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Understanding the formation of the Grade B4 Beef in Alberta

by

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ABSTRACT

One hundred and seventy-nine (179) carcasses were surveyed for *Longissimus thoracis* (LT; rib eye) muscle pH from a commercial abattoir and results demonstrated that specific pH categories existed within the Canada B4 grade carcass population. About 72% of grade B4 carcasses had a muscle pH range of 6.0 to 7.0 and were categorized as classic dark cutters (CL), 21% of carcasses had a muscle pH range of 5.8 to 6.0 and were categorized as borderline dark cutters (BD), and 6% of carcasses had a muscle pH below 5.8 and were categorized as atypical dark cutters (AT). Carcass sides within the studied grade B4 sub-populations demonstrated reduced weights, but adequate muscling and fat cover. Muscle profiling of 12 muscles showed AT carcasses have potential for value recovery. In conclusion, Results indicate that animals within the AT pH category have sufficient energy *ante mortem* to produce beef of normal colour.

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LIST OF ABBREVIATIONS

A	Single A beef
AA	Double Grade A beef
AAA	Triple Grade A beef
AAFC	Agriculture and Agri-Food Canada
ADP	Adenosine diphosphate
AD	*Adductor*
AT	Atypical Dark Cutter
ATP	Adenosine triphosphate
BF	*Biceps femoris*
BD	Borderline Dark Cutter
B4	Classic Dark Cutter
CBGA	Canadian Beef Grading Agency
DCB	Dark Cutting Beef
DFD	Dark, Firm, and Dry
GM	*Gluteus medius*
IF	*Infraspinatus*
LD	*Longissimus dorsi*
LL	*Longissimus lumborum*
Mb	Myoglobin
Metmb	Metmyoglobin
Oxymb	Oxymyoglobin
pH	Measure of acid base scale using hydrogen ions
pHu	Ultimate pH
PM	*Psoas major*
PP	*Pectoralis profundi*
RF	*Rectus femoris*

SAG	Sexually active group
SM	*Semimembranosus*
ST	*Semimembranosus*
TB	*Triceps brachii*
USA	United States of America
Y1	Canada Yield Grade 1
Y2	Canada Yield Grade 2
Y3	Canada Yield Grade 3
USDA	United States Department of Agriculture

1.0. LITERATURE REVIEW

1.1. Introduction

When youthful beef carcasses have a *Longissimus thoracis* (LT; rib eye) muscle face that is dark red or purple, they are graded Canada B4 and their values are reduced by up to $1 per kg. These carcasses, known as 'dark-cutting' carcasses, usually arise when cattle have been physically stressed prior to slaughter such that muscle glycogen has been depleted, which prevents the ultimate pH (pH_U) within the muscle from declining below 6.0. Dark-cutting beef is unacceptable at retail because it is visually unappealing to consumers and it has a high pH that encourages bacterial spoilage thus reducing shelf-life (Ingram, 1948). Dark cutting beef that falls into the pH range of 5.8 to 5.9 postmortem has demonstrated increased levels of toughness when compared to beef with a normal pH_U ranges between 5.4 to 5.6 (Marsh, 1987; Jeremiah *et al.*, 1991). The reduced quality of dark-cutting beef has resulted in grading penalties being applied to dark-cutting carcasses in the United States of America (USA) (Wulf *et al.*, 2002).

Dark-cutting beef (DCB) is a meat quality disorder that has plagued the beef industry for decades, with the earliest observations dating back to 1774 (Lawrie, 1958). It was a well-known and well-described phenomenon until recently when dark beef was observed in carcasses that had rib eye muscles with mean pH values less than 6.0 (Murray, 1989; Page *et al.*, 2001; Robertson *et al.*, 2007). There has also been an increase in the occurrence of DCB carcasses in Alberta, with the prevalence increasing from less than 1% to above 2% within the

last six years (Alberta Beef Producers, personal communication). This increase in DCB carcasses has prompted renewed interest in the phenomenon and in the development of prevention strategies. The purpose of this literature review is to evaluate the causal mechanisms that produce DCB, examine the quality issues of the beef resulting from dark-cutting carcasses, and examine the factors that increase the prevalence of DCB.

1.2. Overview of the Canadian Beef Industry

1.2.1. Overview of the Canadian Beef Grading Agency

The Canadian Beef Grading Agency (CBGA) is the independent third party responsible for assessing grades of Canadian beef carcasses in accordance with national standards. The grading system in Canada is used to evaluate carcasses based on maturity, muscling, masculinity (stagginess), colour and texture of lean, colour and texture of fat, marbling and lean yield. Only youthful (under thirty months of age) carcasses from steers (male castrates) or heifers (young females) can qualify for quality grades. Carcasses eligible for the quality grades are then assessed for marbling of the rib eye muscle (slightly abundant = Prime, small = AAA, slight = AA, and traces = A) and yield (Y1 = 58% or more, Y2 = 54-58%, and Y3 = under 54%) of lean tissue. Yield grades are determined using the grading ruler, which was designed by researchers at the Agriculture and Agri-Food Canada Meat Research Laboratory at Lacombe, Alberta.

The United States Department of Agriculture (USDA) grading requirements for America beef are similar to those of Canada; however, there are some differences. Yield grade is calculated differently by the USDA grading

system, which uses an algorithm that places less selection pressure against overly fat carcasses for calculating lean yield than the Canadian algorithm. As a result Canadian carcasses are leaner than American carcasses. The minimum marbling requirement used for USDA Prime (slightly abundant), Choice (small), Select (slight) and Standard (trace) are the same requirements used to segregate grades in Canada Prime, AAA, AA and A, respectively. Canada's grading system is hierarchical, with grade determined by process of elimination; however the USDA system is weighted and allows for certain quality attributes to offset deficiencies in specific characteristics for muscling, colour, and fat cover. The maximum age requirement allowed into the Canadian grading system is 30 months of age while the USDA system allows 42 months of age into the youthful quality grade categories if carcasses exhibit sufficient levels of marbling. The American system will penalize dark cutters by no more than one full grade, compared to the Canadian system, which has a separate grade category for dark cutters (Canada B4). It is possible then in the USA for dark-cutting beef to be graded Choice, Select, or Standard.

Carcasses that do not qualify for the youthful quality A grades in the Canadian system will qualify for grades B1 through to B4; however these carcasses will be worth considerably less than those of the A grades. A Canada B1 grade carcass will meet all of the grade standards for the A grades but will be deficient in subcutaneous fat (< 2 mm) or devoid of marbling. The Canada B2 grade category captures carcasses with yellow fat, the Canada B3 grade category captures carcasses deficient in muscling, and the Canada grade B4 category

captures carcasses that are dark cutters, assessed subjectively by comparison to a standard colour chit (Canadian Beef Grading Agency, 1992).

1.2.2. Allocation of Canada Grade B4

On January 1st 1986, Canadian beef carcass grading regulations were modified to allocate carcasses that were judged to have dark-coloured muscles into a single grade; at the time of this modification, dark beef was included in the Canadian B2 grade or B3 grade (The Canada Gazette, 1987). Because DCB is such a serious meat quality defect, price penalties were applied to cattle carcasses that have DCB. For ease of applying this penalty, DCB was given its own grade, Canada B4, in 1991 and the grading regulations changed to increase the number of B grades from three to four. The new 1991 regulations outlined the following: B1 carcasses were those that lacked sufficient marbling or external fat thickness, B2 carcasses were those with yellow fat, B3 carcasses were those that lacked adequate muscling, and B4 carcasses were those that had a dark meat colour (Canadian Food Inspection Agency, 1992). Today, Canadian grade B4 carcasses generally exhibit a unique darkened or blackened discolouration of the rib eye muscle (*longissimus thoracis*) exposed at the grading site between the 12th and 13th rib and are known colloquially as "dark cutters". The Canadian Beef Grading Agency (CBGA) uses a colour chit to distinguish between normal coloured and abnormally dark carcasses. The official colour chit was recognized in January 2011 and was designed by researchers at the Agriculture and Agri-Food Canada Meat Research Laboratory at Lacombe, Alberta, as the official colour reference guide for Canadian grade B4 carcasses. When carcasses are

downgraded to Canada grade B4 they are still heavily discounted because of the associated deficits in meat quality.

1.2.3. Economics of Grade B4 Beef

In the first Canadian Beef Quality Audit in 1995 to 1996, Van Donkersgoed *et al.* (1997) surveyed 3,225 carcasses at four processing plants and found the incidence rate of dark cutters was 1% in steers and heifers, 4% in cows, and 17% in virgin bulls. The second audit was conducted from 1998 to 1999 and assessed meat quality of 3,981 carcasses at 5 processing plants and found the prevalence of dark cutters was 1% in steers, 0.5% in heifers, 3% in cows, and 14% in bulls (Van Donkersgoed *et al.*, 2001). Since then the Canadian Beef Grading Agency has been monitoring the frequency of dark cutters and has recently reported an unusually high incidence of dark cutters in Canada. Before 2005, dark-cutting beef in Western Canada occurred at a rate of 0.7% in young slaughtered cattle, however since 2005 the occurrence rate jumped to 1.5 to 2.5%.

Dark cutting once was about three times more common in Eastern Canada than in Western Canada, however statistics show that trend has reversed (Figure 1.1). Since 2005 the incidence of dark cutters has almost doubled in Western Canada while it has fallen below historical levels in the east (below 1%). The incidence of dark cutters was higher in Western than in Eastern Canada in 2009 and the trend appeared to be similar in 2010 (Canadian Beef Grading Agency, 2009).

Alberta has experienced this dramatic increase in the number of beef carcasses grading B4 since 2005, and estimates show the range of carcasses grading Canada B4 was between 1.5 to 2.5%, which is an increase from the historical range of 0.5 to 1.0% between 1999 and 2004 (Canadian Beef Grading Agency, 2009). Industry estimates dark-cutting carcasses are penalized between $0.80 and 1.00/kg carcass weight, costing producers on average $300 dollars a head. The cost of dark-cutting beef accumulates quickly considering the volume of animals slaughtered in Alberta. In 2008, Canada slaughtered 3.4 million head of cattle, the majority of which were slaughtered in the west (Alberta, 67%) as compared to the east (Ontario, 20%). Not only are producers losing money on dark-cutting beef, but the processor is also affected because the most susceptible muscles of dark-cutting carcasses are generally those of highest retail value.

1.3. Meat Quality of Grade B4 Beef

The quality of meat can be described by various terms depending on the sector of the beef industry that is being considered: the processor, the producer or the consumer. The consumer is the final judge and hence has the most important opinion on meat quality. Some factors that influence consumer-purchasing decisions are taste, tenderness, food safety, appearance, and juiciness, but colour is the criterion that consumers rely on most heavily for distinguishing quality and freshness (Renerre and Labas, 1987). Distinguishing meat quality includes subjective and objective evaluation of colour structure, appearance, flavour, juiciness and texture (Adegoke & Falade, 2005). The quality characteristics most

relevant to dark-cutting beef are microbial growth, colour, palatability, and tenderness.

1.3.1. Microbial Growth

Lawrie (2006b) reported that DCB is undesirable because it is aesthetically unpleasant and is more susceptible to microbial growth than beef with a normal, bright red colour. The increased incidence of microbial growth stems from the fact DCB has an abnormally high pH_U; classic DFD carcasses have a pH_U value greater than or equal to 6.0, which is considerably higher than the pH_U of 5.5 to 5.7 of a normally coloured beef muscle. The microbiology of meat with a high pH_U was reviewed by Newton & Gill, (1981), and bacteria that grow on the surfaces of B4 meat tend to produce ammonia and off-odours. The increased microbial growth associated with DCB significantly shortens its shelf life, contributing to the loss of value at retail of this beef (Rey *et al.*, 1976).

1.3.2. Colour

The colour of beef is an economic concern because of the role colour plays in the initial purchasing decisions of the consumer. Kropf (1980) suggested that the single greatest factor determining the purchase of meat at retail was probably muscle colour, however meat colour has a limited relationship to other quality attributes. Research has shown that quality is highly dependent on ultimate meat pH as well. In a meat quality survey in Japan, 58% of all participants (n = 10,941) identified meat colour as the most important factor in selecting beef products (Sanders *et al.*, 1997). If fresh beef colour is not a bright cherry red, the meat may

be considered undesirable or even spoiled (Hood & Riordan, 1973). Clydesdale and Ahmed (1978) argued that colour was perhaps the most important sensory attribute of a food because if it was deemed unacceptable, the food would not be purchased and/or eaten, and consequently all other sensory attributes would lose significance. Colour is highly valued by consumers and contributes to the quality of carcasses in the Canadian beef grading system, which relies upon visual assessment from Canadian meat graders. The only requirement in Canada for carcasses to grade B4 is the dark colour of lean at the grading site.

1.3.3. Palatability

Past research has found that beef from dark-cutting carcasses may have compromised meat quality. Research has shown that the palatability of DCB is reduced when compared to normal-coloured beef (Viljoen *et al.*, 2002). Wulf *et al.* (2002) found that cooked beef palatability was substantially lower for dark-cutting carcasses compared to normal carcasses. Wulf *et al.* (2002) found that 84% of dark-cutting steaks had off-flavours when compared to normal beef steaks, although there were no differences in juiciness or flavour intensity. Dransfield (1981) found no difference in juiciness of dark-cutting steaks when compared to normal beef, but he did find that steaks from DCB carcasses had less flavour and were less acceptable than those from normal carcasses.

Carcasses that grade Canada B4 are not assessed for marbling or yield however, Wulf *et al.* (2002) noted a trend toward DFD carcasses having 23% less (P=0.06) intramuscular fat in the *longissimus* muscle than normal carcasses, but

that they remained in the same marbling score when assessed by a qualified grader. In that study, DFD carcasses with low marbling scores had reduced cooked *longissimus* muscle palatability. Wulf *et al.* (2002) suggested that DFD carcasses appeared to have more marbling than could be detected chemically because of the increased contrast between the flecks of marbling and the darkened lean muscle. This may result in increased visibility of white fat and the perception of increased marbling. This means that DFD carcasses could receive higher marbling scores than normal, bright-red muscled carcasses with the same amount of chemically-detectable intramuscular fat.

1.3.4. Tenderness

Past research assessing the tenderness of DCB is conflicting. Beef has a "cooked toughness pH zone" that is maximized within a muscle pH range of 5.8 to 6.0 (Marsh, 1987). Because of the high pH_U of DCB, steaks from Canada B4 carcasses may fall within this toughness range. Wulf *et al.* (2002) found differences in tenderness when shear force values from normal and DCB steaks were compared, finding values for cooked *longissimus* steaks from DFD carcasses to have a mean shear force value that was 46% higher than that of normal pH beef steaks. However, several researchers have found DCB to be more tender than normal pH beef which has pH values less than 5.8 (Harrell *et al.*, 1978; Hedrick 1965; Marsh *et al.*, 1981; Martin *et al.*, 1971; Redeen *et al.*, 1974; Rhodes 1973). In addition Dransfield (1981) and Jeremiah *et al.* (1991) reported that DFD beef from bulls, which had pH values from 5.8 to 6.19, was more tender than beef of normal pH ($pH < 5.8$) from steers. These conflicting results may be due to the

range of muscle pH found within DCB muscles, with DCB muscles producing the toughest beef when having pH values below 6.0 and more tender beef when pH values are above 6.0.

1.4. Potential factors contributing to the incidence of Grade B4 Beef

The causes of DCB are well documented and low muscle glycogen levels are the basis for the condition. Glycogen levels at slaughter are influenced by two factors: the amount of glycogen stored in the muscle during the pre-slaughter period and the amount of glycogen lost from stress before slaughter. Management can impact the incidence rate of DCB carcasses. Scanga *et al.* (1998) found that mean percentages of DCB per pen differed ($P < .05$) between individual feed yards demonstrating that management strategies or yard design may affect the incidences of DCB. The amount of glycogen stored in the muscles in the pre-slaughter period can be controlled by proper management and is influenced by a variety of factors such as nutrition, gender, stress before slaughter, and hormonal growth implants. Even extreme weather variability (Grandin, 1992; Immonen & Puolanne, 2000; Smith, *et al.* 1993) and seasonality can play a role (Eldridge *et al.*, 1986; Kreikemeier *et al.* 1998; Munns & Burrell, 1965; Tarrant & Sherington, 1980) and tend to increase DCB.

1.4.1. Nutrition

Nutrients provide the necessary energy for the animal to replenish muscle glycogen reserves. High initial levels of glycogen in the muscle increase the likelihood of an animal enduring stressors prior to slaughter before meat quality is

affected. Animals on a low plane of energy such as forage finished beef may have low initial levels of muscle glycogen compared to animals on a high plane of nutrition such as a feedlot steers and can affect the ability of individual animals to moderate stress. Grazing animals on high quality green pastures two to three weeks before slaughter or providing a grain supplement while on pasture to ensure energy intake is adequate may increase muscle glycogen levels before slaughter (Immonen *et al.*, 2000) and may be a strategy to manage muscle glycogen to prevent dark-cutting within forage fed beef animals. The meat from pasture finished beef animals has been shown to be darker than that of meat from beef animals finished on concentrate when measured objectively and subjectively (Priolo *et al.*, 2001). However the majority of beef animals in Alberta will enter the feedlot before slaughter. Feedlot cattle have relatively high-energy intakes because of the energy density of the rations typically fed in feedlots. This means high levels of energy should be reserved within the muscle to combat stress. Ensuring the blood has high levels of glucose means muscles are most likely able to endure stress before slaughter and before glycogen reserves within the muscle are depleted (Immonen *et al.*, 2000). However the rate at which glycogen is metabolized within muscles may differ among individual animals even if levels of energy are optimized before slaughter.

1.4.2. Gender

Bulls pose a high risk for DCB due to their aggressive and dominating behaviour (Warriss, 1984; Kenny & Tarrant, 1988). Heifers can also be more likely to produce a DCB carcass than steers especially if exhibiting estrus as they

typically form what is known as a sexually active group (SAG). The SAG group will demonstrate mounting and excitable behaviour, which is detrimental to weight gains and nutrient intake and will increase the risk of DCB. Scanga *et al.* (1998) found that gender contributes ($P< 0.05$) to the incidence of dark cutters and intact heifers produced higher ($P < 0.05$) mean percentages of dark cutters than steers or spayed heifers across all pens and feed yards.

Carcass composition of different genders may also impact the susceptibility of carcasses to cut dark. Many bulls are excessively lean when slaughtered and will not possess enough fat to meet even the minimum standards for grading. In a study examining the influence of fat thickness on carcass quality traits, Aalhus *et al.* (2001) reported a lightened muscle colour as fat thickness increased from less than 5 mm to greater than 40 mm of grade fat. Thus increased fatness in heifers per se would be expected to contribute to a light meat colour rather than dark meat colour. However, under normal feedlot conditions, the reproductive cycle of heifers is controlled through the use of melengesterol acetate (MGA) (Patterson *et al.*, 1989), which can potentially alter behaviour and impact meat quality. Disruption of the control of the reproductive cycle by any circumstance can result in a synchronized and rapid return to estrus, which is a known cause of dark-cutting (Kenny and Tarrant, 1988).

1.4.3. Pre-slaughter Stress

Temperament may be a contributing factor for animals that exhibit DCB; an excitable or temperamental animal may over-react to stress and may demonstrate behaviours that will exhaust muscle glycogen stores (Voisinet *et al.*

1997). Animals that are more sensitive to stress stimuli are more likely to exhibit flight or fight behaviours, which may determine the level of stress they experience. The idea of the response to stress being known as the fight or flight response was first proposed in 1929 (Cannon, 1929). This response to stress activates neurons in the hypothalamus causing the release of epinephrine from the adrenal medulla. In response to epinephrine, heart rate, glucose availability, blood pressure and blood volume are increased as blood flow is directed away from non-essential organs in preparation to fight or flee (Lay & Wilson, 2001). Emotional stress can be caused when unfamiliar cattle are mixed before slaughter because mixing results in behaviour associated with the re-establishment of hierarchy such as milling, mounting, physical exertion, and fighting (Jones & Tong, 1989). Mixing animals or introducing new cattle to a group can be quite enticing for a feedlot operator if the pen of animals has not grown consistently. To maximize profit a feedlot operator will only send finished animals to slaughter, but this usually does not consist of the whole pen. A group of animals may be mixed before slaughter in order to fill the cattle liners. Mixing cattle before slaughter can increase the stress of animals and can increase the risk of DCB (McVeigh & Tarrant, 1983).

Ante mortem stress depends on a variety of factors such as cattle transport, handling, and abattoir handing. Handling cattle can result in two forms of stress: physiological and physical (Grandin, 1997). Physiological refers to the stress that comes from restraint and handling, while physical refers to stress from hunger, thirst, fatigue, temperature extremes, or injuries from poor driving conditions.

Stress during transport can occur as an animal struggles to keep its balance by tensing its muscles, which depletes glycogen stores. Extreme temperatures while travelling can cause stress through shivering to keep warm or heat exhaustion, and both increase the use of energy and glycogen. All of these factors contribute to exhausting glycogen stores before slaughter and thus affect meat quality (Hall *et al.*, 1944).

The implications of stress from transport are difficult to predict. Stephens (1980) found that a 1 to 4 h transport experience for cattle elevated plasma corticosteroid levels more than other stressful experiences such as castration, dehorning or withdrawal of water over 48 hours. Past research has found that loading, unloading, or mixing of cattle was most likely the critical stressor at time of transport and not the actually physical transportation of animals (Bench, personal communication). Cattle tend to habituate to travel if given appropriate space as observed by Eldridge (1988) who measured stress based on heart rate. They found that heart rate only increased 15% during transport with adequate space compared to the baseline that was measured while cattle were grazing.

1.4.4. ***Stress hormones***

Certain "stress hormones" will become apparent in the blood when an animal is in a stressful situation; these include catecholamines and corticoids (Monin, 2004). Catecholamines are chemical messengers that affect tissues of the sympathetic nervous system (Eckert, 1988). These messengers elicit a series of physiological responses increasing heart and respiration rates, elevating body

temperature, increasing hepatic muscle glycogenolysis and decreasing protein degradation (Knowles & Warriss, 2007). Examples of catecholamines produced by the adrenal gland are epinephrine and norepinephrine. These hormones promote glycolysis and breaking down of skeletal muscle glycogen to glucose-6-phosphate. Glycogen depletion induced by catecholamines has been shown to cause the dark-cutting condition (Apple *et al.*, 2005). Increased levels of epinephrine and norepinephrine were associated with reduced glucose concentration in the *longissimus thoracis* muscles and increased rates of respiration (Bass *et al.*, 2010). Muscle glycogen can be depleted by catecholamine release or through strenuous exercise (Tarrant, 1989). The adrenal gland also produces cortisol during episodes of stress to maintain homeostasis. Plasma levels of cortisol have been used to indicate the levels of behavioural stress in cattle (Stahringer *et al.*, 1990) and increase when cattle where subjected to social isolation and physical restraint (Munksgaard & Simonsen, 1996; Rushen, *et al.* 1999).

The use of adrenaline to induce dark-cutting was first reported by Hedrick *et al.* (1959). Research has noted the pattern of darkened muscles following adrenaline injection does not match the pattern of darkened muscles seen among naturally occurring Canada B4 carcasses. Tarrant and Sherington (1980) found the hind quarter muscles (AD, GM, SM and ST) and the LD muscle were affected by a high pH in dark cutting carcasses collected from the abattoir. When dark-cutting carcasses were experimentally induced via subcutaneous adrenaline injection, the pattern of high pH among muscles changed; there was a generalized increase in

pH_u among all muscles and most notably in the front quarter. The different patterns of elevated pH among muscles demonstrated by "natural" and "induced" dark cutters may relate to the type of stress and the different responses of oxidative and glycolytic muscle fibres to the corresponding stress. The results suggested that different metabolic pathways are being activated in each circumstance. Slow twitch muscle fibres are more responsive to circulating levels of adrenaline as shown by decreased levels of glycogen within slow twitch muscles after adrenaline administration (Tarrant, 1989). The pH differences among muscles post-mortem directly relates to LT muscle pH and impacts the colour of the muscles found among the pH categories.

