LEVEL AND SOURCE OF FAT IN THE DIET OF BEEF COWS

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By

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ABSTRACT

Two studies were conducted to evaluate the effects of fat and the type of fatty acid (MUFA vs. PUFA) inclusion in the diet of beef cows during the pre- and postpartum period on the performance of the dam and the progeny. In study 1, replicated over three years, 36 second- and third-calving lactating Angus cows were stratified by BW (554±15.5 kg) and days postpartum (38±1.5 d), and randomly assigned to 9 paddocks (4 cows/paddock) where cows grazed cool-season grass (CSG) pastures (12.5±2.5% CP and 56.5±2.9% TDN). Each paddock was randomly assigned to one of three replicated treatments: a non-supplemented control (CON), and two supplemented (SUP) treatments where cows were offered either a canola seed (CAN) or a flaxseed (FLX) based pellet targeting 300 g/cow/d of supplemental fat (EE) over 42 d. Data were analyzed as a RCBD with contrasts for the effect of fat supplementation (CON vs. SUP) and source (CAN vs. FLX). Results indicate that CON had greater (P=0.01) forage utilization and tended (P=0.08) to have greater estimated forage DMI compared to SUP, while no difference (P≥0.76) was observed between CAN and FLX. At the end of the trial, all treatments resulted in positive ADG, maintained or increased BCS and SCFT, and reduced serum NEFA concentration with no difference (P≥0.20) among treatments. No differences (P≥0.12) were observed for pregnancy rate, calving distribution and calving to calving interval. In study 2, replicated over 2 years, 75 multiparous (≥3 calving) pregnant Angus cows were stratified by BW (663±21.5 kg) and BCS (2.6±0.12), and randomly assigned to 15 outdoor pens. Subsequently, each pen was randomly assigned to one of three (n=5) treatments: a low-fat diet (LF; 1.4±0.12% EE) and two high-fat diets (HF; 3.3±0.20% EE) which included a CAN or a FLX pelleted feeds similar to those used in study 1. Diets were formulated to meet the requirements of pregnant beef cows during the last two trimesters of gestation (183±4.8 d), and offered such that each pen on average received similar amounts of DE (31.2±2.8 Mcal/cow/d), CP (1.36±0.13 kg/cow/d), and DM (12.9±1.0 kg/cow/d). Data were analyzed as RCBD with contrasts for the effects of level (LF vs. HF) and source (CAN vs. FLX) of fat. After 160 d on trial, conceptus corrected-BW (CC-BW) of LF cows (708 kg) and the proportion of over conditioned cows (13.2%) were greater (P≤0.04) than those of HF, with no difference (P≥0.84) between CAN and FLX. Feeding FLX diet over gestation resulted in subcutaneous adipose tissue (SCAT) with greater (P≤0.01) concentration of

CLnA (0.12 vs. 0.05%) and n-3 (0.58 vs. 0.37%) fatty acids, and a tendency (P=0.09) for CLA concentration (1.05 vs. 0.88%) to be greater when compared to CAN diet. By the end of gestation, serum NEFA concentration of LF cows (592 µEq/L) was lower (P<0.01) than that of HF cows, and FLX cows had greater (P<0.01) serum NEFA concentration than CAN cows (636 vs. 961 µEq/L). Cows receiving the LF diet over gestation gave birth to lighter (P≤0.01) calves compared to those receiving the HF diets (40.2 vs. 42.9 kg), with no difference (P=0.24) between calves born to CAN and FLX cows. No differences (P≥0.21) were found for BW or calving to weaning ADG of cows. The average BCS during the first 42 d of lactation was greater (P<0.01) for LF compared to HF (2.63 vs. 2.51) with no difference (P=0.35) for CAN vs. FLX cows. Subcutaneous fat thickness over the 12/13th ribs was greater (P≤0.01) for LF compared to that of HF cows at calving (5.7 vs. 4.3 mm) and at weaning (4.3 vs. 3.7 mm) with no difference (P≥0.11) between CAN and FLX cows. Over the first 42 d of lactation, no difference (P≥0.23) was observed for 12-h milk yield. However, milk protein concentration was greater (P=0.03) for CAN compared to FLX (3.11 vs. 3.01%) cows while no difference (P≥0.28) was observed for any other milk component. Milk fat from FLX cows had greater (P<0.01) CLA and CLnA concentrations than that of CAN cows during the first 42 d of lactation. Pregnancy rate of HF cows (95.4%) tended (P=0.07) to be greater than that of LF cows with no difference (P=0.77) between CAN and FLX cows. From calving to weaning, ADG of calves born to CAN cows was greater (P=0.03) than that of calves born to FLX cows (1.19 vs. 1.13 kg/d) with no difference (P=0.18) between calves born to LF and HF cows. At slaughter, progeny of HF cows had greater (P≤0.03) shrunk BW (605 vs. 579 kg) and HCW (355 vs. 339 kg) compared to those from LF cows with no difference ($P \geq 0.16$) between progeny of CAN and FLX cows. Expression of evaluated genes in muscle tissue of male calves was not significantly affected by either the level or source of dietary fat during gestation. These results indicate that reproductive performance of lactating young beef cows was not affected by the level or source of fat in their diet likely as a result of sufficient good quality pastures. On the contrary, a prepartum high-fat diet tended to increase the pregnancy rate of beef cows at the end of the breeding season. Also, a prepartum high-fat diet resulted in a reduced amount of subcutaneous adipose tissue in the dam but heavier calves at birth, which suggests a partitioning of the ME dependant on the type of dietary energy. Moreover, a high-fat diet during gestation resulted in improved birth to slaughter performance and superior HCW at slaughter of the progeny.

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DEDICATION

To: Miranda and Aquiles.

For being my inspiration and the reason that keeps me going.

To my mentors and friends: Aquiles Escobar, Gonzalo Martinez and Rosana Figueroa.

For believing in me, trusting me, and showing me the path.

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ABBREVIATIONS

ADF	Acid detergent fibre
AD	Atypical diene
ADG	Average daily gain
AI	Artificial insemination
ALA	α-Linolenic acid
AOAC	Association of Official Analytical Chemists
BHBA	β-hydroxy butyrate
BW	Body weight
CLA	Conjugated linoleic acid
CLnA	Conjugated linolenic acid
CP	Crude protein
d	Day
dL	Deciliter
DDG(S)	Dried distillers' grains (with solubles)
DE	Digestible energy
DIP	Degradable intake protein
DM	Dry matter
DMI	Dry matter intake
EE	Ether extract
FA	Fatty acid
FBW	Final body weight
g	Gram

HCW	Hot carcass weight
IBW	Initial body weight
IVDMD	In vitro dry matter digestibility
kg	Kilogram
LA	Linoleic acid
mL	Millilitre
mm	Milimeter
ME	Metabolizable energy
MP	Metabolizable protein
MUFA	Monounsaturated fatty acid
N	Nitrogen
NDF	Neutral detergent fibre
NE	Net energy
NEFA	Non-esterified fatty acid
NFC	Non-fibre carbohydrates
PUFA	Polyunsaturated fatty acid
SAS	Statistical Analysis Systems
SCAT	Subcutaneous adipose tissue
SCFA	Short-chain fatty acid
SCFT	Subcutaneous fat thickness
SD	Standard deviation
SEM	Standard error of mean
SFA	Saturated fatty acid
TDN	Total digestible nutrients

1. GENERAL INTRODUCTION

Success of cow-calf operations depends on the ability of the cow to conceive a calf every year and the ability of the calf to grow in an efficient manner. These two variables, the reproductive performance of the dam and the performance of the progeny, can be affected by the pre- and postpartum nutrition of the beef cow (Hess et al., 2005; Funston et al., 2010). Since feed cost represents the largest proportion of the production costs in cow-calf operations, an efficient nutritional and/or feeding plan will most likely translate into higher profits for producers.

The nutrient requirements of the beef cow will increase when facing stages other than maintenance such as growth, gestation and lactation. For example, beef heifers are expected to conceive at slightly over 12 months of age and to do it every year for as long as they are part of the herd. For such a level of production, the young beef female needs to successfully overcome the high demands for nutrients that she would experience during the second and third year of her productive life as a result of being pregnant, lactating and still growing. Moreover, beef cows with increased nutrient requirements may be exposed to low-quality forage diets which do not meet those requirements. For example, cows calving later within the calving season will be grazing pastures with advanced stage of maturity during the summer and part of the fall, which might increase their chances of being non-pregnant at the end of the breeding season. Nutrient requirements of beef cows can also increase due to changes in environmental conditions. For example, in western Canada, gestating beef cows are often exposed to extreme low-temperatures during the winter which increases their energy requirement for maintenance (NASEM, 2016). Therefore, meeting the nutrient requirements of beef cows during periods of increased energy demand becomes a challenge for cow-calf operations.

In ruminants, including fat up to 6% of the total DMI increases the energy density of high-forage diets while avoiding negative effects associated with starch supplementation (Palmquist, 1994; Bowman and Sanson, 1996; Hess et al., 2008). Also, feeding beef cows high-

fat diets have resulted in greater plasma estradiol-17β and progesterone concentrations, as well as greater number of follicles during the first and second estrous cycle, compared to those fed low-fat diets with similar energy content (Lammoglia et al.,1997ab). Moreover, the source of fat can induce different responses in terms of reproductive performance of beef cows. For example, including a fat source high in polyunsaturated fatty acids such as flaxseed in the diet of beef (Petit and Berthiaume, 2006; Lopes et al., 2009) and dairy (Petit et al., 2001) cows, has resulted in higher conception and pregnancy rates compared to other fat sources high in saturated fatty acids.

Including fat in the diet of beef cows during gestation does not only have a positive effect on the performance of the dam, but also brings benefits to the performance of the offspring. Parameters such as birth weight (Lammoglia et al., 1999a; Radunz et al., 2010), survival of newborn calves (Lammoglia et al., 1999ab; Dietz et al., 2003), and survival from birth to weaning (Petit and Berthiaume, 2006) have been improved by increasing the fat content in the diet of the dam during gestation. These effects of maternal nutrition status during gestation on performance of the offspring have been attributed to developmental programming effects (Wu et al., 2006; Gicquel et al., 2008; Du et al., 2010a). Also, some evidence has shown that epigenetic systems may play an important role in these changes occurring during fetal development.

Overall, energy requirements of the beef cow increase according to the level and stage of production, as well as changes in environmental conditions. Therefore, increasing the fat level in the pre- and postpartum diet of beef cows can help to overcome these high demands for energy; thus, improving their reproductive performance. However, this improvement might depend on the source of fat being included. Moreover, fat inclusion in the prepartum diet of gestating beef cows might result in improved performance of the progeny.

The objectives of the following literature review are to provide an overview of the energy requirements of beef cows and factors affecting those requirements; to review the digestion, absorption and metabolism of dietary lipids and fatty acids in ruminants; to review the use of fat as a source of energy supplementation for ruminants and its effects on rumen fermentation, reproduction, and fatty acid composition of adipose tissue and milk fat of beef cows; and to review the developmental programming effects of the level and type of dietary energy during gestation on the performance of the progeny as well as the possible epigenetic systems involved.

2. LITERATURE REVIEW

2.1. Nutrient Requirements of the Beef Cow

Nutrition of the cow is one of the most significant factors influencing the success of cow-calf operations including maximizing pregnancy rates of cows and weaning weight of calves. Since feed cost is approximately 60% of the annual cow costs in many cow-calf operations, providing beef cows with their nutrient requirements in a cost-effective manner is a requirement for profitability in cow-calf operations (Taylor and Field, 1995; Hersom, 2007). Therefore, a good understanding of the nutritional requirements of beef cows and the factors affecting those requirements is important for improving the efficiency of cow-calf operations.

