucose (Barendse et al., 2007b). Additionally, Vitamin A (retinoic acid) restriction has been shown to lead to an increase in marbling fat (Kruk et al., 2018).

Mannen et al. (2003) implicated mitochondrial DNA (mtDNA) sequence variation in the displacement loop (D-loop) region to have an impact on marbling phenotype in Japanese Black cattle. The authors characterize the potential for mitochondria to be responsible for marbling variation because the organelles contain their own DNA and are inherited only from the dam (Brown et al., 1989). Additionally, Jiang et al. (2005) have found mitochondrial transcription factor A (TFAM) to play a role in maintenance and biogenesis of mtDNA.

Marbling is difficult to target with genetic selection because it is assessed visually or using a camera grading system, leading to greater error associated with observed degree of marbling. Furthermore, marbling is a trait that is influenced by multiple SNP on multiple genes and heavily influenced by several environmental factors. Tenderness is a trait that is heavily influenced postmortem, whereas marbling is a trait that is impacted throughout the animal's life, from conception to harvest.

1.2.2 Measurement of beef carcass quality

1.2.2.1 USDA Quality Grade

Carcass quality grade is currently determined by USDA standards using amount of marbling and animal maturity. Quality grades consider characteristics of the meat which predict the palatability of the lean of the carcass. The animal's dentition is used to determine its physiological age, and animals that are determined to be less than 30 months old are classified as A maturity. Final quality grade is determined in A maturity carcasses by the degree of marbling (Figure 1.1; USDA, 2017).

1.2.2.2 USDA Yield Grade

USDA Yield Grades (YG) identify the yield of closely trimmed, boneless retail cuts that can be expected to come from major subprimals. YG 1 through 5 are applicable to all classes of beef, with a YG of 1 applying to carcasses with the highest cutability, or the least amount of trimming necessary, and a YG of 5 applying to carcasses with the least cutability, or the most amount of trimming necessary. YG considers adjusted 12^{th}-rib fat thickness, percent kidney, pelvic and heart fat, hot carcass weight, and ribeye area (USDA, 2017). The official USDA Yield Grade calculation is as follows: 2.50 + (2.50 x adjusted fat thickness, inches) + (0.20 x percent kidney, pelvic, and heart fat) + (0.0038 x hot carcass weight, pounds) - (0.32 x area ribeye, square inches) (USDA, 2017).

1.2.3 Heritability of carcass quality traits

Heritability is the ratio of variance that is observed due to both phenotype and genotype (Visscher et al., 2008). In short, it is a measure of how well genotypic differences account for observed phenotypic differences as a way of describing response to selection. Since tenderness is a polygenic trait, meaning it is influenced by multiple genes, it can vary how much selection response will show within a herd. Tenderness in terms of WBSF was shown to be intermediately heritable ($h^2 = 0.53 \pm 0.15$), while calpastatin activity has been shown to be moderately heritable ($h^2 = 0.65 \pm 0.19$) (Shackelford et al., 1994). The authors suggest selection against calpastatin activity could produce a rapid genetic response because of its heritability estimate. Additionally, Mateescu et al. (2015) identified WBSF of the longissimus muscle as an excellent trait to target for genomic selection because of its relationship to improved eating satisfaction.

Marbling has been shown by Utrera and Van Vleck (2004) to be a moderately heritable ($h^2 = 0.37$) trait. MacNeil et al. (2010) evaluated heritability of marbling and observed increased genetic correlations between molecular breeding values (MBV), or values derived from DNA markers that are used to assist in selection for a particular breeding objective (Akanno et al., 2014), and the targeted economically relevant carcass traits that were achieved between the first and second generation of MBV. The correlation between first and second generation MBV was $r = 0.42$ for marbling. The authors concluded that MBV are useful indicators of economically relevant traits in Angus cattle, although they did not evaluate tenderness. Furthermore, they did not evaluate crossbred cattle. Minick et al. (2004) observed a heritability estimate of 0.43 ± 0.28 for Marbling Score (MS).

Mateescu et al. (2015) observed heritability for MS, intramuscular fat content (IMFC), WBSF, tenderness, juiciness, and connective tissue traits to be 0.67, 0.38, 0.19, 0.18, 0.06, and 0.25, respectively. They saw that genetic correlation of MS with tenderness, juiciness, and connective tissue was 0.57 $\pm$ 0.14, 1.00 $\pm$ 0.17, and 0.49 $\pm$ 0.13, respectively. Genetic correlations of IMFC with tenderness, juiciness, and connective tissue were estimated to be 0.56 $\pm$ 0.16, 1.00 $\pm$ 0.21, and 0.50 $\pm$ 0.15, respectively. The authors concluded that similar gene networks may control MS, IMFC, and juiciness or WBSF, panel tenderness, and connective tissue. They confirm that MS used in selection breeding programs has positive genetic correlations with, and is thus a good indicator of, tenderness, juiciness, and WBSF.

1.3 Genetic testing

Genetic testing is used for several reasons in beef cattle including determining parentage, avoiding genetic disorders, or selecting for specific traits (Van Eenennaam, 2016). These tests have been available since the early 2000's. Genetic testing requires a DNA sample (blood, hair follicle, tissue, or semen) to be collected from the animal or animals. This sample is then sent to a lab which extracts DNA and reports back a variety of genetic information, depending on what type of test is requested. Some tests report back a series of MBV's. Many commercially available tests report a series of index number MBV's which correspond to the specific key created by the company selling the test. For example, Igenity® (Neogen®, Lincoln, NE) evaluates genotypes of animals for both maternal traits and carcass quality traits. The company offers three different profiles, each assigning indexes for genetic merit for their own traits. The traits evaluated by the Igenity® Beef profile fall into three categories, which are maternal traits, performance traits, and carcass traits. The maternal traits evaluated are birth weight (BW),

calving ease direct (CED), calving ease maternal (CEM), stayability, heifer pregnancy rate, docility, and milk production. The performance traits assessed are residual feed intake (RFI), average daily gain (ADG), weaning weight (WW), and yearling weight (YW). The carcass traits that are evaluated are tenderness, marbling, ribeye area (REA), cover fat thickness, and hot carcass weight (HCW). The indexes assigned are for general production and general maternal. Genetic tests commercially available to cattle producers are summarized in Table 1.1. These tests can be used by producers to make selection decisions within their herd for several different economically relevant traits. Therefore, using genetic tests to select for carcass quality traits like tenderness and marbling has great potential to benefit beef producers, as these carcass traits are difficult to predict subjectively (Hedrick, 1983; Topel and Kauffman, 1988).

1.3.1 Use of genetic panels to predict carcass quality

Commercially available genetic panel information for beef carcass quality traits have been shown to have a low, yet significant, correlation with the objective measurements of carcasses from purebred animals (Van Eenennaam et al., 2007; DeVuyst et al., 2011). Additionally, selecting animals for their DNA panel tenderness score is thought to be an indirect way to select for greater marbling potential and an improvement in ADG (DeVuyst et al., 2011). For example, McEvers et al. (2012) found that the crossbred cattle that were grouped into their "tough" category (Igenity® panel score 2-5) were significantly leaner than cattle in their "tender" category (Igenity® panel score 7-10). They also found that cattle in their "tough" category had lower marbling scores. It has been well-documented that improvements in marbling improves tenderness, both objectively (McBee and Wiles, 1967; Luchak et al., 1998) and via consumer perception (Millar, 1994; Li et al., 2006).

Van Eenennaam et al. (2007; 2011) validated the TenderGENE, GeneSTAR Quality Grade, and the GeneSTAR Tenderness (Igenity®; Neogen®, Lincoln, NE) genetic panels on commercial cattle to determine their significance, which they concluded were indeed viable sources of genetic information. Quaas et al. (2007) validated the GeneSTAR Tenderness and Quality Grade tests as well as the Igenity® TenderGENE test on crossbred animals; the authors observed a strong association of each marker panel with notable WBSF effects. Among their evaluation of several economically relevant traits, they found that a reduction in the calpastatin single nucleotide polymorphism (SNP) was associated with a decrease of 0.14 kg of WBSF, and each calpain-1 SNP was associated with a decrease of 0.18-0.21 kg of WBSF. These gains were estimated based on use of the least tender genotype, and the authors caution that producers should not expect gains of that magnitude because they will likely have a herd that is more tender genotypically than the population that was used in their study.

Interestingly, DeVuyst et al. (2011) found that the Igenity® genetic panel value for tenderness is positively correlated with improvements in USDA Quality Grade, which is a trait with higher heritability. Minick et al. (2004) found heritability for WBSF to be low to moderate (h^2 = 0.11 for Hereford, 0.16 for Simmental, and 0.33 for Angus), but heritability for marbling score to be moderate to high (h^2 = 0.40 for Angus, and 0.45 for Simmental). This suggests that selecting for high tenderness values has the potential to produce product with more desirable marbling scores. Rusche et al. (2018) observed that the Igenity® Silver, Igenity® Gold and PredicGEN™ tests used on crossbred cattle are positively correlated with their respective carcass traits.

Zoetis™ (Kalamazoo, MI) has released several different genetic panels that test for carcass quality traits. For example, the GeneSTAR test includes a tenderness test which identifies two

variants for the CAST gene, one which is associated with increased tenderness and the other with increased toughness (Quaas et al., 2007). Zoetis™ has an HD50K test that provides genomic information on 18 different production traits and is intended for use on non-registered animals. Additionally, the PredicGEN™ (Zoetis™, Kalamazoo, MI) test is intended for use as a heifer selection tool for commercial cattle; it provides genomic predictions for yield grade, grid merit index scores, marbling, and tenderness.