1.4.5. Implants

Individual groups of cattle can fall into several high-risk categories for DCB, but the emerging cause of low glycogen levels in the muscle may be due to hormonal growth promotants (Scanga *et al.*, 1998; Thompson, 2002). Research has shown mixed results about the impacts of growth promoting implants in the incidence of DCB. Scanga *et al.* (1998) found that combination implants, which contain both estrogen and androgen components, when used upon entry to the feedlot resulted in a 6% increase of DCB when compared to steers implanted with solely estrogenic implants. Implants containing estrogen and testosterone have been shown to increase sexual and antagonistic behaviours (Dykeman, *et al.* 1982). In contrast, implants have also been shown to reduce DCB, with the use of estrogenic implants in steers decreasing the occurrence of DCB from 9.2% per thousand cattle shipped to 2.0 and 0.5% per thousand cattle shipped (Scanga *et*

al., 1998). Implants appeared to modify growth curves affecting rates of gain and fat distribution by altering metabolism, which may pre-dispose beef animals to cut dark. Scanga *et al.* (1998) also found an inverse relationship between the pay-out period and percentage of DCB, observing that as the duration between terminal implants increased the mean percentage of DCB declined. Producers that held cattle 100 days past re-implantation (the pay-out period) could reduce DCB by 38% in heifers and 69% in steers. Implants can accelerate muscle metabolism and contribute to low muscle glycogen levels, which may impact lean colour of beef muscles.

1.4.6. Seasonality

Several researchers have observed a seasonal effect in the incidence of DCB such as increases in autumn (Munns and Burrell, 1966; Tarrant & Sherington, 1980). When the frequencies of dark-cutting beef were assessed by month, Kreikemeier *et al.* (1998) found that DFD carcasses occurred more frequently in August, September, and October than in other months. The Canadian Beef Quality Audit (Van Donkersgoed *et al.*, 1997) found that frequencies of dark-cutting in beef cows were 15% in August, 7% in October, and 0% in March, while Jones and Tong (1989) found March and April had the highest frequencies and December the lowest frequency for DCB. Spring and fall months experience large differences in diurnal temperature, which places stress on the animal to maintain homeostasis.

1.5. Conversion of Muscle to Meat

The basis for the dark cutting condition stems from glycogen depletion within the muscles, which results from ante-mortem stressors and affects the rate and extent of glycolysis within muscles post mortem. To completely understand the connection of muscle glycogen to raw beef colour one must first explore post mortem muscle metabolism, the factors that affect the rate of muscle metabolism, and the state and functions of muscle proteins.

1.5.1. Conversion of muscle to meat

Slaughter is the beginning of the conversion of muscle to meat. Slaughter begins with stunning, shackling, and exsanguination (removal of blood from the system) of the animal. The blood is removed from the circulatory system, which removes the supply of nutrients such as free glucose and oxygen and also the means of waste removal from tissues. Cascades of intracellular events occur in response and the onset of rigor mortis occurs within 12 to 24 h (Bodwell *et al.*, 1965). Rigor mortis or stiffening after death is reached as post-mortem glycolysis proceeds and the muscles become inextensible (Lawrie, 2006a).

1.5.2. Composition of Muscle

Muscle is composed of many muscle fibres (muscle cells), which are composed of parallel sub-fibres called myofibrils. Myofibrils contain repeating units of contractile proteins called sarcomeres that are responsible for the contractibility of muscle. Muscle fibres contain many organelles like the nucleus, mitochondria, the sarcoplasmic reticulum, stored glycogen, as well as enzymes

and contractile proteins in the sarcoplasm or muscle cell cytoplasm (Solomon *et al.*, 1988). Sarcomeres are composed of thick filaments, which consist primarily of myosin molecules, and thin filaments, which are composed of actin molecules and proteins like troponin and tropomyosin. Structural proteins such as titin and nebulin, which keep the thick and thin filaments in register, are also involved in maintaining sarcomere integrity.

1.5.3. Muscle Contraction

Muscle contraction is described by Huxley and Hanson (1954) as the sliding filament hypothesis and describes how chemical energy is converted into movement. Muscle contractions are fueled by adenosine triphosphate (ATP) and begin with a stimulus traveling from the brain or spinal cord, to a nerve, and then to the sarcolemma (cell membrane of the muscle fibre). The stimulus signals a neurotransmitter called acetylcholine to be released which binds to a receptor on the sarcolemma. This produces a depolarization of the muscle fibre membrane, reversing the polarity of the membrane. The inside of a muscle cell is usually at a negative potential due to the high concentration of K+ ions held within the cell and the high concentration of Na+ ions maintained outside of the cell. As the muscle fibre goes through depolarization the balance of potassium and sodium ions is reversed. Depolarization also causes the release of a high concentration of calcium ions from the sarcoplasmic reticulum. High concentrations of calcium are confined to the sarcoplasmic reticulum and are not found freely in the sarcoplasm of relaxed muscle. Calcium ions saturate the troponin and tropomyosin and allow binding sites on the actin molecules to be exposed and attraction to the myosin

heads causing a sliding of the filaments and contraction (Warriss, 2010). The relaxed state is regained the presence of ATP, which is present in the form of Mg^{2+} ions, relaxation is achieved by ATP binding to myosin and causing disassociation from actin thus preventing the binding of actin and myosin (Aberle *et al.*, 2001). As the resting state is re-established, the Ca^{2+}-ATPase proteins, located in the sarcoplasmic reticulum, pump excess calcium from the sarcoplasm back into the sarcoplasmic reticulum and calsequestrin binds calcium in an inactive form. The retrieval of calcium requires energy, which is produced from the enzymatic hydrolysis of ATP to adensosine diphosphate (ADP) and inorganic phosphate (Forrest *et al.*, 1975).

1.5.4. Rigor Mortis

After death, the Mg^{2+}-ATP supply is depleted and calcium is released from the sarcoplasmic reticulum. The free Ca^{2+} concentration in the sarcoplasm binds to troponin-C and initiates muscle contraction. Tropomyosin is relieved of inhibition of cross bridge formation duties and sites for myosin heads to attach with actin chains are exposed as the protein complex actomyosin is formed and cross-bridges develop contractile force. The myosin heads change their angles and climb along the actin molecules causing the filaments to slide past each other (Lawrie, 1998) locking the muscles into the stiffening after death or rigor mortis. ATP is mandatory for the basis of muscle movement, however after death it is available in limited supplies. ATP causes the initial shift in the position of the myosin heads necessary for muscle contraction, but is also necessary for the release the myosin heads from actin filaments and muscle relaxation. The ATP

required for relaxation and pumping calcium ions from the sarcoplasm back into the sarcoplasmic reticulum is not available postmortem. As the carcass cools muscle pH declines, denaturing sarcoplasmic membranes and leaking calcium into the sarcoplasm as calcium concentrations increase stiffening of muscles occur.

1.5.3. Post mortem Muscle Metabolism

Muscle cells remain metabolically active post mortem and try to maintain homeostasis and levels of ATP (energy) necessary for contraction, but as the supplies of oxygen diminish cells shift their metabolism to anaerobically oxidize both glucose and glycogen. Live muscle cells rely primarily on aerobic energy pathways like the citric acid cycle and oxidative phosphorylation for the most efficient ATP production, however post mortem cells are forced to rely upon anaerobic energy pathways such as those that access phosphocreatine stores and glycolysis. Muscle cells rely on anaerobic pathways to produce energy post mortem because limited amounts of energy will be produced from the citric acid cycle and oxidative phosphorylation due to the reliance of oxygen of these pathways (Honikel, 2004). The cells modify the primary pathways used to regenerate ATP and begin to utilize glycogen stores as an energy source rather than glucose that was previously supplied by the blood (Swatland, 1994). Cells utilizing glycogen stores in the absence of oxygen are less efficient and synthesize fewer ATP through glycolysis compared to the combination of glycolysis, the citric acid cycle, and oxidative phosphorylation (Eckert, 1988). The glycogen stores necessary for ATP generation will be available in limited concentrations in

some of the muscles of dark cutting carcasses and will affect post mortem metabolism.

Glycogen phosphorylase is responsible for converting glycogen into glucose-1-phosphate, which will enter glycolysis. Glycolysis is the conversion of glucose to pyruvate in a sequence of phosphorylating reactions that occur in the cytoplasm of the cell (Hinchcliff, *et al.,* 2008). The byproducts of aerobic glycolysis are carbon dioxide and water as pyruvate is consumed in the Krebs cycle and oxidative phosphorylation follows. However during anaerobic glycolysis, pyruvate is converted into lactic acid by lactate dehydrogenase. The lactic acid disassociates into a hydrogen ion and a lactate ion. Lactate and hydrogen ions slowly accumulate and the hydrogen ions cause the acidification of the cell sarcoplasm causing the intramuscular pH to decline. Glycolysis will cease when the acidic environment disables the glycolytic enzymes, with phosphofructokinase being particularly susceptible to low sarcoplasmic pH (Pearson and Young, 1989). The declining muscle pH compromises the structure of the membranes of the sarcoplasmic reticulum, causing a release of calcium ions, which activate numerous actin/myosin linkages (Aalhus *et al.*, 1994). The concentration of ATP in the muscle is insufficient to free all the actin/myosin crosslinks and the actin and myosin become locked together in mortis, which is resolved with enzymatic tenderization that occurs over time.

1.5.4. Post mortem Ultimate pH Decline

The enzymatic activity, rate of temperature decline, and glycogen content within muscles will control the ultimate carcass pH decline after slaughter (Gardener *et al.*, 2005). Healthy, well-fed, rested, unstressed animals have a body pH of approximately 7.2 to 7.0, which falls after slaughter as glycogen is metabolized into the byproducts of hydrogen ions and lactic acid to a pH_U of between 5.4 and 5.7 (Murray *et al.*, 1995). If animals are exposed to stress immediately prior to slaughter the muscle glycogen reserves could be exhausted, which will restrict the production of lactic acid and hence hydrogen ions and result in an elevated pH_U post mortem (Tarrant, 1989). The primary cause of DFD is insufficient levels of muscle glycogen to reduce pH_U postmortem. The pH decline after death varies among muscles and species due to the glycolytic rate. Glycolysis occurs faster in pork, poultry, and lamb than in beef; this is due to the rate of ATP consumption by the muscle cells. The time to reach a pH_U of 5.5 to 5.7 in the *longissimus dorsi* in pigs can take about 6 h in normal carcasses or about 1 h in pale, soft, exudative (PSE) pork compared to about 18 h in normal beef carcasses (Honikel, 2004).

1.6. Fresh Meat Colour Development

1.6.1.Colourimetry and Measurement of Colour

The human perception of colour is based on the light that is reflected from a surface. Colour can be perceived differently by different people hence a standardized measure of colour can be achieved only through objective colour

measurement with a colourimeter that converts colour to numerical values described by International standards. The Commission Internationale de L'Eclairage (CIE) uses the parameters of *L**, *a**, and *b** to describe colour objectively. *L** or lightness differentiates between dull dark colours, which are close to black in the colour sphere and lack luminosity, and bright colours with an apparent glare or reflectance that are high in luminosity and are closer to white within the colour spectrum. The *L** lightness maximum is 100 representing a perfect reflecting diffuser and its minimum is 0 representing black. The *a** value represents the green to red colour spectrum within the colour sphere with negative *a** values indicating green and positive *a** values indicating red or magenta. The *b** values represent shifts of colour between blue and yellow, with negative *b** values indicating blue and positive *b** values indicating yellow (Murray *et al.*, 1995).

1.6.2. Dynamics of Myoglobin State

Colour development, as explained by Young and West (2001), is dynamic because muscle respires post mortem, enzymes and organelles are still active, meat is continually degrading, and the colour of meat is transient and always changing. The perceived colour of meat depends on many factors such as the concentration and chemical state of myoglobin, the morphology of the muscle structure, and the light absorbing or light-scattering properties of the muscle (Walters, 1975). The heme pigment myoglobin (Mb), also known as deoxymyoglobin (Mb), is continually converted between chemical states and

several biochemistry reviews have explored the relevance of myoglobin to meat colour (Cornforth, 1994; Faustman & Cassens, 1990; Renerre, 1990). When myoglobin is oxidized it is termed metmyoglobin (MMb) and when it is oxygenated it is termed oxymyoglobin (OMb). From metmyoglobin it can be reduced to myoglobin. The colour development of fresh meats will depend on the presence, concentrations, and forms of myoglobin (Ouali *et al.*, 2006).

The perceived colour of fresh meat is based on light reflected from the surface of meat. Visible light that escapes from the muscle is reflected and captured by the eye giving muscle its perceived colour. Myoglobin is the pigment within meat that strongly absorbs green light, which means that red, yellow, and orange wavelengths are reflected. Muscle colorimeter values show that high muscle pH values are associated with beef that has blue and green hues, while beef with a low pH is associated with red and yellow hues (Jeremiah *et al.*, 1991; Wulf *et al.*, 1997).

1.6.3. Myoglobin Structure

Myoglobin is a water-soluble protein and is composed of two portions: a protein portion called globin and non-protein portion consisting of a porphyrin ring with a central iron atom. The iron atom plays a primary role in meat colour because the colour is expressed based on the chemical state of the iron and the type of compounds bound to it (Giddings & Solberg, 1977; Mancini & Hunt, 2005). Porphyrin is derived from a Greek word meaning purple and myoglobin is the primary meat pigment responsible for an intense purplish-red pigmentation

when partial pressure is low and oxygen is not bound by the iron atom. A high concentration of myoglobin produces raw meats with an intense red colour when exposed to oxygen and differences of myoglobin concentrations contribute to colour differences in beef muscle compared to pork or lamb muscle. Heme proteins such as hemoglobin and cytochrome c may also play a role in meat colour, but the primary meat pigment in myoglobin.

1.6.4. Function of Myoglobin

Myoglobin is not to be confused with hemoglobin, which differs in biological function. Hemoglobin is responsible for the transport of oxygen in the blood from the lungs to the muscle cell, while myoglobin acts as an intracellular reservoir for oxygen storage within the muscle. Enzymes and organelles, such as mitochondria, within muscle cells consume stored oxygen during the production of energy through the citric acid cycle (Renerre, 1990). Myoglobin can bind one oxygen molecule and has a higher affinity for oxygen than hemoglobin; therefore oxygen transported by hemoglobin is readily transferred to myoglobin. Hemoglobin can bind four oxygen molecules to make transportation of oxygen over long distances efficient (Akers and Denbow, 2008).

1.6.5. Oxymyoglobin

The iron atom within myoglobin stores oxygen and forms the meat pigment oxymyoglobin; the bright red colour of beef, referred to as “bloom”, is produced when oxygen is bound at the sixth coordination site of the myoglobin haem ring. When oxygen is not present and oxygen partial pressure is low Mb is

the dominant meat pigment and fresh meat appears dark purplish red colour; this is the predominant pigment in DCB. It is the presence of oxymyoglobin that reduces the reflectance of blue light (wavelengths around 470nm) and reflects increased red light (wavelengths around >600 nm), which is perceived as bright cherry red (Young and West, 2001).

1.6.6. Metmyoglobin

When oxygen contacts the exposed surface of beef, myoglobin is converted to oxymyoglobin and the iron is in a ferrous oxidative state (Fe2+). This phenomenon is referred to as "bloom" when the meat is fully oxygenated, and this "bloom" is a temporary state. Mb pigments that are constantly exposed to oxygen can be oxidized to metmyoglobin (Fe 3+) and the meat changes to brownish red (Giddings, 1977). In living muscle the concentration of MMb is low due to the activity of metmyoglobin reductase, which is an enzyme that combines with cofactor NADH and coenzyme cytochrome b4 to revert MMb Fe^{3+} back to Fe^{2+} to restore levels of Mb. It is in this state that Mb is capable of binding with oxygen and restoring the red colour. As meat ages post mortem, the Fe^{3+} ions accumulate due to the reduced activity of metmyoglobin reductase and the bright red colour of muscle is slowly replaced by a brown colouration as MMb dominates. The brown colouration becomes noticeable to the naked human eye when about 20% of surface pigments have oxidized, which may result in consumer discrimination (Warriss, 2010).

1.7. Reflectance and Light Scattering

Protein denaturation, an irreversible process, is the occurrence of chemical bonds between and within molecules breaking and is a natural part of the degradation process of muscle postmortem. When chemical bonds break, proteins are released from their complex structures and stretch into straight chains of amino acids, and loss of functionality and water binding ability ensues (Huff-Lonergan & Lonergan, 2005). The sarcoplasmic proteins aggregate as the pH drops causing the cytoplasm to become dense with protein coagulation. The muscular lattice also begins to expel water as proteins approach the isoelectric point, losing their charges and the ability to hold water (Offer & Knight, 1988). An elevated muscle pH postmortem can slow the rate of postmortem degradation and maintain the strength of the bonds between proteins and water. Such is the case in DFD beef, which maintains a high muscle pH postmortem; water remains bound to the intact proteins, and a high water holding capacity is the result. Strong protein structures will mean limited free water is available within and on the surface of meat (Ledward *et al.*, 1992).

1.7.1. Water Holding Capacity and Effects on Colour

The increased water holding capacity of dark-cutting beef has several implications on beef colour. The water holding capacity of meat is the ability of meat to retain water when external force is applied. Normally water is held within meat as bound, immobilized and free water. Charged hydrophilic groups found on muscle proteins, which strongly attract and hold water, are responsible for

retaining moisture within meat. Immobilized water is attracted to the bound water, so the bonds are not as strong as those of water affiliated with the hydrophilic groups of intact proteins. Free water is held by capillary forces and is not affected by the charged groups of intact proteins. Water holding capacity is lowest at the isoelectric point, which is when the number of positively charged groups of the myofibrillar proteins equals the number of negatively charged groups. At the isoelectric point proteins do not have a net charge, thus no charge is available to hold water. The isoelectric point of muscle is at a pH value of about 5.0 or 5.1 (Swatland, 1994). The high pH of DCB increases the pH 'distance' from the isoelectric point and so the overall water holding capacity of DCB meat is much greater than that of conventional beef.

DCB maintains a high level of bound water and low amount of free water because the majority of water is held within the muscle fibres. These saturated muscle fibres swell, creating a tight barrier to oxygen, which results in decreased oxygen for oxymyoglobin formation (Page *et al.*, 2001). Most of the water is bound tightly to proteins within muscle fibres meaning that the proportion of freely bound water, which is responsible for light reflection, is reduced (Walters, 1975). In addition the high pH would prevent further water loss that normally occurs as cell walls disintegrate during pH decline. This also means less free water is available to be lost post mortem which is known as purge or drip loss. The reduced amount of free water reflects less light and increases light absorption both of which contribute to a dark meat surface (Ledward *et al.*, 1992; Lister & Spencer, 1983).

In normal pH beef, the abundance of freely bound water reflects more light than DCB, while the microstructure of the meat disrupts these light pathways randomizing the reflecting light and contributing to light scattering. This light scattering increases the luminosity of the meat surface colour, affecting meat appearance. A high amount of light scattering means the light pathways are short and a minimal amount of light is absorbed, therefore the meat will appear light or pale as selective light absorbance of myoglobin is reduced (Swatland, 2004). Weak scattering, as in DCB beef, will produce long light pathways and myoglobin will be fully visible as translucency of the beef is enhanced and the beef will appear dark. MacDougall *et al.* (1969) stated that pH can affect the translucency of muscle and hence the perceived colour; the abnormal pH_U of DFD beef increases muscle translucency.

1.7. The Role of Mitochondria in Muscle Colour

1.7.1. Colour Development

The interrelationship between mitochondria and myoglobin plays a significant role in both fresh meat colour development and colour stability (Mancini & Ramanathan, 2010). Both pre-rigor muscle and dark-cutting beef are dark purple because of high pH and mitochondrial respiration. The elevated pH of classic DCB (pH of > 6.0) provides a stable environment for enzymes and mitochondria and prolongs survival and activity levels. Lawrie (1958) demonstrated the activity of the mitochondrial enzyme cytochrome oxidase was enhanced at high pH values, reflective of the high pH environment of DCB. Enhancing mitochondrial activity will elevate oxygen consumption within the

tissue and may darken muscle by subsequently reducing oxygenation of myoglobin to oxymyoglobin (Ashmore *et al.*, 1972).

Many processes compete with myoglobin for oxygen, but the primary competitors are likely mitochondria (Mancini & Ramanathan, 2010). If mitochondria have a higher oxygen affinity for oxygen than myoglobin, the dominant pigment will become the de-oxygenated form of myoglobin, which produces a dark beef colour (Ashmore *et al.*, 1972; Cornforth & Egbert, 1985). When partial pressure of oxygen is low and free oxygen is inadequate the favoured meat pigment will be myoglobin and the colour will appear dark purple (Egbert & Cornforth, 1986). Normally the pH of beef post mortem is reduced via byproducts of glycolysis to a pH range of 5.4 to 5.8, which impairs mitochondrial activity over time. The effect of low pH on mitochondrial structure, integrity, and function has been well documented (Ashmore *et al.*, 1972; Cheah & Cheah, 1974; Giddings & Hultin, 1974). Ashmore (1973) reported that oxygen consumption from "dark cutters" continued for at least 5 days post mortem. As pH declines mitochondrial activity will decrease and oxygen will become increasingly available for conversion of myoglobin to oxymyoglobin resulting in the bright red "blooming of the meat". Although mitochondrial concentration plays a role in muscle colour, an understanding of haem pigment interaction is essential when studying the formation of colour within a muscle.

1.8. Chilling, Metabolism, and Muscle Colour

1.8.1. Colour Stability Among Muscles

Faustman and Cassens (1990) observed that muscle metabolism in the live animal influences the characteristics of its muscle post mortem. Muscles will have varying levels of glycolytic or oxidative activity, and these differences among muscles will contribute to colour stability postmortem (Ledward, 1985). Muscles with a high oxidative capacity such as those composed predominantly of red fibres will have high concentrations of mitochondria, and should therefore be darker and have lower colour stability than those composed of white fibres. In support of this, Lanari and Cassens (1991) found that GM muscles were less colour stable than LD muscle. LD muscles, which provide a postural support, were less oxidative than GM muscles, which are used primarily for locomotion. The GM muscle is more oxidative than the LD muscle, which reflects the fact that the GM required more oxygen, energy, and contains more mitochondria than LD muscles based on its primary function.

Muscles will vary in colour based on myoglobin content; muscles that have high levels of myoglobin like the *psoas major* (tenderloin) will bloom quickly. The high concentrations of myoglobin are accompanied by high amounts of iron, which contribute to oxidation and formation of metmyoglobin. The *psoas* muscle blooms quickly upon oxygen exposure and turns brown more rapidly than a muscle like the LD, which has comparatively low levels of myoglobin and iron. Vitamin E has been shown to improve colour stability of meat exposed to air by reducing the amount of lipid oxidation within meat and reducing metmyoglobin.

Concentration of myoglobin, iron, and meat colour are highly correlated; a muscle that is redder than another probably has more myoglobin and thus more iron.

1.8.2. Muscle Fibre Type

Muscle fibres can be categorized into three types: oxidative (sometimes referred to as red fibres), glycolytic (sometimes referred to as white fibres), and intermediate muscle fibres. The reason that these fibres are sometimes distinguished by colour is due to the enzymes and metabolic activity of the corresponding fibre; oxidative muscle fibres have high concentrations of myoglobin (producing a red colour), high capillary and mitochondria density, are not easily fatigued, and predominantly use aerobic metabolism. Aerobic metabolism relies heavily on enzymes and activities that need oxygen such as mitochondria and cytochromes (Warriss, 2010). Cytochromes require oxygen and are activated through oxidative phosphorylation as the cell oxidizes nutrients to produce energy. Both red and intermediate muscle fibres have smaller diameters than white (glycolytic) fibres. Intermediate fibres, as the name states, have a metabolism that is somewhere between glycolytic and oxidative fibres. Glycolytic muscle fibres primarily utilize stores of glycogen for anaerobic metabolism, are easily fatigued, and have a larger fibre diameter. Glycolytic muscles fibres appear white in comparison to red oxidative muscle fibres and contain fewer mitochondria and cytochromes. As explained by Warriss (2010), muscle fibre types can be identified histochemically using dyes that distinguish between cells that have either strong ATP-ase and phosphoylase activity (glycolytic fibres) or

strong cytochrome oxidase and succinate dehydrogenase activity (oxidative fibres).

