IMPACT OF WINTER FEEDING AND VACCINATION STRATEGIES ON CARCASS OUTCOMES IN BEEF CATTLE

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ABSTRACT

Two studies were conducted to determine the impact of management practices on carcass outcomes in beef cattle production systems: a) the impact of corn supplementation of cows (non-supplemented = 23 vs. supplemented = 24), when fed low-quality forage-based diets at d 110 of gestation for 22 wks on carcass outcomes of their offspring; and b) the effect of a needle-free injection device (NFID = 22) versus needle syringe (NS = 20) on injections site reactions in Angus steers vaccinated and boostered with modified live bovine viral diarrhea. Steers from both maternal treatments did not differ in carcass traits and meat quality ($P > 0.05$). Injection site lesions were not observed in the carcass tissue from both NF and NS-steers. In conclusion, maternal corn supplementation does not affect the carcass outcomes of the offspring. Also, the use of NF technology does not cause tissue damage and would help to eliminate the presence of broken needles that can occur with NS injections.

Key words: beef, corn supplementation, carcass outcome, needle-free injection systems

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FOREWARD

This thesis is written in manuscript style and is composed of two manuscripts: i) Impact of maternal corn supplementation on the growth performance and carcass outcomes of their offspring and ii) Impact of needle-free injection device on injection-site reactions in beef sub-primals. Both manuscripts have been formatted based on the Canadian Journal of Animal Science guide for manuscript preparation.

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ABBREVIATIONS

°C	=	degree Celsius
AAFC	=	Agriculture and Agri-Food Canada
AB	=	Alberta, Canada
ADG	=	average daily gain
AF	=	Alexa Fluor
AMSA	=	American Meat Science Association
AOAC	=	Association of Official Analytical Chemists
BCS	=	Body Condition Score
BVD	=	bovine viral diarrhea
BW	=	body weight
CCAC	=	Canadian Council of Animal Care
cm	=	centimeter
CMC	=	Canadian Meat Council
CP	=	crude protein
d	=	days
DDGS	=	dried distiller's grains
g	=	gram
h	=	hours
hd	=	head
HCW		hot carcass weight
IBR	=	Infectious bovine rhinotracheitis

IRT	=	infrared thermography
ITS	=	injection type system
KCl		potassium Chloride
Kg	=	kilogram
KPO_4	=	monopotassium phosphate
LC	=	Langerhans cell
MB	=	Manitoba
MD	=	monitoring days
$MgCl_2$	=	magnesium chloride
MHC	=	Myosin heavy chain
MFI	=	myofibrillar fragmentation index
MLV	=	modified live vaccine
mo		months
N	=	number
NaN_3	=	sodium azide
NBQA	=	National Beef Quality Audit
ND	=	North Dakota
NDSU	=	North Dakota State University
NFID	=	needle-free injection device
NRC	=	National Research Council
NS	=	needle syringe
OCT	=	optimal cutting temperature

PBS	=	phosphate buffer saline
PVC	=	polyvinyl chloride film
SC	=	subcutaneous
SKINR		skin reaction
SSS	=	standard salt solution
TDN	=	total digestible nutrient
TMR	=	total mixed ration
VBP^{+}	=	Verified Beef Production Plus Program
WBDC	=	Western Beef Development Centre
WBSF	=	Warner-Bratzler shear force

1. GENERAL INTRODUCTION

In western Canada, many beef producers have adopted extensive overwintering grazing management strategies such as stock-piled forages, swath and bale grazing (Sheppard et al. 2015) in order to reduce overall feeding costs. However, cattle exposure to extended periods of cold weather (mean daily temperature of -10 ºC to -20 ºC, with temperatures as low as -30ºC) results in increased energy demand (Young 1981). Maintenance energy requirement for beef cattle may increase by 30 to 70% in Canadian winter conditions (Jordan et al. 1968; Lister et al. 1972). As many herds in western Canada calf in March (Sheppard et al. 2015), cows may experience an increase in nutrient demand associated with decreased temperatures and increased fetal growth during the last trimester of pregnancy. Further, forage quality may not be sufficient to meet this increased nutrient demand. A forage survey conducted in Saskatchewan (Government of Saskatchewan 2014) demonstrated that only 38% forage sampled met energy requirements for cows at 6 mo gestation and only 5% of sampled forages provided adequate energy for cows in final months of gestation.

This adverse prenatal environment can permanently affect fetal development, which can result in both short and long-term impacts in the offspring such as increased calf mortality, digestive and respiratory dysfunction, metabolic disorders, decreased post-natal growth rates and reduced meat quality (Wu et al. 2004, 2006; Larson et al. 2009; Underwood et al. 2010; Long et al. 2012). Thus, maternal nutrition during gestation plays a critical role in the pre and post-natal growth performance, as well as carcass outcomes of the offspring. Therefore, positive development of the fetus can be achieved through proper management of cow nutrition during gestation (Wu et al. 2006).

The impact of maternal nutrition on fetal development varies depending on the restricted nutrient, the duration of restriction, and the timing of restriction. Corah et al. (1975) demonstrated energy restriction in gestating heifers during the last trimester produced low birth weight calves and also reduced weaning weight. However, other authors have reported negative maternal energy level during mid-gestation did not influence calf birth weight, weaning weight or carcass outcomes (e.g. carcass weight) in the offspring (Long et al. 2012; Mohrhauser et al. 2015). Protein restriction of dams can also alter growth performance and carcass outcomes in subsequent calves (Du et al. 2017; Funston et al. 2010a) due to reduced skeletal muscle fibre numbers and changes in muscle fibre composition (Du et al. 2010). It has been reported that calves born from protein restricted cows during mid- or late gestation had poor growth performance (lower average daily gain (ADG) and lighter final body weight), lighter carcasses, decreased backfat thickness, lower marbling score (low US Choice grade carcasses) and higher shear force values (less tender meat) compared with those of non-restricted cows (Larson et al. 2009; Underwood et al. 2010). Thus, it is important to examine winter nutritional management strategies of the cows during mid- to late gestation reared in western Canada and the impact on progeny growth performance, carcass composition and meat quality.

In addition to feeding management, vaccination of livestock is also a crucial aspect of management in the cattle industry. Use of a needle syringe (NS) is the standard technique used for vaccination against diseases, as it is inexpensive and simple to perform (Rey et al. 2015). However, this technique can result in tissue damage, broken needle fragments and disease transmission (Kale and Momin 2014). Tissue damage can result in trimming and discarding of meat cuts, as well as reduced meat tenderness; both of which result in

significant economic loss to the meat industry. Injection site lesions cost the beef industry $0.56/head or $1.63 million in 2016 (Beef Cattle Research Council 2018). Therefore, alternative vaccine strategies are needed to minimize injection site lesions, as well as limit animal stress, prevent disease transfer and ensure the safety of the handler and consumer (Chase et al. 2008) should be explored. Hence, a needle-free injection device (NFID), which has been used in the hog industry may be a suitable alternative.

Evaluation of the immune response associated with vaccination using NFID vs NS has demonstrated that NFID produces an antibody response comparable to NS (Van Drunen Littel-van den Hurk 2006; Pires et al. 2007; Rey et al. 2015). More specifically, immune response following IBR and Clostridial vaccination has been shown to be comparable using NFID and NS systems (Rey et al. 2015). However, the presence of injection site reactions following vaccination suggested that pressure trauma associated with vaccination could cause subcutaneous and muscle tissue damages beyond the skin reaction and potentially persist until slaughter.

The objectives of this thesis were to: a) to determine the impact of corn supplementation of cows during mid- to late gestation on growth and carcass outcomes of their offspring, and b) to determine the effect of NFID on tissue damage and beef sub-primal quality.

2. LITERATURE REVIEW

2.1. Maternal winter feeding and fetal programming

According to the Western Beef Development Centre (WBDC 2018), the cost of production for a cow-calf operation is approximately $635/cow/ year. Larson (2013) reported that the annual cost of production for feeding was $1.18/cow per day during winter, where average cost per grazing day was $0.53/pair/day ($109/cow divided by 205 d). The winter feeding season in western Canada ranges between 150 and 210 d (WBDC 2018) and feeding costs for bale grazing, swath grazing and traditional dry lot system could be $0.83, $0.31 and $0.86/cow per day respectively (Kelln et al. 2011). Winter feeding for these days represents approximately 60-65% of total production costs and is three to five times more costly than summer feeding in cow-calf operations in western Canada (Kaliel and Kotowich 2002). Similarly, in the Northern Great Plains (USA), winter feed cost account for 67% of the cost per cow (USDA 2010).

Therefore, many producers have adopted extended grazing strategies to reduce the cost of production. Maintaining beef cattle on pasture beyond the normal grazing season (McCartney et al. 2008) is called extending grazing. Extending grazing methods namely, swath, bale and stockpile grazing are gaining popularity in western Canadian beef producers as they offer economic benefits over conventional confined feeding (Baron et al. 2014; McCartney 2008, 2016). This grazing system helps to reduce or eliminate labour and machinery for harvest and storage of feed (McCartney et al. 2008; Baron et al. 2014; Kulathunga et al. 2016). Also, animals are brought to the field for grazing which aids in

the natural dispersion of manure decreasing manure management cost and enhancing the nutrient profile of the grazed land (Jungnitsch 2011; Kelln et al. 2011).

A survey, which consisted of 1009 operations in the east and prairie region of Canada, states that 58% practiced winter grazing including 44% grazed rolled forages, 42% grazed bale, 29% stockpile forages, 25% swathed cereal crops, 7.1% grazed standing corn and 1.9% other feedstuffs (Shepherd et al. 2015). Likewise, the Western Canadian cow-calf survey (WBDC 2018) reported that out of 261 producers responding to the survey, 62% of producers practice winter grazing including 47% bale grazing, 41% using crop residues, 40% stockpile grazing, 13% grazing standing corn and 28% swath grazing.

Annual forage grazing systems have been used by cow-calf producers as an alternative to mitigate the reoccurring draught and perennial pasture shortage (McCartney et al. 2008). Grazing of standing corn and swath grazing are common practice in Canadian prairies (Baron et al. 2003). Winter grazing of standing corn is becoming more prevalent due to its feasibility and ability to produce large amounts of biomass (McCaughey et al. 2002). However, Western Beef Development Centre (WBDC 2018) reported that 6.4% of the western cow-calf producers choose not to extensively winter graze their cattle due to concerns about feed wastage, losing animal body condition, lack of watering system at pasture, too low temperature and animal welfare.

As indicated above, nutrient intake may be compromised during the winter season due to utilization of low quality forages which results in a negative energy/protein balance (NRC 1996). According to NRC (2016), a maintenance energy requirement for grazing animals is 20% greater than the confined animal. In extended grazing systems, cattle are exposed to the cold ambient temperatures (Sheppard et al. 2015), which is characteristic of

prairie Canada (occasionally reaching -30°C or lower). In addition, animals are exposed to wind (McDonald et al. 2002) without the advantages of a bedding pack which may generate considerable heat (Boadi et al. 2004). These conditions result in increasing nutrient demand (Young 1981) and may further lead to a nutrient deficiency in gestating cows compared to penned cattle.

Inadequate nutrition during the gestation period results in decreased body condition score at calving, as well as a prolonged anestrus period and reduced pregnancy rate (Martin et al. 2007). Low body condition score not only impacts the reproductive performance of cows but also affects prenatal fetal growth, which ultimately affects growth, development and reproductive health of the offspring (Rhind 2004).

Maternal in-utero insults (i.e. nutrient deficiency) during the prenatal development of the fetus may have a long-term impact on the offspring and is known as fetal or developmental programming (Funston et al. 2010a). The idea of fetal programming was first established in humans, and it is known as the 'Barker hypothesis' (Gardner et at. 2008). Barker et al. (2002) reported that poor maternal nutrition impacted birth weight in babies, and had long-term implications for adult health including an increased incidence of diseases like diabetes, heart diseases, and hypertension. This concept of nutrient deficiency in utero has more recently been investigated in terms of impact on cow performance as well as the offspring's productivity and health (Larson et al. 2009).

2.2 Prenatal growth and development

2.2.1 General growth process of the bovine fetus

Growth is defined as an increase in size, which involves a gradual process of hypertrophy (enlargement of existing cells), hyperplasia (multiplication of new cells) and accretionary growth (increase in the non-cellular structural material), as described by Wu et al. (2006). Growth involves an increase in muscle, bone, and adipose tissue, while development involves the cell differentiation process giving rise to the specialized cell types with specific functions in the embryonic, fetal and neonatal stages (Forrest et al. 1975).

The commencement of growth begins with fertilized ovum or zygote and the period from conception to birth is referred as prenatal growth phase involving ovum, embryonic and fetal phases. The ovum phase lasts for 11 d and includes the stage from fertilization to implantation (Evans and Sack 1973). It is characterized by the formation in the blastocyst of three cell types known as the germ layers: the ectoderm, the mesoderm, and the endoderm, from which the embryonic membranes and fetal tissue develop (Forrest et al. 1975). The duration of the embryonic phase is 25-45 d and is characterized by the differentiation of tissue, organ and various systems involving a gradual change in the embryo (Forrest et al. 1975). The remaining period is termed the fetal phase, which ends at birth, and includes development of nervous system, heart, liver, and kidneys (Forrest et al. 1975).

Maximum placental development, blood vessel formation and differentiation occur during the early stages of fetal development (Funston et al. 2010a). The placenta plays a significant role in transportation of respiratory gas, as well as nutrient and waste exchange between the maternal and fetal systems (Reynolds et al. 2009), and therefore, it regulates fetal growth and development (Funston et al. 2010b). In addition to placental development,

fetal organogenesis also occurs in early gestation. In the bovine fetus, sequential development of the organs includes, heart, pancreas, liver, adrenals, lungs, thyroid, spleen, brain, thymus, and kidney (Hubbert et al. 1972; Funston et al. 2010a). Systems including the circulatory and respiratory systems, which have functional importance develop in the early stages of fetal growth, develop first, whereas systems like the digestive tract, which are important only after birth, are developed during the later stages of fetal life (Forrest et al. 1975). In addition, muscle and adipose tissue develop during mid- to late gestation (Du et al. 2010).

2.2.2 Fetal skeletal muscle development

The growth and development of muscle starts with the myogenesis leading to muscle tissue formation during the embryonic stage (Forrest et al. 1975). In the embryo, embryonic skeletal muscle cells become myogenic progenitor cells (somites) which are derived from the mesoderm (germ layers), splitting to form dermomyotomes and sclerotome. The dermomyotomes (dorsal portion of the paraxial mesoderm somite), give rise to skin, muscle and fat cells, whereas the sclerotomes (ventral portion) develop into the skeleton and ribs (Aoyama and Asamoto 1988; Bailey et al. 2001). The dermomyotomes separate and become dermatome and myotome. Myotome is differentiated into the myoblast (Forrest et al. 1975) which proliferates and fuses to form primary muscle fibres (myotubes). In the bovine fetus, primary muscle formation occurs early in the first trimester (conception to 2 mo). Formed primary muscle fibres then act as the template for the formation of the secondary muscle fibres (Du et al. 2010) through

continuous proliferation of the precursor cells surrounding the primary muscle fibres (Beermann et al. 1978). Secondary muscle fibres form from 2 mo until 7 to 8 mo.

At the end of mid- gestation or during late gestation, muscle fibre formation decreases and muscle growth occurs through an increase in muscle length and size. However, proliferation of myogenic cells continues, fusing with the existing muscle fibres and increasing the diameter of previously formed muscle fibres. The length of the muscle fibre increases with myofibrillar protein synthesis (Du et al. 2017). In cattle and sheep, muscle fibre number is fixed at birth and does not increase in post-natal life (Du et al. 2017).

During late gestation, both (primary and secondary) muscle fibres structurally resemble mature, fully formed muscle fibre (Aberle et al. 2001). At this stage, four myosin isoforms can be identified in skeletal muscle: Type I are slow twitch fibres originating from primary muscle fibres, and type II are fast twitch fibres originating from secondary muscle fibres (Brooke and Kaiser 1970). Further, subtypes of the type II fibres include type IIB (fast twitch fibres with limited oxidative capacity), type IIA and IIX (fast twitch fibres with intermediate oxidative and high glycolytic capacity; Klont et al. 1998). The characteristics of the fibre types are given in the Table 1.

Some of the proliferating myogenic cells also form satellite cells. Satellite cells are the quiescent (inactive, no growth and no division) myogenic cells positioned underneath the basal lamellae or connective tissue which are activated in muscle injury and help in regeneration of muscle fibre (Morgan and Patridge 2003). These satellite cells act as reserve cells for post natal muscle growth and are responsible for muscle fibre size increase (Du et al. 2017), which occurs mainly through an increase in the DNA content of the muscle

fibre and increased myofibrillar protein synthesis as the satellite cells fuse with the muscle fibre (Kuang et al. 2007). Thus, postnatally, skeletal muscle development occurs mainly through an increase in muscle fibre size without increase in the muscle fibre number.

Table1. Characteristics of skeletal muscle fibres found in meat animals (Aberle et al. 2001)

Characteristics	Type I	Type IIA	Type IIX	Type IIB
Redness	++++ (red)	+++	+	+ (white)
Myoglobin content	++++ (high)	+++	+	+ (low)
Fibre diameter	+ (small)	+	+++	++++ (large)
Contraction speed	+	+++	+++	++++
Fatigue resistance	++++	+++	+	+
Contractile action	tonic	tonic	phasic	phasic
Number of mitochondria	++++ (high)	+++	+	+
Oxidative metabolism	++++ (high)	++++	+	+ (limited)
Glycolytic metabolism	+ (limited)	+	+++	++++ (high)
Capillary density	++++	+++	+	+

2.2.3 Fetal adipose tissue development

Adipogenesis or adipocyte formation begins at the end of the first trimester of gestation in ruminant animals, and therefore, overlaps with the formation of the secondary muscle fibres (Du et al. 2017). Extensive adipogenesis also occurs during late gestation to early weaning stages (Du et al. 2013).

Adipoblast cells are formed from embryonic mesenchyme of the mesoderm layer (dermatome), which differentiates into pre-adipocyte (Forest et al. 1975; Du and McCormick 2009). Accumulated lipid droplets start to fuse within the pre-adipocyte and develop into a single large droplet of lipid; the cell structure is now termed a "mature adipocyte" (Forrest et al.1975; Hausman et al. 2009). The large lipid droplet is present at the center, pushing cell organelles to the edge of cell. Lipid present in adipocytes is responsible for maintaining the energy of the fetus (Forrest et al. 1975). The initial events of adipose tissue development (adipocyte accumulation) form lobes and lobules which are enclosed by collagenous fibres and supplied with a vascular network (Forest et al. 1975; Alberle et al. 2001; Muhlhausler et al. 2006).

