University of Alberta

Meat Characteristics and Stress of Bison Slaughtered in a Mobile or Stationary Abattoir

by

Jayson Kent Galbraith

A thesis submitted to the Faculty of Graduate Studies and Research

in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in

Animal Science

Department of Agricultural Food and Nutritional Science

Spring 2011

Edmonton, Alberta

Dedication

This thesis is dedicated to my wife and best friend Cheryl Lynn Galbraith.

Abstract

Meat characteristics and physiological stress measurements in bison exposed to different *ante-mortem* treatment groups and reasons behind the rapid discolouration of fresh bison meat (compared to beef) were examined. It was hypothesized that bison slaughtered on farm (dispatched in pen, MLAPEN, or confined then dispatched MLACON) through a mobile location abattoir (MLA), would have favourable meat characteristics and lower stress levels than those transported to a stationary abattoir (LAND). It was also hypothesized that differences in fatty acid profile, vitamin E levels, and oxidative properties of bison meat compared to beef, are related to the observed difference in retail shelf life. A higher incidence of carcasses graded as "dark" were observed in the LAND group. Improved tenderness measured through shear force (MLACON 7.28 kg and MLAPEN 7.40 kg *vs.* LAND 9.43kg) and initial tenderness sensory scores (MLACON 4.95, MLAPEN 4.55, *vs.* LAND 3.93; where 8= extremely tender and 1= extremely tough) was seen in the MLA groups. The lowest blood cortisol level was found in MLAPEN group compared to the MLACON or LAND groups (71.16 nmol/L, 124.17 nmol/L and 139.50 nmol/L respectively; $P<0.01$). Bruising was found in all treatment groups, however less was found on the MLA groups compared to the LAND group. Fatty acid composition was significantly different between bison and beef for all the fatty acids measured. The inherent tissue traits of bison were linked to poorer performance in the retail environment when compared to beef. Bison meat had higher polyunsaturated fatty acid levels, and a lower omega 6:omega 3 ratio than beef. Bison also had a lower total fat and

higher pigment and vitamin E levels. Stepwise regression models included some of these traits and accounted for a significant proportion of the variation in metmyoglobin (R^2 = .689), % discolouration (R^2 = .737) and appearance (R^2 = 0.676) between d 0 and d 3 in retail. An improved understanding of the effects of *ante mortem* handling and the inherent characteristics of bison meat will improve animal welfare and help create an improved eating experience for the consumer.

Acknowledgement

Thank-you: To my supervisor Erasmus Okine who helped start me on this journey by receiving a phone call into his department with me asking about pursuing this degree. He has provided me with behind the scenes support, and several pep talks along the way. To Jennifer Aalhus who has been my mentor and 'go to' person throughout this degree. She has supported and challenged me, has been 'in my corner' from start to finish and has improved my writing immensely. To Al Schaefer who, aside from being a fantastic mountain advocate, also supported and encouraged me along the way. Bodo Steiner for always keeping things 'real', his drive and enthusiasm are motivational. Laki Goonewardene who helped me actually see what SAS was doing to my data. To Wendy Wismer and Martin Zuidhof for their participation on both my candidacy and final exams. Luigi Faucitano for serving on my examining committee as the external examiner. To the funders for this project; the Alberta Agricultural Research Institute and the Diversified Livestock Fund of Alberta.

From Alberta Agriculture, I thank Doug Milligan (retired) who encouraged me to pursue this path and along with Michelle Follensbee helped me set up my education plan. My branch head Rod Carlyon and director Jo-Ann Hall have supported me throughout my degree despite me being in my own world for large chunks of time. Many colleagues supported me with encouragement, laughter, or a listening ear.

The staff at Lacombe Research Centre. Ivy Larsen and Rhona Thacker provided me with patient consistent help at the Lacombe lab throughout. Thanks to Denise Froehlich for working through the cortisol analysis with me. Nigel Cook for helping with edits to the stress chapter. Thanks also to the sensory lab and to Chuck, Darcy, Darryl, Jeremy, Dewey and the rest of the meat cutters and CFIA inspectors. Mike Duggan, Dave Rolland, Manuel Juarez, and Noeli Aldai for their help in fatty acid analysis and reviewing that chapter. Thanks to Stan Landry who showed me how to drive around with no coffee cup lids and was patient with my many 'obvious' questions.

Wayne Robertson (retired) was pivotal to this degree in helping with the application, recruitment of co-operators and logistics both on-farm and at Lacombe. Bert Denning, Slav Heller, Brad MacLeod and Thomas Hettesheimer were all central to the implementation of this project; turning the idea into action. Thanks to Brad for not giving me an easy time in the slaughter plant... I learned lots even though my right hand tended to be working against my left hand (thank goodness for chain-mail gloves).

Thanks to my wife Cheryl and daughters Amy and Kelly for their love and support through this journey. My late father-in-law Rev. Dr. Richard Kraemer for his sound advice on committees, timelines etc. His vast experience in the post secondary world became evident at several important times. To my brother Ken (and family), mom Kathleen Galbraith, Mike Robertson and Janet Kraemer for their encouraging words or useful distractions throughout. To my friend Harley Hakanen for listening to me ramble on about having some 'unfinished business' with my education. Last and most importantly thanks to my Lord and Saviour Jesus Christ for the ability, determination, and opportunity to pursue and complete this degree journey.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

Δ	Change
ATP	Adenosine triphosphate
CP	Creatine phostphate
d	Day
DFD	Dark firm dry
DHA	Docosahaxaenoic acid
EPA	Eicosapentaenoic acid
FG	Fast twitch glycolytic
FOG	Fast twitch oxidative glycolytic
h	Hour
HVES	High voltage electrical stimulation
LAND	Land based abattoir group
LL	*Longissimus lumborum*
LT	*Longissimus thoracis*
Mb	Myoglobin
MbO_2	Oxymyoglobin
MetMb	Metmyoglobin
MLA	Mobile location abattoir
MLACON	Mobile location abattoir confined group
MLAPEN	Mobile location abattoir penned group
n-3	Omega 3 fatty acid
n-6	Omega 6 fatty acid
PUFA	Polyunsaturated fatty acid

SO	Slow twitch oxidative
TBARS	Thiobarbituric acid reactive substance
UTM	Under thirty months
WBC	White blood cell

CHAPTER 1

Introduction and Literature Review

1.1 Background

Bison (*Bison bison*) are raised for their meat and other products in North America. In 2010 in the Canadian province of Alberta there were over 18,000 bison slaughtered in inspected abattoirs (Steenbergen, 2011). The nutrient content of bison meat has shown it to be a leaner meat than beef in both US (Marchello and Driskell, 2001) and Canadian studies (Galbraith et al., 2006), and is reported to have fewer calories compared to beef (McClenahan and Driskell, 2002). There is a perception that consumption of bison meat is healthier than beef (Rule et al., 2002). The healthiness of bison meat has been shown to be an important characteristic for both US (Torok et al., 1998) and Canadian (Gao, 2006) bison consumers.

With consumer trends towards more specific demands for meat attributes, it is increasingly important for the meat industry to provide a consistently good eating experience for their consumers. Adding value to food creates an opportunity to improve the economic return throughout the value chain that distributes food (Catlett, 2006).

Among visual attributes of meat, colour may be considered to be the most significant. Meat with discolouration is perceived to be unwholesome and of lower value (Zhu and Brewer, 1999). Bison meat has been found to have rapid rates of discolouration resulting in a shorter shelf life (Pietrasik et al., 2006;

Dhanda et al., 2002; Janz et al., 2001a). While the colour of the meat may affect the initial purchase decision, tenderness has been identified as the most important meat palatability attribute (Morgan et al., 1991; Miller et al., 2001) and affects repeat purchase decisions. Therefore, consumers are willing to pay more for a guaranteed tender steak (Boleman et al., 1997; Mintert et al., 2000).

Pre-slaughter stress and environmental conditions can influence meat quality. There have been a number of studies examining pre- or post-slaughter influences on the tenderness of meat from cattle (Christensen et al., 1991; Shackelford et al., 1995; Gullett et al., 1996; Field et al., 1997; Moloney et al., 2001; Vote et al., 2003; Meyer et al., 2005). Mixing, loading, transport, time off of feed and water are examples of events that meat production animals typically experience that can influence carcass traits. Bruising (McNally and Warriss, 1997), increased toughness (Jeremiah et al., 1992) and colour abnormalities such as dark firm dry meat (Gregory, 1996), are all examples of consequences of *ante-mortem* management that have been observed in beef cattle.

To minimize the consequences indicated above, the concept of mobile slaughter "facilities" have been developed. For example Trivan truck body (http://www.mobileslaughter.com/index.htm) is a company that builds multi-location abattoirs in Washington. In 2008 a multi-location abattoir was built by Trivan truck body (Appendix 1) and brought to Alberta for a period of field testing and evaluation. Mobile abattoirs have had some appeal with consumers evidenced in Sweden where an increased consumer willingness to pay for meat from pigs slaughtered in a mobile abattoir has been found (Carlsson et al., 2007).

Despite an improved willingness to pay for certain credence attributes such as animal welfare, natural, local production (Carlsson et al., 2007b) and guaranteed produced without genetically modified organisms (Steiner et al., 2010), such price differentials are difficult to retain through the current Canadian commodity systems. Nonetheless, a mobile abattoir system could facilitate the development of low volume, high value livestock products for local markets.

1.2 Research objectives and thesis structure

The purpose of this study was to investigate meat characteristics and physiological stress measurements in bison exposed to different *ante-mortem* treatment groups, and to examine the reasons behind the rapid discolouration of bison meat on the retail shelf. This work was supported by the Animal Compassion Foundation™ (a *Whole Foods Market Foundation)* and the Canadian Bison Association (Appendices 2 and 3). It was hypothesized that bison slaughtered on farm, through the mobile slaughter unit would have superior meat quality and lower stress levels than those transported to a stationary abattoir. Another purpose for this investigation was to examine the reasons behind the rapid discolouration of bison meat on the retail shelf compared to beef. It was hypothesized that differences in fatty acid profile and oxidative properties of bison compared to beef, will be related to this difference.

This thesis will outline significant literature dealing with stress, meat quality (scientific and industry importance), muscle structure, meat attributes, and fatty acids in meat. Throughout the thesis, a consumer or industry perspective will be brought in to highlight the practical importance of topics. Three chapters in

paper format will report on research conducted examining meat quality of bison slaughtered in a mobile or stationary abattoir, physiological stress in bison slaughtered in a mobile or stationary abattoir, and fatty acid composition and appearance of beef and bison in retail display. The fifth and final chapter will summarize key findings, provide insight into implications for the bison industry, and the whole meat industry, and suggest research areas to follow up on this work.

1.3 Literature review

Throughout this literature review, studies on beef and other species will be drawn upon for characteristics of stress, meat structure and function, and on valuation of certain attributes. This is due to the lack of studies done specifically on bison, and because the author does not have reason to believe that, in many cases, the strategies employed to deal with stress, the structure of muscle and quality traits are different in bison. Where there is evidence to highlight differences between beef, or other species, and bison, it will be noted.

1.3.1 Stress

Hans Seyle defined stress as: " the nonspecific response of the body to any demand. A stressor is an agent that produces stress at any time" (Seyle, 1976). Fraser et al. (1975) have a useful definition of stress particularly in animal studies " a state that occurs when an animal is required to make an abnormal or extreme adjustment in physiology or behaviour in order to cope with aspects of its environment". Stress is an inevitable consequence of the process of transferring animals from farm to slaughter. As a result of limited slaughter facilities for bison,

it is common for bison to be transported for several hours (2-12) to get to an abattoir. Transport is often followed by lairage overnight in pens for slaughter the next morning. Assembly, loading, transport, unloading, regrouping, feed and water withdrawal, novel surroundings and temperature fluctuations are all factors that can create both physiological challenges and psychological disruptions which ultimately impact carcass yield and meat quality (Schaefer et al., 2001). It has been noted in pork that fasting duration appears to have a more substantial effect on glycogen metabolism and thus on meat quality than lairage duration (Faucitano, 2010). Ferguson and Warner (2008) reviewed the status of current knowledge of the impact of pre-slaughter stress in ruminants and the consequential effect on meat quality. They found compelling evidence to demonstrate that pre-slaughter stress can have significant deleterious effects on meat quality traits in beef and lamb, and further, concluded that the impact of pre-slaughter stress has been underestimated. In the interest of optimizing animal welfare and minimizing losses in product yield and quality it is important that more research attention be directed to the issue (Ferguson and Warner, 2008).

Environmental stressors like extreme temperatures, pain, thirst, hunger, noise, and confinement, can cause stress in animals. Pale, soft and exudative (PSE) meat and dark, firm and dry (DFD) meat are two examples of extreme changes to quality which can result from pre-slaughter stress. PSE is a term that refers to defects in the meat that all result in paleness and drip (Monin, 2004a). Stress-induced PSE pork occurs as a result of a combination of two conditions: First, glycogen being rapidly utilized just prior to (acute stress) or at the time of

death, resulting in a build up of lactic acid in the muscles and a rapid drop in pH, and secondly this occurring while the carcass is still at a high temperature (before cooling). This results in protein denaturation and precipitation of sarcoplasmic proteins onto the myofibrillar proteins. The increased temperature during the onset phase of rigor can also be detrimental to the myoglobin pigment structure causing protein precipitation and the structure to become "open" causing light to become scattered resulting in a pale colour (Savell et al, 2005). Without the ability to hold water, the meat loses firmness and becomes soft (Monin, 2004a). PSE meat can also occur in pigs due to a recessive gene complex that causes sensitivity to halothane (a gaseous anaesthetic used in human and animal surgery). The gene complex affects ryanodine receptors and affects heterozygous and homozygous muscle tissue for the gene. Weaver et al. (2000) found reduced calsequestrin levels in the brains of pigs heterozygous for the gene compared to wild-type pigs. Muscle cells in animals with this gene complex have been found to exhibit a defect in the Ca^{++} channel protein that results in the loss of control of free Ca^{++} (Monin, 2004a). In response to any of numerous stresses occurring during an animals' life, including mixing strange animals, transportation and slaughter, this defect causes an explosive increase in sarcoplasmic free calcium which induces a rapid drop in the pH leading to drip formation and discolouration (Monin, 2004a).

PSE can occur in beef also, typically in deep muscles of the hip and this condition has been induced by low voltage electrical stimulation (Aalhus et al., 1994). The high temperature and low pH causes protein denaturation and

precipitation of sarcoplasmic proteins onto myofibrillar proteins which decreases the water holding capacity.

DFD (dark firm and dry) or DCB (dark cutting beef) is caused by an incomplete pH drop post-mortem due to a lower than normal level of glycogen in the cells at the time of slaughter as a result of prolonged (chronic) pre-slaughter stress. This results in an insufficient amount of lactic acid being produced, the pH remains high, and the resulting meat can be dry and dark in colour (Adegoke and Falade, 2005). A dark red to almost black lean colour and a dry, often sticky, surface is characteristic of this condition. The high pH meat holds more water, the higher level of intracellular water reflects less light and therefore appears dark (Miller, 2002). DFD beef typically has an ultimate pH of above 5.9 (Swatland, 1994). The darker meat from DFD carcasses will not bloom to a bright red colour when exposed to air since the tissue respiratory activity stays high and less oxygen is available for oxymyoglobin formation (Monin, 2004b). The diminished oxymyoglobin layer results in the purplish-red colour of myoglobin predominating.

An unstressed meat animal contains about 4-10 mmol ATP per kg of muscle at the moment of slaughter depending on animal type and muscle group (Honikel, 2004b). Anaerobic glycolysis keeps the level of ATP constant for a time post-slaughter depending on the amount of glucose present, the pH, and the temperature. The propensity for a muscle to become fatigued pre-slaughter depends on the composition of its fibre types. This was seen in a study comparing varying muscle groups in pigs that experienced a number of pre-slaughter

treatments including fasting and encounters to novel environments with another animal (Fernandez et al., 1995). Fasting significantly enhanced stress-induced glycogen depletion in only fast-twitch glycolytic (FG) fibres of the *Longissimus* muscle (Fernandez et al., 1995).

The Recommended Code of Practice for the Care and Handling of Farm Animals: Transportation (Canadian Agri-Food Research Council, 2001), suggests that the absolute maximum total time in transport and lairage during which time beef cattle have received no food or water is 48 hours (Section 5.5.2) plus an additional 4 hours if the transport is close to the abattoir. This amount of time is likely too long for the prevention of carcass quality defects. Beef bulls that had feed and water withheld from them 36 h prior to slaughter had significantly lower tenderness rating than those that had not had water and feed withheld (Jeremiah et al., 1992).

Meat quality factors, specifically increased toughness, are affected by stress particularly in the *ante mortem* environment (Schaefer et al., 2006). Comparing tenderness among groups of animals given glucose, electrolytes, water, or no water pre-slaughter showed an increasing trend in toughness respectively, but the difference was not statistically significant (Schaefer et al., 1990). A 20% or greater retention of visible appearance of marbling was seen in a study examining the effect of providing *ante mortem* nutrition to beef cattle 12 to 24 hours prior to slaughter (Schaefer et al., 2001; Schaefer et al., 2006). Feeding cattle during transport to slaughter, or while in pens at the plant, could have an effect on the emotional state of the animal thus reducing stress. Managing stress

in animals pre-slaughter through providing pre-transport supplementation, and minimizing situations where animals are too crowded or in a situation where they fight, improves overall animal well-being and decreases financial losses due to DFD meat or carcass loss due to bruising (Gregory, 1996; McNally and Warriss, 1996).

1.3.1.1 Hormones associated with stress

Stressful situations in animals cause physiological and metabolic changes in the body in reaction to the situation. An important change is the increase in blood 'stress hormones' that include catecholamines and corticoids (Monin, 2004b). An elevated body temperature at the time of death in pigs accelerates post-mortem metabolism leading to an increased likelihood of PSE meat (Eikelenboom et al., 2004). Catecholamines are amine compounds that exert a sympathomimetic action on nervous tissues (Eckert, 1988). Two adrenal catecholamines, epinephrine and norepinephrine, have a large number of actions most of which help in the sympathetic flight-or-fight response to stressors. They promote glycolysis; a breakdown of glycogen to glucose-6-phosphate, in skeletal muscles (Eckert, 1988). Cortisol is a corticosteroid hormone that is secreted by the adrenal cortex. During episodes of stress, release of cortisol helps restore homeostasis. When released in response to chronic stress cortisol can reduce an individual's fitness by immunosuppression and atrophy of muscle tissues (Munck et al., 1984). Both could have negative consequences for the meat industry either by having a sick animal or producing an animal with less muscle. Glycogen

breakdown in muscle can occur in response to both catecholamine release or through strenuous muscular activity (Tarrant, 1989).

1.3.1.2 Measuring stress

Non-invasive methods of measuring stress in animals have been accomplished and include measures such as infrared thermography (Stewart et al., 2005) and heart rate variability (Stewart et al., 2008). Fecal corticosteroid measurements have also been used for this purpose in varying species (Miller et al., 1991; Palme et al., 1996; Palme et al., 1999). Frequent fecal collections ensure that peaks in cortisol levels are not missed. There are complications though in using fecal cortisol measurements. The excretion rate and volume of feces are influenced by the psycho-physiological state of the animal, feces are not a homogenous biological compound (as is the case with blood) and there are many bacteria in the feces that may degrade and transform large molecules (Carlsson et al., 2007a).

The concentration of cortisol metabolites in a fecal sample reflects the cortisol production after a time period that varies between species depending on the rate of passage through the digestive tract (ruminants 10-12 hours; Mostl and Palme, 2002). The detection of short peaks of metabolites needs frequent sample collection or the spikes could be missed (Mostl and Palme, 2002). Through a study involving the collection of all excreted feces in pigs for a 24 hour period, ‘spot’ sampling did not give an accurate indication of the corticosteroid level when compared against a composite of the 24 hour collection period (Carlsson et al., 2007a). In contrast, Rabiee et al. (2001) found that variation in grass intakes

did not influence the concentration of fecal progesterone metabolites in dairy cattle. In a study evaluating the biological relevance of fecal cortisol metabolites, Palme et al. (1999) injected adrenocorticotropic hormone (ACTH) and dexamethasone into cattle and found a good correlation (r = 0.77) between the injected dose of ACTH and the increase of fecal cortisol metabolites. No correlation was found, however, between the fecal concentrations of cortisol metabolites and the increase in plasma cortisol concentrations. Blood levels change quickly, whereas fecal concentrations of cortisol metabolites reflect the total amount excreted (Mostl and Palme, 2002). The delay time to detect cortisol metabolites in the feces in cattle was about 10-12 hours (Palme et al., 1999). A drawback to measuring concentration of cortisol in fecal samples is that it may not reflect the total amount of circulating hormones because the amount of material passing through the gut causes a dilution effect possibly skewing the results unless one assumes the volume is the same for all treatment groups (Chelini et al., 2006).

Urine (Lowe et al., 2004), saliva (Cook and Schaefer, 2002), milk (Verkerk et al., 1998), and blood (Schwartzkopf-Genswein et al., 2007) have all been used to measure stress hormone levels in animals. A drawback to these measurements in live animal studies is that the animal must be physically manipulated to retrieve samples. In studies examining meat quality (or other terminal studies), samples can be collected at the time of slaughter. The measurement of corticosteroid (specifically, cortisol) levels in blood samples obtained during exsanguination have been considered a reliable indicator of pre-

slaughter stress in livestock (Pollard et al. 2002) and have been used to assist in the evaluation of transport and abattoir treatments (Shaw and Tume, 1992). Pollard et al. (2002) investigated the effects of pre-slaughter handling on the blood chemistry of red deer (*Cervus elaphus*) which were either paddock shot or commercially harvested after transport to a slaughter house. Plasma cortisol concentrations in the paddock-shot deer (15.5 nmol/L) were consistent with an unstressed state while concentrations in the commercially harvested deer (79.9 nmol/L) were indicative of moderate stress. In another study field shot red deer had plasma cortisol concentrations similar to their pen shot counterparts (5.7 ng/ml and 6.8 ng/ml respectively), while deer from the same farm which were transported a short distance to a slaughterhouse had much higher cortisol concentrations of 29.9 ng/ml (Smith and Dobson, 1990). Buckham Sporer et al. (2008) investigated the effect of transportation of young beef bulls on stress biomarkers. They reported a 321% increase in plasma cortisol following onset of transport compared to levels measured in blood samples collected 24 h prior to transport. Additionally, cattle with more excitable temperaments have been shown to respond more extensively to simulated stress challenges and have higher concentrations of cortisol compared to calmer cattle (King et al., 2006).

White blood cells (WBC) are the mobile units of the body's immune defence system. Changes to the relative percentages of different types of WBC are an endocrine-driven response that occurs over many hours (Cook and Schaefer, 2002). Two particularly pertinent WBC types are the neutrophils (N) and lymphocytes (L), with a higher N/L ratio indicative of a stress response (Eckert,

1988). Neutrophil counts are elevated when high levels of stress are encountered. This stress response can be due to damage or inflammation of tissues, bacterial infection, or can result from nervousness and excessive exercise. N/L ratio values for bison transported and held overnight in a novel environment prior to slaughter have not been previously reported. Hematologic values for bison have been reported from several studies with inconsistent findings. A N/L ratio of 0.82 was obtained for American bison bulls from Yellowstone National Park where blood samples were collected after shooting unrestrained animals (Zaugg et al., 1993) and the ratio of 0.85 was obtained for range bison restrained for blood collection (Vestweber et al., 1991). Sikarskie et al. (1990) reported an N/L ratio of 1.00 for ranched bison and 0.82 for free ranging bison, while Marler (1975) obtained a ratio of 1.10 from two populations of free ranging bison. Stoltenow and Dyer (2001) reported a mean N/L ratio of 1.12 for ranched bison of three different age groups obtained from thirteen herds in eleven states. Mehrer (1976) evaluated haematological values of 163 bison from wildlife refuges and bison ranges from five areas of the USA. His data showed very low numbers of lymphocytes relative to neutrophils resulting in a mean N/L ratio for all age groups of 2.56.

1.3.2 Bruising

Pre-slaughter bruising of animals is of concern for meat scientists as a result of the accumulation of exudates at the injured site during the *ante mortem* period which can cause an elevated pH or a prolonged elevated pH post-mortem (Gregory, 1996). This is important to industry since it can result in losses due to excess trimming. In a study examining bruising in cattle, the incidence of bruising when hauled from live auctions was higher than if hauled from dealers or farms

(McNally and Warriss, 1996). McNally and Warriss (1996) also found the incidence of bruising of carcasses increased with increased travel distance. Bruising has been found to affect between 2.4 and 17.9% of 4473 cattle derived from 21 auction markets delivered to one slaughter plant (McNally and Warriss, 1997). This represents both a financial loss for the packer due to excess trimming and an animal welfare issue. This relationship was also found in a study examining bruising in red deer (Jago et al., 1996). The number of carcasses with a higher ultimate pH increased with the amount of carcass bruising (McNally and Warriss, 1996). Many factors, such as time of year for seasonal animals (Jago et al., 1996), and horn status and class of animal (Wythes et al., 1985) can affect the extent of carcass bruising. Most commercial bison herds are raised with their horns (Kremeniuk, 2010). If a producer chooses to dehorn, typically the entire herd is dehorned, so that there is not a mix of dehorned and horned animals together. Proper pre-slaughter handling can minimize these losses.

The loss due to bruising in bison has been reported as high as 25-35% of a carcass in some situations (Jorgenson, 2008). With bruising rates this high, an examination of the stocking density in the trailer or in holding pens may be warranted. Also an examination of the extent of bruising in bison in a typical commercial situation is needed to determine the magnitude of the problem in the bison industry.

1.3.3 Meat quality introduction

The properties of meat, and ultimately the resulting meat quality, are determined by factors ranging from conception right through to preparation and

consumption of the meat. Understanding the structure of muscle and the conversion of muscle tissues to meat can ultimately help in managing animals and carcasses for optimal meat quality. Indices of meat quality include subjective and objective evaluation of colour structure, appearance, flavour, juiciness and texture (Adegoke and Falade, 2005). All of the attributes combined represent the factors that influence the consumer's overall eating experience.

Meat graded to reflect different eating quality ranges may be valued differently by consumers which counters an argument often put forward by sectors of the meat trade that consumers buy purely on price and will not pay for quality (Polkinghorne and Thompson, 2010). Understanding the mechanisms that affect meat attributes valued by consumers can help the meat industry better provide the product desired by consumers.

Structure and function of muscle and post-mortem changes to muscle will be discussed to better understand how they affect meat quality in general, and the quality of bison specifically where references exist. Drawing from the extensive body of literature related to beef can help in understanding principles that will apply to bison.

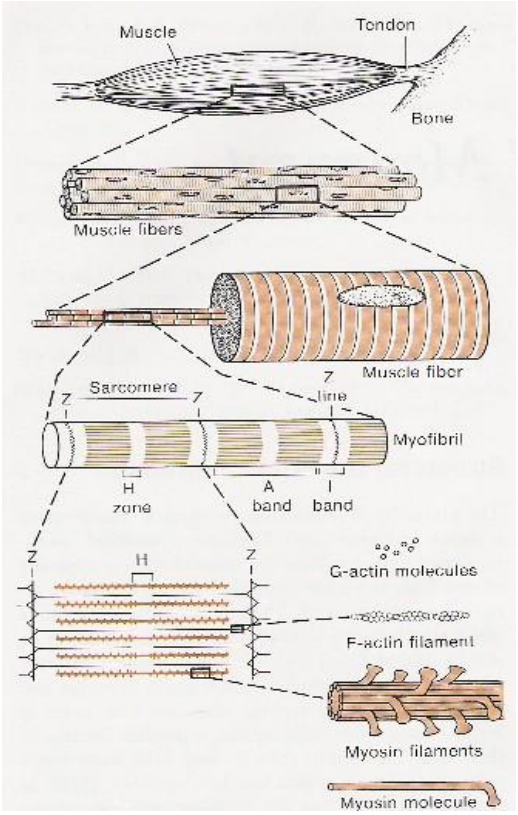
1.3.4 Muscle type and structure

Like other vertebrates, there are three general types of muscle tissue found in bison. Smooth muscle tissue is typically under involuntary control such as muscles in the digestive tract and reproductive tract. Cardiac muscle in the heart is specialized for the continuous pumping of the blood. Skeletal or striated muscle

connects the bones in the arms, legs and spine. Non-organ meat of a commercial beef or bison carcass is skeletal muscle.

Skeletal muscle can be broken down into various components as illustrated in figure 1.1. The largest component is the group of multi-nucleated cells of striated muscle that run from a tendon or other connective tissue to a bone. The muscle bundle is comprised of individual muscle fibres, which are the cellular units of muscle tissue. These cells have up to a hundred nuclei and are filled with bundles of parallel myofibrils. Longitudinally repeating sarcomeres, each about 2 μm long in resting state, bound by the 'Z-lines' make up the myofibrils. The 'myo' prefix specifically refers to muscle so that 'myofilaments' refers to a muscle filament.

Figure 1.1. Hierarchy of skeletal muscle organization



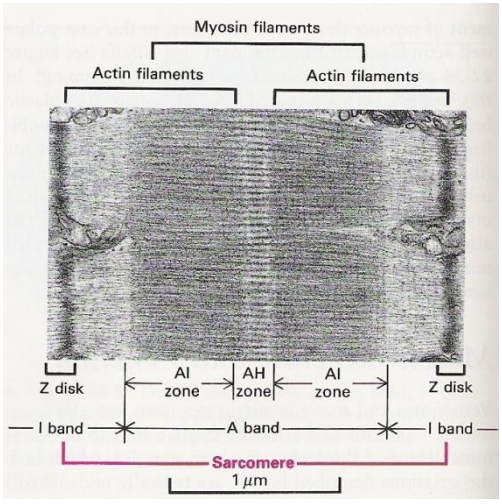
(Bloom and Fawcett, 1968)

Extending from the Z-lines in a myofibril are both thick and thin filaments. The thin filaments consist mostly of the actin, troponin, and tropomyosin proteins, and the thick filaments are made up of the myosin protein with a molecular weight of about 520 kDa (Honikel, 2004a). The Z-line appears as an electron-dense zigzag line about 40-50 nm in width. Z-lines tend to be thicker in fibres with a slow contraction speed and thinner in fibres with a fast contraction speed (Swatland, 1994). While there are different models proposed about the structure of the Z-line, the predominant one is that the filaments of the Z-line that anchor

the ends of the actin filaments are composed of alpha-actinin (Chowrashi and Pepe, 1982).

The A-band is primarily made up of myosin thick filaments 300-500 nm in length (Figure 1.2). The A-band is so labelled because it is anisotropic when viewed in polarized light (Darnell et al., 1990). The 'I' band is comprised of thin filaments and is less anisotropic (more isotropic) hence the label 'I' (Darnell et al., 1990). It is the 'I' band that reduces in width during contraction; this is the part of the actin microfilaments not covered by myosin (Figure 1.2). Contraction takes place when thin filaments slide along the thick filaments reducing the I-band. This action shortens the length of the sarcomere by about 0.7μm from a resting length of 2-3μm (Honikel, 2004a).

Figure 1.2. Striated muscle tissue



(Darnell et al., 1990)

1.3.5 Muscle fibre types

Classification of muscle fibre types is based on the aerobic oxidative capacity using the reference enzyme succinate dehydrogenase has resulted in red, intermediate and white fibre types (Klont et al., 1998). This method reflects differences of mitochondrial content in the tissue with 'white' fibre type having low amounts of mitochondria. Classification based on contraction speed is based on the myosin ATPase isoforms which have a difference in sensitivity to staining after exposure to either high or low pH. This has resulted in I, IIA, and IIB (IIC) nomenclature (Klont et al., 1998).

Another commonly used nomenclature is slow-twitch oxidative (SO; Type I), fast-twitch glycolytic (FG; IIB) and fast-twitch oxidative-glycolytic (FOG; Type IIA) (Renerre, 1990; Darnell et al., 1990; Peter et al., 1972). SO fibres are rich in myoglobin and contain high numbers of mitochondria, rely on aerobic metabolism for the production of ATP, and have a high enzymatic activity. Type I (SO) myofibres are the smallest in diameter (Rosser et al., 1992) and are associated with more blood capillaries, a high lipid, myoglobin, mitochondria and tricarboxylic acid (TCA) cycle enzyme content to suit their high oxidative metabolism (Essen-Gustavsson et al., 1992). Fast twitch glycolytic fibres have limited mitochondria, have a greater level of high energy phosphates and glycogen (Renerre, 1990) and contract and fatigue quickly. They have been found to rely on anaerobic metabolism for the production of ATP (Peter et al., 1972). FOG fibres are intermediate and rely on both anaerobic and aerobic metabolism for production of ATP (Peter et al., 1972). Fibre type composition varies amongst muscles and species based on individual muscle functions. Muscle groups in

different species have varying proportions of fibre types equipped to perform individual muscle functions.