Zuidema et al. (2017) compared Igenity® panel results for carcass quality traits in crossbred beef cattle with PredicGEN™ results to evaluate correlations between similar traits within each test. The authors observed a positive correlation between the PredicGEN™ tenderness and marbling indexes. Additionally, they found a positive correlation between tenderness and marbling scores across both tests. The authors saw that the tenderness tests were highly correlated between tests, suggesting that the two would assign index numbers to animals similarly for tenderness. Though they saw these similarities, they did not perform any other statistical comparisons. Furthermore, there are very few published papers using the PredicGEN™ test in general. Based on the results of the previous studies mentioned that have both evaluated genetic factors affecting carcass quality traits and tests which evaluate those, tenderness and marbling can likely be manipulated using commercially available genetic tests to make animal selection decisions.

Thompson et al. (2014) determined that phenotypic traits were indeed correlated with their genetic panel values, but these tests would be a more economically important test to use for replacement breeding stock. MBV information could be used in an additional way to allow producers to treat cattle that have a high probability to be tender or to produce a more favorable

USDA Quality Grade differently when they market that animal. Using the panel scores in this way would allow producers to capitalize on available carcass quality premiums. Since tenderness is a trait that is currently impossible to predict or measure at line speed and requires substantial product and labor to measure objectively, the use of genetic tests would be a valuable asset to producers trying to achieve these premiums.

If it is possible to use selection tools that can give insight into an animal's genotype, it would be possible to predict what quality of carcasses its progeny could produce. Additionally, producers could provide an environment (i.e. implant strategy) that will capitalize on that genetic ability to produce a desirable product if they are aware of the animal's potential. These tools would be well suited for use as a selection tool for replacement heifers and for seedstock producers. Producers would be able to say their animals have a high chance of producing progeny that will generate a tender or well-marbled product. Furthermore, given the accuracy of these tests, producers could use them as a tool to determine which animals of to retain ownership at the feedlot.

1.4 Economic benefits to improvement in carcass quality

The ultimate goal of producing beef is to provide safe food of high nutritional value and eating experience at a reasonable price. Being able to use relatively inexpensive ($19-$29/hd) DNA panels to make selections early on without having to see the carcass first would greatly reduce the amount of time that it takes to obtain breeding stock that will yield high quality product. This would ultimately reduce the amount of money spent on raising cattle that will not conjure premiums. Therefore, genetic panels are becoming increasingly more popular in the U.S. beef

industry as tools for beef animal selection (Pollak et al., 2012). Selection for desirable traits in the seed-stock sector has the potential to accelerate the rate of genetic gain (Weaber and Lusk, 2010; Van Eenennaam et al., 2011). Weaber and Lusk (2010) determined that selection of bulls based on their genetic merit to improve the genetic pool would produce economic benefits up to $7.6 billion over time. Selecting animals for their DNA panel tenderness score is thought to be an indirect way to select for higher marbling potential and an improvement in ADG (DeVuyst et al., 2011). No research to date, however, has been conducted to evaluate genetic panel marbling index scores in this same fashion. Though the USDA has implemented a tenderness category into their quality grading system, currently there are only a few processing plants which participate in that program (ASTM, 2011; Morris, 2017). There are, however, still a majority of commercial processing plants which offer premiums for USDA Quality Grade, and the Choice-Select spread is expected to continue to hit peaks over $20/cwt seasonally for the foreseeable future (Zimmerman, 2020). This means that improving the chances of producing animals that consistently have favorable carcass quality will ultimately increase the value and marketability of product, because it will add the guarantee that the product will provide a positive eating experience.

Thompson et al. (2016) found that the value of the information that can be derived from genetic tests is not enough to offset the price of the Igenity® test, which was $38/hd at the time of the study. Since that analysis was completed, the price of the Igenity® test has decreased to $29/hd, meaning that using the test at the feedlot level may be more economically feasible. Other literature has found considerable economic value (up to $60/head) to using genetic information for selecting feeder cattle for placement in the feedlot (DeVuyst et al., 2007; Lusk, 2007;

Lambert, 2008; Thompson et al., 2014). Though these evaluations found genotyping animals for their fat deposition was able to generate profit, it would barely break even when offset by the price of the tests. Yet again, these analyses were performed when the cost of that particular test was much higher than it is currently. Van Eenennaam et al. (2011) determined that selection of seedstock bulls using MBV was a good way to improve genetic merit for certain carcass quality traits over time. Lusk (2007) determined that using genotypic information to separate cattle at the feedlot is relatively low, but when the strategy is broadened to select and feed only certain genotypes, the profit greatly improves. Lambert (2008) looked at genotypic information and determined that value can be found when separating animals based on their genotype for intramuscular fat, but they did not use commercial tests to reach that conclusion. Thompson et al. (2016) estimated that the genetic profile is profitable when using it to select and feed cattle based on genetic potential. Thompson et al. (2016) also identify marbling and ADG as the most economically relevant traits. Additionally, they found that using genetic panel information to sort cattle at the feedlot was not a financially viable way to use the tests because of the loss in profit due to the cost of the test itself. They suggested that Igenity® market a test that is reduced in the traits that it evaluates for use on feedlot animals, that way feedlots can separate cattle into management groups more effectively.

DeVuyst et al. (2007) predicted use of genetic panels which genotype cattle for the leptin gene for improvement in quality grade would break even at best, but tests cost $40-$50 at the time of the analysis. Lusk (2007) estimated the profitability of selecting cattle based on their leptin genotype had the potential to generate over $22/hd for steers and heifers.

1.5 Summary

In summary, genetic improvement in beef herds has the potential to improve overall consumer eating experience and therefore economic benefit to beef producers. During times when the Choice-Select spread becomes very large, producers could potentially lose millions of dollars if their cattle grade USDA Select vs. USDA Choice or USDA Prime. If there was a possibility for producers to be able to predict carcass quality grading potential, they could provide an environment that is conducive to capitalizing on that potential. For example, animals that have high marbling fat deposition potential could be on feed for less time. Additionally, animals that do not have that potential could be fed longer in order to improve the chances of reaching the marbling threshold to avoid discounts at the packing plant for unfavorable carcass quality. Alternatively, animals that do not have the potential to grade USDA Choice could be harvested early and therefore the feedlot would save on feed and management costs. Using this technology would specifically benefit producers who retain ownership of their cattle at the feedlot because they could use these tests to decide on which animals, they want to retain ownership.

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Chapter 2. Using genetic panels to predict tenderness in beef cattle

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2.1 Abstract

Genetic panel use as a selection tool has grown in popularity in the beef industry. The objective of the study was to determine if beef cattle genetically selected for tenderness generated a tender product. Igenity® (IT) panel results were provided by a cattle producer for fifty-two steers, which were harvested at a commercial harvest facility. Boneless strip loins (IMPS # 180; USDA Choice, n = 32; USDA Prime n = 20) were collected from the left side of each carcass and transported to the University of Idaho Meat Science Laboratory. Four steaks were cut from each subprimal and assigned to aging periods of 7, 14, and 21 days for Warner-Bratzler Shear Force (WBSF) analysis or 21 days for consumer sensory analysis. Carcasses were assigned to tenderness groups based on their IT tenderness indexes (Low IT, 3-6; n = 30; High IT, 7-10; n = 22). Data were analyzed using the Mixed Model procedure of the Statistical Analysis System (SAS Institute, Inc., Cary, NC). An interaction was observed between tenderness group and USDA quality grade ($P < 0.01$) when analyzing WBSF. All of the cattle had less than 4.14kg of WBSF; however, USDA Prime steers that were in the High IT tenderness group produced more tender steaks than High IT USDA Choice, Low IT USDA Prime, and Low IT USDA Choice steers. Consumers were not able to detect tenderness differences between IT tenderness groups ($P = 0.11$) or USDA quality grades ($P = 0.11$), but they found USDA Prime steaks to be more acceptable ($P = 0.01$), juicier ($P = 0.01$), and more flavorful ($P = 0.02$) than USDA Choice

steaks. In conclusion, regardless of tenderness group, USDA Prime steaks were preferred by consumers over USDA Choice steaks in terms of flavor, juiciness, and acceptability.

Keywords: beef, genetic panel, tenderness, Igenity®

2.2 Introduction

Genetic panel use as an animal selection tool is growing in popularity in the U.S. beef industry due to their complement to sole use of somewhat more traditional expected progeny differences (EPD) (Pollak et al., 2012). Producers can submit deoxyribonucleic acid (DNA) samples in the form of hair, tissue, blood, or semen to companies that produce these genetic panels to obtain genetic information which can be used as a management tool within their herd (Neogen®, Lincoln, NE; Zoetis, Kalamazoo, MI). With advancements in genetic capabilities, panels which predict beef carcass quality traits have been increasing in accuracy for use as a supplemental selection tool. Furthermore, these tests continue to gain in popularity due to their increase in affordability as the field of genomics is further explored (Hocquette et al., 2007; Picard et al., 2015; Van Eenennaam, 2016).