The sequence of fat deposition in cattle fetus occurs in the following order: visceral fat, subcutaneous fat, intermuscular fat and intramuscular fat (Du et al. 2017). The perirenal fat development occurs at 80 d after conception (Bonnet et al. 2010). The formation of intramuscular adipocytes occurs after late gestation and continues until 250 d of age, this period is also known as the 'marbling window' and it provides enough opportunity to increase only intramuscular adipocytes formation which has high commercial value (Du et al. 2013).

2.2.4 Fetal connective tissue development

Connective tissue supports, holds and connects different body tissue and organs together including, skin, muscles, cardio-vascular, tendons and lungs (Forest et al. 1975). Cartilage and bone are supportive connective tissue whereas connective tissue proper is considered as fibrous connective tissue (Forest et al. 1975). Connective tissue consists

mainly of cells and extracellular fibres (Karunaratne et al. 2005). The cell components include fixed cells, fibroblasts (close related to the properties of meat) and wandering cells (eosinophils, plasma cells, mast cells, lymph cells and macrophages, which are involved in injury reaction; Forest et al. 1975). Fibroblasts are responsible for production and maintenance of extracellular fibres (Forest et al. 1975). Fibroblasts synthesize precursors of extracellular components of connective tissue, namely tropocollagen, tropoelastin, and ground substance, which are released into the extracellular matrix, developing collagen and elastin fibers.

Fibroblast and adipocytes share a common pool of progenitor cells called fibro/adipogenic progenitors (FAPs; Du et al. 2017). FAPs originate from the dermomyotomes during the prenatal stage and they are continuously present in adipose tissue in the postnatal phase, however, the density reduces with the chronological age of animal (Yan et al. 2013; Du et al. 2015;). Fibrogenesis occurs during mid- to late gestation as does adipogenesis (Due et al. 2010; Yan et al. 2010). Fibroblasts, along with adipocytes formed during mid- gestation, form connective tissue of the fetal skeletal muscle namely, endomysium, perimysium and epimysium during late gestation (Du et al. 2010).

2.3 Impact of maternal nutrition on prenatal growth and development

2.3.1 Impact of maternal nutrition on fetal skeletal muscle development

As described above, the fetal phase of skeletal muscle development could have irreversible long term consequences for offspring growth performance (Stannard and Johnson 2004; Zambrano et al. 2005). Fetal skeletal muscle development is impacted by maternal nutrition (Zhu et al. 2004). Maternal nutrition helps to regulate the amount of fetal

insulin-like growth factors (IGF) in fetal blood, including IGF-1 and IGF-2, which helps in proliferation of myogenic precursor cells. These precursor cells are important for muscle fibre formation (Gonzalez et al. 2013). Inadequate maternal nutrition can alter normal skeletal muscle development (Du et al. 2017; Gonzalez et al. 2013).

Early to mid-gestation is a critical stage for fetal skeletal muscle development in both cattle and sheep (Yan et al. 2013). Skeletal muscle is vulnerable to low maternal nutrition, because muscle is a low priority tissue during nutrient partitioning compared to vital organs including, brain, heart and liver (Zhu et al. 2006). Zhu et al. (2004) determined the effect of a 50% nutrient restriction (total digestible nutrient) in ewes during early gestation, d 28 to 78, on fetal skeletal muscle development. These researchers observed decreased secondary muscle fibres and a secondary/primary muscle fibre ratios in the fetuses from nutrient-restricted ewes compared to control ewes. Beef cattle fed a diet 40% below nutrient requirements during early to mid-gestation (d 85 to 140), had reduced fetal IGF-1 expression and myogenic progenitor cell density resulting in reduced fetal muscle fibre number (Gonzalez et al. 2013). Decreased fetal muscle fibre numbers have also been observed in offspring from nutrient restricted ewes (50% of NRC requirement) compared to those fed diets to meet requirements (Zhu et al. 2006). Thus, inadequate maternal nutrient in early and mid-gestation results in a decrease in muscle fibre number (Du et al. 2010), leading to negative consequences in post-natal performance and muscle mass of offspring (Stickland, 1978; Zhu et al., 2004).

Muscle fibres mature during late gestation, at approximately 105 d and 210 d in sheep and cattle; respectively (Du et al. 2010). Therefore, inadequate maternal nutrition

during late gestation does not affect the muscle fibre number; however, the muscle fibre size is vulnerable at this time (Du et al. 2010; Greenwood et al. 1999).

Alternatively, excess nutrients through the increasing of feed allowance could impact the number and type of muscle fibers. Extra feed provided to sows from d 25 to d 50 of gestation (during primary fiber formation) increased the number of muscle fibers (Dwyer et al. 1994a; Gatford et al.2003). Cerisuelo et al. (2009) demonstrated the impact of that extra feed to sows (1.5 to 2.0 kg/d more than control) from 45 to 85 d of gestation and reported that the progeny had fewer muscle fibers and fewer estimated primary and secondary fibers than pigs born to control mothers. The smaller number of muscle fibers found in the supplemented pigs was associated with fewer type IIB fibers with greater cross-sectional areas.

2.3.2 Impact of maternal nutrition on fetal adipose tissue development

Fetal adipose tissue formation begins at mid-gestation, but late gestation to early weaning stage (<250 d of age) is considered the most important stage for adipose tissue formation (Du et al. 2017). Therefore, manipulation of maternal nutrition during these period affects the hyperplasia of adipocytes and adipocyte development in different body depots including marbling (Du et al. 2013), which is an important attribute for palatability (flavor and juiciness) of meat and improves meat quality (Du et al. 2017; Yan et al. 2013). This has been shown in 8 mo old offspring born from ewes subjected to a 50% nutrient of total NRC requirements during early to mid-gestation, resulting in increased visceral fat including, kidney and pelvic fat compared to offspring from control ewes (Zhu et al. 2006). Similarly, increased adiposity was reported in the offspring from ewes on a low plane of

nutrition (60% of the required ME) during gestation (Bispham et al. 2003). Further, maternal dietary restriction from d 30 to 70 of gestation (50% of NRC requirements) resulted in increased fat: lean ratio in the lamb compared to the control group (Daniel et al. 2007). Maternal restriction during early to late gestation also resulted in a decrease in intramuscular adipocyte number (Du et al. 2010).

Unlike maternal undernutrition, maternal overnutrition mainly enhances intramuscular adipogenesis or marbling by stimulating peroxisome proliferator-activated receptor (PPAR; a marker of adipogenesis) and inhibiting adenosine monophosphate-activated protein kinase (AMPK) (Tong et al. 2008). Fetuses from ewes fed at 150% of NRC recommended for energy intake during d 60 before conception until 135 of gestation (late gestation) demonstrated increased number and size of intramuscular adipocytes (Yan et al. 2010). Since, intramuscular adipocytes are formed at late gestation at approximately 250 d of age, which can also be called the 'marbling window', as it provides an opportunity to increase intramuscular adipocyte formation (Du et al. 2013).

2.3.3 Impact of maternal nutrition on fetal connective tissue development

Fibrogenesis occurs actively during the prenatal stage and plays a crucial role in meat tenderness and texture (Duarte et al. 2014). Therefore, understanding the impact of maternal nutrition on the prenatal fibrogenesis and its effect on the offspring skeletal muscle is vital to the meat industry (Duarte et al. 2014). However, few studies have provided evidence to demonstrate the relationship between maternal nutrition and fibrogenesis and the impact on collagen content of the skeletal muscle of offspring (Du et al. 2010). Underwood et al. (2010) did not find differences in the amount of total collagen

in steers from dams grazing improved pasture (11% CP) versus steers from dams grazing native range (6.5% CP).

Offspring born from cows fed at 1.5 times maintenance requirement exhibited enhanced collagen deposition in skeletal muscle compared with offspring born to cows fed a control diet (1.0 times maintenance requirement; Duarte et al. 2014). Also, Huang et al. (2012) reported enhanced intramuscular fibrosis related to maternal overnutrition (150% of NRC nutrient recommendations) compared to control fed ewes (100% of NRC nutrient recommendations) from 60 d before conception until parturition. The results from these studies are not comparable due to differences between species. Therefore, further researches regarding the impact of prenatal nutrition on fibrogenesis and collagen deposition in the offspring are required.

2.4 Impact of maternal nutrition on postnatal growth and development

2.4.1 Impact of maternal nutrition on offspring growth performance

One of the first indications of maternal undernutrition in the offspring is low birth weight, which could result in a limited growth trajectory which is evident until weaning and market weight (Greenwood et al. 2017). Low birth weight in lambs from ewes fed at 50% of NRC requirements during late gestation has been reported compared to lambs from ewes in which the restriction was imposed during early to mid-gestation or unrestricted ewes (Fahey et al. 2005). Decreased birth weight was also reported in kids from goats with 40% protein and 40% energy restrictions during late gestation compared to a control group fed at 100% of NRC requirement (He et al. 2013).

Underwood et al. (2010) demonstrated lower average daily gain (ADG) in steers born from cows grazing native range (6.0% CP) during mid- to late gestation compared to cows grazing improved pasture (11.1% CP). Also, lower ADG has been reported in lambs (Nordby et al.1987) and rat pups (Beermann 1983) born from nutrient restricted dams compared to those on higher nutritional planes. In an extensive literature review, Greenwood et al. (2017) indicated cattle within pasture-based systems under severe and chronic nutritional restriction (magnitude not defined by author) from d 80 of pregnancy to calving, resulted in fetal growth retardation with reduced birth weight which continued until slaughter. In contrast, maternal nutrition and fetal growth retardation within pasture-based systems does not appear to contribute substantially to variation in feed intake and efficiency in the feedlot from 26 to 30 mo of age, when BW at feedlot entry is taken into account (Cafe et al. 2009; Robinson et al. 2013; Greenwood et al. 2017).

Muscle fibre number and muscle fibre type could impact growth potential of offspring (Dwyer et al. 1993; Pedersen et al. 2001; Rehfeldt and Kuhn 2006). Reduced fetal muscle fibre number can decrease fetal muscle mass and this can be a major cause of low birth weight (Hediger et al. 1998). Low birth weight and decreased muscle fibre number has been reported in offspring born from undernourished pigs (Dwyer et al.1994b). In addition, protein turnover is greatly dependent on the muscle fibre type. Type I myofibre exhibit reduced growth efficiency due to increased protein turnover whereas, type II myofibres has reduced catabolic rates and are more efficient for growth (Therkildsen and Oksbjerg, 2009). Lambs from dietary restricted ewes (50% of recommended requirements) during early to mid-gestation grew slowly and also had decreased number of type II myofibres compared to control ewes (Daniel et al. 2007). In contrast, Cerisuelo et al. (2009)

provided extra feed to sows (1.5 to 2.0 kg/d more than control) from d 45 to 85 of gestation and found fewer muscle fibers and fewer type IIB fibers muscle type; however, postnatal growth performance was not affected by maternal treatment.

2.4.2 Impact of maternal nutrition on offspring carcass characteristics

Although maternal nutrition may impact growth, it may have limited effects on offspring carcass characteristics. Mohrhauser et al (2015) examined carcass characteristics of offspring from cows in a positive energy status (PES; 100% of the BW maintenance energy required) and negative energy status (NES; 80% of the BW maintenance energy required) during mid-gestation. Although hot carcass weight (HCW), dressing percentage, marbling score, kidney, pelvis and heart fat percent and intramuscular fat percentage were not different, there was a tendency for a larger ribeye area and an improved USDA Yield Grade score in steer carcasses from NES-cows relative to steer carcasses from PES-cows.

A similar result was demonstrated by Long et al. (2012), where offspring from dams with a 70% of control diet (100% of NRC) from d 45 to 185 of gestation did not differ in HCW, backfat but had a significantly greater USDA yield grade compared to control (100% of NRC recommendations) and nutrient restricted with protein supplemented (70% of control + protein supplemented) treatments. Similarly, Larson et al. (2009) reported that steers from non-protein-supplemented cows grazing winter pasture in late pregnancy had lighter final BW and HCW, lower marbling score with fewer steers grading USDA Choice compared with steers from protein-supplemented cows (28% CP cubes). Underwood et al (2010) reported higher back fat thickness and adjusted fat thickness (adjusted based on subcutaneous back fat score) from dams grazing improved pasture (11% CP) than dams

grazing native range (6.5% CP), but no changes in the proportion of animals that met US Yield Grade 3 were observed between dietary treatments.

2.4.3 Impact of maternal nutrition on offspring meat quality characteristics

As indicated above, Underwood et al. (2010) examined the effect of grazing improved pasture (11% CP) or native range (6.5% CP) during mid- to late gestation on carcass outcomes. Muscle samples from cattle on improved pasture had lower Warner-Bratzler shear force value (WBSF; $P = 0.01$; more tender meat) compared to offspring born from the cattle grazing native range. In contrast, Mohrhauser et al. (2015) reported WBSF values of the offspring were not altered due to the maternal energy restriction during mid-gestation in the beef cattle.

The impact of the maternal nutrition on meat color has been evaluated by Andrade et al. (2016), where dietary treatments for ewes fed at 100% and 120% of NRC requirements during the last third of the pregnancy had no significant effect on meat color of the lambs. Similarly, Mohrhauser et al. (2015) found no significant differences in the objective meat color (L*, a* and b* value) of offspring born from positive (100% of BW maintenance energy required) and negative energy (80% of BW maintenance energy required) fed dams. Cerisuelo et al. (2009) did not find differences in meat quality (including pH, meat color and drip loss) of progeny when their dams received 1.5 kg/d to 2.0 kg/d extra feed compared to control sows (100% NRC requirement) during mid-gestation.

2.5 Injection devices and injection-site lesions

2.5.1 Needle syringe: Advantages and Disadvantages

Needle syringe (NS) technique is a common and popular strategy for vaccination as it is easily available, readily portable, simple to operate and inexpensive to purchase (Rey et al. 2015). Another merit is its availability and low cost, which provides flexibility for use by veterinarians and producers (Chase et al. 2008; Rey et al. 2015). However, NS is an invasive method and may lead to tissue damage, broken needle fragments and disease transmission (Kale and Momin 2014). The practice of using the same needle in multiple animals could result in the iatrogenic transmission of the blood borne-diseases like anaplasmosis or bovine leukosis (Hollis et al. 2005). Also, use of multiple shot NS could increase the possibility of disease transmission within and between herds (Makoschey and Beer 2004). Reinbold et al. (2010) demonstrated iatrogenic transmission of *Anaplasma marginale* from infected to uninfected steers when using the same multidose needle and syringe.

In addition, NS may result in inadvertent puncture or needle stick injuries causing potential hazard to the handler (Weese and Jack 2008). Inadvertent puncture can result in infection associated with the inoculation of the blood-borne pathogens like arboviruses, organisms from the animal's skin and fine needle aspirates or modified live vaccine which may result in brucellosis, tuberculosis and other diseases to animal handlers and veterinarians (Collins 1987).

Broken needle and needle fragments are a major food safety issue in the meat industry as they are directly related to the public health and are also responsible for loss of consumer confidence in beef (Dubeski 2001; Stier 2003; BCRC 2019). Although the

incidence is low (0.3%-0.0%; Van Donkersgoed et al. 1997a), it can still be an issue in the beef industry (BCRC, 2018) because a single fragment can cause severe trim losses for individual carcasses or can generate significant negative media attention (Li et al. 2011; Min 2015). Although modern-day larger processing plants have metal detectors installed, the fragments can remain undetected due to the composition of the fragment (non-magnetic alloys), small fragment size and orientation of the fragments to the metal detectors (Sundberg 2000). Thus, undetected fragments in beef sub-primals increases the risk of passing fragments into the food supply, resulting in a food-safety physical hazard, negative media attention and negative public perception (Stier 2003; CBC 2013).

2.5.2 Needle-free injection device

2.5.2.1 Components of a needle-free injection device

The main components of a needle-free injection device (NFID; Figure 1) include an energy source, amplifying system, high-pressure hose and hand piece (Grant 2015). The principle of the NFID is mechanical compression (Chase et al. 2008), which occurs through the energy source, leading to vaccine penetration through skin (Jackson et al., 2001; Mousel et al. 2008). The desired dose of vaccine is administered through a small orifice in the skin by placing the handpiece with nozzle perpendicular to skin (Chase et al. 2008; Grant et al. 2015). Once the trigger is pressed, a high-pressure stream is created, which penetrates the targeted tissue; intradermal, subcutaneous or intramuscular (Jackson et al. 2001; Mousel et al. 2008; Rey et al. 2015).

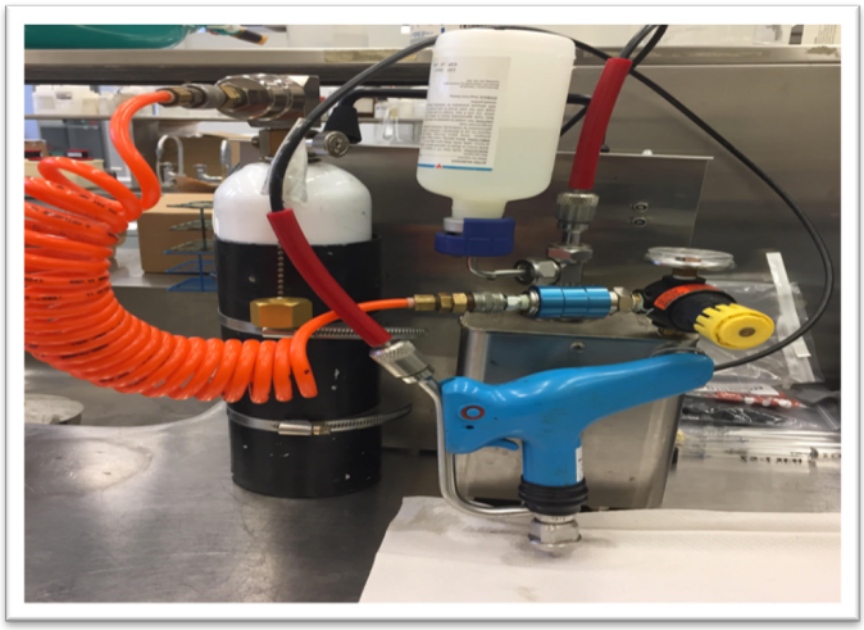


Figure 1. Needle free injection device (Pulse 250) used for vaccination

On the basis of power or energy source of NFID, it can be classified into three types: spring-powered, battery-powered and compressed-gas-powered (Chase et al. 2008). Spring-powered devices are compact, low cost and are mainly used for subcutaneous administration (Chase et al. 2008; Mitragotri et al. 2006). Battery-powered devices are also compact, however, like spring- powered device, they demonstrate a limited range of force and versatility whereas, gas-powered devices have sustained force generation, are more flexible and can administer a large volume of the vaccine (Chase et al. 2008; Mitragotri et al. 2006). The primary disadvantage of gas-powered device includes increased complexity with multiple components and exhaustible energy source (Chase et al. 2008). The total time required for vaccine delivery with NFID is < 1/3 of a second (Chase et al. 2008) including three-phases: Phase 1 or peak pressure phase when the vaccine penetrates the skin with

optimum pressure (< 0.025 sec); Phase 2 or dispersion phase (~0.2 sec) and Phase 3 or drop-off phase (< 0.05 sec) (Diggle et al. 2006).