The beef cow has specific requirements for energy, protein, minerals, vitamins, and water (Kunkle et al., 2000). However, energy is considered the most important nutrient when feeding livestock since a significant portion of the animals' protein requirement is met by microbial protein synthesis. Moreover, when fed to adequate levels, most of the rumen degradable protein is deaminated by the rumen bacteria; but when protein is fed in excess of the requirement, the excess ammonia produced is absorbed from the rumen and converted to urea in the liver causing an expenditure of energy (Cassard and Juergenson, 1971; Cullison, 1975; Ørskov, 1982). Also, nutrient requirements of beef cows can be divided into four categories and classified according to their prioritization as follows: maintenance > lactation > growth > reproduction (Short et al., 1990; Marston et al., 1998). Within and between these categories, nutrient requirements of beef cows can vary significantly due to several factors such as age, BW, breed, weather, physiological state, milk production and body condition (NASEM, 2016).

2.1.1. Energy requirements

2.1.1.1. Energy requirement for maintenance

Maintenance energy requirements include that for the animal to breathe, move, digest feed, maintain body temperature, repair tissues, and maintain BW (Hall et al., 2009).

The energy requirement for maintenance is defined as the amount of energy intake that will result in no net loss or gain of energy from the tissues of the animal (NRC, 2000). The energy requirement for maintenance is considered the most important requirement to be met before nutrients are used for production. According to Ferrell and Jenkins (1984), approximately 70% of the energy required by the non-pregnant, non-lactating cow can be attributed to energy costs for maintenance. The net energy required for maintenance (NEm) is the amount of energy expenditure through heat production and urinary loss by the fasting animal (NRC, 2000). For growing cattle, the NEm has been estimated to equal 0.077 Mcal per kilogram of average empty metabolic BW (Garrett, 1980). However, there are many factors affecting the energy requirement for maintenance of beef cows such as BW, previous nutrition, breed or genotype, age, environmental conditions and physiological state (NRC, 2000).

Since metabolic body weight ($BW^{0.75}$) is used to estimate the NEm of growing beef cattle, it is reasonable to consider live weight of the animal as the main factor affecting energy requirement for maintenance. However, for mature beef cows it is more accurate to account for other parameters such as BCS or previous nutrition. Lemenager et al. (1980) observed that the coefficient of determination (R^2) improved from 0.60 to 0.81 when using the BW and BCS in an equation to predict the TDN requirements of crossbred beef cows rather than using BW alone. Similar findings have been reported by Birnie et al. (2000). Thompson et al. (1983) used the energy retention of Angus × Hereford cows during the winter to estimate their maintenance energy requirements and found that fatter cows had 6.1% lower energy requirements than thinner cows. Houghton et al. (1990), after nutritional manipulation of pregnant beef cows to achieve BCS of 1, 3 or 5, observed that thinner cows required more predicted maintenance energy at parturition and early postpartum than fatter cows. The results from these studies suggest that, at the same metabolic weight, a thin cow needs more energy than a fatter cow due to the higher amount of energy required for protein turnover than for fat accretion. It can be concluded that

both body condition and metabolic weight must be considered to determine energy requirement as there are differences in the energetic efficiency of fat and thin cows.

Breed component and type can also influence maintenance requirements. Purebred beef cattle have been shown to have greater maintenance requirements than their crosses (Solis et al., 1988; Reid et al., 1991). Also, maintenance requirements of dairy breeds are estimated to be 20% greater in comparison to beef breeds (NRC, 2000). Differences in maintenance requirements can also be found within beef cattle breeds. According to the NRC (2000), the maintenance energy requirement of *Bos indicus* breeds is 10% lower than that of *Bos taurus* breeds. Ledger and Sayers (1977) reported that *Bos indicus* steers required 37% less feed than *Bos taurus* steers to maintain BW. Differences in maintenance energy requirement is attributed to reduced levels of internal fat and smaller metabolically active organs in *Bos indicus* cattle compared to *Bos taurus* cattle. Lunt et al. (1986) reported that liver, heart and lung tissue mass of Angus steers was greater compared to that of Brahman and Brahman × Angus steers. In general, there is a positive relationship between maintenance requirement and genetic potential for measures of productivity in adult and growing cattle (NRC, 2000). For example, after plotting the estimates of daily maintenance requirements for nonpregnant, nonlactating cows vs. mean milk yield at peak lactation, Ferrell and Jenkins (1988) observed an increase of 6.2 kcal/kg $BW^{0.75}$ per each kilogram of increase in milk yield.

Physiological state such as pregnancy and lactation can also affect the energy requirements for maintenance. An increase in heat production has been attributed to the productive process of pregnancy (NRC, 2000). According to Hersom (2007), the energy required for gestation is only 0.1% of the total energy requirement at the beginning of the pregnancy, while the energy requirement for gestation during the last month of pregnancy is approximately 56% of the total requirement. Ferrell and Reynolds (1985) found that total heat production of mature pregnant Hereford cows increased 0.64 Mcal/d and 3.57 Mcal/d at 137 and 250 d of gestation, respectively. Similarly, it has been shown that maintenance metabolizable energy requirements increased by 25% as gestation progressed for two-year old pregnant heifers (Warrington et al., 1988). Lactation can also affect the maintenance energy requirements of beef cows. Neville and McCullough (1969) reported increases in maintenance energy requirements of

30% for lactating compared to non-lactating Hereford cows. According to NRC (2000), the maintenance requirements are about 20% greater for lactating compared to nonlactating cows.

Environmental conditions can affect energy requirements for maintenance with temperature being the main environmental factor affecting requirements (NRC, 1981). The thermal neutral zone is defined as the range of ambient temperatures within which the metabolic heat production of an animal is independent of ambient temperature (Forbes, 1995). Cattle exposed to extreme heat or cold stress (above or below the thermal neutral zone, respectively) will need to either dissipate or increase body heat production to maintain homeostasis. In the case of cold stress, the normal heat of fermentation and metabolism produced by the animal can no longer maintain its body temperature (Fox et al., 2004). Therefore, the metabolism of the animal utilizes dietary energy to increase and provide adequate heat to maintain body temperature. As a result, energy requirements for maintenance increase (NRC, 2000). Delfino and Mathison (1991) conducted a study where yearling steers were fed either indoor (16.9ºC of mean temperature) or outdoor (-7.6ºC of mean temperature) from January to April; and concluded that steers exposed to the lower temperature required 18% more NEm than those fed indoors. Similar findings have been reported by Birkelo et al. (1991) who found that cold stress increased the energy requirements for maintenance of Hereford steers.

Age of the animal is another variable affecting the energy requirements for maintenance. Corbett et al. (1985) proposed a model where maintenance decreases 3% every year in sheep and cattle. However, Vermorel et al. (1980) indicated little change in maintenance requirements of cattle between 5 and 34 weeks of age. Overall, the amount of energy required for maintenance per unit of size decreases as the animal ages. Consequently, total maintenance requirements are often greater for mature animals due to increased size (NRC, 2000).

2.1.1.2. Energy requirement for gestation

The nutrient requirements of pregnant beef cows include those necessary for proper fetal growth and development, ensuring an adequate body condition to calve and lactate, a timely return to oestrus, and for continued growth in the case of the 2- or 3-year-old heifers (NRC, 2000). Fetuses of most mammalian species reach at most 25% of their birth weight by mid-gestation, experiencing an exponential increased in fetal weight during the last third of gestation, whereas

the placenta experiences rapid growth during early-gestation practically reaching its total weight by mid-gestation (Eley et al., 1978; NRC, 2000; Redmer et al., 2004). Therefore, the energy requirement of pregnant beef cows also increases exponentially during late stages of gestation. As indicated previously, the energy required for gestation is initially 0.1% of the total energy requirement, while in the last month of gestation the energy requirement is approximately 56% of the total energy requirement (NRC, 2000; Hersom, 2010).

According to NRC (2000), since birth weight of the calf is associated with nutrient requirements for pregnancy, factors that affect calf birth weight have a proportional affect on nutrient requirements during pregnancy. Such factors affecting calf birth weight are: breed of sire and dam, heterosis, age or parity of the dam, number of fetuses, sex of the fetus, environmental temperature, and nutrition of the dam (Ferrell, 1991; NRC, 2000). Therefore, NRC (2000) estimates the energy requirement for pregnancy using expected calf birth weight (CBW) and day of gestation (t) according to the following equation:

$$\text{NEm (kcla/d)} = \text{CBW} \times 4.43 \times (0.05855 - 0.0000996t) \times e^{(0.03233-0.0000275t)t} \qquad \textbf{Equation 2.1}$$

2.1.1.3. Energy requirement for lactation

According to NRC (2000), ME is utilized for lactation with similar efficiency as it is utilized for maintenance. Therefore, the NEm required for milk production is equivalent to the energy content of the milk produced.

The energy (E) content of milk can be estimated from the following regression models proposed by Tyrrell and Reid (1965) based on the fat and solids not fat (SNF) content:

$$\text{E (Mcal/kg)} = (0.097 \times \%\ \text{Fat}) + 0.361 \qquad \textbf{Equation 2.2}$$

$$\text{E (Mcal/kg)} = (0.092 \times \%\ \text{Fat}) + (0.049 \times \%\ \text{SNF}) - 0.0569 \qquad \textbf{Equation 2.3}$$

Assuming milk contents of 4.0% fat and 8.3% SNF, NRC (2000) estimates a daily total NEm required for milk production of 3.58 to 10.03 Mcal/d for 5 to 14 kg/d of milk, respectively, at peak milk yield.

2.1.1.4. Energy requirement for growth

The energy required for growth or gain (NEg) is defined as the energy content of the tissue deposited which depends on the proportion of fat and non-fat organic matter content (NRC, 2000). The retained energy (RE) of tissue deposited is estimated by NRC (2000) according to the following equation:

$$RE\ (Mcal/d) = 0.0635 \times (EQEBW)^{0.75} \times (EBG)^{1.097} \qquad \textbf{Equation 2.4}$$

where: EQEBW is equivalent empty BW (kg) and EBG is empty body gain (kg).

The conceptus weight (CW) of pregnant heifers, which includes fetal and gravid uterine weight, needs to be removed from EQEBW to estimate their growth requirements. The CW can be estimated using the expected calf birth weight (CBW) and day of gestation (t) according to the following equation (NRC, 2000):

$$CW\ (g) = CBW \times 0.01828 \times e^{(0.02-0.0000143t)t} \qquad \textbf{Equation 2.5}$$

Therefore, since they are still growing, lactating young cows (2 to 3-year-old) would have a greater energy requirement than that of mature cows at the same stage of lactation (Freetly et al., 2006). For example, Linden et al. (2014) showed that postpartum DMI (as % of BW) of primiparous beef heifers was greater than that of mature cows, but still not enough to support their requirements for maintenance, growth, and lactation. Moreover, since the nutrient requirements for reproduction stand last in the priority chain of metabolic efficiency and usage, a lactating young cow needs to meet her requirements for maintenance, lactation and growth before starting to make use of nutrients for reproduction. Therefore, a postpartum fat supplementation program, which provides more energy per unit of DM, could help to overcome the nutritional challenge of lactating young cows for a successful breeding season.