Bison have been found to have a greater percentage of FOG fibres (33.3 vs. 26.9%) and lower percentage of FG fibres (40.0 vs. 45.0%) than cattle (Koch et al., 1995; Janz et al., 2002). Bison muscle displayed a more rapid and extensive post-mortem metabolism than beef muscle, even though a lower frequency and smaller total area of FG fibres were found (Janz, 1999). Species differences in muscle fibre type should be considered when optimizing carcass cooling and handling in any abattoir. Pigs have a much higher percentage of FG and a lower percentage of SO muscle fibres than cattle and consequently are more susceptible to PSE and less susceptible to cold shortening (Aalhus et al., 1998). Bison carcasses chilled with an elevated temperature chilling regime (10-12^{o}C for the first 9 hours of chilling) were found to produce more uniformity of tenderness when compared to conventionally chilled (0-2^{o}C for 24 h) carcasses (Janz et al., 2000).

Muscle fibre types can impact colour stability. Beef with lowest oxygen consumption rate, and lamb with the highest have been found to have the best and worst colour stability respectively (Klont et al., 1998).

1.3.6 Muscle contraction

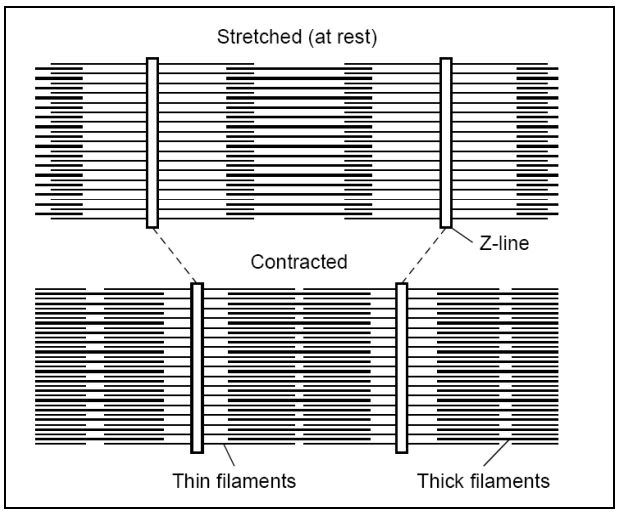
Contraction starts with a nerve impulse from the brain to the muscle cell that causes hormonal and chemical changes to muscle membrane (depolarization) causing a signal to the T-tubules, and then to the sarcoplasmic reticulum (SR).

The SR is a smooth membrane that surrounds each myofibril and is the organelle in the cell that functions as a reservoir of calcium ions. Depolarization causes the SR to release calcium into the sarcoplasm. The cytoplasm inside a muscle cell is called sarcoplasm and the aqueous phase of the cytoplasm around cellular organelles is called the cytosol. In resting muscle the calcium level in the cytosol remains low since the SR reticulum sequesters calcium. Consequently cross bridges between the troponin complex and myosin heads remain inactive since there is very little calcium available to bind with troponin (Eckert, 1988). However, when the calcium is released into the sarcoplasm it binds to troponin C and causes a physical shift in tropomyosin opening up binding sites along the actin filament. As the muscle contracts charged myosin molecule heads attach to the actin molecules (Swatland, 1994). In the process adenosine triphosphate (ATP) is split into adenosine diphosphate (ADP), shifting the myosin head and releasing energy. Troponin is the only protein in the actin myosin complex with a high binding affinity for calcium (Eckert, 1988).

Since ATP supplies energy for a muscle to contract, it might be concluded that removal of ATP would result in a relaxation of muscle. In fact this does not occur since the cross bridges that form during contraction need ATP to relax (i.e. for the actin filaments and the myosin cross bridges to separate) in addition to a lowered calcium concentration in the sarcoplasm (Davies, 2004). During the conversion of muscle to meat ATP reserves are depleted so the myosin molecule remains locked to actin so that even passive filament sliding is not possible (Swatland, 1994). This locked muscle contraction results in rigor mortis. In a

living cell with the presence of ATP, the binding of ATP to myosin weakens the binding of the myosin heads to actin, and results in the relaxing of contracted muscle. Figure 1.3 shows the sarcomere structure at rest and contracted.

Figure 1.3. Sarcomere structure at rest and contracted



(Honikel, 2004a)

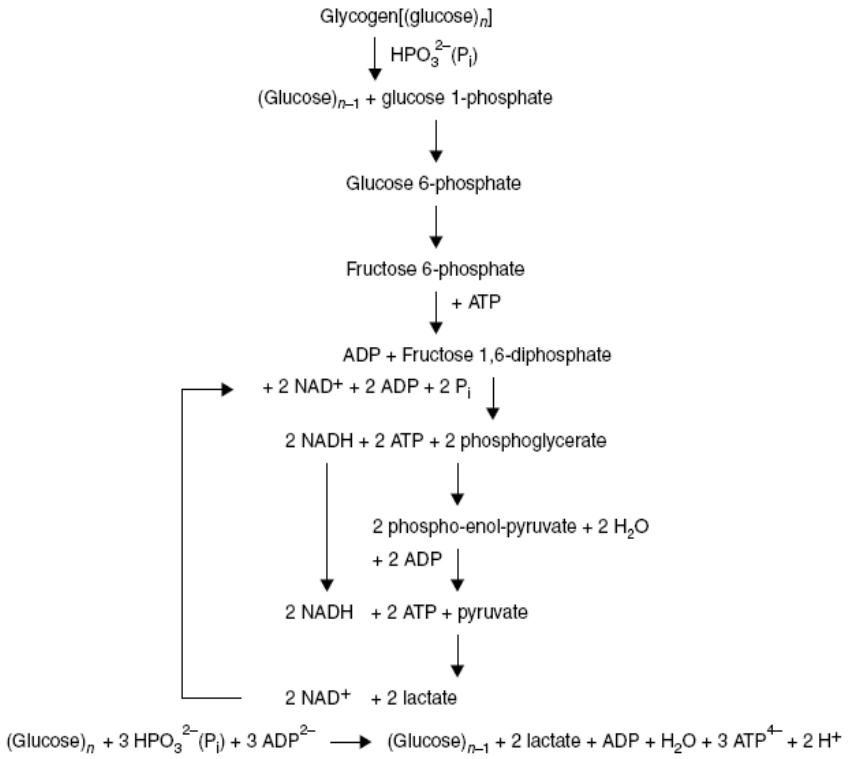
1.3.7 Post-Mortem changes to muscle tissue

Cells in living tissue maintain physiological homeostasis for a balanced internal environment for factors like pH, temperature, O_2 concentration and energy supply. After exsanguination, O_2 supply is cut off to cells, and the pathway for the supply of energy for the cell changes from aerobic to anaerobic metabolism (Swatland, 1994). Cells can produce energy from stored glycogen reserves in the absence of oxygen only for a period of hours. Glycogen is a polysaccharide consisting of many glucose units. Glycolysis (Figure 1.4) is a pathway of reactions that breaks down glucose to lactate and pyruvate, to be used as an energy source for the cell (Honikel, 2004b). Under aerobic conditions pyruvic acid is a substrate for the Tricarboxylic Acid Cycle (TCA), also called the Krebs Cycle. The formation of acetyl CoA and CO_2 is formed from the eight

reactions of the TCA cycle (Eckert, 1988) yielding 34 ATP. In the absence of O_2 after death or in periods of strenuous exercise, anaerobic glycolysis occurs. This produces far less energy (2 ATP net gain) and the lactic acid (H^+ ions) produced in the cell accumulate, causing a drop in the pH. In beef the ultimate pH is between 5.5 to 5.7; from a starting point of around 7 (Honikel, 2004b). The drop in pH is affected by factors such as the amount of glycogen present in the cell prior to death, the enzymatic activity, and rate of temperature decline in the muscle.

Glycolysis continues in the post-mortem period for various times dependant on species. The normal time for muscle to reach a pH of 5.5-5.7 ranges from 6 hours in pork to 18-25 hours in beef, depending on the muscle (Honikel, 2004b). However in pale, soft and exudative (PSE) pork, glycolysis proceeds rapidly and the pH drop can be very rapid, as quick as 1hour *post mortem* in extreme PSE pork (Aalhus et al., 1998) and in slow metabolizing beef pH may still be dropping at 48 hours.

Figure 1.4. The anaerobic glycolytic pathway in muscles



(Honikel, 2004b)

Since there are different pathways to produce energy in the presence or absence of oxygen, it is important to examine why lactate is produced anaerobically ultimately dropping the pH in the meat. In living cells when anaerobic respiration occurs, and there is a lactic acid build-up, when sufficient oxygen becomes available to the cell once again, the unused chemical energy stored in the lactic acid is utilized by oxidation by NAD+ (nicotinamide adenine dinucleotide) and the enzyme lactate dehydrogenase to convert it back to pyruvic acid (Eckert, 1988). Pyruvic acid is converted to acetyl Co-A with Coenzyme-A

releasing 2NADH and 2 molecules of CO_2. However, in a cell from an exsanguinated animal without a blood supply, there is not a replenishing of O_2 and no removal of lactate which results in a drop in muscle tissue pH. Aerobic glycolysis and the Krebs cycle is about 20 times more efficient than anaerobic glycolysis (Eckert, 1988). The result of aerobic respiration results in 34 molecules of ATP whereas the result of anaerobic respiration only results in a net gain of 2.

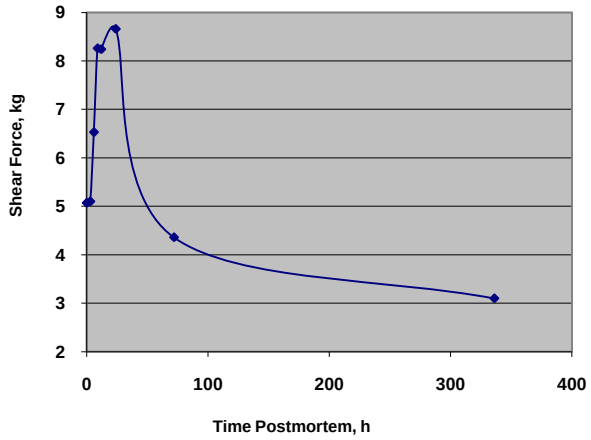
In living cells energy for cellular functions is derived from the Krebs cycle. Glucose is phosphorolated and through a series of steps produces energy in the form of ATP (adenine triphosphate) and NADH. After death, in the absence of oxygen, the glycogen in muscle cells will continue to convert to lactic acid until such time as the pH drops to a level that inactivates enzymes responsible for the conversion (Lawrie, 1998). The conversion of 'muscle' to 'meat' can be considered complete when muscles have depleted their energy reserves or have lost the ability to use these reserves (Swatland, 1994).

The toughening associated with *rigor mortis*, is a result of the normally labile actinomyosin complex becoming tightly associated and the shortening of sarcomeres (Koohmaraie et al., 1996). The shortening also causes the entire tissue framework to become denser. There are three phases to *rigor mortis*, delay, onset and completion (Swatland, 1994). The delay phase is when there is plenty of ATP in the muscle (complexed with Mg^{++}), the muscle will remain in the relaxed state and no cross bridges between the thick and thin myofilaments will occur. During this phase ATP is actively being utilized to maintain membrane stability. The onset phase is when stores of ATP and creatine phosphate (CP) (used to

rephosphorylate ADP to ATP) are depleting, Calcium begins to accumulate in the cytosol and bonds between the thick and thin myofilaments are formed. As more bonds are formed, the muscle loses extensibility. The completion phase is when all of the CP is depleted, the muscle has no way of regenerating ATP and full *rigor mortis* has occurred.

Meat that goes into *rigor mortis* in an extended or non-contracted position has less cross bonding of the actin and myosin filaments and can be tender upon cooking. In meat that goes into rigor mortis in a contracted position, there are more cross bridges across the actin and myosin filaments and the meat is tougher upon cooking (Figure 1.6) (Lawrie, 1998).

Figure 1.5. Tenderness of ovine longissimus measured by Warner-Bratzler shear force, at various times *post-mortem*



(Lawrie, 1998)

1.3.7.1 Chilling

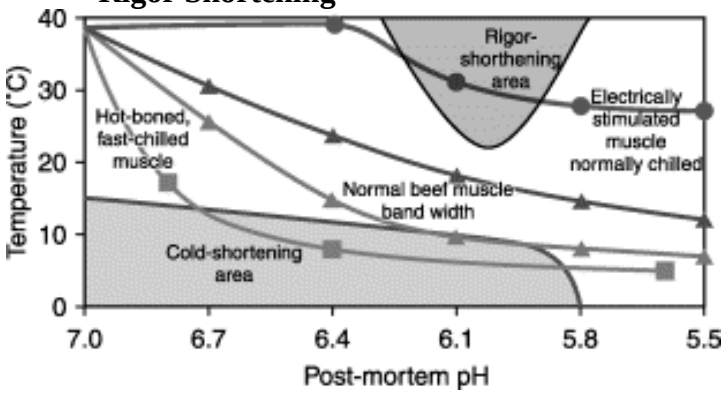
Temperature has a major effect on the rate and extent of rigor contraction. At lower temperatures the calcium accumulating systems work less efficiently and the amount of calcium released by the sarcoplasmic reticulum (SR) is increased (Honikel, 2004a). Below 10^{o}C, the efflux is distinctly higher than the pumping into the SR resulting in an increased concentration of calcium around the filaments while ATP levels are still at levels high enough to fuel contraction (Honikel, 2004a). As temperatures fall, the ATP-driven calcium pump acting from the myofibrillar space into the SR becomes slower (Honikel, 2004a). Troponin binds to the calcium ions, causing a configural shift in tropomyosin opening up sites for binding between actin and myosin heads. Contraction cycles occur provided there is ATP present (Honikel, 2004a). If temperature falls quickly when there is lots of ATP available, then high Ca^{++} and high ATP will result in significant contraction. The lower the temp, the higher the calcium build-up in the myofibrillar space and the stronger the contraction. This is called cold shortening (Figure 1.6).

The calcium in the cytosol, while muscle pH is still high, can activate myofibrillar contraction causing toughness (Locker and Daines, 1963). Three conditions are needed for cold shortening to occur: Temperature below 10^{o}C, pH above 6 and the presence of ATP (Monin, 2004a). Contraction in the muscle is prevented at lower pH and without ATP. The pH in meat at 24 hour *post mortem* ranges from 5.4-6.0 (Swatland, 1994). A moderate chilling rate, or acceleration of glycolysis (thereby depleting ATP before temperatures fall) through electrical

stimulation of the carcass can prevent cold shortening. Hence *post mortem* carcass-chilling regimes can play an important role in the ultimate tenderness of meat. A modified 48 hour chilling time rather than the conventional 24 hour chilling period resulted in reduced shear values across all muscles tested (Janz et al., 2004). The improvement was not such that the modified chilling time alone could be relied upon to produce guaranteed tender meat.

'Very fast chilling' has also been examined for improvements in tenderness (Joseph, 1996). This involves reducing the temperature of the carcass to $-1^{o}C$ after 5 hours *post mortem* throughout its mass (Troy and Joseph, 2001). Koohmarie (1996) demonstrated that by immediately freezing beef carcasses post slaughter the normal toughening phase was not allowed to occur, and meat does not toughen when sarcomere shortening is prevented. Very fast chilling of bison resulted in darker meat muscle colour than conventional chilling, and the meat from the longissimus muscle represented the greatest proportion of samples ranked in either a 'tender' or 'probably tender' category (Janz et al., 2001b). None of the chilling regimes examined by Janz et al. (2001b) resulted in reduced variation in tenderness.

Figure 1.6: Temperature and pH Relationship Showing Cold and Rigor Shortening



(Honikel, 2004a)

Heat shortening occurs when meat is stored at too high a temperature *post mortem* (Figure 1.6). The calcium efflux from the SR into the sarcoplasm works well at elevated temperatures (+18^{0}C) with the pH at 6.0 (Honikel, 2004a). The shortening occurs for essentially the same reason as cold shortening; the calcium efflux into the sarcoplasm is greater than the pump into the SR causing a build-up of calcium, and in the presence of ATP, contraction of the muscle occurs.

Sarcomere lengths in cold-shortened or heat-shortened muscles are reduced by up to 50% (Honikel, 2004a). Sarcomere length can range from <1.5 μm in contracted muscle to 4.5 μm in stretched muscle, and the lengths are closely related to toughness in the meat (Davies, 2004). Bison muscle from conventionally chilled carcasses have been found to have a mean sarcomere length of 1.57± 0.05 (SEM) μm at 24 hours post-mortem (Janz et al., 1999). Shortening, both cold and heat, has detrimental effects on both the ultimate tenderness and water-holding capacity of meat.

Managing *post mortem* slaughter temperatures can pose a challenge particularly in beef carcasses where the gradient between surface and deep tissue temperatures has been shown to be as much as 12^{o}C (Simmons et al., 2006). This could have implications on segments of the bison industry that are marketing lean grass finished animals, and using a custom slaughter plant that has its cooler set for the usual grain finished beef. With less fat insulation covering the carcass, it is reasonable to predict a more rapid temperature decline in the lean carcasses with subsequent negative effects on quality.

1.3.7.2 Ageing

Ageing is the breakdown of muscle tissue *post mortem*. The ageing process has been shown to account for a significant portion of variation in tenderness since optimal tenderness will not be achieved unless ageing is long enough in duration or takes place at elevated temperatures (Devine, 2004). Aging includes the proteolysis of myofibres, denaturation of collagen, destruction of connectins, and disassociation of actinomyosin complex and associated proteins. This is referred to as the tenderization phase, see figure 1.5. It is during this phase that 'ageing' takes place. Ageing is not as important to the final tenderness of a muscle if toughening is prevented in the first place by stopping contraction during rigor. However, much research has examined the mechanism of ageing to better understand how to end up with a more tender meat.

Endogenous peptidases (enzymes) act in a way that results in a weakening of the myofibrillar structure aiding development of tenderness in meat post

slaughter (Sentandreu et al., 2002). Calpains and cathespins are two groups of enzymes thought to be primarily responsible for this process (Koohmarie and Geesink, 2006). The calcium activated neutral protease calpain exist in at least three forms μ-calpain, m-calpain and skeletal muscle-specific calpain, p94 or calpain 3 (Koohmarie and Geesink, 2006). μ-calpain is more sensitive to calcium than m-calpain as it requires 1-30 μmol of calcium and m-calpain requires 100-750 μmol of calcium for half maximal activity (Devine, 2004). Boehm et al. (1998) suggests a possible scenario where μ-calpain is involved in the loss of sarcolemmal integrity in the 24 to 48 hour *post mortem* period, which would lead to 'leakage' of Ca^{++} into muscle fibres, and m-calpain is involved in protein degradation after 48 hours *post mortem*, when Ca^{++} levels would be higher to support its activity. Later articles suggest the μ-calpain is responsible for protein degradation post mortem (Geesink et al., 2006).

Calpastatin inhibits the action of calpains. The inhibition of calpain by calpastatin is pH dependant so that at a pH of 7.5 the calpastatin activity exceeds the calpain activity, and at a *post mortem* muscle pH of <5.8 the activity of calpain is reduced, but the calpastatin activity is reduced more (Devine, 2004). An important characteristic of μ-calpain and m-calpain is that in the presence of calcium they undergo autolysis (Koohmaraie and Geesink, 2006).

The literature is divided on the exact role of the calcium activated proteolytic system. Ouali et al. (2006) have suggested that there are three main lines of thought regarding this process: those that favour the notion that the

calpain system of proteases are solely responsible for *post mortem* tenderization, those that feel it is a combination of the two stated systems, and those that propose a 'new' multienzymatic process (Herrera-Mendez et al., 2006). All seem to agree that the proteolytic system has been found to be integral for the degradation of muscle tissue *post mortem* (Huff-Longergan et al., 1995; Casas et al., 2006; Koohmaraie and Geesink, 2006). In a series of studies investigating early post-mortem tenderization in beef cattle, no evidence to support the theory that post-mortem tenderization was controlled solely by the calcium activated proteolytic system (i.e. μ-calpain) was discovered (Aalhus, 2000). However in a recent study to determine the importance of μ-calpain as a key enzyme involved in proteolysis, it was found that the mice that had the μ-calpain gene deactivated had less proteolytic activity (Geesink et al., 2006). It may be that a combination of calpain activity with other factors contribute to proteolysis. Caspases are a family of cysteine proteases that also may play a role in post- mortem tenderization potentially by proteolysis of calpastatin, which in turn could activate calpains (Kemp et al., 2010). The exact mechanism of post-mortem proteolysis has not yet been determined, but continued understanding in this area will allow for capitalization on factors that promote an appropriate level of proteolysis for a more tender meat.

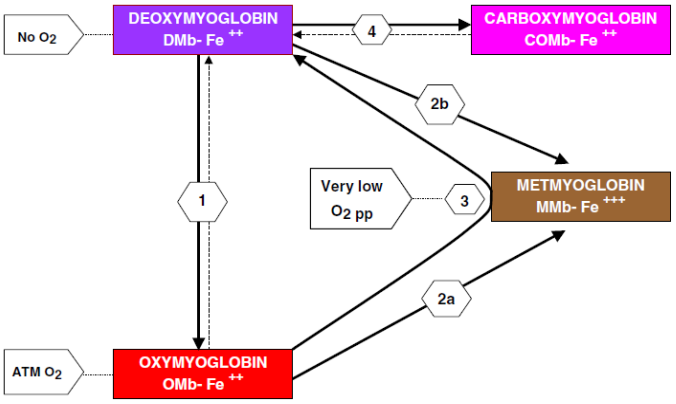
1.3.8 Meat Colour

Meat colour is important because it is used by consumers as an indicator of freshness and wholesomeness (Mancini and Hunt, 2005). The hue, which describes the general type of colour, the chroma, which describes the intensity of a given colour and the luminosity, which refers to the lightness (white to black) of

the colour, are colour properties that can be used to describe the human perception of colour (Swatland, 1994). Factors such as the type and intensity of lighting, temperature, packaging film and the atmosphere within the package will affect the perceived colour impression (Kropf, 1980).

Myoglobin is a single-chain oxygen-binding protein found in muscle tissue of all vertebrates. An important functional property of myoglobin is its ability to reversibly bind oxygen (O_2) to create an intracellular reservoir of O_2 and to facilitate the diffusion of O_2 from the plasma membrane to the mitochondria where O_2 is consumed in production of energy through the Krebs cycle (Renerre, 1990). Myoglobin consists of a protein portion and a non-protein porphyrin ring with an iron atom in the centre. The iron atom is important to meat colour since the colour is defined by the chemical state of the iron and the type of compounds that are attached to it (Giddings, 1977; Mancini and Hunt, 2005). When the surface of meat comes into contact with O_2, the myoglobin becomes oxygenated, and the meat is said to ‘bloom’ as it changes to a bright red appearance (Figure 1.7). Discolouration results from the oxidation of ferrous and myoglobin derivatives to ferric iron (Mancini and Hunt, 2005).

Figure 1.7: Visible Myoglobin Redox Interconversions on the Surface of the Meat



Rx 1 (Oxygenation): DMb + O_2 → OMb
Rx 2a (Oxidation): OMb + [oxygen consumption or low O_2 partial pressure] – e^- → MMb
Rx 2b (Oxidation): [DMb – hydroxyl ion – Hydrogen ion complex]+ O_2 → MMb + O_2^-
Rx 3 (Reduction): MMb + Oxygen consumption + metmyoglobin reducing activty → DMb
Rx 4 (CarboxyMb): DMb + carbon monoxide → COMb

(Mancini and Hunt, 2005)

The colour of meat can be attributed to both myoglobin and hemoglobin pigments, which absorb certain wavelengths and reflect others. The majority of hemoglobin is lost during slaughter as a result of exsanguination (Faustman et al., 1996). The colour of fresh meat is impacted by the amount of each derivative of myoglobin. Myoglobin loses its affinity for O_2 as chronological age increases, and there are differences in the affinity for oxygen across species. A greater myoglobin concentration yields a more intense colour. The derivatives of myoglobin include reduced myoglobin (Mb), red oxymyoglobin (MbO_2), and metmyoglobin (MetMb). Mb is the purple pigment of deep muscle and of meat under a vacuum; MbO_2 is formed when myoglobin is exposed to air forming a bright red colour; the oxidized form of myoglobin forms MetMb, which is brown and undesirable. During storage of meat the rate of MetMb accumulation on the

surface of beef meat is related to many intrinsic factors like pH, muscle type, animal, age, breed, sex, diet; or extrinsic ones like pre-slaughter treatments, chilling mode, electrical stimulation or hot-boning (Renerre, 1990).

1.3.8.1 Measuring colour

Reflectance measurements are close to what the eye and brain see, and can relatively easily be repeated on the same sample multiple times. The CIE (Commission Internationale de l'Eclairage, 1978) colour space contains three axes, L* a* b*, that define locations within the colour sphere. The CIE colour space is recommended over the older Hunter Lab value formulas as the CIE L* a* b* puts more emphasis on the red part of the spectrum (AMSA, 1991). The L* axis defines lightness on a scale of 0 (black) to 100 (white). The a* and b* axes describe the colour directions; +a* is the red direction, -a* is the green direction, +b* is the yellow direction, and –b* is the blue direction (Konica Minolta Senseing Inc., 2003). Values for a* and b* can be converted to the hue (H_{ab}=arctan[b*/a*]) and chroma ($C_{ab}=[a^{*2}+b^{*2}]^{0.5}$) which are colour descriptors that relate more closely to visual perception of colour (Commission Internationale de l'Eclairage, 1978).

As conversion to metmyoglobin occurs, some ferric pigment will be reduced to the ferrous form and in an aerobic environment, oxymyoglobin will result (Figure 1.7). This is dependent on the reducing capacity, oxygen availability and myoglobin autoxidation rate of the muscle, and will differ depending on the age, species and muscle in question (Renerre, 1990).

Bison has been found to discolour more rapidly than beef (Pietrasik et al., 2006; Dhanda et al., 2002; Janz et al. 2000, 2001b) and the reason for this has not been determined. Structurally, beef and bison have identical myoglobin which displays no difference in primary structure, kinetics of oxidation, and themostability (Joseph et al., 2010). Therefore the differing rate of discolouration of bison cannot be attributed to differences in the structure and biochemistry of myoglobin.

The type of fibres in a particular muscle group can affect the colour of the meat. Bison have been found to have a lower percentage of white and a higher percentage of intermediate fibres than cattle with no difference in red fibres (Koch et al., 1995). With increasing numbers of mitochondria and consequently more myoglobin, the fibre composition of bison could be expected to contribute to a darker muscle colour.

1.3.8.2 Significance of meat colour

In beef, fresh meat that has turned brown in the meat case may account for upwards of $1 billion in lost revenue to retailers in the USA, either because the meat has to be discounted, taken off the shelf and ground into burgers, or simply thrown out (Keefe, 2009). In a study examining demographics and beef preferences affecting consumer motivation for purchasing beef steaks, the most frequently cited reasons for steak selection before eating were appearance/fat/packaging (28.5%) and colour (17.2%) (Reicks et al., 2011).

1.3.9 Water holding capacity

The colour of meat can also be affected by water holding capacity. The water holding capacity of meat is the ability of meat to retain its water during application of external forces such as cutting, heating, grinding, or pressing. Water is found in three locations in meat. Bound water is held by charged hydrophilic groups on the muscle proteins that attract water forming a tightly bound layer. Immobilized water that has a less orderly molecular orientation toward the charged group, and free water held only by capillary forces and their orientation is independent of the charged group (Swatland, 1994). The water holding capacity of meat is the lowest when the number of positively and negatively charged groups of the myofibrillar proteins are equal, and is termed the isoelectric point. At this time no charge is available to hold the bound and immobilized water. At its isoelectric point, a protein does not have a net charge and this occurs in meat at a pH around 5.0 to 5.1 (Swatland, 1994). Below a pH of 5 (for example in processed meats where the pH is deliberately lowered) the water binding capacity increases again. At a high ultimate pH, muscle fibres are more tightly packed and the water holding capacity of the muscle protein increases causing a darker colour (Renerre, 1990) due to lower reflectance and lightness.

Osmolality is a measure of the osmoles of solute per kilogram of solvent, and is an indicator of hydration status where higher numbers indicate increased dehydration (Oppliger et al., 2005). During *ante mortem* stress, sodium and potassium cations are lost from intracellular fluid (Schaefer et al., 2001). This change in osmolality could be expected to have an impact on water holding capacity and hence L* values due to a change in light scattering.

1.3.10 Tenderness

Tenderness has been examined and measured for many decades. Its perception is a complex factor to isolate and measure due to the combination of vertical and lateral movements of the jaw, which all together give the impression of tenderness (Pearson, 1963). The techniques for evaluation of tenderness can be categorized into human and instrumental measurements. The consumer evaluation is particularly useful when trying to attribute a monetary value to the enhanced (or worsened) eating experience that a particular piece of meat offers. It gives a 'real world' opinion of quality attributes of meat. The problem occurs in the repeatability due to the complexity of the processes involved in assessing a piece of meat.

Mechanical and chemical methods of analyzing meat offer an objective manner in which to evaluate tenderness. There have been many different attempts to devise a mechanical means to objectively measure tenderness. The earliest recorded attempt was in 1907 when Lehman published a paper (in German) that described a device that looks something like a balance scale. At one end of the scale was a platform for weights to be placed, on the other end are opposing metal blades. A sample of meat was placed between the blades and weights were placed onto the platform until the meat at the other end was sheared as described in English by Pearson (1963). Other devices tested include the cutting gauge and the Penetrometer (Tressler et al., 1932), the Child-Satorius shear (Satorius and Child, 1938), and the Volodkevich tenderness device, which utilized artificial teeth to mimic the chewing motion of the mouth (Volodkevich, 1938).

The most widespread method used in meat quality labs is the Warner-Bratzler shear force (WBSF; Bratzler, 1932; Warner, 1932). It is essentially a modification of the Lehman device (1907) that measures the force required to cut through a core of meat. Since there are varied methodologies for determining shear force, it is imperative to account for differences in methods between institutions when comparing WBSF values or consumer thresholds, done at different labs (Wheeler et al., 1997). Cross tabulation back to the sensory data is important to know how well the WBSF measurements predict the perception of tenderness. When this is done, the values for the force needed to shear a standard core of meat are categorized into ranges where a certain percentage of people are likely to rank the sample a certain way. For Canadian beef, shear force thresholds have been established at the 50, 68 and 95% confidence interval which equate to 7.85, 6.39, and 4.30 kg of shear force. Samples that meet these thresholds have a 1 in 2, 2 in 3, or 19 in 20 chance of being rated acceptable or better (Aalhus et al., 2004). In a study examining various chilling methods and the effect they have on meat quality traits of bison the following categories were used: Tender:<5.6 kg; Probably tender: 5.6-<7.85 kg; Probably tough: 7.85-<9.6 kg; Tough: >9.6 kg (Janz, 1999). Threshold cut-offs should only be used within a standardized laboratory procedure. For example thresholds established using a 1.27cm shear force core (Shackleford et al., 1995) cannot be applied to data collected using a 1.9 cm core (Aalhus et al., 2004).

The relationship between shear force and panel tenderness can be highly variable. A simple correlation between shear force and panel tenderness was

found to be –0.68 in a study that examined a number of measurements and did correlations to taste panel findings (Wulf et al., 1997). Lorenzen et al. (2003) found that the association between WBSF and sensory panels was low with a correlation coefficient of –0.16 ($P < 0.001$). Since there are varied results in the correlation between taste panel determination of tenderness and WBSF many studies make statements about tenderness of meat based on a combination of sensory and WBSF measurements.

There are many pre-slaughter factors that can influence the tenderness of meat. These include breed, diet and feeding strategies at finishing, fattening, the use of implants, pre-slaughter stress during transport or lairage, growth rate, and age of animal at slaughter. Hydration of cells and ion concentration also impact the amount of carcass weight loss and meat quality attributes (Schaefer et al., 1997).

1.3.10.1 Growth rate and age

During periods of rapid growth, the connective tissue, often associated with toughness, is 'diluted' with myofibrillar tissue (Adegoke and Falade, 2005). Also, faster growth rates typically means more labile connective tissue resulting in the formation of fewer mature cross-linkages. Since faster growing cattle reach a desired slaughter weight at a younger age, the effect of growth rate on tenderness may be essentially the same as looking at the relationship between animal age on tenderness (Perry and Thompson, 2005). In their study Perry and Thompson (2005) found that improvements in eating quality was closely related to differences in group-age at slaughter, rather than growth rate.

There are mixed results from experiments examining the effects of age on tenderness. A general trend indicating that tenderness of meat decreases with large gaps of cattle ages (both genders, veal to adult cattle) has been reported for most cuts of meat (Prost et al., 1975). Similarly, eight steers of two ages (18 and 30 months) were found to show differences in tenderness in the four muscles tested, with the younger animals resulting in more tender meat (Simone et al., 1959).