The amount of glycogen within muscle fibres relates to their preferred metabolism. Glycolytic muscle fibres will have a larger reservoir of muscle glycogen than oxidative fibres (Aberle *et al.*, 2001). Muscles are composed of a variety of types of muscle fibres, but some types of fibres may be more prevalent depending on the use of the muscle. The composition of muscle fibre type may be helpful in determining why some muscles and some species are susceptible to the dark-cutting condition. The initial levels of glycogen at death impact the susceptibility of cutting dark. Slow twitch muscle fibres are more responsive to circulating levels of adrenaline than fast-twitch muscle fibres; therefore, levels of glycogen within slow twitch muscles will be lower than in fast-twitch muscles after adrenaline administration (Tarrant, 1989). Lacourte and Tarrant (1985) also demonstrated the differences of fast and slow twitch muscle fibres in response to stress. Fast muscle fibres were more readily depleted when cattle were mixed prior to slaughter than slow fibres, while slow fibres were more readily depleted by adrenaline injection ante mortem than fast muscle fibres. The use of propranolol, an adrenergic-blocking agent, prevented muscle glycogen depletion in animals that were injected with adrenaline (Ashmore *et al.*, 1973), but failed to prevent dark-cutting in animals that were socially re-grouped (McVeigh & Tarrant, 1983). This evidence suggested that muscle fibres will respond differently to different kinds of stress resulting in patterns of glycogen depletion.

1.8.3. Implications of Fat Cover

The amount of back fat that a carcass carries not only indicates the degree of "finish" an animal has, but impacts several meat quality characteristics too. Tatum *et al.* (1982) reported lean carcasses resulted in decreased tenderness and these authors identified a threshold of 0.76 cm of back fat as the tipping point for compromised meat quality. If carcasses carried fat levels below the 0.76 thresholds the consequent steaks were tougher than those from carcasses with greater than 0.76 cm of back fat. The direct link between tenderness and fatness is not completely clear, but the fat insulation effect may be part of the answer. Page *et al.* (2001) argued that the fat insulation affected how quickly the temperature declined within the muscles of the carcass, which would affect the rate of postmortem glycolysis within the muscles. Warm (greater than 25 ºC carcass temperatures) will hasten glycolysis within the muscles, and this is of concern as the rate at which glycolysis occurs also impacts cooked tenderness (Beltrán *et al.*, 1997; Harrell *et al.*, 1978) and the rate of muscle pH decline, which affects colour. Subcutaneous fat cover was found in the past to be negatively related with occurrence of DCB (Savell *et al.*, 1978). The fat is postulated to have an insulation effect that slows the carcass chilling process, which in turn reduces cold shortening, cold-induced toughness, and cold induced darkening of lean.

The tendency of lean cattle to produce dark meat was supported by the results of Wulf *et al.* (1997) who found that *b** values were positively correlated with external fat and *L** values had little relationship to external fat. Page *et al.* (2001) reported that, as quality grade increased meat pH declined, colour values

increased, and variation in pH and colour decreased. These authors also found that USDA Select carcasses were twice as variable in muscle pH as USDA Choice carcasses. Murray *et al.* (1989) found that heifer carcasses produced darker lean than steer carcasses, but considered this a result of an increased cooling rate in the muscles of heifer versus steer carcasses dues to differences in muscle mass.

1.8.4. Chilling

Carcass chilling is one of the most costly and important procedures in the meat industry. Chilling regimes have been designed to avoid carcass meat temperatures falling below 10°C within the first 10 h post-mortem (Aalhus *et al.*, 2001) to prevent quality issues. The effects of very fast chilling (VFC) include darkening of lean, cold shortening, and toughness because of the role temperature plays in the glycolytic rate of muscle postmortem (Marsh, 1954). Van Moeseke *et al.* (2001) measured the colour of VFC beef and found the *L**, *a** and *b** values were always reduced for the VFC samples. Van Moeseke *et al.*, (2001) concluded that the reduced, dark values were probably related to cold shortening of the muscle. Renerre and Dantchev (1987) also found evidence of slow-chilled lamb carcasses having greater lightness values than those rapidly chilled.

Rapid chilling can stimulate muscle contraction in skeletal muscles (Guttman & Ross, 1958; Smith, 1952), and can be associated with toughening of the cooked meat (Marsh & Leet, 1966). Orcutt *et al.* (1984) found in their study on the development of "heat-ring", which is a coarse dark band that occurs on the cut surface of the LD that this dark band was the result of rapid chilling. During

rigor mortis, chilling temperature affects the rate of postmortem glycolysis (Cassens & Newbold, 1967), which is slowest in the coldest parts of the muscles. Muscles will not cool uniformly and this results in different rates of cooling across the carcass depending on fat cover or location of individual muscles, with superficial muscles cooling most rapidly. MacDougall (1982) identified a two-tone colouration of the SM muscle when cooled in an intact hindquarter; the muscle was lightest closest to the femur and the muscle colour began to darken towards the exterior of the SM. This colour difference was attributed to a difference of cooling rate showing rapidly chilled muscle was darker than muscle that cooled slowly. Muscles that are insulated by fat cover or interior muscle tissue will also have an increased rate of anaerobic glycolysis due to a slower chill than peripheral muscle. The translucency of meat depends on both chilling regime and dimensions of the muscles on a carcass (MacDougall, 1977).

The LT and specifically the outer ring of tissue of the LT is prone to rapid chilling because of anatomical location within the carcass. A quick chill can slow anaerobic glycolysis and can result in darkening of muscle tissue. As noted by Aalhus *et al.* (2001) an extreme chilling regime can shift meat colour at grading to a darker, less saturated, purple-red colour (lowered L*, chroma and hue) than the expected bright red colour of muscles from moderately chilled control sides.

1.9. Technologies With Potential to Improve Meat Quality

1.9.1. Electrical Stimulation

Electrical stimulation is a standard meat processing technology that can be used to control the pH decline and accelerate tenderization of commercially slaughtered beef, and it should provide a tool to manipulate DCB. Electrical stimulation induces vigorous muscular contractions, which increase energy expenditures, accelerate muscle metabolism, and hasten the onset of rigor mortis (Hwang & Thompson, 2001a). This dramatically accelerates the decline of muscle pH and can drop the pH by 0.5 units over a period of 60 s of stimulation (Ducasting *et al.*, 1985). Muscle sarcomere contracture is dependent on muscle ATP concentrations and can be limited by encouraging muscle pH decline (Honikel *et al.*, 1983). Hwang & Thompson, (2001b) found a combination of stimulation and chilling treatments were used to cause independent variation in pH and temperature decline. Results showed that, *in situ*, the rate of pH decline had the largest effect on eating quality.

Electrical stimulation has been found to improve the appearance of fresh meats (Eikelenboom *et al.*, 1985; Nortje *et al.*, 1986; Savell *et al.*, 1978; Sleper *et al.*, 1983; Unruh *et al.*, 1986). The likely explanation for this is differing rates of pH decline in stimulated versus non-stimulated carcasses. Electrical stimulation hastens the onset of rigor mortis and will increase the brightness of beef when compared to non-stimulated carcasses (Lawrie, 2006a) by rapidly decreasing pH_U. Orcutt *et al.* (1984) noted that relationship of muscle texture and colour to the rate or extent of anaerobic glycolysis that occurs during rigor mortis development in

the LD. Post mortem glycolysis lowers pH and is the mechanism responsible for making muscle bright and superficially wet (Swatland, 1989). Through electrical stimulation, a rapid decline of muscle pH occurs that coincides with a high carcass temperature in relation to non-stimulated controls. This decreases the water binding properties of myofibrillar proteins, which are partially denatured from the interactions of a high temperature and low pH environment (Hamm, 1986). When the amount of free water released by myofibrillar proteins is increased then the meat appears light as free water reflects light from the surface of meats (Swatland, 1994). Although electrical stimulation will improve fresh beef colour, it will not prevent the dark-cutting condition, but it potentially could be used as a tool to encourage desirable bright red meat colour in slow blooming carcasses or carcasses that are at the threshold for cutting dark.

1.9.2. High Pressure Processing

High pressure processing is a meat processing technology that can be used to inactivate microbes at low to moderate temperatures and extend shelf life of beef products. Packaged foods are placed in a pressure vessel, which is submitted to water pressures from 100 to 900 MPa and because pressure is applied equally at all angles the appearance and sensory aspects of the product are largely unchanged. The majority of meat products being processed using high pressure are cured products such as hams, salami, bacon, or smoked goods. High pressure processing kills or injures bacterial cells by a combination of factors that affect cell membrane, cell wall, protein, and enzymes (Cheftel and Culioli, 1997). Pressurization can also impact colour as noted by Carlez *et al.* (1995) who found

that minced beef meat that was packaged under vacuum, and pressurized at 10°C for 10 min was lighter, less red, and more pink in colour as demonstrated by increasing L* values and decreasing a* values. The research also showed proportions of oxymyoglobin decreased and were being replaced with increased levels of metmyoglobin that resulted in browning. Goutefongea *et al.* (1995) also observed the colour of pressurized minced pork and beef meat lightened after pressurization, which they attributed to coagulation of sarcoplasmic and myofibrillar proteins encouraged from pressure. The high pressure processing technology is being implemented most aggressively for ready to eat products because the technology decreases microbes while leaving product appearance mostly unaffected. Using high pressure processing on dark-cutting beef to improve shelf life and colour quality has not been exclusively studied and might be a method to improve the colour and appearance of dark-cutting beef.

1.10. Expected Results

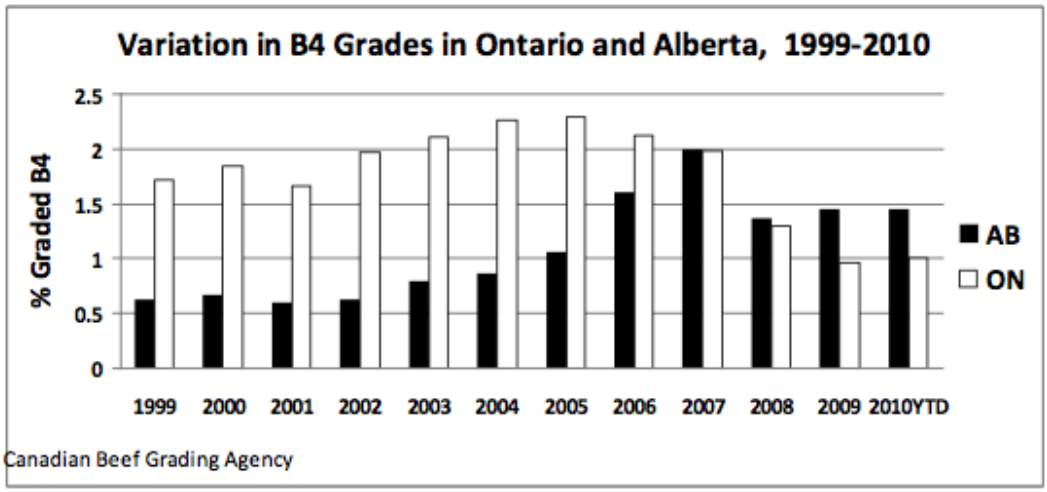
Research has shown there are a variety of causes of DCB; however our knowledge of the pH ranges within the Canada B4 grade and the percentage of the carcass affected by the dark-cutting condition is limited. A new type of dark-cutting carcass within the Canada B4 grade has been identified; a carcass type that maintains a dark muscle colour even though the muscle pH is normal. The rib eye muscle of this type of dark cutter does not brighten with subsequent ageing. These abnormal B4 carcasses have been referred to as atypical B4's (Robertson *et al.*, 2006, 2005) and appear not to conform to historical explanations of the dark-

cutting condition that are based on high muscle pH. In the study of Robertson *et al.* (2006), 25% of the 352 dark-cutting carcasses evaluated had pH values <5.8. Other studies in the literature have also made note of carcasses that have graded dark, but that had pH values < 5.87 (Murray, 1989; Page *et al.*, 2001). The atypical dark cutters have been characterized as cattle that have sufficient glycogen to produce bright red meat, but produce dark beef instead. A recent survey by Agriculture and Agri-Food Canada (Robertson *et al.*, 2007) found that some grade B4 beef carcasses had final post-rigor pH values less than 5.8 in the *longissimus thoracis* muscle, verifying that atypical B4 carcasses exist in the Canadian slaughter cattle population. Past research has attempted to explain colour based on the classification of muscle colour as a function of pH (Korkeala *et al.*, 1986) or attempted to identify colour evolution over a wide pH range (Abril *et al.*, 2001). Within this thesis, the first study will survey the Alberta grade B4 population and classify the different types of DCB carcasses based on muscle pH, and determine the pH and colour of 12 major muscles within these carcasses to determine if the muscles affected are different. The second study will examine the hypothesis that ageing and chilling regimes affect muscle colour of the *longissimus thoracis* muscle and that rapid chilling darkens the LT.

These studies seek to improve understanding of the factors impacting the incidence of DCB in Alberta. Further research stemming from this project may include investigation of the effects of growth promoting hormones and their interactions with muscle glycogen levels and pH_U, the colour evolution of growth hormone implanted beef, the colour development of beef produced with ß-

adrenergic agonists such as Optaflexx™, or a large survey of DCB carcasses to characterize the pH and type of animal that is affected.

Figure 1.1 Incidence of dark cutting beef in Ontario and Alberta between the years of 1999 and 2010.



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2.0. UNDERSTANDING THE EATING QUALITY AND MEAT CHARACTERISTICS OF GRADE B4 *LONGISSIMUS THORACIS* IN ALBERTA

2.1. Introduction

When youthful beef carcasses have a *longissimus thoracis* (LT; rib eye) muscle face that is dark red or purple, the carcass is graded Canada B4 and its value is reduced by up to $1 per kg. These carcasses, known as 'dark-cutting' carcasses, usually arise when cattle have been physically stressed prior to slaughter such that muscle glycogen has been depleted, which prevents post mortem muscle pH from declining below 6.0. Bulls tend to produce dark-cutting beef most frequently particularly if they are mixed prior to slaughter because they engage in fighting and mounting to re-establish social hierarchy, which depletes or exhausts muscle glycogen (Warriss *et al.*, 1994). Heifers produce dark beef if estrus activity is not inhibited by progestin supplementation such as melangesterol acetate. Dark beef is also produced when the muscles of cattle are depleted of glycogen due to bracing during transport (Immonen *et al.*, 2000) or if cattle are excitable and anxious at being handled or separated from other cattle (Voisinet *et al.*, 1997). In Western Canada diurnal temperature variation in the spring and fall may contribute to dark cutting by increasing the energy expenditure as the animal maintains homeostasis.

Dark cutting beef is unacceptable because it is visually unappealing to consumers, is bland in flavour, has variable pH, and a high pH facilitating bacterial spoilage and reducing shelf-life (Ferguson *et al.,* 2001). Also, dark-cutting beef from steers and heifers can be tough if the ultimate pH ranges from

5.8 to 6.0, pH values associated with the toughest post rigor beef (Jeremiah *et al.*, 1991). This has prompted a call for grading penalties applied to dark-cutting beef in the United States of America (USA) to be increased because of the associated reduction in eating quality (Wulf *et al.*, 2002).

Recent research has noted that aggressive implantation of cattle has led to an increase in beef toughness (Schneider *et al.*, 2007) and the proportion of dark-cutting carcasses (Scanga *et al.*, 1998). The use of hormonal growth promotants skews the bovine metabolism toward the production of muscle protein at the expense of fat and glycogen accretion and may lead to cattle being marketed with fewer energy reserves than in the past.

In Alberta, the proportion of beef carcasses that grade Canada B4 has increased noticeably from an average of 0.7% in 2005 to between 1.25% and 2.5% in 2010. The onset of this increase coincided with a dramatic decrease in the number of cattle killed in Alberta in October 2005, suggesting that some production factors related to this shift in cattle kill numbers may have been responsible for the observed increase in the proportion of dark-cutting carcasses. This increase in dark-cutting carcasses in Canada represents about $1.4 million in lost carcass value each year and is of significant concern to the Canadian beef industry.

A new class of dark-cutting carcasses has emerged as well, one where the LT muscle is dark but the ultimate pH is in the normal range (Wulf *et al.*, 2002). This type of dark-cutting carcass may be characterized by having a resting muscle

glycogen similar to classic or traditional dark-cutting beef animals, which have less than 80 mmol/kg of muscle glycogen, but a final muscle pH similar to well-fed and rested beef animals (80 to 140 mmol/kg of glycogen) (Crouse *et al.*, 1984; Immonen & Puolanne, 2000; Immonen *et al.*, 2000; Pethick *et al.*, 1994). There may exist then a class of 'atypical' dark-cutting carcasses that has sufficient glycogen to produce bright red meat, but produces dark beef instead. These carcasses have had limited research within the Canadian slaughter population; therefore, in this the hypothesis that there were no longer atypical Canada B4 carcasses in the Canadian slaughter cattle population was investigated. Also, there may be differences in the number of muscles in the carcass affected by the dark cutting condition in each type of dark-cutting carcass as in the USA, 1/3, ½ or full degree dark-cutting carcasses have been recognized in the industry (Bass *et al.*, 2008); therefore, an additional hypothesis was that there was no difference in the number of muscles that were affected by dark-cutting between the atypical and typical dark-cutting carcasses. Further, as meat quality between atypical and typical dark-cutting carcasses has not been completely characterized, a final hypothesis tested within this study was that there is no difference in the rib eye meat quality between the atypical and typical dark-cutting carcasses.

2.2. Materials and Methods

Twenty Canada Grade B4 carcass sides were selected for study based upon LT pH measurements performed after grading (24 h to 3 d post-mortem), with ten sides having LT pH values less than 6.0 and ten sides having LT pH values of greater than 6.0. Ten Canada Grade AA sides were also selected

without measurement of LT pH as control sides (total study n=30). Carcass sides were selected at five different times from a commercial beef packing plant in Alberta. Selected sides were then transported to the Agriculture and Agri-Food Canada Meat Research Laboratory at Lacombe, Alberta, in a refrigerated truck and were chilled overnight until fabrication. The mean pH values for the LT muscles of the Canada AA and B4 carcasses were again measured upon arrival to AAFC. The muscle pH of each carcass side was measured using a Fisher Scientific Accumet AP72 pH meter (Fisher Scientific, Mississauga, ON) equipped with an Orion Ingold electrode (Udorf, Switzerland) pH meter with a temperature probe used to adjust for muscle temperature. The pH meter was standardized using pH 4 and 7 standards (Fisher Scientific, Nepean, Canada). Rib eye muscle pH was measured between the 12th and 13th ribs three times, with each measurement performed at a different portion of the muscle.

The carcass sides collected aligned with the following pH categories: classic Canada B4 carcass sides (CL; dark colour, pH≥ 6.0), borderline Canada B4 (BD; dark colour, pH> 5.8 <6.0), atypical Canada B4 carcass sides (AT; dark colour, pH ≤ 5.8), and Canada AA (AA; normal bright red colour, pH <5.8).

The LT muscle was removed from each carcass and pH was again recorded. The muscles were then fabricated into two equal portions, which were assigned, controlling for location within grade, to immediate or aged (18 d) evaluation. The sections to be analyzed after 18 d of ageing were weighed, packaged in polypropylene bags, and stored under vacuum at 2 °C. From the LT portion to be immediately evaluated, four steaks were cut from the end closest to

the grade site. The first steak (3 cm) was used for myoglobin assessment and proximate analyses, the second steak (2.5 cm) was used for cooking loss and shear force determinations, the third steak (2.5 cm) was used for objective colour measurements and retail drip (reported in Chapter 3), and the fourth steak was sub-sampled for glucidic potential analysis, fibre type determination and sarcomere length measurement. Following 18 d of ageing at 2 ºC, the LT portion was removed from its vacuum packaging, weighed to determine purge losses and fabricated into four steaks (2.5 cm thick), starting at the end closest to the grade site. The first steak was used for myoglobin pigment assessment. The second steak for taste panel determinations was re-packaged under vacuum and frozen at -20 ºC until sensory evaluation. The third steak was used for shear force measurements, cook loss, cook time, and the fourth steak was used for drip loss.

2.2.1. Glucidic Analysis

Glucidic metabolites were extracted from frozen muscle samples by pulverizing each sample individually with a mortar and pestle in liquid nitrogen. One g of the crushed sample was weighed into 5 mL of 0.6 N perchloric acid, the sample homogenized for 30 seconds (Polytron Homogenizer PT1200 with a 12 mm generator; Brinkmann Instruments Inc., Mississauga, ON), and a 0.20 mL aliquot removed. Potassium carbonate (3M) and amyloglucosidase (0.1 g) were added to the aliquot and it was placed in a 40°C water bath for extraction of glycogen. Following incubation, the glycogen samples were clarified by centrifugation (Beckmann Floor Model J2-MC and JA-14 rotor; Beckmann Instruments Ltd., Mississauga ON) and the supernatant neutralized with

potassium carbonate (3 M, 7.5 pH). Neutralized supernatant was analyzed using a glucose analyzer (YSI 2300 StatPlus; YSI Incorporated, Dayton OH). The remainder of the crushed homogenate was centrifuged and mixed with 0.6 N perchloric acid (5 mL) and used for glucose and lactate analysis. Glucidic metabolites were reported as mmol/L, which is equivalent to μmol/mL. Free glucose, lactate, and glycogen were calculated by multiplying the weight of sample g by the corresponding dilution factor (Dalrymple and Hamm, 1973). Glucidic potential was calculated as the total sum of free glucose, glycogen, and half of lactate measurements ((lactate*2) + glucose + glycogen) to estimate the total amount of energy available at slaughter.

2.2.2. Japanese Meat Grading Agency Colour

Colour was determined subjectively by an experienced Canadian Beef Grading Agency grader on a 1 to 8 point scale (1 = lightest, 8 = darkest, optimum between 3 and 5) according to Japanese Meat Grading Association acrylic colour standards (Japanese Meat Grading Association, 1988). Where colour gradients existed in the muscle, the range in colour was noted and if the colour did not meet a full colour standard, half increments were used.

2.2.3. Myoglobin Pigment Assessment

LT steaks designated for total myoglobin pigment assessment and proximate analysis were each trimmed of all fat and coarsely diced before being homogenized using a food processor (Robot Coupe Blixir BX3, Robot Coupe USA Inc., Ridgeland MS). A portion of the grind was frozen into sample cups for

myoglobin determination and the remainder was reserved for proximate analysis. Frozen grind was thawed when time permitted and was assessed for total myoglobin pigments similar to Rickansrud and Henrickson (1967). Five g portions of the thawed grind were weighed, combined with phosphate buffer, and homogenized. Samples were centrifuged for 10 minutes and the supernatant was recovered and used for absorbance readings using an Evolution 60S UV-Visible Spectrophotometer (Thermo Scientific, Madison, Wisconsin, USA). Spectral reflectance readings were collected at 503, 525, 557, to calculate R1 (A582/A525), R2 (A557/A525), and R3 (A503/A525). R values were then used to calculate total pigments as Mb (CMb/CMb = -0.543R1 + 1.594R2 + 0.552R3 – 1.329), Oxymb (COxymb/CMb = 0.722R1 -1.432R2 – 1.659R3 + 2.599), and Metmb (CMetmb/CMb = -0.159R1 – 0.085R2 + 1.262R3 – 0.520). Total myoglobin pigments were valid if the sum of all values for Mb, Oxymb, and Metmb were within 1.00+/- 0.02. The total concentration of myoglobin was calculated as Mb = ((A525 – A730) x 2.2303) as described by (Krzywicki, 1979).

2.2.4. Proximate Analysis

One hundred g of the grind were weighed into stainless steel beakers and placed in a gravity convection-drying oven at 102°C for 24 hours (Model 1370M, VWR Scientific, Mississauga, ON). Beakers were removed from the oven and final weights recorded for moisture loss determination. The dried sample was pulverized to a fine powder (Grindomix Model GM200; Retsch Inc., Newton, PA) and analyzed for crude fat content (Method 960.39; Association of Official Analytical Chemists 1995) by petroleum ether extraction (Foss Soxtec System

Model 2050; Foss Analytical AB, Hoganas, Sweden). Nitrogen content (Method 992.15; Association of Official Analytical Chemists 1995) was determined from the resulting fat free grind (Nitrogen/Protein Determinator CNS2000, Leco Corp., St., Joseph, MI]) to complete the proximate analysis.