2.2. Lipids

Generally, lipids are defined as organic compounds which are insoluble in water (non-polar) but soluble in organic solvents such as hexane, benzene, chloroform, methanol, and toluene (Attwood et al., 2006; Dickschat, 2017). However, a more specific definition has been proposed by Fahy et al. (2011) who defined lipids as small hydrophobic molecules that originate from carbon-based condensations of ketoacyl thioesters or isoprene units. Oils, fats, waxes, steroid hormones, cholesterol, liposoluble vitamins, and phospholipids are examples of different types of lipids. Lipids play many important roles in animal and plant metabolism such as components of the cell membrane structure, substrate for synthesis of hormones, intracellular messengers, among others. However, storing and providing energy is an essential role of lipids for animal and plant organisms (Nelson and Cox, 2005). After going through complete oxidation, lipids are able to provide an average of 9.45 Kcal/g, or 2.25 times more energy than that provided by carbohydrates and proteins (Arrigoni et al., 2016).

2.2.1. Classification of lipids

As indicated previously, lipids are generally insoluble in water but soluble in organic solvents. However, these chemical features are present in a large range of molecules such as fatty acids, phospholipids, sterols, sphingolipids, terpenes and others; therefore, the necessity for classifying lipids (Fahy et al., 2011). According to Fahy et al. (2005), lipids have been divided into two major groups based on their "building blocks": ketoacyl and isoprene groups. Within these two groups, lipids have been classified into eight categories (Fahy et al., 2011):

2.2.1.1. Ketoacyl derivatives

- ***Fatty acyls or Fatty acids*** are hydrocarbon chains with terminal carboxylic acids synthesized by elongation of an acetyl-CoA primer with malonyl-CoA groups (Nelson and Cox, 2005). According to Hames and Hooper (2005), the major roles of fatty acids are: 1) components of membranes, 2) covalent modification of several proteins, 3) energy stores and fuel molecules, and 4) fatty acid derivatives that serve as hormones and intracellular secondary messengers.

- ***Glycerolipids*** are fatty acid esters of glycerol composed mainly of mono-, di-, and tri-substituted glycerols and essential for the synthesis of membrane lipids. The best known among the glycerolipids are the triacylglycerols or triglycerides. Glycerolipids constitute most of the storage fat in mammalian tissues and is the most abundant component in oils and fats of animals and plants (Donato et al., 2013; Donato et al., 2015).
- ***Glycerophospholipids or Phosphoglycerides*** are the most abundant phospholipids. Glycerophospholipids are the main constituents of membrane bilayers and are comprised of three parts: 1) a three-carbon backbone of glycerol, 2) two long-chain fatty acids esterified to the hydroxyl groups on C1 and C2 of the glycerol, and 3) a phosphoric acid esterified to the hydroxyl group on C3 of the glycerol (Blanco and Blanco, 2017; Pollard et al., 2017).
- ***Sphingolipids*** are vital components of the cell membrane of fungi, plant and mammalian cells (Patton and Lester, 1991). Sphingolipids are compounds that contain a sphingoid base backbone and a long-chain fatty acid. Sphingoids are mainly derivatives of the amino alcohol sphingosine or phytosphingosine which are found in animal and plant tissues, respectively (Gordon, 2003).
- ***Saccharolipids*** are compounds in which fatty acids are connected directly to a sugar backbone. This exclusive structure makes them compatible with membrane bilayers (Yan et al., 2016). In the saccharolipids, a sugar substitutes for the glycerol backbone that is present in glycerolipids and glycerophospholipids (Fahy et al., 2005).
- ***Polyketides*** are polymers of acetate and other carboxylic acids synthesized through linear poly-β-ketones (Attwood et al., 2006; Ziemert and Jensen, 2012).

2.2.1.2. Isoprene derivatives

- ***Sterol lipids*** are ringed molecules with an OH group on the first 6-carbon ring. Sterols, also known as steroid alcohols, occur in the membranes of plants, animals and microorganisms. Cholesterol is the main sterol occurring in animals (Gordon, 2003; Monreal and Schnitzer, 2013).

- ***Prenol lipids*** are molecules synthesized from isopentenyl diphosphate and dimethylallyl diphosphate which are five carbon precursors. Vitamin A and its derivatives are grouped under C_{20} isoprenoids (Fahy et al., 2005).

2.2. Classification of Fatty Acids

Fatty acids can be classified into numerous groups according to their structure, physiological role and biological effects (Tvrzicka et al., 2011). Most commonly, and for the purpose of this document, fatty acids have been classified according to the number of double bonds in their carboxylic chain and according to the number of carbon atoms (Tvrzicka et al., 2011) as follows:

- Saturated (**SFA**): fatty acids with no double bond in the carboxylic chain. According to the number of carbon atoms in the carboxylic chain, SFA are subdivided into (FAO, 2010):
  - ✓ Short-chain: SFA containing 2 to 7 carbon atoms. This category includes acetic (2:0), propionic (3:0), butyric (4:0) and caproic (6:0) acids.
  - ✓ Medium-chain: SFA containing 8 to 13 carbon atoms. This category includes capric (10:0) and lauric (12:0) acids.
  - ✓ Long-chain: SFA containing 14 to 19 carbon atoms. Within these FA, the most common in plant and animal products are palmitic (16:0) and stearic (18:0) acids, respectively.
  - ✓ Very long-chain: SFA containing 20 or more carbon atoms. This category includes arachidic (20:0), behenic (22:0) and lignoceric (24:0) acids.

- Unsaturated: fatty acids with at least one double bond in the carboxylic chain. Unsaturated FA are further subdivided according to the number of double bonds in the carboxylic chain and configuration into:
  - ✓ Monounsaturated (**MUFA**): fatty acids containing one double bond in the carboxylic chain. According to the position of the hydrogens at the double bond, MUFA are divided into:

- ***cis***-MUFA with the two hydrogens at the double bond on the same side of the molecule.
- ***trans***-MUFA with the two hydrogens at the double bond on the opposite side to one another.

✓ Polyunsaturated (**PUFA**): fatty acids containing at least two double bonds in the carboxylic chain. The PUFA can be classified into three families:

- **n-3**: this family of PUFA is comprised of α-linolenic acid (ALA; 18:3n-3) and its derivatives. Examples of n-3 PUFA are eicosapentaenoic (EPA; 20:5n-3) and docosahexaenoic (DHA; 22:6n-3) acids.
- **n-6**: this family of PUFA is comprised of linoleic acid (LA; 18:2n-6) and its derivatives. Arachidonic acid (AA; 20:4n-6) is an example of FA in this family.
- Conjugated FA: these are isomers of PUFA with multiple combinations of numerical, positional and geometrical configurations in double bonds (Nagao and Yanagita, 2005). Isomers of conjugated linoleic acid (CLA) are the most abundant FA with conjugated system of double bonds (Tvrzicka et al., 2011). The isomer most commonly found in meat and dairy products is rumenic acid (*cis*9, *trans*11-18:2).

✓ According to the number of carbon atoms in the carboxylic chain, unsaturated FA are classified as (FAO, 2010):

- Short-chain: unsaturated FA containing 19 or less carbon atoms.
- Long-chain: unsaturated FA containing 20 to 24 carbon atoms.
- Very long-chain: unsaturated FA containing 25 or more carbon atoms.

2.3. Lipid Metabolism in Ruminants

2.3.1. Source and type of lipids in ruminant diets

Domesticated ruminant animals are reared under a wide variety of environmental conditions and production systems which creates substantial variation in the type and quality of their diets (Noble, 1981). Consequently, lipids in the ruminant diet can be derived from different sources such as forage crops, cereal grains, oil seeds or their extracts, and animal oil or tissue. According to Arrigoni et al. (2016), palmitic, stearic, oleic (*cis9*-18:1), linoleic (LA) and α-linolenic (ALA) are the most common FA present in the diet of ruminants.

In forages, the total amount of lipids has been reported to range between 3 to 10% with phospholipids being the most abundant and, along with glycolipids, constitute around 95% of the total lipid fraction (Harfoot and Hazlewood, 1988; Arrigoni et al., 2016). The fatty acid composition of forages is dominated by ALA and LA, with ALA reaching concentrations as high as 75% (Clapham et al., 2005; Elgersma et al., 2007). In cereal grains, the total amount of lipids is somewhat lower than that reported for forages. After analyzing barley, corn, oats, rye, sorghum, triticale, and wheat samples, Price and Parsons (1975) found that the total lipid content in these cereal grains ranged from 2.3 to 6.6%. However, contrary to forages, these authors found that glycolipids and phospholipids represent a minor portion of the total lipids averaging 11.1 and 13.3% respectively; while 76% of lipids in cereal grains are triglycerides. The fatty acid composition in the cereal grains analyzed by Price and Parsons (1975) was mostly oleic and linoleic acids averaging 22.8 and 54.3% of the total fatty acids, respectively. In oil seeds, triglycerides are the most abundant lipids (Arrigoni et al., 2016). The total amount of oil in some of the main oil seeds used in ruminant diets averages 36% for cottonseed, 43% for canola seed, 46% for flaxseed, 38% for safflower seed, and 48% sunflower seed. Linoleic acid is the most abundant FA in cottonseed (50%), safflower (74%) and sunflower (63%) seeds; while oleic (63%) and α-linolenic (57%) acids are the most abundant FA in canola and flaxseed, respectively (McKevith, 2005; Barthet, 2017; Siemens, 2017).

2.3.2. Hydrolysis of dietary lipids in the rumen

Dietary lipids go through major changes in the rumen. Two biochemical pathways are responsible for these changes: hydrolysis and biohydrogenation.

Hydrolysis is the first step in the transformation of esterified lipids reaching the rumen and is mainly carried out by lipolytic bacteria that are capable of producing microbial lipases. Ciliate protozoa and anaerobic fungi have little to no capacity for hydrolysis of esterified lipids in the rumen (Harfoot and Hazlewood, 1988; Dehority, 2003). The main purpose of hydrolysis is to break the ester linkages and release the glycerol backbone for further fermentation and yield of energy mainly in the form of propionic acid. Consequently, unesterified free fatty acids (FFA) are the main product of the hydrolysis process and these are required for biohydrogenation to take place (Harfoot and Hazlewood, 1988; Jenkins, 1993; Buccioni et al., 2012).

2.3.3. Biohydrogenation of unsaturated fatty acids in the rumen

According to Jenkins (1993) and Buccioni et al. (2012), the main purpose of the biohydrogenation in the rumen is to protect the microorganisms from the toxic effect of unsaturated FA. Therefore, unsaturated FA are rapidly hydrogenated by rumen microbes into the corresponding saturated configuration. Biohydrogenation is mainly associated with bacteria attached to feed particles, rather than with those in ruminal liquid. Rumen bacteria are the microorganisms mainly responsible for the biohydrogenation of FA, while the contribution of protozoa is of minor importance (Singh and Hawke, 1979; Harfoot and Hazlewood, 1988). More recent *in-vitro* research has found that linoleic acid can also be hydrogenated by rumen fungi (Nam and Garnsworthy, 2007). However, the extent of hydrogenation by fungi was found to be lower than that of bacteria. Biohydrogenation of unsaturated FA does not occur unless carboxyl groups are released from the glycerol backbone; therefore, hydrolysis is a prerequisite for biohydrogenation (Jenkins,1993).

Oleic, linoleic and linolenic acids are the most abundant unsaturated FA released after the hydrolysis of lipids entering the rumen. Usually, oleic acid undergoes complete hydrogenation which results in stearic acid. In the case of linoleic and α-linolenic acids (Figures 2.1 and 2.2), the first two steps of the biohydrogenation process are similar. Biohydrogenation of linoleic and α-

linolenic acids starts with isomerization of the *cis*-12 double bond by the enzyme isomerase which results in *trans*-11 isomers. After this first step, the hydrogenation of the *cis*-9 bond occurs through microbial reductase and the products are *trans*11-18:1 (vaccenic acid) and *trans*11, *cis*15-18:2 for linoleic and linolenic acids, respectively. The resulting vaccenic acid can go through one more hydrogenation and become stearic acid (18:0), while the *trans*11, *cis*15-18:2 product from α-linolenic acid can go through two different pathways based on the type of bacteria carrying out the hydrogenation process. The first pathway involves one more hydrogenation and the product is either *trans*15- or *cis*15-18:1 acid which does not undergo further hydrogenation. The second pathway involves two more hydrogenation steps resulting in vaccenic and stearic acid as the intermediate and final products, respectively (Harfoot and Hazlewood, 1988; Jenkins, 1993). The extent to which oleic and vaccenic acids are hydrogenated to stearic acid depends on the ruminal environment. For example, complete hydrogenation to stearic acid is inhibited by large amounts of linoleic or linolenic acid (Harvatine and Allen, 2006).