In contrast to these results, no significant difference in tenderness was observed in either WBSF or sensory panel evaluations in Angus or Hereford females ranging in ages from 10 months to 27 years of age slaughtered without grain finishing (Reagan et al., 1976). For this study animal ages were pooled together to match USDA beef grading standards. For example, 8 animals ranging in age from 305-1033 days were pooled together (Reagan et al., 1976).

No significant difference was found between WBSF values of steers of either 12, 17, or 24 months of age (Gullett et al., 1996). However, the age determination in this study was not consistent. They selected 12-month-old steers based on producer data, and the 17 and 24-month age old animals based on dentition. The latter selection method selects for physiological maturity rather than chronological age (Gullett et al., 1996). This study found the meat from the 12-month-old animals to be consistent for leanness and tenderness.

Commercial bison typically finish at older ages than cattle and typically gain slower than cattle (Koch et al., 1995). They are typically slaughtered at 20-30 months of age compared to 18-20 months of age for cattle. Despite this difference

in rates of gain, when BSE was found in an Alberta dairy cow in 2003, bison to be exported from Canada to the United States fell under similar export age requirements as beef cattle (under thirty months; UTM). This caused exporters increased cost and complication due to the requirements for branding, and short timelines for finishing a slower growing animal. Under the current regulations (Canadian Food Inspection Agency, 2007) for export to the USA, animals must be born after 1999 after the ruminant feed ban was implemented in Canada. UTM animals are still achieving a premium over the older animals (Canadian Bison Association, 2011) since youthfulness is reflected in the assessment of bison carcass grade to be categorized in any of the 'A' or 'B' grades (Appendix 4).

1.3.10.2 Electrical stimulation

Electrical stimulation works by propagating muscle contraction throughout a carcass while membranes are still viable thus depleting excess glycogen (ATP) and thereby expedites the process of *rigor mortis* while carcass temperatures are still elevated (Harper, 1999). The electrical current needs to be able to travel through the carcass in viable membranes capable of polarizing and depolarizing. The important part of decreasing ATP while temperature is still high is that it limits temperature induced loss of membrane integrity which causes flooding of calcium into the cell while there is still lots of ATP around to fuel contraction. If there is depleted ATP, then when the membranes fail due to dropping temperature/inactivation of sodium and potassium pumps, the calcium release does not cause contraction. Electrical inputs utilized during processing can also result in significant effects on pH decline as glycogen is converted to lactic acid

and H^+ ions (Simmons et al., 2006). The electrical current causes the muscles to contract increasing the rate of utilization of ATP resulting in an immediate fall in pH (Hwang et al., 2003).

Both high and low voltage electrical stimulation of carcasses have been examined for improved tenderness. High voltage electrical stimulation (HVES) does not require intact nervous system, but still requires the membranes to be physiologically active in order to propagate the current, and can be applied up to one hour *post mortem*. In addition to depletion of glycogen, HVES also results in some microscopic tearing (Savell et al. 1978). Low voltage electrical stimulation requires an intact nervous system, and is therefore applied early *post mortem*. It causes depletion of glycogen and a rapid pH drop in the muscle (Hawrysh et al., 1987).

In Hawrysh et al. (1987) two levels of low voltage electrical stimulation (30 V for either 1 or 2 minutes) were applied to 3 groups of eight mature cows (n=24). In the SM, an improvement in tenderness in both sensory and WBSF measurements was found in the group of carcasses exposed to the longer stimulation time. Twenty-eight Chinese Yellow crossbred bulls (Yanbian x Simmental) were used to compare carcass chilling methods and low voltage stimulation (24 V for 30 seconds) treatments for their effects on various meat quality parameters (Li et al., 2006). The electrically stimulated carcasses that were chilled conventionally had significantly lower WBSF measurements than any other treatment groups. Both low and high voltage stimulation of beef carcasses (n=12, 21 V for 20 seconds, and 470 V for 40 seconds) were found to reduce

shear force values in the SM, with high-voltage stimulation having a greater effect (Aalhus, 2000).

In a study that used higher voltage electrical stimulation (500v for 60 seconds, n=20) tenderness (WBSF and sensory panel) was significantly improved in the stimulated carcasses (Powell, 1991). The effect of electrical stimulation was less after a period of ageing (Powell, 1991), indicating that the benefits to electrical stimulation may occur through stopping toughening (by preventing contraction) which could allow the meat to be in the cooler for a shorter time period. In the only study conducted on bison, low voltage stimulation (21v 20 seconds) had a tendency to improve bison meat tenderness as indicated by reduced WBSF values (Janz et al., 2001b).The application of carcass electrical stimulation capabilities in a multi-location abattoir may be beneficial to reduce time in another facility for chilling prior to carcass break down.

1.3.11 "Guaranteed" tenderness- Meat Standards Australia approach

Meat Standards Australia (MSA) is a voluntary beef-grading program that labels beef sold at the retail level with a standard of eating quality based on consumer perceptions (particularly tenderness scores) with a suggested cooking method (Meat Standards Australia, 2004). The prediction model developed by the MSA provided the tool to develop a Palatability Assured Critical Control Point (PACCP) that assigns a score for each muscle from a given carcass when cooked by various methods (Polkinghorne, 2006). The terminology of a 'critical control'

point is borrowed from the common system in food safety of HACCP (Hazard Analysis Critical Control Point). HACCP is a processed based standard that involves documentation along a process chain, particularly at points that can have significant impact on the safety of the final product.

The focus of the entire PACCP is the acceptance of the piece of meat by the consumer. There are points along the production chain that are monitored that impact what the predicted eating experience will be. Factors including direct movement from farm to slaughter, a prohibition of mixing cattle from different groups during transport, a rib fat depth minimum (3mm), and a requirement for the loin to reach pH 6.0 within a defined temperature range are required for animals to qualify under this scheme (Polkinghorne, 2006). Model inputs affecting the score that a muscle will receive include: bos-indicus%, sex, hormone growth implant use, carcass weight, skeletal ossification, marbling, rib fat depth, ultimate pH, carcass suspension method and days aging by muscle. These inputs are then used to generate a predicted eating experience based on certain cooking methods, and given a 'rating'. The rating system is like a hotel rating with the '5 star' being the highest quality. The '5 star' piece of meat is priced at more than double the '3 star'. The uniqueness in this system is that the consumer does not have to know the anatomy of cattle to select a product that will have a good eating experience. By selecting a high 'star' rating, and preparing the meat in the recommended way, there is assurance that the meat will provide a good eating experience.

The fact that the rating system includes specific cooking recommendations makes it consumer friendly. This allows for a piece of meat that may not be of premium tenderness if barbequed, but will be of superb eating quality if prepared in a slow cooker, to garner a premium price. The two pieces of meat could be priced the same per kg with the appropriate cooking recommendations. Adding cooking methods also takes some of the risk away for the company marketing the meat because if the consumer follows the directions, chances are good that he or she will enjoy the meat. This differs from the traditional way that meat is presented to the consumer where they can easily buy a cut of meat at the store independent from the thought of how they will prepare it.

In this system, the price is determined based on the retail value of the end product, and the likelihood of providing a good eating experience to the consumer. It encourages players throughout the chain to pay attention to factors affecting tenderness pre and post slaughter. If new information about tenderness arises, new requirements can be added or existing ones modified to quickly integrate the new information into the system.

The Australian Palatability Assured Critical Control Point system provides an example of a model by which a ‘guaranteed tender’ product might be marketed by Canadian companies. By utilizing the scientific knowledge that is available to make the conditions favourable, from production to plate, to end up with a tender cut of meat, this attribute could consistently be provided to the consumer along with other attributes such as animal welfare claims and healthy fatty acid profile.

1.3.12 Fatty acids in meat

A fatty acid is a carboxylic acid with a long un-branched aliphatic tail (chain) which is either saturated (have no double bonds) or unsaturated (have at least one double bond). Muscle contains both long chain omega 6 (n-6) and omega 3 (n-3) fatty acids. Dietary changes to alter the fatty acid composition in beef have been examined (Laborde et al., 2002; Nuernberg et al., 2008), and subtle changes to diet have less of an effect in a ruminant animal compared to a monogastric, due to bio-hydrogenation of dietary fatty acids in the rumen (Scollen et al., 2006). The beneficial effects of long chain n-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahaxaenoic acid DHA have been well documented including anti-atherogenic, anti-thrombotic, and anti-inflammatory effects (Givens et al., 2006). Dietary treatment showed an increase in all of these unsaturated fatty acids in bison (Turner, 2005). Inclusion of flaxseed and vitamin E in beef diets were both shown to increase level of n-3 fatty acids in intramuscular fat with the response primarily related to higher levels of α-linolenic, and EPA (Juárez et al., 2011). Comparison of forage and feedlot fed beef to bison show total fat content to be similar between forage fed beef and bison, while the total fat of feedlot fed beef was greater than that of feedlot fed bison (Rule et al., 2002). The n-6 and n-3 fatty acids have implications for human health with a ratio of n-6 to n-3 below 4 recommended (Wood et al., 2008) to contribute toward fulfilling dietary recommendations for improved cardio-vascular health (Gebauer et al., 2005). Greater proportions (P <0.01) of n-3 fatty acids combined with a lack of treatment effect for n-6 fatty led to a decreased n-6:n-3 ratio in grass silage finished Angus cattle (1.20) compared to the high

concentrate group (2.25; Faucitano et al., 2008). Similarly, Leheska et al. (2011) found higher n-3 levels and a lower n-6 to n-3 ration in grass finished beef compared to conventionally grain finished beef.

Another implication of having different fatty acids in muscle tissue is the oxidative stability during processing and retail display. Polyunsaturated fatty acids (PUFA) in phopholipid membranes are susceptible to oxidative breakdown resulting in changes to the colour, smell and taste of the meat (Wood et al., 2008). Lipid oxidation has been defined as the process by which molecular oxygen reacts with unsaturated lipids to form lipid peroxides (Decker et al., 2000). Species differences in susceptibility of meat to oxidation have been linked to the heme iron content (Rhee and Ziprin, 1987; Rhee et al., 1996). Heme iron has been proposed as an initiator and promoter of lipid oxidation in raw meats and H_2O_2-activated metmyoglobin has been seen to promote lipid oxidation in model systems (Decker et al., 2000). Higher levels of iron have been found in raw bison meat compared to those typically found in beef (Marchello and Driskel, 2001; Galbraith et al., 2006). PUFA levels (weight percentage) in range fed and feedlot fed bison were found to be higher than in range or feedlot fed cattle (16.5,10.7, vs. 9.53, 5.04 respectively; Rule et al., 2002). The relatively rapid deterioration of colour quality of bison muscle compared with beef may be related to the significantly higher content of both total PUFA (Rule et al., 2002) and total iron. These characteristics may make bison meat more susceptible to a reduction in display life because of oxidation-related changes in appearance. During the conversion of muscle to meat there is less control of the oxidation of unsaturated

membrane lipids, compartmentalization of metal ions is disrupted, and released iron may catalyze oxidation of both lipids and myoglobin (Morrissey et al., 1998). These reactions could be expected to proceed virtually unchecked in bison tissue, depending on levels of antioxidants present, having relatively high concentrations of PUFA and iron (Rule et al., 2002) which would result in rapid discolouration of fresh meat in retail display.

The fatty acid composition of muscle affects its oxidative stability during retail display, the PUFA's in phospholipid being susceptible to oxidative breakdown at this stage (Wood et al., 2008). To measure the oxidative stability of foods, the thiobarbituric acid reacting substances (TBARS) test of Tarladgis et al. (1960) is commonly used. The TBARS test measures the oxidation product malanaldehyde (Wood et al., 2008). Values above about 0.5 are considered critical since at this level lipid oxidation products which produce a rancid odour and taste that is detectable to consumers are present (Wood et al., 2008).

Lin and Hultin (1977) studied the relationship between lipid oxidation and myoglobin oxidation and found that oxidation of lipids enhanced the latter confirming the positive correlations found between metmyoglobin accumulation and the production of lipid oxidation products (Faustman and Cassens, 1990). The rate of discolouration of meat is believed to be related to the effectiveness of oxidation processes and enzymatic reducing systems in controlling metmyoglobin levels in meat (Faustman and Cassens, 1990).

Antioxidants protect against oxidation by donating a hydrogen atom to a lipid containing a free radical forming a complex between the antioxidant and the lipid chain (see equation below). Anti-oxidants can also act as reducing agents by transferring hydrogen atoms.

$$LOO^{\cdot} + LH \longrightarrow LOOH + L^{\cdot}$$

$$L^{\cdot} + O_2 \longrightarrow LOO^{\cdot}$$

Where LH is a lipid, $LOO^{\cdot}$ is the lipid peroxyl radical and LOOH is the lipid hydroperoxide. The primary role of α-tocopherol is to scavenge the lipid perxyl radical before it is able to react with the lipid substrate as:

$$LOO^{\cdot} + TOH \longrightarrow LOOH + TOO^{\cdot}$$

Where TOH is tocopherol and $TOO^{\cdot}$ is the tocopheroxyl radical (Christie, 2010).

Vitamin E is a term that encompasses a number of tocopherols and trienols that have antioxidant properties (Brigelius-flohe and Taber, 1999). Tocopherols constitute a series of benzopyranols that are present in plant tissues and vegetable oils and are powerful lipid-soluble antioxidants (Christie, 2010). There are four main constituents of tocopherols Alpha, beta, gamma and delta (Christie, 2010). While they are only synthesized by plants, they are essential components of the diet of animals. It has been shown that feeding vitamin E to lambs improved meat colour stability (Lauzurica et al., 2005), and decrease *trans* 18:1 in beef backfat (Juárez et al., 2010). Vitamin E treated pigs produced steaks that were redder and less brown compared to non-supplemented pigs, and showed improved colour

stability (O'Sullvan et al., 2002). In a comparison between pasture and feedlot finished cattle, improved colour stability was observed in the pasture finished groups containing higher levels of α-tocopherol in the diet (Insani et al., 2008). Both α and γ tocopherol levels have been found in bison in a variety of cuts (Table 1.1). Vitamin E levels in pork *longissimus dorsi* (LD) was found to be 26.0 ppm (or µg/100g) (O'Sullivan et al., 2002) which is lower than the levels shown in Table 1.1 in bison. Larrain et al. (2008) reported beef fed a corn diet were found to have α- tocopherol of 188µg/100g, and fed a high-tannin sorgum diet 152µg/100g which is over three times the amount in Table 1.1.

Table 1-1: Reported Tocopherol Components in Various Bison Meat Cuts wet weight basis

Bison Vit E Components	Clod	Ribeye	Sirloin	Top Round	Reference
α – Tocopherol (µg/100g)	46.0	39.0	51.0	52.0	Driskell et al., 1997
α – Tocopherol (µg/100g)	45.8	38.7	50.7	51.9	Marchello et al., 1998
γ – Tocopherol (µg/100g)	14.0	12.0	12.0	14.0	Driskell et al., 1997
γ – Tocopherol (µg/100g)	14.3	12.0	12.1	13.8	Marchello et al., 1998

Lipid oxidation in muscle tissues can promote myoglobin oxidation (Figure 1.7) causing discolouration and rancid odours and flavours (Scollen et al., 2006). It has been suggested by Scollen et al. (2006), that when animals are fed a similar base diet, but fatty acids are altered through dietary supplementation with oils, it is the difference in fatty acid composition and not the amount of antioxidant, that the lipid and colour stability can be attributed to.

An examination of the fatty acid profile of beef, bison, elk and chicken breast concluded that range fed bison and beef cows both provide a lean meat comparable to elk and chicken breast with respect to a fatty acid profile that is currently regarded as healthful (Rule et al., 2002). In their study, the ratio of PUFAs to saturated fatty acids was highest ($P<0.01$) in range fed bison bulls and free ranging cow elk in the *longissimus dorsi* and *semitendinosus* when comparing to range or feedlot fed steers or feedlot fed bison.

With the bison industry growing in North America, producers and marketers are becoming more responsive to consumers preferences. Protocols requiring certain attributes for producers to supply to chains have been developed, such as the premium producer criteria developed and used by Carmen Creek Gourmet Meats (2010). Information about properties of bison meat and opportunities to ensure the best possible quality of meat are desired. Although likely to be similar to beef, there is currently a lack of knowledge specific to bison meat relating to pre-slaughter conditions and the impact on the final product.

This thesis examines meat characteristics and physiological stress of bison exposed to different *ante mortem* treatments. It is hypothesized that bison slaughtered on farm, through the mobile slaughter unit will have superior meat quality and lower stress levels than those transported to a stationary abattoir. Another purpose for this investigation is to examine the reasons behind the oxidative instability and rapid discolouration of bison meat on the retail shelf compared to beef. It is hypothesized that inherent factors such as differences in

fatty acid profiles, myoglobin content and antioxidant content of bison compared to beef will be related to this difference.

Chapter 2 will present meat characteristics of bison slaughtered in a mobile or stationary abattoir. The following chapter will examine the physiological stress response of bison to different pre-slaughter treatments. Chapter 4 will compare bison and beef meat characteristics from fatty acid composition to the performance of fresh steaks in the retail environment. In the last chapter, a summary of conclusions is presented, followed by comments about the mobile abattoir, and future studies to help the bison industry.

1.4 Literature Cited

Aalhus, J. L., Jones, S. D. M., Lutz, S., Best, D. R. and Robertson, W. M. 1994. The efficacy of high and low voltage electrical stimulation under different chilling regimes. Can. J. Anim. Sci. **74:** 433-442.

Aalhus, J. L., Best, D. R., Murray, A. C. and Jones, S. D. M. 1998. A comparison of the quality characteristics of pale, soft and exudative beef and pork. J. Muscle Foods **9:** 267-280.

Aalhus, J. L. 2000. The investigation of early post-mortem tenderization in beef cattle. Alberta Agricultural Research Institute, project: 92M482.

Aalhus, J. L., Jeremiah, L. E., Dugan, M. E. R., Larsen, I. L. and Gibson, L. L. 2004. Establishment of consumer thresholds for beef quality attributes. Can. J. Anim. Sci. **84**: 631–638.

Adegoke, G. O. and Falade, K. O. 2005. Quality of meat. J. of Food, Agriculture and Environment. **3(1)**: 87-90.

AMSA 1991. Guidelines for meat colour evaluation. Savoy, IL: American Meat Science Association. Accessed July 29, 2010. http://www.meatscience.org/Pubs/factsheets/M9110228.pdf

Bloom, W. and Fawcett, D. W. 1968. A textbook of histology. 9th Edition. Saunders. Philidelphia, USA. *In*: Eckert, R., 1988. Animal Physiology Mechanisms and Adaptations. 3rd Edition. W.H. Freeman and Company. New York, USA.

Boehm, M. L., Kendall, T. L., Thompson, V. F. and Goll, D. E. 1998. Changes in the calpains and calpastatin during post-mortem storage of bovine muscle. J. Anim. Sci. **76:** 2415-2434.

Boleman, S. J., Boleman, S. L., Miller, R. K., Taylor, J. F., Cross, H. R., Wheeler, T.L., Koohmaraie, M., Shackelford, S.D., Miller, M. F., West, R. L., Johnson, D. D. and Savell, J. W. 1997. Consumer evaluation of beef of unknown categories of tenderness. J. Anim. Sci. **75:** 1521-1524.

Bratzler, L. J. 1932. Measuring the tenderness of meat by means of a mechanical shear. *Master of Science Thesis*. Kansas State College (KA), USA.

Brigelius-Flohe, R. and Traber, M. 1999. Vitamin E: function and metabolism. FASEB **13:** 1145-1155.

Buckham Sporer, K.R., Weber, P.S.D., Burton, J.L., Earley, B. and Crowe, M.A. 2008. Transportation of young beef bulls alters circulating physiological parameters that may be effective biomarkers of stress. J. Anim. Sci. **86**: 1325-1334.

Canadian Agri-Food Research Council. 2001. Recommended code of practice for the care and handling of farm animals: transport.

Canadian Bison Association. 2010. Market Report. Accessed Feb 14, 2011. http://www.canadianbison.ca/producer/News_Events/news_archives.htm

Canadian Food Inspection Agency. 2007. Exporting cattle, bison and their products under the USDA final rule on BSE risk. Accessed July 28, 2010. http://www.inspection.gc.ca/english/anima/heasan/export/americ/usdae.shtml

Carlsson, H. E., Lyberg, K., Royo, F. and Hau, J. 2007a. Quantification of stress sensitive markers in single fecal samples do not accurately predict excretion of these in the pig. Res. Vet. Sci. **82:** 423-428.

Carlsson, F., Frykblom, P. and Lagerkvist, C. J. 2007b. Consumer willingness to pay for farm animal welfare: mobile abattoirs versus transportation to slaughter. European Review of Ag Econ **34(3):** 321-344.

Carmen Creek Gourmet Meats. 2010. Premium Producer Criteria. Accessed July 29, 2010. http://www.carmencreek.com/producers/producer_alliance.shtml

Casas, E., White, S. N., Wheeler, T. L., Schackelford, S. D., Koohmaraie, M., Riley, D. G., Chase Jr., C. C., Johnson, D. D. and Smith, T. P. L. 2006. Effects of calpastatin and μ- calpain markers in beef cattle on tenderness. J. Anim. Sci. **84:** 520-525.

Catlett, L. 2006. Global perspectives and consumer needs in a changing world. Pages25-27 *in* 52nd International Congress of Meat Science and Technology Proceedings. Wageninggen Academic Publishers, Dublin, Ireland.

Chelini, M. O. M., Souza, N. L., Cortopassi, S. R. G., Felippe, E. C. G. and Oliveira, C. A. 2006. Assessment of the physiological stress response by quantification of fecal corticosteroids. J. of the American Assoc. for Laboratory Anim. Sci. **45**: 8-11.

Chowrashi, P. K. and Pepe, F. A. 1982. The Z-band: 85,000-dalton amorphin and alpha-actinin and their relation to structure. J. Cell Biol. **94:** 565-573. *In* Swatland, H. J., 1994. Structure and development of meat animals and poultry. Technomic Publishing Company Inc., Lancaster, Pennsylvania 17604, USA. pp 246.

Christensen, K. L., Johnson, D. D., West, R. L., Marshall, T. T. and Hargrove, D. D.1991. The effect of breed of sire and age at feeding on muscle tenderness on the beef chuck. J. Anim. Sci. **69**: 3673-3678.

Christie, W. M. 2010. The lipid library- tocopherols and tocotrienols. Accessed July 29, 2010. http://lipidlibrary.aocs.org/Lipids/tocol/file.pdf

Commission Internationale de l'Eclairage 1978. Recommendations on uniform color spaces-color difference equations – psychometric color terms, CIE Publication No. 15 (E-1.3.1) 1971/(TC-1.3), Supplement No.2, pp. 8-12. Paris.

Cook, N. J. and Schaefer, A. L. 2002. Stress responses of wapiti (cervus elaphus Canadensis) to removal of antler. Can. J. Anim. Sci. **82:** 11-17.

Darnell, J., Lodish, H. and Baltimore, D. 1990. Molecular Cell Biology. 2nd Edition. Scientific American Books Inc., W.H. Freeman and Company, New York, USA.

Davies, A. S. 2004. Muscle structure and contraction. Encyclopedia of Meat Science. Elsevier: 882-902.

Decker, E., Faustman, C. and Lopez-Bote, C. J. 2000. Antioxidants in muscle foods: nutritional strategies to improve quality. John Wiley & Sons Inc. New York. 485 pp.

Devine, C. E. 2004. Conversion of muscle to meat- Ageing. Encyclopedia of Meat Science. Elsevier: 330- 338.

Dhanda, J. S., Pegg, R. B., Janz, J. A. M., Aalhus, J. L. and Shand, P. J. 2002. Palatability of bison semimembranosus and effects of marination. Meat Sci. **62:**19-26.

Driskell, J. A., Yuan, X., Giraud, D. W., Hadley, M. and Marchello, M. J. 1997. Concentrations of selected vitamins and selenium in bison cuts. J. Anim. Sci. **75:** 2950-2954.

Eckert, R. 1988. Animal Physiology Mechanisms and Adaptations. 3rd Edition. W.H. Freeman and Company. New York, USA.

Eikelenboom, G., Walstra, P., Huiskes, J. H. and Klont, R. E. 2004. Species of meat animals- Pigs. Encyclopedia of meat science. Elsevier: 1284-1291.

Essen-Gustavsson, B., Karlstrom, K. and Lundstrom, K. 1992. Muscle fibre characteristics and metabolic response at slaughter in pigs of different halothane genotypes and their relation to meat quality. Meat Sci. **31:** 1-11.

Faucitano, L. 2010. Invited review: Effects of lairage and slaughter conditions on animal welfare and pork quality. Can. J. Anim. Sci. **90:**461-469.

Faucitano, L. , Chouinard, P. Y., Fortin, J., Mandell, I. B., Lafreniere, C. L., Girard, C. L. and Berthiaume, R. 2008. Comparison of alternative beef production systems based on forage finishing or grain-forage diets with or without growth promotants: 2. Meat quality, fatty acid composition, and overall palatability. J Anim. Sci. **86:** 1678-1689.

Faustman, C. and Cassens, R. G. 1990. The biochemical basis for discolouration in fresh meat: A review. J. Muscle Foods **1:** 217- 243.

Faustman, C., Chan, W. K. M., Lynch, M. P. and Joo, S. T. 1996. Strategies for increasing oxidative stability of fresh meat colour. Reciprocal Meat Conference **49**: 73-78.

Ferguson, D. M. and Warner, R. D. 2008. Have we underestimated the impact of pre-slaughter stress on meat quality in ruminants? Meat Sci. **80:**12-19.

Fernandez, X., Meunier-Salun, M., Ecolan, P. and Mormede, P. 1995. Interactive effect of food deprivation and agonistic behavior on blood parameters and muscle glycogen in pigs. Physiol. and Behavior **58:** 337-345.

Field, R., McCormick, R., Balasubramanian, V., Sanson, D., Wise, J., Hixon, D., Riley, M. and Russell, W. 1997. Tenderness variation among loin steaks from A and C maturity carcasses of heifers similar in chronological age. J. Anim. Sci. **75**: 693-699.

Fraser, D., Ritchie, J. S. D. and Fraser, A. F. 1975. The term 'stress' in a veterinary context. Brit. Vet. J. **131:**652-662.

Galbraith, J. K., Hauer, G., Helbig, L., Wang, Z., Marchello, M. J. and Goonewardene, L. A. 2006. Nutrient profiles in retail cuts of bison meat. Meat Sci. **74:** 648-654.

Gao, F. 2006. Assessing the consumer acceptance and market potential of Alberta bison meat. M.Sc. thesis. University of Alberta. Edmonton, Alberta.

Gebauer, S., Harris, W. S., Kris-Etherton, P. M. and Etherton, T. D. 2005. Dietary n-6:n-3 fatty acid ratio and health. In: Akoh, C. C. and Lai, O. M. (eds) Healthful lipids, AOCS, Champaign, pp: 221-248.

Geesink, G. H., Kuchay, S., Chishti, A. H. and Koohmaraie, M. 2006. μ-Calpain is essential for post-mortem proteolysis of muscle proteins. J. Anim. Sci. **84:** 2834-2840.

Giddings, G. G. 1977. Symposium: The basis of quality in muscle foods the basis of color in muscle foods. J. Food Sci. **42(2):** 288-294.

Givens, D. I., Kliem, K. E. and Gibbs, R. A. 2006. The role of meat as a source of *n*-3 polyunsaturated fatty acids in the human diet. Meat Sci. **74:**209-218.

Gregory, N. G. 1996. Welfare and hygiene during preslaughter handling. Meat Sci. **43:** s35-s46.

Gullett, E. A., Buttenham, S. and Hore, T. 1996. Effect of age and cut on consistency of tenderness and leanness of beef. Food Quality and Preference **7**: 37-45.

Harper, G. S. 1999. Trends in skeletal muscle biology and the understanding of toughness in beef. Aust. J. Agric. Res. **50:** 1105-1129.

Hawrysh, Z. J., Shand, P. J., Wolfe, F. H. and Price, M. A. 1987. Studies of extra low voltage electrical stimulation of mature beef carcasses. Meat Sci. **21:** 121-135.

Herrera-Mendez, C. H., Becila, S., Boudjellal, A. and Ouali, A. 2006. Meat ageing: reconsideration of the current concept. Trends in Food Sci. and Tech. **17:** 394-405.

Honikel, K.O., 2004a. Conversion of muscle to meat – cold and heat shortening. Encyclopedia of Meat Science. Elsevier: 318-323.

Honikel, K.O., 2004b. Conversion of muscle to meat – glycolysis. Encyclopedia of Meat Science. Elsevier: 314-318.

Huff-Lonergan, E., Parrish, F. C. Jr. and Robson, R. 1995. Effects of post-mortemaging time, animal age, and sex on degradation of titin and nebulin in bovine longissimus muscle. J. Anim. Sci. **73:** 1064-1073.

Hwang, I. H., Devine, C. E., and Hopkins, D. L. 2003. The biochemical and physical effects of electrical stimulation on beef and sheep meat tenderness. Meat Sci. **65:**677-691.

Insani, E. M., Eyherabide, A., Grigioni, G., Sancho, A. M., Pensel, N. A. and Descalzo, A. M. 2008. Oxidative stability and its relationship with natural antioxidants during refrigerated retail display of beef produced in Argentina. Meat Sci. **79:** 444-452.

Jago, J. G., Hargreaves, A. L., Harcourt, R. G. and Matthews, L. R. 1996. Risk factors associated with bruising in red deer at a commercial slaughter plant. Meat Sci. **44(3):** 181-191.

Janz, J. A. M. 1999. Characterization of bison muscle tissue and evaluation of the efficacy of post-mortem carcass treatments designed to influence the quality of bison meat. M.Sc. Thesis, University of Alberta. Edmonton, Alberta, Canada.

Janz, J. A. M., Price, M. A., Hudson, R. J., Schaefer, A. L. and Aalhus, J. L. 1999. Factors affecting quality of bison meat. Alberta Agricultural Research Institute (AARI) Farming for the Future Project #980835.

Janz, J. A. M., Aalhus, J. L., Price, M. A. and Schaefer, A. L. 2000. The influence of elevated temperature conditioning on bison (Bison bison bison) meat quality. Meat Sci. **56:** 279-284.

Janz, J. A. M., Aalhus, J. L., Price, M. A. and Greer, G. G. 2001a. Bison meat quality research: An investigation of spray chilling, very fast chilling and the relationship of grade factors to meat quality. A final report to the Peace Country Bison Association.

Janz, J. A. M., Aalhus, J. L. and Price, M. A. 2001b. Blast chilling and low voltage electrical stimulation influences on bison (Bison *bison bison*) meat quality. Meat Sci. **57:** 403-411.

Janz, J. A. M., Aalhus, J. L. and Price, M. A. 2002. Fibre type characteristics and post-mortem glycolysis of bison (Bison bison bison) longissimus lumborum. Can. J. Anim. Sci. **82:** 259-262.

Janz, J. A. M., Aalhus, J. L., Robertson, W. M., Dugan, M. E. R., Larsen, I. L. and Landry, S. 2004. The effects of modified carcass chilling on beef carcass grade and quality of several muscles. Can. J. Anim. Sci. **84:** 377-384.

Jeremiah, L. E., Schaefer, A. L. and Gibson, L. L. 1992. The effects of ante-mortem feed and water withdrawal, ante-mortem electrolyte supplementation, and post-mortem electrical stimulation on the palatability and consumer acceptance of bull beef after ageing (6 days at 1^{o}C). Meat Sci. **32**: 149-160.

Jorgenson, L. 2008. Mobile slaughter fights government. *in:* The Western Producer March 6: 118.

Joseph, P., Suman, S. P., Li, S., Beach, C. M., Steinke, L. and Fontaine, M. 2010. Characterization of bison (Bison bison) myoglobin. Meat Sci. **84:** 71-74.

Joseph, R. L. 1996. Very fast chilling of beef and tenderness. A report from an EU concerted action. Meat Sci. **43:** S217-227.

Juárez, M., Dugan, M. E. R., Aalhus, J. L., Aldai, N., Basarab, J. A., Baron, V. S. and McAllister, T. A. 2010. Dietary vitamin E inhibits the *trans* 10-18:1 shift in beef backfat. Can. J. Anim. Sci. **90:** 9-12.

Juárez, M., Dugan, M. E. R., Aalhus, J. L., Aldai, N., Basarab, J. A., Baron, V. S. and McAllister, T. A. 2011. Effects of vitamin E and flaxseed on rumen-derived fatty acid intermediates in beef intramuscular fat. Meat Sci. *In press.*

Keefe, L. M. 2009. The color of money [Tech Connection]. Meatingplace. 11.09. pp53-56.

Kemp, C. M., Sensky, P. L., Bardsley, R. G., Buttery, P. J. and Parr, T. 2010. Tenderness- an enzymatic view. Meat Sci. **84:** 248-256.