2.2.5. Cooking Loss and Shear Force

The steaks were removed from the refrigerator (2°C) and were weighed. The steaks were grilled (Garland Grill ED30B, Condon Barr Food Equipment Ltd., Edmonton, AB) to an internal temperature of 35.5°C, turned and cooked to a final temperature of 71°C. The internal temperature was monitored with a spear-point temperature probe (10 cm in length) inserted into the mid-point of the steaks (Hewlett Packard HP34970A Data Logger, Hewlett Packard Co., Boise, ID). Upon removal from the grill, steaks were placed into polyethylene bags, sealed and immediately immersed in an ice/water bath to prevent further cooking. The steaks were then transferred to a cooler (2°C) for 24 h. Following cooling, final steak weight was recorded and six cores (1.9 cm in diameter) were removed parallel to the fibre grain. Peak shear force was determined on each core perpendicular to the fibre grain using an Peak shear force and was determined on TA-XT Plus Texture Analyzer equipped with a Warner–Bratzler shear head at a crosshead speed of 200 mm/min a 30 kg load cell using Texture Exponent 32 Software (Texture Technologies Corp., Hamilton, MA). Peak shear force was expressed as the average of the 6 cores. Raw and final steak weights were used to determine cooking loss (mg/g) and cooking time per g of raw weight (sec/g).

2.2.6. Sarcomere Length and Muscle Fibre Type

Sarcomere lengths were determined from subsamples of the designated steak by hand-mincing with a surgical scalpel 2 g of muscle that had been previously trimmed of connective tissue and large deposits of fat. The minced sample was homogenized in 20 mL of 0.02M EGTA/0.25M sucrose solution (Polytron Homogenizer PT3100 and a 2 cm generator; Brinkmann Instruments Inc., Mississauga ON). A drop of the minced sample in the EGTA and sucrose solution was placed on a microscope slide and covered with a cover slip. Twelve images were captured per animal for length using a phase contrast microscope (Axioscope, Zeiss, West Germany) equipped with a Sony DXC 930 Colour Video Camera (Sony Corporation, Japan) and Image Pro-Plus software (V4.0, Mediacybernetics; Silver Spring, MD). Twelve sarcomere images were captured for each half-carcass and the means were calculated from the best ten images.

Muscle fibre types were determined from a one cm^3 muscle sample from the designated steak, with the sample mounted on cork perpendicular to the muscle grain with mounting compound (Shandon Cryomatrix Mounting Compound Thermo Scientific, Pittsburgh PA) and flash frozen in liquid nitrogen. The frozen block was sectioned (11 μm thickness) onto slides using a cryostat (Cryotome FSE Cryostat, Thermo Fisher Scientific Inc., Runcorn Chesire, UK). Slides were stained using a combined procedure (Solomon & Dunn, 1988) for succinate dehydrogenase (SDH; Humason, 1972) and myofibrillar adenosine triphosphatase (ATPase; Guth & Samaha, 1970) activities to differentiate between fibre types as follows. Samples were pre-incubated in an acid solution at a pH of

4.15 (Solomon & Dunn, 1988) at room temperature for 10 min and then rinsed with running water. Samples were then incubated in the SDH staining solution for one h followed by incubation in a $CaCl_2$-TRIS hydroxymethyl aminomethane solution, pH 7.8, used twice for one minute and repeated in separate wash buckets. Sections were then incubated consecutively three times into an aqueous solution of $CaCl_2$ (0.18M) for 30 sec followed by an aqueous $CoCl_2$ (2%) solution for 4 min. Samples were washed for two min in running water, incubated in aqueous $(NH_4)_2SO_4$ solution for 3 min, and washed again for 4 min in running water. Samples were counterstained using Mayers hemotoxylin for 2 to 3 min and rinsed for 5 min with running water and then samples were placed in successive serial dilutions of reagent alcohol (50%, 75%, 95%, and 100%) for two min each. Samples were then placed in 50% Hemo-De and 100% Hemo-De for 2 min each and were covered with a cover-slip. Images were captured for analysis using a phase contrast microscope (Axioscope, Zeiss, West Germany) equipped with a Sony DXC 930 Colour Video Camera (Sony Corporation, Japan) and Image Pro-Plus software (V4.0, Mediacybernetics; Silver Spring, MD). Fibres were classified as slow oxidative (SO), fast oxidative and glycolytic (FOG), or fast glycolytic (FG). Red fibres were identified as dark with the SDH stain as they contained the highest amounts of mitochondria, which contain the enzyme succinate dehydrogenase. Four images for muscle fibre type and three for muscle were captured and measurement analysis was performed as described above for sarcomere lengths.

2.2.7. Sensory Analysis

Sensory attributes were measured on thirty 18 d aged LT steaks by nine panelists. Steaks were removed from storage at -20 ºC and were thawed overnight at approximately 2 ºC. At time of analysis, taste panel steaks were removed from refrigeration 15 minutes prior to grilling and weighed to determine raw steak weight. Temperature probes were inserted horizontally to the mid-point along the long axis of the steak. Steaks were grilled to an internal temperature of 35.5°C, turned and cooked to a final temperature of 71°C as described previously. After cooling for 5 min, steaks were weighed to determine final cooked weight. Raw and cooked weight differences were expressed as cook loss (mg/g) and total cook time expressed as sec/g. Each steak was cut into 1.3 cm^3 cubes, avoiding connective tissue and large areas of fat. Eight cubes from each sample were randomly assigned to an eight-member trained taste panel to assess. Samples were placed in glass jars in a circulating water bath (Lindberg/Blue model WB1120A-1; Kendro Laboratory Products, Asheville, NC) and allowed to equilibrate to 71°C prior to evaluation. Attribute ratings were electronically collected with Compusense 5 (release 4.6) computer software (Compusense Inc., Guelph, ON) using a nine point descriptive scale for initial and overall tenderness (9=extremely tender; 1=extremely tough), initial and sustainable juiciness (9=extremely juicy; 1=extremely dry), beef flavour intensity (9=extremely intense; 1=extremely bland), off-flavour intensity (9=no off-flavour; 1=extremely intense off-flavour) and amount of connective tissue (9=none detected; 1=abundant). Flavour desirability and overall palatability were rated on a nine point hedonic scale,

(9=extremely desirable; 1=extremely undesirable). Initial tenderness was rated on the first bite through the cut center surface with the incisors; initial juiciness was rated after 3-5 chews with the molars; beef flavour intensity, flavour desirability, off-flavour intensity and amount of connective tissue between 10-20 chews and sustainable juiciness, overall tenderness, and overall palatability were rated prior to expelling. Each panelist also assigned flavour (metallic, off sour, livery, grainy, bloody, others, unidentified or none) and texture (typical beef, mushy, mealy, spongy, rubbery or crumbly) descriptor to each cube, if present. Flavour and texture descriptors were reported as the percentage of panelists attributing that descriptor to that sample. All panel evaluations were conducted in well-ventilated, partitioned booths, under 124 lux red lighting. Distilled water and unsalted soda crackers were provided to purge the palate of residual flavour notes between samples (Larmond, 1977).

2.2.8. Statistical Analyses

Carcass and muscle data were categorized by LT ultimate pH category, with classic (CL) dark cutters having a pH greater than 6.0, borderline (BD) having a pH greater than 5.8 but less than or equal to 6.0, atypical (AT) dark cutters having a pH of less than 5.8 and normal colour (AA). The effect of carcass classification by ultimate pH category was determined on data from putatively categorized carcasses using one-way analysis of variance (ANOVA). When measurements were compared at two different ageing times, data were analyzed using a split plot with carcass side and loin portion as the experimental units in the main and sub plots, respectively. Repeated measures analysis was

used for data collected instrumentally over time. A Chi square analysis was used to determine the probability distribution of the sensory categorical data. Correlations analysis was performed using PROC CORR to calculate the Pearson product-moment correlation coefficient among pH categories and aged LT steaks to calculate linear dependence between variables. The PROC MIXED procedure was used for ANOVA and least square means were separated using the PDIFF option (Statistical Analysis Software Version 9.2; SAS Institute Inc., Cary, NC).

2.3. Results

2.3.1. pH and Colour

Mean pH values of the rib eye muscles at the abattoir indicated that three putative categories of dark-cutting carcass existed: atypical (AT) dark cutters with pH values less than 5.8; borderline (BD) dark cutters with pH values less than 6.0 but greater than 5.8; and classic (CL) with pH values greater than 6.0 (Figure 2.1). With the exception of the Canada AA carcasses, Japanese Meat Grading Association (JMGA) muscle colour scores supported the categorization of all the carcasses into a dark-cutting classification, although one AT carcass side brightened to a similar score as the AA carcass sides 24 h after arrival to AAFC-Lacombe. Additionally one BD carcass was brighter than other carcasses in this group, but still darker than the control carcasses. Graphical representation indicated that CL carcass sides had LT pH values greater than 6.4 by 24 h after collection at the abattoir, while pH values in LT muscles from BD carcasses remained greater than 5.8 and less than 6.0 (Figure 2.1). The highest pH that was

measured was 6.94, which corresponded with an extremely high JMGA colour score of 8.0 and belonged to a CL carcass. The lowest muscle pH in the dark cutting carcass sides was measured in an AT carcass side with a pH of 5.5 and a JMGA colour scores of 6.5.

The mean LT pH values of each putative category of dark-cutting carcass were significantly greater than that of the Canada AA carcasses at the abattoir and 24 h later at the meat laboratory, with the mean rib eye pH value of the CL carcasses being greater than all other categories (Table 2.1). At 24 h after receipt of the carcasses at the laboratory when muscles were fabricated from the half-carcasses, the mean rib eye pH of the AT rib eye was similar to that of the 'normal' Canada AA rib eye muscles (Table 2.1).

2.3.2. Glucidic Potential

Differences between individual metabolites showed free glucose was highest in LT muscles from AA and AT carcass sides when compared to BD and CL carcass sides (Table 2.2). CL carcass sides had the lowest amount of lactate when compared to AA, AT, and BD carcass sides ($P< 0.05$). Lactate was negatively correlated with pH category ($R= -0.736$; $P< 0.05$). Glycogen was present in the highest amounts within muscles from AT carcass sides when compared to BD and CL carcass sides, although only numerically different when compared to control carcass sides ($P< 0.05$). Cumulative calculation of glucose, lactate and glycogen indicated that the glucidic potentials of the LT muscles from

AA and AT carcass sides were the highest, with the BD LT muscles intermediate and LT muscles from the CL carcass sides the lowest ($P < 0.05$) (Table 2.2).

2.3.3. Storage Purge Loss and Proximate Analysis

Purge loss values collected from whole loins demonstrated LT muscles from CL carcass sides had the lowest drip loss values, while muscles from BD carcass sides were intermediate and muscles from AA carcass sides lost the most fluid ($P < 0.05$) (Table 2.3). Proximate analysis values demonstrated no differences amongst treatment groups in the amounts of moisture, fat, and protein within LT muscles ($P > 0.05$).

2.3.4. Muscle Fibre Types, Diameters, and Sarcomere Length

There were no significant differences in mean proportions of different fibres amongst treatment groups ($P > 0.05$) (Table 2.3). The mean measurements for sarcomere length indicated sarcomeres within LT muscles were shorter in CL carcass sides than in AA and AT carcass sides ($P < 0.05$).

2.3.5. Cooking time, Cooking Loss and Shear Force

The cooked LT steaks from BD carcass sides had the highest shear force values (8.87 kg) without ageing and required more force to shear than cooked AA steaks (6.18 kg), AT steaks (5.75 kg), and CL steaks (5.58 kg; $P < 0.05$; Table 2.4). The peak shear force values of all cooked LT steaks decreased with time and there was no effect of pH category on shear force. Mean cook loss values showed that LT steaks from AA carcass sides lost more fluid while cooking compared to

steaks from CL carcass sides. There were no differences in time required to cook the steaks among treatments. LT muscle drip loss increased with ageing time, with drip loss increasing by almost 8 mg/ g of fresh muscle after 18 days of storage at 2 °C.

2.3.6. Pigment Concentrations

Concentrations of oxymyoglobin after two days of ageing were lowest in samples from AA and AT carcass sides and were highest in CL carcass sides ($P< 0.05$) (Table 2.5). In aged samples mean concentrations of oxymyoglobin were higher in samples from AT and CL carcass sides compared to AA and BD samples. Mean concentrations of metmyoglobin were lowest in samples from CL carcasses regardless of ageing and were highest in samples from AA and AT carcass sides. Aged samples from AT carcass sides showed the mean concentration of metmyoglobin was lowest in aged samples. Mean concentrations of myoglobin were very consistent among pH categories and was lowest in aged samples from AT carcass sides.

2.3.7. Sensory

Panelist values for initial tenderness showed LT steaks from BD carcass sides were the toughest when compared to LT steaks from AA and CL carcass sides (Table 2.6). Flavour desirability, beef flavour intensity, and off-flavour intensity were highest for Canada AA and BD LT steaks when compared to LT steaks from CL carcass sides. When panelists assessed initial and overall tenderness they found LT steaks from BD were the least tender, AA and CL

carcass sides were most desirable, and AT were intermediate. There were no differences in initial juiciness, amount of connective tissue, sustainable juiciness, or overall palatability among carcass sides (Table 2.6).

When panelists assessed textural characteristics, the LT steaks from AA and AT dark-cutting carcasses were identified as the most crumbly and as having a classic beef texture (Table 2.7). LT steaks that were from AT and CL carcasses were mushy 13 and 18% of the time compared to the AA and BD carcasses being mushy 1 and 0% of the time. Steaks from CL carcasses were most often identified by panelists as having a spongy texture, while rubbery textures were most often identified by panelists in steaks from BD carcass sides. The steaks from the BD carcasses also had textural properties that could not be identified using the descriptors supplied.

When flavour characteristics were described, panelists identified an off/sour flavour in all but the steaks from the CL carcasses. Other flavours were unidentifiable, with most of these occurring in the CL carcass steaks (Table 2.7). These results indicated that CL steaks could be distinguished by flavour or texture, but panelists could not consistently describe the eating experience. The fewest off flavours were described in steaks from AA and BD carcasses.

2.4. Discussion and Conclusion

Dark cutting carcasses are typically thought to result from stresses prior to slaughter that deplete the glycogen levels in the muscles such that normal postmortem processes do not occur and the muscle pH remains above 6.0 (Wulf *et*

al., 2002). However studies in the literature have made note of carcasses that qualified for the Canadian B4 grade based on a colour standard, but had pH values less than 5.87 (Murray, 1989; Page *et al.*, 2001). These carcasses were identified in the present study when LT muscle pH was measured at three separate occasions disproving the null hypothesis that dark-cutting carcasses do not exist with a muscular pH below 6.0 in the youthful grade B4 population in Alberta.

Fischer and Hamm (1981) reported that DFD beef carcasses with elevated ultimate pH had low levels of glycogen, glucose, hexose phosphates, triose phosphates, and lactate. As expected CL carcass sides had the lowest mean levels of free glucose, lactate, and glycogen, which concurred with the high ultimate pH of the beef. This result is supported by several authors (Fernandez & Gueblez, 1992; Immonen *et al.*, 2000) who found a strong curvilinear relationship existed between muscle glycogen concentrations and ultimate muscle pH; as muscle glycogen levels decreased ultimate muscle pH increased. The relationship between lactate and muscle pH group was also noted by the negative correlation (R= -0.736), which indicated that as concentrations of lactate in the muscle accumulated the pH declined. The normal levels of lactate found within atypical LT steaks and the resulting normal pH agree with this. In addition, the potential energy (glucidic potential which equals the sum of glucose, glycogen, and half of lactate) within LT muscles of CL carcasses was insufficient to produce sufficient H^+ ions to lower the postmortem muscle pH into a normal range when compared to AT and AA carcasses. There were no differences between lactate concentration within AA carcass sides and AT/BD carcass sides suggesting that lactate

production was depressed only in the LT muscles from the CL carcasses. Mean glycogen levels were similar in both AT and AA carcass sides, suggesting that the glycogen in AT muscle was present in normal concentrations but for unknown reason(s) was not being metabolized. The high levels of glucose exhibited in muscles from control and AT carcass sides could be the result a mutation or differences in ante mortem glycogenolysis (the breaking apart of glycogen to glucose molecules), or a mutation in the enzyme which regulates this pathway such as glycogen phosphorylase. Glycogen phosphorylase activity is activated by catecholamine levels or muscle contraction (Tarrant, 1989) and so activity ante mortem will also affect the rate of this pathway. In the case of CL and BD carcasses the level of activity of glycogen phosphorylase might be insignificant because the levels of glycogen are low or the concentrations of blood glucose may be inadequate due to nutrition. Glucose is an essential precursor of muscle glycogen, has been reported to decline progressively in grass-fed cattle during late summer and autumn (Manston *et al.*, 1977) as nutritional plane decreases. The nutritional requirements may not have been met adequate in these animals as muscle glycogen seems to be strongly correlated with the energy profile of the diet fed to animals (Tarrant & Sherington, 1980). However the back fat suggests that the energy profile of diet was adequate, at least long term, before slaughter.

Both objective and subjective sensory data of the present study showed that BD carcasses produced the toughest LT steaks as measured by shear force. These results agree with the findings of Jeremiah *et al.* (1991) where LT muscles with pH values between 5.8 and 6.0 produced the toughest beef. The unique

texture of BD steaks was identifiable by panelists, but was not classified using normal textural descriptors for beef. CL carcass sides produced the least desirable steaks with a unique off-flavour that was not classified using normal off-flavour descriptors for beef. The LT steaks from AT carcasses were as tender as AA LT steaks and were also as acceptable as AA LT steaks for flavour. These results indicated that AT carcasses produced an acceptably palatable product with excellent water-holding capacity. Dransfield (1981) found that dark-cutting beef lacked the flavour of normal beef, an effect that was observed in the BD carcass sides in the present study. Past research has noted that dark-cutting beef may be juicy because of a high water-holding capacity, although there may be compromises in flavour (Epley, 1975; Price & Schweigert, 1971). The colour of LT steaks from AT carcasses may be darker than steaks from Canada AA carcasses at grading, but this did not affect their objective and subjective quality attributes. The ability to sort within the B4 grade by pH would be advantageous and would allow more meaningful separation of carcasses based on eating quality, rather than just on colour.

Table 2.1. *Longissimus thoracis* pH measurements post mortem separated by grade category

pH Values	AA[1]	AT[2]	BD[3]	CL[4]	S.E.M.[5]	Prob[6] > F
N	10	3	7	10	-	-
At packing plant		5.74[b]	6.07[b]	6.55[a]	0.15	0.005
On carcass	5.55[c]	5.60[b]	5.86[b]	6.66[a]	0.05	<.0001
On muscle	5.57[c]	5.54[c]	5.96[b]	6.73[a]	0.05	<.0001

[1] Canada AA carcass muscles.
[2] Atypical dark cutters with a muscle pH below 5.8.
[3] Borderline dark cutters with a muscle pH above 5.8 but below 6.0.
[4] Typical dark cutters with a muscle pH above 6.0.
[5] Standard error of the mean.
[6] Probability of the F test, with significance at $P < 0.05$.
[a,b] Means with different superscripts within a row are significantly different at $P < 0.05$

Table 2.2 *Longissimus thoracis* glucidic potential (µmol/g fresh tissue) separated by grade category

Glucidic Characteristic	AA[1]	AT[2]	BD[3]	CL[4]	S.E.M[5]	Prob[6] > F
n	10	3	7	10	-	-
Glucose	6.71[a]	5.74[a]	3.24[b]	1.25[c]	0.44	<.0001
Lactate	92.13[a]	89.82[a]	84.70[a]	56.02[b]	4.18	<.0001
Glycogen	21.75[ab]	42.77[a]	10.18[bc]	8.47[c]	5.04	0.0016
Glucidic Potential[7]	74.52[a]	93.42[a]	55.77[b]	37.74[c]	5.64	<.0001

[1] Grade AA carcasses.
[2] Atypical dark cutters with a muscle pH below 5.8.
[3] Borderline dark cutters with a muscle pH above 5.8 but below 6.0.
[3] Typical dark cutters with a muscle pH above 6.0.
[4] Standard error of the mean.
[5] Probability of the F test, with significance at $P < 0.05$.
[7] Potential is the sum of all potential energy as calculated in glucose equivalents (glucose + glycogen + ½ lactate).
[a,b] Means with different superscripts within a row are significantly different at $P < 0.05$

Table 2.3. ***Longissimus thoracis*** **purge losses, proximate analyses, muscle fibre type proportions and sarcomere length separated by grade category**

	AA	AT[1]	BD[2]	CL[3]	SEM[4]	Pr > F[5]
n	10	3	7	10		
Purge loss, mg.g^{-1}						
Initial muscle weight, kg	3.18	3.13	2.74	2.62	0.19	0.1704
Muscle purge loss, mg.g^{-1}	33.44^{a}	28.03^{ab}	19.63^{b}	8.19^{c}	4.22	0.0270
Proximate Analysis, mg.g^{-1}						
Moisture	738.3	738.8	735.4	738.3	0.4	0.5030
Fat	31.1	24.7	34.3	32.8	0.39	0.2495
Protein	221.8	224.9	225.2	224.3	0.15	0.1753
Fibre Type Proportion, %						
Slow oxidative	30.56	30.27	27.53	28.24	2.01	0.5898
Fast oxidative glycolytic	23.65	23.13	23.24	25.33	2.20	0.3169
Fast glycolytic	45.79	46.60	49.22	46.43	2.97	0.8124
Sarcomere length, μm	1.74^{a}	1.71^{a}	1.54^{ab}	1.44^{b}	0.08	0.0195

[1] Grade AA carcasses.
[2] Atypical dark cutters with a muscle pH below 5.8.
[3] Borderline dark cutters with a muscle pH above 5.8 but below 6.0.
[3] Typical dark cutters with a muscle pH above 6.0.
[4] Standard error of the mean.
[5] Probability of the F test, with significance at $P < 0.05$.
[7] Potential is the sum of all potential energy as calculated in glucose equivalents (glucose + glycogen + ½ lactate).
[a,b] Means with different superscripts within a row are significantly different at $P < 0.05$.

Table 2.4 *Longissimus thoracis* cooking data and objective shear force after 2 and 18 d of ageing separated by grade category

Measurement	AA[1]	AT[2]	BD[3]	CL[4]	S.E.M[5]	2 d[6]	18 d	S.E.M.	Prob[7] > F		
									Grade	Ageing	Grade x Ageing
N	10	3	7	10							
Shear force, kg	6.18[b]	6.19[b]	8.87[a]	5.47[b]	0.58	8.06[a]	5.29[b]	0.38	0.0008	<.0001	0.4163
SD[8] of cores	1.01	1.16	1.56	1.23	0.16	1.48[a]	1.0b	0.12	0.0789	0.0075	0.1675
Cook loss, mg/g	235.52[a]	185.39[ab]	215.08[ab]	169.25[b]	15.05	197.3	205.3	9.21	<.0001	0.367	0.2053
Cook time, sec/g	4.88	3.69	6.09	5.21	0.66	5.01	4.93	0.48	0.1994	0.907	0.6827
Drip loss, mg/g	52.60[a]	42.16[ab]	44.91[a]	31.35[b]	3.68	38.78[b]	46.73[a]	2.69	<.0001	0.042	0.5390

[1] Canada Grade AA carcasses.
[2] Atypical dark cutters with a muscle pH below 5.8.
[3] Borderline dark cutters with a muscle pH above 5.8 but below 6.0.
[4] Typical dark cutters with a muscle pH above 6.0.
[5] Standard error of the mean.
[6] d = Day.
[7] Probability of the F test, with significance at $P < 0.05$.
[8] Standard deviation of cores.
[a,b] Means with different superscripts within a row are significantly different at $P < 0.05$.

Table 2.5 *Longissimus thoracis* concentrations of metmyoglobin (Metmb), myoglobin (Mb), and oxymyoglobin (Oxymb) pigments as measured 2 and 18 days of ageing separated by grade category.