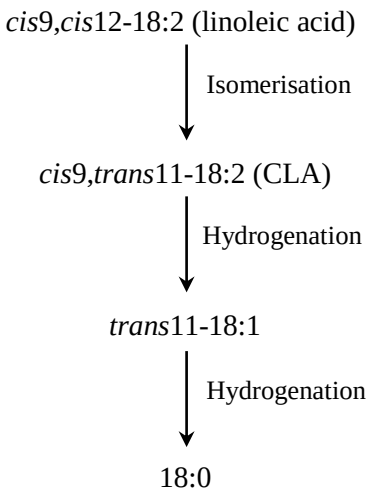

Figure 2.1. Biohydrogenation of linoleic acid in the rumen (Harfoot and Hazlewood, 1988).

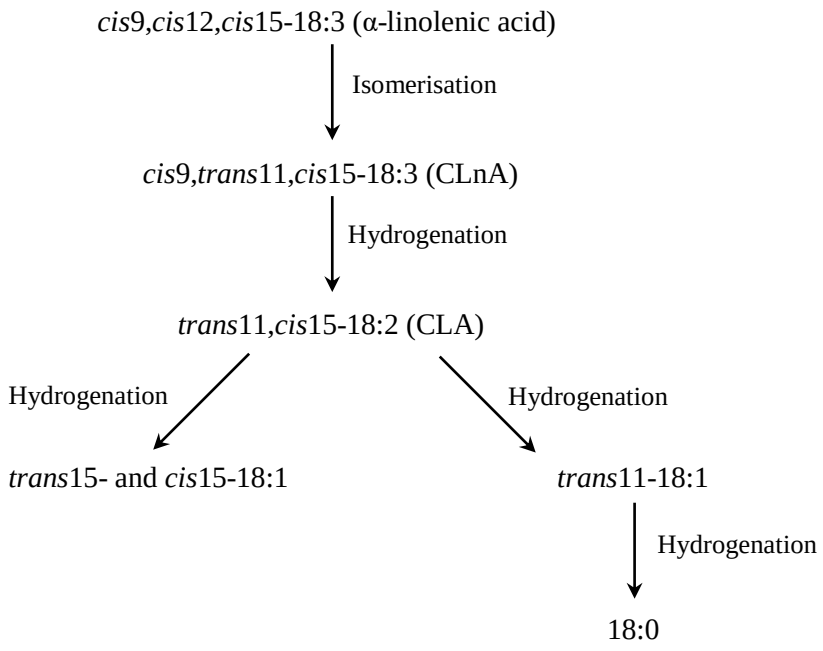

Figure 2.2. Biohydrogenation of α-linolenic acid in the rumen (Harfoot and Hazlewood, 1988).

2.3.4. Absorption of fatty acids in ruminants

In ruminants, the length of the fatty acid determines the site of absorption. It has been estimated that the rumen epithelium absorbs around 70% of the total short-chain fatty acids while very little absorption of long-chain fatty acids takes place in the rumen (Noble, 1981; Bauchart, 1993). The capacity of the rumen to absorb the three major short-chain fatty acids is greater for butyrate, followed by propionate, with acetate having the lowest absorption among these three fatty acids. However, there is evidence that individual SCFA absorption rates vary with changes in ruminal pH or concentration of SCFA (Dijkstra, 1994). Most of the absorbed butyrate (90%) is metabolized in the rumen epithelium (Britton and Krehbiel, 1993; Brockman, 2005). Butyrate is transformed to ketone bodies within the rumen epithelium resulting in the synthesis of β-hydroxybutyrate via the activity of β-hydroxybutyrate dehydrogenase. On the other hand, approximately half of the propionate is metabolized to lactate while little (10%) to no metabolization of acetate occurs within the rumen epithelium (Britton and Krehbiel, 1993). The short-chain fatty acids absorbed by the rumen epithelium and the products of their metabolization

(i.e. β-hydroxybutyrate and lactate) are transported to the portal vein mainly by bicarbonate-dependent mechanisms (Aschenbach et al., 2011); and their passage rate decreases as chain length increases. Kristensen and Harmon (2004) reported that almost all the acetate and propionate that were infused into the reticulorumen of cattle was recovered in the portal blood. Those fatty acids not absorbed by the rumen epithelium enter the omasum where short-chain fatty acids go through a similar absorption to that occurring in the rumen (Noble, 1981).

Lipids that reach the small intestine are mainly non-esterified fatty acids (70 to 90%) attached to feed particles, while the rest of lipids are composed of microbial phospholipids, and small amounts of esterified fatty acids such as triglycerides and glycolipids (Bauchart, 1993; Doreau and Chilliard, 1997). Fatty acids are released from feed particles through the action of biliary salts, lysolecithin, and pancreatic enzymes (Davis, 1990; Arrigoni et al., 2016). Phospholipids, triglycerides and glycolipids are hydrolyzed by intestinal and pancreatic lipases to allow the release of non-esterified fatty acids (Doreau and Ferlay, 1994). Free fatty acids are gradually transfered to the micellar phase as digesta goes through the intestinal tract: 5% of the total transfer occurs in the duodenum, 45% in the jejunum, and 50% in the ileum. Once micelles are formed, they are absorbed by epithelial cells of the small intestine where fatty acids are re-esterified into triglycerides (Noble, 1981; Bauchart, 1993). In ruminant animals, fatty acids that constitute the triglycerides formed in the mucosa cells can differ to those entering in the form of micelles. Desaturation of stearic acid to oleic acid has been observed *in-vitro* within cells from the jejunal mucosa of sheep (Bickerstaff et al., 1972; Wahle, 1974).

After their synthesis in the intestinal mucosal cell, triglycerides are stored in lipoproteins such as chylomicrons and very low-density lipoproteins (VLDL). Chylomicron secretion has been stimulated by increasing dietary fat or dietary PUFA (Harrison et al., 1974; Auboiron et al., 1990). Secretion of VLDL in the intestine has also been enhanced by high-fat diets (Storry et al., 1980). Chylomicrons and VLDL can enter the plasma via the intestinal lymphatic system or through absorption by the portal vein (Noble, 1981). However, absorption of intestinal lipoproteins by the portal vein is a major pathway in calves and in cows fed high-fat diets (Durand et al., 1990; Chilliard et al., 1992). Once in plasma, the main role of chylomicrons is to transport dietary fatty acids (as triglyceride) to tissues for fat storage, milk fat synthesis, or for oxidation; while VLDL will go through lipolysis to produce low density lipoproteins (LDL)

which are implicated in cholesterol distribution to tissues (Bauchart, 1993). High density lipoproteins (HDL) are the major plasma lipoprotein in ruminants (more than 80%); and are synthetized by the liver and small intestine mainly from linoleic acid (Forte et al., 1981; Grummer et al., 1983).

2.3.5. Adipose tissue metabolism

In ruminants, lipogenesis can occur through three pathways: by *de novo* fatty acid synthesis mainly from acetate and to a lesser extent from lactate, by circulating fatty acid uptake, or by hydrolysis of plasma lipoproteins. Acetyl-CoA carboxylase is the enzyme responsible for *de novo* fatty acid synthesis, while the enzyme lipoprotein lipase catalyzes the hydrolysis of plasma lipoproteins allowing the fatty acid uptake by adipocytes (Chilliard, 1993; Chilliard et al., 2000).

Several factors can affect the amount of lipogenesis derived from each pathway. For example, increasing the dietary fat content has been shown to inhibit the activity of acetyl-CoA carboxylase; thus, decreasing *de novo* fatty acid synthesis in growing sheep (Vernon, 1976). However, the degree of inhibition seems to be greater with addition of stearic acid and less effective with addition of linoleic acid (Vernon, 1977). Lipoprotein lipase activity in adipose tissue decreases during fasting; and returns to previous or higher values during refeeding. However, this response of lipoprotein lipase due to under- and refeeding are less in subcutaneous compared to perirenal adipose tissue of adult ruminants (Chilliard et al., 1998; Faulconnier et al., 1999). Synthesis of adipose tissue can also decrease during early lactation in response to the extent of negative energy balance (McNamara, 1989; Vernon, 1998). However, the decrease in adipose tissue synthesis during early lactation has been linked to a decrease in the activity of acetyl-CoA carboxylase rather than lipoprotein lipase (Vernon, 1998). According to Chilliard et al. (1998), the lower insulin secretion during undernutrition regulates the amount and activity of lipogenic enzymes such as lipoprotein lipase.

In ruminants, fatty acid desaturation can take place in adipose tissue through the activity of the enzyme Δ9-desaturase. According to Brooks et al. (2011), most of the monounsaturated fatty acids in bovine and ovine muscle and adipose tissue originate from the desaturation of saturated fatty acids by Δ9-desaturase. For example, desaturation of stearic acid to oleic acid has

been observed in lamb meat (Enoch et al., 1976). Moreover, monounsaturated fatty acids can also go through changes in their saturation and configuration due to the activity of Δ9-desaturase. It has been shown that more than 85% of the conjugated isomer *cis*9, *trans*11-18:2 in mammalian adipose tissue originates from desaturation of *trans*11-18:1 (Ntambi, 1995).

Lipolysis is the release of free fatty acids (non-esterified) from triglycerides in the adipose tissue by hormone-sensitive lipase (Chilliard, 1993; Chilliard et al., 2000). Lipolysis increases during fasting and lactation as a result of the negative energy balance (Dunshea et al., 1988; Chilliard, 2000). This increase in lipolysis and the consequently circulating non-esterified fatty acids during underfeeding can be linked to changes in levels of glucose and insulin in blood (Vernon, 1992; Chilliard et al., 1998). It has been reported (Bonnet et al.,1998) that fasting increased the expression of the hormone-sensitive lipase gene in adipose tissue of cattle.

2.3.6. Synthesis of fatty acids in milk

In ruminants, about 50 to 60% of the fatty acids in milk are derived from *de novo* synthesis occurring within the mammary epithelial cells, while fatty acids taken from circulation are the source of long-chain fatty acids (Dils, 1986; Neville and Picciano, 1997). The substrates for *de novo* synthesis of fatty acids are acetate, as the major carbon source, and β-hydroxybutyrate which provides about 50% of the first four carbons of *de novo* synthesized fatty acids (Bauman and Davis, 1974). Fatty acids utilized for milk fat synthesis are mainly derived from circulating lipoproteins and NEFA originated from the absorption of dietary lipids, while fatty acids derived from lipolysis and mobilization of body fat account for less than 10% (Bauman and Davis, 1974). However, the contribution from mobilized fatty acids increases when cows are in negative energy balance (Bauman and Griinari, 2003).