Improving carcass quality is a goal of many beef producers because of consumer reports of importance of palatability (Schroeder et al., 2013). In the past, producers were only able to make selection decisions within their herd for carcass quality after they received the carcass data back from the abattoir or through the use of expensive, and sometimes labor-intensive, carcass composition predictions (Topel and Kauffman, 1988). Furthermore, if a producer uses a bull for only a few breeding seasons, that bull has been sold and likely harvested before carcass data was collected on any of his progeny. Producers have been able to make subjective

predictions based on visual evaluation, but that information is highly variable depending on the person doing the evaluation (Hedrick, 1983). Even the most experienced visual evaluator is not able to predict important carcass quality traits like tenderness, which Koohmaraie et al. (1995) showed is the most important quality trait that influences a consumer's willingness to purchase that product again. The use of genetic panels to predict carcass quality could therefore help producers gain additional premiums for their beef.

The Igenity® (IT) tenderness index evaluates several single nucleotide polymorphisms (SNP) including but not limited to the following, calpain-1 (CAPN1), a proteolytic enzyme responsible for postmortem muscle breakdown (Geesink et al., 2006), and calpastatin (CAST), a potent inhibitor of CAPN1 (Goll et al., 2003; Kemp et al., 2009). Postmortem proteolysis, and therefore tenderization, is largely affected by levels and activities of calpain and calpastatin within the muscle (Koohmaraie, 1992; 1994; Warner et al., 2010). In addition to calpain and calpastatin activity, tenderness is affected by many environmental factors, including the age of the animal, cooking methods and degree of doneness (Huff and Parrish, 1993; Warner et al., 2010). If it is possible to use selection tools that can give insight into an animal's genotype for tenderness, it would be possible to predict what quality of carcasses its progeny could produce. Additionally, producers could provide an environment (i.e. less aggressive growth promotant strategy), that will capitalize on that genetic ability to produce a tender product if they were aware of the animal's potential. These tools would be well suited for use as a selection tool for replacement heifers and for seedstock producers because these producers would be able to say their animals have a high chance ($h^2 = 0.53 \pm 0.15$ for WBSF tenderness; Shackelford et al.,

1994) of producing progeny that will generate a tender product. The objective of this study was to evaluate tenderness of cattle that were specifically selected for tenderness.

2.3 Materials and Methods

2.3.1 Human subject participation in consumer sensory panel

The University of Idaho Institutional Review Board certified this project as exempt (Appendix A).

2.3.2 Product procurement

IT panel results were provided by a commercial producer for 52 beef steers whose parentage were selected for their tenderness index scores. The steers were harvested at a commercial harvest facility in Toppenish, Washington and allowed to chill for 24 hrs. Carcasses were then assigned USDA quality grades (USDA Choice, n = 32; USDA Prime n = 20) by a USDA grader. Backfat and ribeye area were recorded using a camera grading system (E+V Technology GmbH & Co. KG, Oranienburg, Germany). Following grading, the carcasses were fabricated, and boneless strip loins (IMPS #180) were produced, vacuum packaged, and stored under refrigeration at 4º Celsius (C). The strip loins were transported under chilled conditions 24 hrs post-fabrication to Vandal Brand Meats in Moscow, Idaho where four 2.54 cm thick steaks were cut from the anterior end of each subprimal and subsequently vacuum packaged. Steaks were randomly assigned by order of removal from the strip loin to one of three aging groups (7, 14, and 21 days postmortem) to be evaluated for Warner-Bratzler Shear Force (WBSF) or aged for 21 days postmortem to be evaluated by an untrained consumer sensory panel for subjective tenderness, juiciness, flavor, and acceptability. Strip loins were organized into

tenderness groups based on their IT tenderness indexes. Carcasses were assigned to High IT (n = 22) or Low IT (n = 30), with the High IT group including steaks from carcasses that received an IT tenderness index of 7-10, and the Low IT group with steaks that came from carcasses that received an IT tenderness index of 3-6, similar to McEvers et al. (2012).

2.3.3 Cooking

Steaks were thawed for 24 hrs at 4ºC and then weighed prior to cooking. Steaks were then cooked on a clam-shell style Cuisinart grill (Cuisinart Griddler Deluxe Model GR-150) that was set to 203ºC to a target peak internal temperature of 71ºC. Temperatures were monitored using a type K thermocouple (93230-K EconoTemp, Cooper-Atkins, Middlefield, CT) placed at the geometric center of each steak.

2.3.4 Warner-Bratzler shear force

Steaks were cooked as described above and removed from the grill at 65ºC. Temperature was monitored until it began to decline, at which time the peak temperature was recorded. The cooked steaks were allowed to cool to room temperature on a tray. Once cooled, steaks were weighed again to determine cook loss. At least six cores were cut from each steak parallel to the muscle fibers from the steaks, taking care to avoid connective tissue and excess fat using a Shop Fox W1667 8-1/2” oscillating drill press with a 1.27 cm diameter coring bit attachment. All cores were all sheared using a Warner-Bratzler Meat Shear (G•R Manufacturing, CO, Manhattan, KS, USA, BFG 1000N) machine and the peak shear force of each core was recorded. The average of the shear force values for all cores from each respective steak were analyzed to determine the WBSF of each steak.

2.3.5 Consumer sensory panel

Consumer panelists were given a consent form (Appendix B). They were also asked to fill out a ballot (Appendix C) that asked them to rank each sample for tenderness, flavor, juiciness, and overall acceptability on a scale of 1-9, with 1 being the least favorable, and 9 being the most favorable. Each panelist was randomly assigned a steak sampling order using the Compusense program (Compusense Inc., Guelph, Canada, N1G 4T2), and they were given one sample at a time to evaluate. Steaks were cooked as described above. Samples were cut into 1.27cm x 1.27 cm cubes. Panelists (n = 72) were given tap water and salt-free soda crackers to cleanse their palette between samples. Each panelist evaluated 5 samples. Each steak either had 4 or 5 cubes taken from it, where steaks that had 5 cubes sampled were chosen randomly from each IT tenderness group to keep the samples balanced.

2.3.6 Statistical analysis

WBSF and sensory panel data were analyzed using the Mixed Model procedure in the Statistical Analysis System (SAS Institute, Inc., Cary, NC). WBSF, consumer perception of overall acceptability, tenderness, juiciness, and flavor were used as dependent variables. For all models, USDA Quality Grade, IT tenderness group, and their interactions were fixed effects, and final off temperature was used as a covariate. All significant effects were compared using a least squared means separation test. Significance was determined at $P < 0.05$, and tendencies were determined at $P < 0.10$.

2.4 Results

2.4.1 Cooking

The mean peak internal temperature for WBSF steaks was 73.61 ± 0.41º C. Mean cook loss for all steaks was 20.21 ± 0.31%.

2.4.2 Warner-Bratzler Shear Force

An interaction was observed between tenderness group and USDA quality grade ($P < 0.01$) (Figure 2.1). High IT steaks that graded USDA Prime had lower shear force values than High IT steaks that graded USDA Choice, Low IT steaks that graded USDA Prime, and Low IT steaks that graded USDA Choice.

2.4.3 Consumer Sensory Panel

Consumer sensory panel demographics are summarized in Table 2.1. There were no interactions observed between tenderness group and USDA quality grade when analyzing consumer sensory data ($P = 0.39$). Consumers were not able to detect tenderness differences between IT tenderness groups ($P = 0.11$; Table 2.2). Furthermore, there were no differences between IT tenderness groups in terms of consumer perception of flavor ($P = 0.44$), but there was a tendency for consumers to prefer High IT steaks over Low IT steaks when evaluating juiciness ($P = 0.09$). Furthermore, consumers preferred High IT steaks over Low IT steaks in terms of overall acceptability ($P = 0.02$).

Consumers found USDA Prime steaks to be more acceptable ($P = 0.01$), juicier ($P < 0.01$), and more flavorful ($P = 0.02$) than USDA Choice steaks (Table 2.3), though they were not able to detect tenderness differences ($P = 0.11$) between USDA quality grades.

2.5 Discussion

Though these steers were selected for their genetic propensity to be tender and could thus be expected to all have high IT tenderness indexes, some steers still fell into the Low IT tenderness group (IT index score 3-6; n = 30). This could be because of estimated heritability for tenderness falling between moderate and high (Shackelford et al., 1994; Mateescu et al., 2015), meaning that the genotype of the dam and sire has a high probability of influencing the phenotype of their progeny, but passing the desired phenotypic tenderness trait along is not a guarantee. Additionally, tenderness is polygenic meaning that it is influenced by multiple genes (Page et al., 2002; Goll et al., 2003; Geesink et al., 2006; Kemp et al., 2009). Furthermore, the IT panel does not report genotype as heterozygous or homozygous, it simply assigns an index number for each predicted phenotype (Neogen, Lincoln, NE). Therefore, researchers can speculate about the genes used to evaluate and assign predictions for genetic probability to perform in each category, but the actual SNP used in the panel are proprietary and thus confidential.

High IT steaks that graded USDA Prime exhibited lower WBSF values, making them more tender, than High IT steaks that graded USDA Choice, Low IT steaks that graded USDA Prime, and Low IT steaks that graded USDA Choice. This observation with the findings of Mateescu et al. (2015), who reported a positive genetic correlation between marbling score and tenderness. Similarly, McBee and Wiles (1967) and Luchak et al. (1998) found a significant

decrease in WBSF value as marbling units increased. Magolski et al. (2013) also observed in heifers and steers that were less than 30 months of age that marbling had more influence on variation in WBSF than other carcass traits measured. Miller (1994) described increases in reported tenderness by sensory panels of higher marbled steaks as the reduction of the bulk density of each bite and increased lubrication. Likewise, Li et al. (2006) observed increased disruption of muscle perimysial structure with increased marbling. The current study observed this interaction when evaluating steaks from the *longissimus lumborum*; other muscles of the body, however, could respond differently to genetic selection for tenderness.