	AA[1]		AT[2]		BD[3]		CL[4]		SEM[5]	Pr > F[6]
	2	18	2	18	2	18	2	18		Grade*Age
Oxymyoglobin	0.29^{b}	0.29^{b}	0.28^{b}	0.38^{a}	0.32^{ab}	0.28^{b}	0.38^{a}	0.36^{a}	0.02	0.0362
Metmyoglobin	0.54^{ab}	0.52^{abc}	0.55^{a}	0.47^{cd}	0.49^{bcd}	0.52^{abc}	0.45^{d}	0.46^{d}	0.02	0.012
Myoglobin	0.18^{ab}	0.19^{a}	0.17^{ab}	0.16^{b}	0.19^{a}	0.20^{a}	0.18^{ab}	0.19^{a}	0.01	0.3854
Total	0.99^{c}	1.02^{c}	1.17^{abc}	1.09^{abc}	1.27^{a}	1.22^{ab}	1.07^{bc}	1.10^{abc}	0.06	0.7354

[1] Canada Grade AA carcasses.
[2] Atypical dark cutters with a muscle pH below 5.8.
[3] Borderline dark cutters with a muscle pH above 5.8 but below 6.0.
[4] Typical dark cutters with a muscle pH above 6.0.
[5] Standard error of the mean.
[6] Probability of the F test for interactions of grade and ageing time with significance at $P < 0.05$
[a,b] Means with different superscripts within a row are significantly different at $P < 0.05$.

Table 2.6 *Longissimus thoracis* sensory characteristics of cooked steaks separated by grade category

Panel Attributes	AA[1]	AT[2]	BD[3]	CL[4]	S.E.M.[5]	Prob.[6] > F
n	10	3	7	10		
Initial Tenderness	5.98[a]	5.86[ab]	4.73[b]	6.8[a]	0.4	0.002
Initial Juiciness	5.41	5.45	5.39	5.75	0.23	0.386
Flavour Desirability	5.55[a]	5.22[ab]	5.59[a]	4.72[b]	0.28	0.017
Beef Flavour Intensity	5.61[a]	5.4[ab]	5.58[a]	4.73[b]	0.25	0.015
Off-flavour Intensity	7.98[a]	7.74[ab]	7.97[a]	7.09[b]	0.36	0.015
Amount of Connective Tissue	8.36	8.18	7.77	8.46	0.28	0.053
Overall Tenderness	6.43[a]	6.12[ab]	5.28[b]	7.12[a]	0.40	0.006
Sustainable Juiciness	5.51	5.53	5.55	5.68	0.22	0.855
Overall Palatability	5.21	4.62	4.88	4.36	0.32	0.121

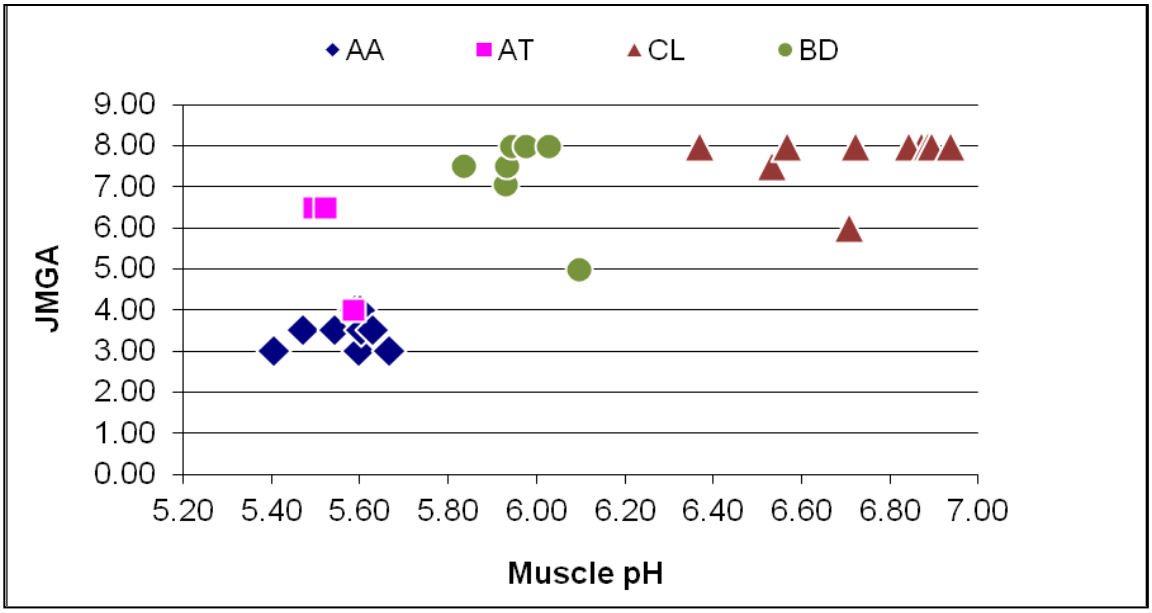
[1] Canada AA carcasses.
[2] Atypical dark cutters with a muscle pH below 5.8.
[3] Borderline dark cutters with a muscle pH above 5.8 but below 6.0.
[4] Typical dark cutters with a muscle pH above 6.0.
[5] Standard error of the mean.
[6] Probability of the F test, with significance at $P < 0.05$.
[a,b] Means with different superscripts within a row are significantly different at $P < 0.05$

Table 2.7 *Longissimus thoracis* texture and off-flavour descriptors separated by grade category

Texture	AA[1]	AT[2]	BD[3]	CL[4]
n	10	3	7	10
Crumbly	38%	21%	11%	14%
Mealy	1%	4%	4%	0%
Mushy	1%	13%	0%	18%
Rubbery	1%	0%	18%	3%
Spongy	8%	8%	5%	44%
Typical Beef Texture	51%	54%	0%	23%
Unidentified	0%	0%	38%	0%
Off-flavours	AA	AT	BD	CL
Bloody/Serum	3%	4%	2%	8%
Grainy	0%	0%	2%	4%
Livery	6%	8%	4%	4%
Metallic	1%	4%	4%	0%
None	44%	33%	46%	28%
Off Sour	35%	21%	20%	3%
Other	1%	0%	2%	3%
Unidentified	10%	29%	21%	53%

[1] Canada AA carcasses.
[2] Atypical dark cutters with a muscle pH below 5.8.
[3] Borderline dark cutters with a muscle pH above 5.8 but below 6.0.
[4] Typical dark cutters with a muscle pH above 6.0.

Figure 2.1 Scatterplot of collected carcasses identified by *Longissimus thoracis* mean ultimate pH showing distribution against Japanese Meat Grading Association (JMGA) colour scores



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3.0. RECOVERING VALUE FROM CANADA GRADE B4 BEEF CARCASSES

3.1. Introduction

The incidence of dark-cutting carcasses has been increasing in Alberta since late 2005. Prior to October 2005, the incidence of dark-cutting beef was three times higher in Eastern Canada than in Western Canada; however, since 2005 Western Canada has experienced a dramatic increase in the number of beef carcasses grading Canada B4. Dark-cutting carcasses may be penalized as much as $1/kg of dressed carcass weight so producers lose on average $300 dollars a head for dark-cutting carcasses. Even though individual muscles other than the rib eye (M. l*ongissimus thoracis*) within a carcass can cut dark and be penalized for it, the bovine carcass as a whole is penalized based on the colour of the rib eye at grading. Recent research (Bass *et al.*, 2008) suggested that not all muscles of a carcass with a dark rib eye are affected by the dark-cutting condition, implying that there is potential for dark cutting carcasses to have some normally coloured muscles within the carcass that could be recovered.

Carcasses exhibiting the 'dark-cutting' condition usually arise after physical stresses prior to slaughter such that muscle glycogen is depleted, which prevents post mortem muscle pH from declining and produces abnormally dark muscles. Carcasses qualify for the Canada B4 grade if the M. *longisimus thoracis* (LT) muscle is the same colour or darker than the standard B4 colour chit used by graders (Canadian Food Inspection Agency, 1992), but this is the only muscle that

is visually assessed at grading. Mixing cattle that are unfamiliar with each other or problems during transport prior to slaughter can lead to sufficient levels of stress to deplete or exhaust muscle glycogen stores (Immonen *et al.*, 2000; Warriss *et al.*, 1994), but usually only in specific muscles (Bass *et al.*, 2008). Bass *et al.* (2008) failed to find any relationships between LT colour and the colour of other muscles measured within beef carcasses. Tarrant and Sherington (1980) observed pH patterns among muscles and found that pH showed the most variable in the LT muscles rather than in other muscles.

Results from this study reported in Section 2 of this thesis and those of Murray (1989) and Wulf *et al.* (2002) indicated that there are different classes of dark-cutting carcasses. There may be a class of dark-cutting carcasses that has been poorly characterized where the *Longissimus thoracis* (LT; rib eye) muscle is dark but the ultimate pH within the LT is within a normal pH range. This type of 'atypical' dark cutter does not completely conform to the theoretical science behind the relationship of muscle pH and colour as these carcasses appear to have sufficient glycogen levels necessary to produce bright red meat but produce dark beef instead (Section 2). The different pH categories of dark-cutting carcasses need to be considered when determining if value can be recovered from Canada B4 carcasses. The number of muscles that are affected by the dark cutting condition may also vary based on as the pH in the LT varies. Dark-cutting carcasses are recognized in the USA industry as 1/3, ½ or full degree of dark-cutting carcasses (Bass *et al.*, 2008) but the effect of muscle pH in relation to the different categories of dark-cutters and the extent of muscles affected throughout

the carcass is unknown. The hypothesis for the current study is therefore that the *Longissimus thoracis* ultimate pH is related to the proportion of muscles affected by the dark cutting condition within the remainder of the carcass. To test this hypothesis, twelve different muscles within the carcass were profiled, their yield and quality described, and the proportion of the carcass that was affected by the dark-cutting condition was assessed.

3.2. Materials and Methods

One hundred and seventy nine Canada Grade B4 (dark-cutting) carcass sides were surveyed for rib eye pH value at five different times at a commercial beef packing plant in Alberta. The muscle pH of one side of each carcass was measured using a Fisher Scientific Accumet AP72 pH meter (Fisher Scientific, Mississauga, ON) equipped with an Orion Ingold electrode (Udorf, Switzerland) pH meter with a temperature probe used to adjust for muscle temperature. The pH meter was standardized using pH 4 and 7 standards (Fisher Scientific, Nepean, Canada). Rib eye muscle pH was measured between the 12th and 13th ribs three times, with each measurement performed at a different portion of the muscle.

From the carcass sides surveyed, six carcasses were selected on each of the five visits: two classic or traditional Canada B4 carcass sides (CL; pH $\geq$ 6.0), two atypical or borderline Canada B4 carcass sides (AT; pH $\leq$ 6.0), and two Canada AA (normal bright red colour). The muscle pH of the *Longissimus thoracis* (LT) muscle was taken at the plant as described and the Canada B4 carcasses were categorized by LT pH as atypical (AT) with pH < 5.8, borderline

with pH between 5.8 and 6.0, and classic with pH above 6.0. Selected sides were then transported to the Agriculture and Agri-Food Canada Meat Research Laboratory at Lacombe, Alberta, in a refrigerated truck. The carcass sides were chilled overnight until fabrication.

At fabrication, the carcass sides were ribbed at the Canadian grade site (between the 12th and 13th ribs), were allowed a 20-minute period of exposure to atmospheric oxygen and were then graded by trained personnel according to Canadian grade standards (Canadian Food Inspection Agency, 1992). Carcass data for each carcass side selected included grade fat, muscle score, cutability estimates, rib eye area (REA), and American Meat Science Association (AMSA) marbling scores (100 – 110; 100 = devoid, 200 = practically devoid, 300 = traces, 400 = slight, 500 = small, 600 = modest; AMSA 1990). A photographic reference scale was used to describe the various levels of marbling (Official USDA Marbling Photographs, National Livestock and Meat Board, Chicago, IL). REA was determined by using a transparent muscling grid sheet to measure the size of the exposed LT muscle. The length and width of the LT muscle was measured using a Canada grade ruler at the grading site to assign muscling scores. Muscle scores range from one to four, with one representing the smallest score designating a small LT muscle (Jones *et al.*, 1991). Following grading, ultimate pH and temperature values were recorded at the rib eye grade site.

3.2.1. Comparison of Carcass Quality

The following muscles were dissected from each carcass side: *longissimus thoracis* (LT); *longissimus lumborum* (LL); *psoas major* (PM); adductor (AD); *biceps femoris* (BF); *gluteus medius* (GM); *semimembranosus* (SM); *semitendinosus* (ST); *rectus femoris* (RF); *infraspinatus* (IF); *pectoralis profundus* (PP); and the *triceps brachii* - long head (TB). Muscles dissected from the carcasses had their pH recorded. Each muscle had one 2.5 cm steak removed from either the proximal end or anterior end depending upon which offered the best portion for colour assessment and determination of retail drip loss.

3.2.2. Retail Display Colour and Drip Loss

The muscle steaks were then weighed and placed onto a polystyrene tray with a soaker pad (Dri-Loc^{TM}, PaperPak, Greenville, USA) and were assessed for colour after a 20 min period of exposure to atmospheric oxygen at 1°C. Colour was determined subjectively by a trained Canadian grader on a 1 to 8 point scale (1 = lightest, 8 = darkest, optimum between 3 and 5) according to Japanese Meat Grading Association acrylic colour standards (Japanese Meat Grading Association, 1988; Jones and Tong, 1989). Where colour gradients existed in the muscle, the range in colour was noted and if the colour did not meet a full colour standard, half increments were used. Samples were wrapped with oxygen permeable film (Vitafilm Choice wrap, permeability 8000 mL m^{-2} 24 h^{-1}, Goodyear Canada Inc., Toronto) and placed into a commercial retail display case at 1°C for 0, 2 and 4 days of retail ageing. All samples were placed into the

display case to ensure as little temperature variation as possible. The Minolta spectrophotometer (Minolta CM700, Minolta Canada Inc., Mississauga, ON) was calibrated using a white tile with plastic overwrap covering the aperture and colour was measured at 0, 2, and 4 d of retail display. On each specific day of retail display, three objective colour axes (CIE L*(brightness), a*(red-green axis), b*(yellow-blue axis); Commission Internationale de l'Eclairage, 1978) were collected on three locations across the steak (Minolta CM700; Minolta Canada Inc., Mississauga, ON). On the final day of retail display (day 4) retail steaks were removed from the oxygen-permeable package and their weights recorded for subsequent determination of retail drip loss.

3.2.3. Myoglobin Pigment Assessment

Myoglobin pigment assessment was measured on the surface of steaks by converting spectral reflectance readings taken with a spectrophotometer (Minolta CM700D, Minolta Canada, Mississauga, ON). Readings were collected and converted to reflex attenuance (A, the logarithm of the reciprocal of reflectance; Shibata, 1966). Interpolation of isobestic points for 473, 525, 572, and 700nm were determined to calculate $a_1 = (A^{572} - A^{700}) / (A^{525} - A^{700})$ and $a_2 = (A^{473} - A^{700}) / (A^{525} - A^{700})$. These values were used to find relative contents of metmyoglobin (Metmb = $1.395 - a_1$), myoglobin (Mb = $2.375 * (1 — a_2)$) and oxymyoglobin (Oxymb=1- (Metmb + myo)) (Krzywicki, 1979).

3.2.4. Gender Determination

DNA was extracted from 10mg of previously frozen ground muscle from

each carcass collected within the study and compared to 200uL of bovine blood collected from a male (bull) and a female (heifer) bovine which served as gender control samples. The standard protocol for Qiagen's DNeasy blood and tissue kit was followed for DNA extraction of the samples. The DNA extraction for the controls used the standard blood protocol from the same kit. DNA was quantified using the NanoDrop ND1000 Spectrophotomer (Thermo Scientific, 2008, Wilmington, DE, USA). Polymerase chain reaction (PCR) was performed using AmpliTaq Gold PCR reagents (AmpliTaq Gold® Fast PCR Master Mix, Applied Biosystems, 2010, Foster City, CA, USA). The PCR conditions in a 25uL reaction volume included 1X PCR buffer, 1mM $MgCl^2$, 200 uM of each dNTP, 10 pmol of each primer (Integrated DNA Technologies, 2010, Coralville, IO, USA), 50 ng of template DNA, and 1.25 Units Amplitaq Gold DNA polymerase. Thermal cycling was done on a GeneAmp PCR System 9700 (Applied Biosystems, 2003, Foster City, CA, USA)

The primers for a portion of the bovine amelogenin locus (AMX/Y) were used to amplify the DNA (Ennis and Gallagher, 1994). The primers amplified the DNA, creating bands that reached 280 bp in females and 217 bp in males. DNA was separated using agarose gel electrophoresis on a 1.5% ultrapure agarose gel (UltrapureTM Agarose, Invitrogen, Life Technologies Inc, Burlington, ON) containing 10% SYBR Safe gel stain (SYBR SafeTM, Invitrogen, Life Technologies Inc, Burlington, ON) and the running buffer used was 1X TBE (Gel loading solutions, Ambion, Life Technologies Inc, Burlington, ON). Electrophoresis was performed for 80 min at 110V in a Sub-Cell Model 192

horizontal electrophoresis system (Agarose Gel Electrophoresis Systems Model 192, Bio-Rad Laboratories Ltd., Mississauga, ON). The molecular weight marker was a 100 bp DNA ladder (Invitrogen Life Technologies Inc, Burlington, ON). Two (2) uL of PCR product was mixed with 8 uL of 1x BlueJuice loading dye (10X Blue JuiceTM Gel Loading Buffer, Invitrogen, Life Technologies Inc, Burlington, ON) and was loaded onto the gel. The gels were imaged on an Alpha Innotech FluorchemSP camera (Alpha Innotech Inc., CA, USA).

3.2.5. Statistical Analysis

The effect of carcass classification by ultimate pH category was determined on data from selected carcasses using a one-way analysis of variance (ANOVA). When measurements were compared at two different ageing times, data were analyzed using a split plot with animal and LT section as the experimental units in the main and sub-plots, respectively. Repeated measures analysis was used for data collected instrumentally over time with time being the repeated variable. Frequencies were constructed for colour scores exceeding the AA grade average or those exceeding the JMGA colour score of 7 and a Fishers test was used to determine significance. Mixed model statistical analysis procedures were conducted using SAS version 9.2 (SAS Institute Inc., Cary, North Carolina).

3.3. Results

3.3.1. Comparison of Carcass Quality

Survey of 179 Canada grade B4 carcasses in the present study indicated that the majority (72%) had *longissimus thoracis* (LT) muscle pH values at the grade site ($12^{th}/13^{th}$ thoracic vertebrae) greater than 6.0 and were categorized as classic dark cutters (CL)(Table 3.1). Atypical dark-cutting carcasses (AT) with LT pH values of less than 5.8 were present as well, but comprised the lowest proportion at about 6% of the population sampled. The remaining carcasses, approximately 21% of the total number of carcasses, had rib eye pH values between 5.8 and 6.0 and were categorized as borderline dark cutters (BD).

Mean carcass side weights (kg), grade fat (mm), muscle score, fat classes, rib eye area (REA) and marbling scores for the sampled population (n=30) are presented for the putative carcass quality categories (AA, CL, AT, BD) described in the previous chapter in Table 3.2. There was no effect of putative grade category on grade fat, muscle score, fat class or rib eye muscle area; however, CL and BD carcass sides weighed significantly less and had higher mean AMSA marbling scores than Canada AA carcass sides ($P< 0.05$). Carcass gender was noted by class; however, this was estimated from DNA samples rather than visual assessment and the gender for one BD and one B4 carcass could not analyzed due to sample contamination. All of the control, Grade AA animals were found to be male, while only one out of seven BD, two out of three AT and four out of ten CL carcasses were male.

3.3.2. Retail Display JMGA Colour

The proportions of the various muscles from each of the putative carcass categories that were given a JMGA colour score of greater than or equal to 6.5 are summarized in Table 3.3. Muscles that were subjectively scored over a JMGA colour score of 6.5 are considered to be abnormally dark and are penalized (Robertson *et al.*, 2007). CL carcass sides had the greatest proportion of LT and LL muscles with JMGA colour score above 6.5, followed by BD and then AT sides (Table 3.3). CL carcass sides also had the greatest proportion of AD, GM, SM, ST and PP muscles with JMGA colour scores greater than or equal to 6.5 (60%). CL carcass sides had 10 of the 13 muscles studied with JMGA colour scores greater than or equal to 6.5, while BD, AT and AA had 11, 5 and 1 out of 13 muscles, respectively, with JMGA scores greater than or equal to 6.5 (Table 3.3).

Carcass sides from the CL carcass groups had the largest portion of muscles that were dark. The LT and LL muscles were consistently dark within CL carcasses (90% and 100%, respectively), while AT carcasses had slightly higher proportions of dark PM muscles compared to CL carcasses (67% versus 60%). The PM accounted for the lowest proportion of dark muscles among the middle muscle cuts for grade B4 carcasses, and was dark in 10% of control carcass sides.

Within the hind quarter cuts, 90% of the AD muscles in the CL category were considered abnormally dark. In contrast, 43% of AD muscles were

considered dark in the BD dark cutters. The BF demonstrated normal colour in all carcasses the majority of the time with only 14% of BD BF muscles and 10% of CL BF muscles abnormally dark. The GM muscles were usually dark within CL carcasses (70%) compared to BD muscles (29%) and those of AT and AA carcasses were unaffected (0%). The RF was sometimes dark, but only in AT (33%) and BD (14%) carcasses. The SM was dark in the CL carcasses (90%) and BD carcasses (57%) and was the only other muscle dark within the hind quarter (33%) of AT carcass sides. The ST was usually dark in the CL carcasses (90%), hardly affected in BD carcasses (14%), and not affected in AT and AA carcasses (0%)(Table 3.3).

The muscles within the front quarter were largely unaffected by the dark-cutting condition. The IF was dark most often in BD carcasses (29%) and to a reduced extent in CL carcasses (20%). The PP was rarely dark and was only affected in 10% of the muscles from CL carcass sides. The TB was also largely unaffected, except in BD muscles, which were dark 29% of the time.

Mean JMGA colour scores for the B4 carcass pH categories are listed in Table 3.4. CL carcass sides had the highest mean JMGA colour scores for all the muscles studied except for the RF, PP, and TB, there being no difference amongst the carcass categories for the latter two muscles. AT and BD carcass sides had the highest JMGA colour score for the RF. The two darkest muscles were found in the middle cut muscles from the CL carcass sides, the LT and the LL (JMGA= 7.72 and 8, respectively), which also had the highest mean pH values (pH= 6.7 and 6.6, respectively)($P< 0.05$). The third muscle of the middle cuts, the PM, was

not affected by the dark cutting condition to the same degree as other muscles in the middle meats.

3.3.3. Drip Loss

Differences were found between drip loss values among Canada B4 pH categories and control carcasses (Table 3.5). Within the middle cuts, the LT muscles of the AA and AT carcass sides had significantly ($P<0.05$) higher drip loss values than those of the CL and BD carcass sides, with the LT muscles from the CL carcasses exhibiting the lowest mean drip loss values. LL muscles showed similar values for drip loss, while those LL muscles from AT and BD carcasses had intermediate values. The PM showed no differences in drip loss values amongst any of the carcass categories.

The four muscles from the hind quarter, the AD, GM, SM, and ST, had differences in mean drip loss values (Table 3.5) with muscles from CL carcass sides having the lowest drip loss values ($P<0.05$). The BD carcass sides demonstrated intermediate drip loss values for most muscles except for the SM muscles, which were the same as AA carcass sides. The BF and RF muscles from the hind quarter and the muscles within the front quarter showed no significant differences in drip loss values.

*3.3.4. CIE L*a*b* Colour Scores*

Changes in objective retail display colour over 0, 2, and 4 d for the middle cuts, hind-quarter, and front quarter are shown in Table 3.6. The LT muscle indicated that pH category had an effect on a* values, which increased over time

in LT muscles from CL carcass sides. Increases in a* values indicated an increase in the redness of the muscle, and were initially highest at 2 d in all control and Canada B4 carcass sides when compared over time, but were lowest in CL carcass sides. The pH category had no effect on L*, a*, and b* values for the LL muscles.

An effect of pH category on the L*, a*, and b* values was observed in the muscles of the hind quarter and there were significant interactions between pH category and storage time for the mean a* and b* values as demonstrated within AD muscles ($P< 0.05$). Within the AD muscles mean redness values decreased over time; a* values decreased in steaks from AA, and AT carcass sides. The BF muscle showed no differences of pH category on colour. The GM muscles showed mean a* and b* values were affected by pH category, showing decreases in redness of steaks from AA and AT carcass sides and increases of redness of steaks from CL carcass sides ($P< 0.05$). The GM muscle exhibited a similar pattern for colour and pH category, showing a decrease in redness (a*) and yellowness (b*) of steaks from AA and AT carcass sides. Colour was relatively consistent among GM muscles from CL carcass sides. The SM muscle showed decreases in redness in AA carcass sides and increased redness of steaks from CL carcass sides. The ST muscles demonstrated interactions of pH category over time showing a decrease in mean redness values of steaks from AA carcass sides and an increase in redness in steaks from CL carcass sides. Mean values for yellowness of ST steaks increased in steaks from BD and CL carcass sides. No effect on colour over time was observed for the RF muscles in any pH category.