King, D.A., Schuehle Pfeiffer, C.E., Randel, R.D., Welsh Jr., T.H., Oliphant, R.A., Baird, B.E., Curley Jr., K.O., Vann, R.C., Hale, D.S. and Savell, J.W. 2006. Influence of animal temperament and stress responsiveness on the carcass quality and beef tenderness of feedlot cattle. Meat Sci. **74**: 546-556.

Klont, R. E., Brocks, L. and Eikelenboom, G. 1998. Muscle fibber type and meat quality. Meat Sci. **49:** S219-S229.

Koch, R. M., Jung, H. G., Crouse, J. D., Varel, V. H. and Cundiff, L. V. 1995. Growth, digestive capability, carcass and meat characteristics of *Bison bison*, *Bos taurus* and *Bos x Bison.* J. Anim. Sci. **73:** 1271-1281.

Konica Minolta Sensing Inc. 2003. Precise Color Communication. [online] Accessed July 15, 2010. http://www.konicaminolta.com/instruments/knowledge/color/pdf/color_communi cation.pdf.

Koohmaraie, M., Doumit, M. E. and Wheeler, T. L. 1996. Meat toughening does not occur when rigor shortening is prevented. J Anim. Sci. **74:** 2935-2942.

Koohmaraie, M. and Geesink, G. H. 2006. Contribution of post-mortem muscle biochemistry to the delivery of consistent meat quality with particular focus on the calpain system. Meat Sci. **74:** 34-43.

Kremeniuk, T. 2010. Personal Communication. Canadian Bison Association Executive Director. Regina, Sask. Canada.

Kropf, D. H. 1980. Effects of retail display conditions on meat colour. 33rd reciprocal meat conf. **33:** 15-32.

Laborde, F. L., Mandell, I., Tosh, B. and Buchanan-Smith, J. J. 2002. Effect of management strategy on growth performance, carcass characteristics, fatty acid composition, and palatability attributes in crossbred steers. Can. J. Anim. Sci. **82**: 49-57.

Larrain, R. E., Schaefer, D. M., Richards, M. P. and Reed, J. D. 2008. Finishing steers with diets based on corn, high-tannin sorghum or a mix of both: color and lipid oxidation in beef. Meat Sci. **79:** 656-665.

Lauzurica, S. Fuente, J., Diaz, M. T., Alvarez, I., Perez, C. and Caneque, V. 2005. Effect of dietary supplementation of vitamin E on characteristics of lamb meat packed under modified atmosphere. Meat Sci. **70:** 639-646.

Lawrie, R. A. 1998. Lawrie's meat science. Sixth edition. Woodhead Publishing Limited Cambridge, England.

Leheska, J. M., Thompson, L. D., Howe, J. C., Hentges, E., Boyce, J., Brooks, J. C., Shriver, B., Hoover, L. and Miller, M. F. 2011. Effects of conventional and grass-feeding systems on the nutrient composition of beef. J Anim. Sci. **86:** 3575-3585.

Lehman, K. B. 1907. Studien über die Zähigkiet des fleisches und ihre ursachen. Archiv. Fur Hygiene **63:** 134. *In:* Pearson, A. M. 1963. Objective and subjective measurements for meat tenderness. Proceedings Meat tenderness Symposium, Camden New Jersey. Campbell Soup Company, USA, pp. 135-160.

Li, C. B., Chen, Y. J., Xu, X. L., Huang, M., Hu, T. J. and Zhou, G. H. 2006. Effects of low-voltage electrical stimulation and rapid chilling on meat quality characteristics of Chinese yellow crossbred bulls. Meat Sci. **72:** 9-17.

Lin, T. S. and Hultin, H. O. 1977. Oxidation of myoglobin *in vitro* mediated by lipid oxidation in microsomal fractions of muscle. J. Food Sci. **42:** 136.

Locker, R. H., and Daines, G. J., 1963. A cold shortening effect in beef muscles. J. Sci. Food and Agr. **26**: 1721-1733.

Lorenzen, C. L., Miller, R. K., Taylor, J. F., Neely, T. R., Tatum, J. D., Wise, J. W., Buyck, M. J., Reagan, J. O. and Savell, J. W. 2003. Beef customer: trained sensory panel ratings and Warner-Bratzler shear force values. J. Anim. Sci. **81:** 143-149.

Lowe, T. E., Devine, C. E., Wells, R. W. and Lynch, L. L. 2004. The relationship between post-mortem urinary catecholamines, meat ultimate pH, and shear force in bulls and cows. Meat Sci. **67:** 251-260.

Mancini, R. A. and Hunt, M. C. 2005. Current research in meat colour. Meat Sci. **71:**100-121.

Marchello, M. J., Slanger, W. D., Hadley, M., Milne, D. B. and Driskell, J. A. 1998. Nutrient composition of bison fed concentrate diets. J. Food Composition and Analysis **11:** 231-239.

Marchello, M. J. and Driskell, J. A. 2001. Nutrient composition of grass and grain finished bison. Great Plains Res. **11:** 65-82.

Marler, R. J. 1975. Some hematologic and blood chemistry values in two herds of American bison in Kansas. J. Wildlife Diseases **11:** 97-100.

Mehrer, C. F. 1976. Some hematologic values of bison from five areas of the United States. J. Wildlife Diseases. **12**: 7-13.

McClenahan, J. M. and Driskell, J. A. 2002. Nutrient content and sensory characteristics of bison meat. NebFacts, Nebraska Cooperative and Extension NF01-502, University of Nebraska, Lincoln, Nebraska.

McNally, P. W. and Warriss, P. D. 1996. Recent bruising in cattle abattoirs. Vet Record **138:** 126-128.

McNally, P. W. and Warriss, P. D. 1997. Prevalence of carcase bruising and stick-marking in cattle bought from different auction markets. Vet Record **140:** 231-232.

Meat Standards Australia. 2004. Accessed Feb. 14, 2011. http://www.msagrading.com/

Meyer, D. L., Kerley, M. S., Walker, E. L., Keisler, D. H., Pierce, V. L., Schmidt, T. B., Stahl, C. A., Linville, M. L. and Berg, E. P. 2005. Growth rate, body composition, and meat tenderness in early vs. traditionally weaned beef calves. J. Anim. Sci. **83**: 2752-2761.

Miller, M. 2002. Beef facts: Dark, firm and fry beef. National cattlemen's beef association and the cattlemen's beef board. USA

Miller, M. F., Carr, M. A., Ramsey, C. B., Crockett, K. L. and Hoover, L. C. 2001. Consumer thresholds for establishing the value of beef tenderness. J. Anim. Sci. **79**: 3062-3068.

Miller, M.W., Hobbs, N.T., Sousa, M.C., 1991. Detecting stress responses in Rocky-Mountain bighorn sheep (Ovis Canadensis Canadensis) – reliability of cortisol concentrations in urine and feces. Can. J. Zool. **69:** 15–24.

Mintert, J., Lusk, J. L., Schroeder, T. C., Fox, J. A. and Koohmaraie, M. 2000. Valuing beef tenderness. Kansas State Agricultural Experiment Station and Cooperative Extension Service. MF-2464. Beef Marketing.

Moloney, A. P., Mooney, M. T., Kerry, J. P. and Troy, D. J. 2001. Producing tender and flavoursome beef with enhanced nutritional characteristics. Proc. Nutrition Soc. **60**: 221-229.

Monin, G. 2004a. Conversion of muscle to meat- Colour and texture deviations. Encyclopedia of Meat Science. Elsevier: 323-330.

Monin, G. 2004b. Conversion of muscle to meat- Slaughter-line operation and pigment quality. Encyclopedia of Meat Science. Elsevier: 338-342.

Morgan, J. B., Savell, J. W., Hale, D. S., Miller, R. K., Griffen, D. B., Cross, H. R. and Schackelford, S. D. 1991. National beef tenderness survey. J. Anim. Sci. **69**: 3274-3283.

Morrissey, P. A., Sheehy, P. J. A., Galvin, K., Kerry, J. P. and Buckley, D. J. 1998. Lipid stability in meat and meat products. Meat Sci. **49:** S73-S86.

Mostl, E. and Palme, R. 2002. Hormones as indicators of stress. Dom. Anim. Endocrinology **23:** 67-74.

Munck, A., Guyre, P.M. and Holbrook, N. I. 1984. Physiological functions of glucocorticoids in stress and their relationship to pharmacological actions. Endocr. Rev. **5:** 25-44.

Nuernberg, K., Fischer, A., Nuenberg, G., Ender, K. and Dannenberger, D. 2008. Meat quality and fatty acid composition of lipids in muscle and fatty tissue of skudde lambs fed grass versus concentrate. Small Rum. Res. **74**: 279-283.

Oppliger, R. A., Magnes, S. A., Popowski, L. A. and Gisolfi, C. V. 2005. Accuracy of urine specific gravity and osmolality as indicators of hydration status. Int. J. Sport Nutr. Exercise Met. **15:** 236-251.

O'Sullivan, M. G., Byrne, D. V., Stagsted, J., Andersen, H. J. and Martens, M. 2002. Sensory colour assessment of fresh meat from pigs supplemented with iron and vitamin E. Meat Sci. **60:** 253-265.

Ouali, A., Herrera-Mendez, C. H., Coulis, G., Becila, S., Boudjellal, A., Aubry, L. and Sentandreu, M. A. 2006. Revisiting the conversion of muscle into meat and the underlying mechanisms. Meat Sci. **74:** 44-58.

Palme, R., Fischer, P., Schildorfer, H., Ismail, M.N., 1996. Excretion of infused ^{14}C-steroid hormones via faeces and urine in domestic livestock. Anim. Reprod. Sci. **43:** 43–63.

Palme, R., Robia, C., Meβmann S., Hofer, J. and Mostl, E. 1999. Measurement of faecal cortisol metabolites in ruminants: a non-invasive parameter of adrenocortical function. Wien Tierarztl Mschr. **86:** 237-241.

Pearson, A. M. 1963. Objective and subjective measurements for meat tenderness. *In:* Proceedings Meat Tenderness Symposium, Camden New Jersey. Campbell Soup Company, USA, pp. 135-160.

Perry, D. and Thompson, J. M. 2005. The effect of growth rate during back-grounding and finishing on meat quality traits in beef cattle. Meat Sci. **69**: 691-702.

Peter, J.B., Bainard, R. J., Edgerton, V. R., Gillespie, C. A. and Stempel, K. E. 1972. Metabolic profiles of three fibber types of skeletal muscle in guinea pigs and rabbits. Biochemistry **11:** 2627-2633.

Pietrasik, Z., Dhanda, J. S., Shand, P. J. and peg, R. B. 2006. Influence of injection, packaging, and storage conditions on the quality of beef and bison steaks. J. Food Sci. **71:**s110-s118.

Polkinghorne, R.J., 2006. Implementing a palatability assured critical control point (PACCP) approach to satisfy consumer demands. Meat Sci. **74:** 180-187.

Polkinghorne, R. J. and Thompson, J. M. 2010. Meat standards and grading A world view. Meat Sci. **86:**227-235.

Pollard, J.C., Littlejohn, R., Asher, G.W., Pearse, A.J.T., Stevenson-Barry, J. M., McGregor, S.K., Manley, T.R., Duncan, S.J., Sutton, C.M., Pollock, K.L. and Prescott, J. 2002. A comparison of biochemical and meat quality variables in red deer (*Cervus elephus*) following either slaughter at pasture or killing at a deer slaughter plant. Meat Sci. **60**: 85-94.

Powell, V. H. 1991. Quality of beef loin steaks as influenced by animal age, electrical stimulation and ageing. Meat Sci. **30:** 195-205.

Prost, E., Pelczynka, E. and Kotula, A. W. 1975. Quality characteristics of bovine meat. II. Beef tenderness in relation to individual muscles, age and sex of animals and carcass quality grade. J. Anim. Sci. **41:** 541-547.

Rabiee, A. R., MacMillan, K. L. and Schwartzenberger, F. 2001. The effect of level of feed intake on progesterone clearance rate by measuring faecal progesterone metabolites in grazing dairy cows. Anim. Repro. Sci. **67:** 205-214.

Reagan, J. O., Carpenter, Z. L. and Smith, G. C. 1976. Age-related traits affecting the tenderness of the bovine *longissimus* muscle. J. Anim. Sci. **43**: 1198-1205.

Reicks, A. L., Brooks, J. C., Garmyn, A. J., Thompson, L. D., Lyford, C. L. and Miller, M. F. 2011. Demographics and beef preferences affect consumer motivation for purchasing fresh beef steaks and roasts. Meat Sci. **87:** 403-411.

Renerre, M. 1990. Review: Factors involved in the discolouration of beef meat. Int. J. Food Sci. and Tech. **25:** 613-630.

Rhee, K. S., Anderson, L. M. and Sams, A. R. 1996. Lipid oxidation potential of beef, chicken and pork. J. Food Sci. **61:** 8-12.

Rhee, K. S. and Ziprin, Y. A. 1987. Lipid oxidation in retail beef, pork and chicken muscles as affected by concentrations of heme pigments and nonheme iron and microsomal enzymatic lipid peroxidation activity. J. Food Biochem. **11:** 1-15.

Rosser, B. W. C., Norris, B. J. and Nemeth, P. M. 1992. Metabolic capacity of individual fibres from different anatomical locations. J Histochem. and Cytochem. **40:** 819-825.

Rule, D. C., Broughton, K. S., Shellito, S. M. and Maiorano, G. 2002. Comparison of muscle fatty acid profiles and cholesterol concentrations of bison, beef cattle, elk and chicken. J. Anim. Sci. **80:** 1202-1211.

Satorius, M. J. and Child, A. M. 1938. Effect of exterior temperature upon press fluid, shear force, and cooking losses of roasted beef and pork muscles. J. Agric. Res. **57:**865-871. *In:* Proceedings Meat tenderness Symposium, Camden New Jersey. Campbell Soup Company, USA, pp. 135-160.

Savell, J. W., Dutson, T. R., Smith, G. C. and Carpenter, Z. L. 1978. Structural changes in electrically stimulated beef muscles. J. Food Sci. **43:** 1606-1609.

Savell, J. W., Mueller, S. L. and Baird, B. E. 2005. The chilling of carcasses. Meat Sci. **70:** 449-459.

Schaefer, A. L., Jones, S. D. M., Tong, A. K. W. and Young, B. A. 1990. Effects of transport and electrolyte supplementation on ion concentrations, carcass yield and quality in bulls. Can. J. Anim. Sci. **70**: 107-119.

Schaefer, A. L., Jones, S. D. and Stanley, R. W. 1997. The use of electrolyte solutions for reducing transport stress. J. Anim. Sci. **75:** 258-265.

Schaefer, A. L., Dubeski, P. L., Aalhus, J. L. and Tong, A. K. W. 2001. Role of nutrition in reducing antemortem stress and meat quality aberrations. J. Anim. Sci. **79 (E. Suppl.):** E91-E101.

Schaefer, A. L., Stanley, R. W., Tong, A. K. W., Dubeski, P., Robinson, B., Aalhus, J. L. and Robertson, W. M. 2006. The impact of antemortem nutrition in beef cattle on carcass yield and quality grade. Can. J. Anim. Sci. **86**: 317-323.

Schwartzkopf-Genswein, K. S., Booth-McLean, M. E., Shah, M. A., Entz, T., Bach, S. J., Mears, G. J., Schaefer, A. L., Cook, N., Church, J. and McAllister, T. A. 2007. Effects of pre-haul management and transport duration on beef calf performance and welfare. Applied Anim. Behaviour Sci. **108:** 12-30.

Scollen, N., Hocquette, J. F., Nuernberg, K., Dannenberger, D., Richardson, I. and Moloney, A. 2006. Innovations in beef production systems that enhance the nutritional and health value of beef lipids and their relationship with meat quality. Meat Sci. **74:**17-33.

Seyle, H. 1976. Forty years of stress research: principal remaining problems and misconceptions. Can. Med. Assoc. J. **115:** 53-56.

Sentandreu, M. A., Coulis, G. and Ouali, A. 2002. Role of muscle endopeptidases and their inhibitors in meat tenderness. Trends in Food Sci. and Tech. **13:** 400-421.

Shackelford, S. D., Koohmaraie, M. and Wheeler, T. L. 1995. Effects of slaughter age on meat tenderness and USDA carcass maturity scores of beef females. J. Anim. Sci. **73**: 3304-3309.

Shaw, F. D. and Tume, R. K. 1992. The assessment of pre-slaughter and slaughter treatments of livestock by measurement of plasma constituents – a review of recent work. Meat Sci. **32**: 311-329.

Sikarskie, J. G. 1990. Comparative blood characteristics of ranched and free-ranging American bison (*Bison bison*). Am. J. Vet. Res. **51**: 955-957.

Simmons, N. J., Daly, C. C., Mudford, C. R, Richards, I., Jarvis, G. and Pleiter, H. 2006. Integrated technologies to enhance meat quality- An Australian perspective. Meat Sci. **74:** 172-179.

Simone, M., Carroll, F. and Chichester, C. O. 1959. Differences in eating quality factors of beef from 18- and 30-month steers. Food Tech. **13:** 337-340.

Smith, R. F. and Dobson, H. 1990. Effect of preslaughter experience on behavior, plasma cortisol and muscle pH in farmed red deer. The Veterinary Record **126**: 155-158.

Steenbergen, J. 2011. Business Development – Elk/Bison, Alberta Agriculture and Rural Development. Personal communication.

Steiner, B., Gao, F. and Undershultz, J. 2010. Alberta consumers' valuation of extrinsic and intrinsic red meat attributes: a choice experimental approach. Can. J. Agric. econ. **58:** 171-189.

Stewart, M., Webster, J. R., Schaefer, A. L., Cook, N. J. and Scott, S. L. 2005. Infrared thermography as a non-invasive tool to study animal welfare. Anim. Welfare **14:** 319-325.

Stewart, M., Stafford K., J., Dowling, S. K., Schaefer, A. L. and Webster, J. R. 2008. Eye temperature and heart rate variability of calves disbudded with or without local anaesthetic. Physiol. And Behavior **93:**789-797.

Stoltenow, C. L. and Dyer, N. W. 2001. Biochemical and hematological parameters in ranch-raised American bison (*Bison bison*). Carrington Research Extension Centre Beef and Bison Field Day. July 19, 2001. Accessed July 29, 2010. http://www.ag.ndsu.nodak.edu/carringt/bison/01%20BISON%20Report.pdf

Swatland, H. J., 1994. Structure and development of meat animals and poultry. Technomic Publishing Company Inc., Lancaster, Pennsylvania 17604, USA.

Tarladgis, B. G., Watts, B. M., Younathan, M. T. and Dugan Jr., L. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. J. Am. Oil Chemists Soc. **37:** 44-48.

Tarrant, P. V. 1989. Animal behaviour and environment in the dark-cutting condition in beef- a review. Irish J. Food Sci. & Tech. **13:**1-21.

Torok, S. J., Tatsch, K., Bradley, E., Mittelstaedt, J. and Mary, G. J. 1998. Identification of bison consumer characteristic dimensions and restaurant marketing strategies. Agribusiness **14 (**1): 33–48.

Tressler, D. K., Birdseye, C. and Murray, W. T. 1932. Tenderness of meat. I. Determination of relative tenderness of chilled and quick-frozen beef. Indus. Engin. Chem. 24: 242. *In:* Pearson, A. M. 1963. Objective and subjective measurements for meat tenderness. Proceedings Meat tenderness Symposium, Camden New Jersey. Campbell Soup Company, USA, pp. 135-160.

Troy, D. and Joseph, R. 2001. Very fast chilling in beef. Teagasc Final Report. Project no. AIR-CT94-1881.

Turner, T. D. 2005. Evaluation of the effect of dietary forages and concentrate levels on the fatty acid profile of bison tissue. MSc. Thesis University of Saskatchewan, Canada.

Verkerk, G. A., Phipps, A. M., Carragher, J. F., Mathews, L. R. and Stelwagon, K. 1998. Characterization of milk cortisol concentrations as a measure of short-term stress responses in lactacting dairy cows. Anim. Welfare **7:** 77-86.

Vestweber, J. G., Johnson, D. E., Merrill, G. L. and Staats, J. J. 1991. Hematological and blood chemistry profiles of American bison grazing on Konza Prairie of Kansas. J. Wildlife Diseases **27**: 417-420.

Volodkevich, N. N. 1938. Apparatus for measurements of chewing resistance on tenderness of foodstuffs. *Food Res.* **3**: 221-225.

Vote, D. J., Belk, K. E., Tatum, J. D., Scanga, J. A. and Smith, G. C. 2003. Online prediction of beef tenderness using a computer vision system equipped with a BeefCam module. J. Anim. Sci. **81**: 457-465.

Warner, K. F. 1928. Progress report of the mechanical test for tenderness of meat. Proc. Am. Soc. Anim. Sci. **21:** 114.

Weaver, S. A., Schaefer, A. L. and Dixon, W. T. 2000. The effects of mutated skeletal ryanodine receptors on calreticulum and calsequestrin expression in the brain and pituitary gland of boars. Mol. Brain Res. **75:** 46-53.

Wheeler, T. L., Shackelford, S. D., Johnson, L. P., Miller, M. F., Miller, R. K. and Koohmaraie, M. 1997. A comparison of Warner-Bratzler shear force assessment within and among institutions. J. Anim. Sci. **75**: 2423-2432.

Wheeler, T. L. and Koohmaraie, M. 1994. Prerigor and postrigor changes in tenderness of ovine longissimus muscle. J. Anim. Sci. **72:** 1232-1238.

Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Sheard, P. R., Richardson, R. I., Hughes, S. I. and Whittington, F. M. 2008. Fat deposition, fatty acid composition and meat quality: A review. Meat Sci. **78:** 343-358.

Wulf, D. M., O'Connor, S. F., Tatum, J. D. and Smith, G. C. 1997. Using objective measures of muscle colour to predict beef longissimus tenderness. J Anim. Sci. **75:** 684-692.

Wythes, J. R., Kaus, R. K. and Newman, G. A. 1985. Bruising in beef cattle slaughtered at an abattoir in southern Queensland. Australian J. Exp. Ag. **25:** 727-733.

Zaugg, J. L., Taylor, S. K., Anderson, B. C., Hunter, D. L., Ryder, J. and Divine, M. 1993. Hematologic, serologic values, histopathologic and fecal evaluations of bison from Yellowstone Park. J. Wildlife Diseases **29**: 453-457.

Zhu, L. G. and Brewer, M. S. 1999. Relationship between instrumental and visual colour in a raw, fresh beef and chicken model system. J. Muscle Foods **10:** 131-146.

CHAPTER 2

Meat Characteristics of Bison Slaughtered in a Mobile or Stationary Abattoir

2.1 Introduction

Bison (*Bison bison*) are raised for their meat and other products in North America. In 2010 in the Canadian province of Alberta there were over 18,000 bison slaughtered in licensed abattoirs (Steenbergen, 2011). As a result of limited slaughter facilities for bison, it is common for bison to be transported for several hours to reach an abattoir. Transport is often followed by lairage overnight in pens for slaughter the next morning. Assembly, loading, transport, unloading, regrouping, feed and water withdrawal, novel surroundings and temperature fluctuations are all factors that can create both physiological challenges and psychological disruptions which ultimately impact carcass yield and meat quality (Schaefer et al., 2006).

Branding and niche market opportunities are increasing for livestock producers (Today's Retail Meat Case, 2007; Paganini, 2004) but in some cases slaughter facilities are unable or unwilling to separate and market these low volume niche products. Despite an improved willingness to pay for certain credence attributes such as animal welfare, natural, organic, and local production (Carlsson et al., 2007) they are difficult to retain through the current Canadian commodity systems. A mobile abattoir system could facilitate the development of low volume, high value livestock products for local markets.

The purpose of the present study was to compare carcass characteristics and meat quality attributes of bison slaughtered in a stationary land-based facility following a period of transport and pre-slaughter handling to animals slaughtered in a mobile-location abattoir (MLA) at their home farm locations. Two levels of on-farm pre-slaughter management were investigated prior to processing through the MLA, penned or confined prior to dispatching.

2.2 Materials and Methods

2.2.1 Animals and slaughter

Four bison farms were recruited for participation on the trial; one farm per week on 4 different weeks. A total of 55 bison were used. On three farms 15 bison were used and on the fourth farm 10 bison were used. The bison ranged in age from 16-40 months of age, and were finished on the diets typically fed on the farm which ranged from grass to barley finishing. On each slaughter week five animals from one farm were shipped to the Agriculture and Agri-Food Canada Lacombe Research Centre (AAFC-Lacombe) (transport time 1.5-3 hours) and held in lairage overnight with free access to water until time of slaughter (LAND). The following day 10 animals from the same farm were slaughtered on site using the MLA. Five animals were confined to a squeeze chute or single animal cell (MLACON) and dispatched, while another five were placed in a pen (approximately 100 x 200 feet) and shot in the pen (MLAPEN). On the last week (farm 4) five animals were slaughtered on site, all of which were in the MLAPEN group. Either a .357 magnum or .303 calibre rifle were used to stun the bison on-farm and a .223 calibre rifle was used at AAFC-Lacombe. Live weights were collected on LAND animals only, prior to slaughter. All animals were stunned

and exsanguinated in accordance with the principles and guidelines established by the Canadian Council on Animal Care (1993).

Following splitting of the carcass, hot side weights were recorded, and initial (1 h) pH and temperature were recorded posterior to the left 11th/12th thoracic vertebrae (on the left *Longissimus lumborum*; LL) using a Fisher Scientific Accumet AP72 pH meter (Fisher Scientific, Mississauga ON) equipped with an Orion Ingold electrode (Udorf, Switzerland). A muscle sample (10g) was removed from the same location at the same time, flash frozen in liquid nitrogen, placed in pre-labelled whirlpak bags and stored at -80°C for analysis of glucidic metabolites. Upon entry into the cooler stainless steel thermocouples (10 cm) were placed in both the loin (LL) and deep hip (*semimembranosus*) muscles and temperatures recorded for 24 h using data temperature loggers (Mark III, MC4000; Sumaq Wholesalers, Toronto, ON).

2.2.2 Carcass treatment

At 24 h, left carcass sides were knife-ribbed at the Canadian bison grade site (between the 11th and 12th ribs) and exposed to atmospheric oxygen for 20 min prior to assessing the carcass grade characteristics including grade fat, rib-eye area, carcass conformation, marbling, fat and lean colour (CFIA, 2007). The left *Longissimus thoracis* (LT) and LL were pulled from the carcass, labelled, and trimmed. Final pH and temperature values were recorded (24 h) in the LL adjacent to the same location as initial recordings. A second muscle core sample was collected, flash frozen, and stored at -80°C to complete glucidic analysis.

Loins from animals slaughtered at the mobile abattoir were packaged, placed into a portable cooler and returned to AAFC-Lacombe for meat quality analysis.

2.2.3 Meat quality

The area of the LL where pH and temperature were recorded and cores removed was discarded. A 12.5 cm portion was removed from the cut end, labelled, vacuum packaged and placed in a cooler at 2°C with wind speeds of 0.5 m/sec for 7 d. The remaining portion of the LL was trimmed of all connective tissue and ground three times (Butcher Boy Meat Grinder Model TCA22 with a 3.2mm grind plate (Lasar Manufacturing Co., Los Angeles, CA). One hundred g of the grind were weighed into stainless steel beakers and placed in a gravity convection-drying oven at 102°C for a 24-h period (VWR Scientific Model 1370FM; Mississauga, ON). Beakers were removed from the oven and final weights recorded for moisture loss determination. The dried sample was pulverized to a fine grind (Grindomix Model GM200; Retsch Inc., Newton, PA) and analyzed in duplicate for crude fat (Method 960.39; Association of Official Analytical Chemists, 1995a) and crude protein (Method 992.15; Association of Official Analytical Chemists, 1995b).

A thin slice was removed from the posterior end of the LT and sarcomere lengths and muscle fibre diameters were determined from the fresh sample as described in Aalhus et al. (1999). Images were captured and measurement analysis performed on an Axioscope microscope (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).

Five steaks (25 mm thick) were then removed from all LT muscles. Following a 20-min period of exposure to atmospheric oxygen, three replicate measurements of objective colour measurements (CIE L*, a*, b*; Commission Internationale de l'Eclairage, 1978) were collected across the first steak using a Minolta CM2002 (Minolta Canada Inc., Mississauga, ON) and subsequently converted to hue (H_{ab}=arctan[b*/a*]) and chroma (C_{ab}=[a^{*2}+b^{*2}]$^{0.5}$). The steak was then pre-weighed into a polystyrene tray with a dri-loc pad, over-wrapped with oxygen permeable film, (8000 ml m^{-2} 24 h^{-1} vitafilm choice wrap; Goodyear Canada Inc.), stored for 4 days at 2°C, removed and final weight of steak recorded to determine gravimetric drip loss.

Controlling for location within ageing time, the second, third and fourth steak were assigned to 2, 7 and 14 d of ageing. Steaks were individually vacuum packaged and placed into the 2°C cooler until the assigned ageing time was complete. The fifth steak was labelled, vacuum packaged, and placed into cooler until 6 d of ageing, then frozen at -35°C for future analysis.

On each specific ageing day, steaks were removed from the cooler, taken out of the package and raw steak weight recorded prior to cooking. A spear point temperature probe (10 cm) was inserted into the mid-point of the steak. Steaks were grilled on an electric grill set to 210°C (Garland Grill ED30B; Condon Barr Food Equipment Ltd., Edmonton, AB) to an internal temperature of 35°C, turned and cooked to a final temperature of 70°C (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. Chilled steaks were then transferred to a cooler to allow standing for a 24 h period. Final steak weight was recorded and six cores, 19 mm in diameter, were removed parallel to the fibre grain. Peak shear force was determined on each core perpendicular to the fibre grain (TA-XT Plus Texture Analyzer equipped with a Warner-Bratzler shear head at a crosshead speed of 200 mm/min and a 30 kg load cell using Texture Exponent 32 Software; Texture Technologies Corp., Hamilton, MA) and the average of the six cores was calculated. The standard deviation of the shear values amongst cores was also determined as the inherent variation in tenderness. Raw and final steak weights were used to determine cooking loss (mg/g) and cooking time (sec/g).

Following the 7 d ageing period, the stored LL piece was removed from the cooler and four steaks (25 mm thick) were removed. The first and second steaks were labelled, individually vacuum packaged and placed into a -35°C freezer until evaluation by sensory panellists. The third steak was placed into a polystyrene tray with a dri-loc pad, over-wrapped with oxygen permeable film and put into a horizontal (chest type) retail display case (Hill Refrigeration of Canada Ltd., Barrie, ON) under fluorescent room lighting (GE deluxe cool white), supplemented with incandescent lighting directly above the display case (GE clear cool beam 150 W/120 V spaced 91.5 cm apart) to provide an intensity of 1076 lux at the meat surface for 12 h per day (Jeremiah and Gibson, 2001). Samples were held at 1°C for retail evaluation after 0, 1, 2 and 3 d. The fourth steak was cut in half and one half was immediately prepared for determination of thiobarbituric

acid reactive (TBAR) substances (0 d in retail) as described by Nielsen (1997). The remaining half was placed on pre-labelled polystyrene tray with a dri-loc pad, over-wrapped with oxygen permeable film and put into the retail display case for an additional 3 d, before determining final TBAR values.

2.2.4 Glucidic analysis

Glucidic metabolites were extracted from frozen muscle samples as described by Dalrymple and Hamm (1973) and Yambayamba et al. (1996) with the exception that free glucose, glycogen as glucose units and lactate content of samples were analysed on the YSI 2300 StatPlus glucose/lactate analyser (YSI Incorporated, Dayton, OH). Glucidic metabolites were reported as μmol/g.

2.2.5 Retail evaluation

Treatment samples were placed into the retail display case controlling for known temperature gradients within the retail case. On each specific ageing day three replicate objective colour measurements were recorded as described previously. Spectral reflectance readings were also collected at the same time to calculate the relative contents of metmyoglobin (MetMb), myoglobin (Mb) and oxymyoglobin (MbO_2) as describe by Krzywicki (1979). Following objective colour measurements steaks were subjectively evaluated for retail appearance, lean colour score, percent surface discolouration, colour of discolouration, amount of marbling and marbling colour by 5 trained panellists using an 8-point hedonic (1=extremely undesirable and 8=extremely desirable), 8-point descriptive (1=white and 8=extremely dark red), 7-point descriptive (1=0% and 7=100% discolouration), 7-point descriptive (1=no browning and 7=black), 6-point

descriptive (1=devoid and 6=abundant) and 5-point descriptive (1=white and 5=red) scales, respectively.

2.2.6 Sensory analysis

At time of analysis taste panel steaks were removed from the freezer and placed in a refrigerator to thaw for 24 h. Fifteen min prior to grilling, the steaks were removed from the refrigerator and weighed to determine raw steak weight. Temperature probes were inserted horizontally to the mid-point along the long axis of the steak and grilled as previously described. Each steak was cut into 1.3 cm cubes, avoiding connective tissue and large areas of fat. Eight cubes from each sample were randomly assigned to an eight-member trained sensory panel. 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 70°C prior to evaluation.

