Relationships between bovine phenotype, production practices, muscle proteins and the incidence of dark cutting beef

by

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Abstract

Most recent reports indicated that the prevalence of dark cutting beef has increased in Canada and that 0.5% reduction in the problem could save CAD$1.77 million per annum. Understanding the underlying factors associated with the persistence and recent escalation in the occurrence of dark-cutting is essential; therefore, a series of studies addressing various hypotheses to highlight the factors linked to dark cutting were executed.

The initial study revealed a trend for increased dark cutting in heifers than in steers. Cattle with increased DMI and having carcass weight greater than 300 kg were less likely to produce dark cutting. Heifers at risk of dark cutting had reduced weaning weight, slaughter weight, and produced carcasses of less weight. Intramuscular fat had no significant association with the manifestation of cutting dark. However, cattle slaughtered at a live weight greater than 550 kg were less predisposed to cut dark. It was revealed that there is a potential to identify ultimate carcass grades and predisposition to cut dark in live cattle by weighing and ultrasonically measuring subcutaneous fat depth, intramuscular fat, and muscle score.

Survey of feedlots in Alberta further substantiated that heifers especially those killed as calf-fed had increased susceptibility to dark cutting whereas steers were less predisposed to produce dark carcasses, irrespective of the rearing system. There was no effect of hormonal-growth implants on the incidence of dark cutting likely because their use was in compliance with manufacturer's instructions. Similarly, beta-agonists that may influence intramuscular fat deposition and carcass grades did not affect the incidence of dark cutting. Slow growing cattle appeared to be at risk of cutting dark but frequent shipping and extended stay in lairage substantially increased the likelihood of dark beef.

The occurrence of typical dark cutting (pH > 5.9) beef was associated with a reduced concentration of muscle glucidic potential. However, such beef was as tender as normal beef after storage at refrigerated temperature for 21 days. Conversely, the atypical dark cutting beef (pH < 5.9) had glucidic potential theoretically sufficient to produce normal-coloured beef, but this beef did not acquire tenderness to the level similar to that of normal beef even after 21 days of storage. Increased toughness of atypical dark beef was independent of cattle sex and carcass phenotype. Extended lairage and/or frequent shipping negatively influenced muscle glycogen essential for post-mortem glycolysis and lowering of carcass pH.

Proteomics of the *longissimus thoracic*, using two-dimensional gel electrophoresis coupled with LC-MS/MS mass spectrometry, revealed reduced abundances of creatine kinase, glyceraldehyde-3-phosphate dehydrogenase, lactate dehydrogenase and glycerol-3-phosphate dehydrogenase [NAD(+)] in atypical beef, implying a compromised glycolytic capability in atypically dark muscles. The atypical dark beef had increased levels of phosphatidylethanolamine-binding protein 1 and small heat shock proteins that might have compromised proteolysis and beef tenderness. The study suggested a disparity in muscle proteins and post-mortem muscle metabolism between normal and dark cutting beef.

Preface

Dark cutting continues to occur in beef despite significant research and is causing economic loss to beef industry around the globe. The causes and prevalence of dark-cutting beef differ across the countries where the associated factors may be numerous. A comprehensive study incorporating a range of potential factors was necessary to establish the most important factors responsible for the recent increase in dark-cutting in Canada specifically and the persistence of dark cutting in the beef industry in general. This thesis incorporates multiple studies to address various hypotheses. For chapter 2 and 3, the data were provided by Dr. John Basarab from Alberta Agriculture and Rural Development Department. These two chapters have been published in peer-reviewed journals where Dr. John Basarab, Dr. Walter Dixon, and Dr. Heather Bruce are co-authors.

For chapter 4, the feedlot information was collected by surveying the feedlots during the months of January and February 2014. The information was recorded using a questionnaire approved by the Human Ethics Board of the University of Alberta. The farmers gave their consent to use the data for statistical analyses and subsequently disseminate the information.

For chapter 5, beef samples were collected from a federally inspected commercial beef abattoir where the cattle were slaughtered in compliance with the Canadian Council on Animal Care guidelines. I actively participated in the whole sampling and lab procedures. Dr. Bimol Roy, Dr. Chamali Das, Jennifer Potter, Melissa Anne Marquez and Ting Ting assisted in doing beef quality and proximate analyses performed at in labs at Agri-Food Discovery place and the Department of Agriculture, Food, and Nutritional Science, University of Alberta, respectively. I performed muscle glucidic analysis with the assistance of Ms. Ivy Larsen and under the

supervision of Dr. Jennifer Aalhus at Lacombe Research Centre of Agriculture and Agri-Food Canada. This chapter has been published in a peer-reviewed journal where Dr. Bimol Roy, Ivy Larsen, Dr. Jennifer Aalhus, Dr. Walter Dixon and Dr. Heather Bruce are the co-authors.

For muscle proteomics (chapter 6), the samples analyzed were collected from a beef abattoir and the details of collection are presented in chapter 5. Nancy Turchinsky assisted in doing the experiment where Dr. Francois Paradis aided in analyzing the gel images. The identified protein spots were sent to Proteomics Platform, Ste-Foy, Quebec, Canada, for protein identification using LC/MS/MS. I executed all the statistical analyses and their interpretation. This chapter has been submitted to a peer-reviewed journal for review and publication, and Nancy Turchinsky, Dr. Francois Paradis, Dr. Walter Dixon and Dr. Heather Bruce are the co-authors.

Shahid Mahmood

To my late father, Sabir Ali

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Abbreviations

2-D gel	Two-dimensional gel electrophoresis
A	Canada beef grade having “traces” of marbling
a*	Redness
AA	Canada beef grade having “slight” marbling
AAA	Canada beef grade having “small” marbling
AB4	Atypical dark (pH < 5.9)
ACTA1	Actin, alpha skeletal muscle
ADG	Average daily gain
AK1	Adenylate kinase isoenzyme 1
B4	Canada dark-cutting beef
b*	Yellowness
BY	Backgrounded-yearling cattle fed dry forage before feedlot
Calf-fed	Cattle slaughtered as calf-fed
CBGA	Canada Beef Grading Agency
CCAC	Canadian Council on Animal Care
CFIA	Canadian Food Inspection Agency
CKM	Creatine kinase M-type
CRYAB	Alpha-crystallin B chain
CW	Hot carcass weight
DES	Desmin
DF	Days to finishing (number of days cattle were fed a concentrate diet)
DFD	Dark, firm and dry beef
DMb	Deoxymyoglobin
DMI	Dry matter intake
DTT	Dithiothreitol
FC	Fall calf cattle entering into feedlot after weaning and killed as calf-fed

FCR	Feed conversion ratio
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
gFD	Grade or carcass subcutaneous fat depth
gMS	Grade marbling score
GPD1	Glycerol-3-phosphate dehydrogenase [NAD(+)]
gREA	Grade rib eye area
GY	Grass-fed yearling cattle grazed on pasture before feedlot
GYG1	Glycogenin 1
HSPB1	Heat shock 27kDa protein 1
Imp1	Cattle receiving one hormonal implant
Imp2	Cattle receiving two hormonal implants
Imp3	Cattle receiving three hormonal implants
L*	Lightness
LDHA	L-lactate dehydrogenase A
LT	Longissimus thoracis
LW	Live weight at slaughter
MGA	Melengestrol acetate (MGA)
MMb	Metmyoglobin
MYL1	Myosin light chain 1/3
MYLPF	Myosin regulatory light chain 2
MYOM1	Myomesin-1
OMb	Oxymyoglobin
Opt	Optaflexx®
PCA	Principal Component analysis
PEBP1	Phosphatidylethanolamine-binding protein 1
pI	Isoelectric focusing point
PMSF	Phenylmethylsulfonyl fluoride

PPM1B	Isoform beta-2 of protein phosphatase
PRDX1	Peroxiredoxin-1
PRDX3	Thioredoxin-dependent peroxide reductase
Prime	Canada beef grade having “abundant” marbling
RAN	RAN, member RAS oncogene family
RFI_{fat}	Residual feed intake adjusted for ultrasound back fat
SA	Slaughter age
SDS	Sodium dodecyl sulfate
SMS	Spermine synthase
TA	Test age or age when cattle started receiving concentrate diet
TB4	Typical dark (pH > 5.9)
TPM1	Tropomyosin alpha-1 chain
uFD	Subcutaneous fat depth measured using ultrasound in live cattle
uMS	Marbling score measured using ultrasound
uREA	Rib eye are measured using ultrasound
USDA	The United States Department of Agriculture
WBSF	Warner-Bratzler shear force
WC fed	Winter calf cattle receiving dry forage before feedlot and killed as calf-fed
WW	Weaning weight
Year-fed	Cattle raised to be slaughtered as yearling-fed
YWHAG	14-3-3 protein gamma

1.0. Introduction

1.1. Dark cutting beef

Beef carcasses are graded based on rib-eye (m. *longissimus thoracis*; LT) characteristics such as fat depth, marbling score and colour of fat and muscle. Beef grading is usually performed 24-48 h post-mortem, by trained personnel, after the carcasses are ribbed between 12^{th} and 13^{th} rib and the cut surface is exposed to air for about 10 minutes. Carcasses from cattle ≤ 30 months old qualifying for white fat and pink or cherry-red lean are further categorized, based upon marbling score, into Canada Prime, AAA, AA, and A which are equivalent to USDA (the United States Department of Agriculture) Prime, Choice, Select and Standard carcasses, respectively. Carcasses having dark red LT, despite normal white fat, are downgraded as Canada B4 (Canadian Agricultural Products Act, SOR/92-541 2014), and are also called dark cutting (Hall et al. 1944). Dark cutting beef carcasses usually have an ultimate intramuscular pH greater than 5.8 and may be dry and firm hence the name of dark, firm and dry (DFD) beef.

Fresh meat with bright red colour is more appealing whereas dark meat is discriminated against by consumers who consider it stale and spoiled (Viljoen et al. 2002). Myoglobin in dark meat is also not fully denatured upon cooking and yields an uncooked appearance (Moiseev and Cornforth 1999). Animal muscles contain about 1% glycogen by weight (Morgan 1997), and dark cutting usually occurs due to pre-slaughter muscle glycogen depletion (Lawrii 1983) that leads to low lactate production during post-mortem glycolysis and consequently increased carcass pH (Wulf et al. 2002). Beef with high pH has high water content (Aberle et al. 2001) and a reservoir of hydroxyl and oxygen ions that may promote lipid peroxidation (Bekhit et al. 2003) and rancidity (Wood et al. 2008). Apart from high pH, dark meat and its products may deteriorate earlier also because of aerobic bacteria, that primarily use glucose, degrade amino

acids in the absence of carbohydrate and produce ammonia and a decay odor (Newton and Gill 1981). Anaerobic bacteria rapidly deteriorate high pH meat packed under vacuum and as a result cause a greenish colour, an off-flavour and a foul smell (Newton and Gill 1981). Dark cutting beef is therefore disliked not only by consumers but also retailers because of its reduced shelf life.

1.2. Economic implications of dark cutting

Dark cutting beef is sold at a discounted price and directly costs beef producers. Historical incidence of dark cutting was 0.8% in the Canadian beef industry (Jones and Tong 1989), but the problem increased to 1% during 1995-96 and 1998-99 (Van Donkersgoed et al. 1997 and 2001) and the most recent survey reported 1.28% dark cutting in Canada (Beef Cattle Research Council 2013). The incidence of dark cutting has thus not only been sustained but has also increased from 0.8% to 1.28% in the Canadian beef industry. In the United States of America, the recorded incidence is as high as 3.2% (Moore et al. 2012) while in Australian it is 10% of the beef carcass population (MSA 2010; Hughes et al. 2014). The price discount applied to dark cutting carcasses is up to 10% in Chile (Gallo et al. 2003), 30 to 60% in Spain (Mach et al. 2008), about AUS$0.50 per kg in Australia (McGilchrist et al. 2012) and CAD$ 0.55 per kg in Canada. Reports indicated that the Canadian beef industry could save CAD$1.77 million per annum if dark cutting is lowered from current 1.28% to a historical 0.8% (Beef Cattle Research Council 2014). For recapturing the lost revenue, further research is obligatory to explore the factors linked to the persistence and current escalation in dark cutting, especially in Canadian beef industry.

1.3. Factors influencing meat colour

Muscle proteins, pre-slaughter animal handling, cattle management and post-mortem interventions have a direct or indirect role in determining final muscle colour and beef quality. Some important factors are being introduced here.

1.3.1. Muscle pigments

Beef color is influenced by cytochromes, myoglobin concentration (Young et al. 1999) and relative proportions of deoxy- (DMb), oxy- (OMb) and metmyoglobin (MMb) (Muir et al. 1998). Myoglobin, the main meat pigment, is an intracellular heme protein having a central heme iron that interacts with four nitrogen atoms of porphyrin ring, the fifth ligand with Histidine (His8) and sixth ligand with molecular oxygen (Pratt and Cornely 2004). Heme iron Fe^{+2} (redox) without a molecular oxygen ligand results in DMb and purple-red color (Mancini and Hunt 2005). Post-mortem muscle exposure to air causes conversion of DMb to OMb, which imparts an appealing bright red colour to meat, in a process termed "blooming" (Egbert and Cornforth 1986; Keeffe and Hood 1982; Mancini and Hunt 2005). At low oxygen concentration, heme iron (Fe^{+2}) can be oxidized (Fe^{+3}), which results in MMb formation (Keeffe and Hood 1982; Mancini and Hunt 2005). At OMb < 50% and MMb ≥ 20%, the colour is unacceptably dark red and meat is termed dark cutting (Keeffe and Hood 1982; Moiseev and Cornforth 1999; Van Den Oord and Wesdorp 1971). Moreover, an increased muscle pigment level also presents a saturated red or opaque and consequently dark appearance (MacDougall and Rhodes 1972).

1.3.2. Post-mortem muscle pH

Muscle ATP reserves quickly diminish post-mortem (Scheffler et al. 2011) and ATP supply is then maintained by phosphocreatine (Pcr) and conversion of ADP to ATP with the help of creatine kinase and myokinase (Bendall 1973; Scheffler et al. 2011). Subsequently, ATP is

sourced from anaerobic glycolysis (Scheffler et al. 2011) that also causes lactate accumulation and lowering of intramuscular pH (Wulf et al. 2002; Rhoades et al. 2005) from about 7.0 to an ultimate (24-48 h post-mortem) pH value 5.74-5.5 (Bodwell et al. 1965; Wulf et al. 2002). The drop in muscle pH is as a result of an increase in H^+ concentration (Orcutt et al. 1984). Mitochondrial respiration, which determines the rate of oxygen consumption (Bendall and Taylor 1972), drops post-mortem due to low pH (Ashmore et al. 1972 and 1973) and the spared-oxygen forms OMb and produces the cherry red colour on meat surface (Ashmore et al. 1972; Egbert and Cornforth 1986).

Post-mortem glycolysis continues until muscles cool to 2.8°C (Bodwell et al. 1965), their glycogen reserves diminish, or the muscle attains a low pH (≈5.5) when glycolytic enzymes inactivate (Wulf et al. 2002). Low pH reduces cooperation among subunits of 6-phosphofructokinase (6-PFK) and their affinity for fructose-6-phosphate (Rhoades et al. 200). Deamination and loss of AMP may also lower post-mortem glycolysis (Bendall 1973). Anaerobic glycolysis results in the conversion of glycogen to free glucose, glucose-6-phosphate, fructose-6-phosphate and lactate where the sum of these metabolites plus residual glycogen remains constant and is called glucidic potential (Bodwell et al. 1965; Rhoades et al. 2005). Beef carcasses with normal colour and low ultimate pH may have glucidic potential up to 100µmol glucose g^{-1} muscle (Wulf et al. 2002), but glucidic potential 41.2 µmol g^{-1} glucose is sufficient to attain ultimate pH 5.57 (Bodwell et al. 1965). Beef has normal colour at ultimate pH 5.40 to 5.59 (Page et al. 2001) but is dark at pH > 5.87 (Page et al. 2001). However, 44% beef carcasses graded dark in Canada had ultimate pH < 5.8 **(**Murray 1989) and intramuscular pH ranged 5.4 to 5.6 in 1 to 5% carcasses graded dark in Australia (Hughes et al. 2014). Holdstock et al. (2014) identified dark carcasses from steers which had pH ≤ 5.8, but glucidic potential similar to Canada

AA, and those carcasses were termed atypical dark (AB4), implying that dark cutting problem is not only related to reduced glucidic potential.

1.3.3. Relationship between temperature, pH, and colour

Dark cutting may be associated with increased myoglobin and high pH (Moiseev and Cornforth 1999), both of which are favourable for a high rate of oxygen consumption (McKenna et al. 2005). The oxygen consumption is reduced at low pH (Tang et al. 2005) due to inactivation of mitochondrial enzymes (Ashmore et al. 1972; Bendall and Taylor 1972). Low oxygen consumption at low pH increases OMb and improves muscle colour (Hanson et al. 2001; Young et al. 1999; Sammel et al. 2002; Wulf et al. 1997). Dark colouration is, however, lowered at low pH and high temperature (Hughes et al. 2014) because low pH along with high temperature enhances denaturation of muscle proteins and consequently increase open structures that lead to increased oxygen penetration and OMb formation (Farouk and Lovatt 2000; Sammel et al. 2002). The post-mortem pH decline is accelerated by high temperature (Bruce and Ball 1990; Young et al. 1999) due to increased rate of glycolysis (Orcutt et al. 1984) and as a result, improves meat colour (Janz et al. 2001). Dark cutting despite low pH could be due to a slow rate of post-mortem pH decline.

1.3.4. Pre-slaughter handling and muscle glycogen

Cattle may be disstressed by social isolation, unfamiliar environment, regrouping, and confinement, (Apple et al. 2005; Terlouw et al. 2012; Sanz et al. 1996). Moreover, transportation (Cook et al. 2009), restraint (Apple et al. 2005), mixing (McVeigh et al. 1982), loading and unloading (Averos et al. 2007) and withdrawal of food and water (Schaefer et al. 2001) are also potential pre-slaughter stressors. Physical and psychological stress may lower muscle glycogen by increasing glycogenolysis (Apple et al. 2005; Barth et al. 2007; Immonen et al. 2000). Low

muscle glycogen may limit postmortem glycolysis, the accumulation of lactate, and the extent of muscle pH decline (Immonen et al. 2000; McVeigh et al. 1982), and lead to dark cutting (Apple et al. 2005; Ashmore et al. 1972; MacDougall and Rhodes 1972). Cattle shipped over a short distance and slaughtered after a brief time in lairage may be at a decreased risk of cutting dark (Murray 1989; Sanz et al. 1996) compared to those transported for long time and distance (Puolanne and Aalto 1981; Jones and Tong 1989). Thus transportation distance and subsequent stay in lairage may have a significant role in determining dark cutting. The hypothesis that lack of glycogen recovery due to fasting (McVeigh and Tarrant 1982) and short lairage time is associated with atypical dark cutting (Holdstock et al. 2014) needs further research.

1.3.5. Nutrition and age

Grains or an energy-rich diet increases carcass weight and fat thickness and lowers carcass pH and improves meat colour (Muir et al. 1998; Bruce et al. 2004). Moreover, increased energy intake increases average daily gain, muscle glycogen level, and post-stress glycogen repletion rate (Apaoblaza and Gallo 2014; Pethick et al. 1999; Knee et al. 2004). A high energy diet also increases muscle glycolytic capacity (Branstetter et al. 199) and protects glycogen depletion due to handling and fasting (Apaoblaza and Gallo 2014; Immonen et al. 2000; Jacob et al. 2001). However, grain feeding over 1.5% of body weight may cause acidosis and lower the secretion of insulin (Brown et al. 2000) required to activate glycogen synthase (John et al. 1997), and consequently glycogen repletion rate. Beef cattle are finished as yearling-fed or calf-fed and slaughtered respectively at ages between 14 and 24 months in Canada. Reports indicated a higher likelihood of dark cutting in older animals than in young beef cattle and veal calves (Hughes et al. 2014; McGilchrist et al. 2012), but young cattle are difficult to handle (Grandin 1993) and

may have decreased muscle glycogen (Miller et al. 1987). The persistence of dark cutting, therefore, could be related to cattle production systems and feeding strategies.

1.3.6. Animal and carcass conformation

Heavy and well-fed cattle are not as prone to cutting dark as light weight cattle (Jones and Tong 1989; McGilchrist et al. 2012; Murray 1989; Puolanne and Alto 1981), but Hughes et al. (2014) did not find any relationship between meat colour and dark cutting with carcass weight and fat depth. Increased fat cover may lower the incidence of dark cutting not only because it represents better nutrition (McGilchrist et al. 2012; Orcutt et al. 1984; Puolanne and Alto 1981) but also because it lowers carcass chilling rate (Aalhus et al. 2001), ultimate pH and improves meat colour (Cliplef et al. 1989; Page et al. 2001). Increased carcass weight and muscling lower chilling rate and the likelihood of dark cutting (Farouk and Lovatt 2000; McGilchrist et al. 2012; Murray 1989; Orcutt et al. 1984). Increased muscling is associated with increased muscle glycogen and reduced glycolytic response to adrenaline (McGilchrist et al. 2011), suggesting that reduced muscling may predispose cattle to deplete muscle glycogen and cut dark as a result of pre-slaughter handling stress.

Muscle marbling score may enhance muscle colour score (Wulf and Wise 1999: Bruce et al. 2004; Moiseev and Cornforth 1999) but has no relationship with ultimate muscle pH (Wulf and Wise 1999). Instead, increased intramuscular fat is associated with increased oxidative metabolism (Jurie et al. 2007; Lefaucher 2010) and reduced muscle glycogen (Underwood et al. 2007) suggesting that increased marbling may limit post-mortem glycolysis and the extent of pH decline.

1.3.7. Effect of cattle sex

Bullocks may have higher pH and darker muscle colour than heifers and steers (Page et al. 2001) likely because bulls have volatile behaviour that may lower muscle glycogen (Immonen et al. 2000). Steer carcasses reportedly had highest colour values where cows and bulls did not differ (Kim et al. 2003), but the dark cutting incidence was greater in bulls (11-15%) than cows (6-10%) (Tarrant 1981). Dark cutting ranges 1-5% in heifers and steers (Tarrant 1981), but heifers may be at greater risk than steers to cut dark (Murray 1989; Scanga et al. 1998). Contrarily, studies also indicated that steers tended to have increased post-mortem intramuscular pH and predisposition to dark cutting (Jones and Tong 1989; Mach et al. 2008). Increased dark cutting in heifers has been associated with their oestrus activity and excitable temperament (Kenny and Tarrant 1988; Scanga et al. 1998; Voisinet et al. 1997). Melengestrol acetate (MGA) feeding at a dose of 0.5mg per animal may suppress oestrus (Meyer 2001), but withdrawal of MGA for more than 48 h prior to slaughter may result in estrus (Hill et al. 1971; O'Brien et al. 1968; Popp et al. 1997) and aggravate dark cutting. Sex effect on dark cutting may be due to oestrus management in heifers but needs to be established.

1.3.8. Season effect

Extreme cold and hot weather may compromise muscle glycogen concentration and consequently increase post-mortem muscle pH (Immonen et al. 2000; Kim et al. 2003; Mounier et al. 2006). Jones and Tong (1989), however, observed the lowest incidence of dark cutting in December (Northern Hemisphere winter) and the highest in March and April (Northern Hemisphere spring). Contrarily, Tarrant and Sherington (1980) reported four times higher incidence in September to January (5.2%, Northern Hemisphere fall/winter) than in February to August (1.2%, Northern Hemisphere winter/spring/summer). The lightness of Hanwoo steer

carcasses was not influenced by season, but cows had darker lean in winter while bulls had dark muscles in both summer and winter compared to spring and fall season (Kim et al. 2003), implying that season may interact with cattle sex. Studies also indicated that seasonal effect is due to variation in availability of nutrition (Knee et al. 2004; Tarrant and Sherington 1980) and attaining adequate animal/carcass weight (Murray 1989). Literature contradictions about season effect could be due to geographical locations, where the studies were conducted, and the availability of nutrition (Knee et al. 2004).

1.3.9. Effect of growth promotants

Hormonal growth implants (androgenic and estrogenic) and beta-adrenergic agonists (β-agonist) are being used in the beef industry, especially in North America. B-agonists increase protein deposition and hypertrophy of skeletal muscles (Johnson and Chung 2007; Williams 1987) without increasing the number of satellite cells or fiber nuclei (Gonzalez et al. 2007) but at the expense of lipogenesis (Strydom et al. 2009). Estrogenic and androgenic implants increase average daily gain, live weight and carcass weight (Bruns et al. 2005; Hardt et al. 1995; Hunter 2010) by increasing insulin-like growth factors (IGFs) (Dikeman 2007; Knetter et al. 2012), satellite cell proliferation (Hunter 2010), retention of essential amino acids (Meyer 2001) and feed efficiency (Bruns et al. 2005). Literature indicated β-agonist use increases animal restlessness, heart rate, respiration rate, lameness, and susceptibility to heat stress (Meyer 2001; Grandin 2010), and lowers muscle glycogen (Williams 1987). Studies also suggested that growth implants can lower carcass colour score (Herschler et al. 1995) and increase the incidence of dark cutting (Scanga et al. 1998; Schneider et al. 2007). The effect of growth implants on dark cutting, however, may be subject to available nutrition, antemortem stress and the length of time between terminal implant and slaughter (Morgan 1997; Scanga et al. 1998; Hunter 2010). These

studies lead to speculation that the persistence of dark cutting may be related to the extensive use of growth promotants coupled with inadequate diet but this postulation needs investigation.

1.3.10. Cattle breed and intrinsic properties of muscles

Cattle breeds differ in carcass pH likely because of difference in their temperament and response to handling (Grandin 1993; Wulf et al. 1997). Calm animals exhibit low-stress response (Fazio et al. 2012) and are less likely to deplete muscle glycogen (Immonen et al. 2000) and cut dark (Kenny and Tarrant 1988; Voisinet et al. 1997). British breeds (Angus and Hereford) are calmer than continental Charolais, Limousin, Simmental and Blonde d'Aquitaine cattle (Hoppe et al. 2010; Terlouw et al. 2012). European cattle (Limousin) in contrast to British breed (Angus) have greater muscle glycolytic metabolism (Jurie et al. 2007) likely because of their increased musculature that is also associated with increased muscle glycogen and myosin ATPase activity (Choi and Kim 2009; Lefaucher 2010; McGilchrist et al. 2011). Increased concentration of muscle glycolytic enzymes has been associated with rapid postmortem pH decline (Gagaoua et al. 2015) and muscle colour stability (Canto et al. 2015), implying that ultimate muscle pH may be influenced by muscle metabolic characteristics, musculature and cattle breeds.

1.4. Effect of dark cutting on beef quality

Dark cutting had no effect on the juiciness of steaks (Wulf et al. 2002; Viljoen et al., 2002) and beef patties (Moiseev and Cornforth, 1999). Dark cutting beef with high pH (6.6) may have sour, bitter and peanut like flavour (Moiseev and Cornforth 1999; Wulf et al. 2002). Flavour may not be influenced by muscle darkness (Bass et al. 2008; Viljoen et al. 2002) likely when there is a low pH of dark cutting, as Holdstock et al. (2014) found flavour desirability of atypical dark beef similar to that of normal beef. However, dark cutting is tougher than normal beef (Lawrii 1983; Wulf et al. 2002) while the appealing colour of meat is an indication of

improved tenderness (Bruce et al. 2004; Wulf et al. 1997). Reports also indicated that beef tenderness was not influenced by the degree of darkness (Bass et al. 2008) and carcasses with increased pH were tender likely because these carcasses entered rigor earlier, before cold shortening, due to rapid loss of glycogen reserves (Davey and Graafhuis 1981). However, the shear force had a negative correlation with pH ranged 5.8 to 6.0 (Teke et al. 2014), and Holdstock et al. (2014) also found increased toughness in atypical dark ($pH \leq 5.8$) beef from steers. Tenderness, however, is also influenced by cattle sex (Choat et al. 2006; Wulf et al. 1997), carcass weight (Muir et al. 1998) and subcutaneous fat depth (Muir et al. 1998; Aalhus et al. 2001). Increased toughness could be a result of reduced proteolysis during postmortem (Pulford et al. 2009; Watanabe et al. 1996), but toughness has also been associated with reduced rate of postmortem glycolysis (Anderson et al. 2014). Dark cutting and associated beef quality, therefore, could be related to muscle proteins as well as carcass phenotype.

1.5. Objectives of the thesis

Continued economic loss by beef producers urges re-examination of the factors potentially associated with the persistence of dark cutting in Canadian beef industry. A comprehensive study incorporating the effect of cattle production practices, animal/carcass phenotype, and muscle proteome is inevitable. My objectives were to **1**) identify the contribution of heifers and steers to the occurrence of dark cutting in the slaughter population, **2**) relate cattle and carcass phenotypic characteristics to the likelihood of dark cutting, **3**) determine the effect of production systems, growth promotants and their interactions in heifers and steers on dark cutting, **4**) analyze the effect of pre-slaughter cattle management and season on the occurrence of dark cutting, **5**) examine the biochemical composition and quality of dark cutting beef from both

heifers and steers, **6**) relate beef quality to cattle sex and carcass phenotype, and **7**) characterize protein compliment of LT muscles from normal and dark cutting heifer and steer carcasses.

I hypothesized that: **a**) the likelihood of a cattle to cut dark can be predicted from live animal measurements such as average daily gain (ADG), feed intake, slaughter weight and rib-eye and subcutaneous fat depth measured by ultrasound; **b**) heifers are at greater risk of cutting dark than steers; **c**) the recent increase in dark cutting in Canadian beef industry is due to extensive use of growth promotants; **d**) yearling-fed cattle are more likely to cut dark than calf-fed cattle; **e**) the tenderness difference between normal and dark cutting beef is also due to the effect of carcass phenotype; **f**) the atypical dark cutting exists in both heifers and steers; and **g**) dark cutting phenomenon and associated beef quality is related to muscle protein abundance.

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2.0. Can potential for dark cutting be predicted by phenotype? Relationship between sex, carcass characteritics and the incidence of dark cutting beef[1]

2.1. Introduction

Carcasses from cattle under 30 months of age that have a purple or dark red rib eye muscle (m. *longissimus thoracis*, LT) 20 minutes after "ribbing" at the 12^{th}-13^{th} rib are considered dark cutting and graded Canada B4, while carcasses with normal rib eye muscle color are sorted by marbling into Canada Prime, AAA, AA and A grades, which are equivalent to USDA Prime, Choice, Select and Standard. Dark cutting beef usually has an ultimate pH greater than 5.8 and is discriminated against by retailers because of its reduced shelf life (Koutsoumanis et al. 2006) and by consumers because of its abnormal appearance. In compliance with the Canadian Beef Carcass Grading Regulations (Canadian Agricultural Products Act SOR/92-541 2014), dark cutting carcasses are downgraded to the Canada dark cutting grade (Canada B4) and their value is usually reduced to that of mature, over thirty month old cattle. This price discounting results in a direct financial loss to producers and can result in an individual carcass being discounted by as much as 40%. Dark-cutting continues to occur and its incidence has increased in Canada within the last 10 years from 0.8 to 1.3% (Beef Cattle Research Council (BCRC), 2013). In Australia the incidence of dark cutting is about 10% (Meat Standards Australia (MSA), 2010), while in the United States of America (US) the proportion of dark-cutting has increased from 1.9% in 2005 (Garcia et al. 2008) to 3.2% in 2012 (Moore et al. 2012). Recently, it has been shown that improved muscling and carcass weight lowered the incidence of dark cutting (McGilchrist et al. 2012) but there are contradictory reports about the effect of the sex of animal on the likelihood of dark cutting (Lorenzen et al. 1993; Mach et al.

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2008). Regardless of the individual animal and the management, it appears that muscle glycogen is insufficient in such carcasses to fuel post mortem anaerobic glycolysis and reduce carcass muscle pH is the accepted cause of dark cutting. The persistence of dark cutting in the beef industry, despite significant research efforts and implementation of prevention strategies, and its concomitant economic loss to cattle owners justifies continued research. The purpose of this study was to relate the effect of sex, carcass conformation and animal phenotypic characteristics to the frequency of dark cutting beef to test the hypothesis that the likelihood of a beef animal producing a dark cutting carcass can be predicted from live animal measurements.

2.2. Materials and methods

The study was conducted on an existing data set with production and carcass measurements available; as a result, no animal ethics approval was required; however, animals in the data set had been cared for according to the Canadian Council on Animal Care (CCAC 1993) guidelines.

2.2.1. Data

Data from 180 cattle with live animal and carcass measurements were used for detailed analysis of the relationships between sex, carcass and production phenotypes and the frequency of dark cutting. Data were collected from cattle on study from 2003 to 2011 on a farm in the province of Alberta, Canada. The cattle under study were Hereford (sire)-Angus (dam) crossbreds (n = 82) and purebred Charolais (n = 98). Cattle were blocked by slaughter lot with each block having data from at least one animal/carcass from each grade. Each slaughter lot was either all male (steers, bovine castrates, n = 136, 4 lots) or all female (heifers, n = 44, 2 lots). All the animals were processed at the same abattoir and assessed by the same graders for muscle

score, grade marbling score, grade fat depth and color. The carcasses in the data set were graded Canada A (n = 28), AA (n = 106), AAA (n = 35) and B4/ dark cutting (n = 11).

Production and carcass data included animal weaning weight (WW, kg), dry matter intake (DMI, kg DM day^{-1}), average daily gain (ADG, kg gain day^{-1}), feed conversion ratio (FCR, kg DMI kg^{-1} gain), residual feed intake adjusted for ultrasound back fat (RFI_{fat}, kg DMI day^{-1}), ultrasound rib eye area (uREA, cm^2), ultrasound subcutaneous fat depth (uFD, mm), ultrasound marbling score (uMS), animal slaughter weight (LW, kg), hot carcass weight (CW, kg), grade fat depth (gFD, mm), grade rib eye area (gREA, cm^2) and grade marbling score (gMS). The data also included animal age at the start of feeding a concentrate diet (test age), days cattle were fed a concentrate diet (days to finishing) and age at slaughter. Production and phenotypic measurements were performed similarly to those described by López-Campos et al. (2012, 2013), while feeding a concentrate diet. Dry matter intake was calculated by multiplying daily feed intake by feed dry matter, with daily feed intake measured using GrowSafe® feeding stations (GrowSafe® System Inc., Airdrie, Alberta, Canada) and feed dry matter was estimated from pooled feed samples dried at 80 ºC in a forced-air oven to a constant weight. Feed conversion ratio was calculated by dividing average daily feed intake by average daily gain. Residual feed intake (RFI_{fat}) was calculated as the deviation of actual feed intake from expected feed intake and adjusted for ultrasound back fat (Basarab et al. 2003, 2007). Ultrasound back fat thickness, rib eye area and marbling score were estimated prior to slaughter using an Aloka 500V diagnostic real-time ultrasound with a 17 cm 3.5 MHz linear array transducer (Overseas Monitor Corporation Ltd, Richmond, BC, Canada) by a certified ultrasound technician as described by Brethour (1992). Ultrasound and carcass subcutaneous fat depth, marbling score and rib eye areas were measured at the 12-13^{th} LT (rib eye) muscle interface, which is the accepted Canadian

beef grading site. Both ultrasound and post mortem rib eye muscle marbling scores were categorized using the United States Department of Agriculture (USDA) scoring system (USDA 1997) where Canada A, AA, AAA and Prime quality grade marbling corresponded to traces (Standard, 300-399), slight (Select, 400-499), small to moderate (Choice, 500-799), and greater than or equal to slightly abundant (Prime, 800-1099) amounts of marbling, respectively. Traces, slight, small to moderate and greater than or equal to slightly abundant marbling equated to ultrasound marbling scores between 1.00 to 3.99, 4 to 4.99, 5 to 7.99, and 8 to 11, respectively. Slaughter live weight (kg) was calculated from initial feed trial weight added to the number of feeding days multiplied by the average daily gain.

The cattle were slaughtered at the Agriculture and Agri-Food Canada Meat Research Laboratory (Lacombe, Alberta, Canada). The carcasses were split and weighed to record hot carcass weight and then chilled for 48 hours. After chilling, the left sides of the carcasses were ribbed at between 12^{th} and 13^{th} ribs for assessment of gFD, gMS, and gREA and assignment of quality grade.

2.2.2. Statistical analyses

All statistical analyses with the exception of principal component analyses were performed using SAS (Version 9.3, SAS Institute Inc., Cary, NC). A generalized logit model was applied using the CATMOD procedure to compute the frequency of dark cutting in cattle in the data set. Analyses performed on phenotypic and carcass data included analysis of variance, Pearson correlations, binomial and multinomial logistic regression and principal component analysis (PCA). Data were used to examine the effects of sex and grade on live animal and carcass characteristics using the MIXED procedure with sex, grade and their interaction as fixed sources of variation. Slaughter lot was confounded with sex and so lot within sex was included as

a random term that served as the error term for sex. Breed was not included in the analysis as not all the treatment combinations were represented when it was included in the design, but it was considered in the PCA. Kenward-Roger approximation was used to compute the denominator degrees of freedom. For analysis of variance of DMI and ADG data, body weight at the start of finishing was included as a covariate.

Where analysis of variance models were significant ($P < 0.05$), differences between means were identified using least square means differences, with significance at $P < 0.05$. Linear relationships between independent variables were investigated using Pearson correlations (PROC CORR) and the Bonferroni correction was used to compensate for the likelihood of type I significance error between measurements that were not independent from each other. There is an estimated 7% likelihood of type I error for 12 comparisons; therefore, a correction was made by dividing $\alpha = 0.05$ by the number of comparisons (15) for a level of significance of $P < 0.003$ (Rice 1989).

Binomial and multinomial logit regression models were used to examine trends in the data. For the binomial logit regression, data of cattle that produced normal (Canada A, AA and AAA) carcasses were combined into one category (NORMAL) and compared to the data of cattle that produced dark-cutting (Canada B4) carcasses (DARK) and the probability of being dark (yes or no) was modeled. In multinomial logistic regression, the response variables were the individual grades (Canada A, AA, AAA and B4). In both binomial and multinomial logistic regressions, covariate analyses of live animal and carcass characteristics were conducted separately with sex as the treatment effect. Where correlations existed between the covariates, only the covariate that produced the highest R^2 in the model was included in the final analysis. Regression models were built using the backward selection procedure ($\alpha = 0.05$) and included a

set of uncorrelated variables with increased R^2 value. Goodness of fit for the binomial model was tested using the Hosmer and Lemeshow Goodness-of-Fit test while graphs for predicted probabilities for both binomial and multinomial responses were obtained in SAS.

Principal component analysis (PCA) was performed using Unscrambler® (Camo Scientific Inc., Woodbridge, New Jersey) to identify the variables accounting for most of the variation between grades. Separate PCA were conducted for live animal and carcass measurements, and all measurements were transformed using the center and scale function of the software. Centering and scaling of data reduces differences in variation between measurements due to measurement numerical range, which can bias proportioning of variation within the analysis (Dijksterhuis 1994).

2.3. Results

2.3.1. Categorical modelling analysis

The overall frequency of dark cutting in the data set was 6.1% and categorical (CATMOD) maximum likelihood analysis of variance indicated a trend (P = 0.106) for heifer carcasses to have a greater probability (11.36 $\pm$ 4.78%, standard error of the mean (SEM)) of dark cutting than carcasses from steers (4.4 $\pm$ 1.76%).

2.3.2. Analysis of variance

Results from the analysis of variance are presented in Tables 2.1 to 2.3. There were no differences due to sex for mean ADG, DMI, FCR, RFI_{fat}, slaughter age, uFD, uREA, carcass weight, gFD and gREA values (Table 2.1). Animal sex influenced age at test (P = 0.0022) and days to finishing (P = 0.0009), with heifers older at the beginning of the test than steers and requiring a shorter period of time than steers to finish (Table 2.1). There was a tendency for steers to produce heavier carcasses than heifers (P = 0.0853; Table 2.1).

Grade had no effect on age at test, ADG, FCR, uREA, days to finishing, or slaughter age (Table 2.2). There was an effect of grade on RFI_{fat} (P = 0.0437), uFD (P = 0.0016), carcass weight (P = 0.0098), gFD (P = 0.0005) and gREA (P = 0.037), with cattle that produced Canada AAA carcasses having a greater RFI_{fat} than cattle that produced Canada A carcasses, and an uFD greater than cattle that produced all other grades of carcasses. Canada AAA carcasses also had greater subcutaneous grade fat at the 12-13th rib (Canadian grading site) than Canada AA and A carcasses (P = 0.0005). Animals producing dark cutting carcasses (Canada B4) had a mean RFI_{fat} value similar to all of the other grades, and were most similar to those producing Canada A and AA carcasses with respect to uFD (Table 2.2). Grade tended (P = 0.059) to have an effect on DMI where animals producing Canada AAA grade carcasses had a mean DMI (adjusted for starting weight at finishing) greater than the cattle that produced Canada B4 and A carcasses while the mean DMI for cattle that produced Canada B4 carcasses was not different from cattle that produced Canada AA and A carcasses (Table 2.2). Moreover, mean DMI calculated as a percentage of LW was greater for cattle that produced Canada AAA carcasses (1.72 ± 0.08%; P = 0.004) than all other grade categories, which were not different from each other (1.62 ± 0.08; 1.56 ± 0.09 and 1.59 ± 0.09%, Canada B4, AA and A, respectively). Dark cutting carcasses (Canada B4) had a lower mean hot carcass weight and a lower mean gREA than Canada A and AA carcasses, but the means for both characteristics were similar to those means of the Canada AAA carcasses (Table 2.2). Also, the mean gFD of dark cutting carcasses (Canada B4) did not differ from that of other carcass grades (Table 2.2).

Heifers producing Canada B4 carcasses tended (P = 0.094) to have a mean ADG (1.23 ± 0.17 kg/day) less than that of heifers producing normal carcasses (1.54 ± 0.18, 1.35 ± 0.15 and 1.41 ± 0.17 kg/day, Canada A, AA and AAA, respectively); however, the interaction between

grade and sex for ADG was not significant ($P = 0.17$) after adjustment for body weight at the start of finishing. Significant interactions between sex and grade were noted for weaning weight, live weight at slaughter, uMS and gMS (Table 2.3). The mean weaning and live weights of steers were unrelated to grade, but the mean weaning and live weights of heifers that produced dark cutting carcasses were lower than those of heifers that produced Canada A and AA carcasses and were similar to those of heifers that produced Canada AAA carcasses (Table 2.3). For uMS, steers that produced dark cutting carcasses (Canada B4) had a lower mean uMS score than heifers that produced a dark cutting carcass (Canada B4), but neither was different from cattle within their sex that produced Canada A and AA carcasses (Table 2.3). Dark cutting carcasses (Canada B4) from steers also had a lower mean gMS than Canada AAA carcasses regardless of sex, which was also lower than that of the dark cutting carcasses of heifers (Table 2.3).

2.3.3. Pearson correlations

Pre-planned correlations identifying linear relationships are presented in Table 2.4 with significance at $P < 0.003$ with the Bonferroni correction. Correlation results indicated that DMI was positively associated with RFI_{fat} ($r = 0.44$, $P < 0.0001$), ADG ($r = 0.71$, $P < 0.0001$), live weight ($r = 0.27$, $P < 0.001$), uREA ($r = 0.53$, $P < 0.0001$), carcass weight ($r = 0.58$, $P < 0.0001$), and gREA ($r = 0.48$, $P < 0.0001$). Carcass weight was correlated with ADG ($r = 0.39$, $P < 0.0001$), live weight at slaughter ($r = 0.60$, $P < 0.0001$), uREA ($r = 0.59$, $P < 0.0001$) and gREA ($r = 0.67$, $P < 0.0001$). Residual feed intake (RFI_{fat}) was unrelated ($P > 0.05$) to live weight, uFD, uREA, uMS, ADG, gFD, gREA and gMS. Feed conversion ratio (FCR) was negatively associated with ADG ($r = -0.67$, $P < 0.0001$) but positively associated with RFI_{fat} ($r = 39$, $P < 0.0001$). Animal uREA, uFD and uMS were respectively correlated ($P < 0.0001$) with carcass

gREA (r = 0.61), gFD (r = 0.76) and gMS (r = 0.46), indicating a moderate prediction of carcass measurements from live animal measurements by ultrasound.

2.3.4. Logistic regression analyses

Binomial logistic regression using a binary logit model indicated no significant relationships (P > 0.05) between dark cutting status (yes, no) and live animal parameters. Binomial logistic regression between dark cutting status and carcass parameters showed that as carcass weight increased, the likelihood of dark cutting decreased (P = 0.0286; Figure 2.1).

Application of a multinomial generalized logit model to live animal parameters indicated that DMI (P = 0.0034) and uMS (P < 0.0001) influenced the predicted grade, with a maximum rescaled R^2 value of 0.24. Predicted probabilities showed that the likelihood of dark cutting decreased as DMI increased when the whole data set mean for uMS (4.94) was used to calculate predicted probabilities (Figure 2.2). The probability of Canada AAA grade increased with uMS but the relationship of dark cutting probability with uMS was less remarkable, showing a slight decrease as uMS increased (Figure 2.3).

Application of a multinomial generalized logit model to carcass parameters showed that carcass weight (P = 0.0145) and grade fat depth (P = 0.0002) were related to the incidence of grade with a maximum rescaled R^2 = 0.20. The predicted probability of dark cutting decreased as carcass weight increased when the data set mean for gFD of 8.89 mm was used in the prediction equation for carcass weight (Figure 2.4). The likelihood of dark cutting did not appear to be greatly affected by gFD when a data set mean for carcass weight of 316 kg was included in the prediction model, but carcasses were most likely to be graded as Canada AAA at gFD greater than 15 mm (Figure 2.5).

2.3.5. Principal component analysis (PCA)

PCA described 51% of the variation in live animal measurements, with 30% and 21% of the variation on the first and second principal components, respectively (Figure 2.6). The first principal component (PC-1) illustrated that uMS and FCR increased as DMI, ADG, and uREA decreased and that DMI, ADG and uREA increased as WW and LW increased. The second principal component (PC-2) showed that FCR and uMS increased as WW and LW decreased (Figure 2.6). Mapping of the individual animal data on to the correlation loadings plot showed that dark cutting (Canada B4) cattle were located in every quadrant of the PCA plot (Figure 2.6), but dark cutting steers tended to be located in the centre and left half of the plot and dark cutting heifers on the right. The results indicated that dark cutting steers tended to have decreased values for DMI, uREA, ADG, WW and LW on PC-2, while dark cutting heifers had increased uMS and FCR on PC-1 (Figure 2.6).

Principal component analysis of the carcass measurements accounted for 76% of the variation in carcass measurements with the first two components, with the first and second principal components accounting for 48 and 28%, respectively (Figure 2.7). Most of the variation in carcass data relative to Canada grade was accounted for by the first component axis (PC-1), with increased CW and gREA associated with reduced gFD and gMS. On the second component (PC-2), increased gFD and gMS were also related to slightly increased CW, suggesting two phenotypic relationship populations existed within the data set. Carcasses from dark cutting heifers tended to be located in the quadrants on the left side of the plot, suggesting reduced gFD, gMS, CW and gREA were associated with dark cutting in heifers, while carcasses from dark cutting steers were located in both lower left and upper right quadrants, indicating that both reduced or increased CW and gREA were associated factors for steers (Figure 2.7). Canada AAA

carcasses were associated with increased gMS and gFD, while Canada AA carcasses were located on the right side of the score plot near increased CW and gREA (Figure 2.7).

2.4. Discussion

Dark cutting continues to occur despite preventive measures, and in the United States of America (US), the proportion of dark-cutting has fluctuated, decreasing from 2.3% in 2002 (McKenna et al. 2002) to 1.9% in 2005 (Garcia et al. 2008) then increasing to 3.2% in 2012 (Moore et al. 2012). The incidence of dark cutting has also increased in Canada within the last 10 years (Jones and Tong 1989; Donkersgoed et al. 1997, 2001; BCRC 2013). The incidence of dark cutting in the province of Alberta, Canada, has been as high as 3.7% (Murray 1989) and the frequency of dark cutting in the data set under study was higher than historical levels. The present study also revealed a trend toward increased frequency of dark cutting in heifers. Contrarily, literature has reported the frequency of carcasses with pH value greater than 5.8 to be greater for males than for females (Mach et al. 2008) and that steers have a greater incidence of dark cutting than heifers (Jones and Tong 1989). Results of the present study, however, agreed with the findings of Murray (1989), Lorenzen et al. (1993), Voisinet et al. (1997a) and Scanga et al. (1998) who also reported that intact heifers had a significantly ($P < 0.05$) higher incidence of dark cutting than steers and spayed heifers. Excitable temperament has been reported to be higher in heifers than steers (Voisinet et al. 1997b), which has been related to an increased stress response (Fazio et al. 2012), lowered muscle glycogen reserves and increased frequency of dark cutting (Lawrie 1958). Increased excitable temperament in heifers may be due to estrus behavior (Voisinet et al. 1997a) as the proportion of carcasses with ultimate pH greater than 5.8 increased in heifers classified as “restless” and the proportion of dark cutting significantly increased when heifers exhibited standing heat (oestrus; Kenny and Tarrant 1988). In that study, during late

estrus the frequency of profound dark cutting (pH > 6.0) was greatly increased by mounting activity, which significantly lowered the glycogen level in the *longissimus* muscle (Kenny and Tarrant 1988). A reduction in the tendency of heifers to cut dark in subsequent years may have been due to the use of melengesterol acetate (MGA; Jones and Tong 1989) which suppresses estrus activity (Meyer 2001). In the data set of the present study, the MGA status of the heifers was not known; therefore, the reasons for the observed increased frequency of dark cutting in heifers in the current study were not able to be confirmed.