Only the PP muscles within the front quarter demonstrated effects of pH category on colour over time. The PP muscle showed redness values decreased after 2 d in steaks from AA and AT carcass sides. There were no effects of pH over time on L*, a*, and b* values for the two remaining muscles, the IF and TB.

3.3.5. Myoglobin Pigmentation

The proportions of the different derivatives of myoglobin pigments from the LT and LL muscles were affected by pH category over 0, 2, and 4 d of retail display for mean levels of deoxymyoglobin (Mb), oxymyoglobin (Oxymb), and metmyoglobin (Metmb) (Table 3.7). Levels of Metmb from LT steaks increased over time for AA, AT, and BD carcass sides; steaks from AT and BD carcass sides had the highest levels of Metmb. Over time levels of Metmb decreased in all steaks. Interestingly, levels of Oxymb decreased in steaks from AA carcass sides and increased in steaks from CL carcass sides over time in retail display. LL muscles demonstrated constant levels of Oxymb amongst pH categories. The PM muscle had higher initial levels of Metmb than the LT and LL muscles for all pH categories. Mb levels decreased over time among all pH categories, except for PM muscles from AT carcass sides.

In the hind quarter muscles, the AD, GM, SM, and ST all showed an interaction of pH category over time for the meat pigments within CL carcass sides. Metmb increased over time for all muscles in the hip among pH categories, except for the ST muscles within the CL carcass sides. Mb levels were constant for the AD and GM muscles, except in CL carcass sides where levels decreased

over time. The levels of Mb within SM and ST muscles were constant in the AA class, but decreased over time for muscles in the dark-cutting carcass sides. The BF and RF showed no interaction of pH category over time for any muscle pigment. Different proportions of pigments were observed on different days of retail display for the front quarter, although Mb concentrations did not differ between dark and control carcass sides.

3.4. Discussion

In the most extensive survey of dark cutting Canadian beef (n=170) Murray (1989) found >45% of dark carcasses had pH values <5.8. In 2007, Robertson et al. reported 25% of the Canada B4 population they surveyed (n=352) had LT pH values below 5.8 and identified them as an atypical type of dark cutter. In the present small survey (n=30) of grade B4 carcasses, only 6% of carcasses were AT, whereas 21% were borderline dark cutters and 74% were the traditional CL carcasses. Munns and Burrell (1966) found a strong correlation between high pH in the LD and the dark-cutting condition and this was also supported by the findings of MacDougall and Rhodes (1972).

In the population of carcasses selected for further study, the CL and BD carcass sides weighed less than the control carcass sides by an average of 33 to 40 kg, respectively. Although not significantly different, the carcass sides from the BD and CL groups were lighter and had numerically higher grade fat, lower rib eye area, and higher marbling scores than control carcass sides. The findings of Kreikemeier *et al.* (1998) also showed evidence that light animals may be more

susceptible to dark cutting. They found that as cattle weight increased the incidence of dark cutters decreased, and that DFD carcasses were lighter and leaner than normal carcasses. Given the small population of animals in the current study, the differences in carcass weights are of interest and may be indicative of several possible explanations for the type of beef animal that is susceptible to the dark-cutting condition.

Light weight, dark cutting carcasses within the Canada B4 grade may be the result of a gender effect (heifers), or from ongoing health challenges during the production phase (so-called "poor doers"), or from a breed effect (small frame-size animals). In addition to production factors, light weight carcasses may chill more rapidly early post mortem, slowing the rate of glycolysis and the rate of pH decline within muscles, and ultimately resulting in darkened beef (Bowling *et al.*, 1987; Murray, 1989).

The large variance of muscle colour and proportion of the muscles affected by the dark cutting condition among the carcass sides studied may be related to the level of activity assumed by each individual muscle *ante mortem*. Only working muscle groups utilize glycogen during physical activity (Bergström *et al.*, 1967) and the muscles that are the most active *ante mortem* could be exhausting the glycogen reserves, which reduces the substrates available for glycolysis. This would affect muscle acidification post mortem and muscle colour. Behaviour of beef animals *ante mortem* (ie. physical exertion, aggression, or mounting behaviour) may be causing exhaustion among muscles of the hindquarter and loin muscles more so than other muscles. This may be the cause

of the elevated muscle pH patterns observed among the different dark cutting carcasses. Another hypothesis suggested by Lister and Spencer (1983) proposes than animals that cut dark are unable to mobilize fats to a sufficient extent and therefore deplete glycogen stores more readily than normal animals.

The superior drip loss qualities demonstrated by some muscles within the CL and BD carcass sides may be useful characteristics for further processed meat products. This finding was not unexpected because dark cutting beef can be described as dark, firm and dry (DFD) beef. The firmness exhibited by dark cutting beef is due to the increased water-holding capacity at high pH. The water holding capacity of dark cutting meat may be well suited for retailers who are further processing their beef and this may be an opportunity to increase the marketability or extract additional value from dark cutting muscles.

Colour values for L*, a*, and b* decreased when muscle pH increased demonstrating an increased saturation and darkened colour among muscles with high pH. Values for a* and b* were affected by muscle pH, which is similar to the findings of past research (Page *et al.*, 2001; Wulf and Wise, 1999). According to Page *et al.* (2001), muscle pH affects muscle colour by altering hue (red, yellow, green, blue) as compared to L* which is most affected by lean maturity and impacts the lightness or darkness of muscle colour. According to L*, a*, b* colour measurements of the current study, increased muscle pH is related to increased green and blue hues, where as reduced muscle pH is related to increased red and yellow hues. The decreased colour values of carcass sides from DFD beef can be explained on the basis of lean tissue colour, which encompasses the reflectivity of

light from free water and the oxygenation of myoglobin. As Ledward *et al.* (1992) explained, meat with a high pH reflects less light than that with low pH because of how tightly water is bound within muscle fibres thus reducing the availability of free water (less light reflection) and contributing to a dark appearance. The increased amount of water held within fibres causes fibres to swell and reducing the amount of space between muscle fibres, which also contributes to a dark colour.

Beef with a high muscle pH will also have a relatively high level of enzymatic activity, which can increase the utilization of oxygen leading to a reduction of available oxygen used to oxygenate myoglobin (Ledward *et al.*, 1992; Price and Schweigert, 1987). The slow oxygenation and increase in redness values over time in some grade B4 muscles could be an indication of oxygen utilizing enzymes declining as time post mortem increases.

The JMGA colour score of 6.5 is used to describe dark cutting beef because it was considered a threshold value by Roberston *et al.* (2007) to compare normal and abnormally dark muscles. The darkest muscles based on average JMGA colour scores were found within the middle cuts, followed by the hind quarter, and the highest proportion of normal-coloured muscles were found within the front quarter for CL carcass sides. These results show there may be opportunities to recover value from AT carcasses and from the front quarters of BD carcasses. Only two muscles in AT carcasses exhibited abnormal colour, the SM and the RF, with the exception of the middle meats, and these muscles were normally coloured in over 50% of carcasses. CL carcass sides exhibited limited

ability to recover costs as the AD, GM, SM and ST muscles within the hindquarter were all very dark in colour and only the TB muscles showed any indication of recovery and normal colour.

If muscles were retained from BD carcasses and allowed into the quality grades for Canadian beef, Canada may severely compromising their commitment to quality beef by allowing beef that is both dark and compromised in tenderness. An inexpensive, accurate and non-invasive method is needed to segregate atypical B4 carcasses and recover normally coloured muscles from Canada B4 carcasses. Some countries such as Australia are already segregating carcasses based on LT pH measurements; in 1996 Australia began using pH measurements as part of their formula for sorting quality beef carcasses setting a maximum pH at 5.7 (Meat Standards Australia, 2010). Carcasses with a pH above 5.7 are ineligible for a Meat Standards Australia (MSA) grade (Meat Standards Australia, 2010). The MSA grading program is designed to guarantee tenderness, and measures a variety of factors other than pH such as tropical breed percentage, ossification, marbling, carcass weight, sex, and hanging method. Canada may find measuring LT pH as a valuable indicator of carcass quality and as a means of effectively measuring carcass quality and sorting accordingly for marketing purposes.

3.5. Conclusion

Grade B4 carcass sides weighed less than control Canada AA carcasses, but exhibited adequate muscling and fat cover. This result suggests that dark cutting animals may be a specific biological type not associated with gender as gender did not appear to contribute to the incidence of dark cutting. Segregation

of the B4 carcasses by pH also demonstrated that the LT muscle pH may be indicative of the colour of the remaining muscles within the carcass and usually the proportion of muscles that will be dark within a Canada B4 carcass can be estimated based on mean pH values of the LT muscle. There are opportunities to recover value from dark-cutting carcasses especially if they belong to the AT group with pH<5.8. Carcasses within the AT pH category demonstrate a large proportion of normally coloured muscles. Carcasses within the BD pH category also demonstrate a large proportion of normally coloured muscles within the front quarter, but tenderness may be an issue. Therefore the largest opportunity for cost recovery of dark cutting carcasses lies within AT carcasses due to the high proportion of normally coloured and palatable muscles. AT carcasses would be an ideal candidate to recover value in comparison to BD and CL carcasses.

Table 3.1. Incidence of atypical, borderline and classic dark cutting carcasses within a survey of 179 Canada B4 carcass sides

Lot number		AT[1]	BD[2]	CL[3]	Total Carcasses
1	frequency	1	2	18	21
	%	5%	10%	86%	
2	frequency	4	12	18	34
	%	12%	35%	53%	
3	frequency	7	17	33	57
	%	12%	30%	58%	
4	frequency	0	3	12	15
	%	0%	20%	80%	
5	frequency	0	4	48	52
	%	0%	8%	92%	
Average		6%	21%	72%	179

[1] Atypical dark cutters with a muscle pH below 5.8.
[2] Borderline dark cutters with a muscle pH above 5.8 but below 6.0.
[3] Typical dark cutters with a muscle pH above 6.0.

Table 3.2 Comparison of carcass characteristics between control (AA) and atypical , borderline and classic dark cutting carcasses

	AA	AT[1]	BD[2]	CL[3]	S.E.M.[4]	Prob > F[5]
Carcass characteristics						
No. of carcasses	10	3	7	10	-	-
Side weight, kg	204[a]	176[ab]	164[b]	169[b]	9.13	0.004
Grade fat, mm	6.4	7.3	9.7	7.4	1.54	0.399
Muscle Score[6]	3.7	3.3	3.4	2.7	0.35	0.109
Fat Class[7]	2.9	3.3	4.4	3.6	0.73	0.423
REA, cm[2, 8]	98.1	96.3	90.7	83.5	5.14	0.103
AMSA Marbling[9]	455[b]	457[ab]	511[a]	494[a]	16.52	0.039
No.[10] of males	10	2	1	4	-	-

[1] Atypical dark cutters with a muscle pH below 5.8.
[2] Borderline dark cutters with a muscle pH above 5.8 but below 6.0.
[3] Typical dark cutters with a muscle pH above 6.0.
[4] Standard error of the mean.
[5] Probability of the F test, with significance at $P < 0.05$.
[6] Muscle scores 1-4 (Canadian Grade Standards) with 1 designating the smallest and 4 designating the largest.
[7] Fat class scores 1-8 (Canadian Grade Standards) with 1 demonstrating the least amount of subcutaneous fat and 8 demonstrating the most subcutaneous fat.
[8] REA = rib eye area, size of *Longissimus thoracis* (LT) as measured by grid.
[9] Marbling, AMSA= American Meat Science Association marbling scores (100= devoid, 200 = practically devoid, 300 = traces, 400 = slight, 500 = small, 600 = modest).
[10] Number = number of animals that were male in gender.
[a,b] Means with different superscripts within a row are significantly different at $P < 0.05$.

Table 3.3 Incidence of Japanese Meat Grading Association (JMGA) colour score[1] greater than 6.5 in control (AA) and atypical, borderline and classic dark cutting carcasses.

	AA	AT[2]	BD[3]	CL[4]
Middle Meats N	10	3	7	10
Longissimus thoracis	0%	67%	86%	90%
Longissimus lumborum	0%	67%	71%	100%
Psoas major	10%	67%	29%	60%
Hind Quarter				
Adductor	0%	0%	43%	90%
Biceps femoris	0%	0%	14%	10%
Gluteus medius	0%	0%	29%	70%
Rectus femoris	0%	33%	14%	0%
Semimembranosus	0%	33%	57%	90%
Semitendinosus	0%	0%	14%	90%
Front Quarter				
Infraspinatus	0%	0%	29%	20%
Pectoralis profundi	0%	0%	0%	10%
Triceps brachii	0%	0%	29%	0%

[1] JMGA scores range from 1 to 8 with one being the very palest and 8 being the very darkest. The cutoff for grade CL beef is considered to be at a JMGA score of 6.5.
[2] Atypical dark cutters with a muscle pH below 5.8.
[3] Borderline dark cutters with a muscle pH above 5.8 but below 6.0.
[4] Typical dark cutters with a muscle pH above 6.0.

Table 3.4 Mean muscle pH and Japanese Meat Grading Association (JMGA) colour scores for 12 muscles from control (AA) and atypical, borderline and classic dark cutting carcasses

	AA[1]	AT[2]	BD[3]	CL[4]	S.E.M.[5]	Prob[6] > F
Middle Cuts n	10	3	7	10		
JMGA score						
Longissimus thoracis	3.10[c]	5.08[b]	6.18[b]	7.55[a]	0.39	<.0001
Longissimus lumborum	3.90[c]	6.67[b]	7.14[b]	8.00[a]	0.28	<.0001
Psoas major	4.75	4.83	6.14	5.9	0.63	0.238
Muscle pH						
Longissimus thoracis	5.57[c]	5.54[c]	5.96[b]	6.74[a]	0.05	<.0001
Longissimus lumborum	5.59[c]	5.52[c]	5.92[b]	6.64[a]	0.05	<.0001
Psoas major	5.74	5.63	5.81	5.88	0.07	0.130
Hind Quarter						
JMGA score						
Adductor	3.15[c]	5.33[b]	5.92[b]	7.6[a]	0.51	<.0001
Biceps femoris	3.15[b]	5.9[a]	4.21[ab]	5.0[a]	0.44	0.006
Gluteus medius	2.70[c]	4.0b[c]	5.29[b]	7.0[a]	0.55	<.0001
Rectus femoris	1.85[c]	4.67[a]	3.50[ab]	2.3[bc]	0.64	0.040
Semimembranosus	3.75[c]	5.83[b]	6.50[b]	7.6[a]	0.42	<.0001
Semitendinodus	1.70[c]	2.5[c]	4.00[b]	7.3[a]	0.4	<.0001
Muscle pH						
Adductor	5.62[c]	5.51[c]	5.87[b]	6.16[a]	0.08	<.0001
Biceps femoris	5.61	5.49[b]	5.68[a]	5.7[a]	0.04	0.026
Gluteus medius	5.63[b]	5.6[b]	5.73[b]	6.03[a]	0.06	<.0001
Rectus femoris	5.70	5.74	5.86	5.81	0.10	0.638
Semimembranosus	5.59[b]	5.51[b]	5.75[b]	6.02[a]	0.07	<.0001
Semitendinosus	5.63[b]	5.51[b]	5.79[b]	6.58[a]	0.09	<.0001
Front Quarter						

JMGA score						
Infraspinatus	4.35[b]	4.17[ab]	5.71[a]	5.6[a]	0.44	
Pectoralis profundi	3.95	4.67	4.86	4.85	0.39	0.028
Triceps brachii	4.2	5.5	5.43	4.65	0.42	0.176
Muscle pH						
Infraspinatus	5.76	5.67	5.86	5.89	0.06	0.149
Pectoralis profundi	5.66	5.69	5.72	5.86	0.08	0.071
Triceps brachii	5.67	5.64	5.77	5.65	0.06	0.113

[1] Canada grade AA carcasses.
[2] Atypical dark cutters with a muscle pH below 5.8.
[3] Borderline dark cutters with a muscle pH above 5.8 but below 6.0.
[4] Typical dark cutters with a muscle pH above 6.0.
[5] Standard error of the mean.
[6] Probability of the F test, with significance at $P < 0.05$.
[a, b] Means with different superscripts within a row are significantly different at $P < 0.05$.

Table 3.5 Retail drip loss (mg/g initial weight) for 12 muscles from control (AA) and atypical, borderline and classic dark cutting carcasses

	AA[1]	AT[2]	BD[3]	CL[4]	S.E.M.[5]	Prob > F[6]
Middle Cuts						
Longissimus thoracis	52.69[a]	42.16[ab]	44.91[a]	31.35[b]	3.68	<.0001
Longissimus lumborum	43.73[a]	28.81[b]	27.17[b]	19.87[c]	2.53	<.0001
Psoas major	35.31	44.12	37.32	39.51	10.02	0.805
Hind Quarter						
Adductor	74.13[a]	66.56[ab]	51.94[b]	37.49[c]	6.31	0.0002
Biceps femoris	41.41	24.65	41.21	37.27	5.01	0.295
Gluteus medius	63.77[a]	50.43[ab]	45.76[b]	45.76[b]	6.62	0.010
Semimembranosus	57.80[a]	43.08[ab]	45.79[a]	29.35[b]	4.9	0.0003
Semitendinosus	53.04[a]	27.27[bc]	43.75[b]	20.34[c]	5.63	0.0002
Rectus femoris	63.60	45.76	62.97	69.76	39.59	0.857
Front Quarter						
Infraspinatus	24.37	21.59	20.97	35.95	7.61	0.506
Pectoralis profundi	35.07	29.88	37.08	34.84	3.04	0.600
Triceps brachii	45.32	35.99	36.80	42.02	5.77	0.689

1 Canada grade AA carcasses.
[2]Atypical dark cutters with a muscle pH below 5.8.
3 Borderline dark cutters with a muscle pH above 5.8 but below 6.0.
4 Typical dark cutters with a muscle pH above 6.0.
5 Standard error of the mean.
6 Probability of the F test, with significance at $P < 0.05$.
a,b Means with different superscripts within a row are significantly different at $P < 0.05$.

Table 3.6 Objective colour measurements (L*, a*, b*) measured at d 0, 2 and 4 of retail display for 12 muscles from control (AA) and atypical, borderline and classic dark cutting carcasses

Middle Cuts

	AA[1]			AT[2]			BD[3]			CL[4]			SEM[5]	Pr > F[6]
	0d	2d	4d	0d	2d	4d	0d	2d	4d	0d	2d	4d		trt*time
Longissimus thoracis														
L*	42.35	41.99	41.66	37.87	39.54	38.13	39.34	38.26	37.78	35.38	34.09	32.67	1.00	0.2696
a*	17.48^{cd}	18.83^{ab}	17.87^{cd}	17.33^{abc}	18.42^{ac}	16.45^{bde}	14.36^{ef}	16.88^{cd}	14.74^{e}	10.30^{g}	12.81^{f}	12.74^{f}	0.74	0.0115
b*	12.39	13.72	13.73	10.75	12.44	12.08	9.44	12.15	11.56	6.1	8.55	8.38	0.58	0.1167
Longissimus lumborum														
L*	40.82	40.75	40.95	35.87	35.39	35.03	38.71	38.18	37.89	33.83	32.9	32.47	0.80	0.7207
a*	15.94	18.06	17.6	15.65	16.53	15.95	12.92	15.19	14.67	8.58	10.76	11.54	0.47	0.1538
b*	11.16	13.1	13.4	9.57	10.58	10.87	9.02	11.93	11.69	4.69	7.23	7.7	0.42	0.1431
Psoas major														
L*	39.93	40.3	39.25	40.12	37.99	38.83	38.05	37.52	37.19	40.73	39.79	39.67	0.90	0.7307
a*	17.15	16.07	13.53	18.07	15.39	11.29	16.12	15.31	12.67	16.16	15.39	13.59	0.73	0.188
b*	10.59	12.37	11.6	11.93	11.5	10.83	10.21	11.37	10.55	10.81	12.08	11.38	0.51	0.1268

Hind Quarter

	AA[1]			AT[2]			BD[3]			CL[4]			SEM[5]	Pr > F[6]
	0d	2d	4d	0d	2d	4d	0d	2d	4d	0d	2d	4d		trt*time
Adductor														
L*	44.06	44.18	43.24	37.65	37.63	36.5	37.58	36.08	36.22	34.56	34	33.07	1.06	0.8066
a*	22.00^{a}	17.64^{b}	14.71^{de}	21.00^{a}	17.22^{bc}	14.39^{def}	15.91^{bcd}	15.94^{bcd}	14.72^{cde}	12.40^{f}	13.73^{e}	13.62^{ef}	0.72	<.0001

b*	15.88^{a}	15.42^{a}	14.61^{b}	12.71^{bc}	12.11^{cd}	11.54^{cde}	10.07^{de}	11.89^{c}	11.42^{c}	7.42^{f}	9.45^{e}	9.71^{e}	0.64	0.0001
Biceps femoris														
L*	41.75	41.94	41.7	39.4	38.65	40.23	40.45	39.47	37.98	40.85	40.61	39	1.05	0.1431
a*	20.32	17.93	14.87	20.59	18.06	14.32	18.9	16.4	13.64	17.97	16.37	15.5	0.92	0.2088
b*	13.49	14.18	13.19	12.94	12.51	12.59	12.19	12.7	11.84	12.37	12.48	12.37	0.67	0.8887
Gluteus medius														
L*	43.62	42.8	42.65	38.12	39.1	38.34	38.75	38.03	37.55	36.37	35.23	34.96	1.12	0.6879
a*	22.39^{a}	17.85^{bc}	14.82^{def}	20.11^{ab}	17.47^{cde}	13.67^{fg}	17.09^{bcd}	15.79^{cdef}	14.32^{ef}	14.00^{f}	15.41^{deg}	15.04^{def}	0.93	<.0001
b*	15.65^{a}	14.76^{b}	13.79^{c}	12.55^{bcd}	12.85^{bcd}	11.97^{cd}	10.86^{d}	11.84^{ce}	11.82^{ce}	8.56^{f}	10.68^{de}	10.58^{de}	0.78	<.0001
Semimembranosus														
L*	39.56	41.04	39.94	35.91	36.78	36.11	36.86	36.49	36.5	34.39	34.57	34.67	0.91	0.7819
a*	19.91^{a}	18.34^{bc}	17.20^{de}	17.87^{abcd}	18.13^{abd}	15.79^{cef}	15.00^{f}	16.60^{bcd}	15.69^{def}	12.08^{g}	14.06^{f}	13.89^{f}	0.77	<.0001
b*	13.80^{a}	13.99^{a}	13.80^{a}	11.01^{cde}	13.04^{ab}	11.21^{cde}	9.66^{e}	12.08^{bc}	11.84^{bcd}	7.78^{f}	10.31^{de}	10.07^{e}	0.63	0.0213
Semitendinosus														
L*	46.33	46.65	46.15	42.13	42.89	42.78	41.79	41.25	41.76	35.86	35.5	34.58	0.93	0.4608
a*	18.07^{ab}	17.90^{ab}	16.29^{cde}	20.01^{a}	18.62^{bc}	15.00^{defg}	15.67^{ef}	17.19^{bcd}	15.90^{e}	10.63^{h}	13.38^{g}	13.99^{fg}	0.73	<.0001
b*	15.00^{bc}	16.29^{a}	15.68^{ab}	14.65^{abc}	15.24^{abc}	14.11^{abcd}	12.02^{d}	14.17^{bc}	13.49^{c}	6.18^{f}	9.37^{e}	9.37^{e}	0.68	0.0019
Rectus femoris														
L*	47.75	46.98	47.59	43.06	40.96	40.37	43.85	43.53	42.28	45.75	45.29	45.41	1.52	0.4794
a*	20.17	16.4	13.77	18.19	17.77	17.29	19.11	15.79	13.97	20.58	16.25	14.18	0.89	0.117
b*	16	15.98	15.46	12.36	13.44	13.98	14.14	14.03	13.69	15.53	14.86	15.23	0.76	0.5512

Front Quarter

	AA[1]			AT[2]			BD[3]			CL[4]			SEM[5]	Pr > F[6]
	0d	**2d**	**4d**	**0d**	**2d**	**4d**	**0d**	**2d**	**4d**	**0d**	**2d**	**4d**		**P trt*time**
Infraspinatus														
L*	42.71	41.56	41.71	40.89	38.91	39.59	40.25	40.27	39.83	41.97	40.45	40.94	0.84	0.8454
a*	16.01	14.19	12.43	17.02	14.28	11.47	15.05	13.95	12.74	15.15	14.47	13.78	0.64	0.1049
b*	11.03	13.42	12.67	12.02	12.79	12.2	10.8	12.7	11.73	10.58	12.62	12.32	0.49	0.4515
Pectoralis profundi														
L*	40.65	41.04	41.25	37.39	38.04	37.87	41.58	40.36	40.31	40.65	42.1	40.41	0.88	0.1468
a*	15.94^{ab}	16.10^{ab}	14.22^{d}	16.37^{ab}	16.79^{ab}	13.72^{cd}	14.70^{abcd}	15.18^{abcd}	14.21^{cd}	14.84$^{b}_{cd}$	15.94^{a}	15.71$^{a}_{bc}$	0.61	0.0155
b*	10.89	12.71	12.29	10.46	12.03	11.24	11.22	12.77	12.46	10.69	13.13	12.84	0.37	0.5014
Triceps brachii														
L*	40.87	40.31	40.48	39.13	37.64	37.99	39.75	38.28	37.58	39.95	39.82	39.45	0.92	0.4101
a*	17.87	17.17	14.65	15.32	17.21	14.29	16.23	15.68	14.24	17.38	16.15	14.96	0.86	0.2971
b*	11.89	13.08	12.68	9.58	12.27	11.98	10.62	11.29	11.17	11.38	12.41	12.62	0.59	0.5875

[1] Canada grade AA carcasses.
[2] Atypical dark cutters with a muscle pH below 5.8.
[3] Borderline dark cutters with a muscle pH above 5.8 but below 6.0.
[4] Typical dark cutters with a muscle pH above 6.0.
[5] Standard error of the mean.
[6] Probability of the F test, with significance at $P < 0.05$.
[a,b] Means with different superscripts within a row are significantly different at $P < 0.05$.