Because of the extent of *de novo* synthesis of fatty acids in the mammary epithelial cells, milk fatty acid composition differs from that derived from the diet. Short-chain fatty acids (4 to 8 carbons) and medium-chain fatty acids (10 to 14 carbons) in milk fat arise almost exclusively from *de novo* synthesis, 16 carbon fatty acids originate from both uptake from circulation and *de novo* synthesis, and long-chain fatty acids (more than 16 carbons) are derived from the uptake of circulating fatty acids (Bauman and Griinari, 2003). Moreover, further changes can occur to the

fatty acid configuration due to desaturation activity within mammary epithelial cells. Saturated fatty acids and some monounsaturated fatty acids, such as *trans*-vaccenic acid (*trans*-11 18:1), can be desaturated through a reaction catalyzed by the enzyme Δ9-desaturase (Ntambi and Miyazaki, 2004). Griinari et al. (2000) showed that infusing *trans*-11 18:1 in the abomasum of Holstein cows resulted in a 31% increase in concentration of *cis*-9, *trans*-11 CLA in milk fat. However, inclusion of protected PUFA in the diet of dairy cows has shown to decrease the activity of Δ9-desaturase in mammary gland tissue (Jacobs et al., 2011).

2.4. Fat Supplementation in the Ration of Beef Cows

The main purpose of supplementing fat to ruminants is to increase the energy density of the diets. However, adding fat to the diet of ruminants can disturb ruminal fermentation, causing a reduction in digestibility of structural carbohydrates, lowering SCFA production, and ammonia concentration in the rumen (Jenkins, 1993; Doreau and Ferlay, 1995). However, these negative effects of fat addition on fibre fermentation in the rumen seems to depend on the amounts of supplemental fat and the concentrate to forage ratio in the diet. In a review of the research conducted on fat inclusion in diets fed to ruminants, Hess et al. (2008) concluded that supplementing fat up to 2% of total DMI has no negative effects on fibre fermentation of high-forage diets; whereas DE does not increase when supplemental fat exceeds 4% of total DMI under high-forage diets. On the other hand, when receiving high-concentrate diets, fat can be supplemented up to 6% of total DMI without negative effects on utilization of other dietary components (Hess et al., 2008).

The detrimental effects of fat on rumen fermentation and fibre digestion have been attributed to inhibition on microbial activity, particularly that of cellulolytic and methanogenic microorganisms (Schroeder et al., 2004). It has been documented that unsaturated fatty acids inhibit rumen fermentation more than saturated fatty acids (Jenkins, 1993; Buccioni et al., 2012). Maczulak et al. (1981) studied the effects of adding low concentrations of palmitic, stearic, oleic, and vaccenic acid to in vitro rumen cultures on the growth of seven species of rumen bacteria; and found that bacterial growth was not affected by either palmitic or stearic acids while cellulolytic species were inhibited by oleic and vaccenic acid. In another study, after including

linoleic or linolenic acid in the diet of dairy cows in the form of soybean or flaxseed oil, respectively, Yang et al. (2009) observed a decrease in the colony count of cellulolytic bacteria and in the total number of *Ruminococcus albus* and *Fibrobacter succinogenes* from the ruminal content. The reason for the greater inhibitory effect of unsaturated fatty acids on rumen fermentation has been attributed to a toxic effect on rumen bacteria (Buccioni et al., 2012). Maia et al. (2007) added PUFA to *in vitro* incubations of 26 ruminal bacteria; and found that toxicity to growth of bacteria was ranked as EPA > DHA > ALA > LA and cell integrity was damaged by LA in all 26 bacteria.

2.4.1. Fat supplementation on reproductive performance of beef cows

Fat supplementation to the beef cow has increased since research has reported improvements in reproductive performance of cows fed supplemental fat (Hess, 2009). After supplementing 125 g/hd/d of calcium soaps of fatty acids to pregnant beef cows over 61 d prepartum, Espinoza et al. (1995) found that supplementing fat resulted in more cows exhibiting estrus at 30 to 90 days postpartum and more cows pregnant during the first half of the breeding season. Moreover, high-fat supplements have shown to have a greater positive effect on reproductive performance of beef cows compared to low-fat supplements with similar energy content. Lammoglia et al. (1997b) offered two iso-caloric and iso-nitrogenous supplements that differed in fat content (3.7 vs. 5.2% fat) to beef cows; and found that high-fat supplemented cows tended to have greater medium-sized follicular populations and plasma prostaglandin metabolite concentrations, as well as increased circulating concentrations of insulin, estradiol, and progesterone. Similar findings were reported by Lammoglia et al. (1997a) working with late gestation beef heifers under the same treatment approach. The reason for this improvement in reproductive performance due to fat supplementation has been suggested to be due to an increase in plasma leptin concentration (Hess et al., 2005). However, while the beneficial effect of circulating leptin on reproductive performance has been reported for non-ruminants (Houseknecht et al., 1998; Barb, 1999) and sheep (Wettemann et al., 2003), it has not been reported for beef cows.

2.4.2. Type of fatty acid supplementation on reproductive performance of beef cows.

The nature of supplemental fat can have different responses in terms of reproductive performance of beef cows. Overall, reproductive performance of beef cows has been improved by the inclusion of n-3 and n-6 PUFA to their diets. Petit and Berthiaume (2006) offered gestating beef cows with two iso-caloric and iso-nitrogenous supplements that differed in the type of supplemental fat (flaxseed vs. calcium salts of palm oil); and found that cows supplemented with flaxseed had higher cumulative pregnancy rates compared to those supplemented with calcium salts of palm oil (92.9 vs. 76.7%, respectively). Lopes *et al.* (2009) supplemented *Bos indicus* beef cows with either a rumen protected PUFA source high in linoleic acid or a rumen protected saturated fatty acid (SFA) source from estrus synchronization until 28 d after artificial insemination (AI); and found that cows offered the PUFA source had greater pregnancy rates compared to SFA cows (47.9 vs. 35.5%). Similar findings were observed by Petit *et al.* (2001) when non-gestating dairy cows were fed during 10 weeks from week 9 of lactation with two high-fat (9.3 and 10.0% EE) diets differing in the type of fat (flaxseed vs. calcium salts of palm oil). These researchers found that conception rate at first AI was significantly higher for cows fed the flaxseed diet (87.5 vs. 50.0%), attributing this response to a lower prostaglandin synthesis. After including either fish or olive oil (n-3 PUFA vs. oleic acid) in the diet of dairy cows, Mattos et al. (2004) observed that plasma concentration of PGF2α was lower in dairy cows receiving a diet high in n-3 PUFA. According to Mattos et al. (2000), PGF2α causes regression of the corpus luteum which leads to the initiation of a new oestrous cycle. Therefore, decreasing the uterine secretion of PGF2α by supplementation with n-3 PUFA, may result in an increased time the corpus luteum stays on the ovary; thus, increasing the progesterone level which may contribute to a reduction in embryonic mortality (Staples et al., 1998; Mattos et al., 2000; Gulliver et al., 2012). Greater conception rates in beef cattle have been reported due to an increase in circulating progesterone level. Werth et al. (1996) measured serum progesterone concentration in primiparous beef cows twice weekly after parturition; and found that conception rate after AI at the first estrus was greater (76 vs. 41%) for cows with a sustained increase (more than 1 week) in plasma progesterone

These positive effects of fat supplementation and, more specifically, PUFA supplementation on reproductive performance of cows might help to decrease the parturition to first estrous interval in beef cows, particularly second and third calf heifers that calve later in the season and subsequently graze more mature forages. According to Funston (2014), late calving beef cows and young (2 to 3-year-old) beef cows represent a major proportion of anestrous females at the onset of the breeding season. Moreover, supplementing a source of fat enriched in n-3 PUFA such as flaxseed, may result in improved conception rates compared to a fat source enriched in MUFA such as canola seed.

2.4.3. Type of fatty acid supplementation on milk fat and adipose tissue composition.

Fatty acid composition in milk fat can be affected by the type of supplemental fatty acid in the diet. In the same study cited previously, Petit *et al*. (2001) found that milk fat of cows fed the flaxseed ration had lower concentration of stearic acid (31.7 vs. 35.6% of total FA) but higher concentration of linolenic acid (1.2 vs. 0.9% of total FA) compared to that observed in milk from cows fed the calcium salt of palm oil ration. Similar effects on fatty acid composition of milk fat due to the type of fatty acid supplementation has been observed in dairy cows (Mattos et al., 2004; Cortes et al., 2010; Jacobs et al., 2011).

The type of fat in the diet has also been shown to affect the fatty acid profile in beef. This has been partially attributed to the fact that ruminal microorganisms *in vitro* are not able to hydrogenate C:20 and C:22 PUFA to any significant extent (Ashes et al., 1992). After reviewing several research works evaluating the fatty acid composition in adipose tissue from finished cattle fed diets containing different sources of PUFA, Mir et al. (2003) concluded that concentrations of conjugated linoleic acid and vaccenic acid in beef were increased by the inclusion in the diet of fat sources high in PUFA including flaxseed. He et al. (2011) fed beef steers during the growing and finishing period with diets either including 5% of a 1:1 blend of flax:sunflower oil or without the inclusion, and reported greater levels of linoleic, linolenic, and conjugated linolenic acids in the intramuscular and subcutaneous fat (from the *Pars costalis diaphragmatic* muscle and brisket, respectively) of the carcass from those animals fed the oil blend diet.

2.5. Developmental Programming

Developmental programming refers to the effects of changes and/or adjustments to nutrient supply during gestation on the later life characteristics of the offspring (Gicquel et al., 2008; Reynolds et al., 2010). Both maternal under and over nutrition as well as nutrient imbalance during gestation have been shown to affect the growth performance of the offspring (Du et al., 2010a; Oksbjerg et al., 2013). However, maternal nutrient deficiency is the main cause of deprived fetal growth and development, and adequate nutrition during gestation has been shown to have a positive effect on the development and performance of the offspring of many mammalian species (Wu et al., 2004; Du et al., 2011).

2.5.1. Overall nutrient intake during gestation

2.5.1.1. Overall nutrient restriction during gestation on fetal growth

The effect of overall nutrient restriction of the dam during gestation on the birth weight of the offspring has been extensively documented for mammalian species. When restriction of nutrient supply from the dam to the fetus occurs during the entire or most of the gestation period, the result is a constant decrease in fetal growth. Osgerby et al. (2002) evaluated the growth of 45, 90, and 135 d fetuses from pregnant ewes fed with either 100 or 70% of requirements from 20 d of gestation until term. After 45 d, it was found that fetal weight was not different between restricted and non-restricted groups (8.4 and 8.6 g), but fetuses from restricted ewes were 2% lighter at 90 d (562 vs. 574 g) and 12% lighter at 135 d (3886 vs. 4395 g) of gestation.

2.5.1.2. Timing of overall nutrient restriction during gestation on fetal growth

When nutrient restriction of the dam occurs during a portion of the gestation period, the effects on the progeny seem to depend on the timing of maternal exposure to nutrient restriction. Vonnahme et al. (2003), Zhu et al. (2004), and Ford et al. (2007) evaluated the effect of nutrient restriction (50% of requirements) from early- to mid-gestation (28 to 78 d) with re-feeding (100% of requirements) from mid-gestation to parturition, vs. no restriction (100% of requirements) during the gestation period of pregnant ewes on both the fetal (78 d) and placental weights, and birth weight of the offspring. It was observed that fetal weight and placental efficiency were

reduced in nutrient restricted ewes compared to those from non-restricted dams at mid-gestation. Also, *longissimus dorsi* muscle of 78 d fetuses from nutrient-restricted dams contained fewer secondary muscle fibres. On the other hand, no difference was observed in birth weight between treatments indicating a compensatory fetal growth from mid- to late gestation in lambs born to nutrient restricted ewes after re-feeding.