Consumers preferred USDA Prime striploin steaks over USDA Choice striploin steaks in all categories except tenderness, likely because all of the steaks were very tender. To review, the USDA tenderness program considers anything that is inherently tender, meaning it has not been processed in any way to make it more tender, with a WBSF value at or below 4.4 kg to fall within the USDA *Certified Tender* category, and anything with a WBSF value at or below 3.9 kg to fall within the *USDA Certified Very* Tender category (ASTM, 2011). The steers from this experiment that graded USDA Choice had an average WBSF value of 3.31 kg, whereas the USDA Prime steers had an average WBSF value of 3.09 kg. This observation, therefore, is consistent with the findings of Miller et al. (1995), who concluded that consumers were unable to detect differences in tenderness when the WBSF values did not exceed 0.5 kg of difference in tenderness. Furthermore, it has been well documented that increases in marbling lead to improvements in palatability (Jones et al., 1991; Hunt et al., 2014, Corbin et al., 2015). These findings support the observations of this experiment that consumers preferred USDA Prime steaks over USDA Choice steaks in terms of overall acceptability, juiciness, and flavor.

Consumers were not able to detect differences in tenderness between High IT and Low IT steaks, which is likely because WBSF was not different between Low IT and High IT groups. Consumers were also not able to detect differences in juiciness or flavor between IT tenderness groups, which may be partially due to the carcass USDA quality grade distribution in each tenderness group. The High IT group had 12 USDA Choice carcasses and 10 USDA Prime carcasses, whereas the Low IT group had 20 USDA Choice carcasses and 10 USDA Prime carcasses. It has been observed several times that consumers prefer USDA Prime beef over USDA Choice beef because of the improvements in juiciness, flavor, and overall acceptability (Smith et al., 1987; and Corbin et al., 2015; Lucherk et al., 2016). This is consistent with the findings of this experiment, where consumers preferred the USDA Prime steaks over the USDA Choice steaks in the same categories, likely because of the higher amount of marbling in the USDA Prime steaks than in the USDA Choice steaks.

Currently, there is a USDA premium available for tender carcasses, but it has only been adopted by just a few beef processors (ASTM, 2011; Morris, 2017). Since consumers consider tenderness to be the most important palatability trait in beef (Koohmaraie et al., 1995), products from animals which have been selected genetically for tenderness may likely receive a premium in the future. Though there is a tenderness premium available, genetic selection goals still need to be centered around marbling to help producers capture USDA carcass quality premiums, or avoid discounts for carcass quality, which focus primarily on marbling.

2.6 Conclusion

Cattle that were selected for a tenderness index were confirmed to have a tender strip loin even though some carcasses still fell into the Low IT group. The authors caution that over-selecting animals for a single trait can eventually lead to unintended deleterious effects. Even though all of the cattle were very tender based on WBSF values of the strip loin, cattle that graded USDA Prime outperformed USDA Choice cattle in terms of flavor, juiciness, and acceptability regardless of whether they were high IT or Low IT scores. In summary, tenderness is an important part of consumer eating experience and can be capitalized upon via genetic selection. Consumers preferred the steaks that graded USDA Prime because of their acceptability, juiciness, and flavor even though they did not report a difference between IT tenderness groups.

Producers who market their breeding animals as having the genetic propensity to produce tender offspring could add value to their animals with a higher chance of being able to gain a premium for their carcass tenderness. With the use of the genetic tools that are available commercially, producers could capitalize on carcass quality traits on which they have not been able to capitalize in the past. This analysis could be made stronger by the provision of dam and sire genetic information as well as evaluation of other muscles. Furthermore, more information is needed on how these tests can be implemented on crossbred cattle that were not selected for tenderness.

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Chapter 3. Assessing outcomes of genetic selection panels to predict marbling in crossbred beef cattle

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3.1 Abstract

The objective of this study was to evaluate the effectiveness of genetic panel marbling indexes [Igenity® (IT) and PredicGEN™ (PG)] to predict marbling of crossbred cattle. Steers (n = 23) were harvested at the University of Idaho Meat Science Laboratory, and blood samples were submitted to Neogen® and Zoetis™ for genetic panel analysis. 48 hours post-harvest, one boneless strip loin was collected from each carcass, and six 2.54 cm thick steaks were cut from each strip loin. Steaks were aged for 14 and 21 days and assigned to consumer sensory evaluation or Warner-Bratzler Shear Force (WBSF) analysis. Results were analyzed using the Mixed Model procedure of the Statistical Analysis System (SAS Institute, Inc., Cary, NC). Carcasses were grouped by marbling index score into Low IT (IT indexes 3-6; n = 16; MS = 410), High IT (IT indexes 7-10; n = 7; MS = 496), Low PG (PG index < 50; n = 9; MS = 398), or High PG (PG index $\geq$ 50; n = 14; MS = 458). Marbling was observed to be greater in High IT steaks than Low IT ($P < 0.01$) and greater in High PG steaks than Low PG ($P = 0.01$). There was a trend observed in WBSF between IT marbling groups ($P = 0.06$), however, no difference in WBSF was observed between PG marbling groups ($P = 0.83$). Consumers did not report differences between IT marbling groups in terms of acceptability ($P = 0.99$) or tenderness ($P = 0.24$). Additionally, consumers could not detect differences between PG marbling groups in terms of acceptability ($P = 0.75$) or tenderness ($P = 0.40$). Consumers consistently preferred

Choice steaks over Select steaks in terms of acceptability ($P = 0.02$) and tenderness ($P = 0.02$). In conclusion, though consumers were not able to tell the difference between steaks from each of the genetic panels, using genetic panels to predict marbling could be a beneficial tool to producers making decisions about retaining ownership at the feedlot.

Keywords: beef quality, genetic panels, Igenity®, marbling, PredicGEN™

3.2 Introduction

Marbling is defined as intramuscular fat (Ferguson, 2004) and is influenced by nutrition (Pethick et al., 2004), management (Meyer et al., 2005; Park et al., 2018), and genetics (Utrera and Van Vleck, 2004; Albrecht et al., 2011). Marbling deposition has been linked primarily to the leptin gene (Bonnet et al., 2007; Buchanan et al., 2002; Geary et al., 2003; Yamada et al., 2003). DeVuyst et al. (2007) observed that cattle with the homozygous 'fat' leptin genotype were more valuable than other genotypes. It has been well-documented that improvements in marbling improves tenderness, both objectively (McBee and Wiles, 1967; Luchak et al., 1998) and via consumer perception (Millar, 1994; Li et al., 2006). It is for these reasons that beef packing facilities utilize services of United States Department of Agriculture (USDA) Agriculture Marketing Service (AMS) grading personnel to assigned carcasses a USDA Quality Grade. This allows beef cattle producers to receive a premium, or avoid a discount, for carcasses with high degrees of marbling, while allowing packers to apply discounts for carcasses with poor marbling (USDA Livestock, Poultry, and Grain Market News Division, 2020).

Additionally, increased marbling improves palatability traits of beef (Smith et al., 1987; Magolski et al., 2013; Corbin et al., 2015; Lucherk et al., 2016). Thompson et al. (2014)

determined that phenotypic traits were indeed correlated with their genetic panel values, but these tests would be a more economically important test to use for replacement breeding stock, not necessarily when separating animals at the feedlot. To date, no research, has been published comparing Warner-Bratzler shear force (WBSF) and consumer sensory panel data with genetic information derived from commercially available genetic tests on crossbred cattle.

The objective of the current study was to evaluate the effectiveness of genetic panels [Igenity® (IT) and PredicGEN™ (PG)] to predict marbling and tenderness of cross-bred beef steers. The hypothesis was that cross-bred steers with higher IT and/or PG marbling indexes would produce carcasses with more marbling and that are more tender than cross-bred steers with lower IT or PG marbling indexes.

3.3 Materials and methods

3.3.1 Human subject participation in consumer sensory panel

The University of Idaho Institutional Review Board certified this project as exempt (Appendix D).

3.3.2 Obtaining DNA samples

Crossbred steers (Angus x Hereford x Simmental; n = 23) were harvested under inspection at the University of Idaho's USDA-FSIS inspected Vandal Brand Meats Laboratory. Blood (1 mL; IACUC 2017-32; Appendix E) was pipetted onto blood cards, one from Zoetis (PredicGEN™) and one from Neogen (Igenity®), for DNA analysis.

3.3.3 Marbling Score and Yield Grade

Marbling score (MS) on each carcass was determined visually by trained University of Idaho research team members using USDA quality grading standards at 24 h postmortem and 1 hour after the carcass was ribbed between the 12^{th} and 13^{th} ribs. Quality Grade was assigned to carcasses using marbling scores (high Select: MS 350-399; low Choice: MS 400-499; USDA, 2017). Also at 24 h postmortem, yield grade (YG) was assigned by trained University of Idaho research team members, to carcasses using the formula 2.50 + (2.50 x adjusted fat thickness, inches) + (0.20 x percent kidney, pelvic, and heart fat) + (0.0038 x hot carcass weight, pounds) - (0.32 x area ribeye, square inches) (USDA 2017).