Table 3.7 Proportions of metmyoglobin (Metmb), myoglobin (Mb), and oxymyoglobin (Oxymb) as measured at d 0, 2 and 4 of retail display for 12 muscles from control (AA) and atypical, borderline and classic dark cutting carcasses

Middle Cuts

	AA[1]			AT[2]			BD[3]			CL[4]			SEM[5]	Pr > F[6]
	0d	2d	4d	0d	2d	4d	0d	2d	4d	0d	2d	4d		trt*time
Longissimus thoracis														
Metmb	0.16^{i}	0.20^{efgh}	0.22^{bcd}	0.19^{dghi}	0.22^{cef}	0.26^{ab}	0.17^{gij}	0.21^{cdef}	0.26^{a}	0.18^{fhij}	0.19^{cdefg}	0.20^{cdeg}	0.01	0.0016
Mb	0.10^{ef}	0.08^{f}	0.08^{f}	0.20^{b}	0.12^{cdef}	0.15^{bcdef}	0.17^{bc}	0.08^{f}	0.10^{def}	0.32^{a}	0.15^{bcde}	0.16^{bcd}	0.03	<.0001
Oxymb	0.75^{a}	0.72^{ab}	0.69^{bcd}	0.61^{ef}	0.66^{bcde}	0.59^{f}	0.66^{bcdef}	0.71^{abc}	0.63^{def}	0.49^{g}	0.66^{cdef}	0.63^{ef}	0.02	<.0001
Longissimus lumborum														
Metmb	0.16^{f}	0.19^{e}	0.21^{cd}	0.21^{bde}	0.24^{ac}	0.27^{a}	0.18^{def}	0.25^{ab}	0.26^{a}	0.19^{de}	0.19^{de}	0.20^{de}	0.01	0.0017
Mb	0.12^{de}	0.08^{f}	0.08^{ef}	0.11^{cdef}	0.10^{def}	0.10^{def}	0.17^{cd}	0.08^{ef}	0.09^{ef}	0.38^{a}	0.22^{b}	0.17^{c}	0.02	<.0001
Oxymb	0.73^{a}	0.73^{a}	0.71^{ab}	0.69^{abc}	0.66^{bc}	0.63^{cd}	0.65^{c}	0.67^{bc}	0.65^{c}	0.43^{e}	0.59^{d}	0.63^{c}	0.02	<.0001
Psoas major														
Metmb	0.2	0.32	0.38	0.23	0.34	0.43	0.23	0.33	0.39	0.24	0.34	0.39	0.01	0.4795
Mb	0.19^{a}	0.10^{de}	0.11^{d}	0.12^{cd}	0.10^{de}	0.12^{cde}	0.14^{bc}	0.10^{de}	0.11^{de}	0.15^{b}	0.09^{e}	0.10^{de}	0.01	0.0014
Oxymb	0.61	0.58	0.51	0.65	0.56	0.46	0.63	0.57	0.51	0.61	0.56	0.52	0.02	0.0722

Hind Quarter

	AA[1]			AT[2]			BD[3]			CL[4]			SEM[5]	Pr > F[6]
	0d	2d	4d	0d	2d	4d	0d	2d	4d	0d	2d	4d		trt*time
Adductor														
Metmb	0.19^{h}	0.31^{c}	0.37^{a}	0.24^{efg}	0.30^{cd}	0.37^{ab}	0.24^{fg}	0.30^{cde}	0.33^{bc}	0.22^{gh}	0.27^{df}	0.30^{ce}	0.02	<.0001
Mb	0.05^{d}	0.06^{cd}	0.06^{bcd}	0.05^{cd}	0.07^{bcd}	0.07^{bcd}	0.09^{bc}	0.06^{bcd}	0.07^{bcd}	0.20^{a}	0.10^{b}	0.09^{bc}	0.01	<.0001
Oxymb	0.77^{a}	0.63^{cd}	0.56^{g}	0.71^{ab}	0.63^{cde}	0.56^{fg}	0.68^{bc}	0.64^{cd}	0.61^{defg}	0.58^{efg}	0.63^{cd}	0.62^{def}	0.02	<.0001
Biceps femoris														
Metmb	0.2	0.28	0.34	0.23	0.28	0.36	0.23	0.3	0.38	0.21	0.29	0.33	0.01	0.0891
Mb	0.07	0.07	0.08	0.05	0.08	0.09	0.08	0.08	0.09	0.1	0.08	0.08	0.01	0.3366

Oxymb	0.73	0.65	0.58	0.71	0.64	0.55	0.69	0.62	0.53	0.69	0.63	0.59	0.02	0.1524
Gluteus medius														
Metmb	0.19^{f}	0.31^{bc}	0.37^{a}	0.23^{def}	0.30^{bc}	0.38^{a}	0.23^{e}	0.32^{c}	0.35^{ab}	0.23^{ef}	0.28^{cd}	0.30^{c}	0.02	<.0001
Mb	0.05^{c}	0.07^{bc}	0.08^{bc}	0.07^{bc}	0.08^{bc}	0.09^{bc}	0.10^{b}	0.07^{bc}	0.07^{bc}	0.17^{a}	0.08^{bc}	0.07^{bc}	0.02	0.0002
Oxymb	0.76^{a}	0.62^{cde}	0.55^{fh}	0.70^{ab}	0.61^{cdef}	0.53^{gh}	0.67^{bc}	0.61^{de}	0.58^{efg}	0.60^{eg}	0.64^{bcd}	0.63^{cde}	0.02	<.0001
Semimembranosus														
Metmb	0.20^{i}	0.27^{cef}	0.29^{abd}	0.24^{fgh}	0.29^{bcde}	0.33^{a}	0.23^{gh}	0.27^{def}	0.30^{abc}	0.21^{hi}	0.24^{fg}	0.26^{efg}	0.01	0.0207
Mb	0.04^{e}	0.04^{e}	0.06^{bcde}	0.05^{bcde}	0.05^{bcde}	0.07^{bcde}	0.10^{b}	0.05^{cde}	0.05^{de}	0.21^{a}	0.09^{bc}	0.08^{bcd}	0.02	<.0001
Oxymb	0.77^{a}	0.69^{b}	0.65^{cd}	0.70^{abc}	0.66^{bc}	0.61^{de}	0.67^{bcd}	0.69^{bc}	0.65^{bcd}	0.58^{e}	0.67^{bcd}	0.66^{bcd}	0.02	<.0001
Semitendinosus														
Metmb	0.16^{h}	0.24^{cde}	0.27^{b}	0.20^{efgh}	0.25^{bcd}	0.32^{a}	0.18^{gh}	0.22^{df}	0.24^{bce}	0.20^{fg}	0.19^{gh}	0.20^{fg}	0.01	<.0001
Mb	0.07^{cde}	0.05^{e}	0.06^{de}	0.07^{cde}	0.06^{cde}	0.08^{bcde}	0.12^{bc}	0.06^{de}	0.07^{de}	0.36^{a}	0.14^{b}	0.11^{cd}	0.02	<.0001
Oxymb	0.77^{a}	0.72^{b}	0.67^{cd}	0.74^{abc}	0.69^{abc}	0.60^{d}	0.70^{bc}	0.72^{abc}	0.69^{bcd}	0.44^{e}	0.67^{bcd}	0.69^{bc}	0.02	<.0001
Rectus femoris														
Metmb	0.16^{e}	0.30^{bc}	0.36^{a}	0.20^{de}	0.24^{cd}	0.28^{c}	0.19^{de}	0.29^{c}	0.35^{ab}	0.18^{de}	0.30^{c}	0.36^{a}	0.02	0.0497
Mb	0.08	0.09	0.09	0.1	0.09	0.08	0.09	0.1	0.1	0.07	0.09	0.1	0.01	0.3632
Oxymb	0.76^{a}	0.61^{d}	0.55^{e}	0.70^{abc}	0.67^{bcd}	0.64^{cd}	0.72^{ab}	0.61^{d}	0.55^{e}	0.75^{a}	0.61^{d}	0.55^{e}	0.02	0.0321

Front Quarter

	AA[1]			**AT[2]**			**BD[3]**			**CL[4]**			**SEM[5]**	**Pr > F[6]**
	0d	**2d**	**4d**	**0d**	**2d**	**4d**	**0d**	**2d**	**4d**	**0d**	**2d**	**4d**		**P trt*time**
Infraspinatus														
Metmb	0.21^{d}	0.37^{c}	0.42^{ab}	0.24^{d}	0.39^{bc}	0.47^{a}	0.22^{d}	0.35^{c}	0.38^{bc}	0.22^{d}	0.34^{c}	0.36^{c}	0.02	0.0331
Mb	0.18^{a}	0.08^{c}	0.09^{c}	0.10^{c}	0.09^{c}	0.09^{c}	0.15^{ab}	0.08^{c}	0.09^{c}	0.15^{b}	0.08^{c}	0.08^{c}	0.01	0.0354
Oxymb	0.61	0.55	0.49	0.66	0.52	0.44	0.63	0.56	0.53	0.63	0.58	0.56	0.02	0.1241
Pectoralis profundi														
Metmb	0.18	0.27	0.33	0.21	0.27	0.34	0.2	0.3	0.33	0.19	0.27	0.29	0.01	0.0525
Mb	0.19	0.09	0.1	0.16	0.09	0.11	0.18	0.09	0.09	0.21	0.09	0.09	0.01	0.1807
Oxymb	0.62^{abc}	0.63^{ab}	0.57^{de}	0.63^{abcd}	0.64^{abcd}	0.55^{e}	0.62^{abcd}	0.61^{abcde}	0.58^{cde}	0.59^{bcde}	0.65^{a}	0.62^{abc}	0.02	0.0248

Triceps brachii														
Metmb	0.19	0.28	0.34	0.21	0.26	0.34	0.21	0.29	0.32	0.21	0.3	0.33	0.02	0.2758
Mb	0.11	0.08	0.09	0.15	0.09	0.1	0.14	0.09	0.09	0.13	0.08	0.09	0.01	0.9363
Oxymb	0.7	0.64	0.57	0.64	0.65	0.56	0.65	0.63	0.58	0.66	0.62	0.58	0.02	0.2657

[1] Canada grade AA carcasses.
[2] Atypical dark cutters with a muscle pH below 5.8.
[3] Borderline dark cutters with a muscle pH above 5.8 but below 6.0.
[4] Typical dark cutters with a muscle pH above 6.0.
[5] Standard error of the mean.
[6] Probability of the F test, with significance at $P < 0.05$.
[a,b] Means with different superscripts within a row are significantly different at $P < 0.05$.

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4.0. UNDERSTANDING THE EFFECTS OF CHILLING ON THE COLOUR OF BOVINE LONGISSIMUS THORACIS

4.1. Introduction

Beef products from cattle that have been treated with aggressive growth implants may suffer from increased toughness (Thompson *et al.*, 2002) and may be at risk of dark-cutting (Scanga *et al.*, 1998). The use of hormonal growth promotants skews the bovine metabolism toward the production of muscle protein at the expense of fat and glycogen accretion and may lead to cattle being marketed with fewer energy reserves than in the past. Late in October 2005 the number of animals being slaughtered in Alberta decreased. This coincided with an increase in the percentage of carcasses that 'cut dark' and graded Canada B4 (Alberta Beef Producers, personal communication). The decrease in the number of cattle slaughtered means there was a decrease in the number of carcasses hanging in the cooler, and unless the coolers were adjusted to accommodate this change in carcass density the cooling rate will be impacted. The impacts of chilling rate on colour have been well documented (Aalhus *et al.*, 2001, 2002; Bowling *et al.*, 1987). Animals marketed with compromised muscle glycogen concentration or low levels of subcutaneous fat may produce meat that is susceptible to meat quality defects such as dark-cutting, cold shortening, or cold induced toughening (reference). Results from the first two dark-cutting beef studies (Sections 2 and 3) showed that dark-cutting carcasses may be defined by a biological type that was characterized by carcasses with reduced weights but adequate muscling and fat cover. Carcass weight has been shown to influence meat colour previously, with dark lean colour being exhibited in the LD muscles

of light weight carcasses (Jones and Tong, 1989). A reduced carcass weight may contribute to an increase in cooling rate, which may slow the rate of glycolysis and darken the beef (Aalhus *et al.*, 2002).

A variety of carcass chilling and cooling systems have been explored with the goal to rapidly reduce carcass temperatures and these systems have been described as "rapid," "ultra-rapid," "blast," "very fast," and "extreme," with no consistent definition among authors. In the present study, our focus was to evaluate muscle colour differences in rapidly and conventionally chilled beef at 24 h and over a 4 d time period, and to note any compromises in quality that might accompany chilling treatments. This study tested the hypothesis that rapidly chilled carcasses are darker in lean colour than conventionally chilled carcass. Also, there may be additional meat quality characteristics affected by rapid chilling and so a subsequent hypothesis was the quality of the *longissimus thoracis* (LT) is compromised from rapidly chilled beef carcasses.

4.2. Materials and Methods

Ten steers from the University of Alberta Kinsella Ranch were slaughtered in two groups of five at the Agriculture and Agri-Food Canada (AAFC) Meat Laboratory in Lacombe, Alberta. Steers were loaded early in the morning at the ranch and were shipped to Lacombe (200 km) and held in lairage for 2 h with free access to water before slaughter. Two h after arrival, steer live weights were recorded and the steers were stunned, exsanguinated, and dressed in a simulated commercial manner in accordance with the principals and guidelines established by the Canadian Council on Animal Care (CCAC, 1993). Following splitting of

the carcasses, hot carcass side weights were recorded. The fat cap covering the LT on the right side of all carcasses was peeled back and removed, exposing the underlying LT muscle to a rapid chill. Temperature data recorders within loin muscles measured the temperature decline at the centre of the LT muscles on the right and left sides of carcasses (TempRecord International Ltd., Auckland, NZ). Temperatures were electronically recorded every 15 min after the initiation of chilling for approximately 24 h until carcass fabrication**.**

4.2.1. Carcass Fabrication

At 24 h post mortem, left and right carcass sides were weighed to determine cooler shrink loss. The left and right carcass sides were ribbed at the Canadian grade site (between the 12^{th} and 13^{th} ribs) and exposed to atmospheric oxygen for a 20-min period. Full Canadian grade data were collected on the left side only and LT areas were determined from tracings of digital images (COHU solid state camera and Jandel Scientific Video Analysis Program V1.31, Jandel Scientific, 1989). Three objective colour measurements (CIE L*(brightness), a*(red-green axis), b*(yellow-blue axis) values (Commission Internationale de l'Eclairage, 1978) were collected from three locations at the grade site for both sides of each carcass using a Minolta CM700D meter equipped with Spectra QC-300 Software (Minolta Canada, Mississauga, ON). Carcass sides were also assessed for colour subjectively using the Japanese Meat Grading Association (JMGA, 1988) colour standards by a certified Canadian beef grader. The LT muscles were removed, weighed, and fabricated.

The LT was split into two halves, with one half assigned to immediate, unaged assessments and the other was kept to determine purge loss over 14 d at 2 °C. The half designated for immediate assessment was fabricated into 2.5 cm steaks from the anterior end and steaks were assigned to the following treatments: shear force evaluation, retail Minolta and drip loss assessment, fibre type determination and sarcomere length measurement, and proximate analysis and glucidic potential analysis. The 14 d aged portions of the LT muscles were weighed, packaged in polypropylene bags and stored under vacuum at 2 °C. Following 14 d of ageing at 2 ºC, the aged LT muscle was removed from its vacuum packaging and weighed to determine purge losses during storage. The muscle was then fabricated into three steaks (2.5 cm), starting at the posterior end. The first steak was assigned to sensory panel determinations and was re-packaged under vacuum and frozen at -20 ºC until evaluation. The second steak was used for shear force measurements, cook loss, and cook time and the third steak was used for drip loss determinations.

4.2.2. Methods

Methods for retail display colour (Section 3.2.2), myoglobin pigment assessment (Sections 3.2.3), sarcomere length, muscle fibre type, and muscle fibre length (Sections 2.3.4), proximate analysis (Section 2.3.3), glucidic potential (Section 2.3.2), shear force and drip loss (Section 4.35) sensory analysis (Section 2.3.6.) were all assessed as described in the previous sections.

4.2.8. Statistical Analysis

The statistical model used was a split plot with two treatments applied at different experimental levels. The first treatment of rapid chill was applied to the carcass side and the second treatment of 14 d of ageing was applied to the LT muscle, which was split into two portions. Repeated measures analysis was used for data collected instrumentally over time, including muscle pigments and objective colour values, with time being the repeated variable. Frequencies were constructed for colour scores exceeding the AA grade average or those exceeding the JMGA colour score of 6.5 and a Fisher's exact test was used to determine significance. A Chi square analysis was used to analyze the categorical sensory data. A mixed model statistical analysis was conducted using SAS version 9.2 (SAS Institute Inc., Cary, North Carolina).

4.3. Results

4.3.1. Carcass Quality

Ten steers from the University of Alberta Kinsella ranch, representative of beef animals present in the Alberta beef industry, were consistent in size and averaged 533 kg live weight with the lightest steer weighing 505 kg and the heaviest weighing 554 kg (Table 4.1). Steers were British-cross genetics with mostly solid red or black coats. Animals were moderately finished and averaged a score of 5.4 for fat class fitting into the middle ranges for degree of fatness (1 = least amount of fat and 8 = highest amount of subcutaneous fat) (Table 4.1). The average muscle score was 2, and muscle scores ranged from 1 to 3 (Table 4.1). Rib eye areas (REA) measured the average size of the LT muscles between the

12^{th} and 13^{th} rib (Canadian grading site) and averaged 78.3 mm^2 (Table 4.1). Steers averaged 11.3 mm for subcutaneous grade fat measurements, which ranged from 7 to 17 mm at the grade site (Table 4.1). Marbling scores averaged 454, which demonstrated slight amounts of intramuscular fat (Table 4.1). Carcasses ranged from a quality grade of Canada A with a marbling score of 370 (traces of marbling) to a quality grade of Canada AAA with a marbling score of 500 (small amount of marbling) (Table 4.2). The majority (80%) of the carcasses graded Canada AA. Canadian yield grades demonstrated that half (50%) of the carcasses had yield grades of 2, and 30% had a yield grade of 1. Purge loss values measured in $mg \cdot g^{-1}$ and cooler loss percentage showed no significant differences between conventionally and rapidly chilled carcass sides (Table 4.3). There were no noticeable differences in colour as denoted by Japanese Meat Grading Association (JMGA) colour scores detected by visual assessment of certified graders, although there was a tendency for colour to be darker for the rapidly chilled than for the conventionally chilled beef ($P = 0.11$).

4.3.2. Sarcomere Length, Muscle Fibre Type, Muscle Fibre Diameter, and Temperature and pH values

After 24 h of chilling, the colour of LT muscles only differed in yellowness as indicated by the lowered b* values for the rapidly chilled muscles (Table 4.3). Average fibre areas and proportion of different fibres showed no differences between slow oxidative (SO), fast glycolytic oxidative (FOG), and fast glycolytic (FG) between conventional and rapidly chilled LT muscles. There were also no differences in sarcomere lengths as measured between control and

treated sides (Table 4.3). LT muscles from rapid and conventional chilled carcass sides did not differ in any proximate analysis measurements of moisture, fat, or protein (Table 4.3). There were no significant differences in pH or temperature values when measurements were taken within the loin from 0.75 to 24 h, although there was a trend ($P = 0.0969$) showing that the LT from rapidly chilled carcass sides tended to be cooler than LT from conventionally chilled sides (Table 4.4).

4.3.3. Glucidic Analysis

There were no differences in levels of glucose, glycogen, or total sum of metabolytes expressed as glucidic potential in the LT (Table 4.5). There was a higher mean concentration of lactate in the conventionally chilled LT muscles than in the LT of the rapidly chilled sides ($P< 0.05$).

4.3.4. Retail Display Colour and Myoglobin Pigment Assessment

Objective retail measurements showed no differences in colour over days 0, 2, and 4 h between conventional and rapidly chilled carcass sides (Table 4.6). The proportions of the surface muscle pigments metmyoglobin (Metmb), deoxymyoglobin (mb), and oxymyoglobin (Oxymb) showed changes over time in retail display (Table 4.7). Levels of Metmb from LT steaks increased over time from 0 to 4 d, showing steaks browned over time. Levels of Mb decreased from 0 to 2 d, decreasing the degree of purple in the LT colour. Levels of Oxymb increased from 0 to 2 d showing steaks continued to bloom after 2 days; however this bloom declined by d 4 to below initial levels. Levels of muscle pigments from steaks from conventionally chilled sides had the same initial levels of Metmb as

steaks from rapidly chilled sides; however, after 4 days steaks from rapidly chilled sides had the highest proportion of Metmb. Initial levels of Mb were highest in steaks from conventionally chilled sides, but rapidly chilled sides reached the same level as conventionally chilled sides after 4 d. When muscle pigments were compared between rapidly chilled and control sides among 14 d steaks there was no significant effects of chill (Table 4.7).

4.3.5. Shear Force, Cooking Loss, and Drip Loss

There were no differences in shear force values, cooking time, or drip loss values when control and rapidly chilled LT steaks were compared (Table 4.8). There was a difference between cook loss values when control and rapidly chilled sides were compared ($P< 0.05$). Although there was a trend for increasing tenderness in steaks that were aged 14 d there were no differences in tenderness, cook loss, cook time, or drip loss between rapidly and conventionally chilled steaks (Table 4.9).

4.3.6. Sensory Analysis

Sensory panelists found no differences between conventional and rapidly chilled LT muscles in terms of initial tenderness, initial juiciness, flavour desirability, beef flavour intensity, amount of connective tissue, sustained juiciness and overall palatability. Panelists did rate conventionally chilled LT steaks as having greater overall tenderness than rapidly chilled LT steaks (Table 4.10)($P< 0.05$). Sensory panelists categorized conventionally and rapidly chilled LT steaks textures and flavours similarly (Table 4.11) as both were identified as

having typical beef texture, some unidentified texture, and being crumbly (13%). Sensory panelists categorized flavours of conventionally and rapidly chilled LT steaks similarly as well, describing them as having no specific flavours and off or sour flavour or unidentified flavour. These results indicated that the sensory qualities of steaks were largely unaffected by chill temperature except for measurements of overall tenderness.