This accelerated or compensatory growth observed in fetuses during re-feeding after being exposed to nutrient restriction during early-gestation can be mainly attributed to three reasons. First, fetuses of most mammalian species reach at most 25% of their birth weight by mid-gestation, experiencing an exponential increase in fetal weight during the last third of gestation, whereas the placenta experiences rapid growth during early-gestation practically reaching its total weight by mid-gestation (Eley et al., 1978; NRC 2000; Redmer et al., 2004). Second, fetal growth depends on the acquisition of nutrients mainly from the placenta; and the placental vascularity and nutrient transporter activity are increased as a response to nutrient restriction during early-gestation (McCrabb et al., 1992; Chung et al., 1998; Lunney 1998; Ma et al., 2011). Therefore, when re-feeding is applied, not only the concentration but also the flow rate of nutrients to the placenta is increased in favour of fetal growth (Heasman et al., 1998; Vonnahme et al., 2006). Finally, muscle fibre development can be divided into three stages: embryonic, fetal, and postnatal; and these stages correspond to primary, secondary, and postnatal myogenesis (Du et al., 2010b). In cattle, primary myogenesis occurs within the first two months post conception whereas secondary muscle fibres are formed between 2^{nd} and 7^{th} or 8^{th} month of gestation (Du et al., 2010ab). Maltin et al. (2001) state that primary fibres appear to be resistant to manipulation due to their genetic programming which seems to be not influenced by exogenous factors. However, more secondary than primary muscle fibres are formed in mammals due to the difference between cross sectional areas of both myofibre types (Oksbjerg et al., 2004; Du et al., 2010a). Thus, the fetal stage or mid-gestation is critical for muscle development and perturbations during this period, including maternal over or under nutrition, will result in an increase or reduction in the formation of secondary muscle fibres with long-lasting and irreversible physiological consequences for the progeny (Dwyer et al., 1994; Zhu et al., 2004).

Although nutrient restriction of the dam during late gestation has resulted in reduced fetal growth, this negative effect can be avoided if the dam is in good nutritional status by the

beginning of the nutrient restriction period. Hough et al. (1990) evaluated the effects of nutrient restriction (57 vs. 100% of requirements) of mature beef cows during the last third (90 d) of gestation on the birth weight of their calves and observed no difference between calves born to both groups. This can be attributed to a loss in BW and nutritional status experienced by restricted cows as a consequence of a negative energy balance and mobilization of body fat. From the start to the end of the experimental period, no negative change was observed in BW and BCS (+35 kg and 0 respectively) for non-restricted whereas both measurements decreased (-22 kg and -0.7 respectively) in restricted cows.

2.5.1.3. Overall nutrient restriction during gestation on survival of newborns

Overall nutrient restriction during pregnancy has also been shown to affect survival of the offspring possibly due to lesser colostrum yield or lower absorption of colostrum components. Angus cows fed 57% of their nutrient requirements during the last third of gestation tended to have greater colostral immunoglobulin G (IgG) concentration than those non-restricted (43.0 vs. 39.5 mg/mL); however, when calves from both pre-natal treatments were fed equal amounts of colostrum from either restricted or non-restricted cows, serum IgG concentration was lower (17.2 vs. 22.0 mg/mL) at 24 h in calves fed colostrum from previously restricted cows (Hough et al., 1990). This has been attributed to a possible constituent of colostrum affecting IgG absorption or to the efficiency of nutrient transfer at the small intestine between calves born to restricted or non-restricted cows (Hough et al., 1990; Funston et al., 2010). Hashemi et al. (2008) found no difference in colostral IgG concentration (125 vs. 120 mg/mL) within the first 24 h after lambing between Karakul ewes that had been fed 110% of nutrient requirements from two months prior to parturition vs. ewes fed on poor rangeland vegetation; however, total colostrum yield was grater in well fed dams (318 vs. 171 g).

2.5.1.4. Overall nutrient restriction during gestation on post-natal performance

Post-natal development/growth and performance of the progeny are also influenced by maternal nutrition during gestation. In cattle, Underwood et al. (2010) reported heavier weaning weights (256 vs. 242 kg) in calves born to cows grazing improved pastures over 60 d during mid-gestation compared to those born to cows placed on native range during the same period. Moreover, no difference was found in birth weights between treatments; thus, birth to weaning

performance cannot be attributed to bias in birth weight. The positive effect of adequate maternal nutrition during gestation on post-partum growth/performance has been attributed to a higher number of muscle fibres. Hyperplasia of muscle cells does not occur to any significant degree after birth in mammal species. The increase in size (hypertrophy) of muscle cells is the only postnatal mechanism for muscle fibre growth (Oksbjerg et al., 2004; Du et al., 2011; Du et al., 2013). In a review of the effects of maternal nutrition during pregnancy on the progeny performance in beef cattle, Robinson et al. (2013) concluded that low birth weight calves with reduced fiber numbers are unable to exhibit postnatal compensatory growth. Moreover, even though nutrient restriction during early-gestation does not have an effect on birth weight and post-natal performance of the progeny, Long et al. (2010b) showed that nutrient restrictions in cows (55% of requirements) from 32 to 83 d of pregnancy increased myofibre cross-sectional area reducing number of myofibres in steer progeny compared to non-restriction (100% of requirements).

However, two important considerations should be mentioned at this point. First, according to Robinson et al. (2013), maternal nutrition during pregnancy, particularly from mid-gestation to parturition, can also have carryover effects on lactation performance affecting early post-natal calf growth. In an early study, Ottinger and Tanabe (1968) fed rats throughout gestation and lactation a balanced meal offered either *ad-libitum* or 50% of the established *ad-libitum* level. Following parturition, all litters were randomly reduced to 8 pups and cross-fostered both within and between pre-natal treatments. Although birth weight was lower for pups born to restricted vs. non-restricted dams, no difference was observed in weaning weight due to pre-natal feeding evidencing a post-natal compensatory growth by pups born to nutrient restriction dams. Second, severe nutrient restriction during early-pregnancy may result in altered energy metabolism. Even though no difference was observed by Ford et al. (2007) in both birth and weaning weights of the progeny born to nutritionally restricted (50% of requirements) vs. non-restricted (100% requirements) ewes during early to mid-gestation (28 to 78 d), wethers from restricted dams were heavier at 120 d of age (26.6 vs. 21.8 kg) and remained heavier until slaughter (61.7 vs. 56.8 kg) where they showed a tendency for greater hot carcass weight (31.6 vs. 28.8 kg). However, the higher growth of wethers from restricted ewes was not translated into greater carcass quality. Wethers from nutrient restricted ewes had greater backfat thickness (0.3 vs. 0.2 cm) at 140 d of

age, greater weight of kidney-pelvic fat (0.68 vs. 0.46 kg) at slaughter, and a tendency to lower ratio of *longissimus dorsi* and *semitendinosus* muscle weight to hot carcass weight.

2.5.1.5. Overall nutrient intake during gestation on finishing performance and carcass quality.

Finishing performance as well as carcass and meet quality have been improved by increasing nutritional status of the dam during gestation. In cattle, Robinson et al. (2013) report that each kg of increase in birth weight results in 3.02 and 4.39 kg of increase at the end of backgrounding and finishing phases, respectively. This is in agreement with findings report by Underwood et al. (2010), who found that steers born to cows grazing improved pastures during mid-gestation had greater average daily gain (1.66 vs. 1.49 kg/d) and total BW gain during the finishing period (200 vs. 180 kg), than those born to cows grazing native range. Moreover, greater hot carcass weight (348 vs. 330 kg) and fat (ether extract) content of the *longissimus dorsi* muscle (6.0 vs. 4.8%), which resulted in higher tenderness measured as shear force (31 vs. 37 N), were observed in steers born to cows grazing improved pastures during mid-gestation. However, as mentioned previously (Long et al., 2010a), nutrient restriction during early-gestation may not influence birth weight or postpartum growth but may cause alterations in muscle fiber development, energy metabolism and/or synthesis of adipose tissue in offspring. According to Long et al. (2010b), all these negative effects of nutrient restriction during early-gestation may result in poor beef quality.

These differences in finishing performance and carcass quality between progeny of dams differentially fed during pregnancy are due to the primary to secondary fibre ratio in muscle tissue, as discussed previously, as well as to the amount of adipose (intra-muscular and sub-cutaneous fat) and connective tissue. In livestock, myocytes, adipocytes, and fibroblasts are all derived from mesenchymal stem cells which are affected by maternal nutrient deficiencies during embryonic and fetal development. Enhancing myogenesis during embryonic and fetal development reduces fat accumulation and increases lean tissue and efficiency of production, whereas enhancing adipogenesis during the same stages increases intra-muscular fat which is desirable for improving meat quality and flavor. Finally, connective tissue is responsible for

background toughness and greater connective tissue content decreases tenderness of meat (Du et al., 2010abc; Hocquette et al., 2010; Du et al., 2011; Du et al., 2013).

2.5.1.6. Overall nutrient intake during gestation on reproductive performance.

Reproductive performance of the progeny has also been affected by maternal nutrition during pregnancy. Testicular volume, as well as testosterone concentrations from birth until weeks 28 and 35 and at seasonal peak, was lower in lambs born to moderate fed ewes compared to those born to high fed ewes during gestation (Da Silva et al., 2001). Gunn et al. (1995) observed that lifetime reproductive performance of the female offspring was improved by supplementation of the ewe during late pregnancy. In cattle, Ireland *et al*. (2010) concluded that reducing nutrient intake in pregnant cows causes alterations in the maternal environment which has a negative impact on ovarian function and optimal fertility in heifer progeny. However, when mature beef cows were fed 75% of their nutrient requirements during the last two thirds of gestation, no negative effects were observed on growth or reproductive performance of female progeny (Cushman *et al., 2*014).

2.5.2. Energy intake during gestation

2.5.2.1. Energy intake during gestation on fetal growth.

Level of energy intake of the dam during gestation has been shown to influence fetal growth of mammalian species in livestock. In ruminants, Tudor (1972) evaluated the daily inclusion of 3.5 kg of barley grain vs. non-inclusion to the diet of Hereford cows receiving the same basal-forage diet (Rhodes grass) during the last third of pregnancy, and found that calves born to non-supplemented cows were lighter (24.1 kg) compared to those born to supplemented dams (30.9 kg). Similarly, Corah *et al*. (1975) fed Hereford first-calf heifers with either a low or a high energy diet (65 vs. 100% of energy requirements) during the last 100 d of gestation, and found that heifers from dams fed the high energy diet produced heavier calves at birth (30.6 vs. 28.6 kg). Coincidently in the last two cited studies, dams receiving low energy diets during gestation experienced greater loss in BW and subcutaneous rib fat thickness. This was translated into a change in body condition score of the dam which is an indicator of energy balance, due to a positive correlation with subcutaneous body fat in mammalian species (Domecq et al., 1995;

Schröder and Staufenbiel 2006). Loss in body condition of the dam has also been shown to affect fetal development in mammalian species. Bielli *et al*. (2002) fed two levels of ME (70 vs. 110% of ME requirements) to Merino ewes from mid-gestation until parturition. It was found that ewes receiving the low level of dietary ME did not gain weight and produced lambs with lower birth weights, whereas ewes fed high level of dietary ME gained 17% over their initial weight and birth weights of their lambs were higher. Similarly, Dwyer (2003) reported that ewes in a positive energy balance through gestation produced heavier lambs at birth. Spitzer et al. (1995) and Renquist et al. (2006) observed heavier calves at birth from primiparous beef cows with greater body condition score at calving. However, fetal growth seems to be negatively affected if the dam experiences a negative energy balance only during the exponential fetal growth phase (last third of gestation). Bispham et al. (2003) fed pregnant ewes either 0.84 or 2.22 Mcal/d of ME from 28 to 80 d of gestation, and after 80 d each group was fed either to meet ME requirements (1.7 Mcal/d) or to appetite until 140 d of gestation. It was found that restricting energy intake early in the gestation period did not make a difference in birth weights of lambs if dams were fed at least to requirements from 80 d to parturition. In contrast, ewes fed to requirements from 80 to 140 d of gestation had lighter lambs at birth (3.9 vs. 4.8 kg) compared to those fed to appetite.