Attribute ratings were electronically collected with Compusense 5, release 4.6 computer software (Compusense Inc., Guelph, ON) using an eight point descriptive scale for initial and overall tenderness (8=extremely tender; 1=extremely tough), initial and sustainable juiciness (8=extremely juicy; 1=extremely dry), flavour intensity (8=extremely intense; 1=extremely bland), off flavour intensity (8=no off flavour; 1=extremely intense off flavour) and amount of connective tissue (8=none detected; 1=abundant). Flavour desirability and overall palatability were rated on an eight point hedonic scale, (8=extremely desirable; 1=extremely undesirable). Initial tenderness was rated on the first bite cut through the centre surface with the incisors; initial juiciness was rated after 3-

5 chews with the molars; flavour intensity, flavour desirability, off flavour intensity and amount of connective tissue were rated between 10-20 chews and sustainable juiciness, overall tenderness and over palatability were rated prior to expelling. Each panellist also assigned one of the following flavour (metallic, off sour, livery, grainy, bloody, others, unidentified or none) and texture (typical, mushy, mealy, spongy or rubbery) descriptors to each cube. Flavour and texture descriptors were reported as the percentage of panellists attributing that descriptor to that sample. All panel evaluations were conducted in well-ventilated, partitioned booths, under 124 lx red lighting. Distilled water and unsalted soda crackers were provided to purge the palate of residual flavour notes between samples.

2.2.7 Statistical analysis

PROC MIXED (SAS Institute, 1989) was used to analyze all variables, with ante-mortem management treatment (MLACON, MLAPEN, LAND) as the main effect. Gender, carcass weight grouping (<200 kg = 1; 200-250.5 kg= 2; >250.5 kg= 3), and farm were included as random effcts. Shear force values were also analysed with carcass temperatures at 5 and 10 h *post mortem* as a covariate. Least-square means were calculated for all main effects and means were separated when the *F*-test for the main effect was significant ($P<0.05$). Frequency tables were generated and Fisher's exact test done for muscling, fat colour and quality, maturity class, muscle colour, degree of marbling and shear force cut offs from previously published bison shear value categories (Aalhus et al., 2000). Frequencies were analyzed by treatment and within time period (for shear 2, 7 and 14 d ageing). The MIXED model repeated ANOVA was used to analyze variables

with multiple time measurements made on the same subject over time. These included shear force (d 2, 7, 14), objective retail measurements (L*, chroma, hue, MetMb, Mb, and MbO_2) and subjective measurements (retail appearance, lean colour score, percent colour discolouration, colour of discolouration, marbling score, marbling colour) on d 0, 1, 2 and 3.

2.3 Results and Discussion

The Canadian bison grading system (CFIA, 2007) includes the evaluation of maturity, muscling, fat depth, fat colour and lean colour (Appendix 4). Carcasses are evaluated for maturity to establish the class of carcass. Muscling refers to the proportionate development of parts of the carcass and to the ratio of meat to bone. There are fat cover and colour requirements to make certain grades. The colour of the lean meat also impacts the grade determination with a brighter red colour being more desirable. The Canadian bison grading system was adapted from the Canadian beef grading system in place in the early 1990's (Robertson et al., 2005).

There were no significant differences (P>0.05) among treatments in hot commercial weight or rib-eye area (Table 2.1). All of the carcasses in MLACON and LAND groups were graded as maturity class 1 (youthful; appendix 4), the MLAPEN group had 5 carcasses grade maturity class 2 (mature; appendix 4). Grade fat in the LAND group was approximately 3 mm less than the MLAPEN group. It is possible that moisture/shrink loss in the LAND cooler may have been higher than in the MLA groups partially explaining this difference. Intramuscular fat (marbling) is deposited in lean tissue close to the blood capillaries

(Goonewardene, 2006) and is typically mobilized first as a source of energy by animals during extended exercise. Marbling score has been measured to be lower in bison than grain finished cattle (Koch et al., 1995) suggesting bison may have fewer immediately available energy reserves. Marbling frequency (ranging from small, slight, trace, partially devoid or devoid) did not differ across treatment groups, and there were carcasses in each marbling category from each treatment, with the exception of the small marbling category which only had a carcass from the LAND treatment group (data not shown).

Table 2-1: Carcass characteristics of bison

	MLACON n=15	MLAPEN n=20	LAND n=20	SEM	*P*
Hot carcass wt. (kg)	227.67	225.44	221.74	28.28	0.65
Grade fat (mm)	7.57^{ab}	8.87^{a}	5.26^{b}	3.93	0.03
Rib-eye area (cm^2)	48.59	48.51	51.04	4.63	0.21

[a, b] Means within same row bearing different letters are significant (*P<0.05*)

Muscling frequency (ranging from excellent, very good, good, medium or poor) did not differ among treatment groups (*P=0.21*). Fat colour frequency (Table 2.2) indicated 50 out of 55 carcasses had acceptable white, firm fat grading in the "good" category. One carcass was assessed as having an orange fat colour, and four carcasses were assessed as having yellow fat colour; visual appearance of orange and yellow fat colour is indicative of a high level of beta-carotene (Strachan et al., 1993) which has been deemed to be a disadvantage for Canadian consumer acceptance (French et al., 2000) as they are used to the white fat associated with barley grain finishing. Half of the animals in maturity class 2 and all of the carcasses grading in either the orange or yellow fat colour category came from farm 4.

Table 2-2: Numbers of carcasses with either good, orange or yellow fat colour

	MLACON	MLAPEN	LAND
Good	15	19	16
Orange	0	1	0
Yellow	0	0	4

There were significant (*P<0.01*) differences amongst treatments in the proportion of carcasses receiving downgraded muscle colour scores (Table 2.3). Of note was the higher proportion (40%) of LAND carcasses that were identified as slightly dark to black in colour, and the identification of 13% of MLACON carcasses as exhibiting a pale-wet colour. Prolonged stress *ante mortem* can result in a pre-harvest depletion of glycogen, an abnormally high pH meat and dark meat colour (Adegoke and Falade, 2005). Beef carcasses exhibiting a pale-wet colour are found much less frequently than pork and generally result from protein denaturation caused by a combination of high temperature and low pH (Aalhus et al., 1998).

Table 2-3: Percentage (number) of carcasses in muscle colour groupings

Muscle Colour	MLACON	MLAPEN	LAND
Good-Bright	73.3 (11)	65.0 (13)	60.0 (12)
Sl. Dark-Black	13.3 (2)	35.0 (7)	40.0 (8)
Pale Wet	13.3 (2)	0.0	0.0

Neither the average total glucidic potential (Table 2.4), nor the average pH_{24} reflect the observed subjective differences in the frequency of colour scores at the time of grading. This is in agreement with a study comparing meat quality and stress between paddock-dispatched and commercially slaughtered red deer, where the ultimate pH level in the muscle tissue was not different based on pre-slaughter treatment (Pollard et al., 2002). The pH_{24} values found in the present

study are similar to the mean value of 5.77 reported for conventionally chilled bison carcasses (Janz et al., 2002).

In MLACON both the pH and lactate levels at 1h *post mortem* indicate a more rapid glycolysis early post-mortem while carcass temperatures are high (Table 2.4). This may indicate that the stress experienced in this group was more acute, occurring immediately prior to exsanguination, compared to LAND and MLAPEN groups that appear to experience a more prolonged stress. In extreme cases accumulation of lactate and rapid pH drops while carcass temperatures are high can cause protein denaturation and can result in quality problems (pale, soft and exudative meat).

Although the total glucidic potential was not different amongst treatments, the residual glycogen at 24 h was significantly higher in MLAPEN than LAND ($P<0.01$). MLACON was not different from either MLAPEN or LAND. A similar pattern was found in the lactate levels at 24 h. The MLAPEN group had lower lactate than the LAND group and the MLACON was not different from either group. The rate and extent of glycolysis is known to have a significant effect on meat quality (Honikel, 2004). Immonen et al. (2000) reported that increasing residual glycogen concentration within the *longissimus* muscle (<25 mmol/kg; 25.1-49.9 mmol/kg, >50mmol/kg) decreased redness (19.9 versus 18.5 versus 17.3) and increased yellowness (10.0 versus 8.5 versus 7.8). Table 2.8 shows the LAND group was rated to be significantly darker in retail appearance than MLACON, and numerically higher than MLAPEN.

Table 2-4: Effect of pre-slaughter treatment of bison on meat characteristics

	MLACON	MLAPEN	LAND	SEM	*P*
Muscle pH_1 h	6.45[a]	6.67[b]	6.69[b]	0.06	0.03
Muscle pH_{24} h	5.71	5.76	5.71		
Temperature (^oC)					
Temp 1 h	36.99	38.18	37.98	0.70	0.30
Temp 5 h	12.84[a]	12.91[a]	7.32[b]		<0.01
Temp 10 h	4.94[a]	5.02[a]	3.72[b]		0.02
Temp 24 h	2.84	2.85	2.82		0.30
Glycolytic metabolites (µmol/g)					
Glycogen 1 h	61.63[a]	73.89[b]	73.64[b]	10.39	<0.01
Glycogen 24 h	39.65[ab]	46.30[a]	32.90[b]		
Lactate 1 h	55.87[a]	42.19[b]	30.84[c]	5.77	<0.01
Lactate 24 h	94.75[ab]	92.34[a]	98.97[b]		
Glucidic potential 1 h	92.44	96.54	90.45	9.18	0.41
Glucidic potential 24 h	92.70	97.06	87.69		

[a,b] Means within same row bearing different letters are significant (*P<0.05*)

In the present study the more rapid glycolysis in MLACON resulted in significantly (*P<0.01*) higher drip losses (Table 2.5) and a tendency towards a lighter, less saturated meat colour (higher L*, *P=0.08* and chroma, *P=0.03*). In addition MLACON had lower protein content compared to the other groups, which may have resulted from an increased loss of sarcoplasmic proteins in the drip. Lower amounts of soluble protein have been seen in PSE pork compared to normal pork, where an increased driploss was observed (Aalhus et al., 1998). Maintenance of fibre diameter is related to intracellular hydration. While there was a difference in moisture among treatments, with the MLACON having higher moisture (*P=0.04*), there was no significant difference in muscle fibre diameter

which suggests no significant difference in intracellular hydration. No measure of extracellular water was conducted. The moisture levels are similar to the levels found in bison by Galbraith et al. (2006) where the *Gluteus medius* (sirloin tip) was found to have moisture of 75.1%. MLACON having a higher driploss suggests that the water was held less tightly due to protein denaturation, and higher moisture levels may suggest greater hydration at the time of slaughter. There was no difference in the crude fat content between treatment groups (Table 2.5).

Table 2-5: Bison carcass and muscle characteristics (colour, drip, proximate and fibre)

	MLACON	MLAPEN	LAND	SEM	*P*
Objective colour (24 h)					
L* (lightness)	34.75	33.36	32.88	1.13	0.08
Chroma (saturation)	20.65[a]	18.57[b]	19.02[b]	1.58	0.03
Hue angle (^{o})	20.81	20.30	20.44	0.80	0.56
Drip loss ($mg.g^{-1}$)	25.6[a]	17.5[b]	13.5[b]	0.34	<0.01
Proximate analysis					
Moisture ($mg.g^{-1}$)	760.4[a]	753.7[b]	755.1[b]	0.71	0.04
Fat ($mg.g^{-1}$)	16.3	17.9	16.9	0.57	0.66
Protein ($mg.g^{-1}$)	213.4[b]	217.6[a]	219.1[a]	0.20	<0.01
Fibre measurements					
Sarcomere length (µm)	1.76[a]	1.61[b]	1.55[b]	0.09	0.01
Fibre diameter (µm)	76.37	78.07	75.22	4.89	0.82

[a, b] Means within same row bearing different letters are significant ($P<0.05$)

Despite the apparent deleterious effects on quality associated with a more rapid glycolysis in MLACON, this group had the lowest shear force overall (Table 2.6), equivalent to MLAPEN and significantly lower than LAND

(*P<0.01*). Not only did LAND have the highest shear force overall (all days combined), this effect was still evident after freezing/thawing and serving to panellists. Initial tenderness was significantly lower (*P=0.01*; Table 2.7) and both overall tenderness (*P=0.06*) and overall palatability (*P=0.07*) tended to be lower in LAND than either MLA group. Initial juiciness, flavour desirability, bison flavour intensity, connective tissue, overall tenderness, sustainable juiciness, and overall palatability showed no difference between treatment groups (*P>0.05*).

Table 2-6: Effect of pre-slaughter treatment of bison on tenderness and cooking properties

	MLACON	MLAPEN	LAND	SEM	*P*
Shear value (kg) d combined	7.28[a]	7.40[a]	9.43[b]	0.88	<0.01
2 d	8.58[a]	10.10[b]	12.0[c]	0.92	<0.01
7 d	7.21[a]	6.71[a]	9.04[b]	0.92	<0.01
14 d	6.06	5.41	7.19	0.92	<0.01
Cook time (sec.g-1)	6.64	6.30	6.62	0.28	0.49
Cook loss (overall) (%)	21.3[a]	19.5[b]	20.2[ab]	0.65	0.04

[a, b] Means within same row bearing different letters are significant (*P<0.05*)

Table 2-7: Sensory evaluation of bison *longissimus lumborum*

	MLACON	MLAPEN	LAND	SEM	*P*
Sensory evaluation[1]					
Initial tenderness	4.95[a]	4.55[a]	3.93[b]	0.39	0.01
Initial juiciness	5.44	5.46	5.37	0.14	0.78
Flavour desirability	4.80	4.81	4.80	0.33	0.99
Bison flavour intensity	4.90	5.07	4.94	0.15	0.26
Off flavour intensity	6.15	6.20	6.14	0.34	0.93
Amt of connective tissue	6.40	6.26	6.11	0.22	0.34
Overall tenderness	4.85	4.64	4.12	0.37	0.06
Sustainable juiciness	5.19	5.28	5.22	0.10	0.68
Overall palatability	4.36	4.25	3.93	0.33	0.07

[1]Sensory scores: 8 = extremely tender, extremely juicy, extremely intense, no off flavour, no connective tissue detected, extremely desirable; 1 = extremely tough, extremely dry, extremely bland, extremely intense off flavour, abundant connective tissue, extremely undesirable.

[a,b] Means within same row bearing different letters are significant ($P<0.05$)

The cooling regime 24h *post mortem* can greatly impact meat tenderness (Savell et al., 2005). Muscle temperature at 5 and 10h (Table 2.4) were higher ($P<0.05$) in the MLA groups compared to the LAND group. While the carcass coolers were set to the same temperature, the heat loads on the MLA cooler were greater than the much larger LAND cooler, resulting in less efficient chilling. However, including carcass temperatures at 5 and 10h *post mortem* in the model as covariates did not account for the shear force differences between the MLA groups and the LAND group. This suggests the cooling rates of the carcasses in the two coolers, while different, were not solely responsible for the observed differences in LL tenderness. Associated with a more rapid temperature decline, LAND had the slowest accumulation of lactate at 1h and significantly shorter sarcomere lengths than MLACON (Tables 2.4 and 2.5). Janz et al. (2002) found

that comparatively longer sarcomere lengths in bison equated to lower shear force confirming previous work published in this area (Bouton et al., 1973; Smulders et al., 1990). However, Smulders et al. (1990) indicated that the relationship between sarcomere length and shear force holds true only for slow glycolysing carcasses, and that in rapidly glycolysing carcasses the relationship between sarcomere length and shear force ceases to exist. This may explain why MLAPEN, despite having sarcomere lengths similar to LAND, had overall shear force values similar to MLACON. When shear values are compared at days 2, 7, 14 for each treatment group there are significant differences at days 2 and 7 with the lower shear force in the MLA groups at both ageing times. After 14 d ageing the differences between shear values cease to exist.

Through extensive work on beef using standardized protocols for calibration of equipment, muscle used, core size and cooking methodology, a set of categories have been developed for AAFC-Lacombe to predict how a consumer would rate the meat based on shear force (Aalhus et al., 2000; Aalhus et al., 2004). The categories are as follows: Tender; <5.6 kg; Probably tender; 5.6-<7.85 kg; Probably tough; 7.85-<9.6 kg; Tough; >9.6 kg (Aalhus et al., 2000). Using this scale, 80% of the LAND group at 2 d would be rated as tough whereas the MLACON and MLAPEN would be 20 and 35%, respectively (Figure 2.1). At 14 d ageing, the LAND, MLACON, and MLAPEN group had 20, 6.7 and 5% in the tough category, respectively. At 14 d ageing, 12/15, 18/20 and 13/20 of the samples from MLACON, MLAPEN and LAND, respectively, were in either the tender or probably tender category.

Figure 2.1: Number of shear values in each tenderness category using thresholds from Aalhus et al. (2000) at various ageing days

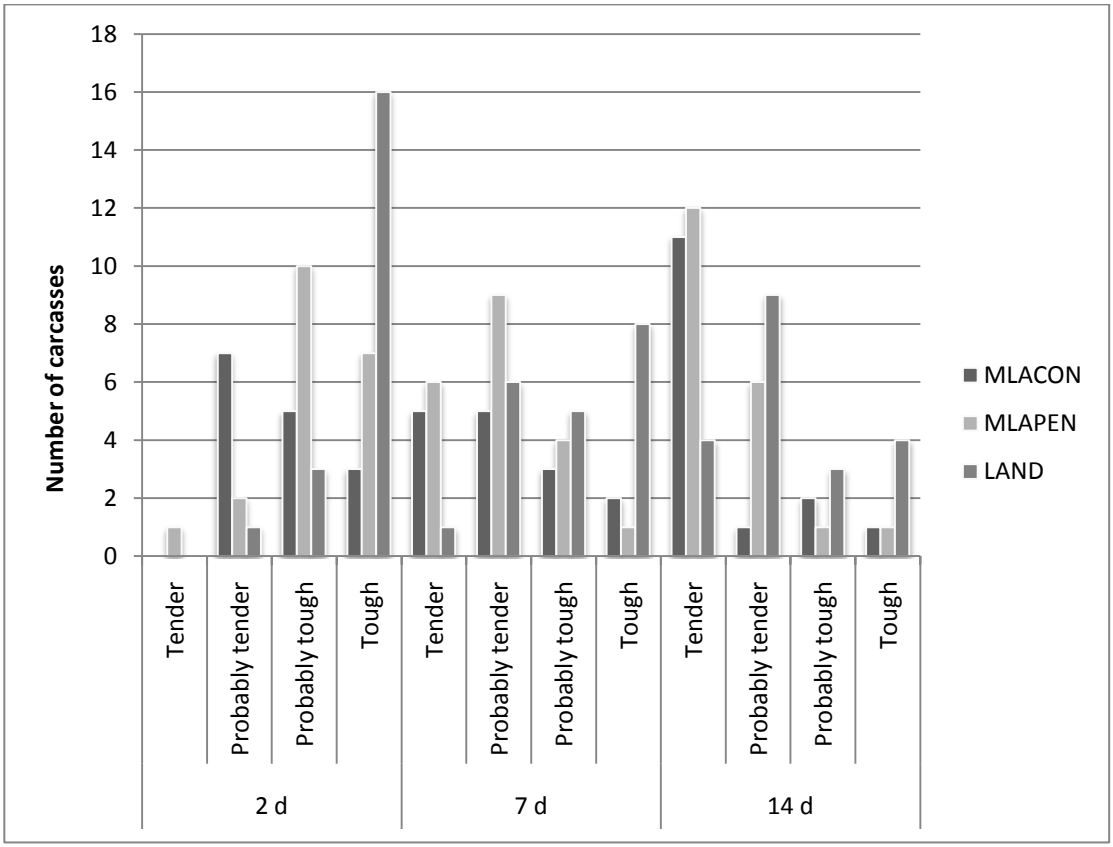


Table 2-8: Bison steak retail measurements days 0, 1, 2, 3 combined (objective and subjective)

	MLACON	MLAPEN	LAND	SEM	*P*
Objective					
L*	36.79	36.39	35.51	0.83	0.09
Chroma	17.83	16.98	16.82	0.63	0.14
Hue	38.0	37.66	36.82	1.41	0.34
Metmyoglobin	0.31	0.30	0.31	0.02	0.76
Myoglobin	0.09	0.10	0.09	0.01	0.39
Oxymyoglobin	0.60	0.60	0.60	0.02	0.97
Subjective					
Retail appearance	4.54[a]	4.79[a]	5.29[b]	0.31	<0.01
Lean colour score	6.05[a]	6.41[ab]	6.58[b]	0.23	0.03
Surface Discolouration	3.72	3.34	3.24	0.40	0.21
Colour of discolouration	2.43	2.37	2.18	0.16	0.18
Marbling Score	2.48	2.57	2.56	0.23	0.73
Marbling colour	1.04[a]	1.03[a]	1.09[b]	0.07	0.01

Steaks were subjectively evaluated for retail appearance, lean colour score, surface discolouration, colour of discolouration, amount of marbling and marbling colour by 5 trained raters using an 8-point hedonic (1=extremely undesirable and 8=extremely desirable), 8-point descriptive (1=white and 8=extremely dark red), 7-point descriptive (1=0% and 7=100% discolouration), 7-point descriptive (1=no browning and 7=black), 6-point descriptive (1=devoid and 6=abundant) and 5-point descriptive (1=white and 5=red) scales respectively.
[a b], Means within same row bearing different letters are significant (*P<0.05*)

Objective retail evaluations showed no differences (P>0.05) between treatment groups (Table 2.8). LAND was assessed as more desirable (retail appearance, d combined) than MLACON or MLAPEN and was redder in colour than MLACON On any given day, however, there were no differences in retail performance between the groups.

Surface discolouration did not differ between treatment groups. Rapid discolouration of bison meat in comparison to beef has been reported by other researchers (Pietrasik et al., 2006; Janz et al., 2001). The amount of discolouration was numerically the highest (d combined) in the MLACON group. This could have implications with respect to the recommended pre-slaughter treatment of bison when slaughtered on-farm through a mobile slaughter facility, suggesting MLAPEN to be the preferred *ante mortem* treatment. The trend for higher glycogen, and lower lactate in the MLAPEN group (Table 2.4) compared to the MLACON group also support the preferred on-farm treatment to be MLAPEN. Further work investigating possible differences in the properties of bison meat may clarify why discolouration occurs more rapidly, and which treatment is most favourable for on-farm use of the MLA.

2.4 Conclusion

This research provided novel information about meat quality of bison slaughtered on-farm in a mobile abattoir compared to following transport in a land-based abattoir. A higher incidence of carcasses graded as “dark” was observed in transported animals, which may have been due to faster chilling rates in the larger land-based coolers. Animals confined prior to dispatch (MLACON), compared to the MLAPEN group showed evidence of higher stress in the immediate pre-slaughter period with 13% of these carcasses having pale, wet meat colour at grading and higher drip losses. Prior to ageing improved meat tenderness was observed in bison slaughtered through the mobile abattoir compared to those requiring transport and slaughter at a land-based plant through

both shear force measurements and sensory evaluation scores. Even following 7 d of ageing meat from LAND slaughtered animals was still significantly tougher than meat from MLA slaughtered animals. Overall the MLAPEN group had superior carcass and meat quality compared to either the MLACON or LAND groups.

2.5 Literature Cited

Aalhus, J. L., Best, D. R., Murray, A. C. and Jones, S. D. M. 1998. A comparison of the quality characteristics of pale, soft and exudative beef and pork. J. Muscle Foods **9:** 267-280.

Aalhus, J. L., Best, D. R., Costello, F. and Jeremiah, L. E. 1999. A simple, on-line processing method for improving beef tenderness. Can. J. Anim. Sci. **79**:27-34.

Aalhus, J. L., Jeremiah, L. E., Dugan, M. E. R., Larsen, I, L, Best, D. R., Thacker, R, Costello, F. and Gibson, L. 2000. Beef tenderness strategy. A final report to the Canadian Cattlemans Association, Calgary, AB.

Aalhus, J. L., Jeremiah, L. E., Dugan, M. E. R., Larsen, I. L., & Gibson, L. L. 2004. Establishment of consumer thresholds for beef quality attributes. Can. J. Anim. Sci. **84**: 631-638.

Adegoke, G. O. and Falade, K. O. 2005. Quality of meat. J. Food, Agric. Environ. **3(1)**: 87-90.

Association of Official Analytical Chemists 1995a. Method 960.39. Fat (Crude) or Ether Extract in Meat Final Action. OAOC. 39:2.

Association of Official Analytical Chemists 1995b. Method 992.15 Crude Protein in Meat and Meat Products Including Pet Foods. Combustion Method. Final Action 1992. OAOC. 39:6-7.

Bouton, P. E., Harris, P. V., Shorthose, W. R. and Baxter, R. I. 1973. A comparison of the effects of aging, conditioning and skeletal restraint on the tenderness of mutton. J. Food Sci. **38**: 932-937.

Canadian Council on Animal Care. 1993. Guide to the care and use of experimental animals. Vol. 1, 2nd ed. E.D. Olfert, B.M. Cross, A.A. McWilliam, eds CCAC, Ottawa, ON.

Canadian Food Inspection Agency 2007. Livestock and poultry carcass grading regulations, part VI section 48-54.1 Schedule I – IV. Accessed July 24, 2010. http://laws.justice.gc.ca/en/showdoc/cr/SOR-92-541/boga:l_VI//en#anchorbo-ga:l_VI..

Carlsson, F., Frykblom, P. and Lagerkvist, C. J. 2007. Consumer willingness to pay for farm animal welfare: mobile abattoirs versus transportation to slaughter. European Rev. Ag. Econ. **34(3):** 321-344.

Commission Internationale de l'Eclairage 1978. Recommendations on uniform color spaces-color difference equations – psychometric color terms, CIE Publication No. 15 (E-1.3.1) 1971/(TC-1.3), Supp. No.2, pp. 8-12. Paris.

Dalrymple, R. H. and Hamm, R. 1973. A method for the extraction of glycogen and metabolites from a single muscle sample. J. Food Tech. **8**: 439-44.

French, P., O'Riordan, E. G., Monahan, F. J., Caffrey, P. J., Mooney, M. T., Troy, D. J. and Moloney, A. P. 2000. Meat quality of steers finished on autumn grass, grass silage or concentrate-based diets. Meat Sci. **56:**173-180.

Galbraith, J. K., Hauer, G., Helbig, L., Wang, Z., Marchello, M. J. and Goonewardene, L. A. 2006. Nutrient profiles in retail cuts of bison meat. Meat Sci. **74:** 648-654.

Goonewardene, L. A. 2006. Opportunities for the production, harvesting and marketing of leaner, lighter and younger beef in Alberta. Alberta Agriculture, Food and Rural Development.

Honikel, K. O., 2004. Conversion of muscle to meat – glycolysis. Encyclopedia of Meat Sci. Elsevier: 314-318.

Immonen, K., Ruusunen, M. and Puolanne, E. 2000. Some effects of residual glycogen concentration on the physical and sensory quality of normal pH beef. Meat Sci **55:** 33-38.

Janz, J. A. M., Aalhus, J. L. and Price, M. A. 2001. Blast chilling and low voltage electrical stimulation influences on bison (Bison *bison bison*) meat quality. Meat Sci. **57:** 403-411.

Janz, J. A. M., Aalhus, J. L. and Price, M. A. 2002. Meat quality of bison (*Bison bison bison*) longissimus thoracis et lumborum following very fast chilling. Can. J. Anim. Sci. **82**: 327-337.

Jeremiah, L. E. and Gibson, L. L. 2001. The influence of packaging and storage time on retail properties and case-life of retail-ready beef. Food Res. Int. **34(7):** 621-631.

Koch, R. M., Jung, H. G., Crouse, J. D., Varel, V. H. and Cundiff, L. V. 1995. Growth, digestive capability, carcass and meat characteristics of *Bison bison, Bos taurus* and *Bos x Bison.* J. Anim. Sci. **73:** 1271-1281.

Krzywicki, K. 1979. Assessment of relative content of myoglobin, oxymyoglobin and metmyoglobin at the surface of meat. Meat Sci. **3:** 1-10.

Nielsen, J. H., Sorensen, B., Skibsted, L. H. and Bertelsen, G. 1997. Oxidation in pre-cooked minced pork as influenced by chill storage of raw muscle. Meat Sci. **46(2)**:191-197.

Paganini, R. 2004. An alternative natural beef production system: A differentiation strategy for California producers and packers. Agric. Res. Econ. Update **8(1)**: 9-11.

Pietrasik, Z., Dhanda, J. S., Shand, P. J. and Pegg, R. B. 2006. Influence of injection, packaging, and storage conditions on the quality of beef and bison steaks. J. Food Sci. **71:**s110-s118.

Pollard, J. C., Littlejohn, R., Asher, G. W., Pearse, A. J. T., Stevenson-Barry, J. M., McGregor, Manley, T. R., Duncan, S. J., Sutton, C. M., Pollock, K. L. and Prescott, J. 2002. A comparison of biochemical and meat quality variables in red deer (*Cervus elephus*) following either slaughter at pasture or killing at a deer slaughter plant. Meat Sci. **60**: 85-94.

Robertson, W. M., Galbraith, J. K., Helbig, L., Gibson, L. L., Aalhus, J. L., Burbidge-Boyd, C. and Landry, S. J. 2005. Bison grading system evaluation: A final report to the diversified livestock fund of Alberta and industry stakeholders. 32pp.

SAS Institute Inc. 1989. SAS/Stat User's guide 4th ed.Version 6 (Vol.2). Cary, NC: SAS Institute Inc.

Savell, J. W., Mueller, S. L. and Baird, B. E. 2005. The chilling of carcasses. Meat Sci. **70:** 449-459.

Schaefer, A. L., Stanley, R. W., Tong, A. K. W., Dubeski, P., Robinson, B., Aalhus, J. L. and Robertson, W. M. 2006. The impact of antemortem nutrition in beef cattle on carcass yield and quality grade. Can. J. Anim. Sci. **86:** 317-323.

Smulders, F. J. M., Marsh, B. B., Swartz, R. L. and Hoenecke, M. E. 1990. Beef tenderness and sarcomere length. Meat Sci. **28**: 349-363.

Steenbergen, J. 2011. Business Development – Elk/Bison, Alberta Agriculture and Rural Development. Personal communication.

Strachan, D. B., Yang, A. and Dillon, R. D. 1993. Effect of grain feeding on fat colour and other carcass characteristics in previously grass-fed *bos indicus* steers. Aust. J. Exp. Ag. **33:** 269-273.

Today's Retail Meat Case 2007. National Meat Case Study. Accessed Aug. 24, 2010 http://www.beefretail.org/uDocs/nmcs_execsum11408.pdf

Yambayamba, E. S. K., Aalhus, J. L., Price, M. A. and Jones, S. D. M. 1996. Glycogen metabolites and meat quality in feed-restricted re-fed beef heifers. Can. J.Anim. Sci. **9:** 157-179.

CHAPTER 3

Physiological Stress in Bison Slaughtered in a Mobile or Stationary Abattoir

3.1 Introduction

Bison (*Bison bison*) are raised for their meat and other products in North America. Stress is an inevitable consequence of the process of transferring animals from farm to slaughter. Ferguson and Warner (2008) reviewed the status of current knowledge of the impact of pre-slaughter stress in ruminants and the consequential effect on meat quality and yield and concluded that pre-slaughter stress has been underestimated and that it is imperative that the issues receive more research attention. Part of this includes a need to optimize animal welfare, and minimize losses in product yield and quality. As a result of limited slaughter facilities for bison, it is common for bison to be transported for several hours to reach an abattoir.

Assembly, loading, transport, unloading, regrouping, feed and water withdrawal, novel surroundings and temperature fluctuations are all factors that can create both physiological challenges and psychological disruptions which ultimately impact carcass yield and meat quality (Schaefer et al., 2006). Transport is often followed by lairage overnight in pens for slaughter the next morning. Fasting duration appears to have a more substantial effect on glycogen metabolism and thus on meat quality than lairage duration (Warriss et al., 1990).

The measurement of corticosteroid (specifically, cortisol) levels in blood

samples obtained during exsanguination have been considered a reliable indicator of pre-slaughter stress in livestock (Pollard et al., 2002) and has been used to assist in the evaluation of transport and abattoir treatments (Shaw and Tume, 1992). Measurements of fecal corticosteroid metabolites have been used as an indicator of stress in dairy cattle (Morrow et al., 2002), where elevated concentrations of fecal glucocorticoid metabolites were evident the day after exposing cows to a new housing environment. Wasser et al. (2000) tested four specific antibodies for detecting fecal metabolites in baboons. They concluded that with well-designed species-specific validations of the antibodies used, fecal glucocortocoids can be used for predictive and explanatory value following a physiological or psychosocial stress .