2.5.2.2 Advantages of the needle-free injection device

There are many benefits of NFID over the conventional NS technique. First, the risk of the broken needle fragments in meat cuts and needle stick injuries are eliminated (Mitragotri 2005; Rey et al. 2015). It also eliminates the need for disposal of used needles, which is considered a biohazard waste product (Mousel et al. 2008).

Second, NFID is the most convenient and efficient method as it increases the speed of vaccine delivery and provides enhanced safety (Giudice and Campbell 2006). Also, NFID does not require changing the needle to inject each animal or group of animals as with conventional NS (Mousel et al. 2008). Mousel et al. (2008) demonstrated an average time of 60.6 s required to vaccinate 7 to 9 sheep with NFID whereas conventional NS required 155.3 s to inject 7 to 9 sheep. This study also demonstrated 60% less time required to vaccinate 100 sheep with NFID compared to NS.

Third, NFID is a precise technique as the pressure used in NFID for vaccine administration is consistent with every injection, which helps to propel the vaccine to a targeted tissue depth in each animal (Chase et al. 2008; Reis et al. 1998). However, in the case of NS, it may vary depending on the needle length and size (gauge) and injection-technique used (Diggle et al. 2006).

Fourth, animal immune response greatly depends on vaccine dispersion (Mitragotri 2005, 2006; Chase et al. 2008). Amplifying vaccine dispersion is important for the stimulation of the immune system and response to antigen present in the vaccine (Baizer

et al. 2002). Use of the NFID technique forms spider web-like dispersion whereas use of NS results in a bolus formation in the tissue residing at the tip of the needle (Chase et al. 2008). The wider dispersion in NFID is associated with the principle of mechanical compression which powers the vaccine in a high-pressure stream with less resistance from the tissue (Grosenbaugh et al. 2004; Diggle et al. 2006;). Therefore, NFID results in a higher ratio of surface area to volume administered compared to NS technique (Grosenbaugh et al. 2004). Large numbers of antigen presenting cells (APC) are present in the dermal and epidermal skin layer, as well as subcutaneous tissue (Chase et al. 2008). Wider dispersion of the antigen provides sufficient opportunity for interaction of the APC with the antigen, thereby enhancing immune response (Hsu et al. 1995; Cui et al. 2003). A direct relationship between immune response and efficient exposure of the immune cells with the antigen has been demonstrated in several studies (Hsu et al. 1995; Baizer et al. 2002; Cui et al. 2003). Comparison of immune response using NFID and NS has resulted in similar and sometimes enhanced response in humans (Williams et al. 2000), swine (Houser et al. 2004), cattle (Hollis et al. 2005) and rabbits (Aguiar et al. 2001) from NFID.

As indicated, vaccination of animals using NS may cause iatrogenic transmission of diseases from infected animal-to-non-infected animal (Otake et al. 2002; Gerlach et al. 2010;). Published literature shows inconclusive results regarding lateral transmission of disease using NFID. Reinbold et al. (2010) indicated no iatrogenic transmission of *Anaplasma marginale* from infected steers to uninfected steers with the use of NFID in comparison with NS technique (transmission in six of ten steers). Whereas, Sweat et al. (2000) used NFID to inject calves and pigs with 0.5 ml saline (in place of vaccine) and collected ejectates in vials after injection. These ejectates might be injected to another animal if not

discarded. This researcher demonstrated that NFID is capable of transferring volume of the blood that can cause infection. In order to conclude that NFID can reduce iatrogenic transmission of diseases, more future study may be required

2.5.2.3 Disadvantages of the needle-free injection device

The greatest disadvantage of NFID is start-up cost which is $3,000 per device (Stuart Webb personal communication). In addition, operational and maintenance costs may also create challenges for producers as it requires maintenance, as well as technical skills for operation (Chase et al. 2008). Another disadvantage is complex structure of the system requiring a power source of exhaustible gas (compressed or CO_2 gas system) for vaccination (Chase et al. 2008).

The NFID can also leave residual vaccine on the skin surface after injection (Jones et al. 2005). A very small volume (0.0004 to 0.0026 mL) is required to make the hide and hair wet (Chase et al. 2008; Schloesser et al. 2008). Further, the appearance of residual vaccine may give rise to concern regarding inappropriate administration of the vaccine and delay acceptance of this technology (Jones et al. 2005).

As described above, use of NFID may result in translocation of bacteria from skin surface to beneath the skin, which may result in disease transfer and abscesses (Gerlach 2010).

Additionally, NFID may cause pain during or following vaccination. Jackson et al. (2001) conducted a study to analyze pain and adverse effects associated with the vaccine administration using NFID (Jet injector, SC or IM) and NS (SC). Doses (0.5, 0.3 and 0.2 ml) of the trivalent inactivated influenza vaccine were administered randomly to 304

healthy young adults. Level of pain (jumping, shying, and vocalization) during vaccine administration and post-vaccination local skin injection site reactions were higher in NFID compared to NS technique. Post-vaccination injection site reactions were reported within 2 d of the NFID administration. However, the immune response was comparable for different doses of the vaccine. In agreement with previous results, other studies (McIntosh et al. 1977; Williams et al. 2000) in chickens and humans have reported similar local reactivity and comparable immune response using NFID. Contrary to these findings, Mousel et al. (2008) reported lower stress in sheep (less jumping, shying, and vocalization) when injected with NFID compared to NS technique. Based on previous contradictory findings, future research may be needed regarding pain associated with NFID.

2.5.3 Injection site lesions and their effect on beef quality

Vaccination or drug administration using needle syringe can damage carcass and decrease its economic value (George et al. 1996). According to a survey conducted from November 1996 to January 1997 in 5 Canadian purveyors, the prevalence of injection site lesions were 18.0%, 22.2%, 4.9%, 1.8%, and 7.6% in top butt, boneless blade, eye of the round, inside round and outside round, respectively (Van Donkersgoed et al. 1997a). According to the Canadian National Beef Quality Audit (NBQA) in 1997 (Van Donkersgoed et al. 1997b), the prevalence of surface injection site lesions in the hanging carcass was 1.3% for fed Canadian cattle whereas it was 1.8-22.2% in sub-primals. In 2011 (BCRC 2015), the prevalence of surface injection site lesions were 0.56% for fed (i.e. steers/heifers finished in feedlot) and 7.34% for non-fed cattle (i.e. culled cattle). For the fed cattle, this data was similar with Canadian (NBQA) 1999 (Van Donkersgoed et al.

2001), whereas for non-fed cattle, prevalence was higher (BCRC 2015). Furthermore, the Canadian NBQA 2016/17 (BCRC 2018) showed increased prevalence of surface injection site lesions compared to the previous audit which reported incidences of 4.45% and 13.69%, for fed and non-fed cattle, respectively. The estimated loss associated with injection site lesions increased from $0.21/head or $662,950 (in 2011) to $0.56/head or $1.63 million (in 2016) as reported by BCRC (2018).

Further, visible injection site lesions result in tough beef impacting meat quality (Van Donkersgoed et al. 2001). Intramuscular injections result in increased variation in shear force values at the center of the lesion and nearby tissue (George et al. 1995ab, 1996; Roeber et al. 2001; Sullivan et al. 2009). George et al. (1996) measured shear force in normal and lesion-affiliated (caused by intramuscular injection) top sirloin butt steaks. Shear force measured on cores obtained from the center, 2.54, 5.08, and 7.62 cm from the center of the lesion in both treatment groups revealed higher shear force values in lesioned steaks compared to normal steaks. Higher shear force values suggests the presence of increased connective tissue causing tough meat (George et al. 1996). George et al. (1995b) also reported higher shear force values in lesioned bottom round steaks compared to normal steaks. The author also reported higher collagen concentration at the center of lesion compared to the 2.54, 5.08 and 7.62 cm distance from the lesion center.

Furthermore, sensory evaluation performed on lesioned and normal steaks demonstrated that lesioned steaks had increased fat (%) and juiciness as well as greater variation in flavor (George et al. 1996). Increased juiciness may be attributed to increased fat, as accumulation of the fat and connective tissue is the main process of repair, indicating damaged tissue (Herenda and Franco 1991; George et al. 1996). The lesions caused by

intramuscular injection can be observed by histopathology (George et al. 1995ab). The center of lesion contains dense sheets of fibroblasts with extensive collagen deposition, which is gradually replaced by adipose tissue as the distance from the lesion center increases. At a greater distance from the lesion center, there is a further reduction in the concentration of connective and adipose tissue, replaced by degenerate muscle fibres (George et al. 1995ab).

Lesion present in the carcass also depends on age at injection. George et al. (1995a) vaccinated cattle intramuscularly against *Clostridia* at branding (48 d of age) and weaning (199 d of age) to examine the potential for chronic lesions in beef meat. These researchers demonstrated that injection lesions may be evident 7.5 to 12 mo after the injection (George et al. 1995a). Interestingly, trimming from the injection site lesion was greater when vaccinated against *Clostridial* at branding compared to weaning (George et al. 1995a).

2.6. Infrared thermography (IRT)

Infrared thermography is a non-invasive and automated technique, which may be used in earlier disease diagnosis through the measurement of the temperature radiated from the animal body (Cook et al. 2015). It absorbs electromagnetic energy of wavelength, 3–12 μm (FLIR 2019a) with temperature changes apparent in images generated with the use of software (Colak et al. 2008). Infrared radiation and visible lights are all part of the electromagnetic spectrum; however, infrared radiation, as indicated above, has longer wavelength (3-12μm) than visible lights (FLIR 2019a).

In veterinary medicine, the practical application of IRT is mainly for recognition of inflammation caused by infection, including mastitis, (Berry et al. 2003; Colak et al. 2008),

subclinical mastitis (Colak et al. 2008), foot and mouth disease and laminitis (Nikkhah et al. 2005; Rainwater-Lovett et al. 2009). Also, IRT has been used as early detection method for infection and inflammation, and early intervention (Schaefer et al. 2004), In addition, IRT is a non-invasive tool for the study of animal welfare (Stewart et al. 2005) and to identify live animals which may have poor meat quality (Tong et al. 2008) and tissue composition (Schaefer and Tong 2004), and for use in determining growth efficiency (Schaefer et al. 2018).

In human medicine, IRT has been used in cancer diagnosis (Colak et al. 2008) and in surveillance of human disease by screening the febrile passenger at airports (Bitar et al. 2009). Thus, IRT is a simple tool that has been of great interest for researchers in veterinary medicine, human medicine and animal husbandry (Poikalainen et al. 2012).

2.7. Conclusion

It is evident that management practices such as feeding and vaccination can impact carcass outcomes in beef cattle. Compromised maternal feeding during gestation can influence fetal muscle and adipose tissue development impacting average daily gain, hot carcass weight, backfat and muscle marbling in progeny. Further, vaccination techniques used may result in injection site lesions causing trimming loss and discarding valuable meat cuts and resulting in economic loss to beef industry. Further research is necessary to determine the impact of adoption of these management practices on carcass outcomes of cattle in Western Canada.

3.0 RESEARCH HYPOTHESIS AND OBJECTIVES

3.1 HYPOTHESIS

In gestating cows, there is an increase in nutrient demand in the third trimester, which is exacerbated due to the increased energy demand during extreme cold during winter. This situation can lead to nutrient deficiencies, and potentially impacting *in utero* fetal muscle and adipose tissue development of their offspring. Moreover, this adverse prenatal environment can permanently affect post-natal growth and development, resulting in reduced growth performance and altered carcass quality and yield. We hypothesized that mid- to late-gestating beef cows receiving corn supplementation over winter have improved nutrient status (i.e. energy and protein balance) thereby giving birth to calves with comparable growth performance with no negative impacts on carcass outcomes compared to offspring from non-supplemented cows.

In addition, carcass outcomes can also be impacted by vaccination strategy. Previous studies evaluating the use of NFID compared to NS vaccination have demonstrated that immune response, following BVD and Clostridial vaccination, were comparable between the two systems; but higher skin reactions were reported with NFID. Pressure trauma associated with vaccination could cause subcutaneous and muscle tissue damage beyond the skin reaction, which could potentially persist until slaughter impacting carcass quality. Therefore, we hypothesized that NFID would impact the incidence of carcass lesion at subcutaneous layer and muscle at the site of injection compared to NS as measured by visual observation, palpation and histopathological method.

3.2 OBJECTIVES

The general objective of the first study was to determine the impact of corn supplementation of mid- to late gestation beef cows during winter on the growth and carcass outcomes of their offspring. Specific objectives include the comparison between maternal feeding treatments on: a) growth performance (ADG, final body weight, feed intake and feed efficiency), b) carcass grading traits such as, ribeye area, backfat thickness (grade fat), marbling score, subcutaneous fat cover score, bone and lean maturity and carcass cutout, c) meat quality traits such as Warner-Bratzler shear force (WBSF), proximal analysis, myofibril fragmentation index (MFI), collagen content, objective color and muscle fibre type.

A second objective was to examine the effect of NFID on the incidence of injection site lesions and possible repercussions on beef sub-primal quality. Specific objectives were to evaluate skin temperature, prevalence of skin injection reactions and subcutaneous and muscle tissue damage, as well as histological and tenderness (WBSF and collagen concentration) evaluation of confirmed lesion.

4.0 MANUSCRIPT I: Impact of maternal corn supplementation on the growth performances and carcass outcomes of their offspring

4. 1 ABSTRACT

To examine the impact of corn supplementation of cows on carcass outcomes of their offspring, 47 multiparous Angus beef cows grazing low-quality forage were assigned to two dietary treatments, corn supplementation at 0.2% BW (SUP; n = 24) vs. non-supplemented (NSUP; n = 23) at d 110 of gestation for 22 wks. Following parturition, offspring were managed as a single group from nursing until the onset of the finishing phase. The steers were placed in the feedlot, assigned to 4 pens (blocks) based on BW and offered 57% corn silage and 37.8% barley grain (76.79% TDN, 11.07% CP) ration with growth performance measured at 28 d intervals. When the steers reached a final BW of 607 kg (~16 mo of age), they were sent to slaughter. Cow data showed that all cows had a positive ME balance regardless of treatment groups; indicating dietary treatments met the energy requirements of cows during the gestation period. Steers from SUP and NSUP cows did not differ in growth performance during the finishing period ($P > 0.05$). No significant difference in carcass quality and yield traits, including sub-primal cut yields, bone, lean and fat trim, was observed between treatments ($P > 0.05$). In addition, differences in the frequency distribution of Canadian quality and yield grades ($P > 0.05$) were not observed. No difference was observed in myosin isoform numbers and dimensions, and meat quality traits; however, WBSF values tended to be higher in steers from SUP dams than steers from NSUP dams ($P= 0.07$). Overall the results indicate that corn supplementation of cows

during the mid- to late gestation does not affect subsequent growth performance and carcass outcomes of their offspring.

Keywords: beef, corn supplementation, carcass outcome and growth performance

4.2 INTRODUCTION

In western Canada, exposure to extended periods of cold weather (with temperatures as low as -30ºC) results in increased energy demand (Young 1981); particularly for gestating cows, which have increased nutrient demand associated with increased fetal growth during the last trimester of pregnancy. Further, forage quality may not be sufficient to meet this increased nutrient demand. A forage survey conducted in Saskatchewan (Government of Saskatchewan 2014) demonstrated that only 38% forage sampled met energy requirements for cows at 6 mo gestation and only 5% of sampled forages provided adequate energy for cows in final months of gestation. This adverse prenatal environment can affect the fetal development. Additional, maternal nutrient restriction during fetal developmental phase can have an impact on the economic traits of offspring including reproductive and growth performance, health, and carcass composition (Vonnahme et al. 2003; Wu et al. 2004; Funston et al. 2010a).

Inadequate maternal nutrition during gestation can impact muscle (Stannard and Johnson 2004; Zhu et al. 2004; Zambrano et al. 2005) and adipocyte (Du et al. 2013, 2017) development. Fetal phase of skeletal muscle development could have irreversible long term consequences for offspring growth performance (Stannard and Johnson 2004; Zambrano et al. 2005). Muscle fibre formation occurs primarily during mid-gestation, when the secondary fibres are formed (Du et al. 2010). Inadequate maternal nutrition during mid- to

late gestation can impact muscle fibre number and can be detrimental for muscle development as muscle fibre number is fixed at birth (Ferrell et al. 1976; Stickland 1978; Zhu et al. 2006). Thus, inadequate maternal nutrient in mid-gestation results in a decrease in muscle fibre number (Du et al. 2010) leading to negative consequences for postnatal performance and muscle mass of offspring (Stickland 1978; Zhu et al. 2004). Likewise, the number of adipocytes is primarily determined during the fetal and early postnatal development and the total number of adipocytes becomes fixed at adolescence (Spalding et al., 2008). Therefore, restricted prenatal nutrition during mid- to late gestation decreases adipocyte number and may reduce intramuscular adipose depots, commonly referred to as marbling (Du et al. 2010).