Moreover, fetal growth depends not only on the energy status but also on the energy source in the pre-partum diet of the dam. In ruminants, Radnuz et al. (2010) and Radunz et al. (2011) fed three iso-caloric diets to mature pregnant cows and ewes from mid-gestation to parturition. Diets differed in primary feed source: hay, corn, and corn-DDGS; where corn and corn-DDGS were limited to achieve similar energy intake across dietary treatments. No difference was found on birth weights between corn and corn-DDGS groups for both calves (43.1 and 41.3 kg respectively) and lambs (6.02 and 6.11 kg respectively), while offspring from hay fed dams had lower birth weight for both bovines (38.8 kg) and ovine (5.46 kg). However, it is not clear if the findings reported in these studies are due to the source of energy or to a difference in protein levels across dietary treatments. Dams fed corn-DDGS diet had a higher CP intake compared to those fed hay and corn diets and, due to the low ruminal degradability of the CP in corn-DDGS (Klopfenstein et al., 2008), the amount of CP available for absorption at the small intestine was also greater in this group. Robinson (1977) reports that the detrimental effect of low maternal CP intake on lamb birth weight is more evident at low levels of energy intake, due to

the ability of the ewe to catabolize protein from her own tissue in order to meet both protein and energy requirements.

2.5.2.2. Energy intake during gestation on survival of newborns.

Survival and vigor of newborn offspring have been positively affected by energy intake of the dam during pre-partum period. Budge *et al*. (2000) fed pregnant ewes during late-pregnancy with either 100 or 150% of their ME requirements, and concluded that neonatal viability was enhanced by increasing the amount of ME provided during late gestation which promoted fetal growth and brown adipose tissue maturation. Lammoglia et al. (1999ab) found that beef females fed a high-fat diet prepartum produced calves with greater tolerance to cold stress due to a greater heat generation. Dwyer (2003) concluded that ewes in positive energy balance during pregnancy produced lambs that stood and suckled faster. Similarly, Dietz et al. (2003) observed that calves from cows that received a high-fat (5%) diet 68 d pre-partum tended to spend less time to stand (40 vs. 56 min) and to nurse (99 vs. 107 min) than those from cows fed a low-fat (2%) diet. Furthermore, not only the level but also the source of energy fed to the dam during gestation has an effect on survival of the progeny. Petit and Berthiaume (2006) reported that calf mortality was four times greater at birth (11.6 vs. 3.0%), and three times greater from birth to weaning (19.7 vs. 7.4%) for calves born to barley supplemented cows than those born to fat (flaxseed or calcium salts of palm oil) supplemented dams. Capper et al. (2006) and Chen et al. (2007) demonstrated that the inclusion of poly-unsaturated fatty acids in the diet of pregnant ewes during late-gestation resulted in lambs with an increased fatty acid oxidation in adipose tissue, increased cold tolerance, and decreased latency to suckle; thus, improving lamb survival rate when compared to those from dams fed diets with inclusion of saturated fatty acids during late-gestation.

Most of the research reporting the effects of energy supplementation during gestation on subsequent performance of the progeny, has been based on trials comparing either energy restriction or oversupply vs. control groups fed to requirements. Few research studies have been carried out to test the effects of diets similar in energy and protein content but differing in the type of energy (i.e. low- vs. high-fat diets). The studies cited previously by Radunz et al. (2010 and 2011) are among the few studies looking at the effects of type of energy. However, due to the discrepancy in CP content of the diets used, it is not clear if the reported responses are a

consequence of either the type of energy or the CP content. Moreover, information is lacking on the effects of the type of fatty acid (i. e. MUFA vs. PUFA) in the diet of gestating beef cows on the performance of the progeny.

2.5.2.3. Energy intake during gestation on lactation performance.

As discussed previously, it is difficult to determine the effect of nutritional plane of the dam during gestation on the post-natal performance of the progeny without accounting for the carryover effects on lactation. Therefore, studies where cross-fostering or milk replacers have been applied to the offspring after birth should provide more reliable results. After Tudor (1972) evaluated the inclusion vs. non-inclusion of barley grain to the diet of Hereford cows during the last third of pregnancy and reported the difference in birth weights previously mentioned, Tudor and O'Rourke (1980) applied an early weaning (4 d) and randomly allocated calves born to both treatments into either a low or high energy diet until 200 d of age. Although calves from the low energy post-natal diet had lower BW at 200 d, within post-natal treatments no effect of the energy intake of the cow during late-gestation was found from 4 to 200 d on average daily gain, feed intake, and final BW of calves. Moreover, calves born to restricted cows had smaller withers (62.4 vs. 65.6 cm) and pelvis (66.9 vs. 70.2 cm) when measured at 3 d of age, but no difference in the same measurements was observed at 200 d (Tudor et al., 1980). As mentioned previously and reported by Ottinger and Tanabe (1968), these results also show that post-natal growth and/or development of offspring born to dams facing nutrient restriction during gestation, can be intrinsically related to lactation performance of the dam and/or level of nutrient supply during post-natal period in mammalian species.

2.5.2.4. Energy intake during gestation on reproductive performance of the progeny.

Reproductive performance in the offspring has shown to be affected by level of energy intake of the dam during pregnancy. Bielli et al. (2002), found that paired testes tended to be heavier, and both absolute volume of testicular cords and the number of Sertoli cells per testis were greater in newborn lambs from ewes receiving 110% of their energy requirements vs. those from dams receiving 70% from mid-gestation to parturition; concluding that depending on the ability of the Sertoli cell population to recover between birth and puberty, this may limit the future capacity for sperm production and fertility. However, when energy intake of the dam was restricted (50 vs.

100% of requirements) from mating to mid-gestation, no effect was observed on reproductive performance of male but reduced ovulation rate in female lambs (Rae et al., 2002). This response of energy restriction of the dam on reproductive performance of the female progeny is in agreement with findings of a study where the timing of the restriction was evaluated and conducted by Rae et al. (2001). In this study (Rae et al., 2001), fetuses were collected at 50, 65, or 110 d of gestation from ewes with non-restriction of energy intake (100% of requirements), and compared to fetuses collected from ewes with restricted energy intake (50% of requirements) applied at one or more critical stages (0 to 30 d, 0 to 50 or 65 d, 0 to 110 d, 31 to 50 or 65 d, and 65 to 110 d) during pregnancy. It was concluded that energy restriction before and during follicular genesis, which occurs during 65-110 d of gestation, can delay fetal follicular development.

2.6. Epigenetics

Many definitions have been proposed for the term *epigenetics* and these have evolved according to new findings/publications within the field of molecular biology. The term *epigenetics* was coined by Waddington (1942) fusing the words *epigenesis* and *genetics*, and defined as "casual mechanisms through which the genes of the genotype bring about phenotypic effects"; but according to Holliday (2006), Waddington had an idea for *epigenetics* rather than a specific definition. Nanney (1958) related the terms *epigenetics* and *gene expression* when defined *epigenetic control systems* as "auxiliary mechanisms with different principles of operation are involved in determining which specificities [genes] are to be expressed in any particular cell". After the DNA methylation models were proposed in the mid-70s and finally demonstrated in the mid-80s, Holliday (1987) published an article titled "The inheritance of epigenetics effects". This publication defined the the term *epigenetics* as "heritable changes that do not involve changes in DNA sequence". Among the definitions arisen during the 90s, the most cited is that proposed by Russo *et al*. (1996) who defined *epigenetics* as "the study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence". Finally, for the purpose of this review, epigenetics will be defined as "stable and heritable alterations in patterns of gene expression that do not involve changes in DNA sequence" as

defined by Wolffe and Matzke (1999), Jaenisch and Bird (2003), Eccleston et al. (2007), and Gicquel et al. (2008).

2.6.1. Characteristics of Epigenetic Systems

Epigenetic processes are necessary for development and differentiation, but they can also occur in mature mammals either by random change or under the influence of the environment (Issa 2004; Reik, 2007). Bonasio et al. (2010) refer to these environmental influences as stimulus, and affirm that if a transcriptional response is self-sustaining after the removal of the initiating stimulus then it can be considered as epigenetic. According to Bird (2007) and Bonasio et al. (2010), a molecular signal is considered an epigenetic system if: *i*) it is heritable, which means that the molecular signal must have a mechanism for propagation; *ii*) is self-perpetuating, which means that the molecular signal is transmitted from one cell to its daughters lasting more than one cell cycle; and *iii*) has an effect on gene expression. Moreover, according to Rhind et al. (2001), changes in gene expression due to epigenetic factors can emerge in successive generations not ending with the present generation. Initially Morgan et al. (1999) and later Waterland et al. (2008) found that the effects of feeding methyl supplements to female mice before and during pregnancy on silencing the agouti gene of the offspring, which causes yellow fur, obesity, and diabetes, can be transmitted from one generation to the next. This indicates that some epigenetic marks of the genome are not completely erased during oogenesis, which may impact the activity of genes in the next generation (Jaenisch and Bird 2003; Wu and Suzuki, 2006). Also, these transgenerational epigenetic effects may include both the physiological and behavioral transfer of information across generations (Youngson and Whitelaw, 2008). In an adoption study, Weaver et al. (2004) found that rat mothers were capable to alter the expression of a glucocorticoid receptor gene promoter in the hippocampus of the offspring, when pups from dams with high maternal care (licking, grooming, and arched-back nursing) were cross-fostered to dams with either high or low maternal care. These alterations in the epigenomic state of the gene appeared over the first week of life and were reversed with cross-fostering, showing that gene expression can be established through behavioral programming, and can also be reversed. This reversal potential, also known as flexibility and/or plasticity, is another characteristic of epigenetic systems allowing transitions among states when the proper signals are delivered. The balance between

stability and plasticity in transcriptional programs presents an inherent regulatory challenge for developing organisms (Reik, 2007; Mohn and Schübeler, 2009; Bonasio et al., 2010).

Three molecular marks are or might be considered epigenetic: DNA methylation, histone modifications, and the influence of small regulatory and non-coding RNA molecules (Lande-Diner and Cedar 2005; Riddihough and Zahn, 2010). Among these three proposed molecular marks, DNA methylation has been the most studied and meets all three independent criteria to be considered as epigenetic mark (Gicquel et al., 2008; Bonasio et al., 2010). On the other hand, whether histone modifications and non-coding RNA are epigenetic marks or not is still up for debate due to the low frequency at which these marks are self-perpetuating and inherited (Wassenegger, 2005; Riddihough and Zahn, 2010). Histone modifications are changes in chromatin organization/structure that occur through post-translational modifications such as such as acetylation, phosphorylation, ubiquitination, and methylation affecting the N-terminal tail of histones (Kiefer, 2007; Gicquel et al., 2008). Nucleosome location is another type of histone modification caused by structural differences between non-corresponding chromatin proteins (variants) affecting accessibility of DNA to transcription factors (Keifer, 2007). Non-coding RNAs are structural, regulatory, and functional RNA molecules that do not encode proteins (Eddy, 1999; Mattick and Makunin, 2006; Kaikkonen et al., 2011). Finally, according to Kiefer (2007), “long-distance chromosomal interactions” has recently been proposed as an epigenetic mechanism which induces alterations in gene activity by direct interactions between chromosomal regions that are distantly positioned from one another.