White blood cells (WBC) are the mobile units of the body's protective system. Changes to the relative percentages of different types of WBC are an endocrine-driven response that occurs over many hours (Cook and Schaefer, 2002). Neutrophils and lymphocytes (and their ratio) are two types of white blood cells that have been used to measure a response to a stressor. A higher N/L ratio is indicative of a stress response; neutrophil counts are elevated when high levels of stress are encountered (Eckert, 1988).

On-farm slaughter through a multi-location abattoir (MLA) system could reduce some of the stressors associated with conventional transport, lairage and slaughter systems. If managed properly on-farm, there would be minimal handling prior to slaughter. Measurements of physiological indices in bison that are processed in the MLA system could provide near 'baseline' values against which

more conventional methods of slaughter can be compared. This will lead to a better understanding of the baseline conditions and how lower stress pre-slaughter handling of bison can result in economic benefits of such improvements.

Pre-slaughter bruising of an animal results in an accumulation of exudates at the injured site during the *ante mortem* period which can cause an elevated pH or a prolonged elevated pH post-mortem (Gregory, 1996). It is also important to industry since it can result in losses due to excess trimming. In a study examining bruising in cattle, the incidence of bruising when hauled from live auctions was higher than if hauled from dealers or farms (7.8% markets, 6.3% dealers, 4.8% direct from farm; McNally and Warriss, 1996). Increased incidence of bruising with increased distance travelled has been found in cattle, (<50 miles 6.9%, >200 miles 9.3%, McNally and Warriss 1997) and Red Deer (<100 km 6%, >200km 11.3%, Jago et al., 1996). Cockram and Lee (1991) report a greater percentage of severely bruised carcasses found in lamb from markets than in those direct from farms (20% vs. 12%, respectively; $P<0.05$). The number of carcasses with a higher ultimate pH increased with the amount of carcass bruising (McNally and Warriss, 1996).

Horn status and class of animal (Wythes et al., 1985) can affect the extent of carcass bruising. Meischke et al. (1974) found the mean bruised tissue trimmed from carcasses weighed 1.59kg for horned as compared to 0.77kg for hornless cattle. Careful pre-slaughter handling can minimize these losses. Most commercial bison herds are raised with their horns (Kremeniuk, 2010). When a producer does dehorn, typically the entire herd is dehorned, so that there is not a

mix of dehorned and horned animals together. The loss due to bruising in bison transported has been reported as high as 25-35% of a carcass in some situations (Jorgenson, 2008). Carcass bruising represents both a substantial financial loss for the packer due to trimming and dark cutters, and is an animal welfare issue.

3.1.1 Objectives

The objectives of this study were to: 1. Compare indices of physiological stress and bruising of bison slaughtered traditionally in permanent facilities following a typical period of transport (LAND) and pre-slaughter handling to animals slaughtered in a multi-location abattoir (MLA) at their home locations. Indices of physiological responses to stress were white blood cell counts, plasma cortisol and fecal corticoid metabolite concentrations. 2. Compare physiological indices of two on-farm pre-slaughter management protocols; dispatched in a pen (MLAPEN) versus held in a confined area (MLACON).

3.2 Materials and Methods

3.2.1 Animals and Slaughter

Fifty-five bison (*Bison bison*) from four farms were recruited for participation in the trial; one farm per week on four different weeks. On three farms a total of 15 bison were used in the trial. On one farm 10 bison were used (Table 3.1). The bison ranged in age from 17-40 months of age. On each slaughter week five animals from a farm were shipped to the land based facility at the Lacombe Research Centre (LRC) (transport time 1.5-3 h) and held in lairage overnight with *ad libitum* access to water until time of slaughter (LAND).

The following day 10 animals from the same farm were slaughtered on-

farm using the MLA system. Animals slaughtered in the MLA were alternated between two treatments. Five animals were confined to a squeeze chute or single animal cell and dispatched in the confined area (MLACON) while another five were placed in a pen (approximately 100 x 200 feet) and dispatched in the pen (MLAPEN). On the last week (Farm 4) only five animals were slaughtered on site, all of which were dispatched in a pen. Either a .357 magnum or .303 calibre rifle were used to stun the bison on-farm and a .223 calibre rifle was used at AAFC-Lacombe. The penned groups were always housed with more than five animals so that there was never a scenario of having a single bison in a pen. All animals were stunned and exsanguinated in accordance with the guidelines established by the Canadian Council on Animal Care (CCAC, 1993).

Table 3-1. Number and genders of animals from each farm location

Treatment	Gender	Farm 1	Farm 2	Farm 3	Farm 4	All
MLACON	F	3	5			8
	M	2		5		7
MLAPEN	F	3	5			8
	M	2		5	5	12
LAND	F	1	5			6
	M	4		5	5	14
Total		15	15	15	10	55

Blood samples were collected post-stunning from all animals into heparinized vacutainer tubes, stored on ice and analyzed for haematology parameters within 24 h of collection. Haematology parameters were measured on a Cell-Dyn 3700 Hematology Analyser (Sequoia – Turner Corp. MountainView, CA).

Plasma samples and fecal samples collected post-stunning were assessed for cortisol and corticosterone levels respectively, using the appropriate Assay

Designs (Ann Arbor, MI, USA) kit. The cortisol kit uses a monoclonal antibody to cortisol to bind, in a competitive manner, cortisol in a sample or an alkaline phosphate molecule which has cortisol attached to it. The corticosterone kit uses a polyclonal antibody to corticosterone to bind, in a competitive manner, corticosterone in the sample or an alkaline phosphate molecule which has corticosterone attached to it.

A 5-point subjective scale was used to evaluate carcasses for extent of bruising ranging from a score of '0', indicating no visible bruising, to a score of '4' which indicated severe and extensive visible bruising throughout carcass. This was a somewhat simplified version of the Australian Bruise scoring system (Anderson 1978) adapted for use in the mobile slaughter facility with less space for additional measurement instruments and people. Carcasses were weighed before and after cutting off bruised tissue to calculate weight lost to bruising.

3.2.2 Statistical Analysis

PROC MIXED was used to analyze all variables SAS Institute (1989), with ante-mortem management treatment (MLACON, MLAPEN and LAND) as the main effect. Some animals from one producer and all animals from another were females (Table 1). Gender, carcass weight grouping, and farm were used as random variables to adjust variances for their effects that could not be included as fixed effects due to small numbers. Fecal corticoid metabolite (FCM) and plasma cortisol values were analyzed with a similar model, with the addition that hot carcass weight was included as a co-variate. The bruising score data were analyzed by generating frequencies tables and using Fisher's exact test (Steel et

al., 2002).

3.3 Results and Discussion

3.3.1 Physiological indices of stress responses

Plasma cortisol levels were significantly (P<0.01) lower in the MLAPEN animals (71.2 nmol/L) compared to either the MLACON (124.2 nmol/L) or LAND (139.5 nmol/L) animals. There was no statistical difference in plasma cortisol levels between the MLACON and LAND groups (Table 3.2). The higher plasma cortisol level in the MLACON and LAND groups reflected the aversion bison have to separation from herd mates, handling, confinement squeezes, transport, overnight lairage in a novel environment and disruption to the social matrix that were associated with these treatments. This pattern of results are similar to those of Pollard et al. (2002) who investigated the effects of pre-slaughter handling on the blood chemistry of red deer (*Cervus elaphus*) which were either paddock-shot or commercially harvested after transport to a slaughter house. Plasma cortisol concentrations in paddock-shot deer (15.5 nmol/L) were consistent with an unstressed state compared to concentrations in the commercially harvested deer (79.9 nmol/L), which were indicative of stress. In another study, field shot red deer had plasma cortisol concentrations which were <19.3 nmol/L, while deer from the same farm that were transported to a slaughterhouse had higher cortisol concentrations of >55.2 nmol/L (Smith and Dobson, 1990). Buckham Sporer et al. (2008) investigated the effect of transportation of young beef bulls on stress biomarkers. They reported a 321% increase in plasma cortisol following onset of transport (117.4 nmol/L) compared to levels measured in blood samples collected 24 h prior to transport (36.5

nmol/L).

The animals in both the MLACON and LAND treatment groups experienced more handling than the MLAPEN group as they were mustered into the confined area or on/off the trailer, into the lairage area, and ultimately to the knock box (LAND). Some of the stress experienced by the MLAPEN group was from unfamiliar people being present and general commotion associated with removing dispatched animals from the pen.

While elevated plasma cortisol levels are snapshots of the immediate physiological condition resulting from stress, the measurement of FCM is a reflection of adrenocortical activity over the period of time that the digesta passes between the bile duct and rectum (Morrow et al., 2002). FCM levels measured in fecal samples collected post-slaughter did not differ among the three treatment groups. There was a positive relationship between sample moisture and fecal cortisol levels. There was a significant negative correlation ($r = -0.56$) between hot hanging weight (HHW) and FCM levels (ng/g). Given that the FCM concentrations were significantly related to both the moisture content of the sample and to the size of the animal (HHW, and by extrapolation live weight) this suggests that the FCM are not entirely dependent on adrenocortical function over the time the feed passes through the gastrointestinal tract. Chelini et al. (2006) stated that a drawback to measuring concentration of FCM is that it may not reflect the total amount of circulating hormones. The amount of material passing through the gut causes a dilution effect possibly skewing the results unless one assumes the volume is the same for all treatment groups. A larger animal with a

larger volume of digesta in the digestive system would dilute the corticosteroid metabolites in a given sample. Thus, although fecal cortisol concentrations were determined the more reliable measure is plasma cortisol concentration. It is possible that fecal cortisol levels are reflecting body size or feed intake.

The mean N/L ratio of the LAND and MLAPEN animals was significantly higher than that of the MLACON group (1.367, 1.145, and 0.791 respectively; $P<0.05$, Table 3.2). Changes to the N/L ratio result from both an increase in neutrophils and a decrease in lymphocytes. MLACON had a neutrophil % not statistically different from MLAPEN and lower than the LAND group.

Hematologic values for American bison have been reported from several studies, primarily for wild animals on range reserves in the USA where blood was collected from live animals restrained in a squeeze (Zaugg et al., 1993; Vestweber et al., 1991). Additionally, some values have been reported for ranched bison restrained in a squeeze, but values for bison transported and held overnight in a novel environment prior to slaughter have not been previously reported. The N/L ratio of 0.79 obtained for the MLACON group is comparable to the ratio of 0.82 obtained for American bison bulls from Yellowstone National Park where blood samples were collected after shooting unrestrained animals (Zaugg et al., 1993) and the ratio of 0.85 obtained for range bison restrained for blood collection (Vestweber et al., 1991). Sikarskie et al. (1990) reported an N/L ratio of 1.00 for ranched bison and 0.82 for free ranging bison, while Marler (1975) obtained a ratio of 1.10 from two populations of free ranging bison.

Stoltenow and Dyer (2001) reported a mean N/L ratio of 1.12 for ranched

bison of three different age groups obtained from thirteen herds in eleven states. Thus the ratio obtained for the MLAPEN group in the current study was similar to the higher values generally reported in the literature, while the LAND animals had an N/L ratio considerably higher as a result of the response to stressors. Mehrer (1976) evaluated hematological values of 163 bison from wildlife refuges and bison ranges from five areas of the USA. His data showed very low percentages of lymphocytes relative to neutrophils resulting in a mean N/L ratio for all age groups of 2.56 which is much higher than what the current study found even for the LAND group. The effects of handling the animals prior to collecting the blood samples was not considered in Mehrer's study and he concluded that resting values could be much different.

Ranched bison in the current study subjected to the LAND treatment appeared to be more able to cope with the stress associated with handling, transport and novel lairage having been exposed to more intensive management. In comparison, the free ranging animals on wildlife refuges would be virtually unexposed to the gathering and handling required to obtain blood samples. There has been limited genetic selection for specific traits such as temperament in farmed bison which may result in a large variation within a population with respect to their ability to cope with a given level of stress. Temperament has been correlated with other physiological measures of stress (such as cortisol) in cattle (Fell et al., 1999; King et al., 2006).

Sporer et al. (2008) attributed their lack of correlations between plasma steroid profiles and neutrophil gene expression to timing of blood collections

relative to the stressor. This difference in timing of the stressors may help explain the differing plasma cortisol and N/L ratio responses between the MLACON and MLAPEN groups. More time elapsed from initial herd disruption in the LAND group than the other two treatment groups since they were held overnight, whereas the MLAPEN group would have had more frequent stress while each dispatched animal was removed from the pen compared to the MLACON group that was only sent into the confinement pen just prior to dispatching. MLACON had higher plasma cortisol than MLAPEN and a lower N/L ratio (Table 3.2). With plasma cortisol response in the order of minutes (Sapolsky et al., 2000) and the expected response time for a change in the N/L ratio to be in the order of hours to days, the MLAPEN (and LAND) groups experienced stressors that affected their N/L ratio that were not detected in the MLACON group. The MLAPEN group had little disturbance immediately prior to being dispatched (compared to the MLACON and LAND group which were worked into either a confinement squeeze or knock box) resulting in the observed lower serum cortisol levels (Table 3.2).

Table 3-2. Effect of pre-slaughter treatment on physiological measures

	MLACON	MLAPEN	LAND	SEM	*P*
White blood cells ($x10^9$/L)	8.68[a]	6.64[b]	7.53[ab]	0.69	<0.01
Neutrophils, %	27.43[b]	37.34[ab]	41.21[a]	5.91	0.04
Lymphocytes, %	43.51[a]	34.51[b]	33.18[b]	2.28	<0.01
Red blood cells ($x10^{12}$/L)	9.06	8.90	8.54	0.29	0.44
Hemoglobin, g/dL	16.1	16.2	15.6	0.7	0.56
Hematocrit, %	44.0	43.9	43.0	1.7	0.82
Neutrophil/Lymphocyte Ratio	0.701[b]	1.145[a]	1.367[a]	0.185	<0.01
Fecal cortisol, ng/g	3897	3855	4601	1559	0.35
Plasma cortisol, nmol/L	124.17[a]	71.16[b]	139.50[a]	22.69	<0.01

[a,b] Means within same row bearing different letters are significant ($P<0.05$).

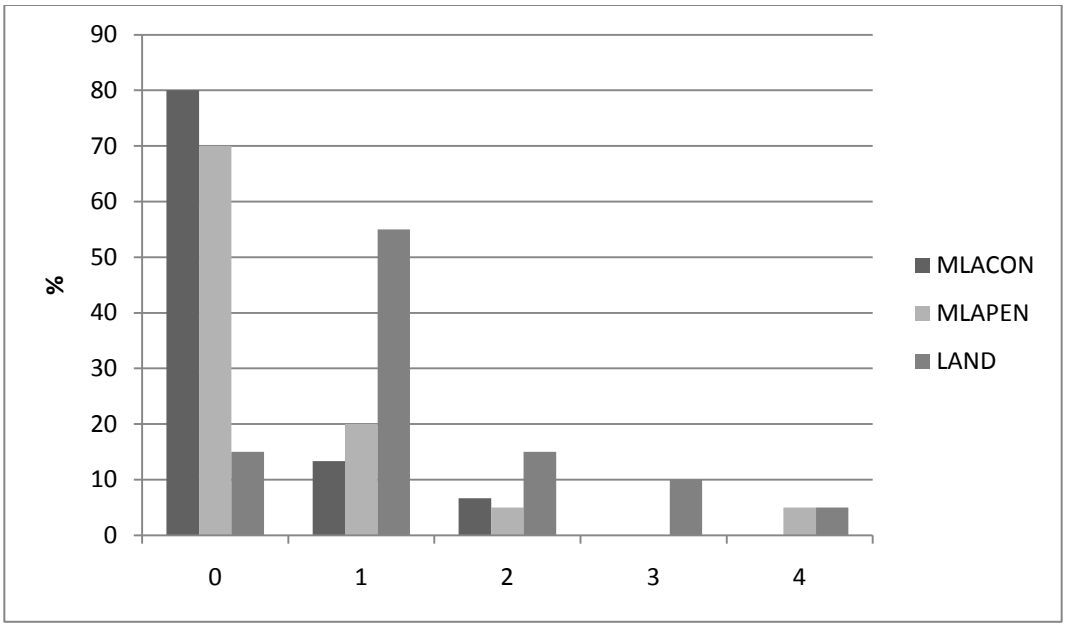
3.3.2 Bruising

The extent of tissues removed as a result of bruising ranged in weight from 0 to 8.5kg. There was no significant difference in the weight of bruising among treatment groups. There were bison with extensive bruising scored as categories 3 and 4 from the LAND and MLAPEN group and not the MLACON group (Figure 3.1). Separating the bison into individual confined areas prior to slaughter, reduced fighting and aggression resulted in the MLACON group exhibiting less serious bruising scores (categories 3 and 4).

Using current hot hanging weight prices of $7.70/kg (Canadian Bison Association, 2011) for the maximum weight of trim lost to bruising in the current study, 8.5 kg, this represents a $65.00 loss. This large economic cost illustrates the importance of optimal *ante mortem* management of bison. Grandin (1981) has shown that cattle sold on a live weight basis had twice as many bruises compared to cattle sold on a carcass basis. This suggests that by shifting the risk to the

producer, there is increased incentive to carefully manage livestock during the pre-slaughter period. Cockram and Lee (1991) report 88% of all bruises in lambs were estimated to have been caused within 24 h of death, i.e. during loading, transit and particularly at markets. The current research shows the MLACON and MLAPEN have a higher percentage (90% *vs* 70%) of animals showing either no bruising or minimal (a '1' score) than the LAND group suggesting that MLA, or on-farm, ante-mortem management reduces bruising severity in bison. As this was the first time a MLA has been utilized on all the participating farms, the pre-slaughter management would be expected to improve further as experience is gained.

Figure 3.1: Percent frequencies of bruising in each of 5 categories.



(0= no visible bruising anywhere on carcass to 4= extensive bruising on several parts of carcass)

3.4 Conclusion

This work provides novel information about the stress response of bison slaughtered on-farm compared to slaughter following transport in a stationary abattoir. Pre-slaughter management of bison can have an effect on their physiological responses to the stressors associated with this process. Blood cortisol was lowest in MLAPEN group compared to the MLACON or LAND groups (71.16 nmol/L, 124.17 nmol/L and 139.50 nmol/L respectively; $P<0.01$) , while MLACON had the lowest N/L ratio (0.701 compared to MLAPEN 1.145 or LAND 1.367, $P<0.01$). Bruising was found in all treatment groups, however less was found in the on-farm slaughtered animals than the transported ones. Slaughter in a multi-location abattoir on-farm resulted in improved indices of animal welfare and will likely also result in quality characteristics that are important to the bison industry for marketing a high value, natural product.

3.5 Literature Cited

Anderson, B. 1978. The Australian carcase bruise scoring system. Proc. Aust. Soc. Anim. Prod. **12:** 242.

Buckham Sporer, K. R., Weber, P.S.D., Burton, J. L., Earley, B. and Crowe, M. A. 2008. Transportation of young beef bulls alters circulating physiological parameters that may be effective biomarkers of stress. J. Anim. Sci. **86**: 1325-1334.

Canadian Bison Association 2011. Monthly market report. Accessed January 27, 2011. http://www.canadianbison.ca/producer/News_Events/documents/Jan212011PriceReport.pdf

Canadian Council on Animal Care. 1993. Guide to the care and use of experimental animals. Vol. 1, 2nd ed. E.D. Olfert, B.M. Cross, A.A. McWilliam, eds CCAC, Ottawa, ON.

Chelini, M. O. M., Souza, N. L., Cortopassi, S. R. G., Felippe, E. C. G. and Oliveira, C. A. 2006. Assessment of the physiological stress response by quantification of fecal corticosteroids. J. of the American Assoc. for Laboratory Anim. Sci. **45**: 8-11.

Cockram, M. S. and Lee, R. A. 1991. Some preslaughter factors affecting the occurrence of bruising in sheep. Brit. Vet. J. **147(2):** 120-125.

Cook, N. J. and Schaefer, A. L. 2002. Stress responses of wapiti (*Cervus elaphus Canadensis*) to removal of velvet antler. Can. J. Anim. Sci. **82**: 11-17.

Eckert, R. 1988. Animal Physiology Mechanisms and Adaptations. 3rd Edition. W.H. Freeman and Company. New York, USA.

Faucitano, L. 2010. Invited review: Effects of lairage and slaughter conditions on animal welfare and pork quality. Can. J. Anim. Sci. **90:**461-469.

Fell, L. R., Colditz, I. G., Walker, K. H. and Watson, D. L. 1999. Associations between temperament, performance and immune function in cattle entering a commercial feedlot. Australiand J Experimental Ag. **39:** 795-802.

Ferguson, D. M. and Warner, R. D. 2008. Have we underestimated the impact of pre-slaughter stress on meat quality in ruminants? Meat Sci. **80**: 12-19.

Grandin, T. 1981. Bruises on southwestern feedlot cattle. J. Anim. Sci. (Suppl. 1) **53:** 213. *In:* **Grandin, T. 1995. Livestock Conservation Institute-Bruise Levels on fed and non-feed cattle.** Proc. Livestock Cons. Institute: 193-201.

Gregory, N. G. 1996. Welfare and hygiene during preslaughter handling. Meat Sci. **43:** s35-s46.

Jago, J. G., Hargreaves, A. L., Harcourt, R. G. and Matthews, L. R. 1996. Risk factors associated with bruising in red deer at a commercial slaughter plant. Meat Sci. **44(3):** 181-191.

Jorgenson, L. 2008. Mobile slaughter fights government. *In:* Western Producer March 6:118.

King, D. A., Schuehle Pfeiffer, C. E., Randel, R. D., Welsh Jr., T. H., Oliphant, R. A., Baird, B. E., Curley Jr., K. O., Vann, R. C., Hale, D. S. and Savell, J. W. 2006. Influence of animal temperament and stress responsiveness on the carcass quality and beef tenderness of feedlot cattle. Meat Sci. **74**: 546-556.

Kremeniuk, T. 2010. Personal communication. Canadian Bison Association Executive Director. Regina, Sask. Canada.

Marler, R. J. 1975. Some hematologic and blood chemistry values in two herds of American bison in Kansas. J. Wildlife Diseases **11**: 97-100.

McNally, P. W. and Warriss, P. D. 1996. Recent bruising in cattle abattoirs. Vet Record **138**: 126-128.

McNally, P. W. and Warriss, P. D. 1997. Prevalence of carcase bruising and stick-marking in cattle bought from different auction markets. Vet Record **140:**231-232.

Mehrer, C.F. 1976. Some hematologic values of bison from five areas of the United States. J. Wildlife Diseases. **12**: 7-13.

Meischke, H. R. C., Ramsay, W. R. and Shaw, F. D. 1974. The effect of horns on bruising in cattle. Aust. Vet. J. **50:** 432-434.

Morrow, C. J., Kolver, E. S., Verkerk, G. A. and Matthews, L. R. 2002. Fecal glucocorticoid metabolites as a measure of adrenal activity in dairy cattle. Gen. And Comp. Endocrinology **126:** 229-241.

Pollard, J. C., Littlejohn, R., Asher, G. W., Pearse, A. J. T., Stevenson-Barry, J. M., McGregor, S. K., Manley, T. R., Duncan, S. J., Sutton, C. M.,

Pollock, K. L. and Prescott, J. 2002. A comparison of biochemical and meat quality variables in red deer (*Cervus elephus*) following either slaughter at pasture or killing at a deer slaughter plant. Meat Sci. **60**: 85-94.

Sapolsky, R. M., Romero, L. M. and Munck, A. U. 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocrine Rev. **21:** 55-89.

SAS Institute Inc. 1989. SAS/Stat User's guide. 4th Edition. Version 6 (Vol.2). Cary, NC: SAS Institute Inc.

Schaefer, A. L., Stanley, R. W., Tong, A. K. W., Dubeski, P., Robinson, B., Aalhus, J. L. and Robertson, W.M. 2006. The impact of *ante mortem* nutrition in beef cattle on carcass yield and quality grade. Can. J. Anim. Sci. **86**: 317-323.

Shaw, F. D. and Tume, R. K. 1992. The assessment of pre-slaughter and slaughter treatments of livestock by measurement of plasma constituents – a review of recent work. Meat Sci. **32**: 311-329.

Sikarskie, J. G. 1990. Comparative blood characteristics of ranched and free-ranging American bison (*Bison bison*). Am. J. Vet. Res. **51**: 955-957.

Smith, R. F. and Dobson, H. 1990. Effect of preslaughter experience on behavior, plasma cortisol and muscle pH in farmed red deer. The Veterinary Record **126**: 155-158.

Sporer, K. R. B., Xiao, L., Templeman, R. J., Burton, J. L., Earley, B. and Crowe, M. A. 2008. Transportation stress alters the circulating steroid environment and neutrophil gene expression in beef bulls. Vet. Imm. And Immunopathology **121:** 300-320.

Steel, R., G., Torrie, J. H. and Dickey, D. A. 1996. Principles and procedures of statistics: A biometrical approach. 3rd ed. McGraw-Hill Company, Columbus, OH 4372, USA.

Stoltenow, C.L. and Dyer, N.W. 2001. Biochemical and hematological parameters in ranch-raised American bison (*Bison bison*). Carrington Research Extension Centre Beef and Bison Field Day. July 19, 2001. Accessed Oct. 27, 2010. http://www.ag.ndsu.nodak.edu/carringt/bison/01%20BISON%20Report.pdf

Vestweber, J. G., Johnson, D. E., Merrill, G. L. and Staats, J. J. 1991. Hematological and blood chemistry profiles of American bison grazing on Konza Prairie of Kansas. J. Wildlife Diseases **27**: 417-420.

Warriss, P. D., Brown, S. N., Bevis, E. A. and Kestin, S. C. 1990. The influence of pre-slaughter transport and lairage on meat quality in pigs of two genotypes. Anim. Prod. **50:** 165-172.

Wasser, S. K., Hunt, K. E., Brown, J. L., Cooper, K., Crockett, C. M., Bechert, U., Millspaugh, J. J., Larson, S. and Monfort, S. L. 2000. A generalized fecal glucocorticoid assay for use in a diverse array of nondomestic mammalian and avian species. General and Comp. Endocrinology **120:** 260-275.

Wythes, J. R., Kaus, R. K. and Newman, G. A. 1985. Bruising in beef cattle slaughtered at an abattoir in southern Queensland. Aust. J. Exp. Ag. **25:** 727-733.

Zaugg, J. L., Taylor, S. K., Anderson, B. C., Hunter, D. L., Ryder, J. and Divine, M. 1993. Hematologic, serologic values, histopathologic and fecal evaluations of bison from Yellowstone Park. J. Wildlife Diseases **29**: 453-457.

CHAPTER 4

Meat Colour Stability and Fatty Acid Profile in Bison and Beef

4.1 Introduction

Bison (*Bison bison*) are raised for their meat and other products in North America. In 2010 in the Canadian province of Alberta there were over 18,000 bison (Steenbergen, 2011) slaughtered in inspected abattoirs. Bison meat composition has been found to be nutrient dense, with high proportion of protein (Galbraith et al., 2006; Marchello and Driskell, 2001). Bison meat is sold as both frozen and fresh product and can be found increasingly at mainstream grocery chains. Bison meat has been found to discolour more rapidly than beef (Pietrasik et al., 2006; Dhanda et al., 2002; Janz et al., 2000) and the reason for this has not been determined. Meat colour is important because it is used by consumers as an indicator of freshness and wholesomeness (Mancini and Hunt, 2005). In beef, fresh meat that has turned brown in the meat case may account for upwards of $1 billion in lost revenue to retailers in the USA, either because the meat has to be discounted, taken off the shelf and ground into burgers, or simply thrown out (Keefe, 2009). Structurally, beef and bison have identical myoglobin which displays no difference in primary structure, kinetics of oxidation, and themostability (Joseph et al., 2010). Therefore the differing rate of discolouration of bison cannot be attributed to differences in the structure and biochemistry of

myoglobin. Early browning in bison meat was also not attributable to a difference in microflora, but rather pigment oxidation (Janz et al., 2001).

Polyunsaturated fatty acids (PUFA) in phopholipid membranes are susceptible to oxidative breakdown resulting in changes to the colour, smell and taste of the meat (Wood et al., 2008). Species differences in susceptibility of meat to oxidation have been linked to the heme iron content (Rhee and Ziprin, 1987; Rhee et al., 1996). Heme iron has been proposed as an initiator and promoter of lipid oxidation in raw meats and H_2O_2-activated metmyoglobin has been seen to promote lipid oxidation in model systems (Decker, 2000). High levels of iron have been found in raw bison meat compared to those typically found in beef (Marchello and Driskel, 2001; Galbraith et al., 2006). PUFA levels (weight percentage) in range fed and feedlot fed bison were found to be higher than in range or feedlot fed cattle, 16.5,10.7, 9.53, 5.04 respectively (Rule et al., 2002).

Thus the relatively rapid deterioration of colour quality of bison muscle compared with beef may be related to the significantly higher content of both total PUFA's (Rule et al., 2002) and total iron. These characteristics may make bison meat more susceptible to a reduction in display life because of oxidation-related changes in appearance. Feeding vitamin E to steers has been found to increase lipid and oxymyoglobin stability in several muscles (Chan et al., 1996). To determine lipid stability in muscle tissue (development of rancidity) the thiobarbituric acid reactive (TBAR) substance test is done. This test measures malonaldehyde production within the product and represents the quantity of

primary oxidation products. Thus, the TBAR values and the extent of oxidation within a product are positively correlated (Nawar, 1996).

The colour of meat can be attributed to pigments, which absorb certain wavelengths and reflect others. The pigments consists largely of two proteins: hemoglobin and myoglobin (Mb). The majority of hemoglobin is lost during slaughter as a result of exsanguination (Faustman et al., 1996) although there is some residual amounts in the muscle. The intensity of the colour (chroma) increases as the concentration of myoglobin increases (Faustman et al., 1996). The colour of fresh meat is impacted by the amount of each derivative of myoglobin. The derivatives of myoglobin include reduced Mb, red oxymyoglobin (MbO_2), and metmyoglobin (MetMb). Mb is the purple pigment of deep muscle and of meat under a vacuum, MbO_2 is formed when myoglobin is exposed to air forming a bright red colour, and the oxidized form of myoglobin forms MetMb, which is brown and undesirable (Mancini and Hunt, 2005).

As conversion to metmyoglobin occurs, some ferric pigment will be reduced to the ferrous form and in an aerobic environment, oxymyoglobin will result. This is dependent on the reducing capacity, oxygen availability and myoglobin autoxidation rate of the muscle, and will differ depending on the age of the animal, species and muscle in question (Renerre, 1990).

Comparative studies to assess differential colour stability between bison and beef based on tissue levels of PUFA levels, tocopherols and pigments, have not been undertaken. The purpose of this study was thus to examine the influence

of fatty acid profile, vitamin E levels, and pigment on the oxidative and colour stability of fresh beef and bison in a retail display environment.

4.2 Materials and Methods

4.2.1 Animals and Slaughter

A total of 20 feedlot steers were fed in feedlot pens. Their diet contained approximately 8% grass hay and up to 80% steam-rolled barley. Animals were finished to a target backfat depth of 8-9 mm then shipped to the Lacombe Meat Research Centre abattoir for slaughter. The steers were also part of another study investigating the effects of vitamin E and flaxseed on rumen-derived fatty acid intermediates and in beef intramuscular fat (Juárez et al., 2011*).* Fourteen intact male bison from three commercial farms were slaughtered at the Lacombe Meat Research Centre abattoir. Bison were grass finished on native grass pasture. Animals were stunned and exsanguinated in accordance with the principles and guidelines established by the Canadian Council on Animal Care (1993).

Following the overnight chill, at approximately 24 h post-mortem, carcasses were knife-ribbed at the grade site (between the 12^{th} and 13^{th} rib for beef and between the 11^{th} and 12^{th} rib for bison) and after being exposed to atmospheric oxygen for 20 minutes were assessed for grade by certified graders (CFIA, 2007). The left *longissimus thoracis* (LT) anterior to the grade site from the beef carcasses and left *longissimus lumborum* (LL) posterior to the grade site from the bison carcasses were pulled, labelled, and trimmed. One steak was removed from the end (closest to the grade site) for subsequent fatty acid and α-

tocopherol determination. The remainder of the muscle was labelled, bagged and aged until 7 d *post-mortem* in a cooler at 2^{o}C.

Following the 6 d ageing period, the stored loin muscle was removed from the cooler and four steaks (25 mm thick) were removed, starting from the grade site. The first and second steaks were utilized for analysis not related to the present study. The third steak was placed into a polystyrene tray with a dri-loc pad, over-wrapped with oxygen permeable film and put into a horizontal (chest type) retail display case (Hill Refrigeration of Canada Ltd., Barrie, ON) under fluorescent room lighting (GE deluxe cool white), supplemented with incandescent lighting directly above the display case (GE clear cool beam 150 W/120 V spaced 91.5 cm apart) to provide an intensity of 1076 lux at the meat surface for 12 h per day (Jeremiah and Gibson, 2001). Samples were held at 1°C for retail evaluation after 0, 1, 2 and 3 d. The fourth steak was cut in half and one half was immediately prepared for determination of TBAR substances (0 d in retail), as described by Nielsen (1997). The remaining half was placed on pre-labelled polystyrene tray with a dri-loc pad, over-wrapped with oxygen permeable film and put into the retail display case for an additional 3 d, before determining final TBAR values.