4.4 Discussion

Although this study showed limited differences between control and rapidly chilled beef carcass sides, past research has shown that rate of chill can affect the lean colour of tissue. Both Aalhus *et al.* (1994) and Bowling *et al.* (1987) reported rapid chilling produced dark muscle within bovine carcasses. Fat cover can also impact lean tissue colour by affecting the rate of muscle temperature decline postmortem. Page *et al.* (2001) reported that fat thickness and muscle temperature were positively correlated due to an insulation effect from the fat cover, which then accelerated the rate of glycolysis. The drop in muscle pH while muscle temperature is still high can cause sarcoplasmic proteins to precipitate and may reduce the ability of muscle to retain water (Lawrie, 1998). These factors can influence the light reflectance and colour of meat post mortem.

Although the treatments did not result in measurable differences in colour as indicated by no differences in lean colour Japanese Meat Grading Association (JMGA) scores, there were differences in objective measurements of colour. Colour differences were observed when retail CIE L*a*b* measurements were taken showing steaks had increased yellowness at 24 h post mortem. The

increased values of yellowness may be due to increased levels of denatured and oxidized heme proteins (Kristinsson *et al.*, 2005) and increased phospholipids (Moayedi *et al.*, 2010) resulting from chilling treatments. This observation is not relevant in the current Canadian grading system where beef carcasses are graded visually, however these observations may become more relevant if the Canadian beef grading system adopts an automated camera system to grade beef colour, which may be more sensitive to colour differences than the human eye.

Surface colorimetry measurements for retail display steaks showed an increased concentration of metmyoglobin (Metmb) levels after 4 d of retail display within rapidly chilled sides, which can be an indicator of meat discolouration (Faustman and Phillips, 2001). Measurements showed that rapidly chilled sides had increased levels of metmyoglobin, which can result from the auto-oxidization of myoglobin and can change meat colour from bright red to brownish red. Levels of myoglobin were the same for LT muscles from both treatments after four days; however, initial levels were higher in the LT from rapidly chilled sides than those from sides chilled slowly. The increased initial levels of myoglobin could be due to decreased rates of glycolysis and increased pH values usually observed in rapidly chilled sides, which were demonstrated by reduced levels of lactate in the chilled carcasses. The reduced muscle temperature of the rapidly chilled carcass sides would provide a buffer against denaturation of myoglobin. Because myoglobin is composed largely of protein it is sensitive to changes in pH and temperature (Faustman and Cassens, 1990).

Past research has found cooling carcasses are sensitive to extreme temperatures, which can impact rate of early post mortem glycolysis (Bate-Smith and Bendall, 1949) and affect colour (Renerre, 1990). Aalhus *et al.* (2002) found similar results when colour was measured on blast chilled sides, finding that LT from blast-chilled carcass sides were darker, less saturated, and more purple-red than conventionally chilled sides. In these cases, colour measurements were almost dark enough to qualify for the Canadian B4 grade (L*~26 and chroma~15) (Murray, 1995) or were defined as "slow bloomers" needing more than 20 minutes of exposure to atmospheric oxygen in order to avoid the dark-cutting grade.

Another abnormal colour that can occur within bovine muscle has been described as "heat-ring" which is development of a dark ring around the outside of the LT muscle upon grading (Calkins *et al.*, 1980). Calkins *et al.* (1980) attributed this effect to rapid chilling in the exterior of the LT muscle, which slows the rate of glycolysis and the related pH decline thus affecting water holding capacity and lean colour. Tarrant and Mothersill (1977) described a similar phenomenon known as two-toning where the deep portions of large muscles appeared lighter than the superficial portions of the same muscle.

The effect of chilling rate on postmortem metabolism is well established in the literature (Cassens and Newbold, 1967). The present study found similar results demonstrating high temperatures increased the rate of pH decline, such that lactate concentration was highest in conventionally chilled sides at 24 h postmortem ($P \leq 0.05$). A decreased rate of pH decline within rapidly chilled beef

can reduce protein denaturation and increase water binding ability of the proteins, increasing the amount of free water and decreasing light reflectivity properties (Offer *et al.*, 1989). Darkened lean colour exposed to rapid rates of chill has also been reported in pork by Jones *et al.* (1988) who found blast chilling of pork before conventional chilling resulted in darkened, firm lean, which is unexpected given that pork usually is prone to having light coloured lean such as within pale, soft, exudative (PSE) pork. The darkening of lean muscles as a result of rapid chill rate has also been shown to occur in rapidly chilled bison carcasses, with the colour improving as the amount of subcutaneous fat increased (Janz *et al.*, 2002). In the present study, although there was an effect on the concentration of lactate between treatments, this effect did not seem to impact colour differences that could be assessed visually, so the treatment may not have been severe enough to impact significantly impact glycolysis; as well the study was limited in scope and significant differences may have been detected with additional animals. Increased rates of pH decline encourage protein breakdown and elevate concentrations of free calcium (Jaime *et al.*, 1992). If energy is still available within the cell, the increase in free calcium concentration may be accompanied by increased muscle contraction, and sarcomere shortening and meat toughening may occur. This theory may explain the differences in tenderness observed by the sensory panel in the current study where steaks were rated as more desirable from control sides. Interestingly no differences in tenderness were detected using Warner-Bratzler shear force. Cooking loss may have confounded the results of tenderness, explaining the differences in tenderness observed by the sensory panel and the

lack of differences measured by shear force. Cooking loss values for steaks destined for shear force revealed control sides lost more fluid than rapidly chilled sides, however, there were no differences in cooking loss values for steaks destined for sensory analysis; this may result from moisture losses and other physical changes during frozen storage prior to sensory analyses. Tenderness can be impacted by a combination of expressible moisture and cooking loss (El Rammouz *et al.*, 2004), both relating to the water-holding capacity of the proteins.

There was no pronounced difference in tenderness between the rapidly and conventionally chilled cooked LT steaks. Continuous monitoring over 24 h demonstrated temperatures within LT muscles from both carcass sides did not fall within the cold shortening danger zone of below 10°C within 10 h postmortem (Bendall, 1972). Mean sarcomere length values did not differ between LT muscles from the different treatments; however, as indicated by Eisenhut *et al.* (1965) sarcomere lengths of intact bovine muscle of ~2.0 μm represent the lower limit of sarcomere length. Bouton *et al.* (1973) demonstrated a strong relationship between Warner-Bratzler shear force values and sarcomere lengths when sarcomeres were shorter than 2.0 μm in mutton. Hostetler *et al.* (1972) indicated that sarcomere lengths within the range of 2-2.5 μm showed significant improvements in tenderness. Cold shortening is also thought to be related to the darkened lean colour produced from very fast chilling (Van Moeseke *et al.*, 2001).

4.5. Conclusion

There was not a pronounced effect of temperature on the colour of bovine *Longissimus thoracis* between rapidly and conventionally chilled carcass sides in

the present study. Although the known impact of temperature decline has been widely documented as mentioned above, the findings of this study indicate that the chilling environment achieved within the present study did not produce measurable differences in beef quality, implying that chilling rate would need to increase substantially in order to produce Canada B4 colour in the LT. It is unknown if the reduced cooler loads experienced due to a reduction in numbers of animals slaughtered would be sufficient to contribute to the increase in carcasses grading B4 in recent years. Nevertheless, small shifts in early post mortem muscle temperature did alter the extent of anaerobic glycolysis, which affected early retail display colour and sensory tenderness, underscoring the importance of proper post mortem management of carcasses to maintain quality.

Table 4.1 Overall carcass characteristics for study steers from the University of Alberta Kinsella Ranch

Variable	N	Mean	SD[1]	Minimum	Maximum
Live weight, kg	10	532.8	13.0	505.5	554.0
Grade Fat, mm	10	11.3	3.5	7.0	17.0
Width	10	2.0	0.8	1.0	3.0
Length	10	1.9	0.3	1.0	2.0
Muscle Score[2]	10	2.0	0.8	1.0	3.0
Fat Class[3]	10	5.4	1.8	3.0	8.0
Cut. Estimate[4]	10	56.8	2.9	52.0	61.0
REA[5]	10	78.3	6.1	69.0	85.0
Marbling[6]	10	454	37	370	500

[1] Standard deviation an estimate of the average variability of a set of data.
[2] Muscle scores 1-4 (Canadian Grade Standards) with 1 designating the smallest and 4 designating the largest.
[3] Fat class scores 1-8 (Canadian Grade Standards) as defined as 1 demonstrating the least amount of subcutaneous fat and 8 demonstrating the most subcutaneous fat.
[4] Cutability estimate.
[5] Rib eye area, cm^2 size of *Longissimus thoracis* (LT) as measured by grid.
[6] American Meat Science Association marbling scores (100= devoid, 200 = practically devoid, 300 = traces, 400 = slight, 500 = small, 600 = modest).

Table 4.2 Quality and yield grades for carcasses of steers from the University of Alberta Kinsella Ranch

Quality Grade[1]	No.[2]	% of total	Chisq P[4]	Yield Grade[3]	No.[2]	% of total	Chisq P[4]
A	1	10%		1	3	30%	
AA	8	80%	0.0074	2	5	50%	0.4966
AAA	1	10%		3	2	20%	

[1] Most valuable quality grades of beef carcasses within the Canadian beef grading system ranges from prime to A and is based on the degree of marbling.
[2] Number of carcasses.
[3] Estimate of lean beef yield of a carcass based on a calculation using a Canadian beef grading ruler.
[4] Pearson's Chi-square test of the independence of two categories, n = 10.

Table 4.3 Meat quality measurements for *Longissimus thoracis* steaks and purge loss measurements from 14 day aged whole loins from 10 steers treated with rapidly chilled right sides and conventionally chilled left sides.

Side Treatment	Conventional	Rapid	S.E.M.[1]	Prob > F[2]
n	10	10		
Cooler loss, $mg.g^{-1}$	1.49	1.45	0.03	0.3503
JMGA score [3]	4.55	4.90	0.38	0.1108
Purge loss, $mg.g^{-1}$	14.59	12.09	1.57	0.1019
Objective colour, Grade site 24h				
L*	37.09	36.53	0.71	0.2944
a*	19.69	19.16	0.41	0.2048
b*	8.90[a]	8.05[b]	0.32	0.0191
Mean fibre area, μm^2				
SO[4]	1714.32	1703.73	120.97	0.9422
FOG[5]	2206.35	2274.26	151.24	0.6043
FG[6]	3435.34	3529.11	302.46	0.7555
Muscle fibre type proportions (%)				
SO	22.41	23.27	1.77	0.6228
FOG	23.97	25.65	1.09	0.2915
FG	53.62	51.09	1.99	0.2709
Sarcomere length, μm	1.69	1.66	0.06	0.6550
Proximate Analysis, mg.g-1				
Moisture	73.63	73.67	0.51	0.9637
Fat	3.22	3.30	0.32	0.6592
Protein	21.33	21.08	0.18	0.1330

[1] Standard error of the mean.
[2] Probability of the F test, with significance at $P < 0.05$.
[3] Japanese Meat Grading Association colour scores range from 1 (the very palest) to 8 (the very darkest).
[4] Slow oxidative muscle fibres.
[5] Fast oxidative glycolytic muscle fibres.
[6] Fast glycolytic muscle fibres.
[a,b] Means with different superscripts within a row are significantly different at $P < 0.05$.

Table 4.4 Mean LT muscle temperature and pH of rapidly and conventionally chilled sides at 0.75, 3 and 24 h postmortem

Measurement	Conventional chill			Rapid Chill			S.E.M.[1]	Prob>F[2]
n		10			10			
Hours post mortem	0.75	3	24	0.75	3	24		
pH	6.71	6.23	5.50	6.76	6.28	5.52	0.05	0.9251
Temperature (°C)	39.23	26.88	1.88	38.89	24.88	1.46	0.47	0.0969

[1] Standard error of the mean.
[2] Probability of the F test, with significance at $P < 0.05$.
[a,b] Means with different superscripts within a row are significantly different at $P < 0.05$.

Table 4.5 Glucidic metabolites of rapidly and conventionally chilled sides.

Glucidic Metabolite, µmole.g^{-1}	Conventional	Rapid	S.E.M.[1]	Prob > F[2]
n	10	10		
Glucose	6.32	6.18	0.19	0.4379
Lactate	104.72[a]	100.22[b]	1.01	0.0012
Glycogen	16.11	16.17	1.10	0.9620
Glucidic Potential[3]	74.79	72.46	1.38	0.1258

[1] Standard error of the mean.
[2] Probability of the F test, with significance at $P < 0.05$.
[a,b] Means with different superscripts within a row are significantly different at $P < 0.05$.
[3] Potential is the sum of all potential energy as calculated in glucose equivalents (glucose + glycogen + ½ lactate).

Table 4.6 Retail colour L*, a*, and b* values for *Longissimus thoracis* steaks (n = 10) for days in retail display by chill treatment

		Conventional				Rapid			S.E.M.[1]	Prob[2]
		0d	2d	4d		0d	2d	4d		
L*		42.18	42.01	43.35		41.39	42.32	43.80	0.56	0.1669
a*		15.44	16.42	16.10		14.80	15.58	15.24	0.33	0.8866
b*		11.63	13.47	13.47		10.75	12.95	13.19	0.26	0.3341

[1] Standard error of the mean.
[2] Probability of the F test, with significance at $P < 0.05$.
[a,b] Means with different superscripts within a row are significantly different at $P < 0.05$.

Table 4.7 Proportion of metmyoglobin (Metmb), deoxymyoglobin (Mb), and oxymyoglobin (Oxymb) surface pigments for *Longissimus thoracis* steaks (n = 10) for days in retail display and chill treatment.

	Conventional			Rapid			S.E.M. [1]	Prob > F [2]	trt*time [3]
	0d	2d	4d	0d	2d	4d			
Metmb	0.13[e]	0.19[d]	0.24[b]	0.13[e]	0.22[c]	0.26[a]	0.01	0.0053	0.0027
Mb	0.17[b]	0.09[c]	0.10[c]	0.21[a]	0.10[c]	0.10[c]	0.01	0.0416	0.3999
Oxymb	0.70	0.71	0.66	0.66	0.68	0.64	0.01	0.6510	0.7256

[1] Standard error of the mean.
[2]Probability of the F test, with significance at $P < 0.05$ for interactions of treatment and time.
[3] P values for interactions of days in retail, treatment, and time.
[a,b] Means with different superscripts within a row are significantly different at $P < 0.05$.

Table 4.8 Tenderness and cooking characteristics of Longissimus thoracis steaks that differ in chilling regime.

Side Treatment	Conventional	Rapid	S.E.M.[1]	Prob > F[2]
n	10	10		
Shear, kg	7.88	8.06	0.78	0.7486
SD of cores[3]	1.65	1.75	0.20	0.6592
Cook loss, $mg \cdot g^{-1}$	206.38[a]	181.20[b]	7.40	0.0209
Cook time, $sec \cdot g^{-1}$	4.22	4.39	0.32	0.5709
Drip loss, $mg \cdot g^{-1}$	32.90	33.89	2.22	0.7541

[1] Standard error of the mean.
[2] Probability of the F test, with significance at $P < 0.05$
[3] Standard deviation of cores showing variance of tenderness between core samples within steaks.
[a,b] Means with different superscripts within a row are significantly different at $P < 0.05$.

Table 4.9 Tenderness and cooking characteristics of *Longissimus thoracis* steaks (n = 10) that differ in chilling regime and days of ageing.

	Conventional		Rapid			
Interactions	2d	14d	2d	14d	Sem[1]	Pr > F[2]
Shear, kg	10.58	5.19	11.14	4.98	0.88	0.4954
SD of cores[3]	2.19	1.11	2.30	1.21	0.25	0.9652
Cook loss, mg·g-1	200.08	212.68	175.23	187.17	10.37	0.9748
Cook time, sec·g-1	4.43	4.01	4.67	4.11	0.38	0.8126
Drip loss, mg·g-1	31.11	34.69	30.66	37.12	3.13	0.6488

[1] Standard error of the mean.
[2] Probability of the F test, with significance at $P < 0.05$.
[3] Standard deviation of cores showing variance of tenderness between core samples within steaks.

Table 4.10 Sensory characteristics for cooked 18 d aged *Longissimus thoracis* steaks including palatability scores, tenderness attributes, cook time, and cook loss.

Side treatment	Conventional	Rapid	S.E.M.[1]	Prob > F[2]
n	10	10		
Initial Tenderness	5.68	5.7	0.31	0.9086
Initial Juiciness	5.65	5.78	0.16	0.2773
Flavour Desirability	5.82	5.81	0.12	0.9449
Beef Flavour Intensity	5.42	5.52	0.1	0.4627
Off-flavour Intensity	8.22	7.99	0.12	0.1758
CT[3]	8.35	8.18	0.12	0.1782
Overall Tenderness	6.15[a]	5.84[b]	0.29	0.0444
Sustainable Juiciness	5.55	5.63	0.11	0.4428
Overall Palatability	5.38	5.25	0.19	0.4112
Cook loss, mg.g-1	269.6	262.38	6.46	0.3742
Cook time, sec.g-1	5.41	5.58	0.21	0.5535

[1] Standard error of the mean.
[2] Probability of the F test, with significance at $P < 0.05$.
[3] Amount of connective tissue.
[a,b] Means with different superscripts within a row are significantly different at $P < 0.05$.

Table 4.11 Proportion of texture and off-flavour attributes detected by sensory panel for cooked 18 d aged *Longissimus thoracis* steaks.

Texture	Conventional	Rapid	ChiSq[1]
Crumbly	13%	8%	0.3458
Mealy	1%	2%	0.5637
Mushy	5%	1%	0.1797
Rubbery	7%	9%	0.593
Spongy	7%	6%	0.763
Typical Beef Texture	54%	59%	0.6153
Unidentified	14%	15%	0.8415
Off-flavours			
Bloody/Serum	1%	4%	0.3173
Grainy	0%	0%	NE[2]
Livery	1%	0%	NE
Metallic	0%	1%	NE
None	45%	45%	1
Off Sour	36%	31%	0.5716
Other(stale)	0%	1%	NE
Unidentified	16%	17%	0.8415

[1] Pearson's chi-square test of the independence of two categories.
[2] NE = not estimable.

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5.0. GENERAL DISCUSSION

5.1. Summary of Findings

The null hypothesis for the first study was that Canada B4 carcasses with a muscle pH below 6.0 do not exist in the youthful Canadian population. The null hypothesis was rejected because results show that Canada grade B4 carcasses with a LT pH below 6.0 existed in the youthful Canadian slaughter population when colour of the LT was evaluated in dark cutting carcasses. The majority of Canada B4 carcasses do have a LT muscle pH above 6.0 in the population surveyed, but there was a large range in pH values in dark cutting carcasses measured.

Canada B4 carcasses may belong to a specific biological type of beef animal that has a smaller frame size and lower carcass weight but a similar level of fat and that the average Canada AA carcass. Grade B4 carcasses did tend to have more detectable marbling than AA grade carcasses, but this finding was likely confounded by the fact that we specified our control carcasses as AA carcasses thus biasing the control group.

Results of the first experiment indicated that Canada B4 carcasses should be segregated into LT pH categories because there were variations in meat quality and the proportion of the carcass that was abnormally dark between the three putative Canada B4 categories. Borderline B4 carcasses (BD), with a pH range of 5.8 to 6.0, had the toughest beef with a rubbery texture, while classic B4 carcasses (CL) were found to have a spongy texture and off-flavours. Both of these groups

of dark cutters had a large proportion of muscles in the middle meats and hind muscles that were dark in colour. The majority of meat that was recoverable was found within the front quarter. However atypical dark cutters (AT) had very few muscles in the hind or front quarter that were dark and segregating these carcasses can recover value by utilizing the significant percentages of normal coloured muscles within the carcasses. Atypical dark cutters have normal levels of glucose and lactic acid and sufficient glycogen stores to produce normally coloured beef and yet yield an LT with an abnormal colour.

The results from the second study showed that chilling carcass sides 2°C faster than conventional chilling does not contribute to the darkening of beef. There was a tendency of rapidly chilled beef to be darker than controls however we found no evidence that increased chilling rate could produce a carcass side that would grade Canada B4. The role chilling plays in lean meat colour is well known and this hypothesis was further supported by the light carcass weights observed in B4 carcasses from the previous study. Rapidly chilled carcasses did demonstrate decreased levels of lactate, which can be an indicator that muscle pH will be different and this can drive lean colour, but we did not find a difference in lean colour between conventional and rapidly chilled sides that could be detected by the naked eye. Rapidly chilled steaks did, however, have an overall lower tenderness compared to control steaks, indicating that tenderness was affected by the increased chilling rate in some manner other than sarcomere length shortening because there was no effect of chill treatment on sarcomere length.

5.2. Future Research

Future research with the abattoir to increase the number of carcasses within this study would greatly increase the validity of our results. If the B4 survey could be continued and the number of carcasses surveyed increased it would provide valuable information about the types of animals that are susceptible to dark cutting. Information regarding the animal's background would be another invaluable measurement such as gender, breed, slaughter weight, temperament, feeding strategy and nutritional plane. There may also be more specific differences relating to carcass quality between dark cutting and normal carcasses that were not apparent due to the small number of carcasses studied. It would also be beneficial to match the grade B4 carcasses with a control group with a similar level of marbling or to measure carcass characteristics separately between quality groups of AAA carcasses, AA carcasses and A carcasses. The results of a similar study with increased replication would be valuable and enable the development of management strategies to decrease the incidence of dark cutters. Investigation of the AT group of dark cutters and the reasons these carcasses exists would further develop our understanding muscle biochemistry and other underlying causal mechanisms.

The specific quality and sensory attributes discovered for each pH category can contribute to the development of meat processing techniques to improve the value and palatability of dark cutting beef. If processing can improve the beef quality of dark cutting beef then then increased value could translate into reduced or diminished penalties for dark cutting carcasses.

5.3. General Conclusion and Industry Perspective

The results of this study do have to be interpreted with caution, as only a small number of carcasses were studied. One proposed conclusion after reviewing the results of this study was to allow entry of borderline B4 carcasses into the quality grades, or to diminish the B4 grade altogether and convert to a system similar to that used by USDA. The overall implications of removing the Canada B4 grade from the Canadian beef grading system could compromise the quality of Canadian beef. Allowing dark cutting beef to enter the quality grades of beef as a means to mitigate the financial losses incurred by those selling Canada grade B4 beef may be short-sighted, because this would allow the toughest carcasses (BD carcasses) to enter to retail beef market and further decrease beef product consistency. Improving beef consistency has been a goal of the Canadian beef industry for several years and allowing any type of Canada B4 beef into the quality grades will diminish product consistency, beef quality, and further blur the differences of Canadian beef compared to American beef.

A suggestion to the Canadian Beef Grading Agency would be to include carcass pH when carcasses are graded within the Canadian beef grading system. Measuring muscle pH would improve the evaluation process and give abattoirs a better estimate of the quality of beef contained within the whole carcass. Carcasses are already being sorted within the Meat Standards Australia program of beef grading. If Canadian carcasses were sorted based on pH and not solely on subjective evaluation of comparison to coloured chits then further processing could be utilized to optimize the beef quality of different classifications of dark

cutting carcasses. As demonstrated by our results there is a large variance of muscle pH, beef quality, sensory, and proportion of the carcass that is affected within Canada grade B4 carcasses.

The data from this study support changing the Canadian Beef grading system to segregate carcasses based on muscle pH to improve the overall quality of Canadian beef and to better utilize B4 carcasses. The segregation of carcasses based on pH will begin the process of sorting carcasses according to quality and not colour. Adoption of this method will strengthen the Canadian advantage by providing distinguishable and marketable qualities of Canadian beef to be used as a means of differentiation for retailers who are trying to capture market space in international markets within or beyond the U.S. The pivotal attributes of the Canadian beef grading system stem from our stringent criteria for yield, age, and lean colour requirements. Adding a pH requirement will continue to create value and maintain Canada's position as a supplier of premium beef. Creative processing solutions in the future and a commitment to premium beef products will ensure a place for all categories of Canada B4 beef and will encourage a fair dollar for Canadian beef in international markets.