There did not appear to be any one live animal phenotypic characteristic that clearly identified the cattle that produced dark cutting (Canada B4) carcasses because at least one of these carcasses appeared in each of the live animal measurements PCA plot quadrants, substantiating that many phenotypes of cattle can produce dark cutting carcasses. These results also implied that the measurements analyzed in the present study, although extensive, may not have adequately captured all the sources of variation in beef quality. For example, data on usage of growth promoters, weather conditions, animal stress response while handling and physiological state of heifers before slaughter were not available in the data set but bear consideration as season, animal temperament, growth promoters and estrus in heifers can affect the incidence of dark cutting (Kreikemeier et al. 1998; Scanga et al. 1998; Voisinet et al. 1997b).

Although no one characteristic appeared to define an animal that would produce a dark cutting carcass, decreased live weights in heifers and decreased carcass weight regardless of sex were related to dark cutting in the present study. Recent research by McGilchrist et al. (2012) indicated that increased hot carcass weight (greater than 220 kg) was associated with decreased incidence of dark cutting. Likewise, Murray (1989) reported that beef carcasses weighing greater than 318 kg had one-half the incidence of dark cutting than those weighing less than 272 kg.

Increased carcass weight may reduce the incidence of dark cutting because heavy carcasses are most likely to have increased muscle glycogen reserves (McGilchrist et al. 2012), a slowed chilling rate and a rapid decline in postmortem pH (Cliplef et al. 1989; Wulf et al. 1997; Aalhus et al. 2001). These results agree with previous research that found that heifer carcasses that cut dark were associated with decreased mean carcass weight (Cliplef et al. 1989; Murray 1989).

In the present study, PCA showed a cohort of dark cutting steers that not only had increased uREA, ADG, and DMI, but yielded large carcasses with increased REA at slaughter, indicating that the dark cutting steers were large cattle. McGilchrist et al. (2012) reflected that increased muscularity and fatness most likely indicated improved nutrition of the cattle and increased muscle glycogen reserves; however increased musculature in the study of Hawrysh et al. (1985) was linked to increased dark cutting and was most likely indicative of rapidly growing cattle with reduced muscle energy reserves. Additionally, muscular animals with an increased proportion of fast muscle fibers (Wegner et al. 2000) may be most at risk of dark cutting with acute activity such as fighting or physical combat before slaughter, which greatly lowers muscle glycogen (Lacourt and Tarrant 1985; McGilchrist et al. 2011). The steers that produced dark cutting carcasses in the present study appeared most similar to the cattle of Hawrysh et al. (1985) that cut dark, and DMI was compromised in dark cutting cattle in the present study when live weight at the start of finishing was taken into consideration, suggesting that these steers may not have been ingesting sufficient feed for glycogen storage relative to their size.

Reduced feed intake has been associated with increased ultimate muscle pH (Pethick and Rowe 1996) as it lowers the muscle glycolytic capacity (Brandstetter et al. 1998; Gardner et al. 2006) available to decrease muscle pH postmortem; however, animals fed a high energy diet tend to maintain muscle glycogen reserves during stress and avoid the risk of dark cutting (Pethick

and Rowe 1996; Immonen et al. 2000; Knee et al. 2004). Muscle glycogen repletion rate in cattle is very slow but repletion during the stay of an animal in lairage may play an important role in meat quality (McVeigh et al. 1982). Also, muscle glycogen repletion rate is lower in cattle that have already been fasted or had low dietary energy intake than in those fed high energy diets (McVeigh and Tarrant 1982; Pethick et al. 1999; Gardner et al. 2001). Thus, reduced DMI may predispose cattle to have decreased hepatic and muscle glycogen contents (Yambayamba et al. 1996) and to cut dark. Feed intake could be depressed by acidosis (Brown et al. 2000), subclinical disease or estrus in heifers (Maltz et al. 1997). Feeding an energetic diet has been reported to increase ADG and the decline in early postmortem pH in beef (Harris et al. 1997).

Logistic regression, PCA and analysis of variance results together supported the conclusion that heifers prone to dark cutting in the present study were small, early-maturing cattle that produced light weight carcasses with increased uMS and FCR and reduced gREA. Increased FCR is associated with reduced animal feed efficiency, which decreases as ADG decreases and body fat increases with age (Berry and Crowley 2013), implying that dark cutting heifers may be early-maturing. This was corroborated by a concomitant increase in back fat but not REA in the dark cutting Canada B4 heifers between time of ultrasound and slaughter, which would be expected of an animal at the end of its rapid growth phase. Improved lean beef color in general has been positively correlated with *longissimus* muscle area as previously noted by Shackelford et al. (1994) and McGilchrist et al. (2012), who found that increased eye muscle area (greater than 70 cm^2) was associated with decreased incidence of dark cutting. Increased muscling is associated with increased proportions of fast twitch muscle fibers (Wegner et al. 2000) which, due to higher glycolytic activity, may result in an increased rate of pH decline and a light red appearance (Dalya et al. 2006; Gardner et al. 2006). Contrarily, dark cutting carcasses

have been reported to have increased rib eye area (Janloo et al. 1998) and data of Hawrysh et al. (1985) indicated that carcasses with rib eye area greater than 80 cm^2 were in the dark cutting pH range of 5.7 to 6.5.

Principal component analysis indicated that both marbling score and fat depth at grading accounted for substantial variation between the grades, and this was expected because quality and yield grading in Canada is based upon intramuscular fat and subcutaneous fat depth. That grade fat depth and grade marbling scores were correlated indicated that energy deposition in these depots is usually related, and this relationship has been reported previously (Muir et al. 1998). Although fat depth at grading was associated with grade and marbling score in the PCA results, the scores plot and analysis of variance indicated that these associations were driven by the 'normal' Canada A, AA, and AAA animals rather than the dark cutting Canada B4 animals. Multinomial logistic regression substantiated that the association between fat depth and dark cutting was not strong, with the probability of a dark cutting carcass (Canada B4) decreasing only slightly with gFD above 20 mm. McGilchrist et al. (2012) also found that increased rib subcutaneous fat (greater than 20 mm) was associated with decreased incidence of dark cutting. Increased fatness has been associated with reduced incidence of dark cutting (Murray 1989), and Page et al. (2001) reported that carcasses with fat thickness below 7.6 mm had darkened rib eyes and increased mean muscle pH. Low ultimate pH in relation to increased fat depth may be due to improved animal nutrition, which increases glycogen reserves along with increasing fat cover (Warner et al. 1998; McGilchrist et al. 2012). Harris et al. (1997) found that live weight, carcass weight, fat depth, marbling score and initial decline in muscle pH early postmortem in carcasses from calves increased as the length of time the calves were fed a concentrate diet increased. In the present study, dark cutting (Canada B4) cattle had a mean fat depth of 8.90 $\pm$ 1.06 mm at

carcass grading, well above the 7.6 mm identified by Page et al. (2001), but analysis of variance results showed clearly that the mean gFD of dark cutting carcasses was not different than that of normal carcasses.

As expected, the level of LT muscle marbling in the present study was strongly related to grade, as marbling score is used to separate youthful beef carcasses into the four quality grades (Prime, Canada AAA, Canada AA, Canada A). The results showed that gMS of dark cutting carcasses was extremely similar to that of the Canada AA carcasses. A low probability of dark cutting with increased carcass marbling score (greater than 500) is in accordance with previous findings that showed that carcasses with low ultimate pH had a higher mean marbling score than those that cut dark (Janloo et al. 1998).

2.5. Conclusions

Results of this study partially supported acceptance of the hypothesis that the risk of dark cutting in cattle may be predicted using phenotypic measurements, particularly those measurements typically performed by producers that incorporate weigh scales and ultrasound into assessment of their cattle. Cattle most at risk of cutting dark in the present study were low body weight heifers and high body weight steers. Dry matter intake greater than 1.72% of animal body weight may lower the incidence of dark cutting beef. Dark cutting cattle, regardless of sex, produced carcasses with USDA grade marbling scores ranging from 410 to 439, decreased rib eye areas and reduced carcass weights. Although correlations between ultrasound and carcass fat values in the present data set were lower than reported in the literature (Brethour 1992; Greiner et al. 2003), improvement in ultrasound technology since the recording of the earlier data may have increased the accuracy of predicting carcass grades and the likelihood of dark cutting.

Incorporation of weather conditions, growth hormone use, estrus activity, pre-slaughter handling, and breed into statistical models also will likely improve prediction accuracy.

Table 2.1. Effect of sex on least square means (± standard error of the means in parentheses) for animal performance and carcass characteristics.

Measurements	Heifer (n = 44)	Steer (n = 136)	P value[1]
Age at test	360 (9)	328 (5)	0.0022
Average daily gain (kg. day^{-1})	1.42 (0.17)	1.49 (0.12)	0.7324
Dry matter intake (kg. day^{-1}) adjusted	9.02 (0.64)	9.19 (0.45)	0.8304
Feed conversion ratio (kg DMI kg^{-1} gain)	6.31 (0.47)	6.29 (0.33)	0.9754
Residual feed intake (kg DMI day^{-1})	-0.152 (0.13)	-0.076 (0.07)	0.6053
Days to finishing	101 (17)	170 (10)	0.0009
Age at slaughter (days)	476 (41)	503 (29)	0.6296
Ultrasound fat depth (mm)	7.13 (0.82)	7.50 (0.54)	0.7158
Ultrasound rib eye area (cm^2)	68.2 (7.8)	72.2 (5.4)	0.6911
Carcass weight (kg)	283 (15)	322 (10)	0.0853
Grade fat depth (mm)	8.55 (0.91)	9.11 (0.61)	0.6220
Grade rib eye area (cm^2)	75.39 (4.57)	78.15 (3.13)	0.6369

[1] Probability of the F test, with significance at $P \leq 0.05$.

Table 2.2. Influence of grade on least square means (± standard error of the means in parentheses) for animal performance and carcass characteristics.

Measurement	Grades[2]				*P* value[1]
	A (n = 28)	AA (n = 106)	AAA (n = 35)	B4 (n = 11)	
Age at test (days)	339 (12.8)	343 (4.80)	344 (7.6)	347 (12.7)	0.9770
Average daily gain (kg. day^{-1})	1.48 (0.11)	1.44 (0.10)	1.49 (0.10)	1.40 (0.11)	0.3107
Dry matter intake (kg. day^{-1}) adjusted	8.90 (0.44)	9.19 (0.39)	9.47 (0.40)	8.88 (0.44)	0.0593
Feed conversion ratio (kg DMI kg^{-1} gain)	6.10 (0.37)	6.52 (0.28)	6.31 (0.31)	6.28 (0.37)	0.3276
Residual feed intake (kg DMI day^{-1})	-0.53^{b} (0.20)	0.008^{a} (0.05)	0.115^{a} (0.09)	-0.042^{ab} (0.17)	0.0437
Days to finishing	140 (26)	137 (10)	142 (15)	123 (26)	0.9409
Age at slaughter (days)	496 (27)	487 (25)	479 (26)	496 (27)	0.2887
Ultrasound fat depth (mm)	6.59^{b} (0.87)	6.98^{b} (0.47)	8.71^{a} (0.55)	6.97^{b} (0.80)	0.0016
Ultrasound rib eye area (cm^2)	71.4 (5.38)	70.6 (4.71)	68.6 (4.82)	70.2 (5.22)	0.5910
Carcass weight (kg)	318^{a} (13)	313^{a} (9)	299^{ab} (10)	283^{b} (13)	0.0098
Grade fat depth (mm)	7.13^{b} (1.07)	8.41^{b} (0.61)	10.88^{a} (0.75)	8.90^{ab} (1.06)	0.0005
Grade rib eye area (cm^2)	81.98^{ab} (4.28)	80.14^{a} (2.73)	74.89^{bc} (3.18)	70.06^{c} (4.26)	0.0370

$^{a, b, c}$ Least Square Means within a row lacking a common letter differ at $P \leq 0.05$.

[1]Probability of the F test, with significance at $P \leq 0.05$.

[2]Canada carcass grades (A, AA and AAA), categorized based upon rib eye muscle marbling using the United States Department of Agriculture (USDA) scoring system (USDA, 1997) (with Canadian equivalency), corresponded respectively to USDA quality grade Standard/Trace, 300-399; Select/ Slight, 400-499 and Choice/Small to Moderate, 500-799 while Canada B4 grade stands for dark cutting.

Table 2.3. Effect of sex and grade interaction on least square means (standard errors of the means in parentheses) for weaning and slaughter weights and ultrasound and grade marbling scores.

Measurement	Heifer Grades[2]				Steer Grades[2]				*P* value[1]
	A	AA	AAA	B4	A	AA	AAA	B4	
n	3	25	11	5	25	81	24	6	
Weaning weight (kg)	231^{ab} (26)	191^{bc} (17)	175^{cd} (19)	154^{d} (23)	232^{ab} (13)	241^{ab} (12)	246^{a} (13)	242^{ab} (18)	0.0229
Live weight at slaughter (kg)	545^{abc} (44)	519^{bc} (30)	487^{cd} (33)	441^{d} (39)	618^{a} (23)	618^{a} (21)	599^{ab} (23)	650^{a} (31)	0.0295
Ultrasound marbling score[x]	4.59^{bc} (0.31)	4.80^{bc} (0.15)	5.78^{a} (0.18)	5.20^{b} (0.25)	4.79^{bc} (0.13)	4.84^{bc} (0.10)	5.23^{b} (0.13)	4.52^{c} (0.22)	0.0080
Grade marbling score[x]	380de (29)	439^{cd} (8)	573^{a} (12)	462^{c} (18)	367^{e} (8)	441^{cd} (4)	530^{b} (8)	410^{d} (16)	0.0239

$^{a, b, c}$ Least Square Means within a row lacking a common letter differ at $P \leq 0.05$.

[1]Probability of the F test, with significance at $P \leq 0.05$.

[2]Canada carcass grades (A, AA and AAA) corresponded respectively to USDA quality grade Standard, Select, and Choice while Canada B4 grade stands for dark cutting.

[x]Both ultrasound and grade muscle marbling scores were categorized using the United States Department of Agriculture (USDA) scoring system (USDA, 1997) with Canadian equivalency as the following, respectively: Standard/Trace, 1 to 3.99 and 300-399; Select/Slight, 4 to 4.99 and 400-499; Choice/Small to Moderate, 5.0 to 7.99 and 500-799; and greater than or equal to Slightly Abundant/Prime, 8.0 to 11 and 800-1099.

Table 2.4. Pearson correlations between production and carcass measurements.

Measurement	TA	SA	DF	LW	uFD	uREA	uMS	ADG	DMI	FCR	RFI_{fat}	CW	gFD	gREA	gMS
Weaning weight	-0.40 ***	0.23 **	0.35 ***	0.82 ***	-0.02	0.15	-0.31 ***	0.15	0.35 ***	0.11	-0.09	0.65 ***	-0.07	0.36 ***	-0.10
Test age (TA)		-0.47 ***	-0.80 ***	-0.46 ***	0.27 **	0.53 ***	0.12	0.42 ***	0.42 ***	-0.13	0.04	0.12	0.24 *	0.24 *	0.07
Slaughter age (SA)			0.90 ***	0.51 ***	-0.20	-0.50 ***	-0.11	-0.08	-0.32 ***	-0.22 *	-0.05	-0.19	-0.10	-0.36 ***	-0.05
Days fed (DF)				0.57 ***	-0.27 **	-0.59 ***	-0.14	-0.26 **	-0.42 ***	-0.08	-0.06	-0.19	-0.18	-0.35 ***	-0.07
Live weight (LW)					-0.12	0.02	-0.33 ***	0.27 **	0.27 **	-0.10	-0.11	0.60 ***	-0.17	0.24 *	-0.19
Ultrasound fat depth (uFD)						0.21	0.31 ***	0.16	0.30 ***	0.08	0.05	0.06	0.76 ***	-0.15	0.39 ***
Ultrasound rib eye area (uREA)							-0.19	0.38 ***	0.53 ***	0.02	0.03	0.59 ***	-0.02	0.61 ***	-0.12
Ultrasound marbling score (uMS)								-0.14	-0.09	0.09	0.09	-0.30 ***	0.33 ***	-0.27 **	0.46 ***
Average daily gain (ADG)									0.71 ***	-0.67 ***	0.03	0.39 ***	0.17	0.29 ***	0.07
Dry matter intake (DMI)										0.03	0.44 ***	0.58 ***	0.17	0.48 ***	0.11
Feed conversion ratio (FCR)											0.39 ***	0.04	-0.07	0.08	-0.02
Residual feed intake (RFI_{fat})												-0.08	0.04	-0.03	0.17
Carcass Weight (CW)													-0.04	0.67 ***	-0.21
Grade fat depth (gFD)														-0.31 ***	0.34 ***
Grade rib eye area (gREA)															-0.18

*, **, *** Asterisks beneath each correlation indicate probability with *** = $P < 0.0001$, ** = $P < 0.001$, and * = $P < 0.003$.

Figure 2.1. Binomial analysis of the relationship ($P = 0.0286$) of the probability of dark cutting with carcass weight (kg).

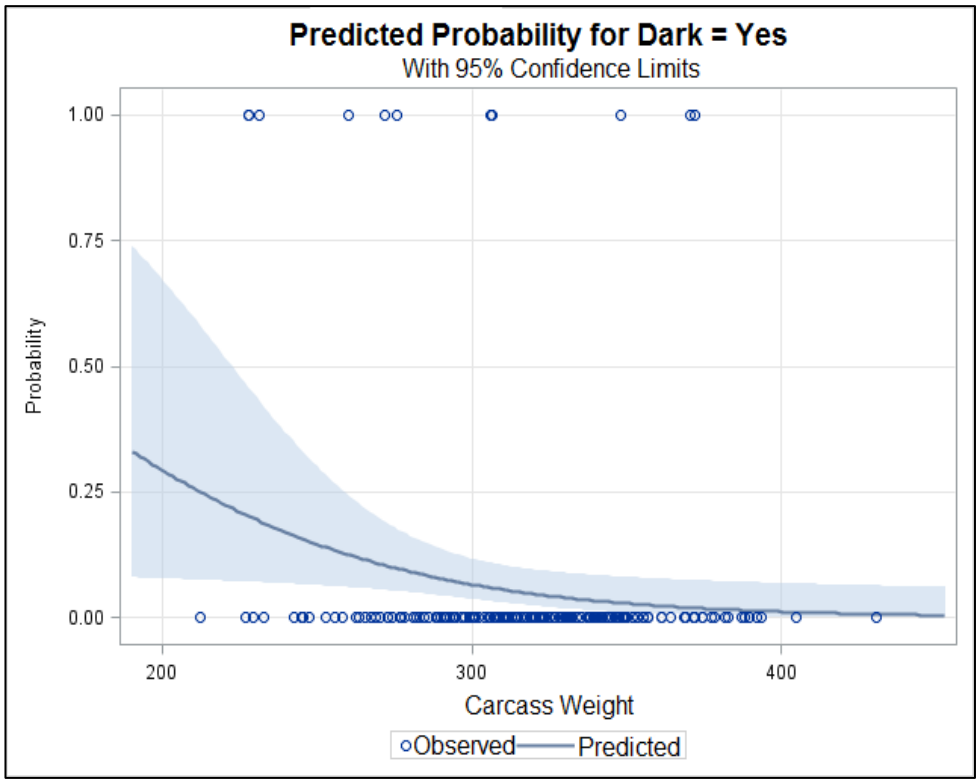


Figure 2.2. Predicted cumulative probabilities from multinomial logistic regression describing the incidence of carcass grades related to DMI (kg day^{-1}) (P = 0.0034) with ultrasound marbling score (uMS) set to the data set mean of 4.939 (Standard/Trace, 1 to 3.99; Select/Slight, 4 to 4.99; Choice/Small to Moderate, 5.0 to 7.99 and greater than or equal to Slightly Abundant/Prime, 8.0 to 11).

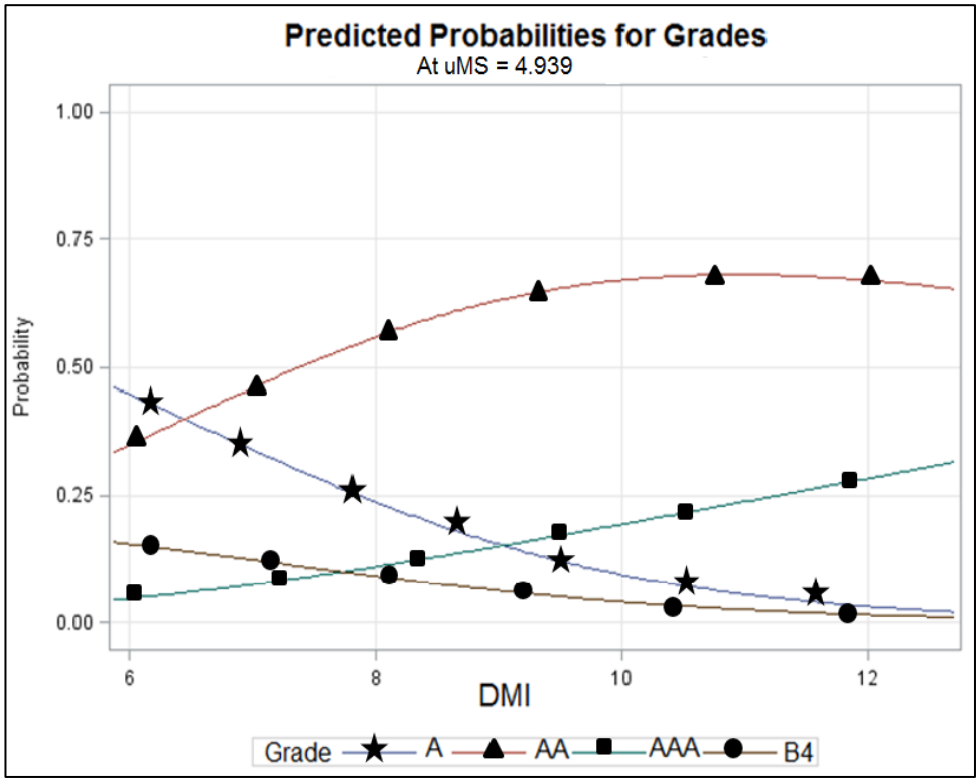


Figure 2.3. Predicted probabilities from multinomial logistic regression describing the incidence of carcass grades as it related to ultrasound marbling score (uMS) (P <.0001). Dry matter intake (DMI) set to overall data set mean of 9.149 kg day^{-1}.

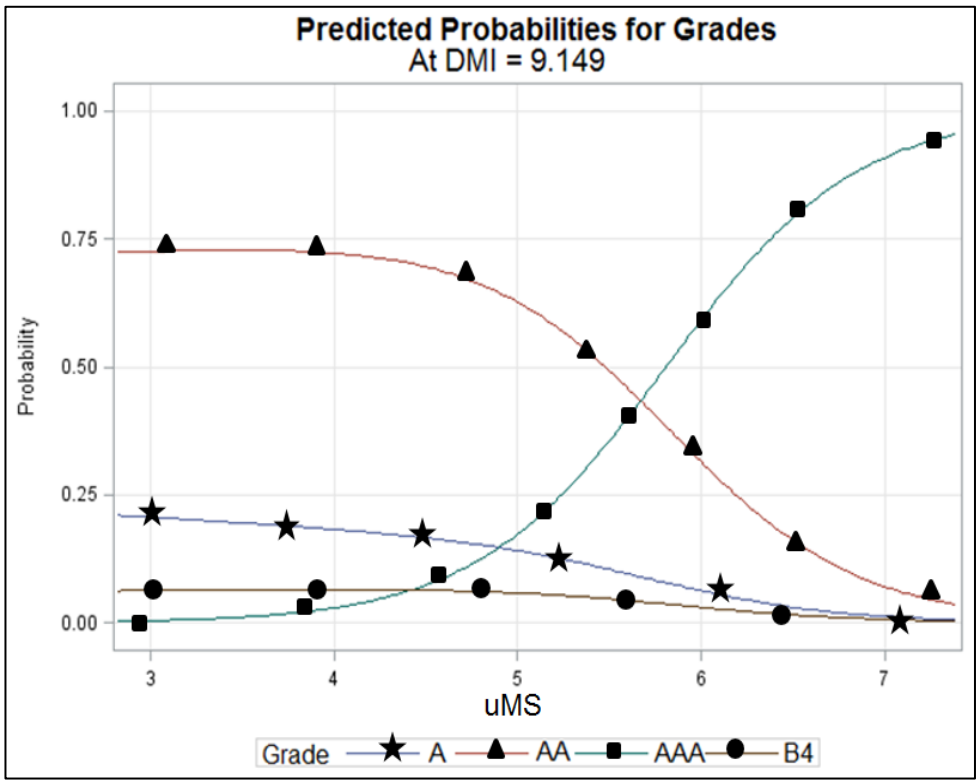


Figure 2.4. Predicted probabilities from multinomial logistic regression describing the incidence of carcass grades as it related to carcass weight (CW, kg) (P = 0.0145). Carcass grade fat depth (gFD) set to data set mean of 8.89 mm.

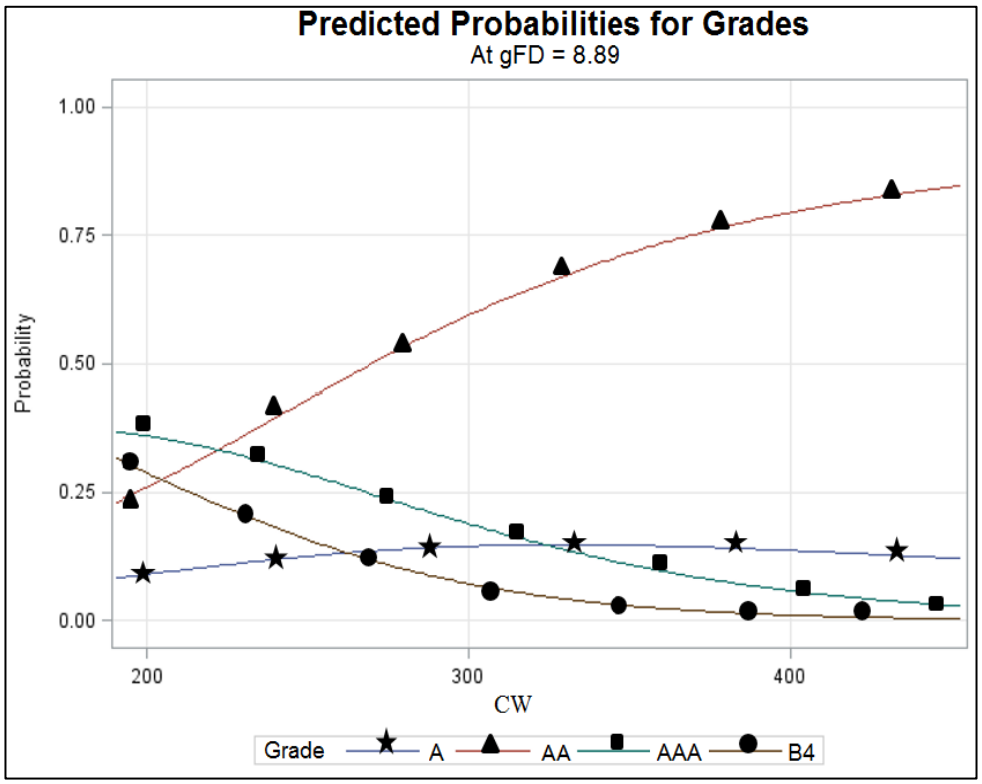


Figure 2.5. Predicted probabilities from multinomial logistic regression by grade describing the incidence of carcass grades as it relates to grade fat depth (gFD, mm) (P = 0.0002). Carcass weight (CW) set to data set mean of 316 kg.

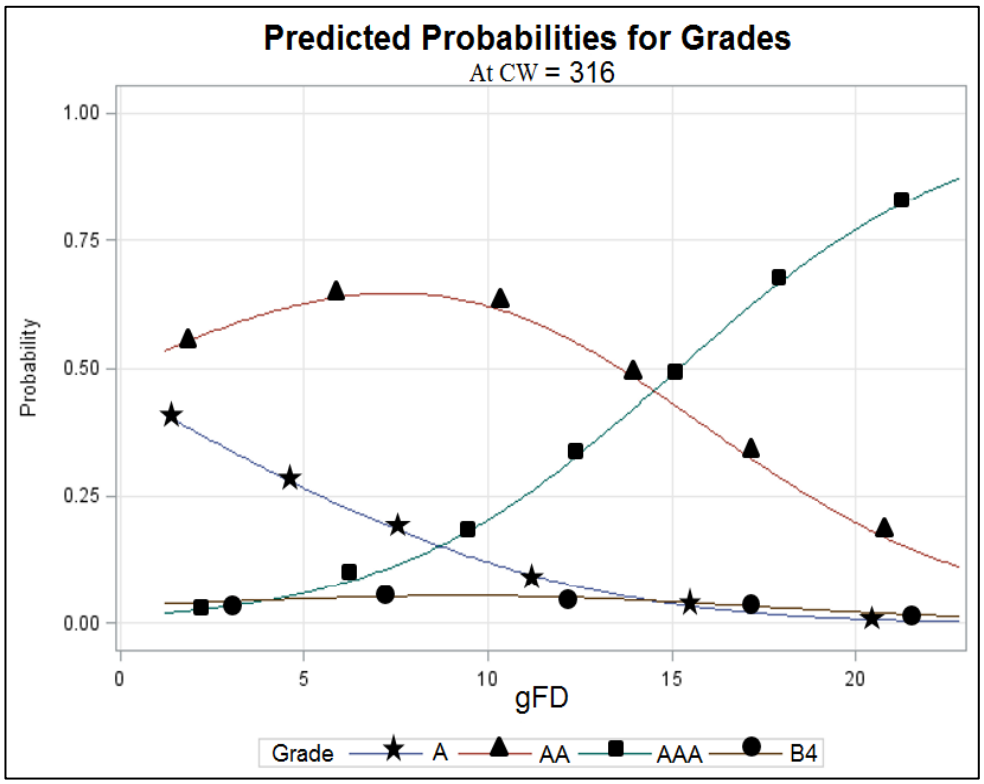
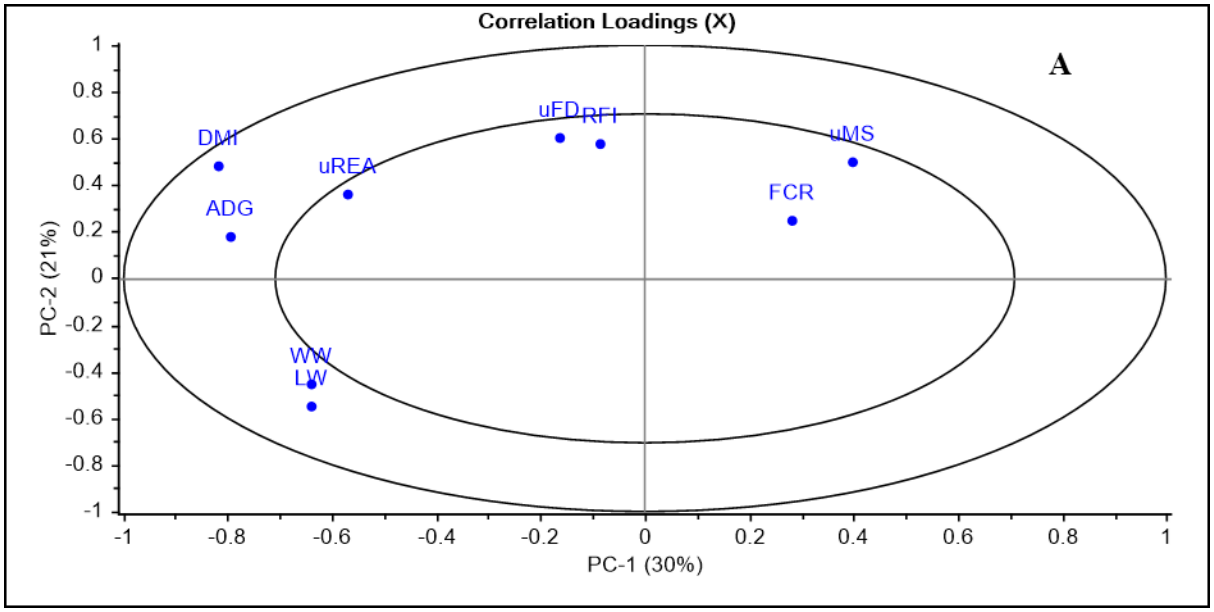


Figure 2.6. Principal component analysis correlation loadings (A) and scores (B) for live animal production data related to animal breed, sex and grade. Score acronyms: HC = dark cutting Charolais heifers, HX = dark cutting crossbred heifers, SC = dark cutting Charolais steers, SX = dark cutting crossbred steers; 3X, 2X and 1X represent crossbred cattle that produced Canada AAA, AA and A carcasses, respectively; and 3C, 2C and 1C represent Charolais cattle that produced Canada AAA, AA and A carcasses, respectively.



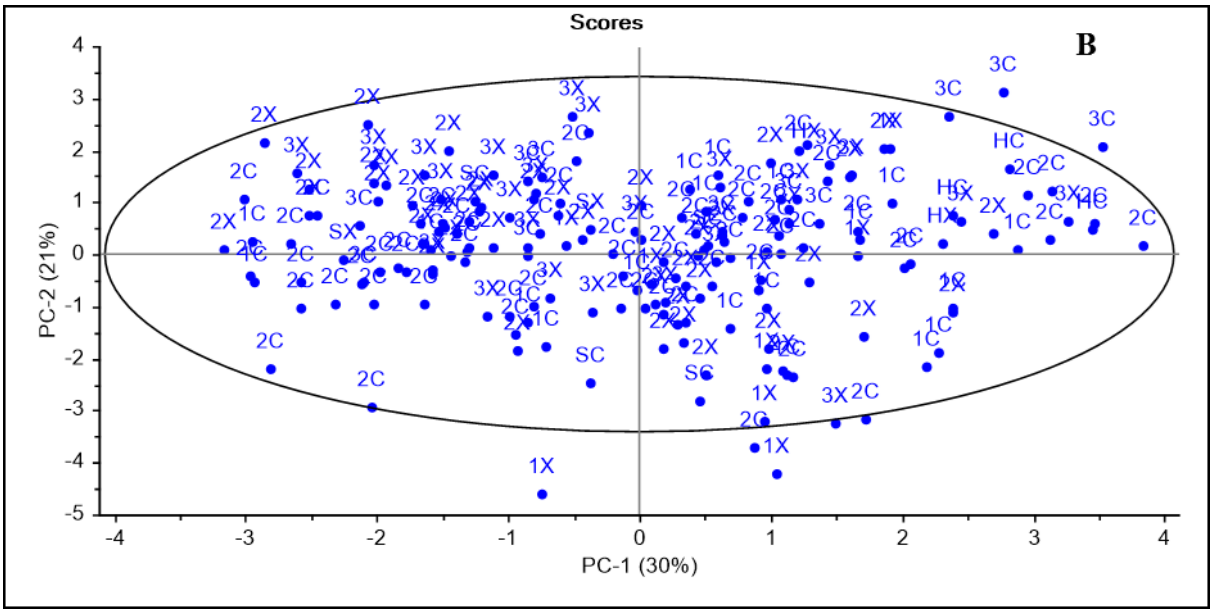
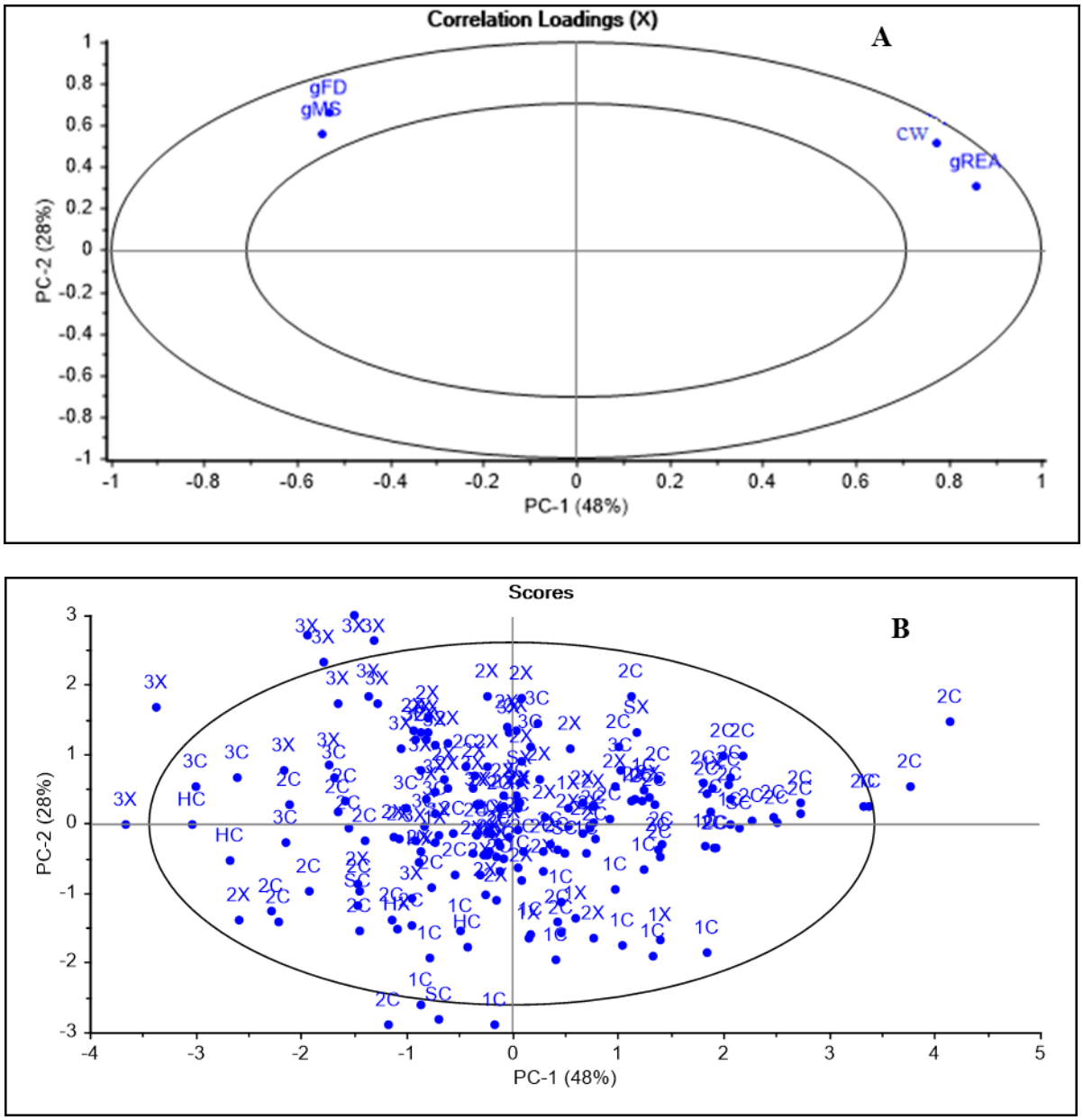


Figure 2.7. Principal component analysis correlation loadings (A) and scores (B) for carcass measurements related to animal breed, sex and grade. Score acronyms: HC = dark cutting carcasses from Charolais heifers, HX = dark cutting carcasses from crossbred heifers, SC = dark cutting carcasses from Charolais steers, SX = dark cutting carcasses from crossbred steers; 3X, 2X and 1X represent Canada AAA, AA and A carcasses from crossbred cattle, respectively; and 3C, 2C and 1C represent Canada AAA, AA and A carcasses from Charolais cattle, respectively.



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3.0. Relationship between phenotype, carcass characteristics and the incidence of dark cutting in heifers[2]

3.1. Introduction

Carcasses from cattle under 30 months of age that have a purple or dark red rib eye muscle (m. *longissimus thoracis*, LT) at grading are considered dark cutting and graded Canada B4 (Canadian Agricultural Products Act SOR/92-541, 2014), while carcasses with normal rib eye muscle colour are graded by marbling score into Canada Prime, AAA, AA and A, which are equivalent to United States Department of Agriculture (USDA) Prime, Choice, Select and Standard. Dark cutting beef usually has an ultimate pH greater than 5.8 and is discriminated against by retailers because of its reduced shelf life and by consumers because of its abnormal appearance. Because of this reduced retail acceptability, dark cutting carcasses are discounted by as much as 40%, resulting in a substantial economic loss to producers whose cattle are affected; therefore identifying dark cutting cattle before slaughter for remediation would be financially advantageous.

Production factors related to dark cutting are manifold and include the use of growth promotants (Schneider et al. 2007), pre-slaughter management (Lacourt and Tarrant 1985; Mach et al. 2008), and time of year/season (Knee et al. 2004; Kreikemeier et al. 1998). The frequency of dark cutting has also been found to be higher in heifer than in steer carcasses (Lorenzen et al. 1993), likely because of their temperament (Voisenet et al. 1997), estrus activity (Kenny and Tarrant 1988) or reduced carcass weight (Murray 1989). Relationships between animal and carcass phenotypes and dark cutting incidence have been identified (McGilchrist et al. 2012) but

[2] This chapter has been published as S. Mahmood, J.A. Basarab, W.T. Dixon, H.L. Bruce (2016) in Meat Science, 121, 261-271.

are contentious, as increased carcass weight, fat depth and rib eye area have been associated with reduced incidence of dark cutting (McGilchrist et al. 2012), while increased rib eye area (Hawrysh et al. 1985; Park et al. 2007), animal growth rate (Młynek and Guliński 2007) and slaughter weight (Vestergaard et al. 2000) have also been associated with increased dark cutting. The relationship between dark cutting and marbling score is also unclear, with increased marbling score either unrelated (McGilchrist et al. 2012) or linked to reduced dark cutting frequency (Park et al. 2007). Similarly, the relationship between dark cutting and muscle fibre type may also be important, as Zerouala and Stickland (1991) found that beef *longissimus dorsi* (LD) at 48 hour post-mortem that had colour which was slightly to vividly dark and pH greater than 6.0 had more oxidative and fewer glycolytic fibres compared to normal LD. Earlier, Hunt and Hedrick (1977) reported dark cutting LD had intermediate fibre percentages greater than that of normal beef LD but similar to that of pale, soft and exudative (PSE) LD, suggesting that dark cutting may supersede PSE as the duration of ante-mortem stress increase and intramuscular glycogen is depleted.

Despite efforts to mitigate the occurrence of dark-cutting such as not mixing unfamiliar cattle, its incidence has increased in Canada within the last 10 years from 0.8 to 1.3% (Beef Cattle Research Council (BCRC), 2013), while in the United States of America (US) the proportion of dark-cutting has increased from 1.9% in 2005 (Garcia et al. 2008) to 3.2% in 2012 (Moore et al. 2012). Finishing cattle on a high plane of nutrition appears to confer some resistance to the depletion of muscle glycogen by pre-slaughter stressors (Warner et al. 1998), although feed efficient animals may be at risk of dark cutting (Baker et al. 2006). The persistence of dark cutting in the beef industry, despite significant research efforts and implementation of prevention strategies, can be a source of substantial economic loss to cattle owners and justifies

further research. The purpose of this study was therefore to relate heifer phenotype and carcass conformation to the frequency of dark cutting to test the hypothesis that the likelihood of a heifer producing a dark cutting carcass can be predicted from live measurements.

3.2. Materials and methods

The study was conducted on an existing data set with production and carcass measurements available. As a result, no animal ethics approval was required. However, cattle in the data set were from previous research studies in which they were cared for according to the Canadian Council on Animal Care (CCAC, 1993) guidelines.

3.2.1. Data

Data from heifers (n = 467) with complete live animal and carcass data were used for detailed analysis of the relationships between carcass and production phenotypes and the frequency of dark cutting. Data were collected from cattle on study from 2003 to 2011 on three farms, designated A, B and C, which contributed n = 44, n = 267, and n = 156 heifers, respectively. The carcasses in the data set graded normal Canada AAA (n = 136), AA (n = 296), and A (n = 14) or dark cutting Canada B4 (n = 21). Notably, because the dark cutting Canada B4 grade consists of Canada Prime, AAA, AA and A carcasses deemed dark, this grade may contain a range of marbling levels. The heifer data from farm A were previously used for the relationship of dark cutting with sex and production phenotype in that farm (Mahmood et al. 2016).

Production and carcass data were as described by Mahmood et al. (2016) and included animal weaning weight (WW, kg), live weight at slaughter (LW, kg), dry matter intake (DMI, kg DM day^{-1}), average daily gain (ADG, kg gain day^{-1}), feed conversion ratio (FCR, kg DMI kg^{-1} gain), residual feed intake adjusted for ultrasound subcutaneous fat depth(RFI_{fat}, kg DMI day^{-1}), ultrasound rib eye area (uREA, cm^2), ultrasound subcutaneous fat depth (uFD, mm), ultrasound

marbling score (uMS), hot carcass weight (CW, kg), grade fat depth (gFD, mm), grade rib eye area (gREA, cm^2), and grade marbling score (gMS). The data also included animal age at test (age at the start of feeding a concentrate diet), days to finishing (DF; number of days the cattle were fed a concentrate diet), and age at slaughter (SA). Live weight at slaughter (kg) was calculated from the initial feed trial weight added to the number of DF multiplied by the ADG. Production and phenotypic measurements were performed similarly to those described by López-Campos et al. (2012 and 2013). Dry matter intake was calculated by multiplying daily feed intake by feed dry matter, with daily feed intake measured using GrowSafe® feeding stations (GrowSafe® System Inc. Airdrie, Alberta, Canada) and feed dry matter estimated from pooled feed samples dried at 80 ºC in a forced-air oven to a constant weight. Feed conversion ratio was calculated by dividing average daily dry matter intake by ADG. Residual feed intake was calculated as the deviation of actual feed intake from expected feed intake and adjusted for uFD (Basarab et al. 2003 and 2007). Ultrasound subcutaneous fat depth, uREA and uMS were estimated prior to slaughter using an Aloka 500V diagnostic real-time ultrasound with a 17 cm 3.5 MHz linear array transducer (Overseas Monitor Corporation Ltd, Richmond, BC, Canada) by a certified ultrasound technician as described by Brethour (1992). Ultrasound subcutaneous fat depth, gFD, uMS, gMS, uREA and gREA were measured at the Canadian beef grading site, which is at the 12-13th LT (rib eye) muscle interface. Both uMS and gMS were categorized using the United States Department of Agriculture (USDA) scoring system (USDA, 1997) where Canada A, AA, AAA and Prime quality grade marbling corresponded to traces (Standard, 300-399), slight (Select, 400-499), small to moderate (Choice, 500-799), and greater than or equal to slightly abundant (Prime, 800-1099) amounts of marbling, respectively. Traces, slight, small to

moderate and greater than or equal to slightly abundant marbling, respectively which, in turn equated to ultrasound marbling scores between 1.00 to 3.99, 4 to 4.99, 5 to 7.99, and 8 to 11.

The cattle under study were Hereford (sire)-Angus (dam) and purebred Charolais at farm A. Cattle at farms B and C were crossbred composite (BeefBooster®, Calgary, Alberta) that originated from the cross of BeefBooster® terminal composite (TX) sires with crossbred cows (British x British-Continental). BeefBooster® terminal composite bulls (TX) were predominantly Charolais-based with infusion of Holstein, Maine Anjou and Chianina breed (http://www.beefbooster.com). Heifers in the data set were not fed melengestrol acetate (MGA) to control estrous cycle. Cattle from each farm were slaughtered separately in two lots and each lot had data from at least one animal/carcass from each grade. Cattle at farm A were fed and processed at the Agriculture and Agri-Food Canada Meat Research Laboratory (Lacombe, Alberta, Canada) where the distance between feeding and the slaughter facility was 3 km. The cattle at farm B and C were fed separately at two commercial feedlots and shipped to a commercial beef abattoir located at a distance of about 85 km and 130 km from farm B and C, respectively. The cattle from all the farms were transported by standard tractor-trailers early in the morning and slaughtered within 2-5 hours after their arrival at the slaughter plants. Cattle were neither moved using electric prods nor were the cattle from pens, at any farm, mixed during and/or post-transportation. Carcasses were not electrically stimulated and were not spray-chilled. The carcasses from animals from all the three farms were split and weighed to record CW and then chilled for 48 hours at 2 ºC with an average wind speed 1.4 m/s. After chilling, the left sides of the carcasses were ribbed at between 12^{th} and 13^{th} ribs for assessment of colour, gFD, gMS, and gREA and quality grade was assigned by certified beef graders. The dark cutting (B4 grade) carcasses were delineated based upon a federally-approved colour standard.