3.3.4 Steaks

Boneless strip loins (IMPS #180; n = 23) were fabricated from each carcass at 48 hours post-harvest and vacuum packaged for subsequent analysis. Carcasses were grouped by marbling index score into Low IT (IT indexes 3-6), High IT (IT indexes 7-10), Low PG (PG index < 50), or High PG (PG index > 50) (Table 3.5), using a technique previously used by McEvers et al. (2012). Steaks were further grouped by their USDA Quality Grade (Table 3.5). Six 2.54 cm thick steaks were cut from the anterior end of each strip loin and randomly assigned to one of six treatment evaluations. Steaks were assigned to either a 14- or 21-day postmortem aging period followed by a consumer sensory panel (14d IT TP, 21d IT TP, 14d PG TP and 21d PG TP) or WBSF (14d WBSF and 21d WBSF) analysis. Steaks were vacuum packaged individually and aged (0º C) for their respective amounts of time before being frozen at -20º C until subsequent analysis could occur.

3.3.5 Cooking

Steaks were thawed for 24 hours at 0º C. They were then cooked on a clam-shell style Cuisinart grill (Cuisinart Griddler Deluxe Model GR-150) that was set to 204º C on both grill plates to a target peak internal steak temperature of 71º C. Temperatures were monitored using a type K thermocouple (93230-K EconoTemp, Cooper-Atkins, Middlefield, CT) placed at the approximate geometric center of each steak. The steaks were removed from the grill at 65º C, temperature was monitored until it began to decline, and the peak temperature was recorded.

3.3.6 Warner-Bratzler shear force

Steaks were cooked as described above. The cooked steaks were allowed to cool to room temperature on a tray. Once cooled, steaks were weighed again to determine cook loss. At least six cores were obtained from each steak parallel to the muscle fibers orientation, taking care to avoid connective tissue and excess fat. Steaks were cored using a Shop Fox W1667 8-1/2" oscillating drill press with a 1.27 cm diameter coring bit attachment. All cores were sheared using a Warner-Bratzler Meat Shear (G•R Manufacturing, CO, Manhattan, KS, USA, BFG 1000N) machine and the peak shear force of each core was recorded. The average of the shear force values for all cores from each respective steak was calculated and were analyzed to determine the WBSF of each steak.

3.3.7 Consumer sensory panel

Steaks were assigned in an incomplete block design to a cooking order and cooked as described above. Panelists were given a consent form (Appendix B), a demographics cover page (Appendix F; Table 3.1) and a questionnaire (Appendix G) that asked them to rank each sample

on an unstructured scale of 1-10, with 1 being the least favorable in its category, and 10 being the most favorable in its category. The rankings were assigned based on each panelist's opinion of the steak's tenderness, flavor, juiciness, and overall acceptability. Each panelist was given 5 samples at the same time and asked to try them in their randomly assigned sampling order. Samples were cut into 1.27 cm x 1.27 cm x 2.54 cm cubes. Panelists (n = 92) were given water and salt-free soda crackers to cleanse their palette between samples.

3.3.8 Statistical analysis

Data were analyzed using the mixed model procedure in SAS assuming a normal distribution. Within each model, aging treatment, genetic panel marbling group, and USDA Quality Grade were fixed effects. The relationship between USDA Yield Grade, WBSF, MS and genetic panel scores was assessed using Pearson correlation analysis. Significance was determined at $P < 0.05$. For significant fixed effects, means were separated using pair-wise comparisons. All statistical analyses were carried out using SAS V9.4.

3.4 Results

3.4.1 Marbling Score

Mean MS was higher in the High IT group than the Low IT group ($P < 0.01$; Table 3.2). Additionally, mean MS was higher in the High PG group than in the Low PG group ($P = 0.01$; Table 3.3).

3.4.2 Warner-Bratzler shear force

Mean final off temperature of the steaks was 70.65 ± 0.30 ºC. There was a trend observed for the High IT group to have higher WBSF values than the Low IT group ($P = 0.06$; Table 3.2). No difference in WBSF was observed between PG marbling groups ($P = 0.83$; Table 3.3) or Quality Grades ($P = 0.88$; Table 3.4). No aging effect was observed ($P = 0.16$).

3.4.3 Consumer sensory panel

Mean final off temperature for consumer sensory analysis was 71.15 $\pm$ 0.22 ºC. Consumers were not able to detect differences between Low IT and High IT groups in terms of acceptability ($P = 0.99$), tenderness ($P = 0.24$) juiciness ($P = 0.20$), or flavor ($P = 0.21$) (Table 3.2). They were also unable to detect differences between PG marbling groups in terms of acceptability ($P = 0.75$), tenderness ($P = 0.40$), or flavor ($P = 0.99$) (Table 3.3). However, there was a trend observed for consumers to consider steaks from the High PG group to be juicier than steaks from the Low PG group ($P = 0.05$). Consumers preferred Choice steaks over Select steaks in terms of acceptability ($P = 0.02$), tenderness ($P = 0.02$), and juiciness ($P < 0.01$) (Table 3.4). Additionally, consumers were not able to detect any flavor differences between USDA Quality Grades ($P = 0.25$). No age effect was observed for acceptability ($P = 0.15$), juiciness ($P = 0.19$), or flavor ($P = 0.71$). Consumers tended to prefer steaks aged 14 days over steaks aged for 21 days in terms of tenderness ($P = 0.06$).

MS was positively correlated ($r = 0.39$) with PG marbling indexes ($P < 0.01$). Additionally, MS was positively correlated ($r = 0.47$) with IT marbling indexes ($P < 0.01$). YG was negatively

correlated (r = -0.39) with PG marbling index ($P < 0.01$), while PG marbling indexes and IT marbling indexes were positively correlated (r = 0.55) with each other ($P < 0.01$).

3.5 Discussion

High IT steaks had significantly greater MS than Low IT steaks. This is consistent with research conducted by Shackelford et al. (1994), Minick et al. (2004), and Utrera and Van Vleck (2004) showing high heritability of marbling. Additionally, High PG steaks had greater marbling than Low PG steaks. Zuidema et al. (2017) found a moderate correlation between the IT marbling score and the PG marbling score, which suggests that the two panels use similar single nucleotide polymorphisms (SNP) to evaluate marbling genotype. Furthermore, PG marbling score and IT marbling score were moderately positively correlated with each other in the present study, further leaning toward that conclusion. Some carcasses that fell into the High IT group, however, did not fall into the High PG group, and vice versa, which suggests that the SNP that are used between the panels are similar, but not exactly equivalent. Additionally, marbling is a trait that is influenced by many different environmental factors, including nutrition (Pethick et al., 2004), management (Meyer et al., 2005; Park et al., 2018), climate (Tume, 2004), and time on feed (Spehar et al., 2009). This supports the observation of the present study, where there were Choice carcasses in the Low IT and Low PG groups and Select carcasses in both High IT and High PG groups. The SNP that are used in the tests are proprietary, so researchers can only speculate about which SNP are used. The objective of this study was not to evaluate the two panels, rather, it was to evaluate the effectiveness of the commercially available genetic panels in a way that a beef cattle producer might apply them profitably in their operation management.

Yield Grade was negatively correlated with PG marbling index, which conflicts with earlier research by DeVuyst et al. (2011), who observed positive correlations between Igenity® marbling index score and YG. Additionally, this observation conflicts with expectations that a greater YG would be positively correlated with marbling due to the greater fatness which has been associated with cattle that have greater marbling score (Jones et al., 1990).

No differences were observed for WBSF between Low IT and High IT or between Low PG and High PG carcasses. Additionally, no difference was observed for WBSF between Choice and Select carcasses. McBee and Wiles (1967), Millar (1994), Luchak et al. (1998), and Li et al. (2006), however, found a significant decrease in WBSF value as marbling units increased. Tenderness is influenced by multiple environmental factors, including cooler temperature (Locker and Haygard, 1963) and degree of doneness (Parrish et al., 1973). In the present study, all group means fell below the threshold for being considered USDA *Certified Very Tender* (WBSF < 3.9 kg; ASTM, 2011). Additionally, consumers were not able to detect differences between High IT and Low IT steaks in terms of tenderness, which aligns with Miller et al. (1995), who found that consumers were not able to detect differences of less than 0.5 kg of WBSF; the difference between the two IT marbling groups was 0.45 kg of WBSF.

Consumers were not able to detect differences between High IT and Low IT groups in terms of overall acceptability, juiciness or flavor. This is likely because mean MS for each group, though significantly different, still fell within the same USDA Quality Grade. Additionally, consumers were not able to tell the difference between High PG and Low PG in terms of acceptability, tenderness, or flavor, but they tended to prefer High PG steaks over Low PG steaks based on juiciness. This is likely because the mean MS difference between the two groups translated to

high Select and low Choice USDA Quality Grades, and consumers are known to prefer the juiciness of Choice steaks over Select steaks (Corbin et al., 2015).

Consumers preferred Choice steaks over Select steaks in terms of acceptability, tenderness, and juiciness. This is supported by the work of Smith et al. (1987), Magolski et al. (2013), Corbin et al. (2015), and Lucherk et al. (2016), who observed improvements in palatability traits with increases in marbling. For example, Corbin et al. (2015) found marbling to be the primary driver of beef flavor acceptability. Consumers tended to prefer steaks aged for 14 days over steaks aged 21 days. This is conflicting with the work of Mitchell et al. (1991), who observed that consumer palatability improved until 10 days of wet aging. Additionally, this conflicts with the observations of Colle et al. (2015), who observed an improvement in consumer perception of tenderness until 14 days. It also conflicts with the research of Huff and Parrish (1993), who observed trained panelist tenderness improvement until 28 days of postmortem aging time.