4.2.2 Lipid Analyses

Lipid extraction was performed using 2:1 chloroform: methanol and with the same solvent to sample ratio as reported by Folch et al. (1957). Approximately 20g of steak from *longissimus* was comminuted using a food processor (Robot Coupe Blixer BX3, Robot Coupe USA Inc., Ridgeland MS) and 1 g was weighed

into 20 × 150mm Pyrex screw capped culture tubes with Teflon-lined caps. To each tube, 6 ml methanol was added and mixed by vortex (Thermolyne M37615 Maximix II; Barnstead Thermolyne, USA) and homogenized (VirTis Cyclone; VirTis Company, Gardiner, NY). To this mixture, 12 ml chloroform were added, mixed by vortex and homogenised. The entire mixture was filtered and 12 ml chloroform were added to the original tube and homogenized for 30 seconds and set aside to rinse the homogenizer. Six ml methanol, 10 ml 0.88% aqueous potassium chloride solution, and 1 drop of 6 N hydrochloric acid were added to the filtrate to create a biphasic system which expels chloroform. The solution was mixed well by shaking and centrifuged (Heraeus Omnifuge RT 3842, Heraeus Christ GmbH, Osterode, Germany) at 1000 x g for 5 min. The bottom chloroform layer was transferred to a pre-weighed 50 ml glass culture tube. The 12 ml chloroform used to rinse the homogenizer were filtered into the centrifuge tube containing upper methanol/water phase through the coarse sintered glass filter funnel containing the original residue to rinse the residue and funnel. The solution was mixed well and the bottom chloroform layer was pooled with previous chloroform layer in the pre-weighed 50 ml glass culture tube.

The lower phase (chloroform) was dried by rotary evaporation (Buchi multivapor P-12 with Buchi vacuum controller –V-800; Buchi, Switzerland) at 40°C for 20 min. The samples were then dried at ambient temperature under high vacuum over night in a vacuum oven (VWR Model 1400, Sheldon Manufacturing Inc.; OR USA) with an attached cold trap (Labonco, Centrivap 78110-00D, Kansas City, MO USA) and vacuum pump (Edwards E-LAB 2, Edwards High

Vacuum International; West Sussex, UK.). After drying, the head spaces of the sample tubes were flushed with nitrogen and the weights of the tubes plus lipids were recorded and lipids were then dissolved in 10 ml chloroform, transferred into 20 ml glass scintillation vials with teflon lined caps and stored at -20 C until methylation.

Lipids were methylated using 1.5 N methanolic hydrochloric acid as described by Kramer et al. (1997). Lipid extract (10 mg) was transferred using a calibrated pipette to a 16×125mm screw top glass culture tube with a Teflon lined cap. The samples were dried under nitrogen using an analytical evaporator (Organomation N-EVAP 111; Organomation Associates, Inc., Berlin, MA, USA). To solubilize the lipid, 1 ml of toluene containing 1 mg tricosanoic acid methyl ester as internal standard was added to the sample and mixed by vortex. To this, 3.0 ml of 1.5N methanolic hydrochloric acid were added, mixed by vortex, the headspace flushed with nitrogen and then capped. Samples were placed in a heating block (VWR 949031; Manufactured by Henry Troemner LLC, Thorofare, NJ USA) set at 80°C for 1 hour. A reagent blank was also prepared in a 16×125mm screw top culture tube containing 1 ml of toluene and placed in the heating block along with the samples. During heating, samples were mixed well using a vortex mixer at 5 and 30 min and caps were tightened. After 1 hour the samples were removed from the heating block and cooled to room temperature. To sample tubes, 1 ml of deionized water was added followed by 3 ml of hexane and the tubes capped again and mixed well with a vortex mixer. The samples were then centrifuged and the upper organic layer was transferred to a 4 ml vial

and dried over 100mg of sodium sulphate. One mg of fatty acid methyl ester (FAME) thus obtained was dissolved in 100 µL 95:5 hexane/diethyl ether and purified using 100mg/1ml Supelclean LC-Si solid phase extraction tubes (Supelco, Bellefonte, PA, USA) conditioned with 1ml acetone and 1ml hexane prior to sample loading and elution with 4 x 1ml 95:5 hexane/diethyl ether. The eluate was then dried under nitrogen and dissolved in 1ml of hexane and analyzed using a Varian CP-3800 GC (Varian Chromatography Systems, Walnut Creek, CA USA) with Model 1079 injector and a flame ionization detector and a Varian CP-Sil88 – 100m column. The GC was operated at constant pressure (25psi) with hydrogen as a carrier gas. One µL samples were injected (1mg FAME/ml hexane) using a 20:1 split and the injector and detector temperatures were set at 250^{o}C. The temperature program used included an initial temperature of 45^{o}C held for 4 min, to 175^{o}C at 13C/min and held for 27min, to 215^{o}C at 4^{o}C/min and held for 35min as outlined by Cruz-Hernandez et al. (2004).

For the identification of FAME by GC, the reference standard #463 from Nu-Chek Prep Inc. (Elysian, MN) was used. Branched-chain FAME were identified using a GLC reference standard BC-Mix1 purchased from Applied Science (State College, PA). The trans-18:1 isomers not included in the standard mixtures were identified by their retention times and elution orders as previously reported (Cruz-Hernandez et al., 2004; Cruz-Hernandez et al., 2007; Kramer et al., 2008). Fatty acid concentrations were reported as percentage of total fatty acids identified.

4.2.3 Pigment

Pigment was determined using a modified procedure from Trout (1991). Ground samples (2.5g) were weighed into centrifuge tubes. Twenty-five ml of phosphate buffer (0.04M) was dispensed into the tubes. The samples were homogenized using a Polytron (Brinkman, Model PT-MR 3100C) for 20 sec. The generator was rinsed between samples and wiped dry. Immediately following homogenization, the entire sample was filtered (Whatman #1 filter paper) into labelled centrifuge tubes. The filtrate was used for pigment assay. Phosphate buffer (3.5ml of 0.04M) and 0.5ml of each filtrate were pipetted into tubes. Triton X -100 (1.4ml) and 0.100 ml of sodium nitrite were pipetted into all tubes. The tubes were vortexed and incubated in a waterbath for 1 h. After tubes were placed in the rack they were run through the spectrophotometer (Pharmacia, Ultraspec 3000 Model 80-2106-20) using wavelengths of 730nm and 409nm.

Pigment concentration was calculated as follows:

Pigment (mg/g) = $A_{409} - (A_{730} * TC)) * (DF * MW_{Myoglobin} / MAC_{409} * 1000))$ where TC is Turbidity Correction = 2.68; DF is Dilution Factor = 14.78; MW Myoglobin is Molecular Weight of Myoglobin = 17,500; MAC_{409} is Molar Absorbance Coefficient at 409 nm = 79.6mM *1000.

Pigment (mg/g) = Average of duplicate samples (Pigment 1 and Pigment 2).

4.2.4 Tocopherol Analyses

Approximately 30g of ground meat was homogenized in a food processor (Robot Coupe Blixir BX3; Robot Coupe USA Inc., Ridgeland, MS) and the homogenate collected in Whirlpak^{TM} bags (Nasco; Salida, CA) and stored in the freezer at -80^{o}C until analysis. Samples were removed from the freezer and thawed overnight at 4^{o}C prior to analysis. Tocopherol content was estimated by high performance liquid chromatography (HPLC; Alliance Waters, Separation module e2695 equipped with a multi-wavelength fluorescence detector 2475; Waters Corporation, Milford, MA) according to methods outlined by Hewavitharana et al. (2004). In summary, approximately 1g of each sample was weighed in duplicate into 50 ml polyethylene screw top centrifuge tubes and capped to avoid oxidation of the samples during the process. Three hundred µl of internal standard (0.2 mg/ml α-tocopherol acetate) was added. The tubes were placed in ice and 4ml of absolute ethanol was added then homogenized. Five ml of reverse osmosis water were added to the tubes and the contents homogenized for 15 seconds, 4 ml aliquot of hexane-BHT was added and then homogenized for an additional 15 sec. Samples were centrifuged (Avanti J-E Centrifuge, Beckman Coulter, Mississauga, ON) for 10 min. The top layer was transferred into an amber HPLC vial containing a 300µl glass insert. From each sample, 50µl were analyzed for α-tocopherol content under fluorescence with an excitation wavelength at 295nm and an excitation wavelength at 295 nm.

4.2.5 Evaluation of Retail Stability

Treatment samples were placed into the retail display case controlling for known temperature gradients within the retail case. On each specific day in retail objective colour measurements (CIE L*[brightness], *a**[red-green axis], *b**[yellow-blue axis] values; Commission Internationale de l'Eclairage, 1978) were collected in triplicate across the face of the steak using a Minolta CR-300 with Spectra QC-300 Software; Minolta Canada Inc., Mississauga, ON). Spectral reflectance readings were also collected at the same time to calculate the relative contents of MetMb, Mb and MbO_2 as describe by Krzywicki (1979). Following objective colour measurements steaks were subjectively evaluated for retail appearance, lean colour score, percent surface discolouration, colour of discolouration, amount of marbling and marbling colour by five trained raters using an 8-point hedonic (1=extremely undesirable and 8=extremely desirable), 8-point descriptive (1=white and 8=extremely dark red), 7-point descriptive (1=0% and 7=100% discolouration), 7-point descriptive (1=no browning and 7=black), 6-point descriptive (1=devoid and 6=abundant) and 5-point descriptive (1=white and 5=red) scales, respectively.

4.2.6 Statistical Analyses

Differences between species (bison and beef) were determined for retail evaluation data on day 0, 1, 2 and 3 using a repeated measures design with PROC MIXED (SAS, 1996). The fixed effects were species, day and their interaction and the experimental unit was the individual animal (species). The model of best fit was determined using the Bayesian Information Criterion (BIC) where the lower BIC indicated a better fit (Wang and Goonewardene, 2004).

A comparison of bison to beef for α-tocopherol, α-Tocotrienol, total vitamin E homologues, pigment and all fatty acids using PROC MIXED least squares means and standard errors for the dependant variables were determined.

PROC STEPWISE was used (SAS, 1996) to examine the effect of inherent tissue levels of total fat, omega-3 (n-3), omega-6 (n-6), PUFA, omega6:3 ratio (n-6:n-3), pigment and vitamin E on the change in MetMb, percent discolouration and appearance scores between d 0 and d 3 in retail display. When a factor was significant ($P<0.15$) it was included in the model towards the overall model R^2 value.

4.3 Results and Discussion

4.3.1 Fatty acids

All the individual fatty acids and indices were different ($P<0.01$) between beef and bison (Table 4.1). Significant differences can be seen between species for saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), PUFA, n-3, and n-6 fatty acids. The long chain SFA 18:0 was significantly higher in bison (18.40mg/100mg compared to 12.52mg/100mg in beef; $P<0.01$) and higher than levels found in an evaluation of the effect of dietary forages and concentrate levels on the fatty acid profile of bison tissue, where forage fed bison were reported to have 18:0 levels of 12.66 mg/100mg (Turner, 2005). In that study intramuscular (*longissimus dorsi; LD)* stearic acid was not different across feeding treatments (12.66 for forage finished compared to 14.03 for feedlot finished bison). However, Rule et al., (2002) found greater proportions of stearic acid within the tissue (LD) of forage fed bison *vs* feedlot grain finished (16.8 mg/100mg compared to 12.6mg/100mg). In the present study, α-linolenic (18:3n-

3) levels were more than 7 times higher in bison than in beef. Diet can alter the fatty acid composition in bison (Rule et al., 2002) and beef (Laborde et al., 2002; Nuernberg et al., 2008), however, changes to diet have less of an effect in a ruminant animal compared to a monogastric, due to bio-hydrogenation of dietary fatty acids in the rumen (Scollan et al., 2006). The level of α-linolenic in forage finished bison in the present study (2.90 mg/100mg) agrees with Turner (2005) where the forage finished bison had a level of 3.08mg/100mg and Cordain et al. (2002) where elk (*Cervus elaphus)* biceps femoris muscle had levels of 2.89mg/100mg. α-linolenic acid is an important precursor to the very long chain fatty acids in particular eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA). The beneficial effects of EPA and DHA have been well documented including anti-atherogenic, anti-thrombotic, and anti-inflammatory effects (Givens et al., 2006). Dietary treatment showed an increase in all of these unsaturated fatty acids in bison (Turner 2005). Inclusion of flaxseed and vitamin E in beef diets were both shown to increase level of n-3 fatty acids in intramuscular fat with the response primarily related to higher levels of α-linolenic, and EPA (Juárez et al., 2011).

The fatty acid composition of muscle affects its oxidative stability during retail display; the PUFAs in phospholipids are susceptible to oxidative breakdown at this stage (Wood et al., 2008). PUFA levels were over 3 times higher in bison than beef.

Table 4-1 Fatty acid content of the *longissimus thoracis* muscle of intact male bison and beef steers.

	Beef	Bison	SEM	*P*
Number of animals	20	14		
Total Fat, %	5.56	1.00	0.39	<0.01
Fatty Acids, mg.100mg^{-1}				
14:0 (myristic)	2.68	1.27	0.17	<0.01
16:0 (palmitic)	27.24	18.92	0.70	<0.01
*c*9-16:1 (palmitoleic)	3.92	1.43	0.16	<0.01
17:0 (margaric)	1.12	1.41	0.07	<0.01
18:0 (stearic)	12.52	18.40	0.47	<0.01
Σtrans 18:1	3.50	2.95	0.55	0.03
*c*9-18:1 (oleic)	37.51	30.22	0.94	<0.01
18:2n-6 (linoleic)	3.00	9.54	0.80	<0.01
*Σ*CLA	0.59	0.78	0.05	<0.01
18:3n-3 (α-linolenic)	0.38	2.90	0.11	<0.01
20:3n-6	0.28	0.38	0.04	0.04
20:4n-6 (arachidonic)	0.80	3.17	0.31	<0.01
20:5n-3 (EPA)	0.14	1.15	0.09	<0.01
22:4n-6	0.13	0.16	0.02	0.04
22:5n-3	0.35	1.72	0.11	<0.01
22:6n-3 (DHA)	0.07	0.41	0.03	<0.01
SFA	44.04	40.59	1.00	<0.01
MUFA	49.52	38.29	0.97	<0.01
PUFA	6.43	21.11	1.44	<0.01
n-3	0.94	6.16	0.32	<0.01
n-6	4.21	13.25	1.14	<0.01
n-6:n-3	4.52	2.11	0.18	<0.01

$^{a-b}$ Least square means in the same row with different letters differ (P<0.05)

Longissimus dorsi samples from bison contained more PUFA than either Hereford or Brahman cattle (26.24, 20.82, and 21.1mg/100mg, respectively; Larick et al., 1989). They also attributed an increased off-flavour and aftertaste to the increased levels of PUFA. In the present study the beef n-3, n-6 and n-6:n-3 was 0.94 mg/100mg, 4.21 mg/100mg and 4.52 respectively compared to feedlot finished cattle on another study at 0.59 mg/100mg, 2.05 mg/100mg, and 3.72

respectively (Basarab et al., 2007). The ‘direct off grass’ cattle in that study had a n-6:n-3 ratio of 1.71 which is lower than the value of 2.11 found in the present study. From a human health perspective, an increaseing omega 6:3 ratio is considered a risk factor in cancers and coronary heart disease, especially the formation of blood clots leading to heart attack (Enser, 2001). The recommendation is for a ratio of less than 4, which the bison had in the present study (2.11) and both the grass and grain finished beef (1.71, 3.72 respectively) had in the study by Basarab et al. (2007).

An examination of the fatty acid profile of beef, bison, elk and chicken breast concluded that range fed bison and beef cows both provide a lean meat, comparable to elk and chicken breast, with respect to a fatty acid profile that is currently regarded as healthful (Rule et al., 2002). In order to obtain a source claim for n-3 fatty acids in food products, the Canadian regulatory authority (Canadian Food inspection Agency, 2003) sets 0.3g or more of n-3 PUFA per reference amount and serving of stated size. If a 6oz steak (170g) is considered at the percentage of total fat levels from the current study this would be 1.7g of total fat, and using n-3 PUFA levels in bison from the current study (6.16 mg/100mg of fatty acid) this would equal 0.105g of n-3 PUFA per serving. Bison would not be over the minimum n-3 PUFA levels required to make a source claim in Canada.

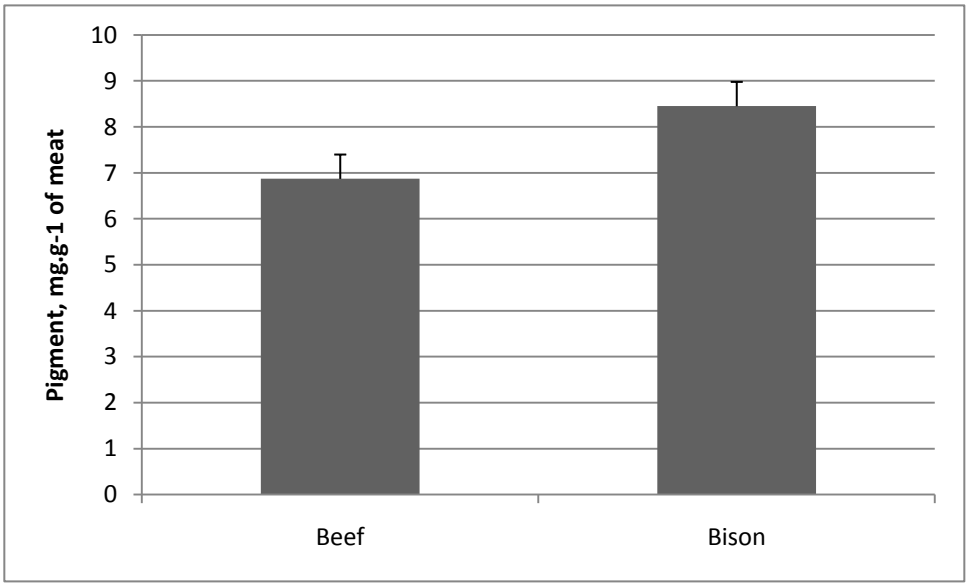
Lipid oxidation in muscle tissues can promote Mb oxidation causing discolouration and rancid odours and flavours (Scollan et al., 2006). Scollan et al. (2006) suggested that when animals are fed a similar base diet, but fatty acids are

altered through dietary supplementation with oils, it is the difference in fatty acid composition and not the amount of antioxidant, that the lipid and colour stability can be attributed to.

4.3.2 Pigment

Mb is the principle protein responsible for meat colour, and is the predominant meat pigment (Mancini and Hunt, 2005). Oxygenation occurs when Mb is exposed to oxygen and is characterized by the development of a bright cherry-red colour. The loss of oxygen from oxymyoglobin and an electron from ferrous ion producing the MetMb, are the changes that occur to alter the absorption properties and the complementary colour which turns from bright red to dark-red and further to brown (Kanner, 1994). Pigment levels in bison can be seen to be significantly higher ($P<0.01$) in bison over beef (Figure 4.1). The iron atom in the centre of the heme ring (in Mb) can form six bonds, the sixth of which can reversibly bind to ligands which dictate muscle colour (Mancini and Hunt, 2005). While the primary structure of Mb between bison and beef have been found to be identical (Joseph et al., 2010), the increased levels of pigment found in bison in the present study combined with high levels of iron found in bison muscle tissue (Galbraith et al., 2006) could contribute to poor colour stability in fresh bison meat.

Figure 4.1 Pigment levels (mg/g) in bison and beef tissue



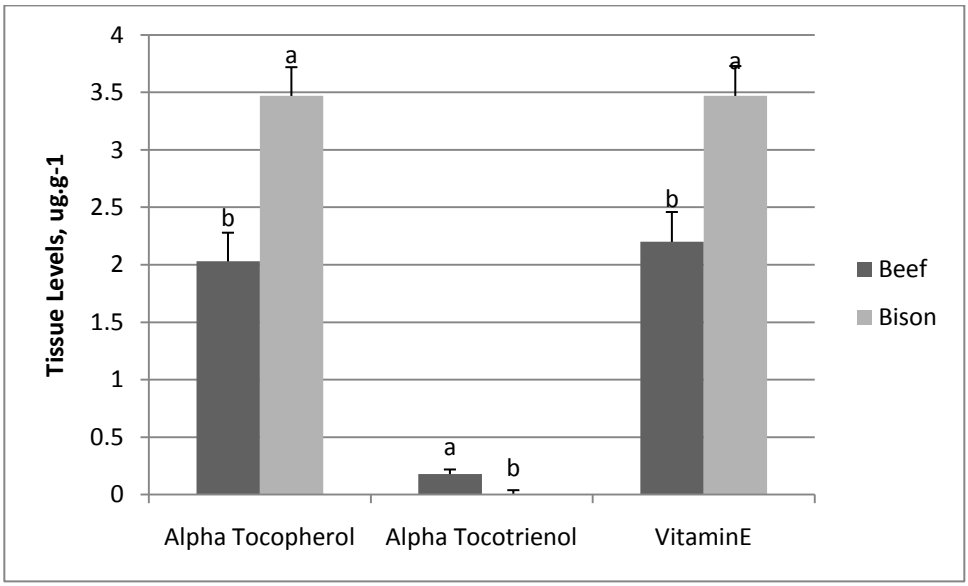
4.3.3 Vitamin E

Vitamin E is a term that encompasses a number of tocopherols and trienols that have antioxidant properties (Brigelius-flohe and Taber, 1999). The efficacy with which they exert their biological action is very low in the case of the tocotrienols, whereas tocopherols, and in particular α-tocopherol, are much more active and potent and account for almost all the vitamin E activity of living tissues (Berges, 2002). Tocopherols constitute a series of benzopyranols that occur in plant tissues and vegetable oils and are powerful lipid-soluble antioxidants (Christie, 2010). In the current study, vitamin E was significantly higher ($P<0.01$) in bison meat than in beef (3.47µg/g *vs*. 2.20µg/g; Figure 4.2). However levels of α-tocotrienols were higher in beef than in bison. In many species, elevated tissue levels of antioxidants have been reported to have a stabilizing effect on meat colour. For example, feeding vitamin E to lambs has been shown to improve colour stability (Lauzurica et al., 2005). Vitamin E treated pigs produced steaks

that were redder and less brown compared to non-supplemented pigs, and showed improved colour stability (O'Sullvan et al., 2002). In a comparison between pasture and feedlot finished cattle, improved colour stability was observed in the pasture finished groups containing higher levels of α-tocopherol in the diet (Insani et al., 2008). Vitamin E levels in pork *Longissimuss dorsi* were found to be 26.0 ppm (or µg/100g) (O'Sullivan et al., 2002) which is much lower than the levels shown in Figure 4.2 in bison (319 µg/100g) or beef (203 µg/100g). Larrain et al. (2008) reported beef fed a corn diet were found to have α- tocopherol of 188µg/100g, which is also lower than the levels reported for both bison and beef in the present study. While it might be expected that the bison would exhibit greater oxidative stability as a result of elevated vitamin E levels, the TBAR measurements did not support this.

Arnold et al (1992) reported that vitamin E supplementation (500IU/head/d) could extend display life of beef LL 2.5 days based on a threshold value of 15% MetMb which corresponded to first detection of discolouration. In the present study MetMb levels started in the display case at d 0 at 15 % for beef and 21% for bison (Table 4.2). An investigation into the effects of vitamin E supplementation in bison may be warranted to slow down conversion to MetMb.

Figure 4.2 Tissue levels (µg/g) of α-tocopherol, α-tocotrienol and vitamin E in beef and bison



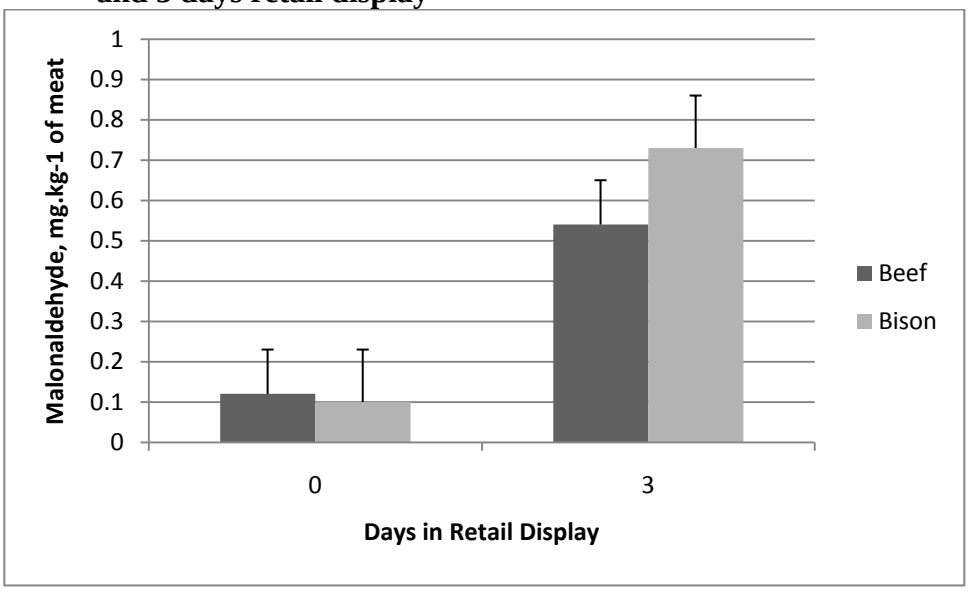
4.3.4 TBARS

To measure the oxidative stability of foods, the Thiobarbituric acid reactive substance (TBARS; Tarladgis et al., 1960) is commonly used. In this procedure, the lipid oxidation product, malonaldehyde, reacts with the TBA reagent to form a red-coloured chromagen. Since other secondary products of lipid oxidation can also react with the TBA reagent, 'reactive substance' is added to the name (Pietrasik et al., 2006). Values above about 0.5 are considered critical since at this level lipid oxidation products which produce a rancid odour and taste, detectable to consumers are present (Wood et al., 2008). In the present study (Figure 4.3), both beef and bison had TBAR values which exceeded this critical level by day 3 of retail display (0.54 and 0.73 respectively). Despite a numerically higher value after 3 d of retail display in the bison compared to beef there was no significant differences (P = 0.28) due to the large range in values. These results are in contrast with Pietrasik et al. (2006) who reported higher TBAR values in

bison compared to beef up to two weeks storage. It may be that had the current study held the steaks for a longer time in retail, differences in TBAR levels between bison and beef would have emerged.

A positive correlation was found between metmyoglobin accumulation and the production of lipid oxidation products (Faustman and Cassens, 1990). The rate of discolouration of meat is related to the effectiveness of oxidation processes and enzymatic reducing systems in controlling metmyoglobin levels in meat (Faustman and Cassens, 1990).

Figure 4.3 Tissue levels of Malonaldehyde in bison and beef after 0 and 3 days retail display



4.3.5 Retail evaluation

Metmyoglobin levels in bison were significantly higher ($P<0.05$) than beef on all retail display days (Table 4.2) confirming the early browning in bison reported previously (Pietrasik et al., 2006; Dhanda et al., 2002; Janz et al., 2000). The beef MetMb level on day 3 was equivalent to the level in bison on day 0 and

by day 3 the levels in the bison were twice that of beef. Over the same time, MbO_2 decreased (P <0.01) slightly in beef (0.78 to 0.71) and substantially in bison (0.70 to 0.55). Hence it appears that the colour stability of bison was already compromised on entry into the retail case after 6 d of ageing.

There was a significant species by day in retail interaction for objective colour measurements, with L* values increasing (P = 0.03) and chroma and hue values (P<0.01) decreasing over time in retail. By day 1 the subjective retail measurements were showing bison to be weaker in the retail environment. Retail appearance, lean colour score, and percent surface discolouration were significantly different for bison than beef, with bison scoring less favourably on all of these measurements.

Table 4-2 Comparison of retail performance between beef and bison steaks at d 0, 1, 2, 3 in retail display

Measure	Species	0 d	1 d	2 d	3 d	SEM	*P*
L*	Beef	42.21 [a]	41.22 [a]	41.31 [a]	41.38 [a]	0.43	0.034
	Bison	37.11 [b]	35.19 [b]	34.55 [b]	36.43 [b]	0.52	
Chroma	Beef	23.89[a]	23.26[a]	23.14[a]	22.89[a]	0.45	<.01
	Bison	18.57[b]	17.49[b]	15.25[b]	14.07[b]	0.54	
Hue	Beef	36.17[a]	36.14[a]	36.62[a]	37.25[a]	0.66	<.01
	Bison	32.39[b]	35.86[a]	38.96 [b]	42.25[b]	0.79	
Metmyoglobin	Beef	0.15[a]	0.18[a]	0.19[a]	0.20[a]	0.01	<.01
	Bison	0.21[b]	0.28[b]	0.34[b]	0.40[b]	0.01	
Myoglobin	Beef	0.07[a]	0.08[a]	0.08[a]	0.09[a]	<0.01	0.04
	Bison	0.10 [b]	0.09 [a]	0.11 [b]	0.11[b]	0.01	
Oxymyoglobin	Beef	0.78[a]	0.74[a]	0.73[a]	0.71[a]	0.01	<.01
	Bison	0.70[b]	0.63[b]	0.55[b]	0.49[b]	0.01	
Retail Appearance	Beef	7.66[a]	7.01[a]	6.69[a]	6.01[a]	0.17	<.01
	Bison	7.40[a]	5.26[b]	3.96[b]	3.26[b]	0.20	
Lean Colour Score	Beef	5.01[a]	4.95[a]	5.02[a]	5.04[a]	0.07	<.01
	Bison	6.00[b]	6.49[b]	6.93[b]	7.19[b]	0.09	
% Surface Discolouration	Beef	1.02[a]	1.33[a]	1.63[a]	2.03[a]	0.19	<.01
	Bison	1.26[a]	3.59[b]	4.77[b]	5.33[b]	0.23	
Colour of Discolouration	Beef	1.04[a]	1.16[a]	1.45[a]	1.72[a]	0.11	<.01
	Bison	1.11[b]	2.13[b]	2.70[b]	2.83[b]	0.13	
Marbling Score	Beef	3.59	3.73	3.80	3.78	0.09	0.57
	Bison	2.29	2.34	2.40	2.43	0.10	
Marbling Colour	Beef	1.00[a]	1.00[a]	1.00[a]	1.00[a]	0.04	<0.01
	Bison	1.23[b]	1.17[b]	1.00[a]	1.00[a]	0.04	

[a-b] Least squares means in the same column within measure with different letters differ ($P<0.05$).

4.3.6 Changes to appearance traits

The current study shows that inherent traits in the muscle tissue of bison influence the lack of retail colour stability. In all retail display days bison had higher MetMb (Table 4.2), and on display days 1, 2 and 3 had a higher percentage of discolouration than the beef steaks. Retail appearance was also rated as less desirable in bison for retail d 1, 2 and 3 (Table 4.2).

Overall for both beef and bison, the change (Δ) in MetMb from d 0 to d 3 was most influenced by the inherent n-6:n-3 levels in the tissue (R^2 =59.30; Table 4.2) with total fat (R^2 =5.40) and vitamin E (R^2 =4.17) bringing the model R^2 to 68.88. Using the b values and the intercept, an equation to describe the influence that the independent variables are having on the dependant variables can be derived. For changes in MetMb from d 0 to d 3 in retail:

Δ MetMb = 0.374 - 0.026(Vit E) - 0.039(omega6:3) – 0.015(Totfat) (P<0.05).

For changes in percent discolouration:

Δ % discolouration = 1.76 – 0.599(Vit E) + 0.698(n-3) (P< 0.05). This relationship shows that as levels of vitamin E decrease and levels of n-3 increase, an increased change in % discolouration will occur between d 0 and d 3.

n-3 fatty acid level in the tissue accounted for the majority of the variance (R^2= 0.68) in the change in percent discolouration between d 0 and d 3.

For changes in appearance scores:

Δ appearance = - 4.55 + 0.512(total fat) (*P< 0.01)*. In this model, as total fat increases, the change in appearance between d 0 and d 3 increases (improves).

These equations show the inherent tissue traits of bison are strongly linked to undesirable changes in fresh meat colour in a retail display environment. Bison had lower fat (Table 4.1), lower n-6:n-3 levels, higher vitamin E levels, and higher n-3 levels than beef and consequently showed a higher Δ in MetMb, % discolouration, and appearance between d 0 and d 3 in retail.