3.2.2. Statistical analyses

The statistical analyses were performed using the Statistical Analysis Software (SAS) system (Version 9.3, SAS Institute Inc. Cary, NC). A generalized logit model was applied using the CATMOD procedure to compute the frequency of dark cutting in three farms while animal and carcass parameters were tested as covariates. Analyses performed on phenotypic and carcass data included analysis of variance, Pearson correlations, and binomial and multinomial logistic regression. Data were used to examine the effects of farm (A, B, C) and grade (Canada AAA, AA, A and B4) on live animal and carcass characteristics using the MIXED procedure with farm, grade and their interaction as fixed effects and slaughter lot within farm was included as a random term that served as the error term for farm. Kenward-Roger approximation was used to compute the denominator degrees of freedom while differences between means were identified using least square means differences, with significance at $P < 0.05$. For analysis of variance of DMI and ADG, body weight at the start of finishing was included as a covariate. Carcass rib eye areas adjusted (adjusted gREA) for each 45.45 kg of CW was also used for the analysis of variance. Where analysis of variance models were significant ($P < 0.05$), differences between means were identified using least square means differences, with significance at $P < 0.05$.

Relationships between independent variables were investigated using Pearson correlations (PROC CORR) and the Bonferroni correction was used to compensate for the likelihood of type I significance error between measurements that were not independent from each other. There is an estimated 7% likelihood of type I error for 12 comparisons; therefore, a correction was made by dividing $\alpha = 0.05$ by the number of comparisons (15) for a level of significance of $P < 0.003$ (Rice 1989).

Binomial and multinomial logit regression models were used to examine trends in the data set. For the binomial logit regression, data of cattle that produced normal (Canada AAA, AA and A) carcasses were combined into one category (NORMAL) and compared to the data of cattle that produced dark-cutting (Canada B4) carcasses (DARK) and the probability of being dark (yes or no) was modeled. In multinomial logistic regression, the response variables were the individual carcass grades (Canada AAA, AA, A and B4). In both binomial and multinomial logistic regressions, covariate analyses of live animal and carcass characteristics were conducted separately with farm as the treatment effect. Where correlations existed between the covariates, only the covariate that produced the highest R^2 in the model was included in the final analysis. Regression models were built using the backward selection procedure ($\alpha = 0.05$) and goodness of fit for the binomial model was tested using the Hosmer and Lemeshow Goodness-of-Fit test. Graphs for predicted probabilities for both binomial and multinomial responses were obtained in SAS.

Principal component analysis (PCA) was used to identify relationships between phenotypic measurements and the dark beef (Canada B4) grade. Principal component analysis was performed using the Unscrambler® (Camo Scientific Inc., Woodbridge, New Jersey) and PCA was conducted separately for live animal and carcass measurements. All measurements were transformed prior to analysis using the center and scale function of the software, as centering and scaling reduces differences in variation between measurements due to measurement scale, which can bias proportioning of variation within the analysis (Dijksterhuis, 1994).

3.3. Results

The results presented in this section elucidated the influence of farm and animal/carcass measurements on the likelihood of dark cutting, and the relationships between and within animal and carcass characteristics. The mean values of animal and carcass measurements, obtained from the analysis of variance, were presented in tables for the main effects of farm and grade, however; when farm and grade interacted, the variables were not presented with the main effects but instead presented in a separate table.

3.3.1. Effect of farm on dark cutting

Farm was identified as significant ($P = 0.0268$) in CATMOD analysis where dark cutting frequency was 11.4 ± 4.8%, 5.2 ± 1.4% and 1.3 ± 0.9%, respectively at farm A, B and C. The effect of farm was confounded with breed type as farm A had a cattle breed different from breeds at farm B and C. Effect of farm was insignificant, however, once LW or CW were included in the analysis, suggesting that the difference between farms was driven by LW or CW that might have been influenced by production practices across the farms.

3.3.2. Effect of farm and grade on animal/carcass measurements

Results of the analysis of variance, indicating how heifers differed between farms and grades, are presented in Tables 3.1 to 3.3. The effect of farm on age at test (age at the start of feeding concentrate diet) approached significance ($P = 0.06$) where heifers from farm B were younger than heifers from farms A and C (Table 3.1). The effect of farm was not significant for ADG, DMI, FCR, RFI_{fat}, and uFD. Mean gFD was greater for heifers from farm C than in heifers from farm B while mean values of farm A heifers were not different from that of farm B and C ($P < 0.05$; Table 3.1). Heifers from farm A had the smallest mean gREA (unadjusted) while heifers from farms B and C were not different ($P < 0.05$).

Grade approached significance for age at test ($P = 0.0682$) and DMI ($P = 0.0601$; Table 3.2), indicating that dark cutting heifer mean age at test and mean DMI were not different from that of heifers that produced normal carcasses. Grade was not significant for mean ADG and FCR but did have a significant effect ($P < 0.05$) on mean values of RFI_{fat}, uFD, gFD and unadjusted gREA (Table 3.2). Results indicated that heifers that produced dark cutting carcasses (Canada B4) had mean RFI_{fat} similar to Canada AA and AAA heifers. Conversely, heifers that produced Canada A grade carcasses had a mean RFI_{fat} less than that of cattle from other grades. Mean uFD and gFD of dark cutting heifers and carcasses, respectively, was similar to mean values of Canada A and AA heifers and carcasses and were greatest for Canada AAA and smallest for Canada A heifers and carcasses, respectively. Dark cutting heifer carcasses had a mean gREA similar to that of Canada AA and AAA heifer carcasses, which was significantly smaller than the mean gREA of the Canada A heifer carcasses.

Significant interactions ($P < 0.05$) between farm of origin and grade were noted for DF, SA, WW, LW, CW, uREA, gREA adjusted for CW (cm^2 45^{-1} kg CW), uMS and gMS (Table 3.3). Results indicated that dark cutting heifers had mean DF similar to the heifers that produced normal carcasses at all three farms. Dark cutting heifers had mean SA similar to those that produced normal carcasses at farm A and B. At farm C, mean SA of dark cutting heifers was less than that of heifers that produced Canada AAA and A carcasses but similar to those produced AA carcasses. At farm A, dark cutting heifers had reduced WW and LW compared to heifers that produced normal carcass grades with the exception of heifers from the same farm that graded Canada AAA. Mean WW, LW and CW of dark cutting heifers were not different from heifers that produced Canada A, AA and AAA carcasses at farms B and C (Table 3.3). The relationship between mean LW and dark cutting did not show a clear pattern, although the interaction was

significant (Table 3.3). For CW, only heifers from farm A that cut dark produced a lighter weight carcass than heifers that produced normal carcasses (Table 3.3). Mean uREA was not different for heifers regardless of grades within farm; however, at farm C the mean uREA of heifers was higher than that of heifers at farms A and B with the exception of heifers that produced Canada A carcasses at farm B (Table 3.3). The interaction for mean gREA adjusted for CW indicated that it was not different due to grade at farm A but was significantly greater for dark cutting (Canada B4) and Canada A carcasses and smaller for Canada AA and AAA carcasses at farm B. At farm C, mean adjusted gREA was significantly greater for Canada A carcasses while the remaining grades were not different from each other (Table 3.3). Mean uMS and gMS were greatest for Canada AAA and smallest for Canada A heifers and carcasses, respectively; however; mean values for dark cutting heifers/carcasses were greater than Canada A heifers/carcasses but similar to that of Canada AA or AAA heifers/carcasses (Table 3.3).

3.3.3. Relationships between carcass and animal measurements

Pre-planned correlations identifying linear relationships between and within carcass and live animal characteristics are presented in Table 3.4 with significance at $P < 0.003$ with the Bonferroni correction. Notably, the results between live and carcass parameters indicated that increased SA was associated with an increased uFD ($r = 0.17$, $P < 0.001$), uREA ($r = 0.26$, $P < 0.0001$) and gFD ($r = 0.36$, $P < 0.0001$). Average daily gain was positively correlated with uFD ($r = 0.23$, $P < 0.0001$), uREA ($r = 0.30$, $P < 0.0001$), LW ($r = 0.60$, $P < 0.0001$) and CW ($r = 0.37$, $P < 0.0001$). Live weight at slaughter had positive correlations with uFD ($r = 0.35$, $P < 0.0001$), uREA ($r = 0.63$, $P < 0.0001$), DMI ($r = 0.61$, $P < 0.0001$), CW ($r = 0.89$, $P < 0.0001$), gFD ($r = 0.14$, $P < 0.003$) and gREA ($r = 0.38$, $P < 0.0001$). Within carcass characteristics, CW had positive correlations with gFD ($r = 0.17$, $P < 0.001$) and gREA ($r = 0.48$, $P < 0.0001$) but

had negative correlation with gMS (r = -0.16, P < 0.001). Increased gREA was negatively associated with gFD (r = -0.17, P < 0.001) and gMS (r = -0.30, P < 0.0001). Moreover, live animal uFD, uREA and uMS had positive correlations respectively with carcass gFD (r = 0.59, P < 0.0001), gREA (r = 0.52, P < 0.0001) and gMS (r = 0.50, P < 0.0001), indicating a moderate prediction of carcass muscle and fat score by ultrasound in live animals.

3.3.4. Effect of animal/carcass measurements on the probabilities of dark and normal carcasses

Binomial logistic regression analysis of the live animal measurements identified significant (P < 0.05) effects of ADG and FCR with backward selection, but the R^2 value for the model was very low (R^2 = 0.057). Individual incorporation of the correlated variables ADG and FCR in the model resulted in no significant effect (P > 0.05) on the probability of dark cutting, therefore; the effects of live parameters on dark cutting were deemed insignificant. Binomial regression identified carcass weight as a significant (P < .0001) indicator of the probability of dark-cutting with an R^2 = 0.12, and a substantial decline in the probability of dark cutting was predicted at carcass weights greater than 325 kg (Figure 3.1).

Multinomial logistic regression indicated significant (P < 0.05) effects of uMS, ADG and farm on the probability of dark cutting and realizing a Canada B4 grade with a max-rescaled R^2 = 0.28. As expected, the likelihood of the grade increasing from A to AAA increased as uMS increased and the likelihood of a carcass dark cutting was greatest although not more than 20% at an uMS of between 5 and 6 for heifers from Farm A only (data not shown). For ADG, the results indicated that the likelihood of a heifer producing a dark cutting carcass (Canada B4) decreased as ADG increased when the data set mean for uMS of 4.7 was used in the regression, but only for heifers from farms A and B (Figure 3.2). Also, the probability of heifers producing Canada AA

carcasses increased with ADG but decreased for Canada AAA and remained unaffected for Canada A heifers, regardless of the farm (Figure 3.2).

Live weight at slaughter was not initially included in the model because it was highly correlated (r = 0.60, P < 0.0001) with ADG; however, replacing ADG with LW in the multinomial analysis indicated a significant effect of LW (P = 0.0247), uMS (P < .0001) and farm (P = 0.0054) without affecting the R^2 (0.28) of the model. The likelihood of heifers producing dark cutting carcasses, regardless of the farm of origin, was appreciably reduced at LW greater than 550 kg when the mean uMS of the data set (4.7) was used in the model (Figure 3.3).

Multinomial analysis with carcass measurements identified significant effects (P < 0.05) of farm, gMS, gREA and CW but correlations existed between these measurements so separate regressions were conducted for each. Regressions with CW and gREA, although significant, had very low R^2 values of 0.057 and 0.093, respectively. Carcasses weighing greater than 325 kg were least likely to cut dark and farm was not significant when included with CW (Figure 3.4). Farm effect, however, was significant when used with gREA as the gREA tended to be associated with decreased likelihood of dark cutting at farm A but there was no association at farms B and C (Figure 3.5). Regression with gMS and farm indicated significant effects of gMS (P <.0001) and farm (P <.0001) on the likelihood of carcass grade and the maximum rescaled R^2 was 0.57. Results indicated that heifer carcasses were least likely to be dark cutting at a gMS of 600 at farm A and at gMS 500 at farm B and C (Figure 3.6). Carcasses at all the farms were most likely to grade Canada A at marbling score below 400 (Figure 3.6).

3.3.5. Relationships between animal/carcass characteristics and dark cutting grade

Principal component analysis (PCA) of the animal data explained 53% of the total variation in live animal measurements (Figure 3.7). The first component (PC-1) described 34% variation and illustrated that DMI, uREA and LW were correlated with each other and that DF decreased as LW, uREA, DMI and uFD increased (Figure 3.7a). PC-2, which described 19% of the data variation, indicated that FCR increased as ADG, WW, LW and DF decreased (Figure 3.7a). Identification of all the individual animals on PCA score plot was impossible due to the number of animals included in the analysis; therefore only dark cutting animals were highlighted by farm of origin in Figure 3.7b. Dark cutting heifers at farm A were mostly present in upper left quadrant of the PCA, indicating reduced LW, DMI and uREA on PC-1 and slightly increased FCR on PC-2. Dark cutting heifers at farm B were positioned left of centre on the origin of PC-2 on the score plot while dark cutting heifers at farm C were located on the right half of the plot. These results showed reduced DMI, LW, uREA and uFD for dark cutting heifers at farm B; however, dark cutting heifers at farm C had increased LW, uREA and DMI (Figure 3.7b).

Principal component analysis with the carcass data identified 42% variation along PC-1 and 29% variation along PC-2 (Figure 3.8). The PC-1 indicated that CW and gREA were positively correlated with each other and both decreased with the increase of gMS (Figure 3.8a). The PC-2 indicated that CW, gFD and gMS could increase with no change in gREA. Dark cutting carcasses from farm A had reduced gREA and CW but increased gMS along PC-1 (Figure 3.8b). Dark cutting carcasses from farm C were not clearly delineated by the PCA factors with only one heifer exhibiting an increased CW and gREA while heifers from farm B were present in all the four quadrants (Figure 3.8b).

3.4. Discussion

The incidence of dark cutting has increased from 0.8 to 1.3% in Canada within the last 10 years (Beef Cattle Research Council (BCRC), 2013) while in the United States of America (US) the proportion of dark-cutting was 2.3% in 2002 (McKenna et al. 2002), decreased to 1.9% in 2005 (Garcia et al. 2008) and again increased to 3.2% in 2012 (Moore et al. 2012). This persistence suggests that the factors contributing to dark cutting are manifold (Mahmood et al. 2016) and most likely cumulative (Scanga et al. 1998). Identification of cattle at risk of dark cutting would be advantageous in that dark cutting and the concomitant financial penalty could be prevented, and the physical condition remedied prior to slaughter. Previous work (McGilchrist et al. 2012) suggested that cattle at risk of dark cutting may have specific phenotypic characteristics, and that establishing a particular phenotype at risk would enable producers to limit incidence of this meat quality defect.

In the present study, results from analysis of variance indicated that growth indicators such as age at the start of feeding a concentrate diet, DMI, ADG, FCR, RFI, and uFD of heifers that ultimately produced a dark cutting carcass were not different from those of heifers that produced normal carcasses. Similarly, according to analysis of variance, carcass measurements such as gFD and gREA were not different between carcasses from at least one normal grade category and those that cut dark. Principal component analysis captured the relationships between heifer characteristics and dark cutting, showing clearly that characteristics related to the incidence of dark cutting varied by farm. Farms differed in the frequency of dark cutting but elucidating the cause was difficult as the effect of farm was confounded with breed, which can influence the incidence of dark cutting (Lorenzen et al. 1993; Shackelford et al. 1994; Mach et al. 2008). The effect of farm was insignificant, however, once LW or CW were included in the

analysis, indicating that the differences in dark cutting incidence due to farm were driven by LW or CW. This result agreed with previous findings where breeds varied in the incidence of dark cutting but also in carcass weight (Lorenzen et al. 1993; Wulf el al. 1997).

Carcass weight appeared to be the most important indicator of the likelihood of a heifer producing a dark-cutting carcass, as binomial analysis showed that the probability of cutting dark declined as CW increased and approached zero at CW greater than 325 kg. This agreed with previous findings where dark cutting heifers (Cliplef et al. 1989; Murray 1989) and steers (Park et al. 2007) had reduced carcass weights. Previous research has indicated that the risk of dark cutting is decreased in carcasses weighing greater than 150 kg (McGilchrist et al. 2012) or 318 kg (Murray 1989), and are in accordance with the observations in the present study.

Carcass weight is usually positively correlated with live weight (Johnson et al. 1986; Wulf et al. 1997) and this was the case in the present study. The risk of dark cutting in heifers in this study also decreased as LW increased when the relationship was described using multinomial logistic regression, with the likelihood of dark cutting substantially reduced at LW greater than 550 kg. That the relationship between LW and dark cutting was not as strong as that between CW and dark cutting suggested that CW was indicative of something more than just weight. Carcass weight was positively correlated to uREA, gREA and DMI, all of which were positively correlated to each other as well, indicating that CW was related to muscularity and feed intake. The contribution of feed intake to dark cutting is well-documented, as increasing the amount and energy of feed for cattle will increase glycogen level in muscle (McVeigh and Tarrant 1982) and in liver (Gardner et al. 2014), thus providing resistance to pre-slaughter stress, the opportunity for repletion of muscle glycogen following exertion (Jacob et al. 2009) and reducing the probability of dark cutting (Knee et al. 2007). In the present study, DMI was not

identified by analysis of variance or PCA as being different for dark cutting (Canada B4) carcasses. According to the analysis of variance, however, gREA was lowest in the dark cutting carcasses and was comparable to that of the Canada AAA carcasses, although dark cutting carcasses had a mean gFD less than that of the Canada AAA carcasses. These results supported the findings of McGilchrist et al. (2012) who noted that reduced fat depth and muscling scores collectively predisposed beef carcasses to cut dark.

Cattle with increased muscling may have reduced likelihood of dark cutting as their muscles tend to have an increased proportion of glycolytic muscle fibres (Wegner et al. 2000; Zerouala and Stickland 1991), which may increase postmortem pH decline and ultimately lightening of meat colour (Dalya et al. 2006; Gardner et al. 2006; Glanc et al. 2015; Shackelford et al. 1994). However, during ante-mortem physical stress the fast-twitch glycolytic as well as fast-twitch oxidative glycolytic (intermediate fibres) rapidly deplete their stored glycogen (Lacourt and Tarrant 1985). Zerouala and Stickland (1991) found increased concentration of slow-twitch oxidative and intermediate fibres in dark cutting beef while Hunt and Hedrick (1977) found a greater concentration of intermediate fibres in both dark and PSE beef and suggested that intermediate fibres were the predictor of dark or PSE condition depending upon the nature of ante-mortem stress. McGilchrist et al. (2016) found that cattle with increased muscling did not in fact have increased glycolytic potential, but did have greater oxidative capacity than cattle with decreased muscling. This suggests that cattle with increased muscling were more likely to completely oxidize glycogen during energy expenditure or to use lipids as a source of energy, thus sparing muscle glycogen, compared to cattle with reduced muscling.

The findings of the current study contrast with previous studies where increased muscle area was associated with increased carcass pH and intensity of muscle colour darkness (Hawrysh

et al. 1985; Janloo et al. 1998). Dark cutting may be associated with increased muscularity and weight in situations where feed energy does not adequately meet animal growth requirements when growth promoting steroids are present (Janloo et al. 1998; Scanga et al. 1998). Animals with increased muscularity may also have decreased subcutaneous fat (Janloo et al. 1998), as also indicated in the current study, which could lead to an increased rate of muscle cooling and decreased rate of pH decline post-mortem, which darkens muscle colour (Aalhus et al. 2001). An increased rate of muscle cooling post-mortem also decreases protein denaturation within muscle organelles such as the mitochondria where high temperature conditions and low pH favour protein denaturation (Sayre and Briskey 1963). Preservation of mitochondrial oxidative activity reduces oxygen residency time with myoglobin post-mortem thus increasing deoxymyogobin concentration (Ashmore et al. 1972; Egbert and Cornforth 1986).

No one live animal or carcass phenotype was associated with dark cutting at each farm, as dark cutting carcasses were found in each quadrant of the PCA plots, suggesting that the causes of dark cutting were manifold and changed with animal phenotype (Mahmood et al. 2016). Multinomial logistic regression indicated that the likelihood of cutting dark decreased as ADG increased for cattle at farms A and B, with no dark cutting exhibited in cattle from any farm when ADG was greater than or equal to 1.5 kg. These findings indicated that heifers at risk of dark cutting were slow growing and had reduced LW. The results, however, contradicted previous findings in bulls (Młynek and Guliński 2007; Vestergaard et al. 2000) where increased growth rate and slaughter weight increased carcass pH and reduced lightness score. The association between ADG and reduced dark cutting observed in the present study may be due to positive correlations between ADG, LW, uREA and CW, but provides a ready indicator for beef producers against which to measure dark cutting risk.

Intramuscular fat did not appear to mitigate the risk of dark cutting; in fact, cattle with gMS between 400 and 600, equivalent to Canada AA (400-499) and AAA (500-799), were most likely to produce dark cutting carcasses according to multinomial logistic regression results. That rib eye marbling did not mitigate the incidence of dark cutting agreed with the results of McGilchrist et al (2012) and Wulf et al. (1997) but opposed the results of other studies (Janloo et al. 1998; Muir et al. 1998; Park et al. 2007). The results supported those of Lorenzen et al. (1993) who found that dark cutting carcasses were similar to USDA Select and Choice carcasses, which are equivalent to Canada AA and AAA, respectively.

3.5. Conclusions

It was hypothesized that the potential of cutting dark in heifers can be predicted from growth performance, live animal phenotype and carcass measurements. The results supported acceptance of the hypothesis as heifers at risk of dark cutting exhibited slow growth, had reduced live weight at slaughter and produced carcasses of decreased weight. Such heifers may need to be sorted by weight and daily gain and managed adequately through provision of a high energy diet in the period leading up to slaughter and through minimizing pre-slaughter stress during handling to mitigate this risk. Initial selection of slaughter heifers for increased growth rate and live weight may reduce the incidence of dark cutting. The results also indicated that marbling was not related to dark cutting and that potential dark cutting heifers were those most likely to have produced Canada AA/USDA Select grade carcasses. Ultrasound measurement of rib eye area, calculation of ADG and monitoring of live weight will assist in predicting carcass grades in heifers and identifying those most at risk of dark cutting.

Table 3.1. Effect of farm on least square means (± standard error of the means within parentheses) for animal performance and carcass characteristics.

Measurements	Farm A (n = 44)	Farm B (n = 267)	Farm C (n = 156)	P value[1]
Age at test (days)	362 (22)	264 (22)	377 (22)	0.0601
Dry matter intake (DMI, kg.day^{-1})	9.17 (0.42)	7.69 (0.40)	8.59 0.43	0.1701
Average daily gain (ADG, kg. day^{-1})	1.43 (0.27)	1.36 (0.26)	1.35 (0.27)	0.974
Feed conversion ratio (FCR, kg DMI kg^{-1} gain)	6.30 (1.50)	5.50 (1.46)	7.43 (1.51)	0.6856
Residual feed intake (RFI_{fat}, kg DMI day^{-1})	-0.152 (0.10)	0.013 (0.052)	-0.057 (0.09)	0.3285
Ultrasound subcutaneous fat depth (uFD, mm)	7.15 (0.64)	5.77 (0.49)	7.48 (0.62)	0.1474
Grade fat depth (gFD, mm)	8.40^{ab} (0.74)	7.44^{b} (0.44)	9.92^{a} (0.92)	0.0454
Grade rib eye area (gREA, cm^2)	75.74^{b} (2.82)	90.84^{a} (2.24)	94.09^{a} (3.15)	0.0217

[a, b, c] Least square means within a row lacking a common letter differ at $P \leq 0.05$.

[1] Probability of the F test, with significance at $P \leq 0.05$.

Table 3.2. Influence of grades on least square means (± standard error of the means within parentheses) for animal performance and carcass characteristics.

Measurements	Grades[1]				P value[2]
	A (n= 14)	AA (n= 296)	AAA (n= 136)	B4 (n= 21)	
Age at test (days)	336 (13)	333 (12)	339 (13)	330 (13)	0.0682
Dry matter intake (DMI, $kg.day^{-1}$)	8.12 (0.30)	8.55 (0.23)	8.69 (0.24)	8.57 (0.30)	0.0601
Average daily gain (ADG, $kg.\ day^{-1}$)	1.30 (0.17)	1.37 (0.15)	1.43 (0.15)	1.41 (0.17)	0.3321
Feed conversion ratio (FCR, kg DMI kg^{-1} gain)	6.38 (1.03)	6.70 (0.85)	6.34 (0.87)	6.23 (1.04)	0.5998
Residual feed intake (RFI_{fat}, kg DMI day^{-1})	-0.393^{b} (0.13)	0.0038^{a} (0.034)	0.0589^{a} (0.050)	0.069^{a} (0.129)	0.0185
Ultrasound subcutaneous fat depth (uFD, mm)	5.82^{c} (0.68)	6.99^{b} (0.29)	7.92^{a} (0.34)	$6.46^{b,c}$ (0.66)	0.00094
Grade fat depth (gFD, mm)	6.33^{c} (1.19)	8.92^{b} (0.28)	11.08^{a} (0.42)	$8.02^{b,c}$ (1.06)	<.0001
Grade rib eye area (gREA, cm^2)	96.59^{a} (3.48)	86.53^{b} (1.35)	81.79^{c} (1.60)	82.64^{bc} (3.13)	<.0001

[a, b, c] Least square means within a row lacking a common letter differ at $P \leq 0.05$.

[1] Canada carcass grades (A, AA and AAA) correspond respectively to USDA quality grade Standard, Select, and Choice while grade Canada B4 indicates dark cutting.

[2] Probability of the F test, with significance at $P \leq 0.05$.

Table 3.3. Interactions between farm and grade (means with standard errors of mean in parentheses) for production and carcass measurements.

Measurement	Farm A				Farm B				Farm C				P value[1]
	A[2]	AA	AAA	B4	A	AA	AAA	B4	A	AA	AAA	B4	
n	3	25	11	5	7	176	70	14	4	95	55	2	
Days to finishing (DF)	110^{b} (24)	115^{b} (23)	114^{b} (23)	117^{b} (24)	176^{a} (23.4)	168^{a} (22.6)	161^{a} (22.6)	178^{a} (23.0)	136^{ab} (24)	111^{b} (23)	130^{ab} (23)	97^{b} (25)	<.0001
Age at slaughter (days)	477^{ab} (36)	474^{ab} (34)	478^{ab} (34)	478^{ab} (35)	440^{b} (35)	427^{b} (34)	431^{b} (34)	443^{b} (34)	513^{a} (36)	494^{ab} (34)	514^{a} (34)	460^{b} (37)	0.0023
Weaning Weight (WW, kg)	230^{a} (23)	191^{b} (16)	175^{bc} (18)	154^{c} (20)	226^{a} (19)	228^{a} (16)	240^{a} (16)	222^{a} (17)	180^{bc} (21)	228^{a} (16)	205^{ab} (16)	198^{abc} (25)	<.0001
Live weight at slaughter (LW, kg)	546^{bc} (33)	519^{cd} (20)	487de (23)	441^{e} (28)	525^{cd} (25)	561^{bc} (18)	583^{ab} (18)	553^{bc} (22)	562^{bc} (30)	618^{a} (18)	597^{ab} (19)	608^{ab} (38)	<.0001
Carcass weight (CW, kg)	316^{bcd} (19)	296de (12)	277^{e} (14)	246^{f} (17)	302^{cd} (15)	317^{bc} (11)	332^{b} (12)	302^{cd} (13)	330^{abc} (22)	354^{a} (11)	337^{ab} (12)	343^{ab} (22)	<.0001
Ultrasound rib eye area (uREA, cm^2)	71.99^{bc} (6.5)	70.09^{bc} (4.07)	65.08^{c} (4.39)	65.88^{c} (5.33)	78.36^{ab} (4.71)	72.98^{bc} (3.81)	74.84^{b} (3.87)	74.46^{b} (4.26)	85.22^{a} (5.33)	87.66^{a} (3.85)	84.39^{a} (3.92)	84.77^{a} (6.47)	0.0224
Grade rib eye area (gREA) adjusted for carcass weight	12.54^{b} (0.83)	12.03^{b} (0.40)	11.78^{b} (0.50)	12.15^{b} (0.68)	14.91^{a} (0.59)	12.72^{b} (0.30)	11.85^{b} (0.33)	13.80^{a} (0.46)	14.50^{a} (1.00)	11.94^{b} (0.32)	11.85^{b} (0.35)	12.13^{b} (1.00)	0.0411
Ultrasound marbling score (uMS)[3]	4.59^{cd} (0.25)	4.80^{c} (0.10)	5.78^{a} (0.14)	5.19^{b} (0.19)	4.08^{e} (0.17)	4.42^{d} (0.07)	4.79^{c} (0.08)	4.57^{cd} (0.13)	4.52^{cd} (0.22)	4.77^{c} (0.08)	5.19^{b} (0.09)	4.65^{cd} (0.29)	0.0179
Grade marbling score (gMS)[3]	379^{d} (23)	438^{b} (9)	573^{a} (13)	461^{b} (18)	301^{f} (15)	343^{e} (6)	408^{c} (7)	357^{e} (12)	240^{g} (23)	331^{e} (7)	404^{c} (8)	358de (28)	0.0002

[a, b, c] Least Square Means within a row lacking a common letter differ at $P \leq 0.05$.

[1] Probability of the F test, with significance at $P \leq 0.05$.

Table 3.4. Pearson correlations between production and carcass measurements (asterisks beneath each correlation indicate probability with *** = $P < 0.0001$, ** = $P < 0.001$, and * = $P < 0.003$).

	DF	SA	WW	DMI	ADG	FCR	RFI	uFD	uREA	uMS	LW	CW	gFD	gREA	gMS
Age at test	-0.68 ***	0.87 ***	-0.05	0.73 ***	-0.15 **	0.54 ***	-0.01	0.33 ***	0.39 ***	0.39 ***	0.21 ***	0.29 ***	0.33 ***	0.03	0.19 ***
Days to finishing (DF)		-0.22 ***	-0.01	-0.77 ***	-0.13	-0.28 ***	0.013	-0.39 ***	-0.40 ***	-0.33 ***	-0.23 ***	-0.32 ***	-0.12	-0.02	-0.18 ***
Age at slaughter (SA)			-0.08	0.45 ***	-0.29 ***	0.53 ***	-0.01	0.17 **	0.26 ***	0.30 ***	0.13	0.18 **	0.36 ***	0.02	0.12
Weaning weight (WW)				0.14 *	0.10	0.03	-0.08	0.12	0.34 ***	-0.07	0.58 ***	0.61 ***	-0.08	0.25 ***	-0.17 **
Dry matter intake (DMI)					0.29 ***	0.26 ***	0.29 ***	0.46 ***	0.54 ***	0.37 ***	0.61 ***	0.63 ***	0.27 ***	0.18 ***	0.11
Average daily gain (ADG)						-0.64 ***	-0.01	0.23 ***	0.30 ***	0.12	0.60 ***	0.37 ***	-0.00	0.11	-0.01
Feed conversion ratio (FCR)							0.11	0.07	0.08	0.11	-0.14	0.03	0.17 **	-0.01	0.08
Residual feed intake (RFI)								-0.01	-0.01	0.01	0.00	0.02	0.07	0.01	0.09
Ultrasound subcutaneous fat depth (uFD)									0.31 ***	0.44 ***	0.35 ***	0.35 ***	0.59 ***	-0.11	0.19 ***
Ultrasound rib eye area (uREA)										0.17 **	0.63 ***	0.66 ***	0.17 **	0.52 ***	-0.22 ***
Ultrasound marbling score (uMS)											0.12	0.09	0.41 ***	-0.21 ***	0.50 ***
Live weight at slaughter (LW)												0.89 ***	0.14 *	0.38 ***	-0.23 ***
Carcass weight (CW)													0.17 **	0.48 ***	-0.16 **
Grade fat depth (gFD)														-0.17 **	0.17 ***
Grade rib eye area (gREA)															-0.30 ***
Grade marbling score (gMS)															1.00

Figure 3.1. Binomial analysis of the relationship (P <.0001) of the probability of cutting dark with carcass weight (CW; kg).

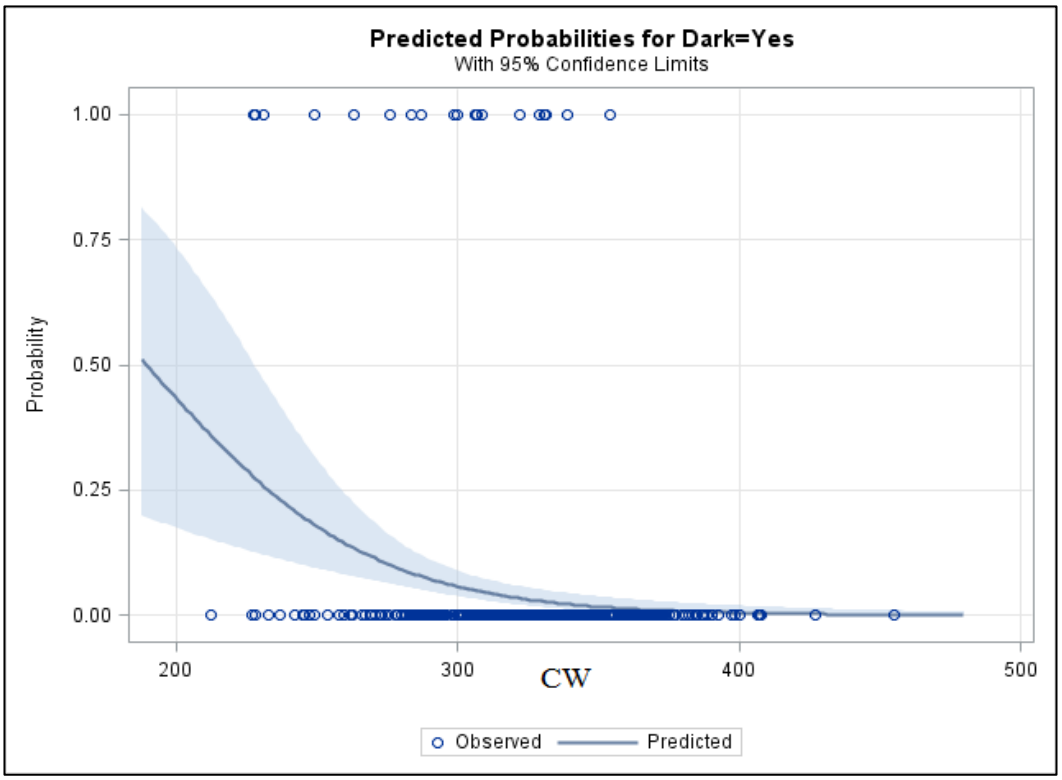


Figure 3.2. Predicted cumulative probabilities from multinomial logistic regression describing the incidence of grades related to average daily gain (ADG, kg day^{-1}) (P = 0.032) and Farm (P = 0.0004).

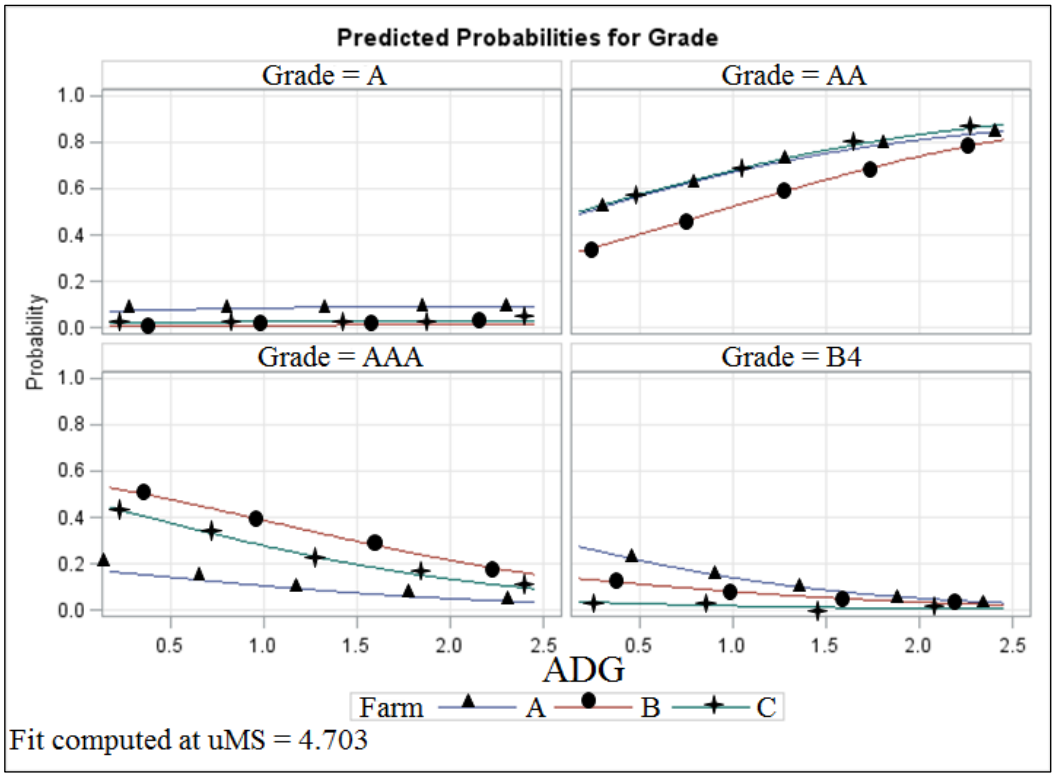


Figure 3.3. Predicted cumulative probabilities from multinomial logistic regression describing the incidence of grades related to live weight at slaughter (LW, kg) (P = 0.0247) and Farm (P = 0.0054).

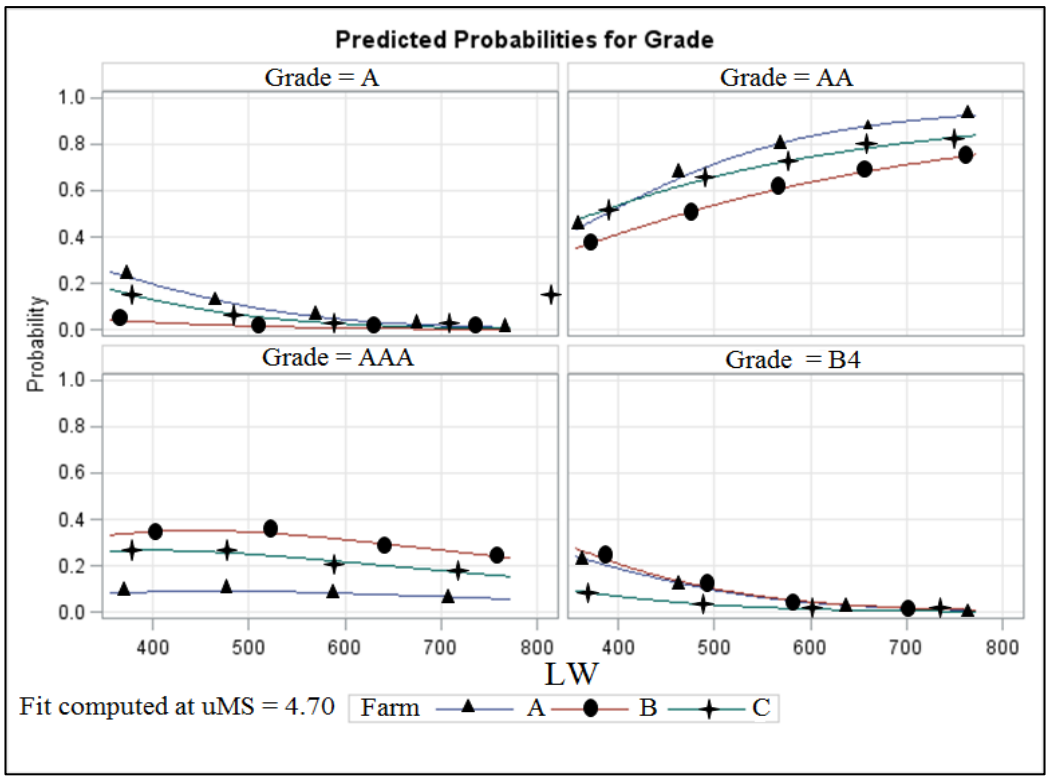


Figure 3.4. Predicted cumulative probabilities from multinomial logistic regression describing the incidence of grades related to carcass weight (CW, kg) ($P < .0001$).

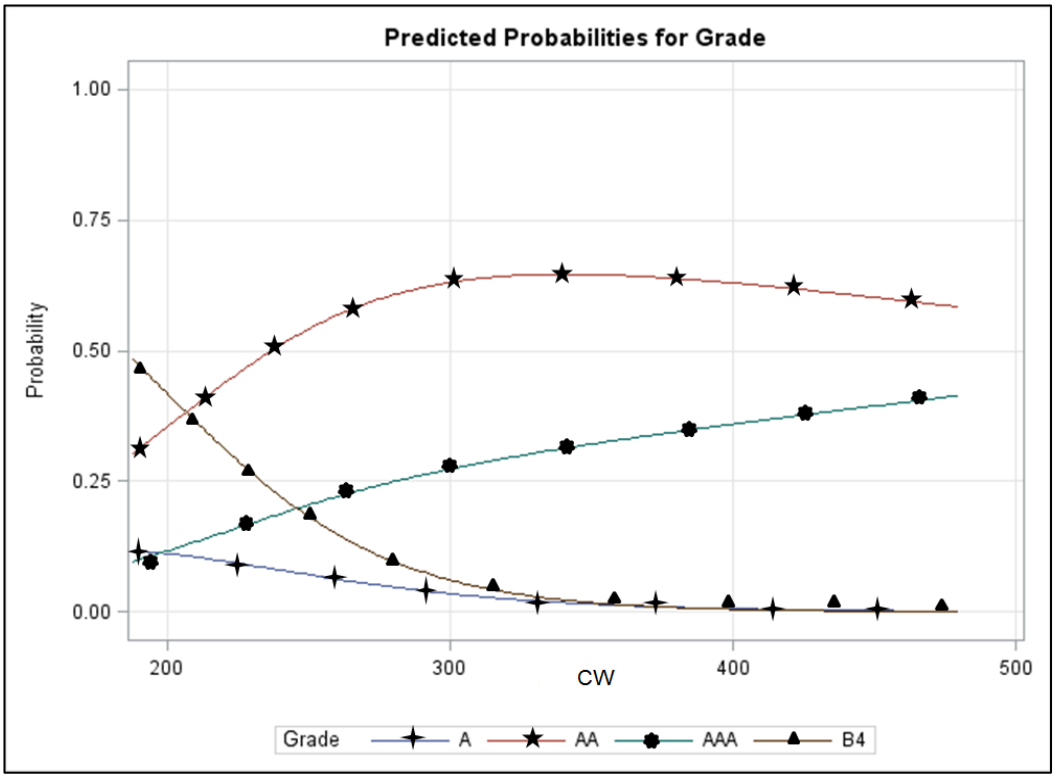


Figure 3.5. Predicted cumulative probabilities from multinomial logistic regression describing the incidence of grades related to grade rib eye area (gREA, cm^2) (P = 0.0002) and Farm (P = 0.0024).

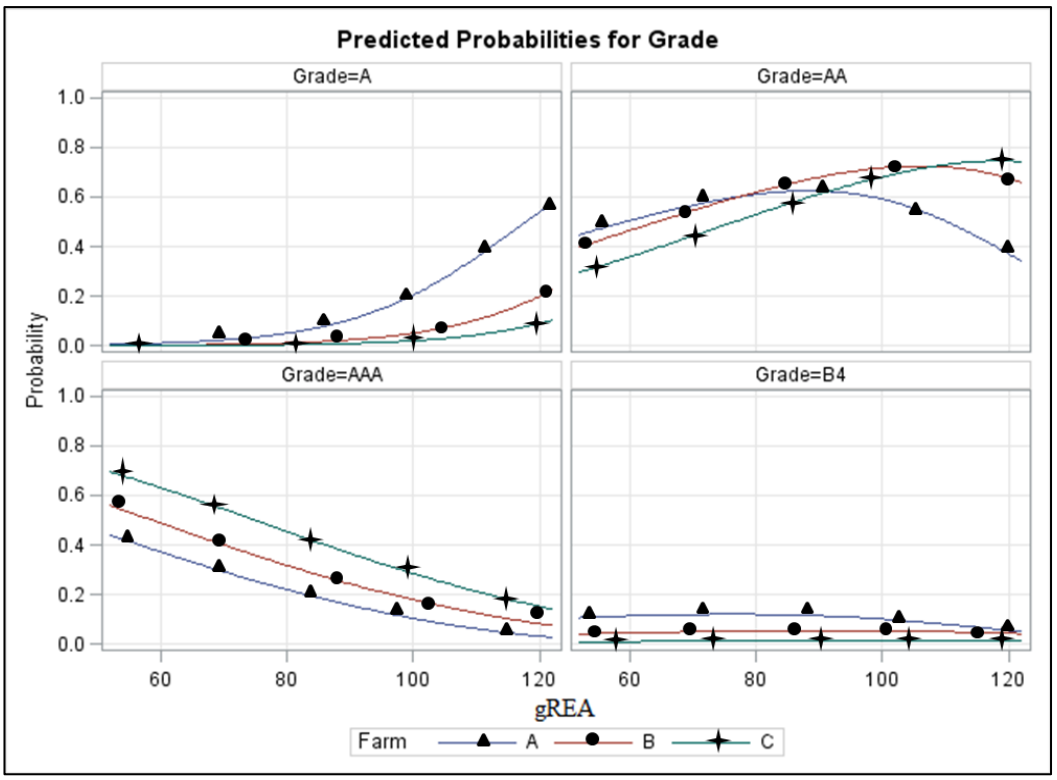


Figure 3.6. Predicted cumulative probabilities from multinomial logistic regression describing the incidence of grades as it related to grade marbling score (gMS; $P < .0001$) and Farm ($P < .0001$).

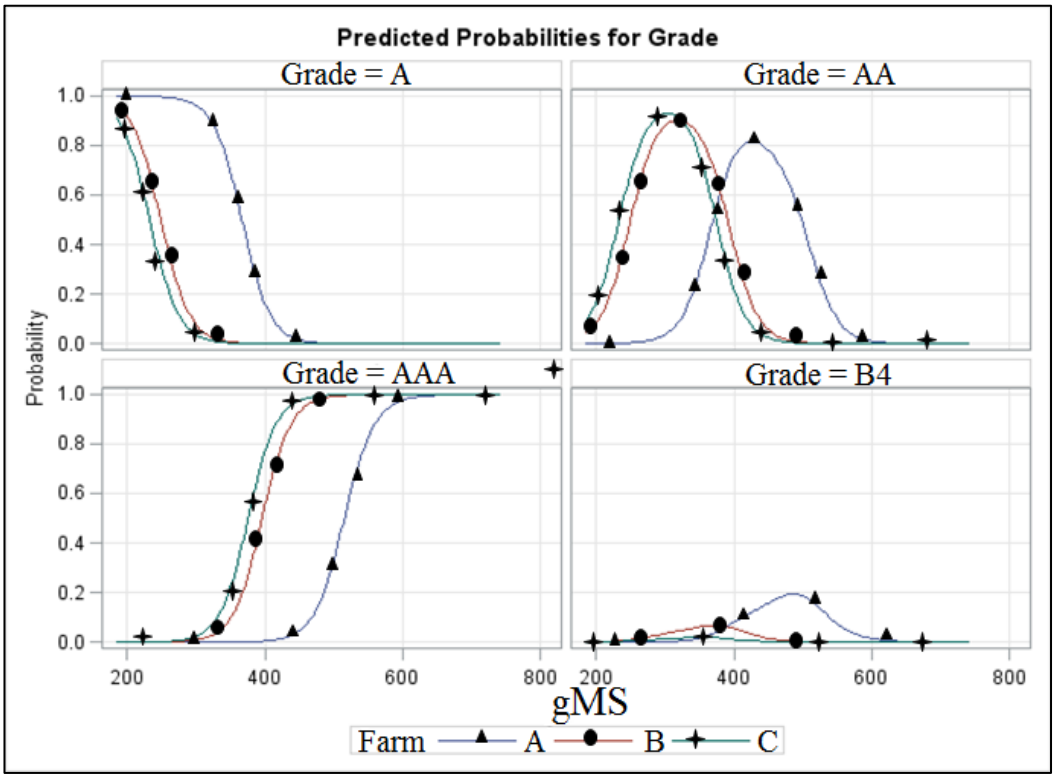


Figure 3.7 (A, B). Principal component analysis correlation loadings (A) and scores (B) for live animal production data related to animal farm and grade. In score plot, dark cutting heifers were identified as A (rectangle) from farm A, B (red circle) from farm B and C (black circle) from farm C while heifers producing normal carcass grades (Canada A, AA and AAA) at all the farms were marked as 1.