The genetic tests evaluated in the present study could be beneficial for use by producers who retain ownership at the feedlot, because they might be able to use them to predict which animals will generate more revenue on a grid-based system by depositing more marbling. Research to compare carcass traits of purebred cattle to commercially available genetic panel scores to determine correlations, as well as validity of the genetic tests, has been completed in cattle of known genetic background (Quaas et al., 2007; DeVuyst et al., 2011). Additionally, the heritability of carcass quality traits have been consistently reported as high when evaluated in beef [$h^2 = 0.67$ (Mateescu et al., 2015); $h^2 = 0.43$ (Minick et al., 2004); $h^2 = 0.37$ (Utrera and Van Vleck, 2004); $h^2 = 0.93$ (Shackelford et al., 1994)].

The sample population in the present study contained 74% Choice carcasses (Table 3.5). When dividing carcasses into High and Low groups based on their panel score, the High IT group contained 86% Choice carcasses, and the High PG group contained 86% Choice. Additionally, the Low IT group contained 69% Choice, and the Low PG group contained 56% Choice. Using genetic tests, the current research was able to predict Choice cattle 86% of the time, thus increasing the percent Choice in the present study by 12%. Therefore, steers in the present study with a high IT or PG marbling index score were observed to be more likely to grade Choice than steers with low IT or PG marbling index scores. If producers were able to improve the percentage of cattle in their herd that produce carcasses of USDA Choice or better, they would be able to avoid discounts for failing to produce at least Choice beef (Smith, 2020).

Commercially available genetic panel information for beef carcass quality traits have been shown to have a low, yet significant, correlation with objective carcass quality measurements in purebred animals (DeVuyst et al., 2011; Van Eenennaam, 2011a; 2011b). Van Eenennaam (2011a; 2011b) predicted that genotyping would decline in price rapidly as more genomic information is gathered, and this has been realized over the last decade. They also predicted that the cost reduction will most likely result in an industry-wide adoption of the practice of using molecular breeding values (MBV), or values derived from genetic information to be used as a selection tool, to make breeding selections. When the analysis was conducted, the price of the Igenity® test was $38/hd, which has since reduced in price to $29/hd (Neogen, Lincoln, NE). The decrease in price allows for more producers to adopt this technology thus improving a producer's opportunity to receive a premium for marbling, which would not only benefit the producer financially, but would also benefit the consumer by providing a more consistent

product and a better eating experience overall. Eventually, these commercially available genetic panel tests may become affordable to the point where commercial producers and feedlot operators use the tests on crossbred market cattle. This would allow managers to make feeding and marketing decisions and tailor implant strategies based on the individual animal's potential to grade USDA Choice or better.

3.6 Conclusion

Based on these results, commercially available genetic tests are a valuable tool for producers to be able to predict marbling by retaining ownership of feedlot steers with high genetic panel indexes. The Choice-Select spread is expected to continue to hit peaks over $20 seasonally for the foreseeable future (Zimmerman, 2020). At times when the Choice-Select spread is high (i.e. during the spring and summer), genetic panels could be cost effective for commercial producers to use at the feedlot level to make decisions about retaining ownership or for feedlot managers to make feeding, implant, and marketing decisions. These decisions would be the most cost-effective for producers to make at weaning time so they can sort their animals into management groups. More research needs to be done to conduct an economic analysis on this data to determine how producers can benefit financially from using these tests to make selection decisions.

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Tables and Figures

Table 1.1. Summary of Commercially Available Genetic Panels

Company		Pfizer/Zoetis™				Neogen®		
Test name		PredicGEN™	GeneSTAR Black	GeneSTAR Horned/polled	HD50K	Igenity® Beef	Igenity® Angus Gold	Tenderness / Leptin
Cost (as of 3/10/2019)		$19.50	$23.00	$29.00	$37.00	$29.00	$29.00	$25.00
Trait								
Maternal Traits	Birth Weight				x	x	x	
	Calving Ease Direct				x	x	x	
	Calving Ease Maternal					x	x	
	Stayability					x		
	Heifer Pregnancy					x	x	
	Docility					x	x	
	Milk				x	x	x	
	Mature Weight						x	
Performance Traits	Residual Feed Intake		x	x		x		
	Dry Matter Intake				x			
	Yearling Height				x			
	Average Daily Gain (ADG)					x	x	
	Residual ADG						x	
	Weaning Weight				x	x	x	
	Yearling Scrotal				x			
	Yearling Weight				x	x		
Carcass Traits	Tenderness	x	x	x	x	x	x	x
	Marbling	x	x	x	x	x	x	x
	Ribeye Area					x	x	
	Fat Thickness					x	x	
	Yield Grade	x						
	Hot Carcass Weight					x	x	

Table 2.1. Demographics of consumer panelists (n = 72)

	n	%
Age		
20-29	30	41.6
30-39	20	27.8
40-49	10	13.9
50+	12	16.7
Gender		
Male	25	34.7
Female	47	65.3
Beef meals/wk		
0-1	7	9.7
2-4	53	73.6
5-7	12	16.7
Most consumed		
Ground	47	65.3
Roast	5	6.9
Steak	18	25.0
Other	2	2.8

Table 2.2. Effects of Igenity® (IT) tenderness score on palatability traits assessed by a consumer sensory panel

Trait	IT Tenderness Group		SEM	*P*-value
	Low (n = 30)	High (n = 22)		
Sensory (n = 72 panelists)				
Tenderness	6.3	6.7	0.2	0.11
Juiciness	6.6	6.9	0.2	0.09
Flavor	7.0	7.1	0.1	0.44
Acceptability	6.7[b]	7.1[a]	0.1	0.02

Scale, 9 = extremely tender, extremely juicy, extremely flavorful, extremely acceptable, respectively; 1= not at all tender, extremely dry, dislike flavor extremely, extremely unacceptable, respectively.

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

Table 2.3. Effects of USDA Quality Grade on palatability traits assessed by a consumer sensory panel

	USDA Quality Grade			
Trait	Choice (n = 32)	Prime (n = 20)	SEM	*P*-value
Sensory (n = 72 panelists)				
Tenderness	6.3	6.7	0.2	0.11
Juiciness	6.5[b]	7.0[a]	0.2	0.01
Flavor	6.7[b]	7.2[a]	0.2	0.02
Acceptability	6.7[b]	7.1[a]	0.1	0.01

Scale, 9 = extremely tender, extremely juicy, extremely flavorful, extremely acceptable, respectively; 1= not at all tender, extremely dry, dislike flavor extremely, extremely unacceptable, respectively.

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

Table 3.1. Demographics of consumer panelists (n = 92)

	n	%
Age		
18-29	66	72
30-39	11	12
40-49	2	2
50+	13	14
Gender		
Male	49	53
Female	43	47
Beef meals/wk		
0-1	10	11
2-4	52	57
5-7	22	24
8+	8	9
Most consumed		
Ground	62	67
Roast	6	7
Steak	23	25
Other	1	1

Table 3.2. Effects of Igenity® marbling score on palatability traits

	Igenity® Marbling Group			
Trait	Low (n = 16)	High (n = 7)	SEM	*P*-value
Marbling	410[b]	496[a]	18	< 0.01
WBSF	2.76	3.21	0.21	0.06
Sensory Traits (n = 92 panelists)				
Acceptability	6.6	6.6	0.2	0.99
Tenderness	6.3	6.6	0.2	0.24
Juiciness	6.0	6.3	0.2	0.20
Flavor	6.3	6.0	0.2	0.21

Scale, 10 = extremely tender, extremely juicy, extremely flavorful, extremely acceptable, respectively; 1= not at all tender, extremely dry, dislike flavor extremely, extremely unacceptable, respectively.

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

Table 3.3. Effects of PredicGEN™ marbling score on palatability traits

	PredicGEN™ Marbling Group			
Trait	Low (n = 9)	High (n = 14)	SEM	*P*-value
Marbling	398[b]	458[a]	17	0.01
WBSF	2.91	2.86	0.18	0.83
Sensory Traits (n = 92 panelists)				
Acceptability	6.6	6.7	0.2	0.75
Tenderness	6.3	6.5	0.2	0.40
Juiciness	5.9	6.3	0.2	0.05
Flavor	6.2	6.2	0.2	0.99

Scale, 10 = extremely tender, extremely juicy, extremely flavorful, extremely acceptable, respectively; 1= not at all tender, extremely dry, dislike flavor extremely, extremely unacceptable, respectively.