Many of the studies examining nutrient restriction have been conducted in more temperate climates which differ from Canadian western winter conditions. Recent survey data suggest that an increasing number of producers across Canada are using corn grazing which accounts for 7.1% of extended grazing strategies in Canada (Sheppard et al. 2015). Similar results were reported by a cow-calf survey conducted in 2017 by the Western Beef Development Centre (WBDC 2018) in which 62% of respondents using winter feeding methods and 13% of western Canadian producers are utilizing standing corn. Utilization of corn as an extensive winter feeding system has been increased compared to 2014 (7%; WBDC 2018). Further, corn may be used as an energy supplement to extend winter feed supply during perennial forage shortages associated with drought conditions. Corn is a high energy warm season annual and potentially a higher biomass yielding crop in comparison to other annual forages (Baron et al. 2003). Corn is becoming a popular strategy among beef producers to extend the grazing season due to following reasons: breeding programs

have led to the development of corn which requires lower heat units for growth and therefore is more suited for western Canada (Baron et al. 2003), as well as development of high yielding corn cultivars like Pioneer, Monsanto, and Hyland (Lardner et al. 2012). These corn cultivars range in TDN from 68.6 to 70.8% and in CP from 6.4 to 8.% (Lardner et al. 2012). Thus, grazing standing corn could meet the nutrient requirements of a gestating cow in second trimester with a body weight of 680 kg that requires 50% TDN and 7.8% CP, corn (Lardner et al. 2012; Sheppard et al. 2015*)* and northern plains of US. However, May et al. (2007) reported that protein content of corn was marginal to meet the nutrient requirements of the gestating cattle during third trimester as protein requirements (DM basis) of the gestating cows are 8-9%.

During periods of drought, there may be limited availability of standing and harvested forages leading to increased price (Wright 2005). In these circumstances, feeding corn grain may be economically viable. However, previous research has shown decreased intake and digestibility of low-quality roughage when supplemented with corn due to a potential energy protein imbalance (Sanson et al. 1990).

Thus, it is important to examine winter nutritional management strategies, including supplementation of corn to cows during mid- to late gestation when exposed to periods of extreme cold that are characteristic of the northern US and central Canada and their impact on progeny growth performance (ADG), carcass grading traits, as well as cutout and meat quality traits (WBSF, proximal composition, MFI, collagen content and muscle fibre type). We hypothesize that the offspring from corn supplemented beef cows would have improved growth performance with no negative impact on carcass outcomes compared to steers from non-supplemented cows.

4. 3 MATERIALS AND METHODS

Care and handling procedures of cows and calves (from birth to weaning) were approved by the North Dakota State University (NDSU) Animal Care and Use Committee (#A16010). In addition, care and handling procedures of steers (from weaning to slaughter) were approved by the University of Manitoba Animal Care and Use Committee, conforming to the guidelines of the Canadian Council of Animal Care (CCAC, 1993).

4.3.1 Maternal dietary treatments

Forty-seven multiparous beef cows (Angus or Angus x Continental) were artificially inseminated with Angus sires. Cows carrying bull calves (confirmed via ultrasonography at d 70 of gestation) were transported from the Central Grasslands Research and Extension Center in Streeter, North Dakota (ND), to the North Dakota State University (NDSU) Beef Cattle Research Complex in Fargo, ND. Details regarding cow management and feeding procedures during the experiment have been described by Tanner (2017). Briefly, on d 110 of gestation, cows (661± 7.8 kg BW; 7.5 ± 0.2 years of age) with an average BCS of 5.2 ± 0.1 (9-point scale; where 1=severely emaciated and 9=very obese), were randomly assigned to two feeding treatments: 1) Non-supplemented (NSUP; n = 23) with ad libitum access to a low quality forage (57.54% TDN, 6.4% CP) TMR and 2) supplemented (SUP; n = 24) access to a corn supplement at 0.2% BW (94.5% TDN, 7.64% CP) along with ad libitum low-quality forage (in order to maintain or slightly increase the BCS of the supplemented beef cows). Corn supplementation was to simulate corn grazing which is high in energy and low in protein. Cows were stratified by BW and BCS across the treatments and housed in 4 adjacent drylot pens (11 or 12 cows per pen; 2 pens per

treatment). After a 3-wk acclimation period, dietary treatments were implemented and intake was monitored and controlled via roughage intake control feeders (Insentec B.V., Markenesse, Netherlands) for a 22-wk period. At d 265 of pregnancy, all cattle were re-alimented to a lactation diet (45 % straw, 30% corn silage, and 25% DDGS) that was higher in protein (11.6% CP) until 3-wk postpartum. Samples of the basal forage diet during both gestation and lactation were collected weekly, processed and analysed as described by Kennedy et al. (2016). All cows had free access to water and a trace-mineralized salt block.

4.3.2 Management of steers from birth to finishing phase

At parturition, calves were weighed and castrated within 24h postpartum. At seven days post-calving, a muscle biopsy was collected on the right side from *longissimus dorsi* of the last rib. Hair was removed from the biopsy site and a local anesthetic (lidocaine HCl; 20 mg/mL) was administered subcutaneously. Flunixin meglumine (e.g. Flunixamine®, Zoetis, NJ, U.S.A) was also administered (1.1-2.2 mg/kg body weight intravenously) to provide analgesia. Penicillin G procaine (3000 units per 100 lbs. body weight) was administered intramuscularly. Thereafter, a 1-cm incision was made with a scalpel, and a sterile Bergstrom biopsy needle (5 mm external diameter) was used to obtain 2 cores from the longissimus muscle. Thereafter, the incision was closed with veterinary tissue glue or surgical staples and sprayed with a wound dressing (Blu-Kote-5 oz, H.W. Naylor Co., Inc., Morris, N.Y. 13808, U.S.A). Samples were immediately embedded in optimal cutting temperature (O.C.T.) compound and snap frozen in liquid nitrogen-cooled isopentane for 30 seconds. Frozen samples were stored on dry ice until transported to the NDSU Nutrition Laboratory for storage at –80 °C. All calves were monitored for swelling for 24 h after the

biopsy. Samples were shipped on dry ice to the University of Manitoba for immunofluorescence analysis.

Fifteen days after calving period ended, the dams with their offspring were transported back to the Central Grasslands Research and Extension Center in Streeter, ND for the nursing period. Dams and calves from both maternal diets were kept in the same grazing area. Calf body weights were recorded at 21 d, 168 d, and 280 d post-calving. Calves were vaccinated subcutaneously at approximately 60 d of age with a modified live bovine viral diarrhea vaccine (Bovishield BVD®, Zoetis). Calves were not implanted with anabolic growth promotant.

At weaning, steers (n=43) were transported from NDSU to Brandon, Manitoba and placed in a meadow brome grass pasture at the Manitoba Beef and Forage Initiative's Johnson farm. Upon arrival, calves were re-vaccinated (at 120 d after the first injection) with BVD. Following a 3 mo grazing period, steers were transported to Glenlea Research Station at the University of Manitoba for backgrounding and finishing. Steers (BW average 310 kg) were stratified by BW and maternal dietary treatment (4 pens total; 10-11 steers/pen; 5-6 steers from each maternal diet/pen). Steers were fed, a backgrounding and finishing diet as shown in Table 2, on an ad libitum basis. Dry matter content of the silage was checked weekly and diets were adjusted accordingly.

Feed intake and feeding behaviour were monitored using the Grow Safe System (GrowSafe Systems Ltd., Airdrie, Alberta, Canada). Steers were weighed on two consecutive days at the start and end of the trial, as well as at 28-d intervals until an average pen BW of 607 kg was reached, and with average days on feed of 69, 83, 96, and 137 d for

pens 1 to 4 respectively. Once the desired BW was achieved, steers were off-feed overnight and shipped to a commercial abattoir (True North Processors, Carman, MB).

Table 2. Ingredients and nutrient composition of diets fed to steers during backgrounding and finishing phase

Items	Backgrounding diet	Finishing diet
Ingredients%, DM basis		
Barley silage	80.9	-
Barley grain	13.6	37.8
Canola meal	1.3	-
32% supp	4.3	5.2
Corn Silage	-	57
Nutrient%,	DM basis	DM basis
CP[1]	11.56	11.07
NDF[2]	41.17	27.6
ADF[3]	25.43	16.08
Ca[4]	0.79	0.75
P[5]	0.36	0.35
$NE_{\text{for gain (Mcal/kg)}}$	1.09	1.24
$NE_{\text{for maintenance (Mcal/kg)}}$	1.71	1.88
TDN	71.56	76.97

[1] Crude protein; [2] Neutral detergent fibre; [3] Acid detergent fibre; [4] Calcium; [5] Phosphorus; [6] Net energy; [7] Total digestible nutrients

4.3.3 Slaughter and tissue sampling

Upon arrival, steers were given ad libitum access to water. Live weight and steer identification were noted before stunning by captive bolt, and thereafter were tracked through exsanguination and carcass dressing procedures. Once each carcass reached the chilling cooler (within 45 min of exsanguination), *longissimus dorsi* muscle biopsy samples were taken from right side of carcass between the 11th and 12th ribs

(approximately 5cm away from the first biopsy taken at 7 d of age) using a disposable sterile biopsy punch (5 mm diameter) and transferred into whirl-pak bags containing gauze wetted with saline to keep muscle tissue moist while transporting for muscle fibre type analysis. Samples were placed on ice and shipped to the Department of Animal Science. Upon arrival, they were immediately embedded with O.C.T. compound in a cryomold, frozen in liquid nitrogen-cooled isopentane for 30 s and stored at -80°C for subsequent muscle fibre type analysis.

4.3.4 Carcass evaluation and grading

At 24h postmortem, complete grade data and instrumental color measurements were collected. Left carcass sides were ribbed between the 12th and 13th ribs (Canada Gazete 1992; CBGA 1998) and data collected included grade fat depth (minimum fat thickness over the rib in 4th quadrant from the spinous process), leg muscle profile (muscle development of round and back; where: 1 = very convex; 2 = convex; 3 = straight; 4 = concave; or 5 = very concave), longissimus muscle area, subcutaneous fat cover score (amount and distribution of subcutaneous fat; where: 1 = very abundant; 2 = abundant; 3 = moderate; 4 = slight; or 5 = devoid), bone and lean maturities, and marbling score. Marbling scores were assessed using United States Department of Agriculture marbling standards (USDA 1989). Leg muscle profile and subcutaneous fat cover were evaluated according to the Venezuelan beef grading system (Decreto Presidencial No. 1896, 1997). Also, the exposed longissimus was allowed to bloom for 20 min, and instrumental color measurements were taken using a Konica Minolta Chroma meter (Chroma Meter CR-410, Minolta Canada Inc., Mississauga, ON).

4.3.5 Carcass fabrication

At 96 h post-mortem, cold carcass weight was recorded, and both sides of the carcass were fabricated into commercial retail cuts (trimmed fat to a maximum thickness of 6.4 mm on the cut), as described by the Canadian Meat Council (CMC 1988). The total yield of product components (i.e., bone-in and boneless cuts and lean trim) and co-products (amounts of trimmed bone and trimmed fat) were computed as proportions (%) of the cold carcass. Strip loins (CMC #180; CMC 1988) from the left side of each carcass were removed, vacuum-packaged, transported to the Pilot Plant of the Food and Human Nutritional Sciences Department at the University of Manitoba, and aged for 14 d at 4 ± 1°C.

4.3.6 Procurement of strip loin samples

Following a 14 d aging period, five steaks (2.5-cm-thick) from each striploin sub-primal of each animal were obtained for shear force, proximal composition, fragmentation index, collagen analysis and objective color evaluation. Samples for retail color evaluation were processed immediately while the samples for the remaining analyses were vacuum packed and frozen at -40°C for subsequent analysis. Proximal composition, myofibril fragmentation index and collagen analysis was conducted at the AAFC- Lacombe Research and Development Centre (Lacombe, AB). People performing all the analysis were blinded.

4.3.7 Evaluation of beef quality

4.3.7.1 Muscle fibre typing

Muscle fibre typing based on myosin heavy chain isoform (MHC) was performed using immunofluorescence analysis (Bloemberg and Quadrilatero 2012). Samples which had been stored at -80°C were placed inside the cryostat (Cryostar NX50 HOVPD,110-120V,10A, 60 HZ, Thermo Fischer Scientific, Ontario, Canada) and maintained at -20°C. Each muscle sample was sectioned into 10 μm thick sections using cryostat and mounted to precleaned colorfrost plus® microscope slides (Thermo Fisher Scientific, Ontario, Canada). Sections from one sample were collected into two microscope slides with 9 sections (10 μm) in each slide. Thereafter, slides were air dried at room temperature for 10 minutes and blocked with 10% goat serum (Jackson Immuno Research Laboratories, INC. West Grove, Pennsylvania, USA) in phosphate buffer saline (PBS) for 1 h. Immunofluorescence was carried out using the primary antibodies BF-F8 (MHC I), SC71 (MHC IIa), 6H1 (MHC IIx), BF-F3 (MHC IIb) which were purchased from Development Studies Hybridoma Bank (University of Iowa) and secondary antibodies (AF 350 IgG2b, AF 488 IgG1, AF 555 IgM) which were purchased from Thermo Fisher Scientific (ON, Canada). Primary antibodies specific to myosin heavy chain expression and its specific secondary antibodies are shown in the Table 3.

Slides were incubated with a primary antibody cocktail for 120 mins, washed with PBS 3 times (5 min each). Thereafter, excess PBS was removed with a tissue and incubated with the secondary antibody cocktail for 60 min (in the dark). Following incubation, the PBS wash was performed as before. Excess PBS was removed and anti-fade mounting medium, Vectashield® (Cedarlane®, ON, Canada) was applied to the slides. Nail polish was applied to the edges of the slide to prevent the leakage of the mounting medium. Once dry, the slides were dried and viewed with a Carl Zeiss: Axioskop 40 FL microscope with

red (Excitation: 550 nm; Emission: 565nm), green (Excitation: 494 nm; Emission: 519 nm), and blue (Excitation: 346 nm; Emission: 446 nm) filters as well as an optronics microfibre camera (Model no. 60808, 100-240V, 50/60Hz). Images were taken exposing the slide to three filters, and then merging together into one image using Image Pro-plus Software version 4 (Media Cybernetics, Silver Spring, MD). The diameter of each fibre type and cross sectional area were measured manually using Image J software (NIH, USA). The proportion of each fibre type was estimated by counting all fibre types in each cross section.

Table 3. Combination of the primary and seconday antibodies for myosin heavy chain (MHC) staining of the *longissimus dorsi* muscle in steers

Primary antibody concentration	MHC reactivity	Secondary antibody Concentration
BA-F8 (1:50)	I	AF 350 IgG2b (1:50) (blue)
SC-71 (1:600)	IIa	AF 488 IgG1 (1:500) (green)
6H1 (1:50)	IIx	AF 555 IgM (1:500) (red)
BF-F3 (1:100)	IIb	AF 555 IgM (1:500) (red)
*BA-F8+ SC-71+ 6H1	I+IIa+IIx	
*SC-71+ BF-F3	IIa+ IIb	
* Antibody cocktail for immunoflourescence analysis		

4.3.7.2 Objective color measurement

Steaks were placed on a white styrofoam tray (foam meat tray, 8.25 x 5.75 x 1, Pack All Manufacturing Inc, Rockland, ON, Canada) containing a soaking pad (Ultra Zap UZ-50, 4.5"x7" premium soaker pads - sealed edges, Paper-95 Pak Industries Inc., Georgia, USA) and overwrapped with polyvinyl chloride film (PVC; 037242 PUR Value Polyvinylchloride Standard Meat Films, AGL, Richmond Hill, Ontario, Canada).

Thereafter, they were placed in a coffin-style retail display cabinets (Model M1, Hussmann) at 3°C under LED lighting (light emitting diodes; Acuity Brands Dimmable Rigid 30-LED Light Strip Board HTG S7 - 94v-0 – 4000k) with an intensity 1240 lx. After 1 h of exposure to atmospheric oxygen in the retail display cabinet, steaks bloomed, and color evaluation, including CIE L* (lightness), a* (redness) and b* (yellowness) values were measured using a Minolta Chroma Meter CR-410 (D65, 2°, A 2.54 cm; Minolta Canada Inc., Mississauga, ON, Canada). The position of the trays was rotated within the retail display cabinet every 24 h to ensure similar light and temperature exposure to all steaks. The objective color evaluation was repeated every 48 h for 5 d (i.e. on d 1, d 3 and d 5).

4.3.7.3 pH measurement and purge loss

The pH of each steak was measured using a pH meter with a non-glass probe (HI 99163 Meat pH meter, Hanna instruments, Carrollton, TX), calibrated using two buffers of pH 7 and 4 respectively. The initial pH and weight (to determine purge loss) of the steaks were obtained on d 1 before overwrapping and placing steaks in the retail display for color measurements. The final pH and weight of steaks were obtained on d 5 after removing steaks from retail cabinet.

Purge loss was determined using following equation:

Purge loss% = [(Initial weight – Final weight) ÷ Initial weight] x 100

4.3.7.4 Warner-Bratzler shear force (WBSF) analysis and cooking traits

Sample preparation and cooking procedures were followed according to Research Guidelines for Cookery, Sensory Evaluation, and Instrumental Tenderness Measurements of Meat (AMSA, 2015). In brief, frozen steaks were thawed at 4°C for 24 h, trimmed for zero fat and initial temperature and weight were measured. Thereafter, steaks were placed on an electric grill (George Foreman GRP99, Spectrum Brands Holdings, Inc. Middleton, Wisconsin, U.S), preheated to approximately 165°C, turned once during cooking (at 35 °C internal temperature) and removed from the grill when they reached the desired internal temperature of 71°C as monitored using meat thermometers (35100-K AquaTuff™ Waterproof Thermocouple Instrument, Cooper 198 Atkins, U.S.A). Once removed from the grill, final internal temperature, cooking time and cooking weight were recorded immediately. Cooking loss (%) was calculated using the following formula: [(thawed weight, g – cooked weight, g) / thawed weight, g] x 100.

Thereafter, steaks were placed on metal trays, covered with polyvinyl chloride film and chilled at approximately 4ºC for 24 h. From each steak, WBSF values were obtained by removing six, 1.27-cm diameter cores parallel to the muscle fibre orientation and shearing across the muscle fibres using WBSF analyzer (TA-XT Plus, Texture Technologies) with a cross-head speed of 200mm/min. Shear force values (kg) were averaged from all six cores per steak.

4.3.7.5 Proximal composition

Frozen steaks were thawed for 24 h, then trimmed of all fat and connective tissue and homogenized (Robot Coupe Blixir BX3 [Robot Coupe USA Inc., Ridgeland MS]). Two, 50 g sub-samples were collected into pre-labelled whirl-pak bags and frozen at -35°C.

One subsample was later thawed for 24 h prior to analysis for moisture and fat (AOAC, 1995; Official Method; 960.39) using CEM rapid analyzer systems (Smart Turbo Moisture Analyzer Model 907990, and Smart Trac Fat Analyzer Model 907955; CEM Corporation, Matthews, NC). The second sub-sample was thawed for 24 h and analyzed for protein (Method 992.15; AOAC 1995) using Elementar Rapid N Cube (Elementar Analysensysteme GmbH, Hanau, Germany).