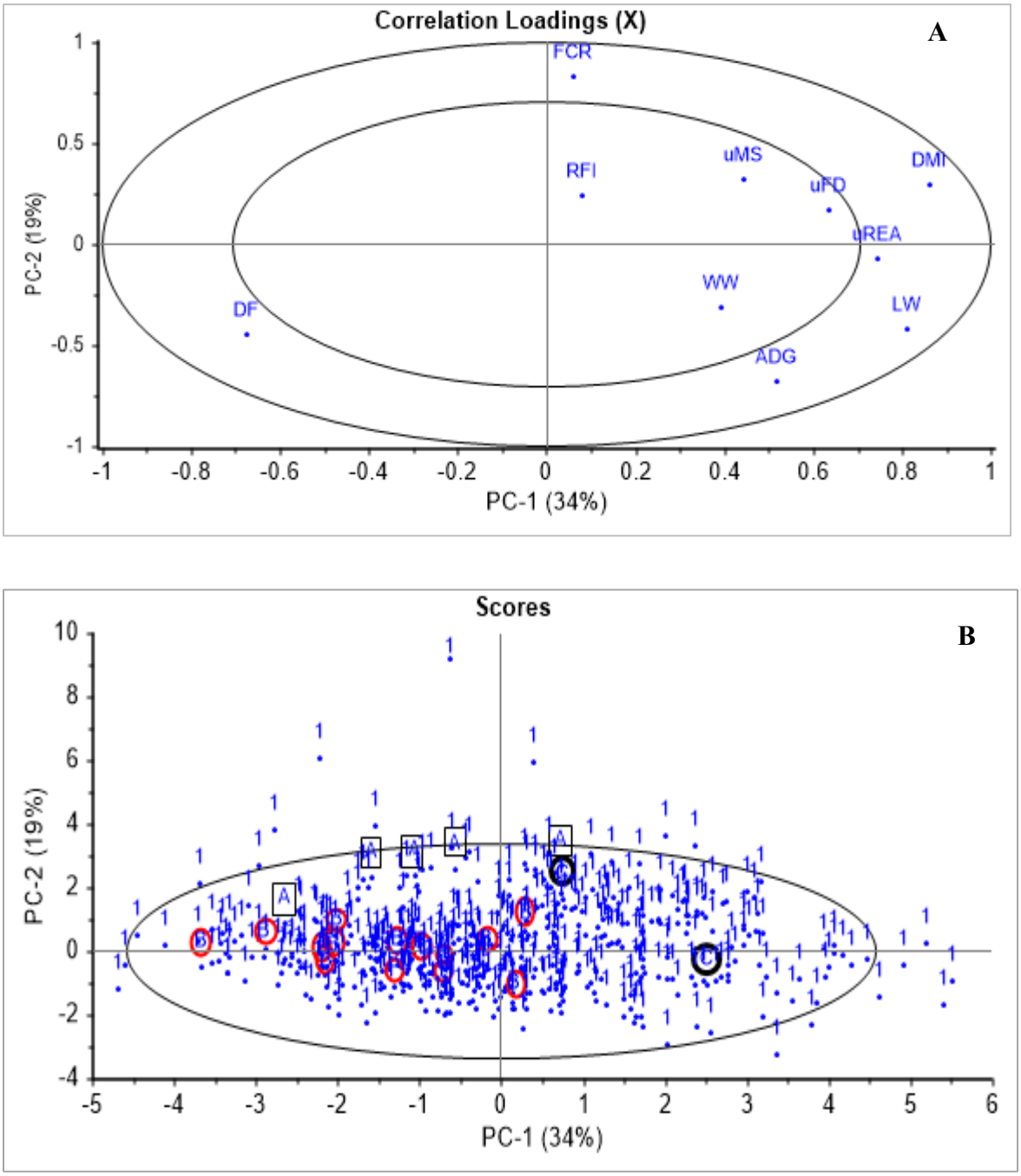
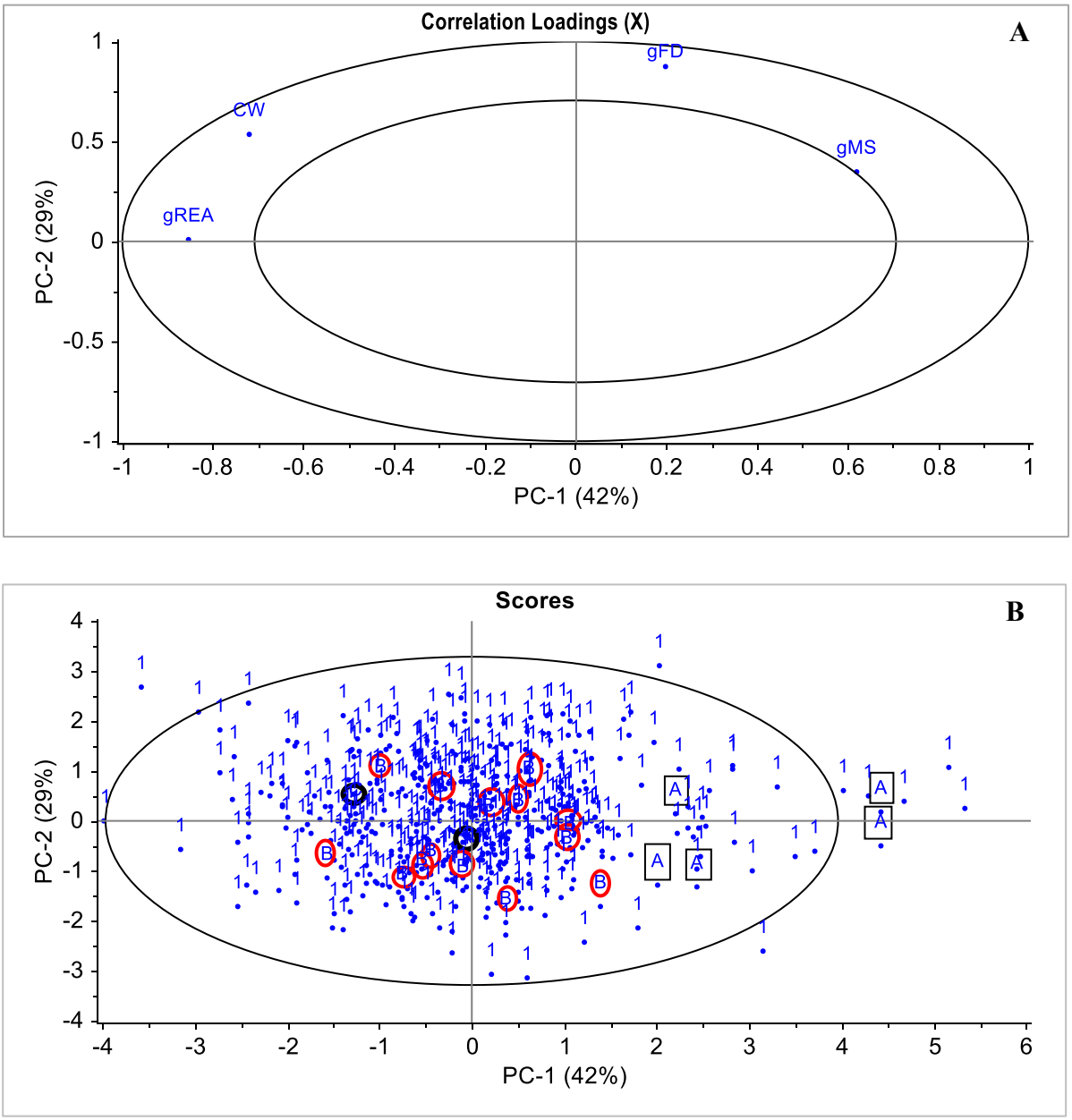


Figure 3.8 (A, B). Principal component analysis correlation loadings (A) and scores (B) for carcass measurements related to Farm and grade. In score plot, dark cutting carcasses were identified as A (rectangle) from farm A, B (red circle) from farm B and C (black circle) from farm C while normal carcass grades (Canada A, AA and AAA) from all the farms were marked as 1.



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4.0. Effect of cattle production practices on the incidence of dark cutting beef

4.1. Introduction

Beef carcasses with a dark red rib eye (*longissimus thoracis*) muscle colour are deemed to be dark cutting and assigned a lower grade in most of the beef producing countries including Canada, the United States of America and Australia. In Canada, dark cutting carcasses are downgraded as Canada B4 (Canadian Agricultural Products Act SOR/92-541, 2014). Inadequate glycogen in cattle muscles at the time of slaughter results in increased post-mortem intramuscular pH and ultimately dark cutting. Other known factors directly or indirectly linked to dark cutting include but are not limited to cattle sex (Lorenzen et al. 1993), animal/carcass weight (Mahmood et al. 2016a, b; Murray 1989), season (Knee et al. 2004; Kreikemeier et al. 1998), and pre-slaughter management (Lacourt and Tarrant 1985; Mach et al. 2008). Increased frequency of dark cutting in heifers (Lorenzen et al. 1993) could be due to oestrus as mounting activity leads to reduced muscle glycogen reserves (Kenny and Tarrant 1988). Heifers may start exhibiting oestrus at slaughter if fed melengestrol acetate (MGA) and when the subsequent gap between MGA withdrawal and slaughter exceeds two days (Hill et al. 1971).

Hormonal growth implants containing estrogens and/or androgens increase cattle average daily gain, body weight, carcass weight (Bruns et al. 2005; Dikeman 2007; Hardt et al. 1995) and the size of the *longissimus* muscle in heifers and steers (Platter et al. 2003; Popp et al. 1997; Schneider et al. 2007; Smith et al. 2007). These responses are greater in Continental than in British breeds when subjected to growth implants (Boles et al. 2009). Beta-adrenergic agonists also increase protein deposition (Williams 1987) and hypertrophy of skeletal muscles (Johnson and Chung 2007). The findings that muscular and heavy cattle are less likely to cut dark (Mahmood et al. 2016b; McGilchrist et al. 2012) suggest that animals receiving growth

promotants may have less propensity to cut dark. Studies, however, have indicated increased susceptibility to cut dark likely because growth implants may result in stress to an animal's physiology and may modify growth curve and nutrition requirements (Scanga et al. 1998; Schneider et al. 2007). Feeding β-agonists may increase an animal's activity (Meyer 2001) and reduce muscle glycogen (Williams 1987) that together may increase the likelihood of dark cutting. Because the provision of high quality nutrition tends to maintain muscle glycogen concentration during handling and extreme weather condition (Immonen et al. 2000; Warner et al. 1998), it can be hypothesized that inadequate or low-energy diets may enhance the threat of dark cutting posed by growth promotants.

Cattle from different production systems may differ in age at slaughter, and the latter may affect the ultimate carcass pH and muscle colour (Miller et al. 1987; Wilkins et al. 2009). Beef carcasses that graded dark in the USDA and Canadian beef grading systems are generally from cattle under 30 months of age, but there can be an enormous variation in age at slaughter depending upon the production system (calf-fed or yearling-fed). In the calf-fed production system, cattle after weaning are finished directly on a concentrate diet; however, in the yearling-fed system (year-fed) the cattle, after weaning, are first backgrounded on a low energy diet to increase their skeletal frame size before brought to the feedlot. Cattle rearing systems, before the feedlot, not only affect carcass weight and musculature but also muscle colour (Durunna et al. 2014). Slaughter season influenced the likelihood of dark cutting, but the literature findings are not consistent (Durunna et al. 2014; Jones and Tong 1989; Tarrant and Sherrington 1980) and seasonal effects have been attributed to the availability of nutrition and the attainment of appropriate live animal/carcass weight.

The effects of the above described factors on dark cutting have been mostly reported individually but their potential interactions especially with cattle sex and production systems are rarely found in the literature. It was hypothesized that the existence of dark cutting in the beef industry was due to use of growth promotants that also interact with cattle sex and production system. The objective of this study was to examine the individual and interactive effects of cattle sex, growth promotants, production systems (year-fed vs calf-fed), MGA, season and pre-slaughter handling on the incidence of dark cutting.

4.2. Materials and methods

The feedlot information was recorded using a questionnaire approved by the Human Ethics Board of the University of Alberta. Because the study was conducted on data collected by commercial feedlots, approval of animal ethics was not required. All cattle were slaughtered in accordance with the Canadian Council on Animal Care (CCAC 1993) guidelines at commercial beef slaughter plants in Canada.

4.2.1. Data set A

Data (n = 2,058) were from a single feedlot containing heifers (young female cattle) and steers (bovine castrates) having predominantly continental breed characteristics. Cattle from both sexes were from calf-fed and year-fed production systems. Cattle from each sex and production system were either fed Optaflexx® (ractopamine hydrochloride, Elanco-Animal Health Division) or were not (control). Production treatments were as follows: calf-fed heifers that received no Optaflexx® (n = 229), calf-fed heifers that received Optaflexx® (n = 210), calf-fed steers that received no Optaflexx® (n = 245), calf-fed steers that received Optaflexx® (n = 358), year-fed heifers that received no Optaflexx® (n = 486), year-fed heifers that received Optaflexx® (n = 193), year-fed steers that received no Optaflexx® (n = 200) and year-fed steers that received

Optaflexx® (*n* = 137). Both calf-fed and year-fed cattle were finished on a diet containing 75% barley grains, 3% supplements and 12% cereal and clover mixed silage on an as fed basis. The calf-fed cattle were finished after weaning while year-fed cattle were backgrounded on pasture or dry forage before receiving the finishing diet. As a result, calf-fed cattle at the start of finishing were younger than year-fed cattle and weighed 250-318 kg while year-fed cattle weighed 385-545 kg. All the cattle were purchased from auction marts or commercial cow-calf operations.

Cattle at the start of finishing were sorted by weight, and calf-fed cattle were provided with tetracycline in their feed for the initial 56 days and implanted with Ralgro® (36 mg Zeranol). Sixty days after the first implant, the calf-fed cattle received a terminal implant of Synovex Choice® (trenbolone acetate 100 mg and estradiol-17β 14 mg). The year-fed cattle received only the terminal implant Synovex Choice® and a minimum 90 days gap was maintained between the terminal implant and slaughter for both calf-fed and year-fed cattle. Optaflexx® treatment was given during the last 28-30 days of finishing, and there was no withdrawal period for Optaflexx®. Heifers were not fed melengestrol acetate (MGA) because the producer had earlier observed an incidence of dark cutting of up to 30% when the time between MGA withdrawal and slaughtering reached 72 h. All the cattle were vaccinated against viral diseases and were treated for internal and external parasites.

Cattle were slaughtered before the age of 30 months at an abattoir located 450 km away from the feedlot. The animals were shipped to the abattoir on a tri-axle trailer and feed was withdrawn two hours before shipment. Cattle were loaded on the trailer by attendants who were familiar to the animals without using electric prods. Mixing of cattle across the lots and sexes was avoided during shipping. Cattle shipped in the morning were slaughtered in the evening of the same day (zero day lairage) while those shipped in evening were slaughtered the next

morning (overnight lairage). One lot of cattle, after initial unloading at the abattoir, was again shipped to a nearby feeding facility because of slaughter being postponed and these cattle were slaughtered four days after the initial transportation from the feedlot.

Measurements collected on these cattle were limited to carcass grade and time in lairage as data involving individual cattle or carcass characteristics were not available. Grading of the carcasses was performed by trained personnel from the Canada Beef Grading Agency (CBGA) at 24 to 48 h post-mortem.

4.4.2. Data set B

Data (n = 86408) were from steers (n = 59813) and heifers (n = 26596) sourced from three feedlots (A, B, and C) located within 50 km from each other and managed by a single producer. The cattle were both from calf-fed and year-fed production systems. Within the year-fed system, cattle were either produced as "grass-fed" yearlings (GY) or "backgrounded" yearling (BY) while calf-fed cattle were fed as Winter (WC) or Fall calves (FC) (Table 4.1). The GY cattle were pasture grazed while BY cattle were either swath grazed or fed dry forage in bunks before entry into the feedlot. The FC cattle entered into feedlot after weaning from their dams on pasture while WC animals received a backgrounding diet like BY cattle but for only a shorter period after weaning and before entering into the feedlot. Both GY and BY cattle were older and heavier than calf-fed cattle at the start of the feedlot period.

Cattle were purchased from auction marts or cow-calf operations and were sorted into pens by weight. All cattle went through a feed adjustment period, after which the cattle were implanted with hormonal growth implants. The calf-fed cattle below 318 kg were implanted with Ralgro® (36 mg Zeranol) at the start of the feedlot, Component TE-100 (100 mg trenbolone acetate + 10mg estradiol) after 45 to 60 days of feedlot, and then Component TE-200 (200 mg

trenbolone acetate + 20 mg estradiol). The calf-fed cattle above 318 kg were implanted with Component TE-100 after 30 to 40 days of feeding and then with a terminal implant Component TE-200 60 days after the first implant. The year-fed cattle that weighed 340 kg to 454 kg and were fed for more than 130 days received Component TE-100 and then Component TE-200 whereas year-fed cattle weighing more than 454 kg and in feedlot for fewer than 130 days were provided with only one terminal implant (Component TE-200). As a result, the year-fed cattle received one (Imp1) or two implants (Imp2) whereas calf-fed cattle received two (Imp2) or three implants (Imp3). The minimum gap between terminal implant and slaughter was 80 days.

The feedlot diet contained 80% barley, 18% corn silage, and 2% supplements on an as fed basis. No antibiotics were given, but cattle were vaccinated against bovine viral diarrhea and infectious bovine rhinotracheitis. Cattle were also treated routinely against external and internal parasites. All steers were fed Optaflexx^{TM} during the last 28-30 days of feedlot while all heifers received melengestrol acetate (MGA) until two days before they exited the feedlot and were shipped to an abattoir about 250 km away. Cattle were neither fasted before shipment nor mixed across pens or sexes. No electrical prods were used, and cattle were loaded by attendants familiar to the cattle on a standard tri-axle cattle liner. Cattle were mostly shipped in the afternoon and slaughtered the following morning after an overnight stay at the abattoir. Carcasses were graded by CBGA personnel at 24-48 h post-mortem, as Canada Prime, AAA, AA, A and B4. Hot carcass weight, grade fat depth (gFD), grade rib eye area (gREA) and grade marbling score (gMS) were recorded for each carcass. Weight at the start of finishing (start weight) and days to finish (number of days the cattle were fed concentrate diet) were also recorded for each animal.

Cattle were slaughtered throughout the year and information about the environmental temperature at the time of transportation at the feedlots was extracted from “Ropin the Web”

(www.agric.gov.ab.ca), an online database. To test the effect of time of year, the slaughter months were grouped into unique four slaughter-seasons as; Winter (Dec, Jan, and Feb with temperature ranged -23ºC to +9 ºC), Spring (Mar and Apr with temperature ranging –10 ºC to 18 ºC), Summer (May to Sep with temperature range of 9-20 ºC) and Fall (Oct and Nov having temperature ranging from -17 ºC to +14 ºC).

4.2.3. Statistical analyses

Datasets A and B were analyzed separately using the procedures of CATMOD, and binomial and multinomial logistic regression in the Statistical Analysis System (SAS Institute Inc., Cary, NC) Version 9.4. The CATMOD procedure was used to apply a generalized logit model for computing the predicted probability of Canada B4 grade versus the normal grade category (Canada Prime, AAA AA and A combined as normal) as well as B4 vs Canada Prime, AAA, AA, and A grade. Treatment effects were tested on the estimate/probability of being dark or a normal grade carcass. For significant interactions of treatments, the analyses were performed by sorting the data by treatment and means were compared using contrasts in CATMOD with significance at $P < 0.05$.

The statistical models using binomial and multinomial logistic regression were built to predict the probability of normal carcass grades and dark cutting. In binomial regression, like the CATMOD procedure, the likelihood of being normal (Yes versus No) was tested while in multinomial regression the probability of the occurrence of each of the five carcass grades (Canada Prime, AAA, AA, A and B4) was computed. The fitness of the binomial regression model was tested using the Hosmer and Lemeshow test. The regression models were built using the backward selection procedure ($P < 0.05$ for data set A; $P < 0.0001$ for data set B) and graphs for the predicted probabilities were obtained in SAS.

4.2.3.1. Sources of variations for data set A

Because cattle slaughtered after four days in lairage were only the calf-fed heifers that received Optaflexx®, the analysis for the effect of time in lairage was thus not possible along with sex, production system, and Optaflexx®. Therefore, the data were initially analyzed to test the effect of time in lairage (zero day, overnight, four days) on the probability of dark cutting using the CATMOD procedure. Subsequent analyses with the CATMOD procedure and logistic regressions were performed after excluding the data of cattle slaughtered after four days in lairage (n = 49). These analyses tested the effects of cattle sex, production system, Optaflexx®, lairage time (zero day vs overnight) and their interactions on the probabilities of carcass grades.

4.2.3.2. Sources of variations for data set B

Cattle were from all the sub-production systems (GY, BY, WC, and FC) and each sex produced at least one carcass from each grade category. Feedlot was not included as a source of variation because feedlot A did not have WC heifers and had only one B4 carcass from WC steers. The sex effect was confounded with MGA and beta-agonist treatment, as there were no control heifers and steers for MGA and β-agonist, respectively. Hence the effects of feeding MGA or β-agonist on carcass grades were not estimable.

The effects of cattle sex, season, implant (Imp1, Imp2, and Imp3), production systems (calf-fed vs year-fed) and sub-production systems (GY, BY, WC, and FC) on the probabilities of carcass grades were sequentially tested. Because implant frequency was confounded with production system, the CATMOD model initially tested the effect of cattle sex and implant nested within production system (calf-fed versus year-fed). Next, the model included the effect of cattle sex and sub-production systems on the probability of dark cutting. All the calf-fed cattle were killed in fall and summer months, therefore, a sub-data set of year-fed cattle slaughtered

throughout the year was used to test the effects of slaughter season, cattle sex and sub-production system (GY and BY) on dark cutting.

For binomial and multinomial logistic regressions, days to finish, carcass weight, gREA, gFD and, gMS were tested as covariates along with main effects that included sex, implant, season and sub-production system. Only the uncorrelated covariates were kept in the final models that were selected based upon greater *R*-square value.

Analysis of variance was performed using GLM procedure in SAS to calculate means for carcass and animal measurements. The sources of variations included cattle sex (steers and heifers), sub-production systems (BY, GY, FC, and WC) and grades (Canada Prime, AAA, AA, A and B4), and analyzed as a 2 x 4 x 5 factorial. For significant ($P < 0.05$) models, the means were compared using Tukey-Kramer adjustment and considered significant at $P < 0.05$. Pearson correlations were also performed, and alpha 0.05 was divided by the number of variables for Bonferroni correction (Rice 1989).

4.3. Results

Results are presented separately for data set A and B. The effects of lairage time and Optaflexx® were tested in data set A only while the effect of cattle sex, production system and grade category on carcass and animal measurements were tested in data set B.

4.3.1. Data set A

The implant frequency was confounded with production system (calf-fed vs year-fed); therefore, the analysis performed tested the effect of cattle sex, production system, Optaflexx®, and lairage time on the probability of Canada B4 and normal carcass grades. Cattle in the data set produced Canada Prime ($n = 38$), AAA ($n = 1302$), AA ($n = 672$), and B4 ($n = 46$) carcasses. The

results indicated a significant ($P < 0.0001$) effect of lairage time on the predicted probability of a Canada B4 grade which was significantly ($P < 0.05$) greater in cattle having four days lairage (14.29 ± 5.0 %) compared to those slaughtered after an overnight (1.72 ± 0.76%) and zero day (1.98 ± 0.34%) stay in lairage. Cattle slaughtered after an overnight stay in lairage had a probability of grading Canada B4 similar to those having zero day lairage.

Results indicated no effect ($P > 0.05$) of Optaflexx® on the probability of being a dark carcass. However, both calf-fed heifers and year-fed heifers had greater ($P < 0.05$) probability of cutting dark than steers from calf-fed and year-fed systems (Figure 4.1). Calf-fed heifers tended ($P = 0.09$) to cut dark more frequently than year-fed heifers (Figure 4.1). Binomial logistic regression also identified Optaflexx® and lairage time (zero day versus overnight) insignificant but the effect of production system approached significance ($P = 0.098$) while there was a significant effect of cattle sex ($P < 0.0001$) on the predicted probability of the Canada B4 grade. The results corroborated with that of the CATMOD procedure, indicating that the likelihood of dark cutting was greatest in heifers, especially those that were calf-fed (Figure 4.2).

The CATMOD procedure indicated a significant ($P < 0.0001$) effect of sex while production system (calf-fed versus year-fed) interacted ($P < 0.0001$) with Optaflexx® for the predicted probabilities of carcass grades (Table 4.2). Results indicated higher probabilities of the Canada Prime, AAA and B4 grades in heifers than in steers (Table 4.2). Conversely, steers had an increased probability for Canada AA than heifers. Year-fed cattle had an increased probability for Canada Prime and AAA than calf-fed cattle. Moreover, year-fed cattle that did not receive Optaflexx® had the highest probabilities for Canada Prime and AAA carcasses (Table 4.2). Optaflexx® did not influence carcass grade category in calf-fed cattle although calf-fed cattle that received Optaflexx® tended to have a greater probability of dark cutting (Canada B4) than year-

fed and calf-fed cattle that did not receive Optaflexx®. Multinomial logistic regression also indicated a significant effect of cattle sex ($P < 0.0001$; Figure 4.3) and an interaction of production system with Optaflexx® ($P < 0.0001$; Figure 4.4 to 4.7) on the probabilities of carcass grades, and the results were as indicated by CATMOD procedure.

4.3.2. Data set B

The objective of the analysis was to investigate the effects of cattle sex, implant frequency, slaughter-season and production systems on the probability of a Canada B4 grade. The effects of sex, sub-production system, grade and their interactions on carcass and animal phenotypic characteristics were also analyzed. The means for the main effects were not presented because all interactions were significant.

4.3.2.1. CATMOD and logistic regression

Results indicated an interaction ($P < 0.0001$) between cattle sex and implant nested within production system (calf-fed vs. year-fed) for the probability of dark cutting. The results (Figure 4.8) indicated increased probability of the Canada B4 grade in calf-fed heifers that received two implants (Calf-Imp2) followed by calf-fed heifers that received three implants (Calf-Imp3) and year-fed heifers that received two implants (Year-Imp2). The year-fed heifers implanted once (Year-Impl) had a lower probability of dark cutting similar to that of steers, which alsohad a reduced likelihood of dark cutting, regardless of the implant and production system (Figure 4.8).

Cattle sex interacted ($P < 0.0001$) with sub-production systems (BY, GY, FC, and WC), and the results showed an increased probability of Canada B4 in heifers from WC followed by FC and BY heifers which, however, were not different from each other (Figure 4.9). The

probability for Canada B4 in GY heifers was lower than in rest of the heifers but similar to or greater than in steers, depending on the production system. Steers from BY system had the highest probability of dark cutting while GY steers had the lowest propensity to cut dark (Figure 4.9).

CATMOD indicated a three-way interaction (P = 0.043) between cattle sex, sub-production system (GY versus BY) and slaughter season for Canada B4 incidence. The predicted probability of dark cutting was greater in BY heifers than in GY heifers, within a slaughter season (Table 4.3). Heifers from BY had the highest probability of Canada B4 in summer, followed by fall and spring, and lowest probability in winter. Similarly, GY heifers also had the highest probability of dark cutting in summer but lowest in spring (Table 4.3). Steers from BY had increased dark cutting in fall and winter compared to summer and spring where GY steers had reduced dark cutting, regardless of the slaughter season (Table 4.3).

Multinomial logistic regression indicated a significant (P < 0.0001) effect of covariate carcass weight and gMS and interaction between sex and sub-production system (BY, GY, FC, WC) for dark cutting. The predicted probability for a carcass grading Canada Prime, AA, and A was not influenced by production system and carcass weight in both steers (Figure 4.10) and heifers (Figure 4.11), at a mean gMS score of 434. The probability for a Canada AAA grade was smallest in FC steers and tended to increase with carcass weight of steers from all the sub-production systems (Figure 4.10). The probability of being graded Canada B4 decreased with increased carcass weight, and was highest in BY but lowest in GY steers (Figure 4.10).

The probability for Canada AAA grade was highest in GY, followed by BY and FC but lowest in WC, and Canada AAA probability increased with carcass weight in heifers (Figure

4.11). The predicted probability of a carcass being graded Canada B4 was greater in WC heifers, followed by FC and BY and lowest in GY heifers. The likelihood of cutting dark tended to decrease as carcass weight increased ($P < 0.0001$; Figure 4.11).

4.3.2.2. Analysis of variance

The analysis of variance indicated a significant ($P < 0.0001$) three-way interaction between cattle sex, grade and sub-production system for mean start weight, days to finish, carcass weight, gREA, gFD, and gMS. Means for the interactions have been presented in Tables 4.4 and 4.5 and were compared within and across the tables.

Results indicated greater mean start weights for steer grades from BY and GY production systems than the corresponding heifer grades from all production systems (Table 4.4 and 4.5). Mean start weights for BY and GY heifers were greater compared to FC and WC heifers (Table 4.4). Canada B4 heifers from the BY system had a mean start weight greater than that of Canada A but similar to that of Canada Prime, AAA and AA from the BY system. The GY heifers that produced Canada B4 carcasses had a mean start weight less than that of heifers that produced Canada AAA and AA carcasses but similar to those that produced Canada Prime and A carcasses (Table 4.4). Contrarily, the mean start weight for Canada B4 heifers from FC was similar to that of the rest of the FC heifers regardless of carcass grade. Heifer grades from the WC system were not different from each other for mean start weight but were heavier than heifers from FC (Table 4.4). The BY and GY heifers had fewer mean days to finish than FC and WC heifers. Mean days to finish for heifers that produced Canada B4 carcasses in the BY system was not different from rest of the heifers from the same system but greater than that for heifers in the GY system that produced Canada AAA, AA and A carcasses. Canada B4 heifers from the GY system had mean days to finish greater than that of Canada AA but similar to other grades from GY system.

Canada B4 heifers from FC had fewer mean days to finish than Canada Prime and AAA but more than that of the remaining FC heifers. However, Canada B4 heifers from WC had mean days to finish similar to that of heifers that produced Canada AAA, AA and A carcasses but fewer than those that produced Canada Prime carcasses (Table 4.4).

Mean carcass weight for Canada B4 heifers from BY was less than that for heifer carcasses that graded Canada Prime and AAA, but was similar to heifer carcasses that graded Canada AA and greater than for Canada A (Table 4.4). Similarly, Canada A carcasses from GY heifers had reduced carcass weight while the mean values for Canada B4 carcasses were similar to those of Canada AAA and AA carcasses but smaller than that of Canada Prime carcasses from GY heifers. Carcasses from all grades, except those graded Canada A from WC heifers had mean carcass weights greater than for FC heifers. Canada B4 from FC heifers had mean carcass weight heavier than Canada A carcasses but lighter than carcasses from Canada Prime and AAA FC heifers. Conversely, Canada B4 carcasses from WC heifers were as heavy as those from the rest of the grades within WC heifer carcasses, except Canada A.

Unlike carcass weight, mean gREA for Canada A carcasses was greater than rest of the grades but mean gREA for Canada AA from FC and BY heifers was not different from A grade within the production system (Table 4.4). Mean gREA was smallest for Canada Prime especially within a production system whereas the mean value for Canada B4 was similar to that for Canada AA but greater than that for Canada AAA from BY and GY heifers. Mean gREA for Canada AAA was similar to that of Canada B4 carcasses from FC and WC heifers. Mean gFD and gMS for heifer grades was similar or greater than that for respective grades from steers (Table 4.4. and 4.5). Mean gFD and gMS was highest for Canada Prime and lowest for Canada A where the mean values for B4 carcasses were similar to that for Canada AAA but greater than

Canada AA with the exception of GY heifers where mean gMS was similar for Canada AA and B4 carcasses (Table 4.4).

Like heifers, mean start weights for BY and GY steers were higher than for FC and WC steers (Table 4.5). Canada B4 steers from BY had a mean start weight heavier than Canada Prime but similar to Canada AAA, AA and A grade steers. Canada B4 steers from GY were as heavy as rest of the GY steers. Mean start weight for Canada B4 from FC and WC steers was similar to rest of the grades from FC and WC except Canada AA from FC that had a mean start weight greater than Canada B4 from FC system (Table 4.5). Mean days to finish for Canada Prime were greater than the rest of the heifers from BY and GY but similar to that for Canada AAA and B4 within FC and WC system. The mean values for Canada B4 were similar to those for Canada AAA and AA from BY and GY systems (Table 4.5).

Carcass grades from steers were heavier than corresponding heifer grades within a production system except B4 carcasses from WC that were not different from each other (Table 4.4 and 4.5). Mean weights for Canada Prime, AAA and AA carcasses from FC and WC steers were higher than the rest of the grades except Canada Prime and AAA from GY and Canada Prime from BY system (Table 4.5). Mean carcass weight for Canada B4 was similar to Canada AA and A but lower than for Canada Prime and AAA from BY system. Conversely, Canada B4 carcasses were as heavy as Canada A but lighter than rest of the grades from GY system. Similarly, Canada B4 from FC and WC system had mean carcass weight lower than the rest of the grades but were as heavy as Canada A carcasses (Table 4.5).

Canada A carcasses had mean gREA greater than all the grade categories, especially within a system (Table 4.5). Canada B4 carcasses across the systems had similar mean gREA but

greater than the values for Canada Prime and AAA. Mean gREA for Canada AA was not different from the mean values for Canada B4 carcasses within a system except FC where Canada AA tended to have greater value than for Canada B4 (Table 4.5). Mean gFD and gMS was lowest for Canada A and highest for Canada Prime followed by Canada AAA, with the exception for Canada AAA from BY steers that had mean values similar to Canada B4 from that system. Canada B4 had mean gFD similar to that for Canada AA but had gMS greater than Canada A carcasses (Table 4.5).

4.3.2.3. Pearson correlations

The relationships between carcass and animal phenotypic characteristics, available in the data set, identified by Pearson correlations (PROC CORR) are presented in Table 4.6. At the adjusted alpha value of 0.007, all the relationships were significant except between carcass weight and days to finish. The results indicated that as start weight increased the number of days to finish decreased ($P < 0.0001$, $r = -0.76$) and carcass weight increased ($P < 0.000$, $r = 0.18$). The linear relationship between start weight and gREA was significant but weak ($P < 0.0001$, $r = -0.025$). Similarly, days to finish had a significant ($P < 0.0001$) but poor linear relationships with gFD ($r = 0.07$), gREA ($r = 0.099$) and gMS ($r = 0.029$). Carcass weight had a positive linear relationship with gFD ($P < 0.0001$, $r = 0.10$) and gREA ($P < 0.0001$, $r = 0.32$) but a trivial relationship with gMS ($P < 0.0001$, $r = 0.014$). Grade fat depth was negatively correlated with gREA ($P < 0.0001$, $r = -0.28$) but had a positive association with gMS ($P < 0.0001$, $r = 0.37$). Grade marbling score, like gFD, had a negative linear relationship with gREA ($P < 0.0001$, $r = -0.21$; Table 4.6).

4.4. Discussion

Dark cutting is still ongoing at levels as high as 1.28% in Canada (Canada Beef Quality Audit 2013), 3.2% in the United States of America (Moore et al. 2012) and 10% in Australia (Hughes et al. 2014). According to the Canada Beef Cattle Research Council (2014) report, the Canadian beef industry can save about CAD$1.77 million per year if the current level of dark cutting (1.28%) is reduced to a lower historical value (0.8%). The objective of this study was to examine if the persistence of dark cutting is due to the individual or interactive effects of cattle sex, growth promotants, production systems, slaughter seasons and pre-slaughter management.

4.4.1. Lairage and dark cutting

Earlier it was found that lairage may compromise the likelihood of beef with normal pH and colour (Kreikemeier et al. 1998; Murray 1989; Teke et al. 2014). Literature findings that indicated that dark cutting increased in cattle slaughtered after an overnight stay compared to those killed on the day of shipment (Murray 1989) were not supported by the current study likely because the interval between unloading at the abattoir and slaughter was not different for the cattle slaughtered on the day of shipment and those after an overnight stay. In 2014, Teke et al. reported reduced dark cutting (40 %) in bulls fed and rested for 72 h, following a long hauling, in the lairage compared to those rested for 24 (90 %) or 48 h (60 %). Cattle in the current study slaughtered after four days of initial shipment had increased dark cutting likely because these cattle experienced additional transportation events. Increased likelihood of dark cutting in these cattle was also probably because these cattle were heifers, which have a greater propensity to cut dark (Lorenzen et al. 1993; Mahmood et al. 2016a) likely because they are more susceptible to heat stress (Busby and Loy 1997). One of the reasons for the persistence of dark cutting in the beef industry thus could be an inappropriate schedule of shipment and slaughter.

4.4.2. Growth promotants and dark cutting

It was reported that beta-agonists lowered intramuscular glycogen (Williams 1987) perhaps by causing restlessness (Meyer 2001) and making animals susceptible to lameness and heat stress (Grandin 2010). However, the results of the current study did not indicate an effect of the beta-agonist (Optaflexx®) on dark cutting. Earlier, Warriss et al. (1989) found dark coloration and increased pH in carcasses from sheep fed beta-agonists (Clenbuterol and Cimaterol) and fasted for 48 h before slaughtering. Clenbuterol also lowered colour of carcasses from steers (Schiavetta et al. 1990) but neither ractopamine nor zilpaterol influenced pH or colour of steer carcasses (Avendaño-Reyes et al. 2006). Optaflexx® lowered the carcass quality grade, especially in year-fed cattle likely because beta-agonists enhance protein deposition (Williams 1987) by increasing lipolysis and decreasing lipogenesis (Strydom et al. 2009). However, Lopez-Campos et al. (2012) found the effect of Optaflexx® on marbling neither in calf-fed nor in year-fed cattle.

Increased dark cutting due to increased frequency of hormonal growth implants (Scanga et al. 1998; Schneider et al. 2007) was not confirmed in the current study as calf-fed heifers that received two implants indeed were more prone to cut dark than those that received three implants although year-fed heifers implanted twice had a greater probability of dark cutting compared to those implanted once. Moreover, implant frequency had no effect on dark cutting in steers. These results suggested no clear tendency of implant effect on dark cutting likely because implant treatment was confounded with sub-production systems and the latter appeared to have a greater effect on dark cutting. Furthermore, implant frequency in the current study probably was not high enough to increase dark cutting, and the gap between terminal implant and slaughter appeared to be as recommended by the manufacturers (Scanga et al. 1998). In 2013, Lopez-

Campos et al. found dark cutting in year-fed steers that received five implants but no dark cutting in calf-fed cattle. The results, however, appeared to agree with Foutz et al. (1997) who did not find a difference in the colour of LT from carcasses of steers implanted once, twice or thrice. Additionally, cattle in the current study were provided with adequate nutrition, which may have shielded against the glycogen depletion that may occur in cattle receiving growth promotants and experiencing pre-slaughter handling and environmental stress (Scanga et al. 1998; Immonen et al. 2000; Warner et al. 1998).

4.4.3. Role of cattle sex and production systems

The results from both data sets (A and B) indicated increased incidence of dark cutting in heifers compared to steers as reported in numerous studies (Mahmood et al. 2016a; Murray, 1989; Lorenzen et al. 1993; Voisinet et al. 1997a). Increased dark cutting in heifers has been attributed to their excitability (Voisinet et al. 1997b) and oestrus activity (Kenny and Tarrant 1988). Cattle during oestrus become more susceptible to environmental stimuli and engage in mounting activities (Schein and Fohrman 1955). Heifers may start exhibiting oestrous after 48 h of MGA withdrawal (Hill et al. 1971) whereas heifers from the dataset B were fed MGA that was withdrawn two days before they exited the feedlots and were stayed overnight at the plant, implying that dark cutting in those heifers was most likely because of the onset of oestrous.

Interestingly, the problem of dark cutting was more prevalent in calf-fed heifers than in year-fed heifers. The difference in the incidence of dark cutting between calf-fed and year-fed heifers could be due to differences in behaviour during oestrus of cattle of different ages (Hafez and Bouissou 1975). Heifers during oestrus engage in antagonistic activities regardless of their social hierarchy (Hafez and Bouissou 1975) and cattle having previous oestrus experience may

be less likely to engage in combative activities because a former experience may alter future response to a stimulus (Broom and Jhonson 1993).

The difference in dark cutting attributed to sex and production systems could be related to muscle fibre characteristics; however, there are conflicting reports about the relationship between fibre types and dark cutting (Zerouala and Stickland 1991; Hunt and Hedrick 1977; Lacourt and Tarrant 1985). Dietary energy has a positive relationship with fast glycolytic fibres but a negative one with intermediate fibres (Johnston et al. 1981). Similar results were reported by Greenwood et al. (2009) but these authors did not find carryover effects of diet during backgrounding on the muscle fibre types after finishing for an extended period on pasture. Fast glycolytic fibres are reportedly more prevalent in heifers than in steers (Johnston et al. 1981) and at the ages below 15 months (Jurie et al. 2005). It can be argued that increased dark cutting in calf-fed heifers could be due to an increased percentage of glycolytic fibres that deplete glycogen to a greater extent as a result of mounting and antagonistic activities (Lacourt and Tarrant 1985). However, literature findings about muscle fibre types in heifers and steers are not consistent (Johnston et al. 1981; West 1974) and further research may be required to investigate the effects of production systems and sex on muscle fibre characteristics and their response to pre-slaughter handling stress.

Apart from age difference, rearing system prior to the feedlot appeared to influence the occurrence of dark cutting as BY heifers produced dark carcasses as much as FC heifers whereas BY steers also had the highest incidence of dark cutting compared to steers from the other sub-production systems. Forage diets can maintain muscle glycogen levels as high as grain feeding (Immonen et al. 2000) and Pordomingo et al. (2012) did not find a difference in pH or lightness score of carcasses from cattle grazed on pasture or fed hay before finishing on pasture. These

studies, however, did not represent the present one where cattle were finished on concentrate diets, and pasture-grazing, before finishing, appeared to lower the likelihood of dark cutting.

Cattle exclusively fed pasture may have a higher incidence of dark cutting than grain fed cattle (Hughes et al. 2014) but the effect of diet before finishing on grains is debatable. In 2014, Durunna et al. found an appealing colour of carcasses from steers subjected to an extensive feeding before finishing on a concentrate diet compared to those fed intensively after weaning. Earlier, Miller et al. (1987) studied the effect of feeding an accelerated diet (2.61Mcal/kg of metabolizable energy; ME) and pasture before finishing on high energy diet (3.04 Mcal/kg ME), and reported a darker LT from cattle fed the accelerated diet. Restricted growth, inflicted by low-quality pasture along with some concentrates during backgrounding, resulted in an increased colour score of beef carcasses compared to fast growth on quality pasture, despite similar finishing diet and the weights at the start of finishing (McKiernan et al. 2009; Wilkins et al. 2009). These authors indicated that improved colour was due to the older age of slow growing cattle while Miller et al. (1987) found that muscle glycogen in cattle was not related to the diets during backgrounding but rather was due to increased age of cattle. The findings that older cattle had a higher incidence of dark cutting than young cattle and veal calves (Hughes et al. 2014) appeared not aligning to the current study. It has also been reported that cattle age had a positive correlation with GAPDH and CKM but a negative correlation with carcass pH (D'Alessandro et al. 2012), suggesting that increased dark cutting in calf-fed and BY cattle could be due to their being young than GY cattle.

4.4.4. Animal/carcass measurements and dark cutting

Cattle fed low energy diets during backgrounding have a compensatory growth during finishing (Kumar et al. 2012; Pordomingo et al. 2012) likely because lighter weight cattle have

higher growth rates while in feedlot (Lancaster et al. 2014). Diets during backgrounding may have no effect on subsequent carcass measurements after feedlot finishing (Kumar et al. 2012) but carcass weight, gREA, and gFD remained lower for cattle fed a low energy diet during backgrounding and then finished on pasture (Pordomingo et al. 2012). In the current study, information about possible grain supplement before entry into feedlot was not available, however, but cattle from GY and BY had similar weights but heavier than WC and FC cattle before feedlot. The observation that feeding to a similar fat depth increased carcass weight and gMS more in year-fed than calf-fed cattle (Lancaster et al. 2014) was also not supported in the current study.

Increased weights exhibited by carcasses that received "normal" A grades appeared to be a result of either increased start weight or increased number of days on finishing diet. Mean weight for Canada B4 carcasses from FC heifers, FC steers, WC steers and GY steers was lower than that of Canada Prime, AAA and AA carcasses from the same production systems despite having similar initial weights and similar or longer finishing period, suggesting a slow rate of gain in Canada B4 cattle during finishing. Dark cutting has been associated with reduced carcass weight but Canada B4 carcasses in the current study had mean weights equal to or greater than the reported values for normal carcasses (Mahmood et al. 2016b; McGilchrist et al. 2012; Murray 1989) suggesting that growth rate may be more important than animal/carcass weight in lowering dark cutting. In 1999, Smith et al. found little effect of carcass weight on pH but reported reduced risk of dark cutting in rapid growing steers. These authors hypothesized that rapid growing cattle were less sensitive to stress, had increased muscle glycogen and differed in using muscle glycogen than slow-growing cattle. It can be advocated that establishing a

threshold weight for Canada B4 carcass may be impractical but cattle gaining less weight than their cohort mates may be predisposed to cut dark.

Canada B4 carcasses from FC heifers, FC steers, WC steers and GY steers were as heavy as Canada A carcasses but the latter had an increased rib eye area that might have protected against dark cutting as reported by McGilchrist et al. (2012). Increased rib-eye area is associated with reduced marbling but increased muscle glycogen (McGilchrist et al. 2011; Underwood et al. 2007) and glycolytic potential (Wegner et al. 2000) essential to lower carcass pH and attain a normal colour. Cattle having increased LT area also had lower glycolytic response to exogenous adrenaline (McGilchrist et al. 2011), implying that such cattle are less likely to deplete muscle glycogen during a fight or flight reaction. Thus it can be argued that cattle having a slow growth rate and reduced marbling but increased rib-eye produce Canada A carcasses whereas slow growing cattle with a tendency toward increased marbling are prone to cut dark. The results also indicated that BY steers, BY heifers, GY heifers and WC heifers that produced Canada B4 carcasses had phenotypic measurements similar to that of normal grades, suggesting that factors other than phenotype also influenced the occurrence of dark cutting.

4.4.5. Season effect on dark cutting

Literature findings about seasonal (months of year) effects on dark cutting are inconsistent likely because of the differences in geographical locations where the studies were performed. The current study showed a higher incidence of dark cutting in year-fed heifers than year-fed steers during summer and fall. High temperature, humidity and extreme cold can be stressful to animals and may lower muscle glycogen and increase the likelihood of dark cutting (Immonen et al. 2000; Scanga et at. 1998). The literature suggesting hot and fluctuating temperature increased dark cutting especially in heifers (Scanga et at. 1998) agreed with the

current study where summer and fall season respectively had high and fluctuating temperatures. Seasonal effects were not analysed in calf-fed cattle that were slaughtered in the summer and fall; however, only the calf-fed (FC and WC) heifers were at greater risk of cutting dark. Increased dark cutting in both calf-fed and year-fed heifers, especially during the summer, could be because mounting activity increases with temperature (Gwazdauskas et al. 1983), and also causes an additional heat stress (Brown-Brandl 2013). Increased dark cutting could also be because heifers are more susceptible to heat stress than steers (Busby and Loy 1997; Brown-Brandl 2013). Reduced dark cutting in heifers during winter appeared to agree with Jones and Tong (1989) but contrasted with Immonen et al. (2000) and Scanga et al. (1998).

The literature indicating highest incidence of dark cutting in March and April (Jones and Tong 1989) partially agreed with the current study as only BY heifers tended to have a higher incidence of dark cutting during those months. The seasonal effects, reported in the literature, have been ascribed to the availability of nutrition as Tarrant and Sherington (1980) associated increased dark cutting in September to January with inadequate diet whereas Knee et al. (2004) explained that reduced dark cutting in spring compared to summer and winter seasons was a result of good quality forage in spring. Increased dark cutting in November (fall) was attributed to the lighter weights of the carcasses (Murray 1989) and in the current study Canada B4 carcasses from FC steers, WC steers, and FC heifers, slaughtered in summer and fall, also had lighter weights. The results suggested that season augmented the effects of cattle sex and production systems on dark cutting and that season had a smaller effect in steers.

4.5. Conclusions

The hypothesis that growth promotants influenced the incidence of dark cutting was not accepted. The study however indicated a greater effect of cattle sex on dark cutting where

heifers, especially calf-fed, were more predisposed to cut dark than steers regardless of the production system and hormonal-growth implants. Increased and fluctuating temperatures further escalated the incidence of dark cutting in heifers. Slow growth and reduced carcass weight may increase the likelihood of dark cutting but extended lairage coupled with multiple loading and unloading events may exacerbate the problem. Moderate implant strategies may not cause dark cutting provided that there is an adequate gap between terminal-implant and slaughter and also that the cattle are well-nourished. Rearing system during the growing phase appeared to influence dark cutting likely by attenuating muscle biochemical characteristics and subsequent growth during the feedlot stage of production, but further research is required. The underlying mechanism of increased dark cutting in heifers than in steers during hot and fluctuating temperature needs investigation.