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

Table 3.4. Effects of USDA Quality Grade on palatability traits

	USDA Quality Grade			
Trait	Select (n = 6)	Choice (n = 17)	SEM	*P*-value
WBSF	2.96	3.00	0.21	0.88
Sensory Traits (n = 92 panelists)				
Acceptability	6.4[b]	6.9[a]	0.2	0.02
Tenderness	6.2[b]	6.7[a]	0.2	0.02
Juiciness	5.9[b]	6.5[a]	0.2	< 0.01
Flavor	6.0	6.3	0.2	0.25

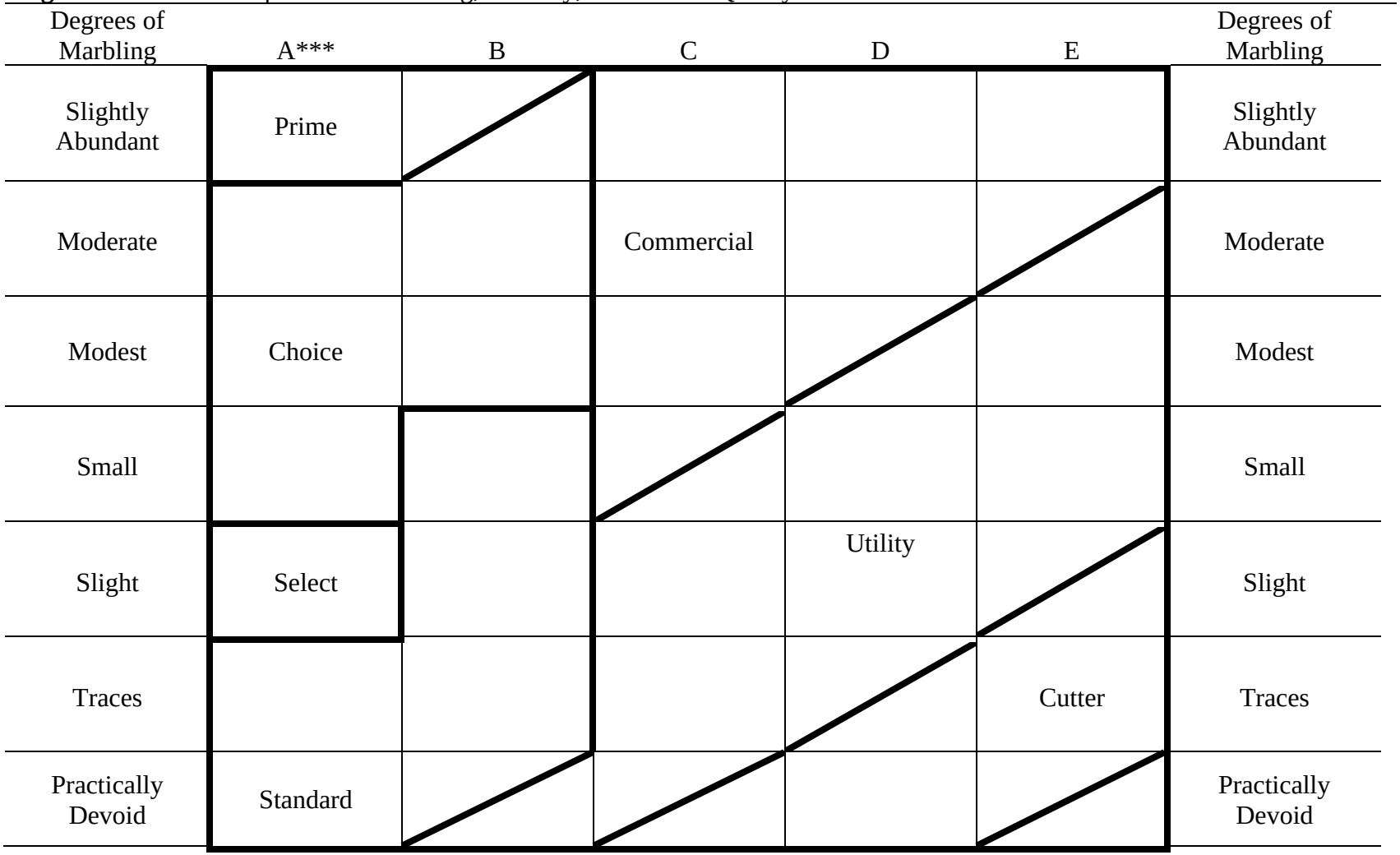
Scale, 10 = extremely tender, extremely juicy, extremely flavorful, extremely acceptable, respectively; 1= not at all tender, extremely dry, dislike flavor extremely, extremely unacceptable, respectively.

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

Table 3.5. Frequency of USDA Quality Grade within each marbling group

	Low IT	Low PG	High IT	High PG
Choice	11	5	6	12
Select	5	4	1	2
Total	16	9	7	14
% Choice	68.8	55.6	85.7	85.7

Figure 1.1. Relationship between marbling, maturity, and carcass Quality Grade*



* Assumes that firmness of lean is comparably developed with the degree of marbling and that the carcass is not a "dark cutter."
** Maturity increases from left to right (A through E).
*** The A maturity portion of the Figure is the only portion applicable to bullock carcasses.

Adapted from USDA, 2017.

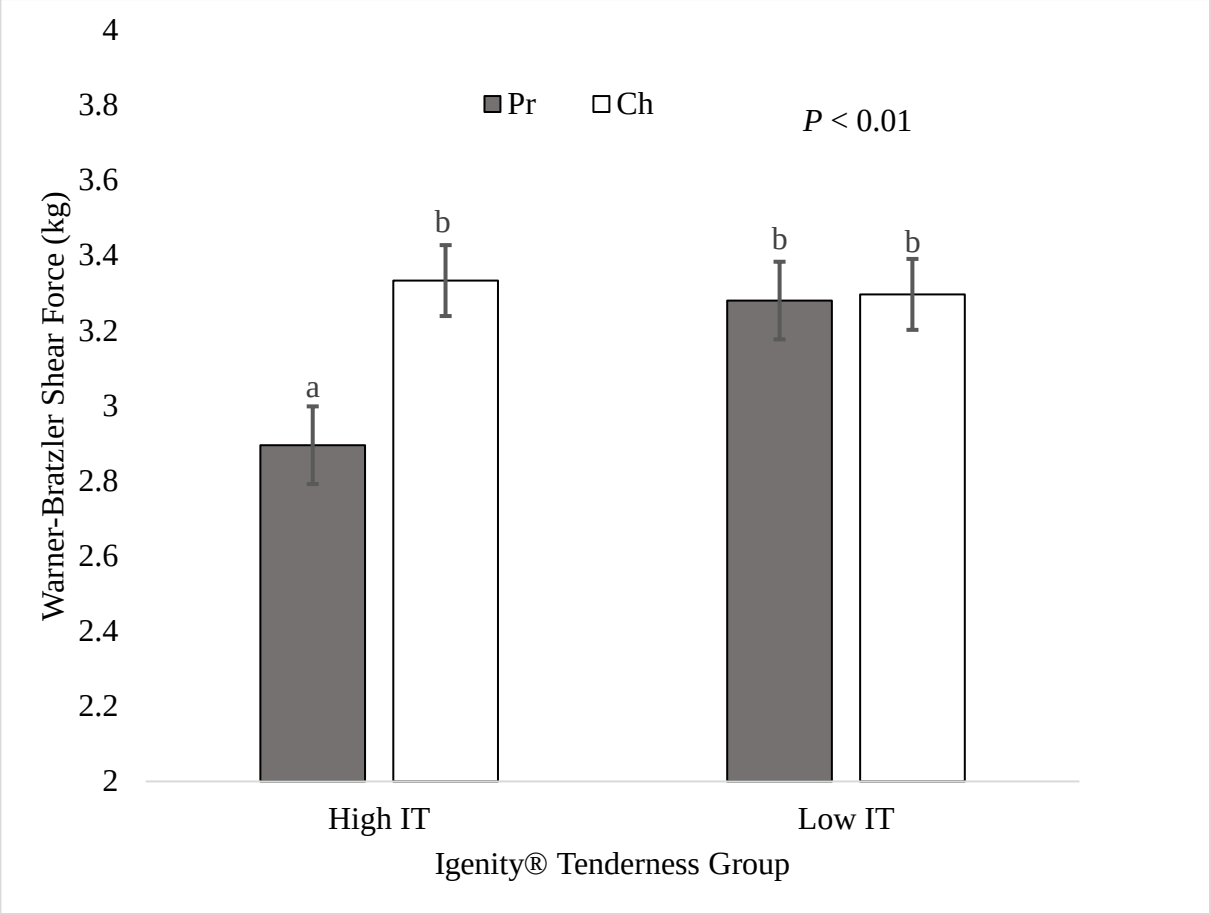


Figure 2.1. Warner-Bratzler Shear Force (WBSF) values for Igenity® (IT) tenderness group [Low IT (IT tenderness index scores 3-6) and High IT (IT index scores 7-10)] x USDA Quality Grade. The High IT group had 12 USDA Choice carcasses and 10 USDA Prime carcasses, whereas the Low IT group had 20 USDA Choice carcasses and 10 USDA Prime carcasses. Gray bars show USDA Prime carcasses, and white bars show USDA Choice carcasses.

Appendices

Appendix A. Exempt certification for IRB project number 18-158

University *of* Idaho
Office of Research Assurances
Institutional Review Board
875 Perimeter Drive, MS 3010
Moscow ID 83844-3010
Phone: 208-885-6162
Fax: 208-885-5752
irb@uidaho.edu

To:	Phillip Bass, Ph.D.
Cc:	Michael James Colle, Tanya Weber
From:	Jennifer Walker, IRB Coordinator
Approval Date:	September 20, 2018
Title:	Evaluation of beef loin strip loin steaks selected from beef carcasses of known tenderness values.
Project:	18-158
Certified:	Certified as exempt under category 6 at 45 CFR 46.101(b)(6).

On behalf of the Institutional Review Board at the University of Idaho, I am pleased to inform you that the protocol for the research project Evaluation of beef loin strip loin steaks selected from beef carcasses of known tenderness values. has been certified as exempt under the category and reference number listed above.

This certification is valid only for the study protocol as it was submitted. Studies certified as Exempt are not subject to continuing review and this certification does not expire. However, if changes are made to the study protocol, you must submit the changes through VERAS for review before implementing the changes. Amendments may include but are not limited to, changes in study population, study personnel, study instruments, consent documents, recruitment materials, sites of research, etc. If you have any additional questions, please contact me through the VERAS messaging system by clicking the 'Reply' button.

As Principal Investigator, you are responsible for ensuring compliance with all applicable FERPA regulations, University of Idaho policies, state and federal regulations. Every effort should be made to ensure that the project is conducted in a manner consistent with the three fundamental principles identified in the Belmont Report: respect for persons; beneficence; and justice. The Principal Investigator is responsible for ensuring that all study personnel have completed the online human subjects training requirement.