4.3.7.6 Collagen content

Frozen steaks were thawed for 24 h in the cooler with airflow speed of 0.5m/sec. After 24 h, steaks were unpackaged, trimmed of all fat and connective tissue and homogenized (Robot Coupe Blixir BX3, Robot Coupe USA Inc., Ridgeland MS). Duplicate samples, each of 4g, were pre-weighed into vials, vacuum packaged and stored in -35°C until time of analysis. Soluble and insoluble collagen contents were quantified by determination of the hydroxyproline content as described by of Bergman and Loxley (1963) and Hill (1966). Hydroxyproline content was multiplied by a factor of 7.52 to determine soluble collagen content (Cross et al. 1973) and by a factor of 7.25 to determine insoluble collagen content (Goll et al. 1963).

4.3.7.7 Myofibril fragmentation index (MFI)

Steaks were removed from the freezer and placed into a cooler with airflow speed of 0.5m/sec and thawed for 24 hours. Immediately after 24 hours, steaks were removed from the package and trimmed off all fat and connective tissue. Myofibrils were extracted using the method described by Goll et al. (1963). In brief, in a 4ºC cold-room, 10 g of

sample was hand minced with a surgical scalpel blade and placed into 100 ml of standard salt solution (SSS; 100mM KCl, 20mM KPO_4 2mM $MgCl_2$, 2 mM EGTA, 1mM NaN_3, pH 6.8). The sample was homogenized for 10 seconds (Osterizer 10 [Sunbeam Corp. Ltd., Canada]), and centrifuged for 10 minutes at 1000G and 4ºC (J2-MC [Beckman Instruments, Mississauga, ON]). Supernatant was discarded and the above steps were repeated with resuspension of the pellet in 60 ml of SSS, followed by 2 x 80 ml resuspension in SSS. For the latter two steps, the sample was passed through a nylon mesh strainer to remove any residual connective tissue or fat before centrifuging. The pellet was then resuspended in 60 ml of Triton-X (1% Triton-X, 100mM KCl, 20mM KPO_4, 2mM $MgCl_2$, 2 mM EGTA, 1mM NaN_3 pH 6.8) followed by homogenization for 10 seconds and centrifugation at 1500G and 4ºC. A series of 2 x 80 ml resuspensions of the pellet in 0.1M KCL, homogenization for 3 seconds, centrifugation at 1500G and 4ºC. The final pellet was resuspended in 40 ml of KCl and 0.130 ml of a 2% NaN_3 to obtain purified myofibrils. Total protein concentrations of the purified myofibrils were analyzed using the BCA Protein Assay Reagent Kit supplied by Pierce Chromatographic Specialties (Rockford, IL). Once the protein concentration was determined, the myofibrillar suspensions were diluted to 0.5 mg/ml of protein, poured into a 4 ml cuvette, read spectrophotometrically at 540 nm (Ultraspec 3000 [Pharmacia Biotech, England]) and multiplied by 200 to give a MFI value for each steak.

4.3.8 Statistical Analysis

As there was a wide variation in forage and supplement intake reported in Tanner (2017), data were reanalysed to examine the metabolizable protein and energy balance in cows, regardless of treatment.

The feed chemical composition and intake, as well as biological and environmental variables from five 28-d periods during gestation were used to estimate metabolizable protein (MP) balance and metabolizable energy (ME) balance of control and supplemented cows using NRC (2016). The five sampling periods were from d 110 to d 266 of pregnancy which coincided with BW and BCS measurements of cows described above. In addition to ME and MP balance, were the metabolizable protein to metabolizable energy ratio (PER) and MP as percentage of requirement (MPP) were estimated. Accordingly, PER was used to segregate the cows as positive or negative during the trial; thus, cows with one or more negative PER values in the five periods, were assigned in the negative balance group (PER-NEG) while all other cows (were assigned to the positive balance group (PER-POS). Mean, minimum, maximum, standard deviations (SD), and coefficient variation (CV) values were estimated for ME balance, MP balance, MPP, PER, roughage intake, and corn intake (kg/d) for each PER balance group of cows within each feeding treatment.

Statistical analysis of the steer data including growth performance, carcass and meat quality traits were analyzed using the MIXED model procedures of SAS (SAS Inst. Inc., Cary, NC) version 9.4 (SAS, 2012). Analysis of variance was a randomized block design using the strategy described above (PER-POS and PER-NEG), as well as with maternal feeding treatment as the main effect and initial body weight of the steers entering the finishing period as the block effect. The former did not result in statistically significant differences between treatments and was not pursued further. For the latter, sire was

included in the model as a random variable. As well, effect of age of animal and days required to reach finish BW were explored as covariates, but were not significant for any of the dependent variables. Separate statistical analyses were performed for color retail evaluation and fibre typing. Main effects for objective color evaluation were maternal feeding strategy, days of the retail display period and their interaction (using day as a repeated measure with autoregressive covariance structure). For fibre typing, each sampling period (7d and 18 mo. of age) was analyzed separately because different management strategies were used at each age. Thus, analysis of variance was a completely randomized design having maternal feeding strategy as fixed effects in each age. Least squares means were separated (F test, $P < 0.05$) using least significant differences generated by the PDIFF option. The degrees of freedom in the denominator were adjusted using the Kenward-Roger procedure. Additionally, Chi-square analysis (Fisher's exact test) was used to test differences between maternal feeding treatments and the proportion of samples in each of the Canada quality and yield grades.

4.4 RESULTS AND DISCUSSION

4.4.1 Estimate metabolizable energy and protein balance in cows

The central tendency and dispersion statistics for ME balance, MP balance, PER, roughage and corn intake for each PER balance group within each feeding treatment are presented in Table 4. All cows had a positive ME balance regardless of treatment groups; indicating dietary treatments met the energy requirements of cows during the gestation period. The ME balance ranged between 3.1 and 28.1 Mcal/d for NSUP cows and, 0.9 to 26.2 for SUP cows (CV 19.8 to 36.7%). A wide range for MP, MPP and PER were observed; particularly in cows with PER-NEG in both feeding treatments (CV > 200%)

As indicated in Table 4, corn supplemented cows in the PER-NEG had numerically the lowest mean roughage intake; while PER-POS control group had the highest intake. However, overall, roughage intake had moderate variation (CV from 10.4 to 15.0%) in PER balanced cows from both feeding treatments. Corn supplemented cows from both PER balance groups had similar mean corn intake, however, PER-NEG had higher variation (CV = 58.3%) than PER-POS cows (CV = 42%).

It was expected that all NSUP cows would have a negative ME, MP and PER balance; while all corn supplemented cows have a positive balance. However, the large variation in corn and forage intake, as described above, led to animals that were above and below requirements in both dietary treatment groups. One possible explanation between non-supplemented and corn supplemented groups could be in their roughage/corn intake pattern. Klein et al. (2014) observed greater hay intake among cows fed with distiller's grain supplement daily compared with those cows fed only on alternate days. In the current study, PER-POS corn supplemented cows had higher roughage intake than PER-NEG

supplemented ones. It is worth noting that roughage intake decreased in the PER-NEG supplemented cows with no increase in corn intake from d 110 to d 265 of pregnancy; suggesting that cows did not supplement corn for forage, commonly referred to as the substitution effect (Klein et al. 2014). Alternatively, PER-POS control cows had higher roughage intake than PER-NEG control ones, leading to a positive balance. As this strategy did not result in statistically significant differences between (PER-POS and PER-NEG) treatments in terms of steer carcass outcomes, it was not pursued further and results will focus on analysis based on maternal treatment effects. However, this data suggests that moderate supplementation (0.2% of BW) of a high energy feedstuff such as corn may serve to reduce the impact of low quality forage, maintain body condition score and calf birth weight. Lack of response to supplementation in terms of calf birth has been observed by several other researchers who examined effect of supplementation under limited forage conditions (Cunningham et al. 2005; Engel et al. 2008; Klein et al. 2014).

Table 4. Descriptive statistics for roughage and corn intake, metabolizable energy balance (ME), metabolizable protein (MP), and protein to energy ratio (PER) of cows segregated based on protein to energy ratio balance[A] during d 110 to 22wks of gestation[B]

		NSUP		SUP	
		Negative (n=11)	Positive (n=10)	Negative (n=9)	Positive (n=12)
Roughage intake (kg/d)	Mean	16.7	18.4	14.8	16.4
	Min	11.3	13.1	10.8	12.5
	Max	22.1	23.9	19.2	19.9
	STD	2.5	2.3	2.0	1.7
	CV	15.0	12.5	13.5	10.4
Corn intake (kg/d)	Mean	.	.	1.2	1.9
	Min	.	.	0.1	0.8
	Max	.	.	3.1	4.1
	STD	.	.	0.7	0.8
ME (Mcal/d)	Mean	12.8	17.1	13.8	19.2
	Min	3.1	10.6	0.9	12.1
	Max	22.1	28.1	23.2	26.2
	STD	4.7	4.1	4.8	3.8
	CV	36.7	24.0	34.8	19.8
MP (g/d)	Mean	15.0	87.3	32.1	111.5
	Min	-144.9	1.1	-151.5	0.6
	Max	146.9	243.9	163.6	212.6
	STD	71.5	60.5	74.3	57.1
	CV	476.7	69.3	231.5	51.2
MPP (%)	Mean	2.8	16.4	6.7	20.9
	Min	-23.5	0.2	-26.3	0.1
	Max	26.0	44.4	32.5	43.1
	STD	12.7	11.1	13.9	11.0
	CV	453.6	67.7	207.5	52.6
PER (Ratio)	Mean	-1.6	4.6	-3.2	5.4
	Min	-46.1	0.1	-166.5	0.0
	Max	6.7	8.7	7.5	8.4
	STD	9.4	2.5	26.2	2.1
	CV	-587.5	54.3	-818.8	38.9

NSUP= Non-supplemented; SUP= corn supplemented

[A]: Cows which had negative protein balance at least in one of the five periods were assigned as "negative"; whereas, cows which had positive protein balance in all the periods were assigned as "positive".
[B]: from d 110 to d 266 of pregnancy. Every 28 d (five periods), energy and protein balance were determined for each cow using NRC (2016).
ME=Metabolizable energy balance; MP=Metabolizable protein balance;
MPP=MP as percentage of requirement based on NRC (2016);
PER=Metabolizable protein to metabolizable energy ratio

4.4.2 Growth performance of steers during finishing phase

Growth performance traits of the steers based on maternal treatment (NSUP and SUP) are shown in Table 5. The feeding treatments of the dams during mid- to late gestation did not alter the growth performance of the progeny (steers). Steers from cows of both treatments entered the feedlot with similar body weights (P =0.20), and during the finishing period they did not differ in DMI, ADG or feed efficiency ($P > 0.05$). Final body weight was not expected to differ as this was the basis upon which animals were sent to slaughter.

Table 5. Growth performance during finishing period of steers from non-supplemented (NSUP) and corn supplemented (SUP) cows

Variable	NSUP	SUP	SEM	*P*-value
Initial body weight, kg	442.68	449.32	17.98	0.20
Average daily gain, kg/d	1.67	1.71	0.08	0.40
Dry matter intake, k/d	11.80	12.09	0.42	0.52
Feed efficiency, G:F	0.14	0.14	0.01	0.92
Final body weight, kg	602.25	610.17	4.67	0.24

Greenwood et al. (2017) indicated that cattle grazing pasture (preponderance of *Axonopus follicipeda, Imperata cylyndrica, Bothrichloa spp. Paspalum notatum*) which represented < 63% organic matter digestibility and < 6% crude protein from d 80 of pregnancy to calving resulted in reduced birth weight which was still evident at slaughter,

with no evidence of compensatory growth at different stages (for every 1 kg decrease in birth weight, there was a decrease of 4.4 kg in feedlot exit BW). Low planes of nutrition in dams, especially protein, can alter growth performance and carcass outcomes in subsequent calves due to reduced skeletal muscle fibre numbers changes on muscle fibre composition (Du et al. 2010). Larson et al.(2009) and Stalker et al.(2006) indicated calves from cows supplemented with protein (20% CP cubes and 42% CP respectively) in late gestation had heavier BW at weaning, at the time of feedlot entry and at the end of the finishing period compared to calves form non-supplemented cows. However, DMI, ADG, and G:F were not affected. Further, Underwood et al. (2010) reported lower ADG and lighter final body weight in steers born from dams grazed on a native range (6.5% CP) compared to those grazing improved pasture (11.1% CP) during mid- to late gestation. Similarly, lower ADG was reported in lambs (Nordby et al. 1987) fed a diet containing 70% of energy and protein requirements (NRC 1975) and rat pups (Beermann 1983) fed 50% of protein requirements.

As described by Tanner (2017), birth and weaning weight of the calves in the current study were not affected ($P > 0.5$) by the dietary treatments of their mothers. Thus, the additive or compensatory effects were not observed. Data presented by the Radunz et al. (2012) is in agreement with the present study. They demonstrated no alteration in the ADG ($P \geq 0.28$) in steers from cows fed with different energy sources (isocaloric intake with grass hay, corn or dried distillers grain) from mid- to late gestation.

4.4.3 Carcass traits

As indicated in Table 6, there were no significant differences in carcass quality and yield traits, and objective color grade site between steers from NSUP and SUP cows ($P >$

0.05). Additionally, no significant differences between maternal feeding treatments were found in the frequency distribution of quality and yield grades ($P > 0.05$) based on Canadian Beef Grading Standards (CBGA 1998).

Radunz et al. (2012) reported greater dressing percentage for offspring born from hay-fed cows (during late gestation) than corn and dried distillers grain-fed cows. However, the isocaloric intake of different energy sources did not result in significant alteration in the BF thickness and ribeye area of the offspring, but marbling score was lower for the progeny from corn-fed dams, which increased the proportion of USDA Select (equivalent to Canadian quality grade, AA) carcasses. However, no difference was observed for the USDA yield grade distribution (Radunz et al. 2012). Mohrhauser et al. (2015) explored the impact of maternal energy status (100 vs 80% of the energy requirements for BW maintenance) during mid-gestation and observed no differences in offspring HCW, dressing percentage, loin muscle area, marbling score, percent kidney, pelvic and heart fat or intramuscular fat percentage. However, an improved USDA Yield Grade score and a tendency for a larger ribeye area were observed in the steer carcasses from cows fed 80% of energy requirements for BW maintenance relative to steer carcasses from cows fed 100% of energy requirements for BW maintenance.

In addition to energy supplementation, several studies have reported that cows supplemented with protein during gestation resulted in changes in carcass traits (Larson et al. 2009; Underwood et al. 2010; Long et al. 2012). Larson et al. (2009) indicated steers from cows assigned to non-protein-supplemented pasture grazed in winter during late pregnancy had lighter final BW and HCW, lower marbling score and proportion of carcasses that graded USDA Choice compared with steers from protein-supplemented

cows. Underwood et al. (2010) reported higher backfat thickness (12^{th} rib fat thickness) and adjusted fat thickness in steers from dams grazed improved pasture (11% CP) compared to dams grazing native range (6.5% CP), with no significant differences in US yield grade proportions between dietary treatments. Long et al. (2012) found increased yield grade in carcass steers from nutrient-restricted dams (70% of NRC requirements; NR) compared to the nutrient restricted dams with protein supplementation (70% of NRC requirements + protein supplement; NRP) and control dams (100% NRC requirements, CON) provided with maternal dietary during early to mid-gestation. Furthermore, these same authors reported increased adipocytes diameter ($P < 0.05$) in subcutaneous, mesenteric, and omental adipose tissue in NR offspring compared to NRP and CON offspring. Greenwood and Café (2007) demonstrated lighter HCW and decreased rib fat depth in the offspring from cattle fed a low plane of nutrition (pasture 6% CP) from d 30 to d 90 of gestation (early gestation), compared to a high plane of maternal nutrition (pasture 9-15%). However, dressing percentage and USDA marbling score did not differ according between treatments.

In contrast, other studies (Stalker et al. 2006; Meyer et al. 2017) have reported no change in carcass traits associated with either an energy or protein restriciton during gestation. Stalker et al. (2006) indicated that carcass traits of offspring (HCW, dressing %, marbling score, *longissimus dorsi* area, yield %) were not affected by cow prepartum protein supplementation (0.45 kg/d of 42% CP) for grazing cows for 3 mo. The findings in our study are in agreement with Meyer et al. (2017). These authors did not find any difference in the carcass weight, yield grade, marbling score, back fat thickness, ribeye area

and dressing % of the offspring fed a 12.3% CP diet compared to those fed a 6.9% CP diet during late gestation.

Table 6. Carcass traits of steers slaughtered at finish from non supplement (NSUP) and corn supplemented (SUP) cows

Variable	NSUP	SUP	SEM	*P*-value
Hot carcass weight, kg	320.58	322.97	2.93	0.56
Dressing, %	53.19	52.96	0.57	0.53
Leg muscle profile score[a]	2.63	2.69	0.12	0.69
Ribeye area, cm^2	77.58	76.28	1.73	0.55
Subcutaneous fat cover score[b]	2.57	2.73	0.14	0.17
Back fat thickness, mm	10.42	10.15	1.00	0.79
Bone maturity[c]	179.54	178.09	7.40	0.68
Lean maturity[c]	172.38	169.05	3.74	0.53
Marbling score[d]	435.32	446.23	20.53	0.54
Estimated lean yield, %[e]	56.28	56.71	1.10	0.51
Canada quality grade, %(n)[e]	AA 28.57 (6) AAA 71.43 (15)	AA 14.29 (3) AAA 85.71 (18)		0.16
Canada yield grade, %(n)[e]	Y1 4.76 (1) Y2 76.19 (16) Y3 19.05 (4)	Y1 28.57 (6) Y2 57.14 (12) Y3 14.29(3)		0.11
Objective color grade site				
Lightness	41.27	40.73	0.38	0.24
Redness	25.58	25.25	0.41	0.33
Yellowness	11.71	11.29	0.33	0.09

[a] 1= very convex, 2= convex, 3= straight, 4= concave and 5= very concave
[b] 1= very abundant, 2= abundant, 3= moderate, 4= slight and 5= devoid
[c] 100-199= A maturity (younger maturity; < 30 mo of age and < 50% ossification), 200-299 = B maturity (30 to 40 mo)
[d]100-199= practically devoid, 200-299= trace, 300-399= slight, 400-499= small
[e] Carcass quality and yield grade according to Canada Gazette (1992). Quality grade AA equivalent to US Select; AAA equivalent to US Choice. Yield grade Y1= lean yield 59 % or more, Y2= lean yield 54-58%, Y3= lean yield 53% or less. Chi-square analysis indicated the carcass grade distribution was not different by for steers from NSUP and SUP cows.