Table 4.1. Structure of Dataset B. Winter calf (WC), fall calf (FC), grass-fed yearling (GY) and backgrounded yearling (BY).

Heifers				Steers			
Calf-fed		Year-fed		Calf-fed		Year-fed	
WC (*n*=5052)	FC (*n*=2706)	GY (*n*=10117)	BY (*n*=8721)	WC (*n*=10336)	FC (*n* = 17619)	GY (*n* = 18924)	BY (*n* = 12933)

Table 4.2. Predicted probabilities% (± standard error) of carcass grades for animal sex *(P* < 0.0001) and the interaction (*P* < 0.0001) of production system x Optaflexx® (Dataset A).

	Sex		Production System x Optaflexx®			
Carcass grade	Heifers	Steers	Yearling-fed[1]		Calf-fed[1]	
			Optaflexx®	No Optaflexx®	Optaflexx®	No Optaflexx®
Prime	2.9[a] (0.52)	0.6[b] (0.26)	1.2[b] (0.6)	4.7[a] (0.81)	0.19[c] (0.19)	0.2[c] (0.21)
AAA	66.4[a] (1.44)	60.5[b] (1.59)	64.5[b] (2.63)	76.4[a] (1.62)	57.42[c] (2.17)	51.5[c] (2.3)
AA	27.2[b] (1.36)	38.5[a] (1.59)	32.4[b] (2.58)	17.0[c] (1.44)	40.08[a] (2.15)	46.6[a] (2.29)
B4	3.4[a] (0.55)	0.3[b] (0.18)	1.8[b] (0.74)	1.9[b] (0.52)	2.31[b] (0.66)	1.7[b] (0.59)

[1]Production systems (yearling versus calves).

[a, b, c] Predicted probabilities with different letter within an individual row for sex and the interaction of production system with Optaflexx® differ at $P \leq 0.05$.

Table 4.3. Predicted probabilities of the likelihood of a carcass grading Canada B4 for the interaction ($P < 0.05$) between sub-production system, slaughter season and sex (Dataset B).

Sub-production system	Heifers				Steers			
	Fall	Spring	Summer	Winter	Fall	Spring	Summer	Winter
Background yearling (BY)	2.35[b] (0.39)	1.99[b] (0.25)	4.3[a] (0.88)	1.02[c] (0.17)	1.16[a] (0.2)	0.11[b] (0.11)	0.4[b] (0.11	0.84[a] (0.12)
Grass earling (GY)	1.13[cd] (0.36)	0.06[e] (0.06)	1.74[c] (0.33)	0.38[d] (0.08)	0.29[b] (0.12)	0.08[b] (0.05)	0.37[b] (0.09)	0.33[b] (0.06)

[a, b, c, d,] Probabilities within a row and column for each sex lacking a common superscript differ at $P < 0.05$.

Table 4.4. Effect of heifer grade and production system on least square means (±SEM in parenthesis) for animal and carcass measurements (Data set B).

Measurements	Backgrounded yearling (BY)					Grass-fed yearling (GY)					Fall calves (FC)					Winter calves (WC)				
	A	AA	AAA	Prime	B4	A	AA	AAA	Prime	B4	A	AA	AAA	Prime	B4	A	AA	AAA	Prime	B4
n	21	1639	6535	370	156	21	2184	7549	301	62	28	1029	1573	17	59	19	962	3662	226	183
Start weight (kg)	357^{ef} (11.2)	397^{d} (1.3)	400^{cd} (0.64)	395^{d} (2.7)	393^{d} (4.1)	387^{de} (11.2)	405^{b} (1.1)	405^{b} (0.59)	397^{d} (2.9)	390^{d} (6.5)	269^{j} (9.74)	280^{j} (1.6)	273^{j} (1.3)	255^{j} (12.5)	273^{j} (6.7)	320^{fg} (11.8)	318^{fg} (1.7)	311^{g} (0.85)	307^{gh} (3.4)	320^{fg} (3.8)
Days to finish	174^{ghi} (5.67)	155^{ik} (0.64)	158^{ik} (0.32)	168^{hi} (1.35)	166^{hik} (2.08)	145^{klmn} (5.7)	140^{lmn} (0.56)	148^{jm} (0.29)	155^{ik} (1.49)	156^{ijk} (3.3)	218^{de} (4.9)	229^{c} (0.81)	238^{b} (0.65)	274^{a} (6.3)	247^{ab} (3.38)	212^{de} (5.9)	206^{e} (0.83)	210^{e} (0.43)	221^{d} (1.7)	204^{e} (1.92)
Carcass weight (kg)	350^{i} (5.9)	378^{efg} (0.67)	385^{d} (0.34)	386^{d} (1.4)	376^{g} (2.2)	354^{i} (5.9)	373^{g} (0.58)	377^{fg} (0.31)	382^{de} (1.6)	375^{g} (3.5)	338^{j} (5.1)	361^{hi} (0.85)	364^{h} (0.67)	372^{efgh} (6.6)	355^{i} (3.5)	361^{hi} (6.3)	382^{de} (0.88)	384^{d} (0.45)	382^{de} (1.8)	385^{d} (2.0)
Grade rib eye area (cm^2)	103^{ab} (2.5)	98.6^{bc} (0.27)	93.8^{f} (0.13)	89.2^{ij} (0.57)	95.9^{cde} (0.88)	103^{ab} (2.5)	94.9^{ef} (0.24)	89.9^{i} (0.13)	84.9^{k} (0.64)	96.4^{cde} (1.4)	102^{ab} (2.2)	99.2^{bc} (0.35)	91.9^{g} (0.28)	84.6^{jk} (2.7)	91.7^{fgh} (1.4)	109^{a} (2.5)	101^{a} (0.35)	92.1^{g} (0.18)	84.5^{k} (0.74)	98.2^{bcd} (0.83)
Grade fat depth (mm)	6.86^{hi} (0.9)	10.4^{ef} (0.1)	13.1^{cd} (0.05)	15.9^{ab} (0.21)	12.4^{cd} (0.32)	6.19^{hi} (0.9)	9.87^{ef} (0.08)	12.7^{cd} (0.05)	15.7^{ab} (0.23)	11.7^{de} (0.52)	7.2^{hi} (0.79)	9.8^{efg} (0.12)	12.5^{cd} (0.10)	14.8^{abc} (0.98)	12.4^{cd} (0.53)	6.52^{hi} (0.92)	10.5^{ef} (0.13)	13.7^{c} (0.06)	16.9^{a} (0.27)	12.3^{cd} (0.30)
Grade marbling score	328^{i} (12.9)	380^{fg} (1.43)	487^{bc} (0.71)	687^{a} (3.02)	495^{b} (4.62)	297^{i} (12.9)	375^{g} (1.25)	482^{c} (0.67)	691^{a} (3.37)	472^{cd} (7.31)	309^{i} (11.3)	368^{gh} (1.81)	460^{d} (1.46)	677^{a} (14.0)	449^{d} (7.58)	318^{i} (13.3)	375^{fg} (1.9)	484^{bc} (0.96)	681^{a} (3.9)	474^{cd} (4.37)

Leas square means within a row of Table 4.4 and across Table 4.5 lacking a common superscript differ at $P < 0.05$.

Table 4.5. Effect of steer grade and production system on least square means (±SEM in parenthesis) for live animal and carcass measurements (Data set B).

Measurements	Backgrounded yearling (BY)					Grass-fed yearling (GY)					Fall calves (FC)					Winter calves (WC)				
	A	AA	AAA	Prime	B4	A	AA	AAA	Prime	B4	A	AA	AAA	Prime	B4	A	AA	AAA	Prime	B4
n	74	4442	8221	101	95	208	7655	10885	122	54	292	8023	9144	73	87	134	4400	5687	72	43
Start weight (kg)	432^{a} (5.99)	425^{a} (0.77)	419^{a} (0.57)	399^{d} (5.13)	416^{ab} (5.29)	430^{a} (3.57)	426^{a} (0.59)	422^{a} (0.49)	417^{ab} (4.67)	432^{a} (7.01)	297^{hi} (3.02)	298^{h} (0.57)	291^{i} (0.54)	282^{ij} (6.03)	285^{ij} (5.53)	334^{f} (4.45)	331^{f} (0.78)	326^{fg} (0.68)	320^{fg} (6.08)	326^{fg} (7.86)
Days to finish	121^{o} (3.02)	130^{o} (0.39)	138^{n} (0.29)	159^{ik} (2.58)	133^{no} (2.66)	128^{o} (1.8)	138^{n} (0.29)	148^{jm} (0.24)	164^{hik} (2.35)	139^{mn} (3.53)	222^{d} (1.52)	227^{cd} (0.29)	235^{b} (0.27)	246^{b} (3.04)	241^{b} (2.78)	175^{gh} (2.24)	182^{g} (0.39)	190^{f} (0.34)	201^{ef} (3.06)	189^{fg} (3.96)
Carcass weight (kg)	382^{de} (3.17)	392^{c} (0.4)	395^{b} (0.3)	399^{a} (2.71)	389^{cd} (2.8)	386^{d} (1.89)	395^{b} (0.31)	399^{a} (0.26)	398^{a} (2.47)	388^{cd} (3.71)	388^{cd} (1.59)	397^{a} (0.3)	400^{a} (0.28)	402^{a} (3.19)	380^{def} (2.92)	383^{de} (2.35)	397^{a} (0.41)	401^{a} (0.36)	399^{a} (3.22)	388^{cd} (4.1)
Grade rib eye area (cm^2)	97.8^{bcd} (1.28)	91.4^{g} (0.17)	87.5^{j} (0.12)	84.2^{k} (1.1)	92.4^{fg} (1.14)	103^{a} (0.77)	91.9^{g} (0.13)	87.8^{j} (0.1)	80.4^{k} (1.01)	92.9^{efg} (1.51)	104^{a} (0.65)	97.4^{c} (0.12)	90.9^{gh} (0.11)	84.2^{k} (1.29)	94.5^{ef} (1.18)	100^{ab} (0.98)	95.6^{de} (0.17)	90.5^{h} (0.15)	84^{k} (1.3)	94.6^{ef} (1.72)
Grade fat depth (mm)	8.01^{hi} (0.47)	10.2^{ef} (0.06)	11.9^{d} (0.04)	15.2^{ab} (0.4)	11.4^{de} (0.42)	6.89^{i} (0.28)	9.39^{fg} (0.05)	11.5^{d} (0.04)	13.7^{c} (0.37)	10.7^{def} (0.55)	6.89^{i} (0.24)	9.59^{fg} (0.04)	11.8^{d} (0.04)	14.3^{bc} (0.47)	9.27^{fgh} (0.43)	6.95^{i} (0.36)	9.35^{g} (0.06)	11.6^{d} (0.05)	14.5^{bc} (0.48)	9.49^{fgh} (0.63)
Grade marbling score	313^{i} (6.71)	369^{gh} (0.87)	458^{d} (0.64)	670^{a} (5.8)	453^{d} (5.96)	306^{i} (4.04)	363^{gh} (0.66)	463^{d} (0.56)	680^{a} (5.34)	413^{e} (7.94)	306^{i} (3.45)	363^{h} (0.65)	456^{d} (0.6)	685^{a} (6.76)	401^{ef} (6.19)	300^{i} (5.15)	363^{h} (0.89)	458^{d} (0.78)	685^{a} (6.85)	416^{e} (9.02)

Least square means within a row of Table 4.5 and across Table 4.4 lacking a common superscript differ at $P < 0.05$.

Table 4.6. Pearson correlations identifying the relationships between carcass and animal characteristics (Data set B).

	Days to finish	Carcass weight	gFD	gREA	gMS
Start weight	-0.76 <0.0001	0.18 <0.0001	-0.012 0.0003	-0.025 <0.0001	0.038 <0.0001
Days to finish		0.056 <0.056	0.070 <0.0001	0.099 <0.0001	0.029 <0.0001
Carcass weight			0.101 <0.0001	0.32 <0.0001	0.014 <0.0001
Grade fat depth (gFD)				-0.28 <0.0001	0.37 <0.0001
Grade rib eye area (gREA)					-0.21 <0.0001

Figure 4.1. Predicted probabilities of dark cutting for production systems ($P = 0.09$) and sex ($P < 0.05$). (Data set A).

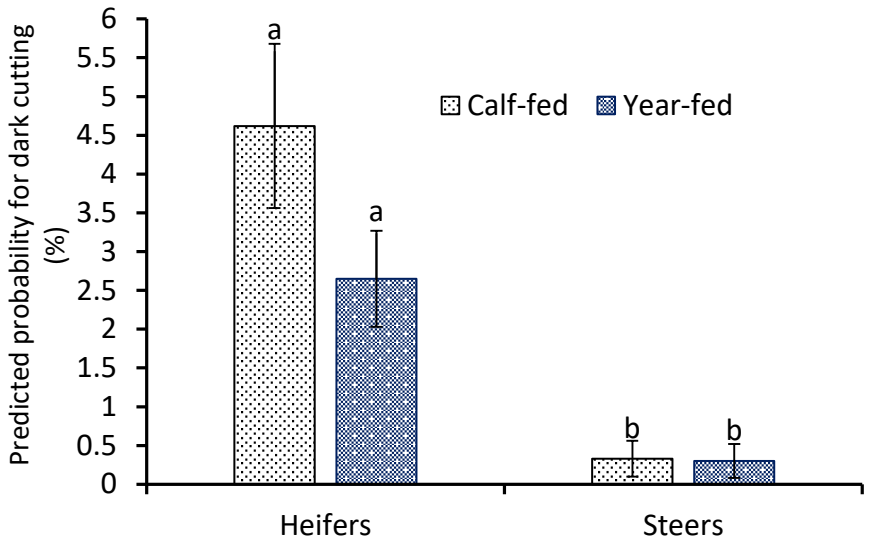


Figure 4.2. Relationship of predicted probability of dark cutting with cattle sex and production system (Calf-fed vs Year-fed). (Data set A).

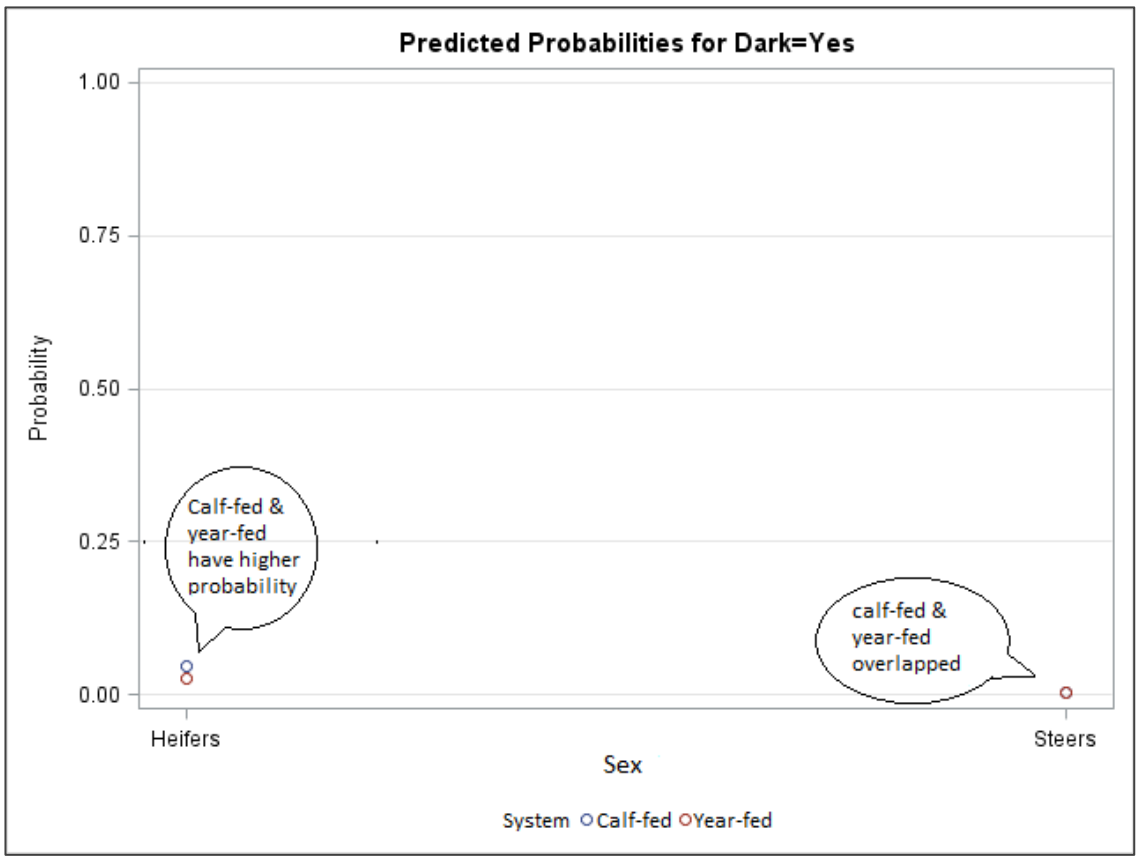


Figure 4.3. Predicted probabilities for carcass grades for the effect of cattle sex ($P < 0.0001$). (Data set A).

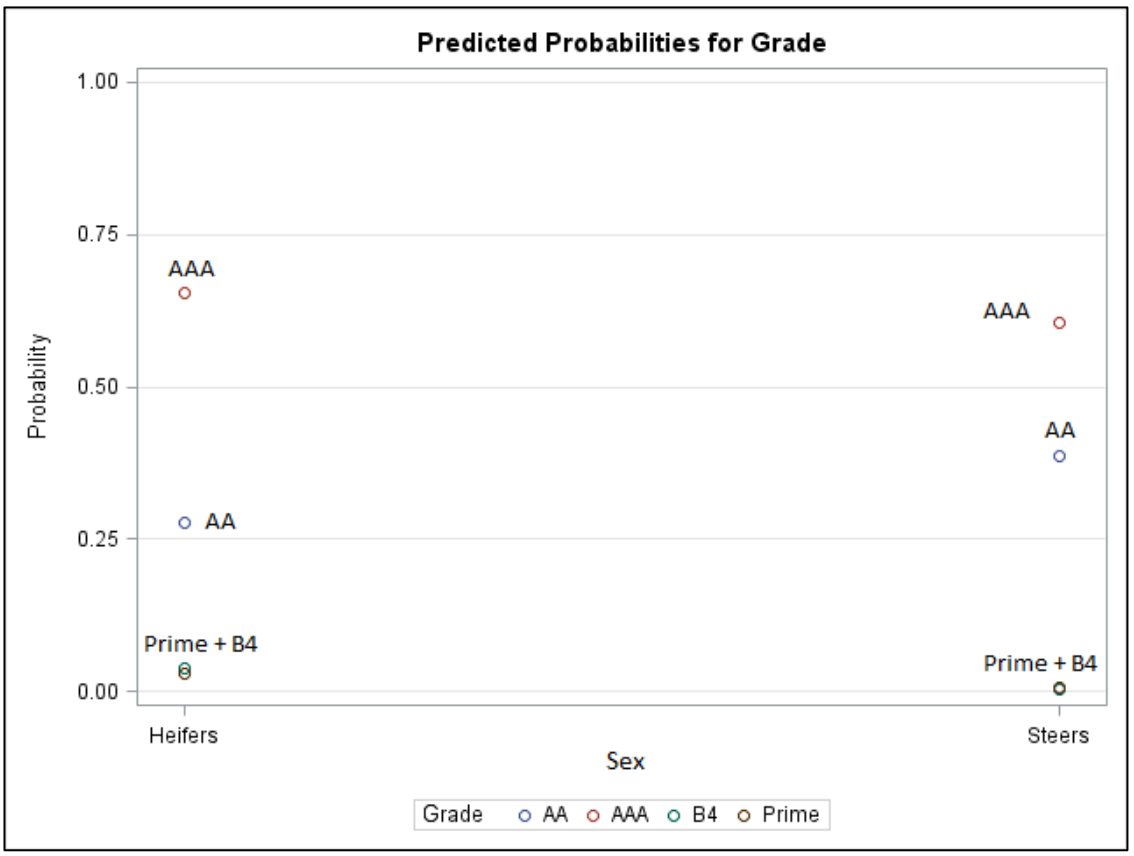


Figure 4.4. Predicted probability for Canada AA for the interaction ($P < 0.0001$) between production system (Calf-fed, Year-fed) and Optaflexx® (Opt) feeding (Yes or No). (Data set A).

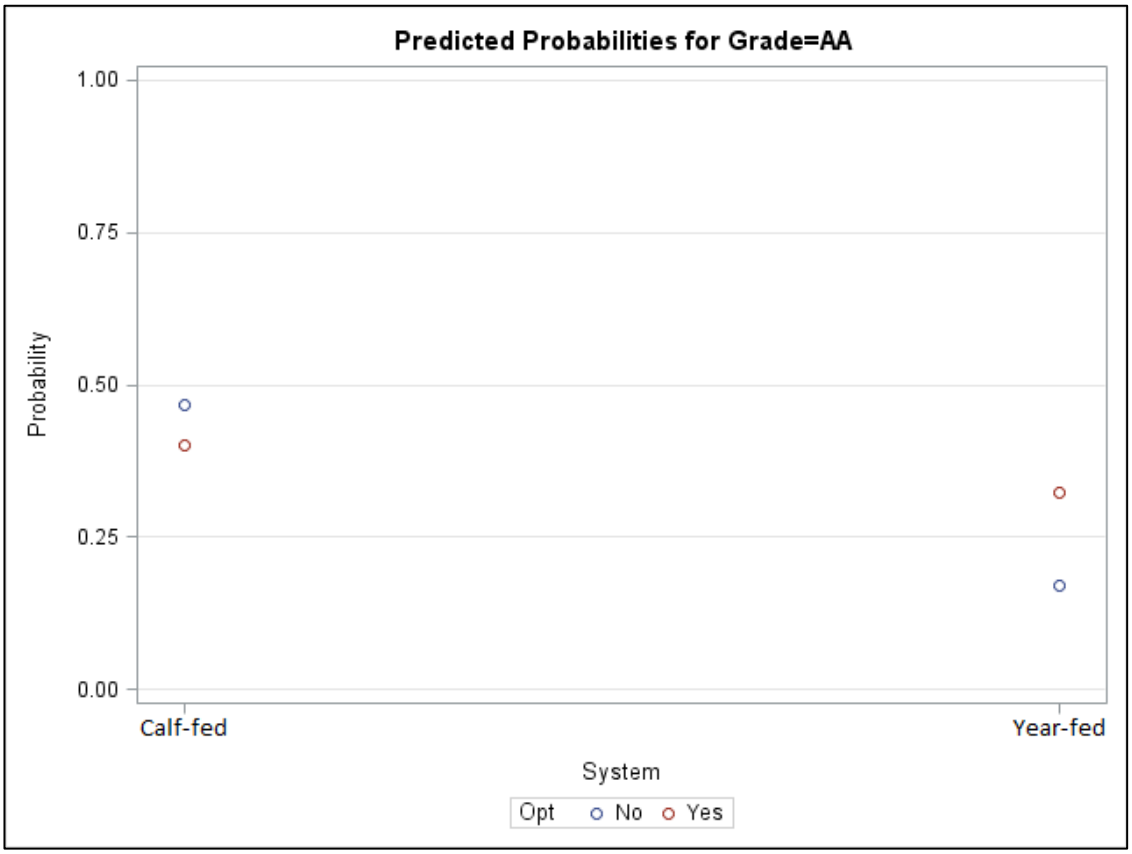


Figure 4.5. Predicted probability for Canada AAA for the interaction ($P < 0.0001$) between production system (Calf-fed, Year-fed) and Optaflexx® (Opt) feeding (Yes or No). (Data set A).

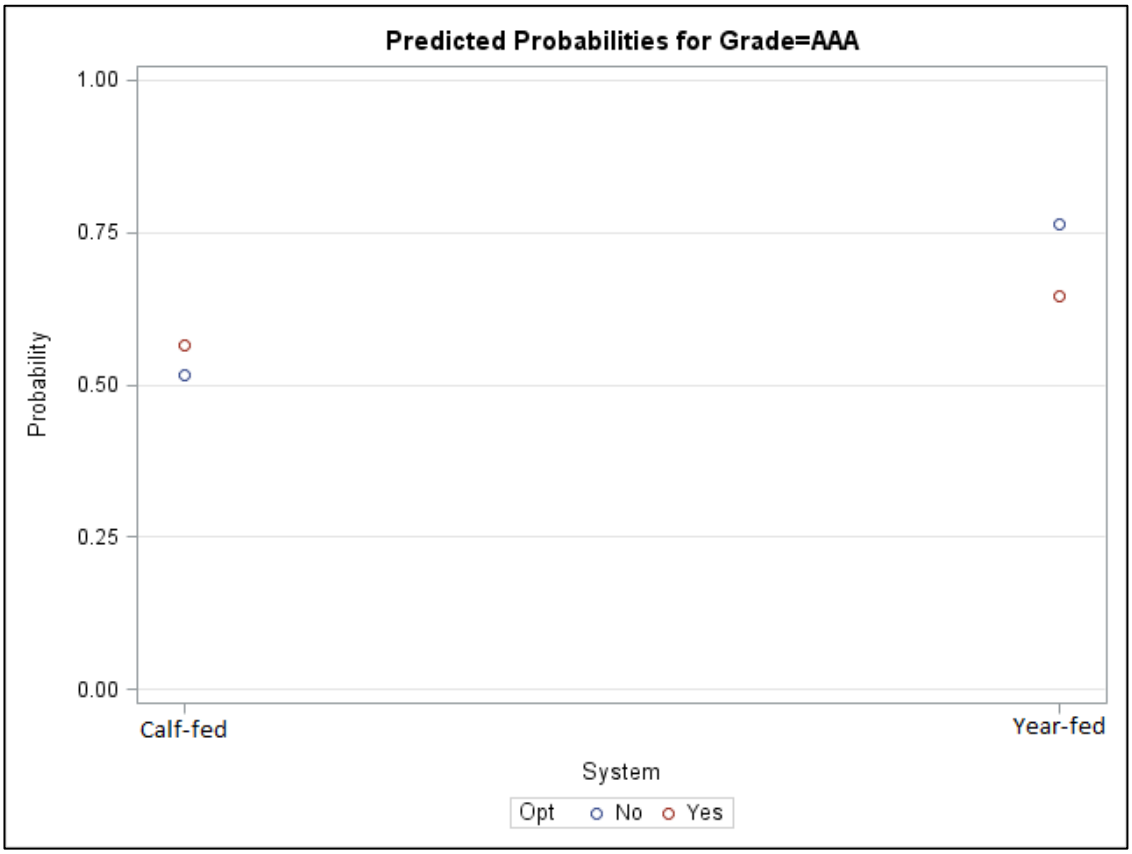


Figure 4.6. Predicted probability for Canada Prime for the interaction ($P < 0.0001$) between production system (Calf-fed, Year-fed) and Optaflexx® (Opt) feeding (Yes or No). (Data set A).

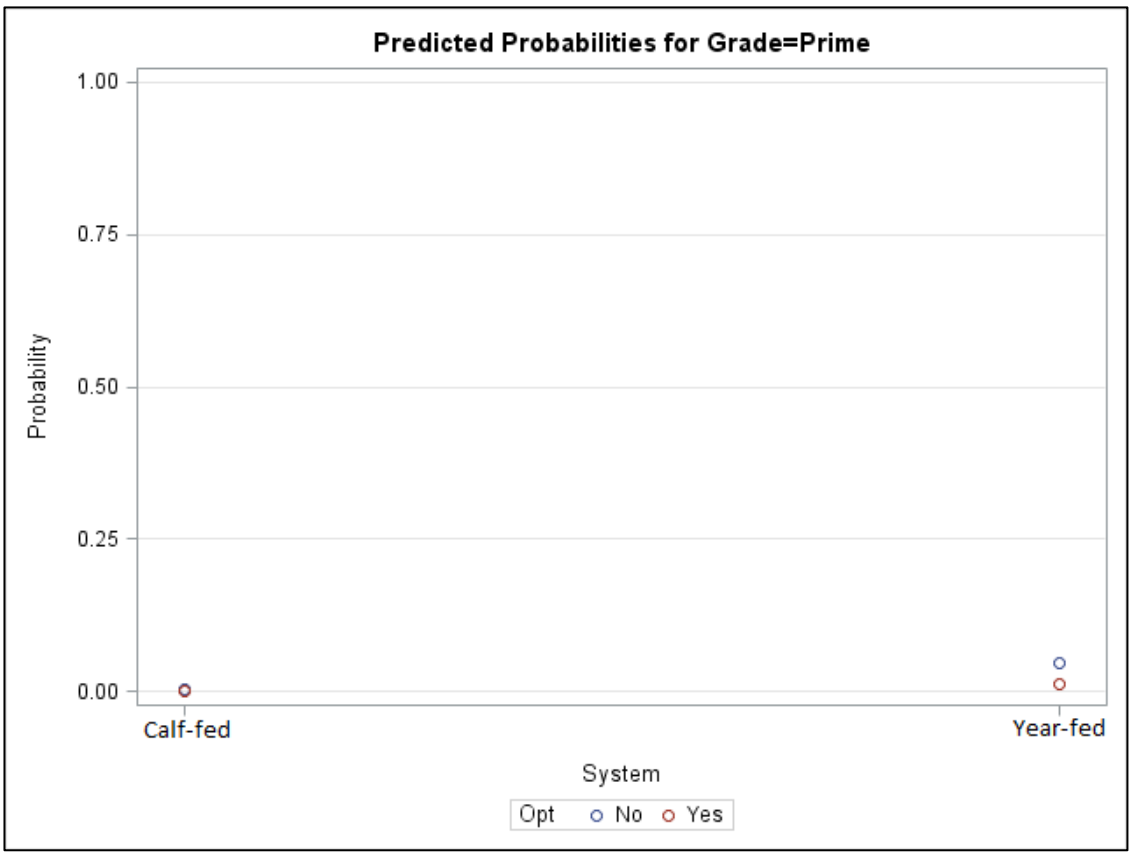


Figure 4.7. Predicted probability for Canada B4 (dark cutting) for the interaction ($P < 0.0001$) between production system (Calf-fed, Year-fed) and Optaflexx® (Opt) feeding (Yes or No). (Data set A).

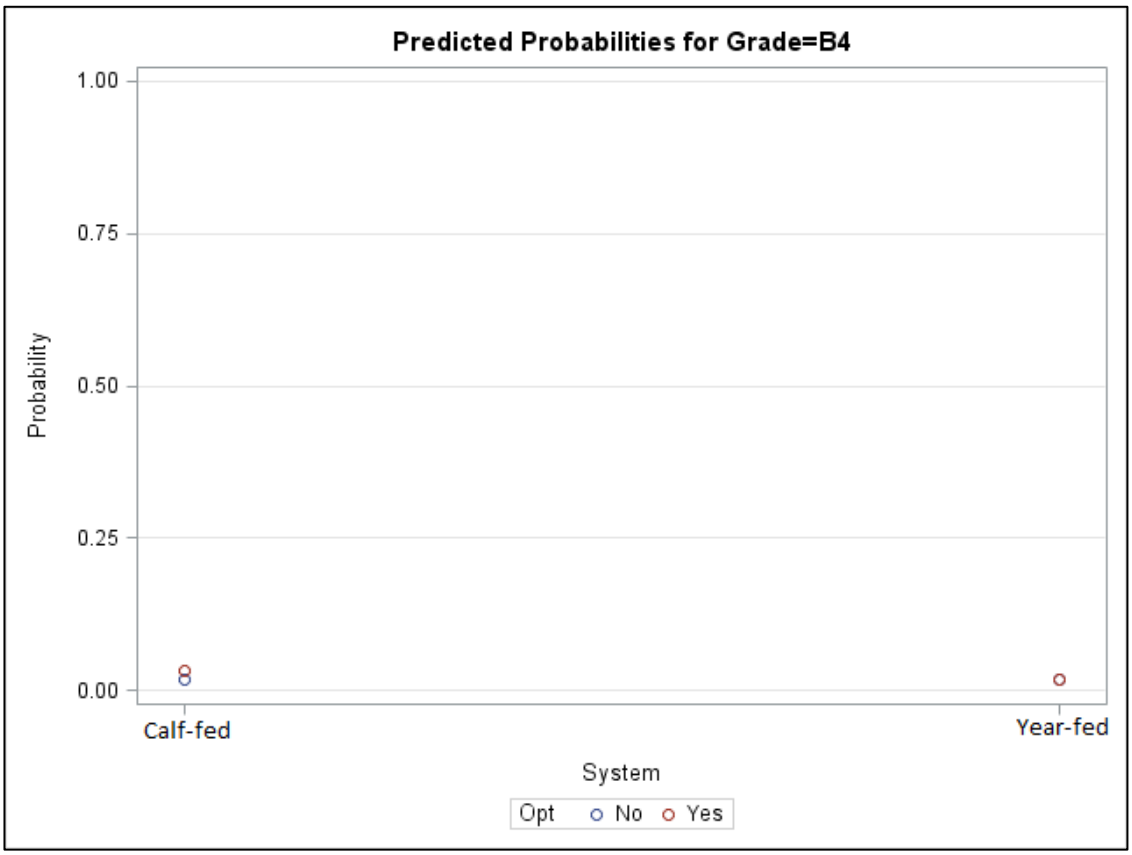


Figure 4.8. Predicted probability for the incidence of dark cutting (Canada B4) for the interaction between cattle sex and implant within production system (Data set B).

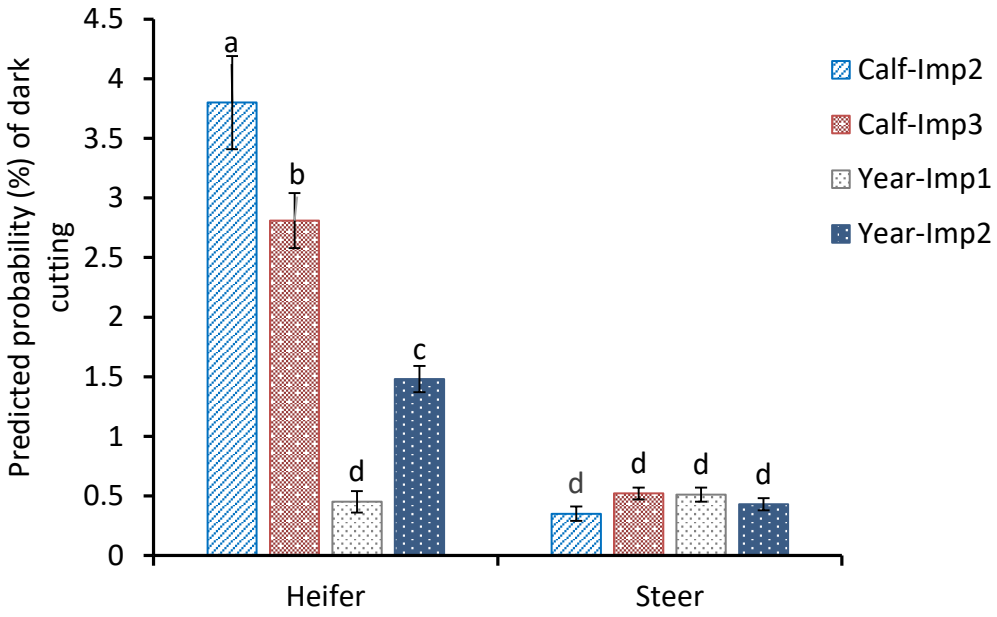


Figure 4.9. Predicted probabilities of dark cutting (Canada B4) for the interaction (*P* <0.0001) between sex and sub-production systems (BY, backgrounded yearling; FC, fall-calf; GY, grass-fed yearling; WC, winter-calf). (Data set B).

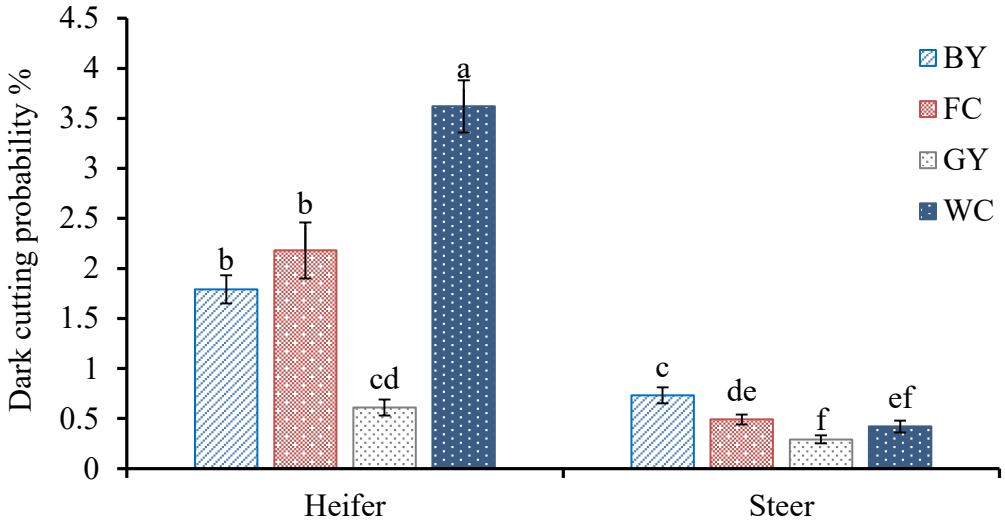


Figure 4.10. Relationship of production systems (BY, backgrounded yearling; FC, fall-calf; GY, grass-fed yearling; WC, winter-calf) and carcass weight for the probabilities of carcass grades in steers (Data set B).

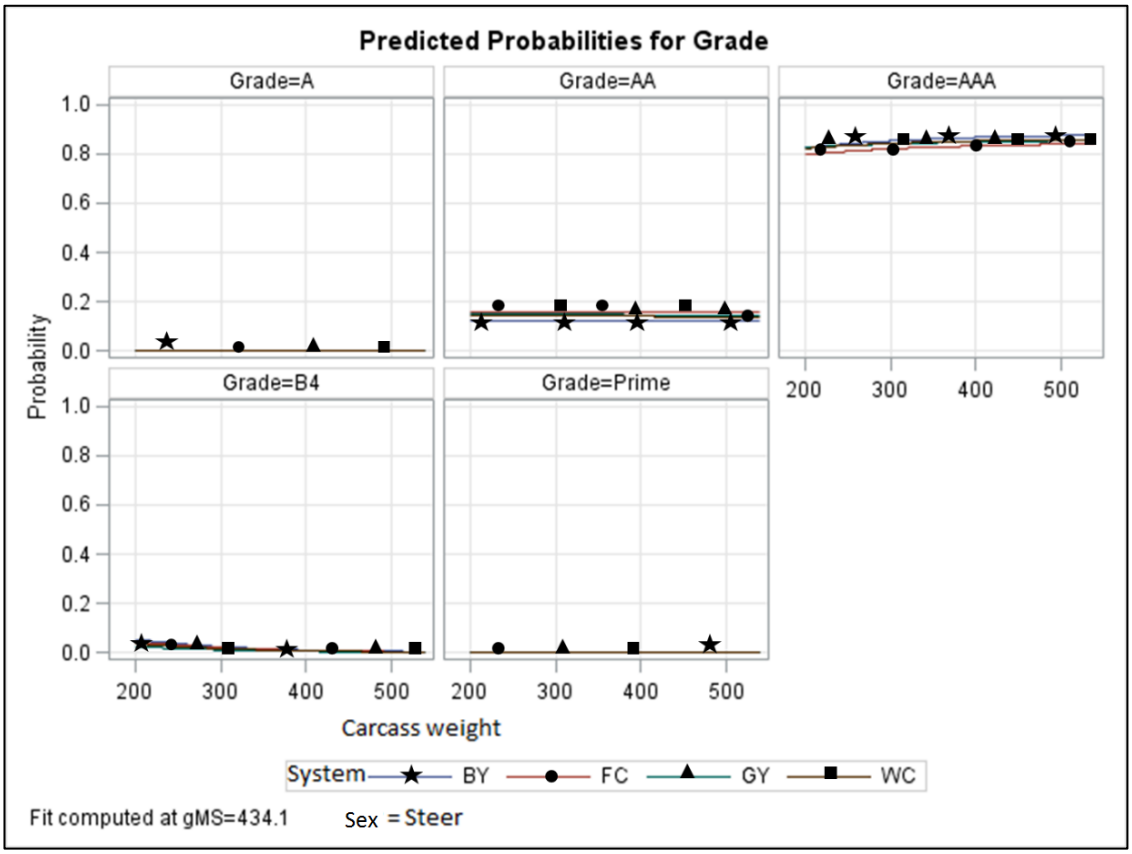
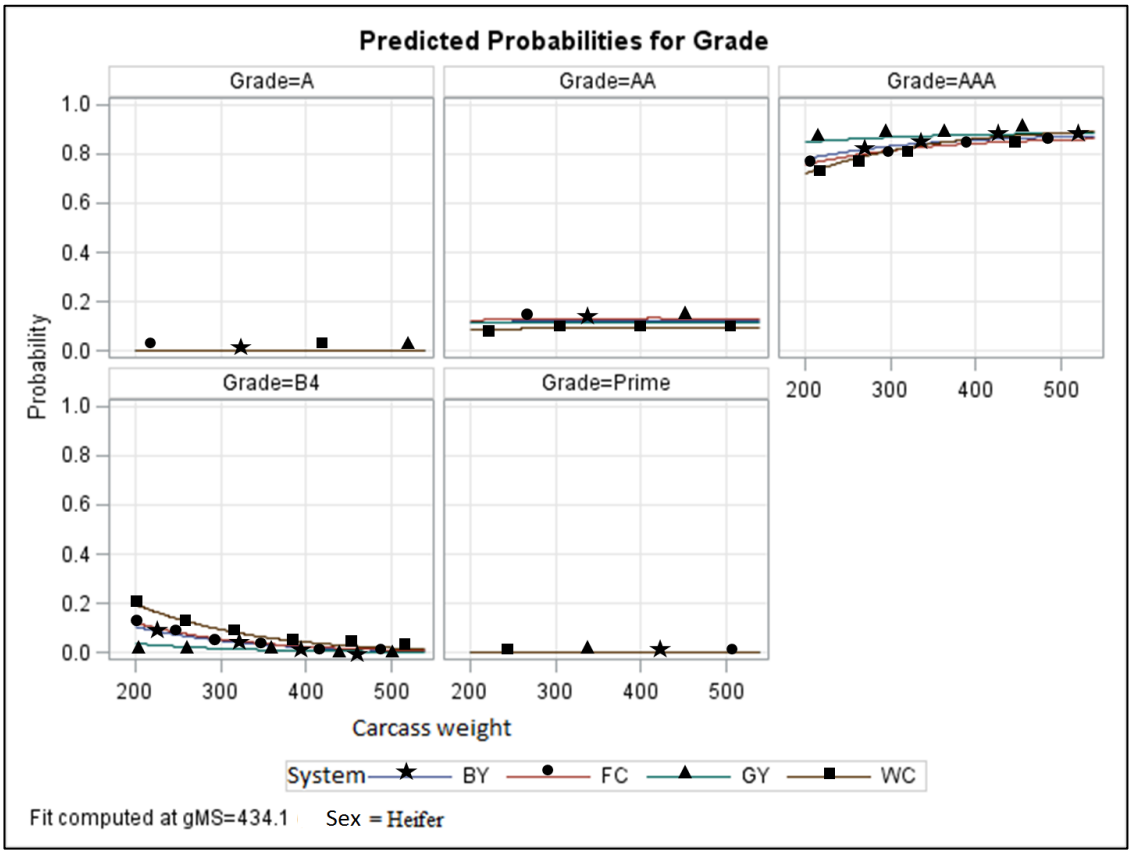


Figure 4.11. Relationship of production systems (BY, backgrounded yearling; FC, fall-calf; GY, grass-fed yearling; WC, winter-calf) and carcass weight for probabilities of carcass grades in heifers (Data set B).



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5.0. Understanding the quality of typical and atypical dark cutting beef from heifers and steers[3]

5.1. Introduction

Carcasses from cattle under 30 months of age that are deemed through having a dark red rib eye muscle (m. *longissimus thoracis,* LT) to be dark cutting are graded Canada B4 in the Canadian beef grading system (Canadian Agricultural Products Act SOR/92-541, 2014). Dark cutting beef is discriminated against because of the reduced aesthetic value (Viljoen et al. 2002) and shelf life (Newton and Gill 1981), and increased toughness and spongy or rubbery texture (Holdstock et al. 2014). Bacteria in meat with reduced glycogen concentration, characteristic of dark-cutting beef, degrade protein and produce ammonia that causes off odours and flavours (Newton and Gill 19881). The widely accepted cause of dark cutting is the depletion of muscle glycogen reserves prior to slaughter, which increases post-mortem pH. The depletion of muscle glycogen ante mortem has been associated with pre-slaughter stressors such as animal handling (MacDougall and Rhodes 1972), transportation (Immonen et al. 2000), mixing of unfamiliar animals (Jones and Tong 1989), lairage time (Mach et al. 2008), extreme weather (Immonen et al. 2000) and animal excitability or aggressiveness (Kenny and Tarrant 1988; Voisenet et al. 1997a). Literature findings regarding lairage effects on dark cutting are equivocal as Gallo et al. (2003) found increased frequency of dark cutting with increased lairage time but Teke et al. (2014) showed that cattle which spent 72 h in lairage were less likely to produce carcasses with LT pH > 5.8 than those rested for 24 h. Further, it has also been indicated that the effect of lairage on dark cutting may depend upon cattle sex (Marenčić et al. 2012).

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Muscles transform from opaque to translucent at pH 5.9 and above this meat is moderate to very dark (MacDougall and Jones l98l). The pH for normal, cherry-red colour beef ranges from 5.40 to 5.59 (Page et al. 2001), while meat that has pH value greater than 5.7 may be dark (Orcut et al. 1984) although pH 5.87 is also considered a threshold below which the colour may (Page et al. 2001) or may not be normal (Bass et al. 2008). Murray (1989) found that most of the carcasses subjectively graded as dark in the Canadian beef industry had pH values below 5.8 while in New Zealand 40% of beef bulls were most likely to be graded as dark at pH > 5.7 (Pulford et al. 2009).

Intramuscular glycogen concentration, like pH, has been reported to vary greatly within normal and dark beef. Glycogen below 66 µmol glucose g^{-1} of muscle may result in dark cutting beef with a pH greater than 6.0 (Hanson et al. 2001); however, glycogen in muscles from bull carcasses with an ultimate pH values of 5.4 to 5.75 could be lower than 55 µmol glucose g^{-1} tissue (Immonen and Puolanne 2000). Additionally, beef with increased pH may have sufficient residual glycogen to further reduce muscle pH (Immonen and Puolanne 2000). Earlier it was reported that meat with pH 5.57 had 82.4 µmol lactate g^{-1} of tissue (Bodwell et al. 1965) suggesting that a glucose concentration as low as 41.2 µmol glucose g^{-1} muscle would allow meat to attain normal pH. Holdstock et al. (2014) reported the existence of atypical dark (pH < 6.0) steer carcasses that had glucidic potential similar to normal Canada AA/USDA Select carcasses. These authors hypothesized that steers that produced atypical dark muscle having pH < 6.0 and adequate glucidic potential had inadequate lairage time to recover from shipping stress.

Dark cutting beef is usually tougher than normal beef and increased toughness is considered to be associated with reduced glucidic potential (Wulf et al. 2002) but may (Pratt et al. 2013) or may not be related to the degree of darkness (Bass et al. 2008). Meat toughness has a

negative (Teke et al. 2014) or curvilinear relationship with intramuscular pH (Watanabe et al. 1996). Holdstock et al. (2014) reported increased toughness in atypical dark beef from steer carcasses compared to that from Canada AA steer carcasses, but these authors did not consider the influence of carcass phenotypic characteristics such as grade fat depth and grade rib eye area, or sex on cooked beef toughness (Aalhus et al. 2001; Choat et al. 2006; Muir et al. 1998; Wulf et al. 2002). Recently it was found that dark cutting cattle/carcasses had phenotypic characteristics similar to cattle/carcasses that produced normal Canada AA carcasses (Mahmood et al. 2016a, b) suggesting that any variation in tenderness between Canada AA and dark cutting beef may not be related to carcass phenotype. Voisinet et al. (1997b) reported increased toughness of beef from borderline dark cutting heifer carcasses whereas Murray (1989) reported dark cutting carcasses with pH $\leq$ 5.8 in heifers. The glucidic potential of such carcasses from heifers has never been reported and it is unclear if those carcasses truly represented atypical dark as indicated by Holdstock et al (2014) in steers. The objective of this study was to investigate potential relationships among muscle glucidic potential, pH, beef quality, lairage time and phenotype of Canada AA and dark cutting carcasses from heifers and steers. It was hypothesized that atypical dark cutting exists in both heifer and steer carcasses, the likelihood of dark cutting is related to carcass phenotype, and that atypical dark cutters yield the toughest beef, regardless of the carcass phenotype.

5.2. Materials and methods

Approval from an Animal Care and Use Committee was not required for this study because LT muscles were purchased from a commercial beef abattoir regulated by Canadian Food Inspection Agency (CFIA), where cattle were slaughtered humanely in compliance with Canadian beef slaughter regulations (Canada Meat Inspection Regulations SOR/90-288).