You are required to timely notify the IRB if any unanticipated or adverse events occur during the study, if you experience and increased risk to the participants, or if you have participants withdraw or register complaints about the study.

Appendix B. Consumer sensory panel consent form

SENSORY PANEL CONSENT FORM

1. The University of Idaho Institutional Review Board has reviewed and found this study to be exempt.

2. The objective of this study is to evaluate the ability to predict tenderness based on genetic markers. The samples will be prepared under the Research Guidelines for Cookery, Sensory Evaluation, and Instrument Tenderness Measurements of Fresh Meat, as outlined by the American Meat Science Association.

3. You will be asked to evaluate 3 samples (approximately 1" x ½" x ½") per session for tenderness (1 = extremely tough to 10 = extremely tender), juiciness (1 = dry to 10 = juicy), and flavor (1 = bland to 10 = intense) using a 10 point scale. It is not necessary that samples be ingested. The study should take approximately 15 to 20 minutes.

4. Although there are no or minimal risks associated with the project, it is possible that some samples will have one or more qualities that may not be appealing to you (e.g. tenderness or juiciness that is less than you would prefer).

5. With your help, society can benefit from our attempt to improve the understanding of genetic prediction technology.

6. We anticipate that samples will be well received by panelists. However, if we find during the course of the taste panel that samples are unappealing, we will stop the evaluation process.

7. To maintain anonymity of the data collected during this evaluation, all the information you provide will be placed in a locked file with Dr. Bass.

8. If you have questions about the taste panel, you can ask the investigator during the evaluation, when the evaluation is complete or at a time you feel is appropriate.

9. Contact information for the University of Idaho faculty member leading this research:
   Dr. Phil Bass
   University of Idaho
   Department of Animal and Veterinary Science
   Moscow, ID 83844
   208-885-0990

10. During the course of this taste panel, you may terminate participation at any time. If you choose to do so, please notify the investigator that you no longer wish to participate.

11. If you choose to terminate participation in this evaluation, there will be no penalties associated with your withdrawal.

I have reviewed this consent form and understand and agree to its contents.

Participant Name: _____ Date: _____

Signature: _____

Appendix C. Compusense sensory panel ballot

Generated by Compusense Cloud

Welcome to our Steak Tasting Panel!

Click the *next* button to begin

Please enter your wsuguest number (for example, 10):

Is your guest number (?)

If the number on the screen does not match the guest number on your consent form, please signal the experimenter.

123

Please indicate your gender.

- Male
- Female
- Prefer not to answer

What is your age (in years)?

Please indicate the number of meals a week in which you consume beef

- 0-1
- 2-4
- 5-7
- 8+

Please indicate the form in which you most commonly consume beef

- Ground
- Roast
- Steak
- Other

You will be evaluating 3 samples of steak today.

When you are ready for your first sample, press the blue button to signal the experimenter and click next to begin

How much do you like or dislike the sample OVERALL?

Sample: BC111

Dislike Extremely	Dislike Strongly	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Strongly	Like Extremely

How much do you like or dislike the TENDERNESS of the sample?

Sample: BC111

Dislike Extremely	Dislike Strongly	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Strongly	Like Extremely

How much do you like or dislike the JUCINESS of the sample?

Sample: BC111

Dislike Extremely	Dislike Strongly	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Strongly	Like Extremely

How much do you like or dislike the FLAVOR of the sample?

Sample: BC111

Dislike Extremely	Dislike Strongly	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Strongly	Like Extremely

Do you detect an off flavor in the sample?

Sample: BC111

- ○ NO
- ○ YES

Would you be willing to purchase this product?

Sample: BC111

- ○ NO
- ○ YES

If applicable, please select the trait you liked least about the product.

Sample: BC111

- [ ] Flavor
- [ ] Tenderness
- [ ] Juciness
- [ ] Texture/ Mouthfeel
- [ ] Not Applicable

If applicable, please select the trait you liked most about this product.

Sample: BC111

- [ ] Flavor
- [ ] Tenderness
- [ ] Juciness
- [ ] Texture/ Mouthfeel
- [ ] Not Applicable

Overall comments on the product:

Sample: BC111

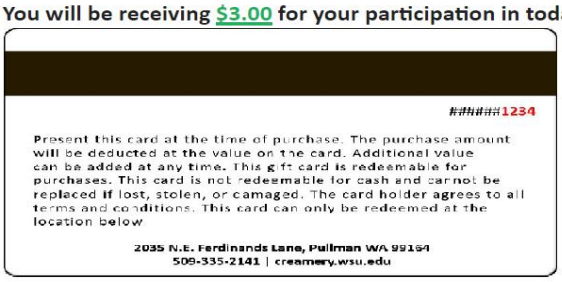
Please take a few seconds to rinse your palate with water and crackers before the next sample. Signal the experimenter when you are ready to proceed.

0:30

Please press the Blue Button

If you have your Ferdinand's Card enter the last four digits on the back of the card.
If you don't have a card with you, we will provide you with one. Then please enter the last four digits.

You will be receiving $3.00 for your participation in today's panel.



Thank you for completing this test!

Please: 1) select 'finish' at the bottom of the screen.

2) select 'sign out' on the next page.

3) Push the blue switch to signal your experimenter.


Finished
Back

Appendix D. Exempt certification for IRB project number 19-182

University of Idaho
Office of Research Assurances
Institutional Review Board
875 Perimeter Drive, MS 3010
Moscow, ID 83844-3010
Phone: 208-885-6162
Fax: 208-885-6014
Email: irb@uidaho.edu

To: Michael James Colle

From: University of Idaho Institutional Review Board

Approval Date: September 05, 2019

Title: Using Genetic Panels to Predict Tenderness in Beef Cattle

Project: 19-182
Certified: Certified as exempt under category 6 at 45 CFR 46.104(d)(6).

On behalf of the Institutional Review Board at the University of Idaho, I am pleased to inform you that the protocol for this research project has been certified as exempt under the category listed above.

This certification is valid only for the study protocol as it was submitted. Studies certified as Exempt are not subject to continuing review and this certification does not expire. However, if changes are made to the study protocol, you must submit the changes through VERAS for review before implementing the changes. Amendments may include but are not limited to, changes in study population, study personnel, study instruments, consent documents, recruitment materials, sites of research, etc.

As Principal Investigator, you are responsible for ensuring compliance with all applicable FERPA regulations, University of Idaho policies, state and federal regulations. Every effort should be made to ensure that the project is conducted in a manner consistent with the three fundamental principles identified in the Belmont Report: respect for persons; beneficence; and justice. The Principal Investigator is responsible for ensuring that all study personnel have completed the online human subjects training requirement. Please complete the *Study Status Check and Closure Form* in VERAS when the project is completed.

You are required to timely notify the IRB if any unanticipated or adverse events occur during the study, if you experience and increased risk to the participants, or if you have participants withdraw or register complaints about the study.

Appendix E. IACUC 2017-32 approval letter

University of Idaho
Institutional Animal Care and Use Committee

Date: July 25, 2017
To: Anne Hermen Laarman
From: University of Idaho
Institutional Animal Care and Use Committee
Re: IACUC-2017-32 *Controlling Salmonella spp. invasion in deep lymphoid tissue in beef carcasses*

Your animal care and use protocol for the project shown above was reviewed and approved by the Institutional Animal Care and Use Committee on 07/24/2017.

The original approval date for this protocol is: 07/24/2017
This approval will remain in effect until: 07/23/2018
The protocol may be continued by annual updates until: 07/23/2020

Federal laws and guidelines require that institutional animal care and use committees review ongoing projects annually. For the first two years after initial approval of the protocol you will be asked to submit an annual update form describing any changes in procedures or personnel. The committee may, at its discretion, extend approval for the project in yearly increments until the third anniversary of the original approval of the project. At that time, the protocol must be replaced by an entirely new submission.

Craig McGowan, IACUC Chair

Appendix F. Consumer demographics questionnaire

CONSUMER EVALUATION OF BEEF QUALITY

Panelist #: _____ Date: _____

Age: _____ Gender: _____

Please indicate the number of meals a week in which you consume beef:

0-1 2-4 5-7 8+

Please indicate the form in which you most commonly consume beef:

Ground Roast Steak Other

Appendix G. Beef consumer sensory panel questionnaire

CONSUMER SENSORY PANEL QUESTIONAIRRE

Sample ID #:_____

1. **OVERALL ACCEPTABILITY OF SAMPLE:** This is based on your overall acceptability of the sample

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

(Dislike extremely) (Like extremely)

2. **TENDERNESS:** This is based on your overall opinion of the sample's tenderness

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

(Dislike extremely) (Like extremely)

3. **JUICINESS:** This is based on your overall opinion of the sample's juiciness

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

(Dislike extremely) (Like extremely)

4. **FLAVOR:** This is based on your overall opinion of the sample's flavor

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

(Dislike extremely) (Like extremely)

5. **OFF-FLAVOR:** This is based on your ability to detect an off-flavor of the sample

NO YES

6. **CONSUMER SATISFACTION:** Would you be willing to purchase this product?

NO YES

7. **IF APPLICABLE,** please circle the trait you liked **least** about this product.

Flavor Tenderness Juiciness Texture/Mouth Feel

8. **IF APPLICABLE,** please circle the trait you liked **most** about this product.

Flavor Tenderness Juiciness Texture/Mouth Feel

9. **Overall Comments on Product:**

Thank you for taking the time to participate in this sensory panel