4.4.4 Carcass cutout of the steers

The total yield of product components (i.e., bone-in and boneless cuts and lean trim) and co-products (amounts of trimmed bone and trimmed fat) are shown in Table 7. The percentage of carcass cutout traits did not differ ($P > 0.01$) between maternal feeding treatments. In sheep, lambs from ewes fed diets restricted to 50% of the recommended dietary allowance throughout pregnancy relative to metabolic BW ($BW^{0.73}$) during gestation (d 30 to d 70) resulted decreased lean:fat ratio compared to the control group (recommended daily allowance relative to metabolic BW; (Daniel et al. 2007).

Table 7. Carcass cutout traits in steers from non-supplemented (NSUP) and supplemented (SUP) cows

Variables	**NSUP**	**SUP**	**SEM**	***P*-value**
Cold carcass weight, kg	309.36	311.06	4.24	0.68
Total sub-primal cuts yield, %[a]	45.02	44.53	4.35	0.91
65/35 trim, %	12.15	12.46	2.60	0.90
86/14 trim, %	16.88	16.15	2.65	0.79
Total fat trim, %	9.15	8.67	0.46	0.34
Total bone, %	16.71	18.18	0.83	0.12

[a] = inside round, outside round, eye of round, center cut sirloin, top sirloin cap, tenderloin, strip loin, ribeye, knuckle, tri-tip, ribeye, shoulder clod, top blade, chuck tender, short ribs, flap, shoulder tender, brisket, heel of round, fore shank, hind shank, chuck roll, hump, flank steak, outside skirt, inside skirt, rib plate.

Similarly, Zhu et al.(2006) reported decreased lean:fat ratio in lambs born from ewes fed at 50% of NRC requirements compared to the control group (100% of the NRC requirement; protein or energy not specified) suggesting fatter lambs were born from nutrient restricted ewes. Increased lean:fat ratio in offspring has been reported with protein supplementation during early to mid-gestation (Underwood et al. 2008). Further,

Mohrhauser et al. (2015) reported enhanced fat deposition (intramuscular and subcutaneous) in offspring from cows fed to meet energy requirements (100% of the NRC energy requirement) compared to cows fed at 80% of BW maintenance energy. Nutrient restriction (100% vs 50% of maintenance energy requirements) in other species such as rabbit, did not result in significant differences in bone, meat and fat weight (Symeon et al. 2015).

4.4.5 Evaluation of beef quality

4.4.5.1 Muscle fibre type

Maternal feeding treatment had no effect on the proportion of each fibre type or dimensions in steers at 7 d or 18 m of age ($P > 0.05$; Table 8). However, the proportion of fibre type IIA decreased while type IIAX increased in samples from steers at 18 m of age compared to samples from steers at 7 d of age. In addition, all the fibre types increased in size as age increased.

Growth potential of offspring is greatly dependent on the muscle fibre number. There is direct relationship between the number of muscle fibres at birth and daily body weight gain (Dwyer et al. 1993; Pedersen et al. 2001; Rehfeldt and Kuhn 2006). In addition, growth is also dependent on the muscle fibre type or the composition. Type I myofibres have lower growth efficiency (i.e. lower ADG and lighter BW) due to increased protein turnover rate whereas, type II myofibres have lower catabolic rates (Therkildsen and Oksbjerg, 2009).

Impact of muscle fibre type on tenderness has not been consistent among studies. Ouali and Talmant (1990) showed both calpain and calpastatin (calpain inhibitor) are

present in the greatest quantity in slow twitch red muscles (Type I). In cattle, high calpastatin reduces proteolysis causing a slower aging rate in post-mortem (Koohmaraie 1996). In contrast, Taylor (2004) reported reduced tenderness in muscle with higher proportion of Type II muscle fibres. In addition, muscle fibre size is also an important factor regarding tenderness at the early postmortem stage. Crouse et al. (1991) reported a correlation between average muscle fibre size with tenderness and shear force at d 1 and d 3 of postmortem storage. Therefore, there are confounding results regarding the tenderness and muscle fibre type in the literature. The relationship between muscle fibre size and tenderness in beef remains elusive (Aalhus et al. 2009).

In an extensive literature review, Aalhus et al. (2009) indicated that meat color (L*, a*, b*) greatly depends on the muscle fibre type composition. Color is associated with differences in myoglobin content, where type IIA and IIX muscle fibre present lower myoglobin content (less red) than type I. Furthermore, fibre type differences can affect the tendency of pigments to oxidize to metmyoglobin (browning formation) under postmortem conditions, consequently less red color (a*) is observed on the muscle surface. Metmyoglobin formation rate is faster in type I than type IIA and IIX muscle fibre because of its greater intrinsic cytochrome oxidase and succinate dehydrogenase enzyme activities at the surface, which results in the faster uptake of the oxygen. Also, type I fibre has a higher concentration of the mitochondria, which may compete with the myoglobin content for oxygen uptake.

The type of MHC isoforms found in our study is in agreement with studies in the beef cattle (Underwood et al. 2010; Underwood et al. 2007) and goats (Argüello et al. 2001) where type IIB was not reported. Meanwhile, Bloemberg et al. (2012) reported Type I,

Type IIA, Type IIB and Type IIX myofibres in rat muscle cross-section along with type I/IIA, IIAX, and IIXB hybrid myofibres using immunofluorescence analysis. Type IIB was also reported in sheep (Zhu et al. 2006) and pigs.

Differences among studies regarding the impact of nutrient restriction on fibre type may be attributed to the type of nutrient deficiency (protein vs energy), time of the gestation and species of the animal. Underwood et al. (2010) reported no alteration in the Type I/type II ratio in the offspring born from cows grazing improved pasture (11%CP) vs native pasture (6.5% CP). In contrast, Zhu et al. (2006) examined offspring born from the nutrient restricted ewes (50% of the NRC requirement during d 28 to d 80 of gestation; protein or energy not specified), and observed a significant increase in type IIx myofibre in comparison to the type I myofibre. Lambs born to nutrient-restricted dams (70% NRC, 30 d before breeding to 100 d of gestation; protein or energy not specified) were found to have larger muscle fibre diameter in the semitendinosus muscle with shorter sarcomere lengths compared to lambs born to the control-fed ewes (100% NRC requirement, 30d before breeding to lambing; Nordby et al. 1987). Similarly, Cerisuelo et al. (2009) reported a reduction in the number of myofibres in the progeny of the maternally-supplemented pigs (1.5 to 2.0 kg/d more than control) compared to the progeny of control sows (100% NRC requirement; protein or energy not specified).

Table 8. Fibre type proportion and dimension in steers from non-supplemented (NSUP) and supplemented (SUP) cows collected 7 d and 18 m of age.

Variables	7 d		SEM	*P*-value	18 mo		SEM	*P*-value
	NSUP	SUP			NSUP	SUP		
Proportion of different fibres, %								
Type I	30.99	33.57	1.59	0.26	32.06	31.44	0.75	0.57
Type IIA	37.00	35.76	1.12	0.44	26.26	28.78	1.20	0.15
Type IIA/X	32.00	30.67	1.44	0.52	41.67	39.78	1.23	0.29
Mean fibre area, μm^2								
Type I	527.95	455.04	32.88	0.13	1665.26	1569.68	93.39	0.47
Type IIA	732.33	669.49	46.37	0.35	1879.13	1886.91	131.74	0.97
Type IIA/X	1124.68	1046.62	74.69	0.47	3025.09	2752.56	168.93	0.26
Mean fibre diameter, μm								
Type I	26.24	24.61	0.82	0.17	47.82	45.00	1.46	0.19
Type IIA	31.42	29.56	1.09	0.23	49.02	48.96	1.67	0.98
Type IIA/X	37.64	36.95	1.28	0.71	62.05	59.73	1.68	0.34

4.4.5.2 pH, purge loss and objective color evaluation at retail display

Maternal feeding treatment had no impact on steer muscle pH, purge loss and objective color evaluation at retail display (Table 9; $P > 0.05$). However, those traits were affected by days of retail display ($P < 0.01$), where pH in all steaks increased over time, becoming slightly alkaline at d 5 of the retail display period, as well as diminished lightness, redness, yellowness over time (not shown in tabular form). Objectives traits were not affected by maternal feeding treatment x display time interaction ($P = 0.92$).

Table 9. pH, purge loss and objective color evaluation at the retail display of steers from non-supplemented (NSUP) and supplemented (SUP) cows

Variables	NSUP	SUP	SEM	*P*-value
Initial pH	5.57	5.60	0.08	0.71
Final pH	5.80	5.79	0.11	0.95
Purge loss, %	8.94	8.76	2.43	0.94
L*	45.62	45.53	0.51	0.87
a*	21.61	20.99	0.63	0.34
b*	10.08	9.74	0.45	0.46

L*: 0 = black and 100= white;
a*: negative values = green and positive values = red
b*: negative values = blue and positive values = yellow

pH influences the water holding capacity (WHC; e.g. purge loss or cooking loss), color development and stability of meat (Alberle et al. 2001). In the current study, the range in pH (pH 5.6-5.8) was deemed acceptable for WHC and color (Alberle et al. 2001). In rabbits, offspring of does fed at different levels of maintenance energy requirements (100%, 75% and 50%) did not have significantly different pH, WHC or objective color measurements (Goliomytis et al. 2016; Symeon et al. 2015). In sheep, Andrade et al. (2016)

reported ewes fed at 100 vs 120% of the nutrient requirements in the last third of pregnancy did not have significantly differences in lightness, redness, and yellowness values of offspring *longissimus dorsi.*

4.4.5.3 Warner Braztler shear force, myofibrillar fragmentation index, proximate analysis, collagen content, and cooking traits

As indicated in Table 8, no differences were observed in MFI ($P = 0.29$), proximate composition ($P > 0.8$) and collagen content ($P = 0.98$); however, WBSF values tended to be higher in steers from SUP cows than steers from NSUP dams (P= 0.07).

Warner Braztler shear force measures the tenderness of meat with low WBSF indicating improved beef tenderness. Meat tenderness depends on multiple factors including, muscle contraction, proteolysis of myofibrillar proteins, fat and moisture content (Youssef et al. 2007). Proteolysis of myofibrillar proteins improves beef tenderness (Koohmaraie 1992; Sentandreu et al. 2002; Maltin et al. 2003), and is attributed to the presence of catheptic enzymes and the calpain/calpastatin system (Boehm et al. 1998; Taylor et al. 1995; Wheeler et al. 2000). The activity of these enzymes can be measured indirectly by MFI, with higher MFI values indicating greater post-mortem proteolytic activity. In the current experiment, WBSF values tended to be higher in steers from SUP dams; however, this difference could not be attributed due to differences in postmortem proteolysis as MFI was not significantly different between treatments. Several researchers have indicated no adverse effect associated with a restricted maternal (energy or protein) diet on WBSF (Underwood et al. 2010; Radunz et al. 2012; Meyer et al. 2017; Mohrhauser et al. 2017). In contrast, Underwood et al. (2010) reported that steers from dams grazing

improved pasture (11% CP) had lower shear force values of the longissimus dorsi compared with the dams grazing native range (6.5% CP). However, calpastatin content did not differ between samples from steers born to mothers grazing improved or native pasture.

Table 10. Meat quality and cooking characteristics of steers from non-supplemented (NSUP) and supplemented (SUP) cows

Variables	NSUP	SUP	SEM	*P*-value
Warner Braztler shear force, kg	2.70	2.93	0.12	0.07
Myofibrillar fragmentation index	257.79	241.17	15.56	0.29
Moisture, %	70.60	70.47	0.89	0.89
Fat, %	6.24	6.42	1.02	0.86
Protein, %	21.80	21.75	0.26	0.86
Total collagen, mg/g	2.24	2.23	0.16	0.98
Insoluble collagen, mg/g	1.94	1.97	0.15	0.85
Soluble collagen, mg/kg	0.30	0.27	0.03	0.30
Soluble collagen, %	13.18	11.85	0.87	0.14
Cooking loss, g	34.87	38.38	3.44	0.31
Cooking loss, %	11.19	11.62	0.57	0.45
Cooking time, min	13.55	14.60	1.77	0.57

The amount of intramuscular fat contributes to beef tenderness and there is a general agreement that high levels of intramuscular fat lead to juicy and tasty meat (Hocquette et al. 2010). Maternal nutritional management during late gestation affects adipocyte hyperplasia, which alters overall adipose tissue development as well as intramuscular adipocyte density and thus marbling fat (Du et al. 2013). In the current experiment, maternal feeding treatment did not change fat content, which suggests that the number of adipocytes in the muscle did not differ. Collagen is a structural protein which is the major component of connective tissue (Karunaratne et al. 2005). Collagen and intramuscular fat play an important role in the meat industry because an increase in these parameters impacts meat tenderness and meat quality (Karunaratne et al. 2005). In utero,

adipogenesis and fibrogenesis occur simultaneously because they share a common pool of progenitor cells called fibro/adipogenic progenitors (Du et al. 2017). In nutrient-restricted (restricted to 60% NE requirements) beef cows, reduced adipogenesis and increased expression of fibrogenic markers, resulted in an increase in connective tissue formation (Gonzalez et al. 2013). Therefore, tenderness can be improved by promoting intramuscular marbling through high energy maternal diets which reduce fibrogenesis and ultimately decrease intramuscular connective tissue (Du et al. 2017). Previous studies of nutrient restriction during gestation agreed with the results of our study, where proximal analysis of meat from offspring was not affected by the maternal feeding treatment (Nordby et al. 1987; Radunz et al. 2012; Meyer et al. 2017; Mohrhauser et al. 2017). In contrast, Underwood et al (2010) reported that steers from dams grazing improved pasture (11% CP) had more chemical ether extract content (tendency), compared with the dams grazing native range (6.5% CP).

Publications addressing the effect of maternal nutrient restriction on cooking traits are very limited. In rabbits, does fed at 100% vs 75% of the maintenance energy requirement from the 7 to the 26 d of gestation, had no observed changes in cooking loss (%) of the *longissimus lumborum* muscle from the progeny (Goliomytis et al. 2016). Similar results were demonstrated in rabbit offspring when maternal nutrition was restricted to 50% of maintenance energy requirements in comparison to the 100% maintenance energy requirements (Symeon et al. 2015).

4.5 CONCLUSIONS

Corn supplementation in cows during mid- to late gestation did not impact the growth performance, carcass quality, carcass yield traits, muscle fibre type characteristics, and meat quality traits of their offspring. This suggests that corn supplementation may be used in cows during periods of feed shortage without negative impacts on the carcass outcomes of their offspring.

5. MANUSCRIPT II: Evaluation of a needle-free injection device on the incidence of injection-site reactions in skin and muscles of the chuck and their implication on beef tenderness

5.1 ABSTRACT

To determine the effect of a needle-free injection device (NFID) on injection site reactions in skin and muscles of the chuck and their implication on beef tenderness, Angus steers were vaccinated and boostered subcutaneously using a needle syringe (NS; n = 20) or a NFID (n=22). The primary vaccination for bovine viral diarrhea vaccine occurred at 60 d of age (right side of the neck), with a booster administered 120 d after first vaccination (left side of the neck). The vaccination site for both injection systems was in the neck triangle zone. Infrared thermography (IRT; model T450sc;FLIR Comp. Boston.MA; high resolution 320 x 240 pixels) was performed on d 0, 2, 5 and 7 post-vaccination to measure skin temperature and assess skin reaction (SKINR). Steers were backgrounded on pasture, finished in a feedlot and slaughtered, when reaching a final BW of 607 kg, and evaluated for further carcass visual tissue damage and histopathological evaluation. Visual inspection of the post-vaccination SKINR was performed 120 d after the last vaccination. Skin temperature measured via IRT at the injection site was higher in NS-vaccinated steers than NFID-vaccinated steers ($P < 0.02$). In both vaccinations periods, NFID-steers had a greater proportion of SKINR compared to NS-steers (NFID 83 vs. NS 0%; $P < 0.01$). “Suspected lesions” were identified by visual inspection in the subcutaneous and seam fat (~ 2. 6 x 3. 8 cm size; NFID = 6 and NS = 3) of the carcass. Core samples obtained from NFID and NS from “suspected lesions” samples based on visual examination at slaughter did not

possess lesions upon histopathological examination. Thus, further analysis of the shear force and the collagen content was not performed. In conclusion, although the use of NFID technology resulted in an increased incidence of skin reactions, there were no detectable injection site lesions in the neck muscles. Therefore, it can be concluded that NFID technology can be used to reduce the incidence of tissue damage and the risk of broken needles with no impact on carcass outcomes.

Keywords: beef, injection lesion and injection system

5.2. INTRODUCTION

Vaccination of livestock is an important management strategy to prevent disease and maintain herd health which ultimately enhances production. Conventional NS technique is the standard approach practiced in the cattle industry for vaccination and administration of antibiotics as it is inexpensive and simple to operate (Rey et al. 2015). However, this simple injection technique can result in broken needle fragments in meat cuts. Although modern day processing plants have metal detectors installed, broken needle fragments can remain undetected due to the composition of some of the needles (alloy composition) used for vaccination, small fragment size and orientation of the needle fragments relative to the metal detectors (Sundberg 2000). Thus, undetected needle fragments in the beef sub-primals, increase the risk of broken needles entering the food supply, resulting in a potential food-safety physical hazard for consumers (Stier 2003). In addition, vaccination may cause injection site lesions resulting in tissue damage, reducing meat tenderness and finally impacting meat quality and decreased carcass value (Dexter et al. 1994; George et al. 1996; Sullivan et al. 2009; Van Donkersgoed et al. 2000).

According to the Canadian Beef Quality Audit 1995/96 (Van Donkersgoed et al. 1997b), the prevalence of surface injection site lesions in the hanging carcass was 1.3%, whereas it was 1.8-22% in the sub-primals (top butts, boneless blades, eye of rounds, inside rounds and outside rounds; Van Donkersgoed et al. 1997a). In the Canadian NBQA 1998/99, the incidence of surface injection site lesion in whole carcass decreased to 0.2% compared to 1995/96 audit (Van Donkersgoed et al. 2001). According to the Canadian NBQA 2011 (BRCR 2015), for fed and non-fed cattle, the prevalence of surface injection site lesions were 0.56% and 7.34%, respectively. Furthermore, the Canadian NBQA 2016/17 (BCRC 2018) showed an increased incidence of surface injection site lesions for fed and non-fed cattle compared to the previous audit, which was 4.45% and 13.69% respectively. Injection site lesions cost the Canadian beef industry $0.56/head or $1.63 million in 2016 compared to $0.21/hd or $662,951 in 2011 due to higher prevalence rates (BCRC 2015, 2018).