5.2.1. Carcass sampling and design of experiment

Beef rib samples (*muscular* longissimus thoracic; LT; $n = 64$) from Canada AA ($n = 24$) and B4 ($n = 40$) heifers and steers were collected from a commercial beef abattoir over two visits (batches). The LT muscles were collected 24-48 h post-mortem after the carcasses were graded. Grading of the carcasses was performed at the 12^{th} and 13^{th} rib interface by trained personnel from the Canada Beef Grading Agency. The LT muscles, collected as rib primals, were not from a particular side of the carcasses because both sides of beef carcasses represent the same mean muscle pH at 24 and 48 h post-mortem (Jelenikova et al. 2008). The rib primals were wrapped in polyethylene bags, boxed and shipped in a refrigerated truck to the meat laboratory of the University of Alberta.

About 96 h post-mortem, the rib primals were removed from packaging, weighed, de-boned, weighed again, and approximately 1 cm at the 12^{th} rib (posterior end) was faced. The rib sections were pierced three times approximately 1.5 cm from the posterior end and intramuscular pH was measured using an Accumet AP71 (Fisher Scientific, Mississauga, Ontario) pH meter fitted with a pH electrode (Cat No. 655-500-30, FC210B, Canada-wide Scientific, Ottawa, ON) calibrated to pH 4.0 and 7.0 with commercial standards (Fisher Scientific, Ottawa, ON). From the posterior end, a 1.5 cm thick steak was removed, vacuum packed and stored at -20 ºC for sarcomere measurement. Another 1.5 cm steak was removed and stored at -20 ºC for another project while a third 2 cm thick steak was removed, frozen in liquid nitrogen and stored at -80 ºC for glucidic analysis. A fourth 2 cm thick steak was cut, frozen in liquid nitrogen and then stored at -80 ºC for proteomic analysis in a subsequent project. The remainder of each LT was split into two halves (anterior and posterior), packaged under vacuum in a polypropylene bag and randomly assigned to storage at 4 ºC for 7 or 21 days post-mortem.

On day 7 post-mortem, the LT samples were again assessed for pH, and Canada B4 LT were sorted, as outlined in Table 5.1, into atypical (AB4, pH < 5.9) or typical B4 (TB4, pH > 5.9) and balanced for sample location (anterior/posterior). Intramuscular pH values of AB4 muscles ranged from 5.41 to 5.89 while that of TB4 ranged from 5.93 to 6.77. Treatments were allocated on day 7 instead of 96 h post-mortem to ensure that the pH value used to assign muscles to treatment was truly "ultimate". After appropriate ageing, the anterior and posterior LT were cut starting from the posterior end of each for quality analyses with the first 5 cm steak designated for cooking loss and shear force, the next 2.5 cm steak for colour, the third 2.5 cm steak for drip loss and the fourth 2.5 cm steak for proximate analysis. The steaks used for colour measurement on day 7 were subsequently packaged under vacuum in polypropylene bags and stored at -20 ^{o}C for assessment of myoglobin state and concentration.

5.2.2. Carcass phenotype and cattle management

Grade fat depth (gFD), hot carcass weight (CW) and grade rib-eye area (gREA) data for carcasses from which LT muscles were collected were obtained from the abattoir. The sampled carcasses were from 25 different lots where a lot represented a group of cattle of the same sex from the same feedlot which was transported and rested together in lairage. Information about lairage time (h), cattle sex and the total number of dark and normal carcasses in each of the 25 lots was also recorded by and obtained from the abattoir. Heifers (n = 2379) and steers (n = 3632) in all the lots were grouped by the following lairage times: 4 h (n = 539); 5 h (n = 2541); 6 h (n = 1393); 8-10 h (n = 1105); and 72 h (n = 433). Each lairage time contained both heifers and steers and each sex produced both normal and Canada B4 carcasses. Moreover, each lairage time category for each gender had cattle from at least two different lots; however, there was only one lot of steers for the 4 h and 72 h lairage times. The cattle in the 72 h lairage time category, after

unloading at the abattoir, were shipped to a feeding facility 2 km away from the abattoir to be fed and were again shipped to the abattoir on the day of slaughter. Cattle in all other lairage-times were rested at the abattoir and had access to water only. Information about producers, transportation distance to the abattoir and length of feed withdrawal before shipment from the feedlots was not available.

5.2.3. Measurement of sarcomere length

Muscle sarcomere length was measured using an existing technique (Cross et al. 1980-1981) where the frozen steaks were tempered at 4 $^{\circ}$C until softened slightly. Five (5) g tissue were removed from the center of the tempered steak and homogenized in 0.25 M sucrose solution for about two minutes in a 50 mL laboratory blender (Waring, Fisher Scientific). A small drop of the homogenate was placed on a glass microscope slide with cover and observed under phase contrast using a microscope (Axio Scope.A1, Carl Zeiss Microscopy, Thornwood, NY, USA) fitted with a camera (AxioCam MRm, Carl Zeiss Microscopy, Thornwood, NY, USA), under 100x oil immersion. Thirty myofibrils from each sample and five consecutive sarcomeres in each myofibril were measured. The average of the five sarcomeres was taken as the length of the sarcomere for each myofibril and the average of 30 myofibrils was considered the sarcomere length for each sample.

5.2.4. Glucidic analysis

Muscle glucidic analysis was executed as described by Dalrymple and Hamm (1973). Frozen muscles were powdered in liquid nitrogen using a mortar and pestle, and one gram of the crushed sample was homogenized for 30 seconds with 5 mL 0.6 N perchloric acid using a Polytron Homogenizer (PT1200, Brinkmann Instruments Inc., Mississauga, ON). From the homogenate, 200 µL was aliquoted for glycogen estimation while the remainder was centrifuged

at 9000 rpm at 4 °C for 10 minutes. Following centrifugation, 2.3 mL supernatant were removed and neutralized with 3.0 M potassium carbonate. The neutralized supernatant was centrifuged at 3000 rpm for 10 minutes and then assessed for free glucose and lactate concentrations using a glucose analyzer (YSI 2300 StatPlus; YSI Incorporated, Dayton, OH). To the aliquot for glycogen assessment, potassium carbonate (3.0 M) and 0.1 g amyloglucosidase were incorporated, and the resulting mixture was incubated at 40 °C for 2 hours. Subsequently, 200 µL perchloric acid were added to the aliquot, mixed, and then centrifuged at 9000 rpm for 10 minutes at °C. The supernatant (1.2 mL) was collected and neutralized with 3 M potassium carbonate, and the resulting mixture was again centrifuged at 3000 rpm for 10 minutes and then analyzed for glucose as described. Glucose and lactate values were calculated with standard dilutions by regression and then converted to µmol g^{-1} of raw meat. The glucidic potential was calculated as:

Glucidic potential (µmol glucose g^{-1}) = Free glucose + [Lactate/2] + Glycogen (extracted as glucose).

5.2.5. Beef quality analyses

For cooking loss and Warner-Bratzler shear force, the steaks were trimmed to 200 ± 5 g, weighed and a temperature probe (Tinytag View 2 TV-2040; Gemini Data Loggers Ltd. West Sussex, UK) inserted in the geometric center of each steak. Each steak was then placed in a polypropylene bag and cooked to an internal temperature of 71 °C in a 73 °C water bath. The cooking time of the steak was recorded, and cooked steaks were cooled in ice water for 20 minutes before being stored overnight at 4 °C. Following overnight storage, the steaks were removed from the bags, blotted dry with paper towel and weighed to record cooking loss. For WBSF, six 4 x 1 x 1 cm cores were removed from the steaks parallel to the direction of the

muscle fibres. The cores were sheared perpendicular to the muscle fibre direction using a trianglular Warner-Bratzler-like shear blade in a materials testing machine (Lloyd Instrument LRX plus, AMETEK®, Digital Measurement Metrology Inc. Brampton, ON) at a pre-load force of 2 N and a crosshead speed of 200 mm per min during shear. Peak shear values were recorded from the six cores and then averaged to calculate WBSF for the sample. Standard deviation within each steak was also calculated for WBSF and recorded.

The objective colour (Commission Internationale de L'eclairage; CIE) was measured, after exposing the cut steaks to air for 30 minutes, using a Minolta Chroma Meter (CR-400, Konica Minolta, Osaka, Japan) calibrated against a white calibration tile supplied by the manufacturer. Three readings, taken at three locations on each steak, were averaged for statistical analysis.

For water holding capacity, 2 cm thick steaks (100 ± 5 g), trimmed of epimysium, were suspended in inflated bags using steel hooks for 24 hours at 4 ºC (Honikel 1998). The steaks were then removed from the bags, blotted dry and weighed to calculate water loss by subtracting final weight from initial weight and expressed as mg g^{-1} of the initial weight.

5.2.6. Myoglobin estimation

Myoglobin estimation was performed as described previously (Tang et al. 2004; Warriss 1979) using 10 ± 0.5 g tissue, free from fat depots and connective tissues, in duplicate from each sample steak thawed at 4 ºC for 2 h. The tissue was homogenized for 40 seconds in 50 mL ice-cold phosphate buffer (pH 6.8) and the mixture was first stored at 4 ºC for 1 hour and then centrifuged for 10 min at 6500 × g at 4 ºC. The homogenates were filtered with Whatman® filter paper No. 4 (GE Healthcare Life Sciences, Mississauga, ON) and the supernatants collected for measuring the absorbance at 503, 525, 557, 582 and 730 nm against a 0.04 M phosphate buffer

blank. Absorbances were measured using a spectrophotometer (Evolution 60S, UV-Visible Spectrophotometer, Thermo Scientific, USA). The ratios of oxy-myoglobin (OMb), deoxy-myoglobin (DMb) and met-myoglobin (MMb) were calculated as described by Tang et al. (2004) where total myoglobin concentration was also calculated using the following equation:

Mb (mg/g) = (Absorption at 525 – Absorption at 730) × 2.2303 × [dilution factor].

The volume of the buffer (mL) was divided by mass (g) of the sample used to compute the dilution factor.

5.2.7. Proximate analyses

For proximate analyses, 100 ± 10 g steak from each loin portion was chopped into small cubes, packed in a pre-weighed aluminium tray, stored at -20 ºC and then freeze dried in a VirTis freeze dryer (SP Industries/SP Scientific, Warminster, PA, USA). The samples were weighed before and after freeze drying to calculate moisture lost. The freeze dried samples were then pulverized in a laboratory blender with three pellets of dry ice, packed in Nasco Whirl-Pak® (Zefon International, Inc. Ocala, FL) bags and stored at -20 °C until further analysis. The crude fat was measured in 2 ± 0.003g of pulverized sample, in duplicate, where fat extraction was performed using petroleum ether (Method 960.39; Association of Official Analytical Chemists (AOAC) 1995) in a Foss Soxtec™ 2050 (Foss Analytical, Hilleroed, Denmark). For crude protein, 100 ± 5 mg of freeze-dried muscle in duplicate were analysed for nitrogen content (Method 992.15; AOAC 1995) using a TruSpec® Carbon/Nitrogen Determinator (LECO ® Corporation, St. Joseph, MI, USA). The crude fat and crude protein contents were then calculated to percentages in raw meat. For total moisture, 2 ± 0.005 g of freeze-dried sample was heated in a glass vial at 100 °C in an oven for 18 hours and then weighed to calculate total moisture in the raw meat. The oven dried samples were then reduced to ash at 490 °C for 48

hours in a muffle furnace (Kejia Furnace Co. Ltd. Henan, China) and weighed to calculate ash percentage.

5.2.8. Statistical analyses

The statistical analyses were performed using SAS (Version 9.3, SAS Institute Inc. Cary, NC). The CATMOD procedure with contrasts was used to test the effect of sex, lairage time and their interaction on the incidence of dark cutting. Lairage time for LT grades (AA, AB4, and TB4) was computed using the Monte Carlo test where means were compared using contrasts within the Permutation Procedure in SAS. Carcass phenotype, glucidic potential, sarcomere length and myoglobin data were analyzed using the MIXED procedure where animal sex, grade, and their interaction served as fixed effects while batch was deemed random. Post-mortem sampling time, CW, gREA, gFD, LT weight and lairage time were individually tested for significance as covariates in the glucidic and myoglobin analyses.

For beef quality data, a split-plot design was used where animal sex and grade were tested in the main plot and post-mortem ageing time in the sub-plot. Mean muscle pH values at 96 h, and at days 7 and 21 post-mortem were compared using the MIXED procedure with cattle sex, grade, post-mortem time and their interactions as fixed effects and batch and its interactions as random effects. For significant ($P < 0.05$) models, the means were compared using least square difference and considered significant at $P < 0.05$. Lairage time was used as a covariant for drip loss while the initial weight of the cooked steak was tested as a covariant for cooking time and cooking loss. Pearson correlations were performed using SAS to investigate relationships between carcass conformation, and biochemical and beef quality measurements from day 7 post-mortem. A Bonferroni correction was applied by dividing alpha 0.05 by the number of variables (Rice, 1989). Linear and polynomial regressions were performed in Excel (Microsoft® Office

2013, Microsoft Corporation, Redmond, WA, USA) for the relationships between CW and glucidic potential and between LT pH and WBSF.

5.3. Results

Results are presented in Tables 5.2 to 5.9 and Figures 5.1 to 5.5. For models having a significant interaction ($P < 0.05$), only the mean values from the interaction were considered and presented.

5.3.1. Lairage time

Results indicated significant ($P < 0.0001$) effects of sex and lairage time on the incidence of dark cutting with no interaction. The mean probability of dark cutting was greater in heifers (3.78 ± 0.39%) than in steers (0.8 ± 0.15%). Cattle that spent 72 h in lairage had the greatest mean probability of dark cutting followed by those that spent 4 and 8 h in lairage, while cattle rested for 5 and 6 h had the lowest mean probability of dark cutting, which was not different from the 8 h lairage group (Table 5.2).

The LT grade categories were significantly ($P < 0.0001$) different for mean lairage time, which was greater for TB4 (45 ± 7.50 standard error of mean h) than for AB4 (13 ± 4.53 h) and Canada AA (7.83 ± 2.79 h) while AB4 and AA LT were not different from each other. Lairage time varied considerably within grade category as evidenced by large standard errors. Most of the AA and AB4 carcasses were mainly from the cattle that spent 5 h in lairage while TB4 were mostly from cattle held for 72 h (Table 5.3).

5.3.2. Carcass phenotype, sarcomere length, and myoglobin

Grade category was a significant source of variation for mean CW, LT weight with and without bones, oxy-, deoxy- and met-myoglobin proportions, and myoglobin concentration. Means for CW and LT weight bone-in were significantly greater for TB4 carcasses than for AB4

carcasses but similar to those from Canada AA carcasses (Table 5.4). For LT weight boneless, Canada AA carcasses were not different from AB4 and TB4 carcasses but the latter two were different from each other (Table 5.4). Mean OMb percentage was significantly greater for Canada AA LT than for AB4 and TB4 LT, while mean DMb and MMb percentages for Canada AA LT were lower than those of TB4 LT. Mean myoglobin concentration was significantly greater for TB4 LT, intermediate for AB4 LT and lowest for Canada AA LT (Table 5.4).

There were no sex and grade category effects for gREA, gFD or sarcomere length (Table 5.4). The sole effect of sex was on mean percentage of DMb, where mean percentage of DMb was greater in LT from steers than LT from heifers (Table 5.4).

5.3.3. Glucidic potential and 96 hour pH

Grade category was significant for pH at 96 h, glucidic potential, and muscle lactate, residual glucose and glycogen concentrations (Table 5.5). Mean pH was greatest for TB4 LT followed by AB4 LT and lowest for Canada AA LT (Table 5.5). The mean values for glucidic potential and lactate were significantly greater for LT from Canada AA followed by those of AB4 and TB4 LT (Table 5.5). Mean residual glucose and glycogen concentrations were significantly greater for Canada AA LT while those of AB4 and TB4 LT were not different from each other (Table 5.5). Analysis of variance indicated sex was also a significant source of variation for residual glycogen concentration only (Table 5.5), with LT from heifer carcasses having a greater concentration than LT from steer carcasses ($P = 0.016$). Notably, the interaction between sex and quality category also approached significance ($P = 0.056$) for residual glycogen concentration, where the mean values for LT from heifer and steer Canada AA carcasses were significantly greater than the mean values for LT from AB4 steer carcasses and TB4 carcasses regardless of sex. Within the AB4 category, LT from heifer carcasses had a greater residual

glycogen concentration than that from steer carcasses, while there was no effect of sex in the TB4 category (Figure 5.1). The effect of sex on glucidic potential also approached significance ($P = 0.095$), with LT from heifer carcasses tending to have a higher mean glucidic potential than LT from steer carcasses.

5.3.4. Meat quality and proximate

5.3.4.1. Effects of grade category and sex

There were significant ($P < 0.05$) effects of grade category on mean muscle pH, redness (a*), yellowness (b*), chroma, hue (angle), cooking loss, WBSF standard deviation and percentages of moisture and crude fat (Table 5.6). Because there was no effect of ageing on these measurements, the mean values are from combined day 7 and 21 postmortem data. As planned, mean pH was greatest for TB4 LT, intermediate for AB4 LT and lowest for Canada AA LT (Table 5.6). Mean a*, b* and chroma were greatest for Canada AA, intermediate for AB4 and smallest for TB4 LT. Mean hue angle for TB4 LT was greater than that of Canada AA and AB4 LT (Table 5.6). The mean cooking loss was lower for TB4 LT than for the other two categories while Canada AA and AB4 LT were not different from each other. The standard deviation in WBSF was smaller for Canada AA LT than for TB4 and AB4 LT, which were not different from each other (Table 5.6). Proximate analysis indicated that moisture and fat content of the AB4 LT were lower and higher, respectively, than that of the Canada AA and TB4 LT with no differences between categories in protein or ash contents (Table 5.6).

Sex was not significant for beef quality characteristics with the exception of cooking time, cooking loss, and moisture and fat contents (Table 5.6). The LT steaks from steers had a shorter mean cooking time, but a greater mean cooking loss than LT steaks from heifers (Table 5.6). The initial weight of the cooked steaks was included as a covariant in these analyses, and

was significant ($P < 0.0001$) for cooking time only. LT from steer carcasses had more moisture and less fat than that from heifer carcasses, again with no differences in protein and ash contents (Table 5.6).

Sex interacted ($P = 0.038$) with grade category for mean lightness (L*) score. Mean L* was greater for Canada AA LT than for AB4 and TB4 LT from both heifers and steers (Figure 5.2). However, mean L* for steer TB4 was greater than for heifer TB4 but similar to AB4 LT regardless of sex (Figure 5.2).

5.3.4.2. Effect of post-mortem ageing

Post-mortem ageing approached significance ($P = 0.096$) for mean LT pH which tended to be greater on day 21 than on day 7 post-mortem (Table 5.7). Post-mortem ageing had no effect on mean colour score values, cooking time and loss, or proximate composition (Table 5.7). Mean drip loss, adjusted for lairage time ($P = 0.034$), was greater on day 7 than day 21 post-mortem. The WBSF standard deviation on day 21 was lower than that on day-7 post-mortem and the difference between the two values approached significance ($P = 0.0727$).

Post-mortem ageing interacted with grade category ($P = 0.0432$) for mean WBSF values, with Canada AA LT having the lowest mean peak shear force values at days 7 and 21 post mortem (Figure 5.3). Mean peak WBSF value was greatest for AB4 LT at day 7, and was greater than that of TB4 and Canada AA LT. Beef LT categorized as TB4 had a greater mean peak WBSF than Canada AA LT (Figure 5.3). By day 21 post mortem, the mean peak WBSF values for LT from AB4 and TB4 were similar, and only AB4 LT had a greater mean peak WBSF than Canada AA LT (Figure 5.3). These results indicated that LT from all categories experienced a

reduction in peak WBSF value with ageing, with the AB4 LT experiencing the largest decrease in WBSF, and Canada AA LT the least.

The pH data at 96 h, days 7 and 21 post-mortem indicated significant effects of grade and time post-mortem on mean LT pH. The mean pH was greatest for TB4, intermediate for AB4 and lowest for Canada AA LT (Table 5.8). Mean pH was greater at 96 h post mortem than at day 7 post mortem but similar to the value at day 21 post-mortem (Table 5.8).

5.3.4.3. Correlations and regressions

Pre-planned Pearson correlations identified linear relationships between carcass phenotype, and biochemical and beef quality measurements assessed on day 7 post-mortem (Table 5.9) with significance at $P < 0.0024$ after Bonferroni correction. Results indicated carcass weight was positively correlated with gREA ($r = 0.43$, $P < 0.001$) and hue angle ($r = 0.39$, $P < 0.001$). Intramuscular pH values at 96 h and day 7 were negatively correlated to glucose, lactate, glycogen concentrations, glucidic potential, oxymyoglobin proportion and L*, a*, b* values and chroma, and positively correlated to metmyoglobin proportion and myoglobin concentration, hue angle and time in lairage ($P < 0.0001$) (Table 5.9). Notably, pH at 96 h was positively correlated with sarcomere length ($r = 0.38$, $P < 0.0024$). Residual glucose, lactate and residual glycogen were positively correlated to each other and to oxymyoglobin proportion, L*, a* and b* values, and chroma, and negatively correlated with metmyoglobin proportion and myoglobin concentration, hue angle and time in lairage ($P < 0.0024$, Table 5.9). Myoglobin concentration was negatively correlated with L*, a*, b* and chroma (r = -0.60, -0.43, -0.54, and -0.43, respectively) values, and positively correlated with hue angle ($r = 0.59$) and time in lairage ($r = 0.49$). Sarcomere length was also correlated with time in lairage ($r = 0.42$, $P < 0.001$). Colour

measurements exhibited various correlations with each other, as did myoglobin species proportions (Table 5.9).

Non-linear relationships were explored using polynomial regression. Glucidic potential and lactate concentration had non-linear relationships with CW. Results indicated that glucidic potential and lactate concentration escalated with CW and reached a plateau at between 350 and 400 kg CW and then declined (Figure 5.4a, b). Polynomial regression also indicated a curvilinear relationship between WBSF and LT pH measured on day 7 post-mortem (Figure 5.5a), where WBSF increased with pH until pH 6.0, at which it subsequently decreased. The relationship between LT pH and WBSF on day 21 post-mortem, however, was not considerable (Figure 5.5b).

5.4. Discussion

The ability to predict the occurrence of dark cutting in beef cattle would be advantageous, as it would allow mitigating management practices to be developed. Recent research (del Campo et al. 2010; McGilchrist et al. 2012; Holdstock et al. 2014; Mahmood et al. 2016a, b) indicated that small, lightly muscled cattle were most likely to produce dark cutting carcasses, yet cattle that do not conform to this phenotype continue to produce dark cutting carcasses (Mahmood et al. 2016a). This was observed in the present study, with the cattle producing typical and atypical dark cutting carcasses having heavy, well-muscled carcasses, similar to that of Canada AA carcasses, with only typical dark cutting carcasses having a mean bone-in rib eye weight heavier than that of Canada AA carcasses. Contrarily, Holdstock et al. (2014) reported that the mean weight of Canada AA carcasses was greater than that of typical and atypical dark carcasses from steers. Earlier it was reported that heavy, muscular cattle had increased muscle glycolytic potential (Jurie et al. 2007; Wegner et al. 2000) but these cattle were at risk of producing

carcasses with increased muscle pH upon experiencing pre-slaughter handling stress (Lacourt and Tarrant 1985; Shackelford et al. 1994; Tarrant 1989; Warriss et al. 1995). To further complicate elucidation of the relationship between animal phenotype and the likelihood of dark cutting, animal sex provides an influencing effect with heifers more likely to produce a dark cutting carcass than steers (Mahmood et al. 2016a) and this was also observed in the present study.

Categorization of the experimental LT muscles by pH on day 7 post mortem indicated that atypical dark cutting carcasses were among those sampled at a large commercial abattoir over two visits. Atypical dark cutting carcasses have not been well-characterized in the literature, but the LT from such carcasses appear to have an ultimate pH of less than 6.0 whereas typical dark cutting LT have a pH of greater than or equal to 6.0 (Wulf et al. 2002; Page et al. 2001; Murray 1989). In the present study, the muscles were sorted by pH on day 7 post mortem to ensure that ultimate pH had been achieved by the muscles and that they were therefore truly representative of the pH category.

As expected, the typical dark cutting (TB4) LT muscles with pH values greater than 5.9 produced dark steaks with reduced oxy-myoglobin, increased deoxy- and met-myoglobin proportions, and decreased lightness, redness and yellowness. The atypical dark cutting (AB4) LT muscles produced darkened steaks as well which had pH values lower than that of the TB4 LT, although these steaks were not as dark as those from TB4 LT. Interestingly, AB4 LT pH was numerically higher at 96 h post mortem than at days 7 and 21 (combined value), suggesting that glycolysis may have continued for the AB4 LT subsequent to 96 h post mortem. A decline in LT pH with time post mortem was not observed in the study of Holdstock et al. (2014); however, the time post mortem that had elapsed before collection of the LT by Holdstock et al. (2014) from

those carcasses was not reported and may have been long enough to allow the LT pH to stabilize. A reduced rate of glycolysis and hence pH decline may account for the formation of AB4 LT as meat colour at 24 h post-mortem, when grading is performed, is a function of the rate of pH decline early post mortem (Hwang and Thompson 2003). At this time, a small difference in the extent of pH decline can greatly affect muscle colour (Poso and Puolanne 2005). Post mortem glycolytic activity can be abridged in muscles devoid of glycogen (Wulf et at. 2002) or having reduced pH (England et al. 2014; Rhoades et al. 2005; Wulf et al. 2002); however; AB4 LT at sampling had neither attained a minimum pH nor catabolized all intramuscular glycogen. Slow glycolytic activity could be due to reduced carcass or muscle weight (del Campo et al. 2010; Murray 1989; Holdstock et al. 2014) or extreme chilling temperature (Yla-Ajos et al. 2006; Bodwell et al. 1965; Kyla-Puhju et al. 2005) but AB4 carcasses had chilling conditions and mean gFD, CW, gREA and LT weights similar to those of the normal, Canada AA carcasses. Holdstock et al. (2014) hypothesized that AB4 carcasses were a result of inactive glycogen phosphorylase at slaughtering due to active glycogen replenishment. In the current study both AB4 and TB4 LT exhibited reduced glucidic potentials, and decreased lactate, residual glucose and residual glycogen concentrations compared to those of normal Canada AA LT, substantiating that depletion had occurred pre-slaughter. As a result, glycogen phosphorylase activity may have been affected in AB4 and TB4 LT, but this was not substantiated in the current study.

The TB4 LT had glucidic potential lower than that required for normal ultimate pH (Ashmore et al. 1972; Bodwell et al. 1965; Hanson et al. 2001; Holdstock et al. 2014; Tarrant 1989) but the mean values for AB4 LT should have been sufficient to attain normal ultimate pH (Bodwell et al. 1965; Tarrant 1989; Immonen and Puolanne 2000). Moreover, the glucidic

potential for heifer AB4 was greater than the minimum values for Canada AA LT from both sexes and the values reported by Holdstock et al. (2014) for Canada AA muscles from steers. Interestingly, the AB4 LT had numerically a higher pH at 96 h post-mortem than at days 7 and 21 pooled value and the latter was comparable to pH values associated with normal subjective colour (MacDougall and Jones l98l; Orcut et al. 1984; Page et al. 2001) although a darkened colour persisted. Increased rate of pH decline at early post-mortem, when muscle temperature is high, may lower the water-binding capacity by partial denaturation of myofibril proteins and increase light reflectance and lighter appearance of meat (Faustman and Cassens 1990). Low pH and high temperature can also denature mitochondrial proteins (Sayre and Briskey 1963) that otherwise may remain functional and utilize oxygen, and prevent formation of the normal colour of muscles (Ashmore et al. 1972; Egbert and Cornforth 1986).

What caused the depletion of glycogen in the muscles sampled is not clear as many factors such as transport distance (Immonen et al. 2000; Mach et al. 2008), growth promotant use (Scanga et al. 1998), handling practices (Apple et al. 2005), and mixing of unfamiliar cattle (Jones and Tong 1989; McVeigh et al. 1982) may deplete glucose and glycogen in bovine muscles. Furthermore, the status of these factors as they pertained to the cattle that produced the carcasses sampled in this study was unknown and these details were not recorded by the abattoir. To clarify the relationships between carcass phenotype and the likelihood of dark cutting, the length of time cattle were in lairage was considered in the present study because delivery and kill times were recorded by the abattoir. The current study indicated the highest probability of dark cutting in cattle held for 72 h in lairage and this contrasted with del Campo et al. (2010), Mounier et al. (2006), Teke et al. (2014), and Warris et al. (1984) but agreed with Gallo et al. (2003), Kreikemeier et al. (1998), Mach et al. (2008), Murray (1989), and Puolanne and Alto

(1981). In 2010, del Campo et al. reported that 4 h transportation followed by a 17 h stay in lairage during a noiseless night lowered the risk of carcasses having a LT pH greater than 5.7. In the current study, information about hauling distance was not documented but studies have indicated that although transportation distance influences muscle glycogen and the incidence of dark cutting (Immonen et al. 2000; Mach et al. 2008), the increase in the likelihood of dark cutting due to extended lairage is independent of the distance travelled (Gallo et al. 2003). The observation that 72 h lairage lowered the frequency of dark cutting for Teke et al. (2014) was likely because cattle in that study were provided a high energy diet containing molasses immediately after shipment. Pethick et al. (1999) found glycogen recovery of 80-90% in *semimembranosus* muscle of cattle rested for 72 h in lairage when they were fed an energy-rich diet as well. Contrarily, cattle in the current study that were held for 72 h were not fed immediately after being received at the abattoir but were instead unloaded and rested without feed before being loaded onto a truck again and shipped to a nearby feeding facility. Prolonged removal from feed has been reported to reduce phosphofructokinase (PFK) activity and the rate of post-mortem glycolysis in muscles of fasted cattle (Daly et al. 2006). Also, Jones et al. (1988) reported darkened muscle in beef carcasses despite low ultimate pH (5.63 to 5.72) when cattle were fasted. Mahmood et al. (2016a) found that as dry matter intake (DMI) decreased, the probability of dark cutting increased. Also, feed intake is greatly influenced by type of feed (Allen 2000) and cattle held for 72 h at the abattoir may receive feed different from that to which they were accustomed. Additionally, they would be handled by unfamiliar personnel and faced an unfamiliar environment which also may contribute to diminished appetite.

Repletion of muscle glycogen would be enhanced in lairage if cattle were not fasted before shipment (McVeigh and Tarrant 1982) because prolonged feed withdrawal can make

cattle reactive to novel environments and may reduce feed intake (Terlouw et al. 2012). Glycogen synthase activity and recovery of muscle glycogen were greater in men who were provided glucose immediately after stressful exercise than in those who received glucose two hours after exercise (Ivy et al. 1998a, b). Prolonged lairage may help to restore muscle glycogen if cattle are fed a concentrate diet until shipment (Apaoblaza and Gallo 2014; McVeigh and Tarrant 1982; Pethick et al. 1999), provided an energetic diet, such as containing molasses (Pethick et al. 1999; Tarrant 1989), immediately after shipment (Ivy et al. 1988a) and that cattle have minimum disturbances in the lairage (del Campo et al. 2010; Teke et al. 2014). In the current study, cattle that spent 72 h in lairage may have had muscle glycogen depleted further by repetitive trucking stress, as the cattle would be loaded and unloaded and transported in a truck at least 6 times within 96 h. Loading and unloading has been associated with stress in cattle (Pettiford et al. 2008).

The results of the current study indicated that cattle rested for 5, 6 or 8 h in lairage had a lower incidence of dark cutting than those rested for 4 or 72 h. Puolanne & Alto (1981) also observed a lower frequency of dark cutting in bulls slaughtered after 5 to 8 h in lairage than in those slaughtered after 2 or 50 h of lairage. In 2010, del Campo et al. observed a higher frequency of dark cutting in cattle after 3 rather than 17 hours of lairage. In the current study, 5, 6 or 8 h in lairage may reflect the time slaughter cattle require to achieve physiological homeostasis but prolonged lairage (greater than 8 hours) of cattle has been associated with mobilizing stored energy in the absence of feed intake (Tadich et al. 2005). Regardless of the mechanism, the results of the present study indicated a detrimental effect of frequent shipping and prolonged lairage whereas resting cattle for 5 to 8 h decreased the probability of dark cutting.

Without considering the time in lairage, the results of this study would suggest that cattle with carcass weights similar to that of Canada AA are most likely to cut dark. The significant effect of grade category on time in lairage and carcass weight and the correlation between lairage time and carcass weight however indicated that lairage was confounded with phenotype. Time in lairage also was positively correlated to pH and negatively correlated to LT glucidic potential and colour values; therefore, using animal/carcass phenotype to predict the likelihood of dark cutting (Mahmood et al. 2016a, b) appeared to be superseded by time and conditions in lairage. These results also necessitate the conclusion that the likelihood of dark cutting is increased at 4 h lairage or after 72 h of lairage that was confounded with additional transport events.

This study supported the discrimination against dark cutting carcasses within the Canadian beef grading system, as both atypical and typical LT steaks were tougher and had greater variation in toughness than those produced by Canada AA LT on day 7 post mortem. These results agree with the existing literatures (Grayson et al. 2016; Wulf et al. 2002). The current study also indicated that AB4 was the toughest beef, and this agreed with Voisinet et al. (1997b) and Holdstock et al. (2014) who found increased toughness for beef with mean pH values of 5.79 and 5.83, respectively. Furthermore, findings that cattle sex has an influence on beef toughness (Choat et al. 2006), and that dark beef with reduced marbling has increased WBSF (Wulf et al. 2002) were not supported by the present study. Also, the relationship between cooked LT toughness at day 7 post mortem and muscle ultimate pH appeared curvilinear, agreeing with the results of Bouton et al. (1971). In contrast, Bass et al. (2008) did not find *longissimus* muscles from full, half and 1/3 degree dark carcasses with mean pH values 6.06, 5.76 and 5.72, respectively, differing from each other and from normal muscles for tenderness. Recently Grayson et al. (2016) found increased toughness for beef categorized as shady dark

compared to moderate and severely dark where mean pH values were 6.10, 6.59 and 6.89, respectively, at 36 h post-mortem. Increased variation as indicated by standard deviation of WBSF within dark cutting grades in the present study also agreed with former studies (Hannula and Puolanne 2004; Holdstock et al. 2014; Wulf et al. 2002).

Ageing the beef for 21 days not only lowered the variation within grade category but also the mean WBSF in all the grade categories. Improved tenderness in aged beef including that which is dark is well documented (Bruce et al. 2004; Holdstock et al. 2014) and is probably due to continuous proteolysis (Colle et al. 2015; Mohrhauser et al. 2011). The increased toughness of the AB4 LT relative to Canada AA LT persisted even after post mortem ageing, and the curvilinear relationship between pH and WBSF observed at day 7 post mortem was no longer evident at 21 days post-mortem, which agreed with the results of Watanabe et al. (1996). Pulford et al. (2009) found reduced WBSF by 4 day post mortem in beef *longissimus* with pH 5.5 and 6.54 but not in those with pH 5.93. Reduced tenderness because of short sarcomere length (Weaver et al. 2008) was not observed in the current study likely because LT tenderness is mainly influenced by proteolysis unlike that of *psoas major* and *semimembranosus* where tenderness is respectively associated with sarcomere length and amount of connective tissues (Koohmaraie et al. 2002). This suggested inadequate proteolysis as a cause of increased toughness of AB4 LT (Pulford et al. 2009; Watanabe et al. 1996). Although not confirmed in the present study, slowed glycolysis and a reduced rate of pH decline might have delayed proteolysis, causing increased toughening of AB4 muscles (Hwang and Thompson 2001; Poso and Puolanne 2005).

Higher L* and lower myoglobin concentration in Canada AA compared to TB4 and AB4 LT indicated that lightness may not only have been influenced by pH but also by myoglobin

concentration (MacDoughal and Rhodes 1972; Moiseev and Cornforth 1999). However, reduced lightness is more related to increased pH than myoglobin concentration in dark beef (MacDoughal and Rhodes 1972) likely because increased water retention in muscle at higher pH lowers the lightness (Faustman and Cassens 1990; Hughes et al. 2015). AB4 LT in the current study had a mean myoglobin concentration 5.07 ± 0.16 mg g^{-1}, similar to the mean 4.56 ± 0.23 mg myoglobin g^{-1} reported for *longissimus lumborum* that had normal pH but reduced colour stability (Canto et al., 2015); however beef *longissimus* with myoglobin concentrations of 4 to 6 mg g^{-1} at normal pH has a normal, bright red colour (Melvin, C. Hunt; personal communication). This suggested that the dark appearance of AB4 LT was unlikely to be due to myoglobin concentration. Increased DMb and reduced OMb in AB4 and TB4 LT may be due to greater mitochondrial oxygen consumption, a characteristic of dark cutting muscles (Moiseev and Cornforth 1999; Tang et al. 2005; Tarrant 1989).

Lack of grade category effect on drip loss in the current study contradicted Holdstock et al. (2014) likely because of the difference between the studies in post-mortem ageing time that had elapsed before the muscles were assessed for drip loss. Reduced drip loss on day 21 compared to day 7 post-mortem agreed with Colle et al. (2015) because moisture lost during early storage/ageing minimizes the available free water to be subsequently lost. The observation that grade category effect was significant for mean cooking loss and not for cooking time agreed with literature findings (Holdstock et al. 2014). Reduced cooking loss in TB4 muscles was most likely due to increased pH (Hawrysh et al. 1985; Holdstock et al. 2014; Pulford et al. 2009); however, AB4 LT was not different from Canada AA LT for mean cooking loss. Holdstock et al. (2014) found greater cooking loss in Canada AA LT than in atypical dark beef LT (pH 5.83), and similarly Pulford et al. (2009) reported increased cooking loss in beef *longissimus dorsi* at pH

5.55 than at pH 5.93. The finding that AB4 had cooking loss similar to Canada AA in the present study was likely because the pH difference between AB4 and Canada AA LT was of insufficient magnitude to result in a difference.

5.5. Conclusions

The hypothesis that atypical dark cutting exists in both heifers and steers and that atypical dark cutters yield the toughest beef was accepted. However, the study indicated no relationship between the incidence of dark cutting and phenotypic characteristics available in the present study. Considering previous experiments supported phenotype as indicating the likelihood of dark cutting (Holdstock et al. 2014; Mahmood et al. 2016a, b), time and management of animals in lairage appear to supersede any predisposition toward dark cutting indicated by phenotype. Reduced glucidic potential and increased pH of typical dark appeared to be associated with frequent transportation events and extended lairage. Investigation of strategies to minimize impact on muscle glycogen in cattle held 72 h in lairage with frequent transport events is required to mitigate the high incidence of dark cutting in this population of slaughter cattle.

Table 5.1. Experimental sampling in two batches (visits).

Batch	1						2					
Sex	Heifers			Steers			Heifers			Steers		
Grade	AA	AB4	TB4	AA	AB4	TB4	AA	AB4	TB4	AA	AB4	TB4
n	10	4	11	10	3	8	2	8	0	2	5	1

Table 5.2. Effect ($P < 0.0001$) of lairage on the mean probability of dark cutting.

Lairage time[1]	*n*	Dark-cutting probability (%)	Standard error of mean (± %)
4 hours	539	1.86[b]	0.58
5 hours	2541	0.51[c]	0.14
6 hours	139	0.93[c]	0.26
8 hours	1105	1.0[bc]	0.3
72 hours	433	16.63[a]	1.79

[1]Time spent at the slaughter plant after shipment from the farms.

[a,b,c]Probabilities (%) lacking a common superscript differ at $P \leq 0.05$.

Table 5.3. Lairage population within each grade category.

Grade Category	4 Hour	5 Hour	6 Hour	8 Hour	72 Hour
AA	5	15	1	2	1
AB4	1	7	5	5	2
TB4	4	1	1	2	12

Table 5.4. Effect of grade and sex on least square means (± standard error of mean in parenthesis) for carcass measurements, sarcomere length and myoglobin concentration.

Measurements	Grades				Sex		
	AA[1] (*n* = 24)	AB4[2] (*n* = 20)	TB4[3] (*n* = 20)	Pr > F[4]	Heifers (*n* = 35)	Steers (*n* = 29)	Pr > F[4]
Carcass weight (kg)	389.1[ab] (10.15)	378.6[b] (11.35)	416.5[a] (11.18)	0.0545	395.37 (8.41)	394.10 (9.37)	0.919
Grade fat depth (mm)	10.46 (1.66)	10.19 (1.56)	10.90 (1.88)	0.939	11.4 (1.42)	9.59 (1.46)	0.15
Carcass rib-eye area (cm^2)	76.38 (3.34)	78.39 (2.84)	81.68 (4.13)	0.3503	80.45 (2.51)	77.09 (2.58)	0.24
Rib-eye weight with bones (kg)	12.06[b] (0.353)	11.81[b] (0.395)	13.19[a] (0.389)	0.0339	12.42 (0.29)	12.29 (0.33)	0.78
Rib-eye weight without bones (kg)	6.24[ab] (0.182)	5.98[b] (0.20)	6.72[a] (0.20)	0.0383	6.29 (0.15)	6.33 (0.17)	0.86
Sarcomere length (μm)	1.96 (0.07)	1.97 (0.07)	2.02 (0.07)	0.375	1.99 (0.067)	1.98 (0.067)	0.86
Oxy-myoglobin	0.76[a] (0.01)	0.70[b] (0.02)	0.67[b] (0.02)	0.0004	0.73 (0.012)	0.69 (0.014)	0.111
Deoxy-myoglobin	0.09[b] (0.01)	0.11[a] (0.01)	0.12[a] (0.01)	0.0147	0.095[b] (0.011)	0.117[a] (0.011)	0.029
Met-myoglobin	0.15[b] (0.02)	0.18[ab] (0.02)	0.21[a] (0.02)	0.0015	0.178 (0.015)	0.187 (0.016)	0.495
Myoglobin (mg g^{-1} fresh muscle)	4.20[c] (0.15)	5.07[b] (0.16)	5.76[a] (0.16)	<.0001	5.165 (0.12)	4.856 (0.13)	0.095

[1]Canada AA grade equivalent to USDA Select carcasses.

[2]Atypical dark cutting (AB4) muscles categorized on day-7 post-mortem.

[3]Typical dark cutting (TB4) muscles categorized on day-7 post-mortem.

[4] Probability of the F test, with significance at $P \leq 0.05$.

[a,b,c]Least Square Means within a row lacking a common letter differ at $P \leq 0.05$.

Table 5.5. Effect of grade and sex on least square means (± standard error of mean) for 96 h pH and glucidic values.

Measurements	Co-variant lairage[1]	Grade				Sex		
	Pr > F[2]	AA[3] (*n* = 24)	AB4[4] (*n* = 20)	TB4[5] (*n* = 20)	Pr > F[2]	Heifers (*n* = 35)	Steers (*n* = 29)	Pr > F[2]
pH 96 h	-	5.58[c] (0.15)	5.93[b] (0.15)	6.28[a] (0.16)	<.0001	5.95 (0.15)	5.90 (0.15)	0.464
Glucidic potential (µmol glucose g^{-1} fresh muscle).	0.0145	88.69[a] (7.33)	65.51[b] (7.19)	55.32[c] (7.49)	<.0001	72.45 (6.98)	67.23 (7.04)	0.095
Lactate (µmol g^{-1} fresh muscle).	0.0013	102.32[a] (4.47)	82.89[b] (4.05)	67.57[c] (4.70)	<.0001	83.89 (3.74)	84.63 (3.84)	0.806
Residual glucose (µmol g^{-1} fresh muscle).	0.0251	6.16[a] (0.57)	2.94[b] (0.56)	2.01[b] (0.58)	<.0001	3.79 (0.54)	3.61 (0.54)	0.48
Residual glycogen (µmol glucose g^{-1} fresh muscle).	-	31.90[a] (5.13)	21.51[b] (5.12)	18.35[b] (5.28)	<.0001	26.56 (4.98)	21.28 (5.01)	0.0164

[1]Lairage was used as a co-variant when significant.

[2] Probability of the F test, with significance at $P \leq 0.05$.

[3]Canada AA grade equivalent to USDA Select carcasses.

[4]Atypical dark cutting (AB4) muscles graded on day-7 post-mortem.

[5]Typical dark cutting (TB4) muscles graded on day-7 post-mortem.

[a,b,c]Least Square Means within a row lacking a common letter differ at $P \leq 0.05$.

Table 5.6. Least square means (± standard error of mean) of the combined days 7 and 21 post-mortem data for the effect of grade and sex.

Measurements	Grade				Sex		
	AA[1] (*n* = 24)	AB4[2] (*n* = 20)	TB4[3] (*n* = 20)	Pr > F[4]	Heifers (*n* = 35)	Steers (*n* = 29)	Pr > F[4]
pH	5.58[c] (0.04)	5.75[b] (0.05)	6.27[a] (0.04)	<.0001	5.85 (0.034)	5.88 (0.038)	0.4689
Redness (a*)	23.44[a] (0.44)	20.70[b] (0.49)	16.28[c] (0.49)	<.0001	20.10 (0.37)	20.17 (0.41)	0.9045
Yellowness (b*)	6.52[a] (0.33)	3.30[b] (0.37)	0.30[c] (0.36)	<.0001	3.32 (0.27)	3.43 (0.30)	0.7934
Chroma	24.43[a] (0.47)	21.08[b] (0.52)	16.47[c] (0.52)	<.0001	20.66 (0.39)	20.65 (0.43)	0.992
Hue	15.24[b] (15.68)	48.10[b] (17.53)	185.83[a] (17.26)	<.0001	89.85 (12.99)	76.27 (14.47	0.487
Drip loss (mg g-[1] muscle)	7.84 (0.58)	8.04 (0.65)	7.76 (0.64)	0.9537	7.65 (0.48)	8.10 (0.54)	0.535
Cooking time (Sec g^{-1} muscle)	17.14 (0.29)	16.87 (0.34)	16.93 (0.33)	0.8077	17.67 (0.24)	16.29 (0.27)	0.0004
Cooking loss (mg g-[1] muscle)	254.4[a] (10.87)	241.2[a] (12.16)	200.9[b] (11.97)	0.0053	217.75 (9.01)	246.61 (10.04)	0.0367
WBSF standard deviation (N)	6.73[b] (0.58)	10.05[a] (0.65)	8.50[a] (0.64)	0.0014	8.6580 (0.48)	8.2012 (0.53)	0.5272
Total moisture (%)	75.69[a] (0.29)	74.66[b] (0.33)	76.20[a] (0.32)	0.0051	74.90[b] (0.24)	76.13[a] (0.27)	0.0013
Crude fat (%)	4.35[b] (0.26)	5.38[a] (0.29)	3.98[b] (0.28)	0.0029	5.05[a] (0.21)	4.09[b] (0.24)	0.0038
Crude protein (%)	22.56 (0.15)	22.33 (0.17)	22.49 (0.16)	0.6007	22.53 (0.13)	22.40 (0.14)	0.5131
Ash (%)	1.033 (0.007)	1.018 (0.008)	1.030 (0.008)	0.3915	1.021 (0.006)	1.033 (0.007)	0.1929

[1]Canada AA grade equivalent to USDA Select carcasses.

[2]Atypical dark cutting (AB4) muscles graded on day-7 post-mortem.

[3]Typical dark cutting (TB4) muscles graded on day-7 post-mortem.

[4] Probability of the F test, with significance at $P \leq 0.05$.

[a,b,c]Least Square Means within a row lacking a common letter differ at $P \leq 0.05$.

Table 5.7. Effect of post-mortem ageing on least square means ± SEM (standard error of mean) for quality and proximate.

Measurements	Post-mortem ageing		±SEM	Pr > F
	Day 7 (n = 64)	Day 21 (n = 64)		
pH	5.83	5.90	0.034	0.0965
Lightness (L*)	34.07	34.43	0.29	0.3311
Redness (a*)	19.94	20.34	0.39	0.4635
Yellowness (b*)	3.25	3.50	0.28	0.5075
Chroma	20.38	20.93	0.41	0.3407
Hue	74.50	91.62	12.52	0.2818
Drip loss (mg g^{-1} muscle)	9.21	6.55	0.51	0.0003
Cooking time (Sec g^{-1} muscle)	17.23	16.73	0.25	0.1557
Cooking loss (mg g^{-1} muscle)	225.16	239.20	9.18	0.2647
WBSF standard deviation (N)	8.86	7.99	0.43	0.0727
Total moisture (%)	75.67	75.37	0.22	0.2188
Crude fat (%)	4.44	4.70	0.18	0.137
Crude protein (%)	22.40	22.53	0.13	0.4671
Ash (%)	1.032	1.022	0.006	0.300

Table 5.8. Effect of grade and post-mortem ageing on mean (± standard error of the mean) LT pH.