Table 4-3 R^2 values (x 100), intercept, and *b*-values for the effect of tissue levels of total fat, vitamin E, PUFA*, n-3, n-6* and n6:n3 ratio on the change in metmyoglobin (MetMb), % discolouration and appearance between day 0 and day 3 in retail display.

	Δ MetMb	Δ% discolouration	Δ appearance
Total model R^2	68.88	73.69	67.63
Intercept	0.374	1.76	-4.55
Total fat R^2	5.40		67.63
b-value	-0.015		0.512
Vitamin E R^2	4.17	5.59	
b- value	-0.026	-0.599	
n- 3 R^2		68.11	
b-value		0.698	
n- 6:3 R^2	59.30		
b-value	-0.039		

*Not in table since PUFA and n-6 were not significant for the model at *P*<0.15

4.4 Conclusion

Fatty acid composition was significantly different between grass-finished bison and grain-finished beef for all the fatty acids measured. Bison meat had a lower total fat and more favourable fatty acid profile with PUFA levels over three times that of beef, a higher n-3 and n-6 fatty acid level than beef, and a lower n-6:n-3 ratio (2.11 compared to 4.52 for beef). Bison also had higher tissue levels of pigment and vitamin E. These meat traits of bison were linked to poorer performance in the retail environment when compared to beef indicated by increased MetMb levels, increased percentage discolouration and lower appearance scores. Models to predict the change in Metmb (n-6:n-3 ratio, vitamin E and total fat included), % discolouration (n-3 and vitamin E included) and retail appearance (total fat included) over three days of retail display accounted for 69, 74 and 68% of the variation respectively.

Despite higher levels of vitamin E, the higher level of PUFA and pigment are interacting with the iron in the pigment to more rapidly destabilize the bison meat and is propagating oxidation resulting in early browning. It would be of interest to investigate if vitamin E levels can be supplemented in bison through feeding at a level that would have a stabilizing effect on rapid browning. If not, there needs to be other strategies developed for stabilizing bison meat prior to, and in the retail environment.

Consumer education about inherent differences with bison meat might make what is deemed ‘acceptable’ in terms of fresh bison meat differ from beef,

and allow the industry to promote another unique aspect of bison further differentiating it from beef.

4.5 Literature Cited

Arnold, R. N., Scheller, K. K., Arp, S. C., Williams, S. N., Buege, D. R. and Schaefer, D. M. 1992. Effect of long- or short-term feeding of α-tocopherol acetate to Holstein and crossbred steers on performance carcass characteristics, and beef color stability. J. Anim. Sci. **70:**3055-3065.

Basarab, J. A., Mir, P. S., Aalhus, J. L., Shah, M. A., Baron, V. S., Okine, E. K. and Robertson, W. M. 2007. Effect of sunflower seed supplementation on the fatty acid composition of muscle and adipose tissue of pasture-fed and feedlot finished beef. Can J. Anim. Sci. **87:** 71-86.

Berges, E. 2002. Importance of vitamin E in the oxidative stability of meat: organoleptic qualities and consequences. CIHEAM- Options Mediterraneennes. http://ressources.ciheam.org/om/pdf/c37/99600036.pdf Accessed: Feb. 11, 2011.

Brigelius-Flohe, R. and Traber, M. 1999. Vitamin E: function and metabolism. FASEB **13:** 1145-1155.

Canadian Council on Animal Care. 1993. Guide to the care and use of experimental animals. Vol. 1, 2nd ed. E.D. Olfert, B.M. Cross, A.A. McWilliam, eds CCAC, Ottawa, ON.

Canadian Food Inspection Agency, 2003. Guide to food labelling and advertising. Accessed Feb. 14, 2011. http://www.inspection.gc.ca/english/fssa/labeti/guide/ch7be.shtml

Canadian Food Inspection Agency 2007. Livestock and poultry carcass grading regulations, part VI section 48-54.1 Schedule I – IV. http://laws.justice.gc.ca/en/showdoc/cr/SOR-92-541/boga:l_VI//en#anchorbo-ga:l_VI

Chan, W. K. M., Hakkarainen, K., Faustman, C., Schaefer, D. M., Scheller, K. K. and Liu, Q. 1996. Dietary vitamin E effect on color stability and sensory assessment of spoilage in three beef muscles. Meat Sci. **42:** 387-399.

Christie, W. M. 2010. The lipid library- tocopherols and tocotrienols. Accessed July 29, 2010. http://lipidlibrary.aocs.org/Lipids/tocol/file.pdf

Commission Internationale de l'Eclairage 1978. Recommendations on uniform color spaces-color difference equations – psychometric color terms, CIE Publication No. 15 (E-1.3.1) 1971/(TC-1.3), Supp. No.2, pp. 8-12. Paris.

Cordain, L., Watkins, B. A., Florant, G. L., Kelher, M., Rogers, L. and Li, Y. 2002. Review article fatty acid analysis of wild ruminant tissues:

evolutionary implications for reducing diet-related chronic disease. Europ. J. Clinic. Nutr. **56:**181-191.

Cruz-Hernandez C., Deng Z., Zhoua J., Hill A.R., Yurawecz M.P., Delmonte P., Mossoba M.M., Dugan M.E.R. and Kramer J.K.G. 2004. Methods to analyze conjugated linoleic acids (CLA) and trans-18:1 isomers in dairy fats using a combination of GC, silver ion TLC-GC, and silver ion HPLC. J. AOAC **87:**545-560.

Cruz-Hernandez, C., Kramer, J. K. G., Kennelly, J. J., Glimm, D. R., Sorensen, B. M., Okine, E. K., Goonewardene, L. A. and Weselake, R. J. 2007. Evaluating the conjugated linoleic acid and trans 18 : 1 isomers in milk fat of dairy cows fed increasing amounts of sunflower oil and a constant level of fish oil. J. Dairy Sci. **90:** 3786–3801.

Decker, E., Faustman, C. and Lopez-Bote, C. J. 2000. Antioxidants in muscle foods: nutritional strategies to improve quality. John Wiley & Sons Inc. New York. 485 pp.

Dhanda, J. S., Pegg, R. B., Janz, J. A. M., Aalhus, J. L. and Shand, P. J. 2002. Palatability of bison semimembranosus and effects of marination. Meat Sci. **62:**19-26.

Enser, M. 2001. The role of fats in human nutrition. In B. Rossell (Ed), Oils and fats Vol 2. Animal carcass fats (pp. 77-122). Leatherhead, Surrey, UK: Leatherhead Publishing.

Faustman, C. and Cassens, R. G. 1990. The biochemical basis for discolouration in fresh meat: A review. J. Muscle Foods **1:** 217- 243.

Faustman, C., Chan, W. K. M., Lynch, M. P. and Joo, S. T. 1996. Strategies for increasing oxidative stability of fresh meat colour. Reciprocal Meat Conference **49**: 73-78.

Folch, J., Lees, M., Sloane Stanley, G. H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. **226:** 497-509.

Galbraith, J. K., Hauer, G., Helbig, L., Wang, Z., Marchello, M. J. and Goonewardene, L. A. 2006. Nutrient profiles in retail cuts of bison meat. Meat Sci. **74:** 648-654.

Givens, D. I., Kliem, K. E. and Gibbs, R. A. 2006. The role of meat as a source of *n*-3 polyunsaturated fatty acids in the human diet. Meat Sci. **74:**209-218.

Hewavitharana, A. K., Lanari, M. C. and Becu, C. 2004. Simultaneous determination of Vitamin E homologs in chicken meat by liquid chromatography with fluorescence detection. J. Chrom. A 1025:313-317.

Insani, E. M., Eyherabide, A., Grigioni, G., Sancho, A. M., Pensel, N. A. and Descalzo, A. M. 2008. Oxidative stability and its relationship with

natural antioxidants during refrigerated retail display of beef produced in Argentina. Meat Sci. **79:** 444-452.

Janz, J. A. M., Aalhus, J. L., Price, M. A. and Schaefer, A. L. 2000. The influence of elevated temperature conditioning on bison (Bison bison bison) meat quality. Meat Sci.**56:** 279-284.

Janz, J. A. M., Aalhus, J. L., Price, M. A. and Greer, G. G. 2001. Bison meat quality research: and investigation of spray chilling, very fast chilling, and the relationship of grade factors to meat quality. A final report to the Peace Country Bison Association.

Jeremiah, L. E. and Gibson, L. L. 2001. The influence of packaging and storage time on retail properties and case-life of retail-ready beef. Food Res. Int. **34(7):** 621-631.

Juarez, M., Dugan, M. E. R., Aalhus, J. L., Aldai, N., Basarab, J. A., Baron, V. S. and McAllister, T. A. 2010. Dietary vitamin E inhibits the *trans* 10-18:1 shift in beef backfat. Can. J. Anim. Sci. **90:** 9-12.

Juarez, M., Dugan, M. E. R., Aalhus, J. L., Aldai, N., Basarab, J. A., Baron, V. S. and McAllister, T. A. 2011. Effects of vitamin E and flaxseed on rumen-derived fatty acid intermediates in beef intramuscular fat. Meat Sci. *In press.*

Joseph, P., Suman, S. P., Li, S., Beach, C. M., Steinke, L. and Fontaine, M. 2010. Characterization of bison (Bison bison) myoglobin. Meat Sci. **84:** 71-74.

Kanner, J. 1994. Oxidative processes in meat and meat products: quality implications. Meat Sci. **36:** 169-189.

Katsanidis, E. and Addis, P. B. 1999. Novel HPLC analyses of tocopherols, tocotrienols and cholesterol in tissue. Free Radic. Biol. Med. **27:**1137-1140.

Keefe, L. M. 2009. The color of money [Tech Connection]. Meatingplace 11.09. pp53-56.

Kramer, J. K. G., Fellner, V., Dugan, M. E. R., Sauer, F. D., Mossoba , M. M. and Yurawecz , M. P. 1997. Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total trans fatty acids. Lipids **32:** 1219-1228.

Kramer, J. K. G., Hernandez, M., Cruz-Hernandez, C., Kraft, J. and Dugan, M. E. R. 2008. Combining results of two GC separations partly achieves determination of all cis and trans 16:1, 18:1, 18:2 and 18:3 except CLA isomers of milk fat as demonstrated using Ag-Ion SPE fractionation. Lipids **43(3):** 259-273.

Krzywicki, K. 1979. Assessment of relative content of myoglobin, oxymyoglobin and metmyoglobin at the surface of meat. Meat Sci. **3:** 1-10.

Laborde, F. L., Mandell, I., Tosh, B. and Buchanan-Smith, J. J. 2002. Effect of management strategy on growth performance, carcass characteristics, fatty acid composition, and palatability attributes in crossbred steers. Can. J. Anim. Sci. **82**: 49-57.

Larick, D. K., Turner, B. E., Koch, R. M. and Crouse, J. D. 1989. Influence of phospholipid content and fatty acid composition of individual phospholipids in muscle from bison, Hereford and Brahman steers on flavor. J. Food Sci. **54(3):** 521-526.

Larrain, R. E., Schaefer, D. M., Richards, M. P. and Reed, J. D. 2008. Finishing steers with diets based on corn, high-tannin sorghum or a mix of both: color and lipid oxidation in beef. Meat Sci. **79:** 656-665.

Lauzurica, S. Fuente, J., Diaz, M. T., Alvarez, I., Perez, C. and Caneque, V. 2005. Effect of dietary supplementation of vitamin E on characteristics of lamb meat packed under modified atmosphere. Meat Sci. **70:** 639-646.

Mancini, R. A. and Hunt, M. C. 2005. Current research in meat colour. Meat Sci. **71:**100-121.

Marchello, M. J. and Driskell, J. A. 2001. Nutrient composition of grass and grain finished bison. Great Plains Res. **11:** 65-82.

Nawar, W. W. 1996. Lipids *in*: Food Chemistry. Fennema, O. R. (ed) and Dekker, M. inc. New York: 225-320.

Nielsen J. H., Sorensen B., Skibsted L. H. and Bertelsen G. 1997. Oxidation in Pre-cooked Minced Pork as Influenced by Chill Storage of Raw Muscle. Meat Science. Volume 46. Number 2. 191-197.

Nuernberg, K., Fischer, A., Nuenberg, G., Ender, K. and Dannenberger, D. 2008. Meat quality and fatty acid composition of lipids in muscle and fatty tissue of skudde lambs fed grass versus concentrate. Sm. Rumin. Res. **74**: 279-283.

O'Sullivan, M. G., Byrne, D. V., Stagsted, J., Andersen, H. J. and Martens, M. 2002. Sensory colour assessment of fresh meat from pigs supplemented with iron and vitamin E. Meat Sci. **60:**253-265 p. 253-265.

Pietrasik, Z., Dhanda, J. S., Shand, P. J. and peg, R. B. 2006. Influence of injection, packaging, and storage conditions on the quality of beef and bison steaks. J. Food Sci. **71:**s110-s118.

Renerre, M. 1990. Review: Factors involved in the discolouration of beef meat. Int. J. Food Sci. and Tech. **25:** 613-630.

Rhee, K. S., Anderson, L. M. and Sams, A. R. 1996. Lipid oxidation potential of beef, chicken and pork. J. Food Sci. **61:** 8-12.

Rhee, K. S. and Ziprin, Y. A. 1987. Lipid oxidation in retail beef, pork and chicken muscles as affected by concentrations of heme pigments and nonheme iron and microsomal enzymatic lipid peroxidation activity. J. Food Biochem. **11:** 1-15.

Rule, D. C., Broughton, K. S., Shellito, S. M. and Maiorano, G. 2002. Comparison of muscle fatty acid profiles and cholesterol concentrations of bison, beef cattle, elk and chicken. J. Anim. Sci. **80:** 1202-1211.

SAS Institute Inc. 1996. SAS User's guide: Statistics. Version 6.11. SAS Institute Inc. Cary, NC.

Scollan, N., Hocquette, J. F., Nuernberg, K., Dannenberger, D., Richardson, I. and Moloney, A. 2006. Innovations in beef production systems that enhance the nutritional and health value of beef lipids and their relationship with meat quality. Meat Sci. **74:**17-33.

Steenbergen, J. 2011. Business Development – Elk/Bison, Alberta Agriculture and Rural Development. Personal communication.

Tarladgis, B. G., Watts, B. M., Younathan, M. T. and Dugan Jr., L. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. J. Am. Oil Chemists Soc. **37:** 44-48.

Trout, G. R. 1991. A rapid method for measuring pigment concentration in porcine and other low pigmented muscles. Int. Congress Meat Sci. Tech. **37:**1198.

Turner, T. D. 2005. Evaluation of the effect of dietary forages and concentrate levels on the fatty acid profile of bison tissue. MSc. Thesis University of Saskatchewan, Canada.

Wang, Z. and Goonewardene, L. A. 2004. The use of mixed models in the analysis of animal experiments with repeated measures data. Can. J. Anim. Sci. **84:**1-11.

Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Sheard, P. R., Richardson, R. I., Hughes, S. I. and Whittington, F. M. 2008. Fat deposition, fatty acid composition and meat quality: A review. Meat Sci. **78:** 343-358.

Yang, A. , Brewster, M. J., Lanari, M. C. and Tume, R. K. 2002. Effect of vitamin E supplementation on α-tocopherol and β-carotene concentrations in tissues from pasture- and grain-fed cattle. Meat Sci. **60:** 35-40.

CHAPTER 5

Summary and Conclusions

5.1 Summary of conclusions from studies

The working hypothesis for this body of work was bison meat quality traits from animals processed through the mobile location abattoir (MLA; MLAPEN animals dispatched in a pen, MLACON animals confined prior to dispatching) would exhibit superior meat quality traits compared to those transported, and processed at a land based facility (LAND). A higher incidence of carcasses graded as "dark" was observed in transported animals potentially due to faster chilling in the land based abattoir resulting in slower *post mortem* metabolism. Improved meat tenderness (ageing days combined) was observed in bison slaughtered through the MLA compared to those transported for slaughter at a land-based plant. This was evidenced through both shear force measurements (MLACON 7.28 kg and MLAPEN 7.40 kg compared to LAND 9.43kg) and the initial tenderness sensory evaluation scores (MLACON 4.95, MLAPEN 4.55, compared to LAND 3.93; where 8= extremely tender and 1= extremely tough). The MLAPEN group exhibited superior carcass and meat quality traits compared to either the MLACON or LAND group.

We also hypothesized that bison slaughtered on-farm through the MLA would have lower stress levels. This was not exhibited clearly in either the blood cortisol or neutrophil/lymphocyte ratio (N/L). The lowest blood cortisol level was found in MLAPEN group compared to the MLACON or LAND groups (71.16

nmol/L, 124.17 nmol/L and 139.50 nmol/L respectively; $P<0.01$). The MLACON group had the lowest N/L ratio (0.701 compared to MLAPEN 1.145 or LAND 1.367, $P<0.01$). Bruising was found in all treatment groups, however a lower incidence of bruising was observed in carcasses from the MLA groups compared to the LAND group. The value of lost trim on the carcass with the most bruising was $65.00.

Examining possible reasons for the observed difference in rates of discolouration between beef and bison was carried out. It was hypothesized that the fatty acid profile, vitamin E levels, and pigment levels would be linked to this observation. Bison and beef were found to have significantly different performance and stability in a retail environment. Bison meat had a favourable fatty acid profile from a human health perspective with PUFA levels over 3 times that of beef, a higher n-3 and n-6 fatty acid level than beef, and a lower n-6:n-3 ratio. Bison also had a lower total fat and higher pigment and vitamin E levels. Stepwise regression models included some of these traits and accounted for a significant proportion of the variation in MetMb ($R^2 = .689$), % discolouration ($R^2 = .737$) and appearance ($R^2 = 0.676$) between d 0 and d 3 in retail. The inherent tissue traits of bison were linked to poorer performance in the retail environment when compared to beef.

5.2 Mobile location abattoir

5.2.1 How did it work?

In total 55 bison were slaughtered for this project, 35 of which were processed through the MLA. The design of the MLA was sufficiently robust to

process the 10 bison per d utilized in the present study. One of the issues was the cost incurred to cool the carcasses. Cooling the carcass in the MLA unit to the required temperature of 4^{o}C (Meat Inspection Act, 2005) before offloading costs money, particularly if the MLA unit is powered by its diesel generator instead of being plugged in. It was estimated that the generator consumed up to 200 litres of diesel (McLeod and Dening, 2009) through the course of a slaughter day plus the cooling of 10 carcasses (approximately $180.00). In the present study, the bison were off-loaded 24-48 hours post slaughter (Mcleod and Dening, 2010). The larger and fatter the carcass, the longer required to cool (Appendix 5). The range of measured grade fat on the carcasses from this study was from a minimum of <1mm to a maximum of 35mm (Appendix 5) which covers the full range expected through different commercial bison production systems. Animals with grade fat levels over 10mm in the present study, came from a grain finishing production facility. There is approximately 7 hours difference in the elapsed time to reduce the carcass temperature of a fat carcass to 4^{o}C compared to a lean one (Appendix 5). This could represent a $25.00 difference in the cost to bring a fat vs. lean carcass down to the temperature where it could be offloaded from the truck. Some bison producers/marketers sell grass-finished but the majority sell grain-finished animals.

Throughput of the MLA unit is a concern with only 10 head per day which would be considered a low number for an abattoir. The capacity is limited by refrigeration rather than the slaughter/dressing procedure. Hence, throughput could be increased by transferring carcasses directly onto an additional

refrigeration truck through the MLA unloading door. This strategy would increase the daily capacity reducing individual unit costs. The estimated cost of running the MLA unit for a day is $2850.00 (Mcleod and Dening, 2010). If only 10 head of bison were processed then just to recover cost alone, an operator would have to charge at least $280.00 per head. This compares to a kill and disposal fee (for bison) of approximately $140.00 in a provincially inspected stationary plant. For a 260 kg carcass, this would translate to a price premium of approximately $0.77 per kg of meat (bone out) averaged across high and low end cuts. Steiner et al. (2010) found Alberta consumers' willingness to pay for farm origin traceability was between $1.30 and $2.90 per kg. The extra cost required to operate the MLA would need to be retrieved through a premium charged to the end consumer, and investigation into what the willingness to pay for a 'no transport' attribute would need to be undertaken to determine if this is feasible.

5.2.2 On farm recommendations

The data from this work support the pen shot scenario for on-farm slaughter of bison. The MLAPEN group had little disturbance immediately prior to being dispatched (compared to the MLACON and LAND group which were worked into either a confinement squeeze or knock box) resulting in observed lower serum cortisol levels. Also, animals confined (MLACON) prior to dispatch, compared to pen-shot (MLAPEN) animals showed evidence of higher stress in the immediate pre-slaughter period with 13% of these carcasses having pale, wet meat colour at grading and higher drip losses. However, the drawback to MLAPEN scenario is the increased risk of shooting into a pen of animals which increases the risk of injury to animals or humans through possible ricochet from a

missed shot. A compromise between the two scenarios could be to run an animal into a confined space and immediately dispatch it without delay negating welfare concerns which develop when herd animals wait alone in a confined area. The disturbance to the remaining animals from hazing one out could be seen as a similar disturbance to that of removing a pen shot animal with a tractor. Where possible, having more animals in the pen than what is needed for the day's slaughter prevents the last animal from being alone. This is how the MLAPEN group was handled in the current study.

Other factors that were not investigated in this study, but which could impact relative stress levels in a bison herd when using the MLA unit include placement of the tractor unit in proximity to where the animals are being held, the familiarity of animals to a tractor and people, and the number of 'strangers' visible to the herd. It was evident through personal observation of the animals in the current study that there is a variation in response to people in different bison herds; some are quite used to human contact, and others initiate a flight response from the slightest movement.

5.2.3 Opportunity

In the province of Alberta in 2010 there were approximately 4,100 bison, 400 elk, 31,000 beef, and 27,000 sheep slaughtered in provincially inspected abattoirs (Steenbergen, 2011; Emunu, 2011). Meat from provincially inspected abattoirs can only be sold within Alberta. Alberta Agriculture and Rural Development employ meat inspectors to do the ante- and post-mortem inspection at these facilities. The MLA used in this study had Alberta provincial inspection

level, but had smaller throughput than most land based provincial abattoirs. Based on the number of head processed in AB in provincially inspected abattoirs, there should easily be room for one or more MLA unit, particularly in regions where there isn't a land based abattoir nearby. The MLA could be used in low volume high value sales, utilizing the zero transport, improved animal welfare and improved quality attributes to add value for the discerning consumer. However, on-farm handling facilities and methods will need to be standardized in order to provide welfare and quality guarantees.

5.3 Limitations of the experiments

There were some issues trying to conduct scientifically controlled studies utilizing commercial operations. One reason was that the MLA was provincially inspected and there were few bison producers dealing in provincially inspected meat who were able to handle 15 bison carcasses in one week. Typically they would process fewer numbers spread over more time. One potential producer couldn't complete construction of his handling facility in time, and notified us 10 days prior to the planned harvest day. Another co-operator was recruited and while he could accommodate the project on short notice, he had only heifers available which compromised the original scientific design which was to use exclusively male bison. Also, one of the farms supplied a mixture of male and female bison despite being recruited on the basis of supplying only male bison.

5.4 Future studies

Consumer education about inherent differences with bison meat and possibly mitigating packaging strategies should be investigated for bison meat. Bison meat has different properties than beef, and consequently should be treated differently with different expectations of colour, fat level etc. While it is necessary to draw on literature from other livestock, the results from this work support that there are differences between beef and bison such that more research specific to bison is required to fully understand how to best present their bison meat products to consumers.

A study to determine the stress levels and any resulting meat quality aberrations with varying amounts of transport times would assist the commercial industry understand how transport affect bison, and could support the development of more mobile abattoirs for certain meat marketing business structures. Also critical for the development of a branded product using the MLA would be further work into the standards for on-farm facilities needed specifically for optimal handling prior to processing.

A study comparing fatty acid profiles and shelf life of bison finished on a variety of diets (grass, grain, silage, etc.) and supplemental levels of vitamin E would provide a better understanding of how to best feed for improved shelf life stability. This could help in understanding how bison differs from, or reacts similar to, cattle in the manipulation of fatty acid profiles and could overlay work in developing packaging strategies best suited for the characteristics of bison meat.

The Alberta bison industry could benefit from an investigation into factors affecting the end consumer's satisfaction of bison meat. A model similar to the Australian Palatability Assured Critical Control Point (Polkinghorne, 2006) could be developed, particularly for the bison processed through provincial abattoirs where there is no carcass grading. An inspected mobile slaughter facility could play an important role in improving *ante mortem* animal welfare in such a model. In addition, information about the effects of feeding, carcass suspension, chilling, and cooking methods for different cuts, could be put together with the end goal of ensuring the consumer is consistently satisfied with the product.

5.5 Literature Cited

Emunu, J. P. 2011. Livestock statistician, Alberta Agriculture and Rural Development. Personal communication.

McLeod, B. and Dening, B. 2010. Multi location abattoir final report. Alberta Agriculture.

Meat Inspection Act, Meat Inspection Regulation. AR 42/2003 with amendments up to and including Albert Regulation 244/2005. Alberta Queen's Printer, Edmonton, AB. Canada. **68:**30.

Polkinghorne, R.J., 2006. Implementing a palatability assured critical control point (PACCP) approach to satisfy consumer demands. Meat Sci. **74:** 180-187.

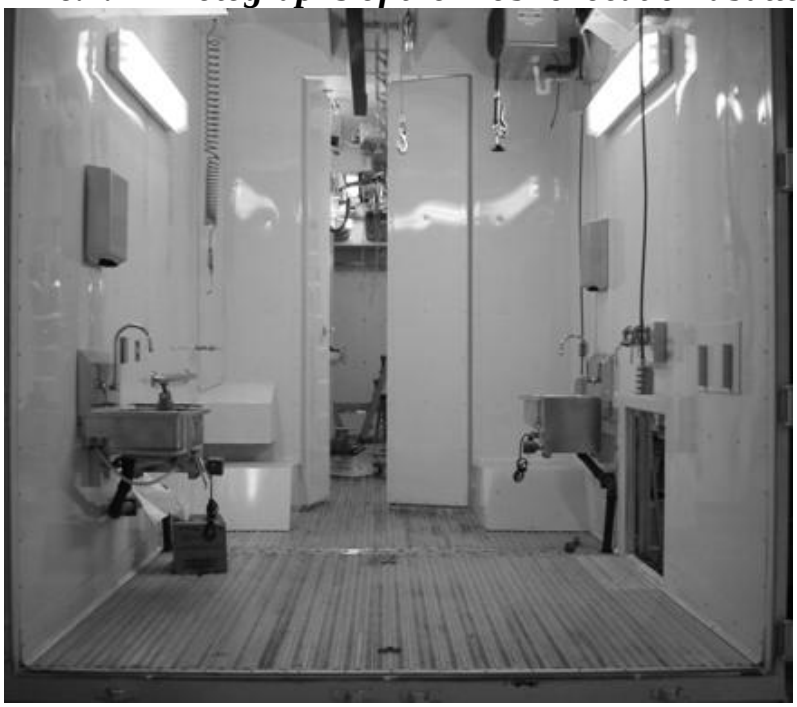
Steenbergen, J. 2011. Business Development – Elk/Bison, Alberta Agriculture and Rural Development. Personal communication.

Steiner, B., Gao, F. and Undershultz, J. 2010. Alberta consumers' valuation of extrinsic and intrinsic red meat attributes: a choice experimental approach. Can. J. Agric. econ. **58:** 171-189.

APPENDIX

6.1 Appendix 1

6.1.1 Photographs of the Mobile location abattoir:



6.2 Appendix 2

6.2.1 Whole Foods letter of support

Animal compassion FOUNDATION

January 8, 2008

Jayson Galbraith
Alberta Agriculture & Food
Agri-Food Business Centre
6547 Sparrow Drive
Leduc, AB, Canada
T9E 7C7

Dear Mr. Galbraith,

This is a letter of support for the multi-phase project *Optimizing slaughter strategies (multi-location abattoir) to manage ante-mortem physiological stress, carcass and meat quality* being conducted by the research team which includes yourself, Wayne Robertson, Dr.'s Al Schaefer, Jennifer Aalhus, Nigel Cook at Alberta Agriculture as well as Staff at Old's College.

The Animal Compassion Foundation™ (*a Whole Foods Market Foundation*) is a producer education and research center dedicated to improving the lives of farm animals. Founded in 2005, the Foundation supports a worldwide network of producers and researchers, leads and funds on-farm research and develops producer workshops. The Foundation has 501(c)(3) status as a private operating foundation.

The objectives of this project reflect the Foundation's main goal; to learn and share best practices that promote farm animal well-being using evidence-based research. It is well documented in the scientific literature that handling and long distance transport of animals can have significant detrimental effects to both their welfare and to meat quality. The utilization of a mobile slaughter plant could improve animal welfare and meat quality by reducing handling stress, hunger, thirst, injuries caused from mixing with unfamiliar animals and those that could be sustained during loading, unloading and/or from the vessel. While multi-location abattoirs have been developed for testing in Texas, Wyoming and New Mexico, this project is unique in that it will compare three different slaughtering scenarios thereby facilitating a direct comparison between treatments. With the Canadian Food Inspection Agency currently reviewing transport times of animals, and with

550 Bowie Street, Austin, Texas 78703 Tel: 512-542-0640 Fax: 512-482-7640
www.animalcompassionfoundation.org

the proposed revised transport times being equal to or exceeding 24 hours for pigs and ruminants, this project could have a significant impact on the millions of animals slaughtered in Canada each year.

The Animal Compassion Foundation™ has produced 11 workshops in the US over the last 12 months that are designed specifically for producers. The workshops offer expertise, updates and practical on-farm solutions in small, informal setting at a low-cost to the producer. As well, the Foundation has funded 10 on-farm collaborative projects in four countries on topics covering many aspects of farm animal production. The Foundation plans to use the results and recommendations from this project to create educational materials, and would also consider becoming a funding partner on this project.

As a research and education stakeholder, the Animal Compassion Foundation™ fully supports this project and believes that the results of these findings will promote positive farm animal welfare.

Yours sincerely,

Anne Malleau
Director of Research & Education

550 Bowie Street, Austin, Texas 78703 Tel: 512-542-0640 Fax: 512-482-7640
www.animalcompassionfoundation.org

6.3Appendix 3

6.3.1 Canadian Bison Association letter of support

Canadian Bison Association

Association Canadienne Du Bison

Suite 200-1660 Pasqua Street

P.O. Box 3116, Regina, SK S4P 3G7

(306) 522-4766 Phone

(306) 522-4768 Fax

November 7, 2007

Wayne Robertson, Meat Quality Biologist
Lacombe Research Centre, 6000 C & E Trail
Lacombe, Alberta, Canada T4L 1W1

RE: Researching the Impact of Stress on Bison Meat Quality

Dear Mr. Robertson;

The bison industry continues to work with stakeholders to minimize those factors that may have a negative impact on the quality of bison meat. To this end, the Canadian Bison Association endorses the project being proposed by Wayne Robertson, Al Schaefer, Jennifer Aalhus, Nigel Cook, and Jayson Galbraith.

The objectives of the study will be to compare indices of physiological stress, carcass characteristics and meat quality attributes of animals slaughtered traditionally in land-based facilities following a period of transport to animals slaughtered in a multi-location abattoir at their home location .
This research will evaluate if on-farm slaughter using a multi-location abattoir is physiologically less stressful to bison than slaughter preceded by transport and lairage, and if on-farm slaughter results in improved meat quality. The Canadian Bison Association sees value in the knowledge that is expected to be gained from the proposed research for not only the many marketers of bison meat to local markets, but also for the larger marketers for information about the impacts of handling, and transport on meat quality factors. The extension of this research, to evaluate the market opportunity for marketing branded bison meat that informs the consumer about the use of a multi-location abattoir, will also be valuable to assist in developing marketing strategies for the industry.

We look forward to the results of this research.

Yours truly, Mark Silzer, President Canadian Bison Association

6.4Appendix 4

Canadian Bison Grades

AGE	Quality Factors	A				B			D		
		A1	A2	A3	A4	B1	B2	B3	D1	D2	D3
Maturity Class I	Muscling	Excellent to Good				Excellent to Good	Excellent to Medium	Deficient to point of emaciation			
(Youthful)	Fat Colour	White to Amber and Firm				White to Amber and Firm	White to Yellow	No Requirements			
Caps ossified 80% or less	Meat Colour	Bright Red and Firm				Bright Red and Firm	Bright to Dark Red	No Requirements			
	Fat Measure	2 - 6 mm	7 - 12 mm	12-18 mm	> 18 mm	<2 mm		No Requirements			
Maturity Class II	Muscling								Excellent to medium	Excellent to medium	< Medium
(Mature)	Fat Colour								No Requirements		
Caps ossified over 80%	Meat Colour								No Requirements		
	Fat Measure								2-6 mm	6 + mm	< 1 mm

(http://laws-lois.justice.gc.ca/eng/SOR-92-541/index.html)

6.5Appendix 5