Furthermore, in a large cattle herd, use of the conventional needle syringe injection technique may also increase risk of lateral transmission (from animal-to -animal) of blood-borne diseases including anaplasmosis or bovine leukosis (Giudice and Campbell 2006; Hollis et al. 2005). Likewise, another crucial factor associated with the use of the NS technique is the inadvertent puncture or the needle stick injuries resulting in a potential hazard to the cattle handler (Giudice and Campbell 2006; Weese and Jack 2008). This type of injury increases the risk of transmission of blood-borne pathogens, like arboviruses, from the animal's skin and fine needle aspirates to humans resulting in infection and other diseases including brucellosis and tuberculosis (Collins 1987). Local irritation and the

systemic reactions may also occur from the NS injection technique due to potential skin lacerations (Weese and Jack 2008).

An NFID may be a viable alternative to NS and has been used in several developed countries (Aguiar et al. 2001; Houser et al. 2003; Pires et al. 2007; Rey et al. 2015). A study conducted by Rey et al. (2015) concluded that the use of the NS and the NFID system resulted in a similar immunological response in the beef calves in the western Canadian conditions. Similar studies conducted in beef heifers (Pires et al. 2007), swine (Houser et al. 2003) and rabbits (Aguiar et al. 2001) demonstrated a comparable or enhanced immune response from the NFID injection. However, several studies have demonstrated a greater frequency of SKINR from NFID vaccination compared to NS vaccination. (Jackson et al. 2001; Rey et al. 2015). Based on the increased presence of injection skin site reactions with the NFID at post-vaccination, we hypothesized that pressure trauma associated with immunization could cause subcutaneous and muscle tissue damage beyond the skin reaction that could potentially persist until slaughter. Therefore, the objective of this study is to determine the effect of NFID on tissue damage and possible negative impacts on beef sub-primal quality.

5. 3 MATERIALS AND METHODS

Animal care and handling procedures were approved by the University of Manitoba Animal Care Committee (F16-023/AC11199), in compliance with the guidelines of the Canadian Council of Animal Care (CCAC, 1993).

5. 3. 1. Animal management

The study was conducted on calves supplied by Tanner (2017), and their feeding management was described in Manuscript I of this thesis. Briefly, forty-three Angus-based calves, along with their dams, were transported from the Beef Cattle Research Complex in Fargo to the Central Grasslands Research and Extension Center in Streeter for the nursing period (both are facilities of North Dakota State University). Dams and calves were kept in the same grazing area.

To determine the impact of injection techniques (NS vs NFID), 21 calves (60 d of age) were vaccinated subcutaneously (SC) with a modified live bovine viral diarrhea vaccine (Bovishield MLV BVD®, Zoetis) using a NS (non-supplemented =10 and supplemented = 11) while the remaining calves (n = 22) were vaccinated using an NFID system (non-supplemented =11 and supplemented = 11). Needle-free vaccination (n=22) was administered with an NFID (Pulse 250 Needle-Free Injection System; Pulse Needle-Free Systems, Lexena, Kansas, USA) at pressures of 45 to 55 PSI in order to deliver an SC injection. Needle-based vaccination (n=21) was administered SC with a multi-dose, pistol-grip syringe (Kane Veterinary Supplies, Edmonton, Alberta) fitted with an 18-gauge, 1-inch needle (Partnar Animal Health, Ilderton, Ontario), using a skin-tenting technique. Vaccination for both injection systems was on the right side of the neck 4-5” behind the

ear in the triangle zone. The presence of vaccine residue at the skin surface was recorded immediately following vaccination. Infrared thermography (hand-held camera, model T450sc; FLIR Comp. Boston.MA; high resolution 320 x 240 pixels) was used to capture an image of the vaccination site at day 0, 2, 5, 7 post-vaccinations to measure skin temperature and assess skin reactions.

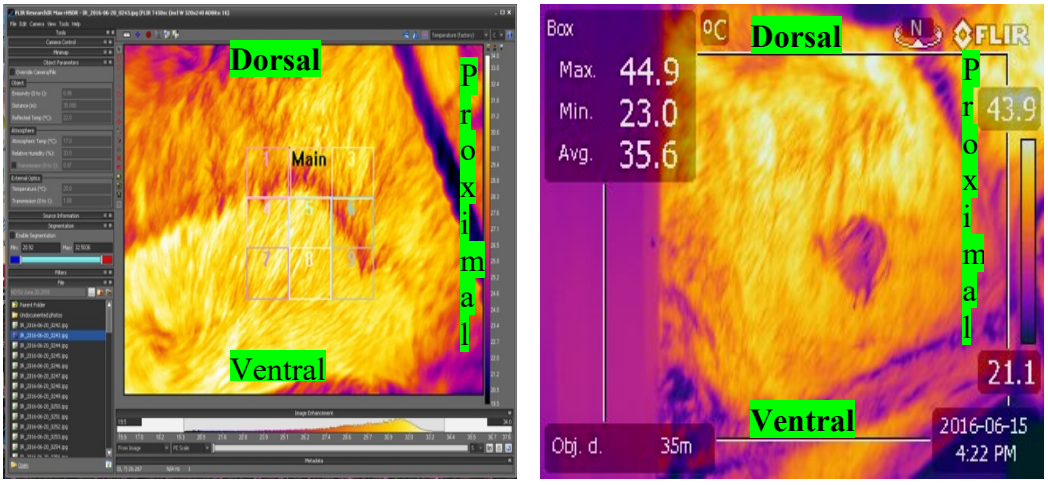
At weaning, steers were transported from North Dakota State University to Manitoba Beef and Forage Initiatives, Brandon, Manitoba. Calves were backgrounded on pasture and re-vaccinated (booster; at 120 d after the first injection) with a modified live bovine viral diarrhea vaccine (Bovishield MLV BVD®, Zoetis) on the left side of the neck using the same injection treatments and techniques as in described. As well, infrared thermography images were captured as described on above paragraph. After a 3 mo grazing period, steers were transported to Glenlea Research Station, the University of Manitoba for backgrounding and finishing as described in Manuscript I. Briefly, steers (initial BW ≈ 310 kg) were stratified by BW and assigned to 4 pens (blocks). Steers in each pen were also balanced by injection treatment (4 pens total; 10-11 steers/pen). Feed intake and feeding behaviour were monitored using Grow Safe System (GrowSafe Systems Ltd., Airdrie, Alberta, Canada). Animals were weighed every 28 d to evaluate their growth performance. Visual inspection of the post-vaccination area to assess skin reaction was performed 120 d after the last vaccination.

5.3.2 Infrared thermography

Infrared thermography (IRT) was performed using an infrared thermography hand-held camera model FLIR T450sc (FLIR Comp., Boston, MA), which is a high resolution

(320 X 240 pixel) instrument with a sensitivity of ±0.1 °C. Thermographic images were taken by restraining the animal in the chute before vaccination (Fig 2, Image A) and images were captured on d 0, 2, 5 and 7 d post-vaccination to access skin reactions associated with the injection technique. Ambient temperature, humidity and air resistance were recorded after every ten animals and were used to adjust thermographs in subsequent analysis. Thus, obtained images were subsequently analyzed using the FLIR® ResearchIR™ software.

Images with acceptable focus, position, angle, clarity and lack of motion were selected to display mean temperature and standard deviation at injection site region. Image processing software was used to place a box (11536 pixels) on the injection site, which was subsequently subdivided into 9 sub-boxes (1124 pixels each) and the average temperature was obtained as shown in Figure 2.



A) Before vaccination B) After vaccination, NFID

Figure 2. Thermographic images of the injection sites on cattle taken before and immediately after the vaccination

5.3.3 Slaughter and evaluation of injection-site reactions on muscles and fat tissues

The finishing period ended when average animal BW per pen reached approximately 607 kg BW with average days on feed of 69, 83, 96, and 137 d for pens 1 to 4 respectively. Once the desired BW was achieved, steers were off-feed overnight and shipped to a commercial abattoir (True North Processors, Carman, MB) for further determination of injection-site reactions in muscles, subcutaneous or seam fat tissues on both sides of the neck area.

Following carcass splitting and before washing, carcasses were inspected by a meat inspector from the Canadian Food Inspection Agency who observed and palpated both side neck areas for the presence of injection-site reactions on carcass tissues. Carcasses were moved to the chilling cooler. After 96 h postmortem, before fabrication, both sides of the neck were sliced (2.5 cm thick), visually inspected and palpated for lesions in the *serratus ventralis*, *splenius*, *rhomboides,* as well as subcutaneous and seam fat between muscles.

If lesions were suspected following visual and palpation observation, the center of 'suspected lesions' was identified, and additional slices (2.5 cm thick) were obtained from 2.54 and 5.08 cm away from the lesion center. The slices were placed in labelled plastic bags and transported on ice to the University of Manitoba for subsequent processing. The 'suspected lesions' were scored according to George et al. (1996) using the following 5-point scale: 1 = cystic (lesion with fluid), 2 = scar with nodules (central foci of necrosis, surrounded by granulomatous inflammation), 3 = mineralized scar (scar lesion containing mineralized remnants of muscle cells callused lesion), 4 = clear scar (white, fibrous scar tissue), and 5 = woody callus (older lesions that are characterized by the injection-site blemish being replaced by organized connective tissue and fat). The slice containing the center of the lesion was measured, and a core was removed from the center, and at radial

distances of 2.54 and 5.08 cm from the lesion location. The cores were placed in separate labelled containers with 10% neutral buffer formalin and shipped to the Veterinary Diagnostic Service Laboratory (Winnipeg, MB) for histopathological examination. The core samples were stained with hematoxylin and eosin, and microscopic examination was performed to confirm the 'suspected injection-site lesions.' If lesions were present, a second stain process would have been performed using Masson's trichrome connective tissue stain to differentiate collagen fibre, muscle fibre, and fibrin (Dey 2018) which differentiates between acute or chronic lesions.

A) Suspected injection lesion B) Core sample collection

Figure 3. Image of a suspected injection lesion. A) Suspected injection lesion, B) Core sample collection from the center of the suspected lesion for histopathology (core sample collected at center, 2.54 cm and 5.4 cm from the center)

5.3.4 Statistical analysis

Data were analyzed using the MIXED model procedures of SAS (SAS Inst. Inc., Cary, NC) version 9.4 (SAS, 2012). A randomized block design model with the factorial arrangement was used to study the effect of vaccination time (primary vs booster), injection treatment (NS vs NFID) and monitoring days post-vaccination (0, 2, 5 and 7 d). Individual steers (block) were incorporated into the model as a random effect. Least squares means were separated (F test, $P < 0.05$) using least significant differences generated by the PDIFF option. The degrees of freedom in the denominator were adjusted using the Kenward-Roger procedure. Additionally, Chi-square analysis (Fisher's exact test) was used to test differences between injection system treatments based on the proportion of injection site lesions.

5.4 RESULTS AND DISCUSSION

5.4.1 Skin surface temperature variation

Vaccination time (primary vs booster; $P < 0.01$), injection treatment (NS vs NFID, $P = 0.02$) and monitoring days (MD, $P < 0.01$) had a significant effect on the skin surface temperature when observed independently. However, the interaction between injection type system and vaccination time was non-significant ($P = 0.06$), as well as injection type system and monitoring days ($P = 0.6$). Steers vaccinated first time (primary; 60 d of age) presented higher skin surface temperature than re-vaccinated steers (booster; at 120 d after the first injection)($P < 0.01$). Steers vaccinated using the NS system had higher skin surface temperature at the injection site than NFID vaccinated steers ($P < 0.02$) in both primary and booster vaccination. Also, regardless of the injection technique, the skin surface temperature increased at d 2 and then diminished at d 5 and d 7 ($P < 0.01$; Fig 1).

The difference in skin surface temperature between primary and booster vaccinated steers could be related to the environment temperature at the moment of vaccination occurred rather than localized immnune response due to re-exposure to the antigen. The primary vaccination occurred during summertime (30 °C); while booster occurred during fall season (5 °C). However, differences between vaccination time could happen. In human, Keital et al. (1994) reported higher local reactions (i.e. erythema and/or induration > 1 cm in diameter) following booster intramuscularvaccination against *Salmonella typhi*.

The increased skin surface temperature, observed with both injection techniques, can be attributed to an inflammatory process (White et al. 2013) as a response of the immune system against foreign bodies (vaccine). Inflammation is characterized by the five cardinal signs: redness, hot, swelling, pain and loss of function (Tracy 2006). Redness and

heat occur due to increased vasodilation and blood supply at the injury site (Tracy 2006). Induction of the vaccination causes innate system to quickly act against the foreign body and activate adaptive immune response through the production of the proinflammatory cytokines tumor necrosis factor-α and interleukin-1 and activates Langerhans cells (LC). Langerhans cells (LC) which are the bone marrow derived DCs that reside in the outer epidermal layer of skin in the immature state (Belyakov et al. 2004). Langerhans cells are the professional antigen presenting cells (APC) which get activated upon contact with proinflammatory cytokines (Zhou et al. 2012). These cells are capable of engulfing the antigen, processing proteins of the antigen to peptides and presenting the antigen complexed with major histocompatibility complex on their surface (Guermonprez et al. 2002; Trombetta and Mellman 2005). Thus formed APC migrate to the draining lymph nodes (kidney) where priming of the specific immune cells or adaptive immune cells, lymphocytes (B cells and T cells) occurs, producing antigen-specific antibodies (Bodey et al. 1997; Belyakov et al. 2004; Liang and Loré 2016). Briefly, T cell receptors of the naïve T lymphocytes (T helper cells) bind with the antigen complexed with MHC present at surface of APC. Once activated, T helper cells release cytokines- interferon gamma, interleukin -2, and tumor necrosis factor which set a chemical alarm for macrophage activation, phagocytosis and humoral immune response (Mosmann and Coffman 1989; Romagnani 1994).

The observed increase in surface temperature can be related to the transient inflammation resulting from the reaction of the immune system against the antigen (foreign body). The observed increase in the skin surface temperature after vaccination is in agreement with a study conducted by White et al. (2013) in which Black Angus heifer

calves were given one of the following 3 treatments: 1) control (injected with sterile saline), 2) Clostridium vaccine, C 7 (Calibar R 7; Boehringer Ingelheim) or 3) Clostridium vaccine, V7 (VisionR 7 with spur; Merck Animal health Summit NJ). All treatment groups were injected SC with NS (1.5 inches, 18 gauge needle). Thermography was performed pre- and post-vaccination and skin surface temperature were measured as the ratio of the injection site area and the control site area. A significantly higher skin surface temperature was observed for the treatment group compared to the control.

The increased skin surface temperature observed in the NS-injected steers compared to the NFID-injected steers may also be associated with the vaccine deposition pattern in the targeted tissue. The vaccine deposition pattern associated with the NS was focalized whereas, a wide dispersed pattern (bolus) is evident when using an NFID (Fisch et al. 1996; du Châtelet et al. 1997; Williams et al. 2000; Grant et al. 2015;). The wide dispersion pattern of the NFID may result in lower resistance during tissue penetration (Grant et al. 2015). The decreased temperature in NFID steers may be attributed to faster heat dissipation from the wider dispersion (large surface area) area in the target tissue. As shown in Figure 1, the temperature for both injection treatments, NFID and NS, increased following vaccination and peaked at 24 h. Thereafter, the antigen-antibody reaction is likely to progress with a reduction in inflammation and return to normal blood flow pattern.

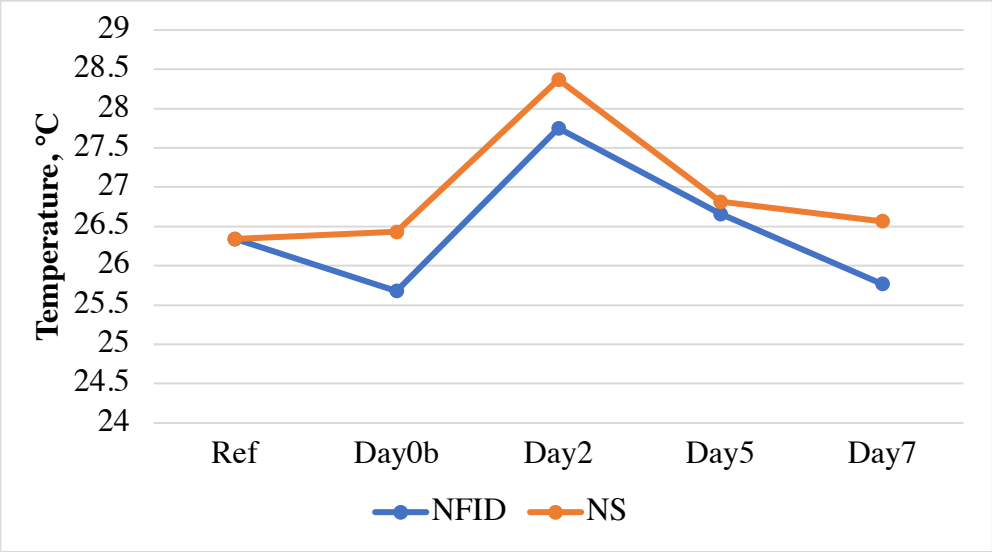


Figure. 4 Pre and post vaccination temperature variation based on injection system used. Injection system $P = 0.02$ (ITS); monitoring day $P < 0.01$ (MD); ITS x MD P = 0.6; SEM= 0.14

5.4.2 Incidence of injection site skin reaction

Injection site skin reactions occurred in 83.36% of the NFID-injected steers following primary vaccination and 64% of the steers following the booster vaccination ($P < 0.01$). These results coincide with the results from the study conducted by the Rey et al. (2015), where the author reported an increased ($P < 0.05$) frequency of skin reactions at the vaccination site of NFID-injected calves compared to the NS-injected calves. Although, injection site skin surface temperature was lower for NFID steers compared to NS, the increased incidence of injection skin reactions associated with the NFID could be because of the pressure trauma as high pressure is used to inject vaccine under the skin. Also, the translocation of skin surface microorganism beneath the skin may have also resulted in increased skin reaction. Similarly, increased injection skin reactions due to NFID have been observed in pigs (Houser et al. 2003). Conversely, Woolums et al. (2011) reported injection

site swelling with both NFID and NS injection systems in cattle vaccinated with multivalent clostridial vaccine; however, swellings were significantly smaller in cattle vaccinated with NFID. Gerlach et al. (2009) demonstrated greater number of abscess in neck and ham of swine injected with NFID compared to NS (injection site contaminated with *Arcanobacterium pyogenes*).