Grade				Post-mortem ageing			
AA (n = 24)	AB4 (n = 20)	TB4 (n = 20)	Pr > F[1]	96 Hour (n = 64)	Day7 (n = 64)	Day21 (n = 64)	Pr > F[1]
5.54[c] (0.12)	5.83[b] (0.12)	6.22[a] (0.12)	<.0001	5.94[a] (0.12)	5.78[b] (0.12)	5.86[ab] (0.12)	0.0029

[1]Probability of the F test, with significance at $P \leq 0.05$.
[a,b,c]Least Square Means lacking a common letter differ at $P \leq 0.05$.

Table 5.9. Pearson correlations indicating the relationships between carcass phenotype, glucidic potential, myoglobin and day-7 beef quality.

	gREA	gFD	pH96	pHD7	Glu	Lac	Gly	GP	DMb	OMb	MMb	Mb	Sarc	WBSF	L*	a*	b*	Chroma	Hue	Lairage
CW	0.43 **	0.14	0.28	0.34	-0.20	-0.25	-0.27	0.28	0.07	0.13	0.15	0.34	0.16	0.052	-0.07	0.12	-0.10	-0.10	0.39 **	0.30
Grade rib-eye area (gREA)		0.03	0.19	0.15	-0.16	-0.20	-0.02	-0.13	0.01	-0.09	0.12	0.18	-0.07	0.18	-0.05	-0.15	-0.15	-0.14	0.29	0.10
Grade fat depth (gFD)			0.15	0.02	-0.19	-0.134	-0.18	-0.18	0.04	-0.03	0.024	0.15	0.22	0.06	0.031	0.013	0.007	0.010	0.062	0.11
pH 96 hour (pH96)				0.69 ***	-0.80 ***	-0.88 ***	-0.52 ***	-0.79 ***	0.19	-0.46 ***	0.50 ***	0.53 ***	0.38 *	0.077	-0.50 ***	-0.63 ***	-0.66 ***	-0.63 ***	0.59 ***	0.65 ***
pH Day-7 (pHD7)					-0.75 ***	-0.77 ***	-0.54 ***	-0.74 ***	0.28	-0.61 ***	0.67 ***	0.59 ***	0.17	-0.06	-0.57 ***	-0.79 ***	-0.79 ***	-0.782 ***	0.81 ***	0.66 ***
Residual glucose (Glu)						0.91 ***	0.66 ***	0.90 ***	-0.28	0.54 ***	-0.57 ***	-0.63 ***	-0.31	-0.16	0.62 ***	0.75 ***	0.79 ***	0.75 ***	-0.63 ***	-0.59 ***
Lactate (Lac)							0.59 ***	0.90 ***	-0.22	0.54 ***	-0.61 ***	-0.62 ***	-0.34	-0.06	0.59 ***	0.73 ***	0.77 ***	0.73 ***	-0.70 ***	-0.66 ***
Residual glycogen (Gly)								0.88 ***	-0.23	0.40 **	-0.42 **	-0.41 **	-0.23	-0.045	0.26	0.50 ***	0.47 ***	0.49 ***	-0.50 ***	-0.44 **
Glucidic Potential (GP)									-0.25	0.54 ***	-0.58 ***	-0.59 ***	-0.32	-0.07	0.50 ***	0.70 ***	0.71 ***	0.69 ***	-0.67 ***	-0.62 ***
Deoxy myoglobin (DMb)										-0.75 ***	0.38 **	0.12	-0.03	0.08	-0.20	-0.22	-0.23	-0.22	0.09	0.049
Oxy myoglobin (OMb)											-0.89 ***	-0.30	-0.12	0.15	0.29	0.50 ***	0.47 ***	0.49 ***	-0.44 **	-0.32
Metmyoglobin (MMb)												0.34	0.19	-0.27	-0.27	-0.57 ***	-0.51 ***	-0.54 ***	0.57 ***	0.42 **
Myoglobin concentration (Mb)													0.15	0.14	-0.60 ***	-0.43 **	-0.54 ***	-0.43 **	0.59 ***	0.49 ***
Sarcomere length (Sarc)														-0.20	-0.09	-0.006	-0.037	0.0004	0.27	0.43 **
WBSF															-0.15	-0.21	-0.23	-0.23	-0.08	-0.09
Lightness (L*)																0.53 ***	0.74 ***	0.56 ***	-0.48 ***	-0.46 ***
Redness (a*)																	0.95 ***	0.99 ***	-0.73 ***	-0.49 ***
Yellowness (b*)																		0.96 ***	-0.72 ***	-0.54 ***
Chroma																			-0.71 ***	-0.49 ***
Hue																				0.68 ***

Asterisks beneath each correlation indicate probability with *** = $P < 0.0001$, ** = $P < 0.001$, and * = $P < 0.0024$.

Figure 5.1. Least square means of the sex and grade interaction ($P = 0.056$) for residual glycogen concentration.

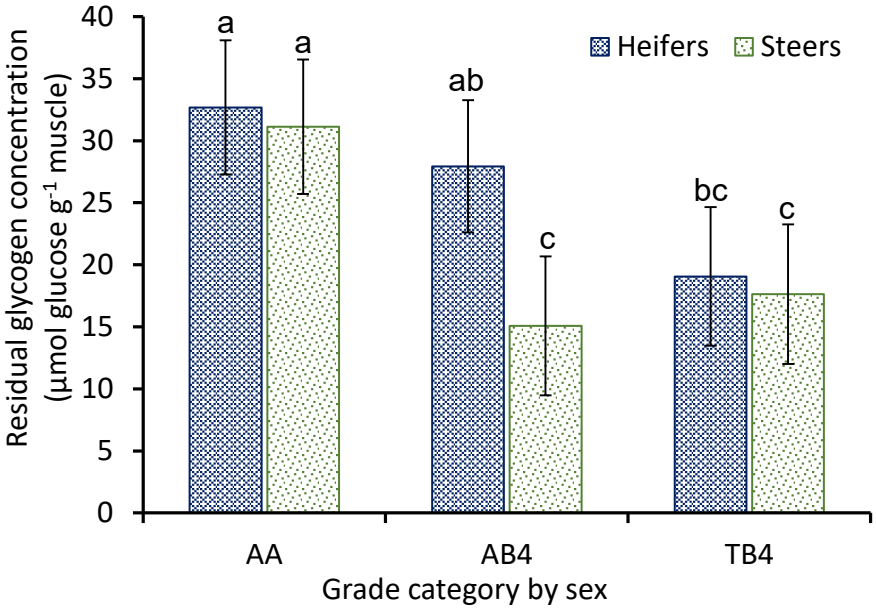


Figure 5.2. Least square means of the sex and grade interaction ($P = 0.038$) for muscle lightness (L*).

Figure 5.3. Least square means of the grade and post-mortem ageing interaction (*P* = 0.0432) for WBSF (N).

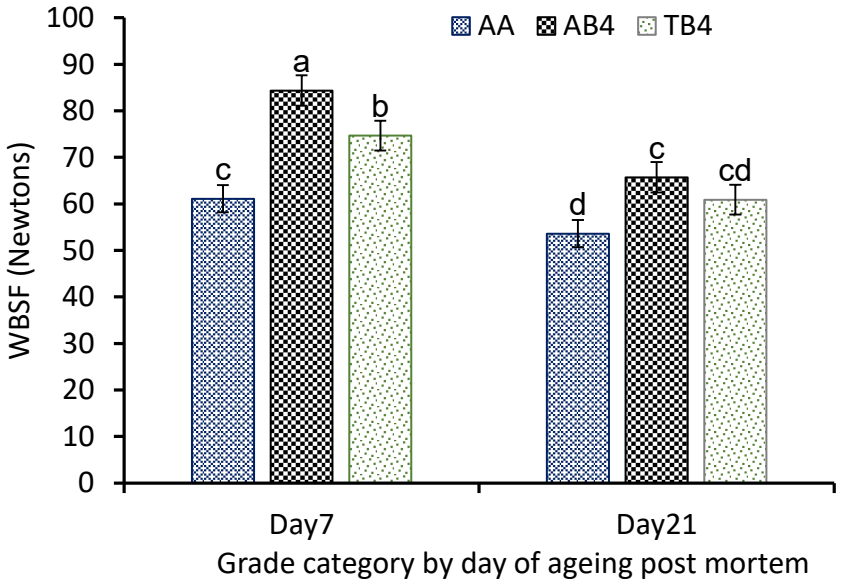
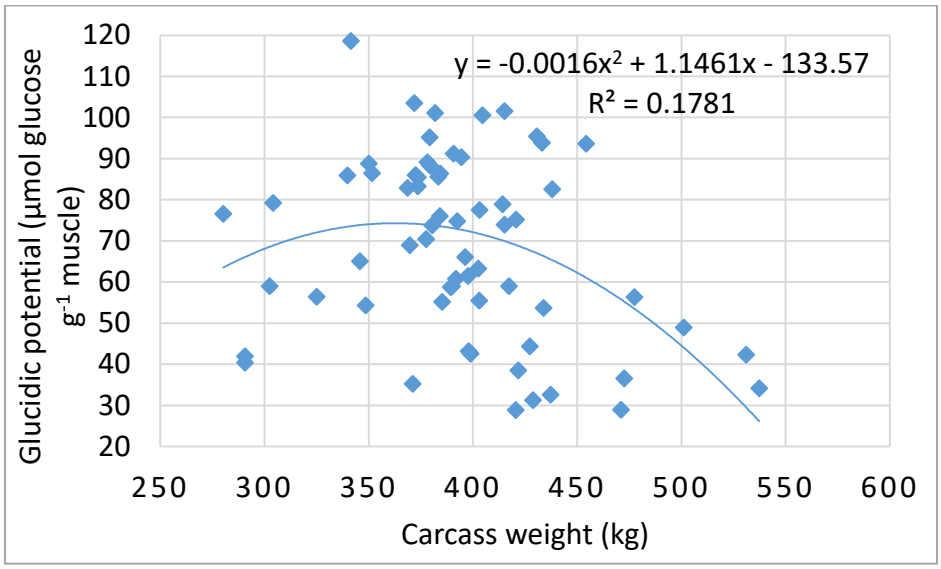
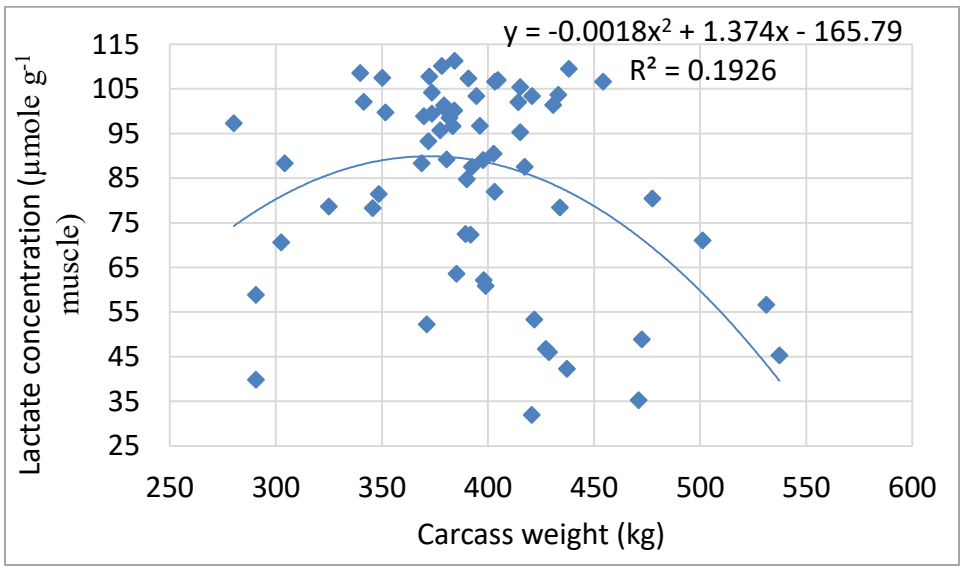


Figure 5.4 (A, B). Polynomial relationships between carcass weight (kg) and glucidic potential (A) and lactate concentration (B).

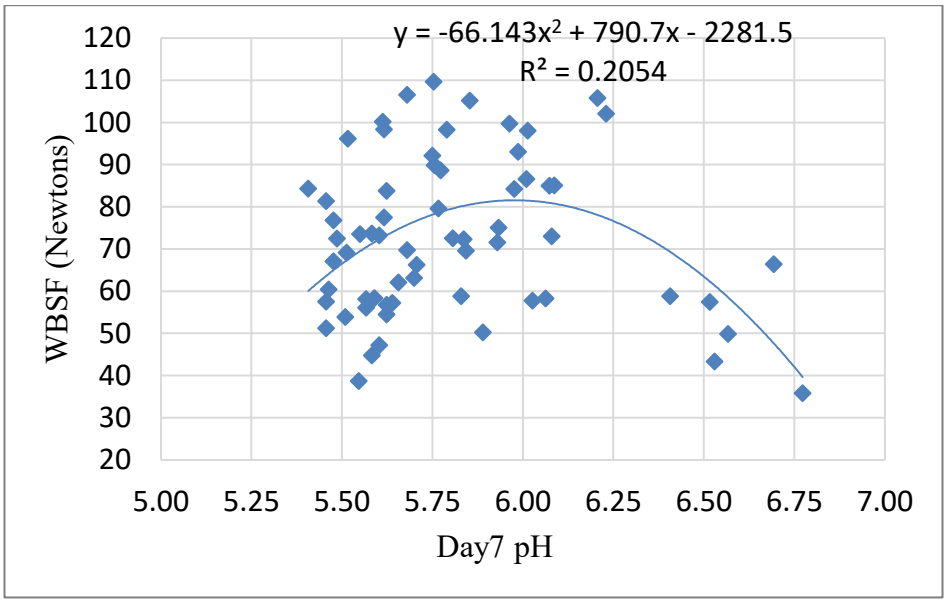


(A)

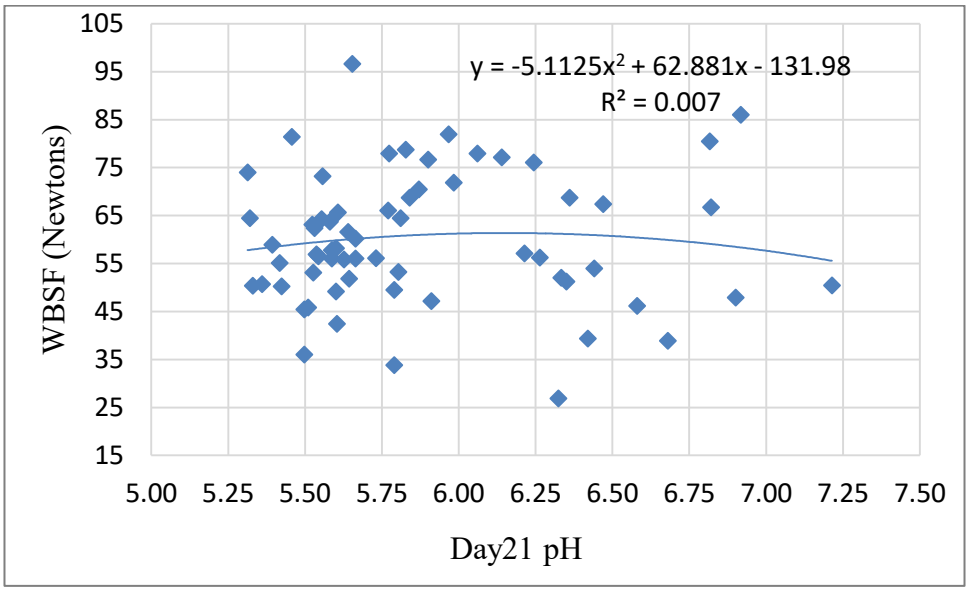


(B)

Figure 5.5 (A, B). Polynomial relationships between muscle pH and WBSF at days 7 (A) and 21 (B) post-mortem.



(A)



(B)

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6.0. Proteomics of dark cutting *longissimus thoracis* muscle from heifer and steer carcasses[4]

6.1. Introduction

Pink or cherry-red colour is one of the prerequisites for beef to be considered normal, whereas aberrant lean colour during display or at grading critically influences purchase decision of the customers and concurrently meat price. Meat discoloration during retail display is disadvantageous to the vendors but dark cutting at grading 24-48 h post-mortem usually removes beef from retail consideration. Dark cutting carcasses, characterized by dark red rib-eye muscle (m. *longissimus thoracis*, LT), are specifically identified in major beef producing countries such as Australia, Canada, Brazil, Uruguay and the United States of America (USA). In Canada, dark cutting carcasses are downgraded to the Canada B4 grade and this is representing a significant economic loss to the beef producers.

Dark cutting beef is usually associated with an ultimate muscle pH greater than 5.8 as a result of insufficient muscle glycogen at slaughter. However, atypical dark (AB4) carcasses, characterized by sufficient glucidic potential to attain normal pH, have been identified in the Canadian beef industry (Holdstock et al. 2014). Increased activity of phosphofructokinase (PFK) (England et al. 2014) as well as increased concentration of lactate dehydrogenase-B have been associated with rapid postmortem pH decline (Gagaoua et al. 2015) while increased concentrations of glyceraldehyde-3-phosphate dehydrogenase and lactate dehydrogenase-A have been also associated with increased colour stability of beef *longissimus lumborum* (Canto et al. 2015). Although, Apaoblaza et al. (2015) reported differences in enzymatic activities between low and high pH muscles but these authors considered steer muscles only, and investigated specific proteins. Also, these authors did not have the atypical dark cutting muscles identified by

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Holdstock et al. (2014) and Mahmood et al. (2017, submitted). In 2015, Canto et al. found increased concentration of glycolytic enzymes in colour stable muscles; however, these authors neither found a difference in the pH between colour stable and colour labile muscles nor did they report the glucidic potential of those muscles. These studies suggested that the phenomenon of dark cutting, especially that observed in the atypical dark muscle, is not only related to reduced glucidic potential but may also be due to reduced concentration and/or activity of muscle glycolytic enzymes.

The AB4 carcasses having LT pH $\leq$ 5.8 have been shown to be tougher than normal-coloured Canada AA (equivalent to USDA Select) beef (Holdstock et al. 2014) although the dark cutting, Canada B4 carcasses/cattle had phenotypic characteristics resembling those of Canada AA carcasses/cattle (Mahmood et al. 2016a, b). This suggested that variation in tenderness between normal and dark beef is not related to carcass phenotype. Lack of relationships between beef toughness and cattle sex, carcass weight, subcutaneous fat depth, and marbling has been indicated in a companion study that reinforced AB4 as the toughest LT (Mahmood et al. 2017; submitted). Although beef tenderness is related to levels of muscle proteolytic enzymes (Koohmaraie 1996; Lamare et al. 2002), it has also been linked with post-mortem muscle glycolytic activity (Anderson et al. 2014). It is therefore hypothesized that dark cutting and its associated beef toughness, especially in AB4, is related to muscle glycolytic proteins. Since the underlying proteomics of dark cutting beef is largely unknown in the literature, the objective of this study was to characterize the protein complement of Canada AA (normal), typical dark cutting Canada B4 (pH > 5.9) and atypical dark cutting Canada B4 (pH < 5.9) LT from heifer and steer carcasses to gain insight into the potential mechanism of dark cutting and associated beef quality.

6.2. Materials and methods

Approval from an Animal Care and Use Committee was not required because the carcass samples were collected from a commercial beef abattoir where the slaughter procedure was in accordance with Canada beef slaughter regulations (Canada Meat Inspection Regulations SOR/90-288).

6.2.1. Muscle sampling and beef quality analyses

Beef LT samples used were from both heifers (AA, n = 4; AB4, n = 4 and TB, n = 3) and steers (AA, n = 4; AB4, n = 4 and TB, n = 4). The sampling procedure has been described previously (Mahmood et al. 2017, submitted) where beef LT muscles (n = 64) from normal and dark-cutting carcasses were used for detailed biochemical and beef quality analyses. The current study used a subset of LT samples (n = 23) from Mahmood et al. (2017, submitted) in an attempt to relate muscle protein profile with beef quality and biochemical composition. Because lairage was identified as a potential stressor that lowered LT glucidic potential (Mahmood et al. 2017, submitted), the selected LT samples were from cattle rested in lairage for about 10 h.

As described by Mahmood et al. (2017, submitted), beef carcasses were graded at the 12^{th}-13^{th} rib LT interface by Canada Beef Grading Agency trained personnel and LT samples were collected 24-48 h post-mortem from a commercial abattoir. Each LT sample was evaluated for pH at 96 h post mortem and processed as described by Mahmood et al. (2017), with two 2 cm thick steaks removed each for the measurement of glucidic potential and the muscle proteome. Steaks were frozen immediately in liquid nitrogen, and stored at -80 °C until analysis. The remainder of each LT was cut into equal halves (anterior and posterior), packaged under vacuum and aged at 4 °C for 7 and 21 days post-mortem. On day 7, Canada B4 LT were sorted into AB4 (pH < 5.9) or typical (pH > 5.9, TB4) dark, and were either aged for an additional 14 days (aged

21 days) or not (aged 7 days). The pH value of 5.9 was used as the delineation point for typical and atypical dark cutting LT because this pH has been associated with extreme toughness (Lomiwes et al. 2013). Muscles were allocated by pH on day 7 post-mortem to ensure that the pH measured was truly the ultimate pH. After each ageing treatment, the samples were assessed for colour (L*, a*, b*) (Commission Internationale de L'Eclairage), Warner-Bratzler shear force (WBSF) and proximate analysis.

6.2.2. Extraction and preparation of muscle proteins

The LT muscle samples previously flash frozen in liquid nitrogen and stored at -80 ºC within 96 h post-mortem were pulverized under liquid nitrogen. Approximately 100 ± 5 mg of pulverized sample from each muscle were homogenized with a pellet pestle (Kimble® Kontes, Sigma-Aldrich) in a buffer containing 40mM Tris, 2mM dithiothreitol (DTT; Fischer Scientific, USA), 1mM phenylmethylsulfonyl fluoride (PMSF; serine peptidase inhibitor; Catalog # P7626, Sigma-Aldrich, Saint Louis, USA), protease inhibitor cocktail (Catalog # P2714, Sigma-Aldrich) and phosphatase inhibitor cocktail tablets (PhosStop™, Roche Diagnostics, Germany). Each homogenate was incubated for 1 h on ice and then centrifuged at 10,000 × g at 4 ºC for 10 minutes. The supernatant was collected and the process was repeated twice. The resulting supernatants, designated "soluble proteins", were pooled for each muscle, aliquoted into multiple tubes, and stored at -20 ºC until analyzed.

The remaining pellet was mixed with protein extraction buffer containing 7 M urea, 2 M thiourea, 4% (w/v) CHAPS, PMSF, protease inhibitor cocktail and phosphatase inhibitor tablets. The pellet was crushed using the pellet pestle (Kimble® Kontes) and the mixture was incubated on ice for 1 h with intermittent mixing. The mixture was then centrifuged at 10,000 × g and 4º C for 10 minutes. These supernatants, designated as "insoluble proteins", were collected and stored

at -20 ºC while the remaining debris was discarded. The protein concentrations in both soluble and insoluble fractions were measured in duplicate using 2-D Quant Kit (Catalog # 80-6483-56, GE Healthcare, NJ, USA) and a Spectra MaxM3 (Molecular Devices, LLC, USA) spectrophotometer.

6.2.3. Two-dimensional gel electrophoresis

The soluble and insoluble proteins were analyzed separately using a two-dimensional gel electrophoresis (2-D gel) procedure as previously described (Novak et al. 2010; Laville et al. 2009). For isoelectric focusing, 200 µg proteins were mixed with 4 µL immobilized pH gradient (IPG) buffer (pH 3-10; Cat # 17-6000-87, GE Healthcare, Sweden), 6 µL DeStreak™ Reagent (Cat # 17-6003-18, GE Healthcare, Sweden) and 2 µL bromophenol blue (2% w/v). Twenty four (24) cm long Immobiline™ Dry Strips (Catalog # 17-6002-44, GE Healthcare, Uppsala, Sweden), pH range 3-10, were rehydrated with the protein mixture overnight. Isoelectric focusing was performed using an isoelectric focusing unit (Ettan™ IPGphor II™, Amersham Biosciences) using the following parameters; 500 V step and hold for 8 h, 1000 V gradient for 1 h, 8000 V gradient for 3 h, 10000 V gradient for 3 h, and 100 V step and hold for 15 h. After focusing, the strips were equilibrated for 15 minutes in 1% (w/v) DTT dissolved in sodium dodecyl sulfate (SDS) buffer, containing 6M urea, 75 mM TRIS (pH 8.8), 29.3% glycerol, 2% (w/v) SDS and 0.002% bromophenol blue and then alkylated for 15 minutes with 2.5% (w/v) iodoacetamide (Acros Organics, New Jersey, USA) in SDS buffer.

The strips were subsequently loaded on second dimension precast Tris-Glycine gradient (4-20% acrylamide) gels (Jule Biotechnologies, Inc. Milford, CT, USA) sealed with agarose solution. BenchMark™ Protein Ladder (Invitrogen Tech-Line℠, USA, Catalog # 10747-012) was used for molecular weight standards. The second dimension was performed using Ettan™

Daltsix large vertical electrophoresis unit (Amersham Biosciences) with the lower chamber filled with 1x Laemmli SDS electrophoresis buffer containing 25 mM Tris, 192 mM glycine and 0.1% (w/v) SDS, while the upper chamber was filled with 2x Laemmli SDS electrophoresis buffer. Proteins were separated initially at 5 W per gel for 40 minutes and subsequently at 17 W per gel, but at a constant 400 mA current, for about 4 h. Sets of six samples representing all the treatments were used at once for both first and second dimension to avoid variability between the treatments.

After the second dimension, the gels were fixed with 40% (v/v) ethanol and 10% (v/v) glacial acetic acid for 60 minutes. The gels were then washed with double distilled water and stained with fluorescent Krypton^{TM} Protein Stain (Pierce Biotechnology, Inc., Rockford, IL, USA) overnight. The following day, the gels were de-stained with 5% (v/v) acetic acid, rinsed with distilled water and then scanned using a Typhoon^{TM} FLA 9500 (GE Healthcare Biosciences, Canada) laser scanner. The images were analyzed using Progenesis SameSpots PG240 (Nonlinear Dynamics, Newcastle, U.K.) software after verifying appropriate resolution and saturation of the spots. A reference gel image was selected, and protein spots on the gel images were visually examined and matched for location with the spots on the reference gel. Protein spots in the images were edited, split or merged as required, and any non-spot was deleted. The volume of each detected and matched spot on a gel image was normalized against the total spot volume of that gel. The background subtraction was performed by the software using the smallest pixel intensity at the boundary of a spot as background for the spot. The gel images were compared in the software to identify the fold change of the spots across the grouping of gel images by grade (AA, AB4, and TB4) and sex (heifers and steers) as well for their interaction. The entire procedure was performed separately for soluble and insoluble proteins.

6.2.4. Statistical analyses

The analyses were performed in Statistical Analysis Software (SAS, Institute Inc., Cary, NC, USA) Version 9.3. The beef quality data were analyzed using a split plot where sex and grade effects were tested in the main plot and post-mortem ageing time in the split plot. Glucidic and protein data were analyzed using the generalized linear model (GLM) procedure where the sources of variation included animal sex, grade, and their interaction. The spot volumes, extracted from Progenesis PG240 software, were transformed into a log (x + 1) (Morzel et al. 2006) for the statistical analysis. A protein spot was considered significant when it demonstrated at least 1.5 fold intensity difference between the treatments in Progenesis PG240 and GLM procedure at alpha 0.05.

Principal component analysis (PCA) was performed using Unscrambler® (Camo Scientific Inc., Woodbridge, New Jersey) to demonstrate the relationship between identified proteins, quality measurements at day 7 post-mortem and 96 h pH values. For PCA, all the measurements were transformed, using the center and scale function of the software, to minimize the differences in variation between measurements due to measurement scale (Dijksterhuis 1994).

6.2.5. LC-MS/MS identification of soluble and insoluble proteins

For protein identification, preparatory 2D gels were performed with soluble and insoluble protein fractions loaded at 700 µg protein each. After the second dimension, the gels were rinsed with water and fixed with HPLC grade methanol (40%) and acetic acid (10%). Following fixing, gels were rinsed again with water, and the gels were washed overnight with 50% ethanol at 4 ºC to minimize gel background. The following day the gels were rinsed with distilled water and stained with Bio-Safe^{TM} Coomassie Blue (Bio-Rad Labs, Hercules, CA) for 1 h. Following

staining, the gels were rinsed with water until the protein spots were visually clear. Spots of interest were extracted and proteins identified using LC-MS/MS mass spectrometry (Proteomics Platform, Ste-Foy, Quebec, Canada). The identified proteins were matched with their respective gel spots based upon isoelectric values and molecular weight from UniProt (www.uniprot.org). The identified and matched proteins had 100% identification probability and peptide sequence coverage greater than 25%. Protein-protein interactions were explored using the STRING protein network server (version 10.0, http://string-db.org).

6.3. Results

The results presented here indicated the relationship of carcass grades with beef quality and muscle proteins. When sex and grade interacted, the mean values for the main effect of sex and grade were not presented. Biochemical and meat quality results from the muscles investigated in the present study were included in means originally presented in Mahmood et al. (2017, submitted). As the data in the present study are from a subset of the muscles originally presented in Mahmood et al. (2017, submitted), means presented in this chapter are unique.

6.3.1. Beef quality and glucidic potential

Beef quality and glucidic potential results are presented in Tables 6.1 and 6.2 and Figure 6.1. Mean pH values for both Canada AA and AB4 were lower than for Canada TB4 at 96 h post-mortem as well as after ageing (Table 6.1). Despite similar pH, mean L* and b* values for AB4 were lower than those for Canada AA LT. Mean a* for AB4, however, was similar to that for Canada AA and greater than that of TB4. The colour values for TB4 LT were lower than for Canada AA (Table 6.1). Mean muscle lactate concentration, measured at 96 h post-mortem, for AB4 LT was as high as for Canada AA LT, while the mean value for TB4 was the lowest (Table 6.1). Grade interacted with sex for mean residual glycogen concentration, glucidic potential, and

glucose (Table 6.2). Mean residual muscle glycogen concentration was greater for AB4 heifer LT than for the other grade categories regardless of sex. The mean glucidic potential was lower (P = 0.031) for TB4 than for AA LT irrespective of the sex whereas the mean value for heifer AB4 was not different from Canada AA LT. Similarly, residual muscle glucose for heifer AB4 was similar to that for Canada AA (Table 6.2).

Grade interacted with post-mortem ageing for mean WBSF (N) and indicated that WBSF was greater for AB4 and TB4 LT than Canada AA LT on day-7 (Figure 6.1). On day-21 post-mortem, however, the mean values for AB4 remained higher than that for Canada AA whereas TB4 was not different from Canada AA and AB4 (Figure 6.1).

6.3.2. Soluble proteins

Twelve differentially ($P < 0.05$) expressed soluble protein spots having at least a 1.5-fold change are shown in Figure 6.2 and detailed in Table 6.3. The results indicated a greater abundance of isoform alpha-1 actin (ACTA1), glycogenin-1 (GYG1) and isoform beta-2 of protein phosphatase (PPM1B) in TB4 LT than in Canada AA LT (Table 6.3). Canada AA LT had a greater abundance of myomesin-1 (MYOM1) compared to both TB4 and AB4. Likewise, Canada AA had a greater abundance of adenylate kinase isoenzyme 1 (AK1) and peroxiredoxin-1 (PRDX1). Alpha-crystallin B chain (also known as αB-crystallin; CRYAB) and protein DJ-1 were in greater abundance in AB4 than in both TB4 and Canada AA LT. The abundance of desmin (DES) and spermine synthase (SMS) was greatest in Canada AA whereas TB4 and ABT LT were not different from each other.

Grade and sex interacted for levels of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and L-lactate dehydrogenase-A (LDHA), and these proteins appeared to be greater in abundance in steer AA than AB4 LT for both sexes and TB4 LT from heifers. Discounting the

interaction term, the results indicated reduced abundance of glyceraldehyde-3-phosphate dehydrogenase and L-lactate dehydrogenase-A in AB4 compared to Canada AA LT. GTP-binding protein RAN, a member of the RAS oncogene family, had greater expression in both AB4 and TB4 than in Canada AA LT. Like glyceraldehyde-3-phosphate dehydrogenase and L-lactate dehydrogenase-A, levels of cytoplasmic glycerol-3-phosphate dehydrogenase [NAD(+)] (GPD1) and creatine kinase (CKM) were also greater in Canada AA than in AB4 LT, while expression of these proteins in the TB4 LT was not different from that in the Canada AA and AB4 LT samples (Table 6.3).

Phosphatidylethanolamine-binding protein 1 (PEBP1) level was greater in AB4 LT than in Canada AA LT, whereas AB4 and TB4 LT samples were not different from each other for the level of this protein. Small heat shock 27kDa (HSPB1) proteins appeared as two different spots on the gel indicating that they slightly differed in molecular weight and isoelectric point values. The results indicated a greater concentration of heat shock 27kDa protein 1 as well as thioredoxin-dependent peroxide reductase (PRDX3) in AB4 LT compared to Canada AA LT whereas TB4 had these proteins at levels similar to that of Canada AA and AB4 LT. A significant sex effect indicated a greater abundance of αB-crystallin, protein DJ-1, phosphatidylethanolamine-binding protein 1, heat shock 27kDa protein 1 and thioredoxin-dependent peroxide reductase in heifers than in steers (Table 6.4).

6.3.3. Insoluble proteins

Results informing how insoluble proteins were differentially modulated across the treatments are presented in Figure 6.3 and Table 6.5. The results indicated reduced abundance of myosin regulatory light chain-2 (MYLPF), a fast isoform, in TB4 LT while Canada AA and AB4 were not different from each other. Creatine kinase, identified in two spots slightly differing in

isoelectric point, was more abundant in Canada AA than in both TB4 and AB4 LT (Table 6.5). Tropomyosin alpha-1 (TPM1), fast isoform, and 14-3-3 protein gamma (YWHAG) levels were lower in TB4 than in both Canada AA and AB4 LT. Like creatine kinase, αB-crystallin also appeared as two different spots; however, one spot was in lower abundance in TB4 than in both Canada AA and AB4 LT, whereas the other spot had a lower intensity in AB4 compared to both Canada AA and TB4 LT muscles. Results indicated a reduced abundance of myosin regulatory light chain 1/3 (MYL1) in AB4 than in both Canada AA and TB4 LT. Canada AA also had a greater abundance of heat shock 27kDa protein 1 than in TB4 and AB4 LT. Additionally, a significant sex effect indicated increased alpha crystallin B chain in heifers than in steers (Table 6.4).

6.3.4. Relationships between proteins and beef quality

The principal component analysis (PCA) explained 60% of the total variation where the first component (PC-1) described 38% while the second component (PC-2) contributed to describing 22% of the variation (Figure 6.4a, b). The PC-1 indicated that WBSF increased with an increase in PEBP1, RAN, and PPM1B abundance but had a negative correlation with AK1, CKM, L*, b*, MYOM1, TPM1 and YWHAG (Figure 6.4a). Muscle pH had a positive relationship with ACTA1, GYG1, and PPM1B but was negatively associated with GAPDH, LDHA, and insoluble HSPB1. PC-2 indicated that HSPB1, DJ-1, and soluble CRYAB tended to increase with MYLPF (Figure 6.4a). The score plot showed AB4 LT in the upper left quadrant of the PCA, indicating increased abundance of soluble CRYAB, DJ-1, HSPB1, PEBP1, and WBSF but reduced L*, AK1, PRDX1, MYOM1, GAPDH, LDHA and insoluble CKM, TPM1 and HSPB1 (Figure 6.4b). The TB4 LT samples were positioned in the lower left quadrant while Canada AA LT samples were located on the right half of the score plot. These results showed

that L*, a* and b* increased with increases in AK1, PRDX1, CKM, GAPDH, LDHA, MYOM1 and DES, and all these were greatest for Canada AA LT and lowest for TB4 where the latter had increased pH, ACTA1, and GYG1.

6.4. Discussion

Despite intensive research spanning decades, the issue of dark cutting still exists in beef producing countries including Canada (Beef Cattle Research Council 2013), the United States of America (Moore et al. 2012) and Australia (Hughes et al. 2014), and is causing significant economic loss to beef producers. The factors leading to dark cutting could be manifold but cattle predisposed to cutting dark may have specific phenotypes (McGilchrist et al. 2012) and be similar to those that produce Canada AA/USDA Select carcasses (Mahmood et al. 2016b). Recent findings that LT from dark cutting carcasses (atypical dark) had glucidic potential similar to normal Canada AA carcasses (Holdstock et al. 2014) suggested that the dark cutting phenomenon could be related to muscle proteins identified previously (Canto et al. 2015; Gagaoua et al. 2015). Moreover, disparity in tenderness between Canada AA and AB4 LT, regardless of the carcass phenotype, (Holdstock et al. 2014; Mahmood et al. 2017 submitted), could also be associated with specific muscle proteins. The aim of the study was to perform a proteomic analysis of dark cutting beef LT to test the hypothesis that dark cutting and its associated beef quality was related to muscle glycolytic proteins.

6.4.1. Muscle glycolytic and energy related proteins

Results indicated that AB4 LT had a glucidic potential theoretically sufficient to attain normal pH (Bodwell et al. 1965; Immonen and Puolanne 2000) and the mean pH of AB4 LT at 96 h post mortem spanned values when beef is either normal or starts to appear dark (Orcut et al. 1984; Page et al. 2001). However, despite the mean pH values for AB4 LT on day 7 and 21 post-

mortem being similar to that of normal Canada AA beef (Bass et al. 2008; Page et al. 2001), the colour values for AB4 remained lower than those of Canada AA LT. Although not able to be confirmed in the present study, the carcasses producing AB4 LT may have had reduced rates of glycolysis early post-mortem. This is implied by the low concentrations of glycerol-3-phosphate dehydrogenase, lactate dehydrogenase A, cytosolic glycerol-3-phosphate dehydrogenase [NAD (+)], and energy-related proteins such as creatine kinase and adenylate kinase isoenzyme 1. Apart from its effect on glycolysis, increased glyceraldehyde-3-phosphate dehydrogenase may also disrupt mitochondria and release cytochrome-*c* (Tarze et al. 2007), which otherwise may have sustained the consumption of oxygen by the electron transport chain, thus allowing myoglobin to retain oxygen and the muscle to attain desirable meat colour.

Increased abundance of creatine kinase, a cellular energy sensor associated with increased phosphocreatine/creatine ratios (D'Alessandro et al. 2012), was reported in muscles having increased glycolytic metabolism (Joseph et al. 2012). Increased levels of glyceraldehyde-3-phosphate dehydrogenase and creatine kinase may have represented increased glycolytic activity because these proteins are associated with a rapid and increased extent of post-mortem pH decline (D'Alessandro et al. 2012; Joseph et al. 2012; Picard et al. 2010). The literature also indicated that increased concentration of lactate dehydrogenase-B is associated with accelerated post-mortem pH decline (Gagaoua et al. 2015) while increased abundances of glyceraldehyde-3-phosphate dehydrogenase and lactate dehydrogenase A were associated with increased colour stability of bovine *longissimus lumborum* (Canto et al. 2015). The results of these studies are in accordance with that of the current one which indicated a trend for a positive relationship of the aforementioned enzymes with muscle lightness but a negative association with LT pH. Deficiency of lactate dehydrogenase A, as exhibited in AB4 LT, may have hindered the second

phase of the glycolytic cycle and thus slowed the conversion of pyruvate to lactate and the synthesis of NAD, the latter being required by glyceraldehyde-3-phosphate dehydrogenase to perpetuate the glycolytic cycle. Why lactate dehydrogenase A was decreased in AB4 LT and not TB4 LT is unclear, but may indicate a difference in muscle physiology, as reduced levels of glycolytic proteins in AB4 LT may be associated with increased proportion of slow-twitch oxidative muscle fibres (Picard et al. 2010). Muscle fibre type proteins are influenced by physiological demand of muscles (Goll et al. 2008), which may differ based upon production system and whether cattle were backgrounded in a non-feedlot situation (Johnston et al. 1981).

The protein AKI plays an important role in energy homeostasis by converting ATP to AMP and ADP while the latter may accelerate the conversion of phosphoenolpyruvate to pyruvate, a rate limiting step of the glycolytic cycle. Also, glycerol-3-phosphate dehydrogenase [NAD(+)], a cytoplasmic protein that responds to cellular cAMP concentration, binds NAD and upregulates both gluconeogenesis and glycolysis. Glycerol-3 phosphate dehydrogenase has been associated with muscle lightness (Sayd et al. 2006). Thus, reduced levels of GAPDH, LDHA, AK1 and glycerol-3-phosphate dehydrogenase [NAD(+)] might have collectively lowered the rate of post-mortem pH decline in AB4 LT and as a result compromised muscle colour.

Results indicated that TB4 LT had reduced glycogen concentration, increased pH, and lowered colour values (Bodwell et al. 1965; Hanson et al. 2001). Reduced glycogen and increased pH in TB4 LT could be due to slow oxidative muscle fibre characteristics (Cusso et al. 2003; Hansen et al. 2000) as indicated by reduced concentration of fast myosin regulatory light chain-2, tropomyosin alpha-1 (fast isoform) and creatine kinase. Hansen et al. (2000) found increased concentration and activity of glycogenin 1 in slow-twitch red fibres compared to fast-twitch white fibres of rat. The current study also indicated increased abundance of glycogenin-1

in TB4 LT. Reduced glycogen in TB4 LT could be ascribed to increased oxidative muscle fibres, which are prone to depletion of glycogen in the event of stress (Lacourt and Tarrant 1985). Increased glycogenin-1 in TB4 LT could be an adaptive response to low level of glycogen (Kraniou et al. 2000) although glycogenin-1 reportedly had no role in determining maximum glycogen concentration in rat muscles (Hansen et al. 2000). Moreover, the TB4 LT had increased abundance of isoform beta-2 of protein phosphatase which may dephosphorylate catalytic subunits (PRKAA1 and PRKAA2) and lower AMPK activity. Reduced AMPK activity is also associated with oxidative muscle fibres (Lee-Young et al. 2009). Reduced muscle glycogen and increased pH in TB4 muscles therefore could be associated with increased oxidative characteristics of muscle fibres but this was not substantiated in the present experiment.

6.4.2. Tenderness and myofibril proteins

Both TB4 and AB4 LT had increased toughness compared to Canada AA on day 7 post-mortem. The finding that meat with increased intramuscular pH (≥6.2) is tender early post mortem (Lomiwes et al. 2013, 2014) was not supported by the current study. Post-mortem meat tenderization is an enzymatic process that degrades myofibril proteins and softens muscle architecture. Increased tenderness of Canada AA was associated with increased expression of desmin and myomesin in the soluble fraction, suggesting increased proteolysis (Koohmaraie 1996; Mohrhauser et al. 2011). Increased tenderness associated with desmin degradation in Canada AA LT could be a result of increased activity of μ-calpain early post-mortem or of cathepsins due to low muscle pH (Lomiwes et al. 2013, 2014). Guillemin et al. (2012) did not find a significant association of calpains with variation in tenderness of LT muscles ranked low and extreme tender. The proteolytic activity of μ-calpain, primarily accounting for meat tenderization, greatly reduces after 24-48 h post-mortem (Koohmaraie 1996; Liu et al. 2016)

suggesting that the disparity in tenderness between grade categories could be due to factors other than calpains.

The results indicated that TB4 LT was as tender as Canada AA on day 21 postmortem, and this was likely because of continued proteolysis during ageing. There was an increased level of α-actin in the soluble fraction of TB4 LT. Actin release from the muscle cytoskeleton suggests progression of apoptosis where caspases activate and initially cleave actin (Goll et al. 2008; Lana and Zolla 2016; Ouali et al. 2013). Muscle tenderness has been associated with degradation of α-actin (Lana and Zolla, 2016; Picard et al. 2014), and the onset of apoptosis may be triggered earlier in TB4 LT due to reduced glycogen concentration although the degradation of α-actin was not associated with tenderness similar to that of Canada AA prior to ageing. Increased degradation of desmin during ageing has been reported in beef with increased pH (Lomiwes et al. 2013) which is also favourable for proteasome activity (Dutaud et al. 2006; Lamare et al. 2002). Proteasomes degrade proteins after they are ubiquinated by ubiquitin protein ligase, a process which continues post-mortem (Lecker et al. 2006; Liu et al. 2016). Studies indicated that the proteasome enhanced disruption of Z disc, I band and M line and degradation of actin, myosin, tropomyosin, troponin and alpha-actinin in myofibrils previously denatured and degraded likely by calpains (Goll et al. 2008; Liu et al. 2016; Robert et al. 1999). Proteasomes and calpains have synchronized activity and proteasomes are crucial to the degradation of calpain-activated proteins (Smith and Dodd 2007), and proteasomes may sustain proteolytic activity of calpains through degradation of calpastatin, as identified in cardiac cells (Pedrozo et al. 2010). Thus, increased degradation of Z disc, I band and M line by proteasomes, identified by Robert et al. (1999), could be due to increased degradation of calpastatin. The μ-calpain activates late post-mortem in beef longissimus with increased ultimate pH (Lomiwes et al. 2013)

suggesting that improved tenderness of TB4 LT after extended ageing was likely a result of continued proteolysis by calpains and proteasomes.

The fact that AB4 LT, unlike TB4 LT, remained tougher than Canada AA even after 21 day post-mortem, and this agreed with literature indicating that dark meat with low pH was the toughest (Grayson et al. 2016; Holdstock et al. 2014). Proteolytic activity of μ-calpain and cathepsin B is lower in muscles with intermediate pH, which tends to produce tough meat that requires extended ageing to reduce shear values (Lomiwes et al. 2013; Pulford et al. 2008). The AB4 LT had a mean pH similar to that of Canada AA LT which was numerically greater at 96 h post-mortem. Improved tenderness of TB4 could be due to sustained synchronized proteolysis by calpains and proteasomes, which have increased activity at high pH (Dutaud et al. 2006; Lamare et al. 2002; Sentandreu et al. 2002). Proteolytic activity of proteasomes might have been compromised by increased concentration of phosphatidylethanolamine-binding protein 1 (PEBP1) (Chen et al. 2006), a serine protease inhibitor, in AB4 LT. Sentandreu et al. (2002) stressed the importance of proteolytic inhibitors in determining muscle tenderness but any decisive role of proteasomes and their inhibitors has yet to be established in skeletal muscles. PEBP1 also inhibits (Zhao and Wenzel 2014; Figure 6.5) matrix metalloproteinase-1 (MMP1), an interstitial collagenase capable of dismantling collagen. Structural weakness and reduced shear force of beef connective tissues following 14 days post-mortem (Nishimura et al. 1995, 1998) suggests improved tenderness due to collagen breakdown. Thus increased toughness of AB4 LT could be due to reduced activity of μ-calpain (Lomiwes et al. 2013) coupled with increased concentration of PEBP1 reducing activities of both MMP1 and proteasomes (Chen et al. 2006; Pedrozo et al. 2010).

D'Alessandro et al. (2012) found higher concentrations of CKM and GAPDH in tender muscle, which had a rapid pH decline and a mean 48 h pH 5.48, than in tough *longissimus* with a slow pH decline and an ultimate pH value of 5.61. In the current study, the AB4 LT appeared to have a slow, prolonged pH decline and reduced abundances of CKM and GAPDH but increased toughness compared to Canada AA LT. D'Alessandro et al. (2012) hypothesized that increased CKM may delay or slow ATP depletion and subsequent contraction of the sarcomere (D'Alessandro et al. 2012) whereas GAPDH may be pro-apoptotic, enhancing protein degradation and improving tenderness (Tarze et al. 2007).

Other proteins allegedly contributing to tenderness variation that were detected in this study included 14-3-3 gamma and RAN where the former was lower in TB4 LT while the latter was greater in both TB4 and AB4 LT compared to Canada AA. Increased abundance of 14-3-3 gamma (YWHAG) in tender *longissimus* (D'Alessandro and Zolla 2013) agreed with PCA results of the current study as WBSF decreased with increase in YWHAG abundance. The current study indicated increased WBSF with increased RAN, a member of the RAS oncogene family, but D'Alessandro et al. (2012) found a greater concentration of RAS-related protein Rab-21 in tender meat; however, such cancer-related proteins may enhance actomyosin complex formation during transformation of muscle to meat by increasing the availability of integrin alpha 5 (D'Alessandro et al. 2012; Hooper et al. 2010).

6.4.3. Antioxidant and heat shock proteins

Antioxidant and small heat shock proteins have been related to meat colour and tenderness. Results for the crystallin protein were not definitive because of its presence in more than one area on the gel and it being found in both the soluble and insoluble fractions. Expression of the same protein in different gel positions could be due to differences in post-

translational modification of the proteins (D'Alessandro et al. 2012). Moreover, αB-crystallin is redistributed at rigor in muscles varying in ultimate pH where low pH muscles have less αB-crystallin in their soluble fraction than high pH muscles; however, total concentration remained unaffected (Pulford et al. 2008). In the current study, these proteins were greater in the soluble fractions in AB4 and TB4 LT but had greater abundance in the insoluble fraction of Canada AA LT which had low mean pH values at 96 h post-mortem, and this agreed with Pulford et al. (2008). At early post-mortem, αB-crystallin and HSP27 have been reported to be higher in beef longissimus with intermediate and high pH than in muscles having low pH (Lomiwes et al. 2013; Pulford et al. 2008). The differential abundance of heat shock proteins (HSP27 and αB-crystallin) in the various grade categories in the current experiment may be a result of degradation and precipitation depending on LT pH (Lomiwes et al. 2013; Pulford et al. 2008). This also suggested caution in correlating beef quality with the levels of heat shock proteins.