5. 4. 3 Incidence of injection site reaction in muscle and fat, and histopathology evaluation

On visual inspection and palpation of fat and muscle tissues of the carcass, no lesions were observed in the muscles, but in the subcutaneous and seam fat, and those "suspected lesions" were identified and scored as woody callus (approximately 2.6 x 3.8 cm size). The NFID calves had 5 and 1 "suspected lesions" from primary and booster vaccinations, respectively and NS calves had 2 and 1 "suspected lesions" from primary and booster vaccinations respectively.

Injection site reactions in muscle and fat may be attributed to animal's sensitivity to a vaccine, injury during the vaccination, adjuvants used in a vaccine to enhance the vaccine reaction and contamination of the injection site during vaccination (Straw et al. 1986). Adjuvants such as oil added (such as Alpha 7) to the vaccine are responsible for more persistent lesions in muscle than aluminum hydroxide, which is the most commonly used chemical adjuvant. (Straw et al. 1986),

Histopathological examination of the core samples (center, 2.5 cm and 5.4 cm at radial distance from the center of suspected lesion) for subcutaneous and seam fat indicated that there were no evidence of lesions for either injection system. The cores obtained at

center of lesion were characterized by adipose tissue surrounded by collagen and non-compromised vascular structures. Whereas the characteristics of the skeletal muscles present in cores obtained at 2.5 and 5.4 cm from centre were characterized as muscle fibres with uniform size and shape separated by a band of collagen, cross sections of vessels and nerves embedded in the adipose tissue within perimysium.

As a result, further analysis of shear force and collagen content were not performed from the preserved samples. Although the thermograph pattern indicated that an inflammatory response was present, it can be concluded that it was a benign reaction at skin level, as it did not persist in the muscle and fat tissue of the carcass at the time of slaughter.

Visible lesions present in the muscle are not desirable in the meat industry as the presence of a lesion results in increased variation in shear force values in the center of the lesion and nearby tissue (George et al. 1995ab, 1997; Roeber et al. 2001; Sullivan et al. 2009) due to collagen formation up to 7.62 cm from the centre of the lesion (McFarlane et al. 1996). Histopathological characterization indicates that the center of a lesion contains dense sheets of fibroblasts with extensive collagen deposition, which are gradually replaced by adipose tissue as the distance from the lesion center increases (George et al. 1995ab). At a greater distance from the lesion center, there is a further reduction in the concentration of connective and adipose tissue, replaced by degenerate muscle fibres (George et al. 1995ab). Acute and chronic lesions are mainly determined by healing time and process (Mohan et al. 2010). The healing process for an acute lesion is of short duration, mainly characterized by acute inflammatory response which may last less than 2 weeks whereas chronic lesion takes longer to heal (months to years; Mohan et al. 2010).

The features and infiltration of inflammation cells vary depending on the type of lesion which can be determined through histopathological characterization (Mohan et al. 2010).

According to McFarlane et al. (1996) a chronic lesion is characterized by increased fibrous connective tissue at the center which is extended to the surrounding skeletal muscle, with minimal signs of regeneration seen in the surrounding skeletal muscle (McFarlane et al. 1996). Characterization of a chronic lesion described above differs from the characteristics of the "suspected lesion" as described in our study. The results from our study are similar to those reported by Houser et al. (2004), who demonstrated that both injection methods (NS vs NFID) produced no lesions (visual or histological) in pork carcasses. Similarly, Gerlach et al. (2010) demonstrated through visual evaluation and histopathology that there were no significant differences in granulation formations in neck injection sites of pigs following vaccination using NFID and NS.

5. 5 CONCLUSION

Use of needle-free injection system did not cause tissue damage, when compared with traditional needle injection and therefore can be used effectively to administer BVD vaccine when injected in the triangle zone of the neck as recommended by the Canadian Verified Beef Production Plus program (VBP 2019). The utilization of the needle-free injection system would eliminate the possibility of broken needles and needle fragments in beef carcasses.

6.0 GENERAL DISCUSSION

Management of the beef cattle herd includes many components including economics, herd management, nutrition and feeding, animal health, castration of calves, implanting, handling facilities and information management to keep current (Manitoba Agriculture 2017). Gestational feeding and management strategies along with calf health has become an important topic in today's cattle industry due to the fact that offspring carcass quality is influenced by maternal gestational feeding and management.

Proper management of cow nutrition during critical periods of gestation can improve long-term progeny performance and health (Funston et al. 2012). However, it is more challenging particularly during the winter season in which gestating cows have increased nutrient demand that may not be met by low quality forage diets. Nutritional insufficiencies during fetal development may negatively impact offspring birthweight, health, growth, reproduction, carcass weight and carcass quality (Funston et al. 2010a). Mitigating negative effects involves feeding management to ensure proper protein and energy intake of cows when the basal diet is deficient (Funston et al. 2012). Feeding supplemental corn grain during mid- to late gestation seems to be an effective strategy to meet the nutrient demand of gestating beef cattle.

Vaccination is a regular husbandry practices to improve animal health; however, vaccination strategy using conventional needle syringe may results in broken needle and injection site lesion, and NFID could be viable alternative to NS.

6.1 Impact of maternal corn supplementation on growth performance and carcass outcomes of their offspring

In the western Canadian production environment, the practice of the extended grazing (stock-piled forages, swath and bale grazing) has increased (Sheppard et al. 2015) in order to reduce the cost of production during the winter season. The prolonged exposure to the cold weather increases demand for maintenance energy (Jordan et al. 1968; Lister et al. 1972; Young 1981), which combined with the feeding of low quality forage may result in nutrient deficiencies in gestating cows, thereby potentially impacting fetal muscle and adipose tissue development (Jordan et al. 1968; Lister et al.1972). This adverse maternal environment (i.e. nutrient deficiency) affects prenatal fetal growth, which ultimately affects the post-natal growth performance and carcass outcomes (e.g. marbling; Underwood et al. 2010; Funston et al. 2012). To mitigate these negative effects, corn supplementation may be a viable to maintain cow BCS and calf birth weight (Tanner 2017) without potential negative impacts on growth performance and carcass outcomes in their offspring.

In the current study, corn supplementation of pregnant cows did not result in significant differences in the growth performance, carcass grading traits and meat quality traits of their offspring compared to the non-supplemented cows. Therefore, we accept our hypothesize that the offspring from corn supplemented beef cows would have comparable growth performance with no negative impact on carcass outcomes compared to steers from non-supplemented cows. Non-significant differences observed in Manuscript I can be attributed to positive ME balance regardless of treatment groups. Further, although ME, MP and PER were negative in some periods for some animals, the protein limitation did not appear to be severe enough to negatively influence offspring growth performance and

carcass outcomes. By contrast, protein supplementation to beef cows in late gestation (d 180 to 246) influenced calf birth weight positively (Kennedy et al. 2016). In addition to calf birth weight, maternal protein supplementation has increased offspring's marbling and backfat at slaughter (Shoup et al. 2015). In a study by Underwood et al. (2010), cows on improved pasture (11.1% CP Vs 6.5% CP) during mid-gestation produced steers with increased HCW.

It appears that benefits of corn supplementation as energy source cannot be obtained fully unless supplemented protein is included (Tanner 2017). Further, not all cows consumed the corn, leading to large differences of ME, MP and PER between animals within this treatment. Lack of response in carcass outcomes to maternal corn supplementation in the current study suggests that producers can use corn as a supplement during periods of limited forage availability (drought).

6.2 Impact of needle-free injection device on injection-site tissue damage in beef sub-primals

Visual assessment of skin reaction on the injection site at 120 d after the booster vaccination during the finishing period revealed that NFID calves had a significantly higher proportion of skin reactions compared to the NS calves. At the time of slaughter, examination of muscle and fat tissues in the carcass of both NFID and NS steers revealed 'suspected lesions', which were deemed to be non-significant upon histological examination. Thus, meat quality was not comprised. Our research revealed no continuation of pressure trauma associated with NFID beyond the skin reaction. Thus, we reject our hypothesis as there was no differences between the two injection techniques in terms of the

incidence of lesions at slaughter. Therefore, this study demonstrates that beef producers can administer BVD vaccines in the triangle zone of the neck, as recommended by the Canadian Verified Beef Production Plus Program (CCA: Verified Beef Production Plus, 2019), through NFID to eliminate the potential for broken needles without compromising carcass quality.

6.3 Future research

As mentioned previously, a forage survey conducted in Saskatchewan (Government of Saskatchewan 2014) demonstrated that only 38% of the forage sampled met energy requirements for cows at 6 mo gestation and only 5% of sampled forages provided adequate energy for cows in final months of gestation. Based on our detailed examination of forage and corn intake compared to NRC requirements, the dietary treatments in the current study fulfilled the energy requirements of cows during the gestation period; however, almost half of the gestating cows in each dietary treatment could not meet protein requirement and presented PER negative balance. Thus, further research should be conducted to determine the effect of a specific nutrient (i.e. protein) at a different levels of restriction in cows at mid- and late gestation in the Western Canadian production environment, including evaluation of physiological compensatory mechanisms of pregnant cows and fetus, as well as carcass outcomes of their offspring. At the same time, balancing CP and ME that allows efficient conversion of feed to fetus tissue without detrimental effect in gestating cows (e.g. BCS) and their future lactation and reproduction performance.

The initial and maintenance costs of the system possibly resulted in delayed adoption of NFID in cow-calf industry, particularly since vaccination is performed only

three to four times a year (Rey et al. 2015). The ways to avoid this cost could be shared ownership by grazing clubs or commodity groups and rental by either group of producers or feed companies. However, in feedlot cattle operations, thousands of cattle are injected upon arrival so the investment may be justified. Multiple vaccination in cattle is standard practice on cow-calf and feedlot operations and with NFID, we can inject only one vaccine at a time as this system (Pulse 250) has only one vaccine bottle attachment, one pneumatic amplifier and one CO_2 canister (Rey et al. 2015). Thus, we need to move cattle through the handling system twice to administer two vaccines which causes additional stress on animals and also requires additional labours and time. Also, from food safety perspective, it is also important to know the vaccine withdrawal period using NFID. To the author's knowledge, there is no paper which report withdrawal period of vaccine using NFID.

Finally, further research should be performed to determine: i) the effect of intramuscular injection using NFID, ii) the potential to design a system in which two vaccines can be administered using the same unit, iii) the potential to design a system which can be used to administer larger volumes such as that required with antibiotics and iv) vaccine withdrawal period using NFID.

7.0 GENERAL CONCLUSIONS

We accept our hypothesis for study I, as we did not find significant differences in the growth performance and carcass outcomes of steers born from supplemented and non-supplemented cows. More specifically, corn supplementation of the cows during mid- to late gestation did not impact carcass quality, carcass yield traits, myosin isoform characteristics, and meat quality traits of their offspring.

In our research we observed dietary negative balance in PER in the cow diet (both corn supplemented and non-supplemented); however, we did not find any impact of the corn supplementation on offspring. The interrelationship between protein and energy within the rumen can have tremendous effects on the overall pattern of nutrient use and determines the partition of available nutrients to be used in tissue development (Drouillard et al. 1991). When dietary imbalances between protein and energy exist, suppression of intake and digestibility of high energy diets can be reduced because of the metabolic limitations to processing energy, and become even more exacerbated when protein is limiting (Sanson et al. 1990; Fisher 2002). To the author's knowledge, it has not been determined the proper range of protein energy ratio that allows efficient conversion of feed, performance of pregnant beef cows and its impact on offspring.

We reject our hypothesis for the study II as an increased incidence of injection site lesions at the carcass of steers from NFID vaccination was not observed via visual observation, palpation or histopathological examination. Compared to traditional injection via needle and syringe, the NFID system does not result in tissue damage and could provide a comparable immune response as demonstrated by Rey et al. (2015). Furthermore, adoption of this system would eliminate the drawbacks of the conventional needle syringe,

including the presence of broken needles. Thus, needle free injection device can be used to effectively to administer BVD vaccine in the triangle zone of the neck of the beef cattle.

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APPENDIX

Appendix 1 Carcass evaluation form
Project: Fetal Programming
NAME:…… DATE…….

Animal ID	Carcass ID	Type B/D	Bone Maturity	Lean Maturity	Marbling	Fat Cover			Grade fat	Ribeye				REA inchs	Fat cover	P.Y.G Adjusted	Muscling Leg Profile (1-5)	S/C fat cover (1-5)	Obj.color No.
						Top, mm	Mid, mm	Bot, mm		W,S	L,S	W mm	L mm						

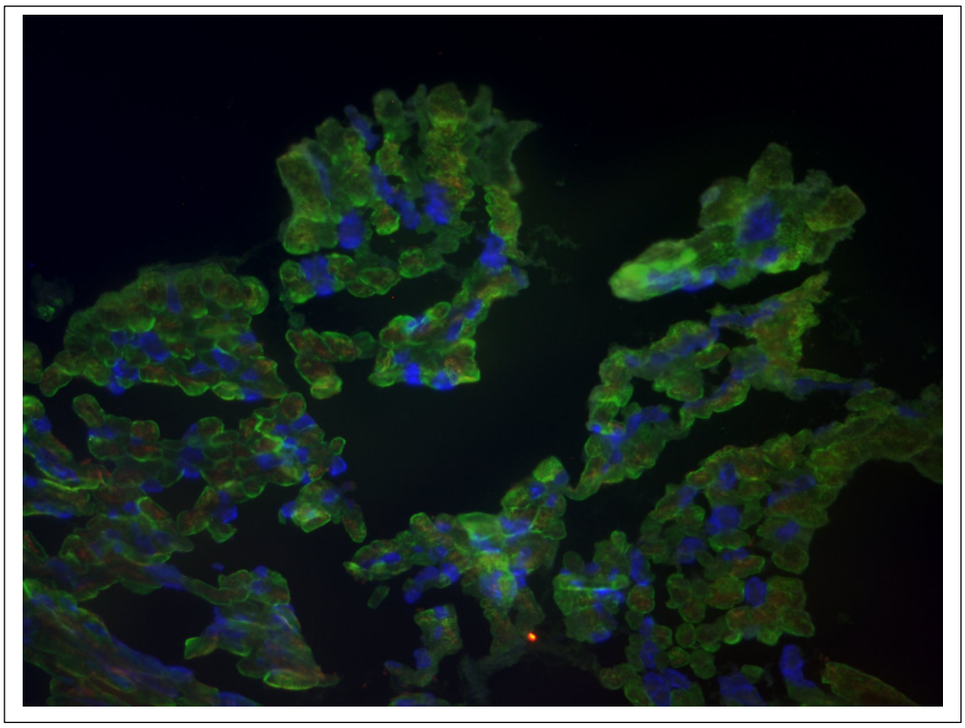
Note: W,S=Weight score; L,S=Length score, W=Width; L=Length; REA=ribeye area

Appendix 2. Injection site evaluation form
Project: Fetal Programming
NAME:…… DATE……

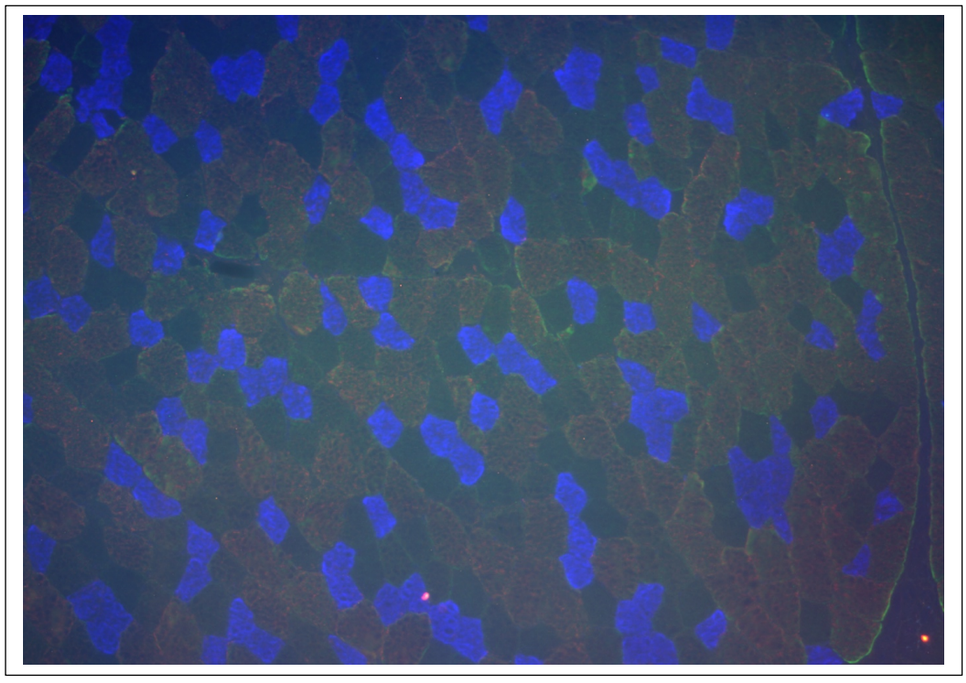
Animal ID	Sequence	R/L	Position	Depth(cm)	Width(cm)	Score
			Centre			
			2.5cm			
			5.4cm			
			Centre			
			2.5cm			
			5.4cm			
			Centre			
			2.5cm			
			5.4cm			
			Centre			
			2.5cm			
			5.4cm			
			Centre			
			2.5cm			
			5.4cm			

Appendix 3 Fibre type image analysis form
Project: Fetal Programming
NAME:…… DATE……

Animal ID	Lab ID	Treatment	Age	Section	Notes
				☐☐☐ ☐☐☐	
				☐☐☐ ☐☐☐	
				☐☐☐ ☐☐☐	
				☐☐☐ ☐☐☐	
				☐☐☐ ☐☐☐	
				☐☐☐ ☐☐☐	
				☐☐☐ ☐☐☐	
				☐☐☐ ☐☐☐	
				☐☐☐ ☐☐☐	
				☐☐☐ ☐☐☐	



Fibre type of 7d of age



Fibre type of 18mo of age

Figure A1. Image acquired using image pro-plus software by observing the slides under Carl Zeiss: Axioskop 40 FL microscope provided with the Red (Excitation: 550 nm; Emission: 565nm), Green (Excitation: 494 nm; Emission: 519 nm), and blue (Excitation: 346 nm; Emission: 446 nm) filters along with the optronics microfire camera
Blue=Type I, Green=Type IIA ,Green and red mixed= Type IIA/X

Figure A2.Carcass evaluation of beef cattle