Increased peroxiredoxin-1 levels in Canada AA compared to TB4 and AB4 LT appeared to agree with Joseph et al. (2012) who found an increased abundance of peroxiredoxin-2 in colour-stable muscles. Moreover, spermine synthase, an endogenous muscle antioxidant (Bekhit et al. 2013), was highly expressed in Canada AA LT. Beef L* and b* had a negative correlation with αB-crystallin abundance (Gagaoua et al. 2015) and PCA in the current study also indicated this trend. Small heat shock proteins had increased expression in AB4 LT and the PCA indicated these proteins and DJ-1 were negatively associated with glycolytic and energy related proteins such as LDHA, GAPDH, AK1, and CK. Increased HSP27 and αB-crystallin have been linked with oxidative metabolism and dark colour in pork muscles (Bekhit et al. 2013; Sayd et al. 2006). Picard et al. (2014) also found a greater abundance of αB-crystallin in oxidative LT muscles than in fast glycolytic ST muscles of cattle, implying a compromised glycolytic potential in AB4 LT.

Apart from PEBP and RAN, the proteins Hsp27, αB-crystallin and DJ-1, which were all highly expressed in AB4 LT, tended to have a positive relationship with shear values. Contrarily, Picard et al. (2014) reported improved tenderness accompanied by increased abundance of Hsp27, Hsp20 and αB-crystallin as well as antioxidant peroxiredoxin-6 and DJ-1 in beef LT while D'Alessandro et al. (2012) also reported upregulation of Hsp27 in tender muscles. Heat shock proteins may behave anti-apoptotic by inhibiting the caspases and protecting proteins from damage or by repairing damaged substrate proteins (Ouali et al. 2013; Picard et al. 2010). Lomiwes et al. (2013) reported increased levels of small heat shock proteins in beef having intermediate pH and increased toughness. Contradictions in the literature regarding the relationship of tenderness with small heat shock proteins could be because of inconsistencies in the conditions of protein extraction (Picard et al. (2010) although genes encoding Hsp27, Hsp40, and αB-crystallin were interrelated and also downregulated in tender beef LT (Bernard et al. 2007). These authors hypothesized that increased Hsp27 can stabilize actin filaments whereas αB-crystallin may reduce the dismantling of desmin (Bernard et al. 2007) and in support of this desmin and actin had reduced expression in the soluble fraction of AB4 LT. As for antioxidant proteins such as peroxiredoxin, Ouali et al. (2013) stressed that further investigation was needed to understand the relationship of these proteins with meat tenderness. The tenderness phenomenon is thought to be multifactorial (Picard et al. 2010), and reduced tenderness of AB4 LT could be because of the slow apoptotic process as a consequence of increased concentration of small heat shock proteins.

6.5. Conclusions

The study supported acceptance of the hypothesis that dark cutting was related to post mortem muscle glycolytic protein abundance as AB4 LT appeared to have reduced glycolytic

capacity. Increased pH and reduced glucidic potential in TB4 LT was likely because of upregulation of oxidative myofibril proteins as muscle physiological demand may change myosin isoforms (Goll et al. 2008). However, establishing absolute muscle fibre type was not possible because muscle fibre typing was not performed in the present study. Also proteomic analysis was not performed on the pellet, which may contain some myofibril proteins. Increased toughness of atypical dark LT appeared to be related to increased concentration of proteolytic inhibitors. The results of this study should be viewed cautiously, as sample numbers were not large although they were comparable to other studies investigating muscle proteins (Anderson et al. 2014; Lomiwes et al. 2014). Genomic testing of dark cutting cattle may help to confirm the findings of the current study and to identify the candidate genes for cattle at risk of cutting dark.

Table 6.1. Effect of grade on least square means (± standard error of mean in parentheses) for beef quality measurements.

Measurements	Grade			Pr > F[4]
	AA[1] (*n* = 8)	AB4[2] (*n* = 8)	TB4[3] (*n* = 7)	
96 hour pH	5.68[b] (0.08)	5.74[b] (0.08)	6.31[a] (0.09)	0.0001
pH (day7 & 21)	5.61[b] (0.06)	5.58[b] (0.06)	6.16[a] (0.07)	<.0001
Light (L*)	37.25[a] (0.57)	33.24[b] (0.57)	32.38[b] (0.62)	<.0001
Red (a*)	23.94[a] (0.80)	21.8[a] (0.80)	17.45[b] (0.86)	<.0001
Yellow (b*)	6.8[a] (0.58)	4.01[b] (0.58)	1.30[c] (0.63)	<.0001
Chroma	24.94[a] (0.86)	22.20[b] (0.86)	17.68[c] (0.92)	0.0001
Hue	15.53[b] (27.15)	17.71[b] (27.15)	122.43[a] (29.23)	0.026
Total moisture %	75.36 (0.50)	74.96 (0.50)	76.10 (0.54)	0.3277
Crude fat %	4.72 (0.49)	5.35 (0.49)	4.03 (0.54)	0.2218
Crude protein %	22.58 (0.21)	22.17 (0.21)	22.695 (0.23)	0.2327
Ash%	1.026 (0.009)	1.023 (0.009)	1.026 (0.010)	0.9793
Myoglobin (mg g^{-1} fresh tissue)	4.21[b] (0.21)	5.06[a] (0.21)	5.44[a] (0.23)	0.0034
Lactate (µmol g^{-1} fresh tissue)	102[a] (4.7)	94[a] (4.7)	68[b] (5.1)	0.0004

[1]Canada AA grade carcasses equivalent to USDA Select carcasses.

[2]Atypical dark cutting (AB4) beef muscles.

[3]Typical dark cutting (TB4) beef muscles.

[2] Probability of the F test, with significance at $P \leq 0.05$.

[a,b,c]Least Square Means within a row lacking a common letter differ at $P \leq 0.05$

Table 6.2. Least square means (± standard errors of mean in parentheses) for the interactions between grade and sex for glucidic measurements.

Measurements	Heifers			Steers			Pr > F[4]
	AA[1] (n = 4)	AB4[2] (n = 4)	TB4[3] (n = 3)	AA (n = 4)	AB4 (n = 4)	TB4 (n = 4)	
Glycogen (µmol glucose g^{-1} fresh tissue)	26.11^{b} (3.08)	40.96^{a} (3.086)	15.94^{b} (3.56)	23.08^{b} (3.08)	16.48^{b} (3.08)	15.23^{b} (3.08)	0.0025
Glucidic potential (µmol glucose g^{-1} fresh tissue)	81.58^{a} (5.54)	93.29^{a} (5.54)	48.95^{b} (6.39)	82.08^{a} (5.54)	67.63^{b} (5.54)	53.81^{b} (5.54)	0.0313
Glucose (µmol g^{-1} fresh tissue)	5.82^{a} (0.38)	5.24^{a} (0.38)	1.32^{c} (0.44)	5.86^{a} (0.38)	3.66^{b} (0.38)	2.06^{bc} (0.38)	0.0261

[1]Canada AA grade carcasses equivalent to USDA select carcasses.

[2]Atypical dark cutting (AB4) beef muscles.

[3]Typical dark cutting (TB4) beef muscles.

[2] Probability of the F test, with significance at $P \leq 0.05$.

[a,b,c]Least Square Means within a row lacking a common letter differ at $P \leq 0.05$.

Table 6.3. Soluble protein spots having lower (↓) or higher (↑) expression (≥ 1.5 fold) in TB4 and AB4 LT relative to Canada AA.

Gel spot	Fold change	Expression in TB4/AB4	Proteins	Peptide count	Sequence coverage%	Observed MW/PI	Theoretical MW/PI	Gene	Accession number
14	3.5	↑TB4	Actin, alpha skeletal muscle	15	34	45/5.25	41.8/5.23	ACTA1	ACTS_BOVIN
14	3.5	↑TB4	Glycogenin 1	15	30	45/5.25	37.5/5.17	GYG1	Q29RN2_BOVIN
15	3.5	↑TB4	Isoform Beta-2 of Protein phosphatase	19	41	45/5.12	42.8/5.06	PPM1B	PPM1B_BOVIN
16	3.5	↓TB4, AB4	Myomesin-1	41	30	165/5.75	165/6.13	MYOM1	F1MME6_BOVIN
30	2.6	↓TB4, AB4	Adenylate kinase isoenzyme 1	44	92	22/8.25	21.66/8.4	AK1	KAD1_BOVIN
30	2.6	↓TB4, AB4	Peroxiredoxin-1	12	47	22/8.25	22.2/8.59	PRDX1	PRDX1_BOVIN
34	2.4	↑AB4	Alpha-crystallin B chain	21	56	22/6.8	20/6.76	CRYAB	CRYAB_BOVIN
34	2.4	↑AB4	Protein DJ-1	20	81	22/6.8	20/6.84	PARK7	PARK7_BOVIN
50	2	↓TB4, AB4	Desmin	66	77	50/5.10	53.5/5.21	DES	DESM_BOVIN
50	2	↓TB4, AB4	Spermine synthase	19	42	50/5.10	41/4.96	SMS	B0JYM7_BOVIN
68	1.7	↓AB4	Glyceraldehyde-3-phosphate dehydrogenase	36	68	35/7.75	35.7/8.52	GAPDH	G3P_BOVIN
68	1.7	↓AB4	L-lactate dehydrogenase A	26	52	35/7.75	36.5/8.17	LDHA	LDHA_BOVIN
72	1.7	↑TB4, AB4	RAN, member RAS oncogene family	13	34	25/7.5	24.4/7	RAN	B0JYN2_BOVIN
78	1.6	↓AB4	Glycerol-3-phosphate dehydrogenase [NAD(+)]	38	78	35/7	37.6/6.42	GPD1	GPDA_BOVIN
78	1.6	↓AB4	Creatine kinase	23	52	35/7	43/6.63	CKM	KCRM_BOVIN
179	2	↑AB4	Phosphatidylethanolamine-binding protein 1	12	63	22/7.37	21/7.39	PEBP1	PEBP1_BOVIN
192	1.8	↑AB4	Heat shock 27kDa protein 1	12	52	24/5.5	22.4/5.98	HSPB1	E9RHW1_BOVIN
360	1.5	↑AB4	Heat shock 27kDa protein 1	11	52	26/6	22.4/5.98	HSPB1	E9RHW1_BOVIN
360	1.5	↑AB4	Thioredoxin-dependent peroxide reductase	8	28	26/6	21.52/5.73	PRDX3	PRDX3_BOVIN

Table 6.4. Soluble and insoluble protein spots having lower (↓) or higher (↑) expression (≥ 1.5 fold) in heifers relative to steers.

Assigned Gel spot	Fold change	Protein fraction	Expression	Proteins	Peptide count	Sequence coverage%	Observed MW/PI	Theoretical MW/PI	Gene
34	2.3	Soluble	↑Heifers	Alpha-crystallin B chain	21	56	22/6.8	20/6.76	CRYAB
34	2.3	Soluble	↑Heifers	Protein DJ-1	20	81	22/6.8	20/6.84	PARK7
179	2.9	Soluble	↑Heifers	Phosphatidylethanolamine-binding protein 1	12	63	22/7.37	21/7.39	PEBP1
192	2.4	Soluble	↑Heifers	Heat shock 27kDa protein 1	12	52	24/5.5	22.4/5.98	HSPB1
360	1.8	Soluble	↑Heifers	Heat shock 27kDa protein 1	11	52	26/6	22.4/5.98	HSPB1
360	1.8	Soluble	↑Heifers	Thioredoxin-dependent peroxide reductase	8	28	26/6	21.52/5.73	PRDX3
38	1.7	Insoluble	↑Heifers	Alpha-crystallin B chain	49	86.90	22/6.8	20/6.76	CRYAB

Table 6.5. Insoluble protein spots having lower (↓) or higher (↑) expression (≥ 1.5 fold) in TB4/AB4 relative to Canada AA LT.

Gel spot	Fold change	Expression in TB4/AB4	Proteins	Peptide count	Sequence coverage%	Observed MW/PI	Theoretical MW/PI	Gene	Accession number
6	4.8	↓TB4	Myosin regulatory light chain 2	47	95.30	18/4.3	19/4.88	MYLPF	MLRS_BOVIN
14	3.2	↓TB4, AB4	Creatine kinase M-type	52	66.70	40/7.5	6.6/43	CKM	KCRM_BOVIN
18	2.5	↓TB4	Tropomyosin alpha-1 chain	31	66.20	28/4.3	33/4.69	TPM1	TPM1_BOVIN
22	2.4	↓TB4	14-3-3 protein gamma	41	76.50	28/4.5	28/4.8	YWHAG	A7Z057_BOVIN
25	2.3	↓TB4, AB4	Creatine kinase M-type	64	74.80	40/7	43/6.63	CKM	KCRM_BOVIN
38	1.9	↓TB4	Alpha-crystallin B chain	49	86.90	22/6.8	20/6.76	CRYAB	CRYAB_BOVIN
51	1.7	↓AB4	Myosin light chain 1/3	14	56.20	25/5.3	21/4.9	MYL1	MYL1_BOVIN
54	1.7	↓AB4	Alpha-crystallin B chain	32	75.40	22/6.75	20/6.76	CRYAB	CRYAB_BOVIN
61	1.6	↓TB4, AB4	Heat shock 27kDa protein 1	7	44.30	25/6.75	22/5.98	HSPB1	E9RHW1_BOVIN

Figure 6.1. Least square means of the interaction ($P = 0.041$) between grade and post-mortem ageing for WBSF (N).

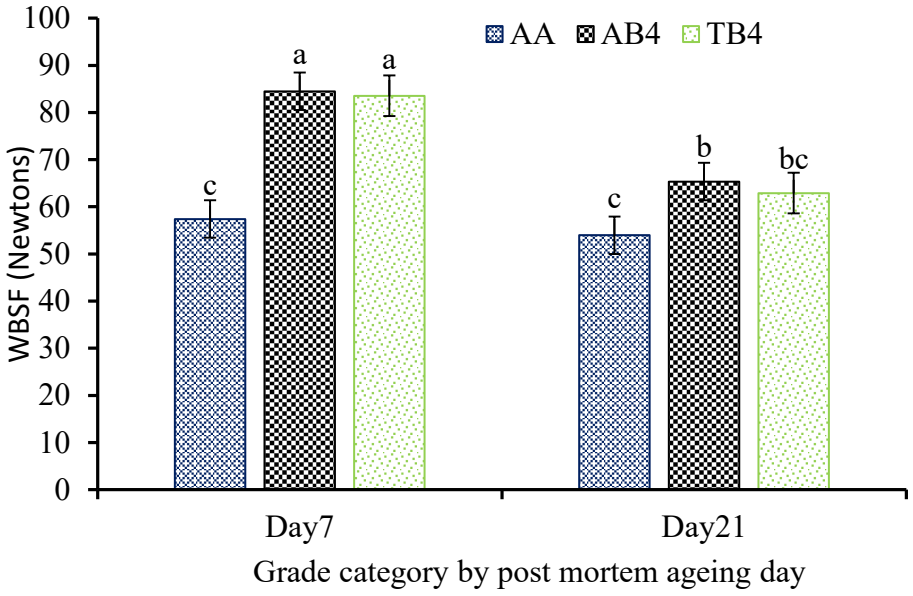


Figure 6.2. Typical two dimensional gel image of soluble proteins with significant spots highlighted (see Table 6.3 for identification of protein by gel spot number).

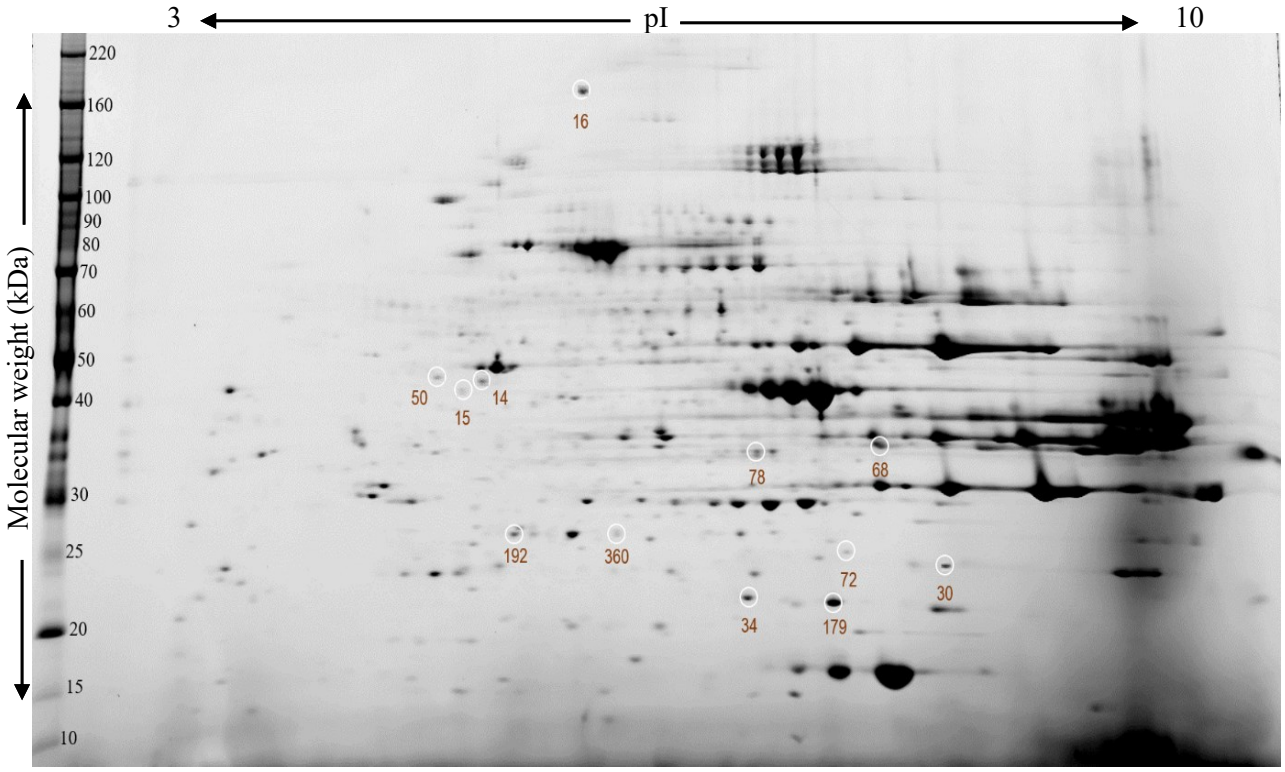


Figure 6.3. Typical two-dimensional gel image for insoluble proteins highlighting the significant spots (see Table 6.4 for identification of protein by gel spot number).

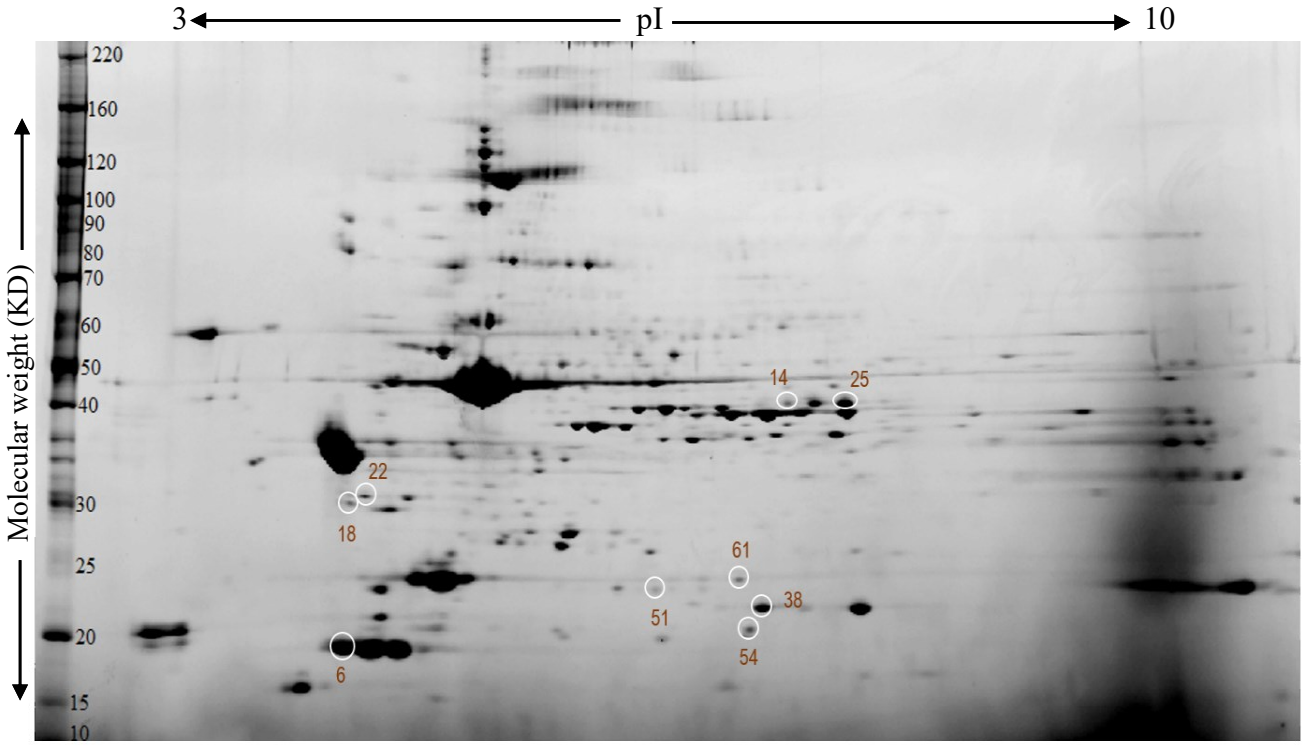


Figure 6.4. Principal component analysis correlation loadings (A) and scores (B) for proteins (indicated by genes' names) and quality measurements. In correlation loadings, blue, red and black text corresponded soluble proteins, insoluble proteins, and beef quality, respectively. In score plot, the grades were encircled as; AA red, AB4 black and TB4 green.

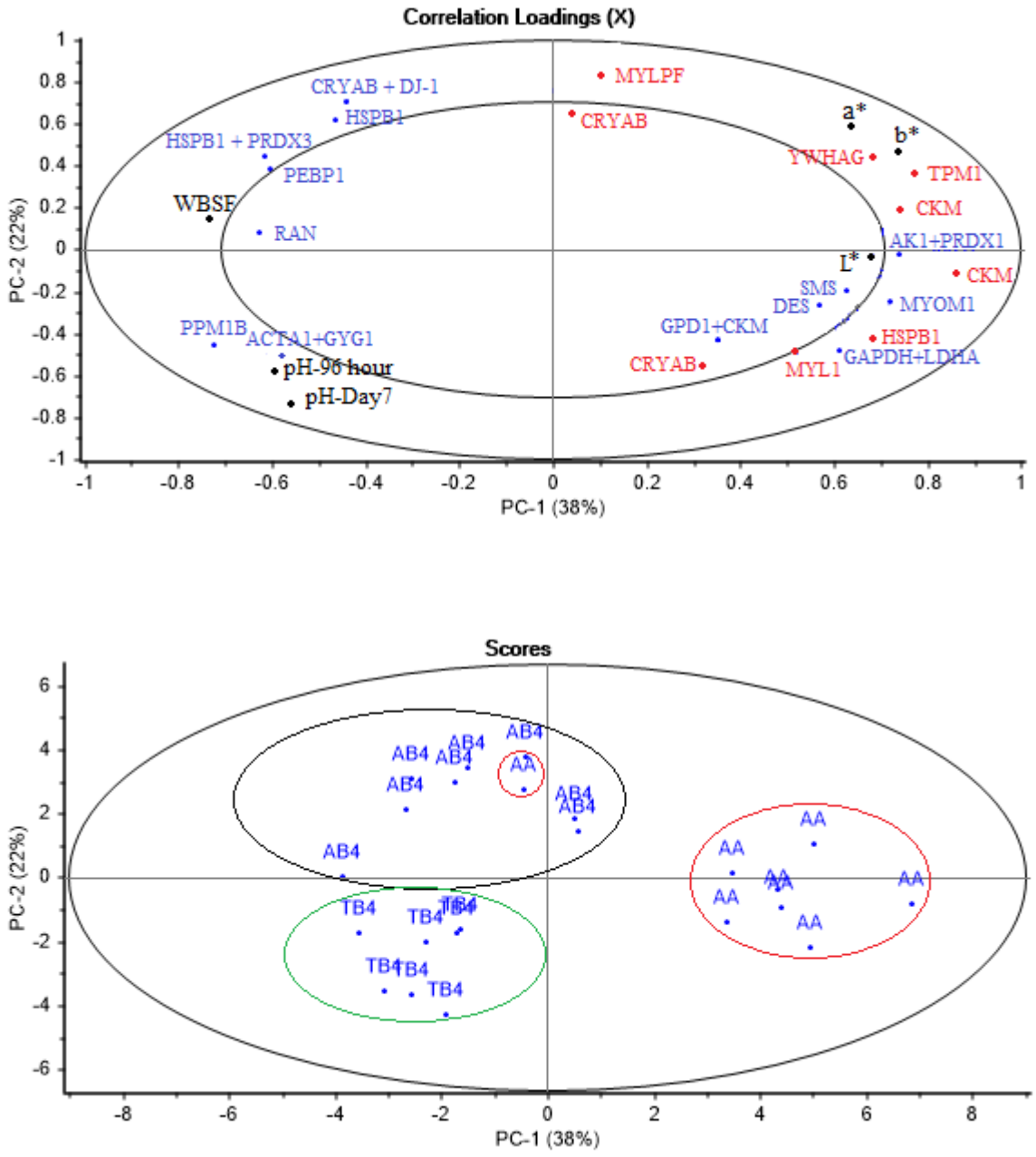
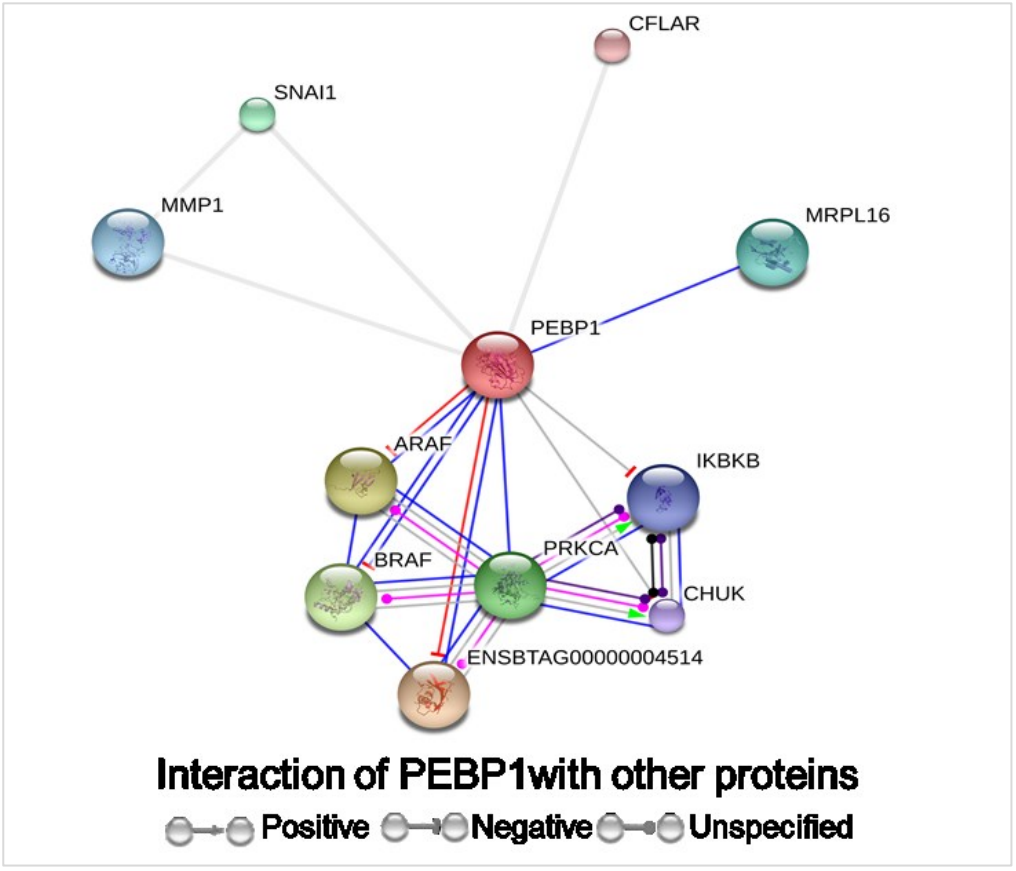


Figure 6.5. Interaction of phosphatidylethanolamine-binding protein 1 (PEBP1) with other proteins (http://string-db.org).



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7.0. General summary and conclusions

7.1. Overview

Dark cutting carcasses, identified by their dark-red or purple rib eye (*longissimus thoracis*; LT) muscles, generally have an increased intramuscular pH, unfavourable for shelf life (Koutsoumanis et al. 2006). Although the phenomenon of dark cutting has been under investigation for decades, the problem still exists at levels as high as 1.28% of the beef carcasses in Canada (Beef Cattle Research Council (BCRC, 2013), 3.2% in the United States of America (Moore et al. 2012) and 10% in Australia (MSA 2010). The widely understood cause is inadequate intramuscular glycogen at the time of slaughter for subsequent post-mortem glycolysis and lowering of muscle pH.

The occurrence of dark cutting, however, has been directly or indirectly linked to carcass marbling score (Park et al. 2007), subcutaneous fat depth, rib eye area and carcass weight (Hawrysh et al. 1985; Park et al. 2007; McGilchrist et al. 2012). Predisposition to cut dark based upon live phenotypic characteristics has only recently been reported in the literature. Moreover, increased carcass weight and ribeye area (Bruns et al. 2005; Schneider et al. 2007) should be related to a reduced likelihood of dark cutting, yet such a relationship may not be straightforward because dark cutting may occur in large and muscular cattle in response to growth promotants when available energy does not meet the demand for rapid growth along with environmental stress (Scanga et al. 1998; Schneider et al. 2007). Literature findings are not consistent about the effect of cattle sex on dark cutting (Lorenzen et al. 1993; Mach et al. 2008) most likely because of different cattle rearing strategies including the use of growth promotants and melengestrol acetate (MGA) and consequently exhibition of oestrus activity in heifers (Hill et al. 1971; Kenny and Tarrant 1988).

Literature findings suggested that a concentrate diet may lower the incidence of dark cutting (Warner et al. 1998; Immonen et al. 2000), and this ascertains the importance of nutrition during feedlot. Because cattle age reportedly influences muscle pH and colour (Miller et al. 1987; Wilkins et al. 2009), and because there are two ages at which cattle are slaughtered for entry into the human food chain, it begs the question whether dark cutting is greater in calf-fed or yearling-fed cattle. The potential interaction between production system and growth promotant use in heifers and steers has not been widely reported in the literature.

Literature findings about lairage effect on dark cutting are inconsistent (Gallo et al. 2003; Teke et al. 2014), and the existence of dark cutting in the Canadian beef industry may be associated with inappropriate lairagemanagement. Slaughter season is another variable potentially affecting the likelihood of cutting dark but the literature citing a season effect is probably more due to the availability of nutrition rather than environmental temperature (Knee et al. 2004). Moreover, different studies have been conducted in different countries, which may vary greatly in climate temperature compared to Canada. Thus a comprehensive study incorporating the effect of animal physical characteristics, production practices, usage of growth promotants, pre-slaughter management and season was needed to establish the relative contribution of these factors to dark cutting incidence in Canadian beef industry.

Studies indicated that muscles at pH below 5.87 may have normal beef colour (Page et al. 2001) but subjectively identified dark carcasses had pH below 5.8 (Murray 1989). Such carcasses produced by steers also had glucidic potentials similar to normal Canada AA and were termed atypical dark cutting carcasses (Holdstock et al. 2014). However, the existence of atypical dark cutting carcasses in heifers needed to be explored. Dark cutting beef with lower pH was reported as extremely tough (Holdstock et al. 2014) but the effect of carcass weight and fat

depth on tenderness of such beef has not been studied. Additionally, what made muscles dark despite sufficient intramuscular glycogen storage was unknown. Studies have suggested that muscle glycolytic enzymes have important role in determining muscle pH, colour and tenderness. Therefore, investigating the effect of cattle sex, phenotype and muscle proteins was necessary to elucidate the underlying mechanism of dark cutting and its associated beef quality.

7.2. Dark cutting influenced by cattle sex, phenotype and production practices

The first two studies, in chapter 2 and 3, were executed to analyze the effects of cattle sex, phenotype, and performance during finishing on dark cutting. These studies tested the intriguing hypothesis that dark cutting can be predicted from physical measurements of cattle. The unique feature of these studies was that dark grade carcasses/cattle were compared with normal carcasses/cattle categorized, based upon marbling score, into Canada AAA, AA, and A grades, instead of comparing dark cutting solely with a single normal category. The results indicated a potential for predicting ultimate carcass grades such as Canada AAA, AA, and A from live-cattle measurements which could be beneficial in the live cattle trade. These studies also revealed that dark cutting cattle were those that otherwise would most likely have produced Canada AA/USDA Select grade carcasses. These results also validated the literature findings that marbling had no significant role in cutting dark.

Results suggested that cattle having feed intake lower than 1.72% of the body weight were at greater risk of cutting dark; however, dark cutting cattle were as feed efficient as those that produced Canada AA and A grade carcasses but more efficient than those produced Canada AAA carcasses. Literature revealing the effect of feed efficiency on dark cutting was limited and had not established any relationship between dark cutting and feed efficiency. The results of chapter 3 indicated that reduced weight at slaughter increased the likelihood of cutting dark

where reduced weight could be related to production practices as determined in chapter 4. The results, however, stressed the potential for sorting cattle at risk of cutting dark by measuring rib-eye area, calculating daily gain and weighing cattle before slaughter.

The results of chapter 4 corroborated the findings of chapter 3 maintaining that dark cutting cattle may have a slow growth rate depending upon production system. However, results of chapter 4 also indicated that dark cutting carcasses from all the production systems were either heavier or had weight similar to the values reported for normal carcasses in the literature. These results advocated that establishing a threshold of weight for a carcass/cattle predisposed to cut dark was challenging. However, the results suggested that the problem of dark cutting could be related more to growth rate than the ultimate weight of cattle or carcasses, validating Smith et al. (1999).

Variability in the incidence of dark cutting due to cattle sex, season, production systems, growth promotants, and lairage time was also tested in chapter 4. Findings of chapter 4 together with that of chapter 2 mitigated the confusion about sex effects and validated earlier studies indicating that heifers were more prone to cut dark than steers. Unfortunately, the data did not have control heifers to compare against those subjected to MGA treatment but one producer shared his observations that up to 30% heifers cut dark when the gap between MGA withdrawal and slaughter reached 72 h. Mounting activity in heifers during oestrus reduces muscle glycogen; however, cattle tend to utilize more glucose than fat during heat stress (Baumgard et al. 2006). Moreover, feed intake drops during oestrus (Rhoads et al. 2009) and heat-stress that also lowers absorption of nutrients from intestinal tract (Baumgard et al. 2006), suggesting a reduced muscle energy reserves as a result of oestrus during hot temperatures and thus increased risk of dark cutting in heifers in summer as indicated by the results of chapter 4. The exclusive finding of

chapter 4 was that the problem was highest in calf-fed heifers while steers were least likely to produce dark cutting beef, regardless of finishing as calf-fed or yearling-fed.

The study has indicated that frequent shipping and extended lairage is an effective model for creating dark cutting. Extended lairage may increase the occurrence of dark cutting beef, as indicated by the results of chapter 4 and 5. However, prolonged lairage was confounded with frequent shipping and the latter might be responsible for increased dark cutting. Increased dark cutting due to extended lairage could also be due to lack of timely provision of diet after shipment to the abattoir. Such practices can also obscure dark cutting prediction using animal phenotypic characteristics such as weight and growth rate. Lack of coordination between shipping and slaughtering schedule or delay in slaughter due to any unexpected interruption in slaughter may prolong lairage time. To avoid such problems, cattle from the nearest farms may be scheduled for delivery and slaughter by the end of the day. In the event of any disruption at the abattoir, delivery of such cattle should be halted and planned accordingly. Heifers, especially those fed MGA, should not be shipped to an abattoir on a Friday or prior to a holiday to avoid a potential stay over a weekend/holiday in the event of any unexpected delay in slaughter.

Pre-slaughter lairage adjustment appears invaluable to minimize the risk of dark cutting. Lairage is more controllable than the hauling distance and short transportation followed by slaughter within five hours may have a less drastic effect towards dark-cutting. Cattle hauled over a long distance may be rested for a longer time provided the cattle have minimum disturbance in the lairage and have access to energetic and palatable diet immediately after shipment. If cattle are planned to be slaughtered in 10 to 72 h after shipment, the stay should be at the abattoir to avoid repetition of loading and unloading.

Although hormonal-implant frequency was confounded with production systems, there was no clear effect of implant treatment on dark cutting. Literature indicated that moderate implant frequency may not cause dark cutting provided the cattle are well-nourished and an adequate gap is maintained between terminal-implant and slaughter. Lack of implant effect could be because the usage of growth implants is strictly regulated in Canada where provision of growth promotants is subject to feeding of a grain diet. Literature indicating increased dark cutting due to increased implant frequency could be misleading if cattle, those not reaching to optimum slaughter weight compared to their herd mates, are re-implanted because in such case the problem could be related to animal physiology/growth rate. Another finding was that grazing cattle before finishing on grains may lower the problem of dark cutting in both heifers and steers compared to feeding dry forage or silage along with grains before finishing. Such relationships need to be validated in a Canadian context and may need further research.

7.3. Beef quality influenced by carcass grade and muscle proteome

The literature extensively cited comparisons of quality of dark cutting and normal beef; however, beef carcasses graded as normal can greatly vary in physical characteristics, such as marbling score, grade fat depth and grade rib eye area, and all these may also influence beef quality including colour. Results of chapter 5 not only established that dark cutting beef carcasses and their LT had phenotypic characteristics similar to that of Canada AA/USDA Select but also validated Holdstock et al. (2014) substantiating the existence of atypical dark cutting in both heifers and steers in the Canadian beef industry. It is ascertained that the atypical dark LT not only had glucidic potential sufficient to attain normal pH but some LT had glucidic potentials greater than for Canada AA LT. The atypical dark LT appeared to have a slow rate of post-mortem pH decline despite subcutaneous fat depth and carcass weight similar to that of Canada

AA. To investigate the underlying mechanism, a proteomic analysis of Canada AA, typical and atypical dark LT muscles from both sexes was performed. This study revealed downregulation of glycolytic enzymes in AB4 LT and supported early studies showing the relationship of muscle colour and glycolytic proteins (Canto et al. 2015). The results also established that dark cutting may exist despite sufficient intramuscular glycogen concentration.

The typical dark LT had persistently higher intramuscular pH and increased myoglobin concentration, and these muscles were from cattle that experienced prolonged stay in the lairage accompanied by frequent shipping. It can be suggested that prolonged lairage/repeated trucking not only lowered muscle glycogen but also resulted in excessive fluid loss, because of low water intake, and subsequently saturated muscle myoglobin. Results of chapter 6, where Canada AA, TB4 and AB4 had similar lairage time, indicated increased myoglobin in both TB4 and AB4 LT. Proteomic results further indicated compromised glycolytic potential and increased oxidative muscle proteins in AB4 and TB4 LT, respectively, implying that dark cutting was also associated with changes in the abundance of muscle proteins.

The results indicated increased toughness of beef from AB4 carcasses and validated Holdstock et al. (2014). The results also showed that increased toughness was not related to carcass weight, subcutaneous fat depth and cattle sex. The results substantiated the effectiveness of the Canadian beef grading system at identifying dark beef which may have had an atypically low pH but the increased toughness of this beef does not make it worthy of being sold with normal beef. The results, however, suggested an opportunity of recovering value from dark cutting carcasses by further sorting after they are identified as dark. The AB4 LT having intermediate pH may be utilized in making processed products that may not have concerns with shortened shelf life due to increased microbial growth in meat with a pH value greater than 6.0

(Newton and Gill 1981). Moreover, atypical beef LT had intermediate colour values while metmyoglobin development similar to Canada AA, therefore, steaks of such beef may be marketed after mechanical tenderization.

7.4. Gaps and hypothesis

The findings that dark cutting cattle/heifers had reduced weaning weight suggested reduced birth weight and subsequently slow growth rate in those cattle. The relationship of cattle birth weight with dark cutting has not been reported in the literature, so it can be hypothesized that calves having reduced weight at birth may be susceptible to cut dark. The results indicated that vulnerability to cutting dark was greater in cattle having a slower growth rate but there is a need to investigate if the reduced rate of gain and subsequently dark cutting are due to increased stress susceptibility. Moreover the relationship between growth rate and muscle physiology including muscle fibre types also needs to be established.

Although no effect of RFI, adjusted for ultrasound fat depth, was found on the incidence of dark cutting in the current study, how feed efficient cattle respond to handling and environmental stress has not yet been established. Feed efficient cattle may effectively preserve muscle glycogen/energy during any stress becasue of their reduced utilization of energy for maintenance and heat production (Herd & Arthur, 2009). However, reduced size of liver in feed efficient or cattle having low DMI (Basarab et al. 2003) is likely to slow down the rate of gluconeogenesis after any exertion and may leave the animal with low glycogen in muscles at the time of slaughter and consequently in danger of cutting dark. Slow muscle glycogen repletion has been associated with fasting (McVeigh and Tarrant 1982) and the latter can also lower glycogen concentration in the liver (Cart et al. 1973) but the effect of fasting on the rate of muscle glycogen restoration and enzymatic activities in feed efficient animals also needs to be

explored. Moreover, time required for physiological recovery after long hauling may also depend upon cattle intrinsic factors such as sex and breed along with pre- and post-shipping management but such information is not available from the literature.

The exact mechanism associated with reduced incidence of dark cutting in cattle grazed prior to finishing than in those fed dry forage/silage along with grains before finishing was unknown. However, it can be hypothesized that backgrounding on dry forage/silage along with grains and then finishing on a diet predominantly comprised of grains may result in acidosis and liver abscess. The effect of liver abscess and acidosis on muscle glycogen level especially during and after handling/shipping indeed has not been documented and needs investigation. Moreover, it can be hypothesized that feeding and keeping cattle in confinement for an extended period may reduce their ability to cope with the stress of handling and trucking and as a result there is increased depletion of energy reserves.

Results from the cattle slaughtered the same day and following an overnight stay at the abattoir also indicated a greater incidence of dark cutting in heifers than steers when those heifers did not previously receive MGA. This suggested that increased dark cutting in heifers is not only linked to oestrus activity prompted by withdrawal of MGA but also because heifers experience oestrus even without use and subsequent withdrawal of MGA. Moreover, loading and transportation can also induce onset of oestrus (Dr. Michael Dyck; personal communication). The sex effect on dark cutting could also be linked to muscle physiology but potential differences in muscle energy utilization and response to stress across the sexes is poorly understood. Johnston et al. (1981) found increased fast types of muscle fibres in heifers than in steers but heifers tend to deposit more fat, which represents an increased oxidative metabolism. Future research on dark cutting, therefore, needs to be focused on muscle physiological response

to pre-slaughter handling in heifers and steers. Additionally, a comparison between on-farm and commercial slaughtering of cattle, across the sexes, breeds and weight categories, may help to establish the effect of transportation and phenotype on dark cutting.

Furthermore, increased dark cutting in calf-fed heifers could be due to their greater response to any disruption in feeding and social hierarchy during oestrus than in experienced and mature (yearling-fed) heifers. Increased dark cutting in calf-fed heifers could also be due to a greater effect of reduced rumination time and dry matter intake during oestrus in primiparous heifers than in mature cows (Halli et al. 2015; Reith and Hoy 2012). These hypotheses, however, need validation in beef cattle because literature evidence is not definitive and is from dairy cattle. Sorting cattle especially those approaching oestrus before shipment to a slaughter plant is challenging. Infrared thermography, however, may be helpful to identify cattle in oestrus and those that are more reactive to handling so these cattle could be managed accordingly. The application and usefulness of such technique, however, has not been validated on commercial basis.

Moreover, the rate of postmortem pH decline could be related to cattle breed and so too could be the beef quality. A comparative study revealing the post-mortem pH decline in carcasses from different breeds finished on pasture and grains may help elucidate if breed is important. Establishing the relationship of cattle age and muscle fiber type within each beef breed as well as the effect of muscle fibre type on muscle physiology under normal circumstances and after stress is obligatory. Studies indicated that post-stress glycogen recovery rate depends upon muscle fibre types (Fournier et al. 2004; Lacourt and Tarrant 1985), suggesting that breeds may differ in rate of glycogen recovery if muscle fibre types differ across the breeds.

Because increased myoglobin is associated with increased oxidative type of muscle fibres, so it can be hypothesized that dark cutting could be related to increased predominant oxidative properties of muscle fibres. Proteomic results suggested increased oxidative characteristics of typical dark LT while downregulation of glycolytic enzymes in atypical dark LT. These characteristics could be due to the intermediate fibre type (IIA) having predominantly an oxidative nature. Downregulation of glycolytic enzymes in atypical dark LT could be related to cattle breed, gene polymorphism or post-translational modification but needs investigation. Proteomic of dark cutting carcasses from each breed may help identifying breed contribution to typical and atypical dark population. A genomic study could help identifying potential gene polymorphism across the breeds and subsequently develop genetic markers to sort the cattle at risk of cutting dark.

Increased toughness of atypical dark beef could be related to increased concentrations of phosphatidyl ethanolamine binding protein 1 (PEBP1) that reportedly lowered proteasome activity. However, the role of proteasomes in determining meat tenderness is poorly understood. Moreover, the inhibitory effect of PEBP1 and small heat shock proteins on proteolytic systems and subsequent meat tenderness also needs to be established.

Beef patties made of dark cutting beef with increased pH, had increased firmness, cooked yield and binding strength (Moiseev and Cornforth 1999), implying that processing suitability of dark cutting beef may depend upon ultimate pH. The processed products from atypical dark beef may be similar to that from normal beef but this needs further research. Although post-mortem ageing improved the tenderness of dark cutting beef, the effect of freezing and then thawing on tenderness of atypical dark beef should be examined. pH values in most of the muscles increase with darkness of *longissimus* muscles (Bass et al. 2008), suggesting that muscles, other than

longissimus, from atypical dark carcasses may have pH similar to those from normal carcasses and can be segregated for marketing as normal beef cuts. However, colour score and cooked beef quality of those muscles needs to be explored.

Although dark cutting is associated with many factors that may be confounded, each influencing factor may be reduced to two aspects of animal status: physical activity and/or dietary energy intake. Table 7.1 summarizes how specific factors that affect the incidence of dark cutting relate to physical activity and dietary energy intake, with increased physical activity and decreased dietary energy intake varying with each situation. For example, oestrus provides the worst scenario for dark cutting with physical activity increasing and dietary energy intake decreasing, thus doubly decreasing muscle energy retention and increasing the risk of dark cutting. Additionally, high environmental temperatures may decrease activity which should decrease the likelihood of dark cutting but also may reduce dietary energy intake enough to lead to negative energy balance and increased dark cutting. Like with oestrus, cattle finished on pasture may have inadequate energy intake and increased activity while grazing, which may reduce muscle energy reserves and consequently may increase the likelihood of dark cutting. Optimum dietary energy, specifically one that does not cause acidosis, combined with minimum physical activity especially before transport to the abattoir, may therefore lower the risk of cutting dark.

The data presented in this thesis indicate the relative contribution of various factors to dark cutting. The thesis has not only contributed to the existing scientific-knowledge about dark cutting beef but has also highlighted the gaps in the pre-slaughter management most likely responsible for the persistence of dark cutting in the Canadian beef industry. Although the causes of dark cutting are manifold, the most devastating factor appeared to be frequent trucking and

poor management in the lairage. Contribution of heifers to dark cutting has been previously documented but this thesis has further added to the research indicating that the problem is greatest in young heifers. Data generated also suggested sorting of dark cutting carcasses based upon pH to recover value from those carcasses. The results also ascertained that the problem of dark cutting is not only caused by reduced glycogen in the muscles but also due to downregulation of muscle glycolytic proteins.

Table 7.1. Relationship between physical activity and dietary energy intake and factors known to increase the risk of dark cutting in cattle

Factors	Physical activity	Energy intake	Dark cutting
Oestrus	Increased	Decreased	Increased
Hot temperature	Decreased	Decreased	Increased
Pasture grazing	Increased	Decreased	Increased

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