2.6.2. DNA Methylation

DNA methylation is a biochemical process, catalyzed by DNA methyltransferases, during which a methyl group is transferred to a nucleotide sequence; and is essential for the development of mammals (Jones and Takai, 2001; Kiefer, 2007). Discovered by Hotchkiss more than six decades ago (Hotchkiss, 1948), nowadays it is known that DNA methylation regulates genome functions through influencing gene transcription and chromatin formation (Bird and Wolffe, 1999; Nakao, 2001). DNA methylation can take place in different nucleotide sequence contexts: CG, CCG, CTG, CAG, CCC, CCT, CCA, CTT, CTA, and CAA (Feng et al., 2010). However, methylation of cytosines at the carbon 5 position of CpG dinucleotides is the most common among many

eukaryotic genomes (Ng and Bird, 1999; Richards, 2006). Approximately 60 to 90% of all CpGs are methylated in vertebrates, leaving a minor part of the genome methylation free, and many of the non-methylated CpGs are found in CpG islands which usually include functional promoters (Antequera and Bird, 1993; Ng and Bird, 1999; Nakao, 2001).

As mentioned previously, DNA methylation is a heritable and self-perpetuating process that has an effect on gene expression; thus, meeting all three independent criteria required to be considered epigenetic. First, DNA methylation can be reproduced after DNA replication through biochemical pathways. In a review of the cytosine methyltransferases in eukaryotic cells, Goll and Bestor (2005) state that a DNA sequence carrying symmetrical methylation marks on both strands can replicate to two hemi-methylated double strands, which can be restored to fully methylated status by maintenance methyltransferases. Second, methylated DNA is transmitted to its daughter cells and, considering that passes on for more than 100 cell generations, DNA methylation is classified as very stable modification (Du et al., 2011). Wigler et al (1981) tested the hypothesis that DNA methylation patterns are replicated in the somatic cells of vertebrates and found that the *in-vitro* methylated DNA of the bacteriophage φX174 RF and the cloned chicken thymidine kinase gene, were able to remain methylated after several rounds of *in-vivo* DNA replication using cultured mouse cells. Finally, the way DNA methylation regulates transcription has a clear effect on gene expression. During the mid-1980s, Bird (1984) reviewed the role of DNA methylation on gene expression and concluded that despite the lack of physical evidence, there was a relationship between the presence of 5-methylcystosine at certain CpGs and the way how genes were expressed; however, the nature of this relationship could be repression of some genes or expression of some others independently of the methylation state. Later in another review, Razin and Cedar (1991) concluded that the role of DNA methylation as silencing mechanism for gene expression in somatic cells was well established; and this silencing effect is stable when methylation occurs at the promoter region of the gene (Suzuki and Bird, 2008). Such statement was in agreement with the evidence that cytosine methylation, on both eukaryotes and prokaryotes, is principally a system for deactivating transcription of invading DNA (Bestor, 1990). According to Reik and Walter (2001), transcription of some genes is repressed by DNA methylation in most cases. This can be attributed to the fact that both core promoter and transcription start site are included within the CpG island and gene expression is completely repressed when this region becomes hypermethylated (Nakao, 2001). Nowadays, the

conventional view that DNA methylation inhibits gene expression is being challenged; furthermore, recent evidence shows that intragenic methylation of some genes, such as IGF2R, is positively correlated with gene expression (Suzuki and Bird, 2008; Lan et al., 2013).

Although being a very stable epigenetic system, DNA methylation can be reversed being DNA demethylation the most likely mechanism of epigenetic plasticity in methylated DNAs; and these alterations in methylation status are essential for normal development (Wolffe et al., *1*999; Bonasio et al., 2010). For example, during early embryo development and germline specification in mammals, the paternal genome is silenced through hyper-methylation at fertilization and reactivated later through DNA demethylation (Mayer et al., 2000). Two mechanisms are proposed by Bonasio et al. (2010) as possible for genome-wide DNA demethylation: active demethylation through oxidation, and base excision repair. However, there is evidence supporting that DNA demethylation in plants can occur through base excision repair, while the relevance of this pathway in animals remains unclear (Zhu, 2009; Bonasio et al., 2010). This discrepancy on the knowledge about DNA demethylation process of plants and animals also applies for DNA methyltransferases (DNMT) and is reported by Ooi and Bestor (2008) concluding that while the evidence for active demethylation in plants is convincing and the mechanism is well understood, the evidence for mammalian DNA demethylases remains contradictory. Two studies (Kangaspeska et al., 2008; Métivier et al., 2008) show that DNA demethylation is initiated by the same enzymes (DNMT3A and DNMT3B) that establish the methylation mark in the first place at the promoter region of the estradiol-estrogen receptor, proposing that cyclical methylation and demethylation occur in mammalian cells.

2.6.3. Epigenetics in developmental programming of livestock species

In order to discuss if epigenetic changes are involved in fetal programming, we need to first review some of the main genes acting on growth, muscle and adipose tissue development of mammalian species.

2.6.3.1. Growth genes.

Insulin-like growth factors 1 and 2 (IGF1 and IGF2) are single chain proteins produced in the liver, released to plasma, bound to IGF binding proteins (IGFBP), and target IGF tissue receptors

(IGF1R and IGF2R) to mediate the growth stimulating effect of growth hormone (GH) which has a major impact on muscle fibre development (Oksbjerg et al., 2004). IGFs and IGFBPs are nutritionally regulated in the fetus and growth-retarded fetuses show abnormalities in the GH-IGF axis (Holt, 2002). According to Randhawa and Cohen (2005), and Duan et al. (2010), IGFs not only play a crucial role in muscle differentiation and development during embryo and fetal stages, but also play an important part in adult muscle regeneration and hypertrophy. IGF1 mediates many of the postnatal effects of GH, while the growth promoting function of IGF2 is restricted to the embryo stage with low levels of expression in adults (Humbel, 1990; Baker et al., 1993; Liu et al., 1993). Moreover, IGF2 is subject to parental imprinting; thus, the paternal allele is highly expressed whereas the maternal allele is silent in most tissues (Haig and Graham, 1991). De Chiara et al. (1991) report that neonatal mice that received a paternal null-mutation of the IGF2 gene were smaller compared to those receiving the maternal null-mutation of IGF2 gene.

2.6.3.2. Myogenic genes.

Muscle mass depends on the number and size of muscle fibres which are the functional units of differentiated muscle (Maltin et al., 2001; Oksbjerg et al., 2004). Mesenchymal stem cells (MSCs) are the precursor of muscle cells and myogenesis is the process through which this transformation occurs (Du et al., 2010b). Myogenesis, or muscle cell formation, is a multistep process controlled by a set of transcription factors such as Wingless and Int (Wnt), and Sonic hedgehog signals; paired box PAX3 and PAX7; and myogenic regulatory factors (MRFs) MYOD, MYF5, myogenin (MYOG), and MYF6 (Maltin et al., 2001; Buckingham et al., 2003; Du et al., 2010a). The myogenesis process starts when the embryonic mesoderm is segmented to form somites that contain MSCs; then, the differentiation of MSCs into myogenic precursor cells initiates after receiving signals (Wnt and Sonic hedgehog) from adjacent tissues. Finally, Wnt and Sonic hedgehog signals regulate the expression of PAX3 and PAX7 which then initiate the expression of MRFs (Oksbjerg et al., 2004; Du et al., 2010ab).

The MRFs are a group of basic helix-loop-helix proteins that play a fundamental regulatory function in the skeletal muscle development (Maltin et al., 2001). When each of the four MRFs (MYOD, MYF5, MYOG and MYF6) has been introduced into non-muscle cell types, myogenic differentiation has been induced (Olson, 1990; Wright, 1992; Emerson, 1993; Rudnicki

et al., 1993). The functions of MRFs during embryogenesis are diverse and seem to be influenced by the cellular environment (Valdez et al., 2000). According to Rawls et al. (1995), and Sabourin and Rudnicki (2000), MYOD and MYF5 appear to be important in the specifications and survival of MSCs into myoblasts, whereas MYOG and MYF6 are required for the control of differentiation in the myotubes. However, studies show that functions of MRFs are not necessarily indispensable. Valdez et al. (2000), report that mice carrying null mutation of both MYOD and MYF5 resulted in mutants with complete absence of muscle tissue; mice carrying null mutation of MYOG resulted in mutants with presence of myoblasts but only a small proportion of these cells differentiated into myotubes; and mice carrying triple mutation of MYOD, MYOG, and MYF6 resulted in mutants with a normal number of myoblasts but differentiation into myotubues did not occur at any extent.

Muscle fibre formation is regulated not only by promoting but also by adverse acting factors such as transforming growth factor-β (TGF-β) and fibroblast growth factor which negatively regulate myoblast differentiation. Among the TGF-β superfamily, there is the growth differentiation factor-8 (GDF-8) or myostatin which acts as an inhibitor of skeletal muscle growth (Langley et al., 2002; Lin et al., 2002). Myostatin was first identified by McPherron et al. (1997), who observed that null mutation of myostatin in mice caused an increase in both hyperplasia and hypertrophy of muscle fibers resulting in a widespread increase in skeletal muscle. Muscle phenotype observed in these mutant mice is similar to the one observed in double muscling cattle breeds, such as Belgian Blue and Piedmontese, which also present mutations in the coding region of myostatin suggesting that this myoblast regulation factor performs the same biological function in both species (McPherron and Lee, 1997). Also, null mutation of myostatin in mice has shown not only to increase myogenesis but also decrease adipogenesis. Lin et al. (2002) observed that 12-week mice carrying null mutation of myostatin had decreased fat depots, and both serum leptin and leptin mRNA expression in inguinal adipose tissue were decreased compared to those from wild mice. The reduction in fat depots and leptin levels in mutant mice are attributed by the authors to an association between myostatin and adipogenesis. Nevertheless, Du et al. (2010b) state that muscle is a major tissue utilizing energy; therefore, a larger muscle mass may require more energy resulting in less fat to store. Finally, when the expression of candidate genes contributing to the regulation of skeletal muscle development in cattle such as MYOD, MYOG, MYF5, and myostatin, Crosier et al. (2002) concluded that myostatin was the

gene whose expression may contribute to the observed changes in muscle development of bovine fetuses produced *in-vitro*. However, studies have shown that manipulation of bovine embryos during the preimplantation period affects the development of the fetus, resulting in heavier calves due to an abnormal increase in muscle mass (Kruip and Den Dass, 1997; Farin et al., 2001).

2.6.3.3. Adipogenic genes.

Adipose tissue is a connective tissue whose main function is energy storage, but also works as endocrine organ secreting cell signalling proteins (adipokines) which regulate energy metabolism (Kershaw and Flier, 2004; Trujillo and Scherer, 2006; Hausman et al., 2009). In livestock, visceral, sub-cutaneous, and intra-muscular fat are the major adipose tissue depots, but only intra-muscular fat is desired due to enhancement of meat quality and flavor whereas adipose tissue accumulation elsewhere increases production costs (Du et al., 2013). Intra-muscular fat corresponds to the amount of fat within muscle, and originates from the same pool of mesenchymal stem cells that also give rise to muscle cells and fibroblasts during fetal development (Du et al., 2010a; Hocquette et al., 2010). Visceral and sub-cutaneous adipose tissues grow later in life due to both hypertrophy and hyperplasia. In contrast, few adipocytes located within muscle are generated later in life thus the amount of intra-muscular fat is determined mostly by hypertrophy of adipocytes set at birth (Hocquette et al., 2010; Du et al., 2010bc).

Adipogenesis is a process through which *de novo* synthesis of adipocytes occurs, and includes the proliferation and differentiation of cells (pre-adipocytes) into lipid-assimilating cells (mature adipocytes) found within adipose tissue (Hausman et al., 2009; Du et al., 2013). Adipogenesis differentiation is regulated by several hormones and transcription factors. After receiving hormonal stimulation, levels of cyclic adenosine monophosphate (cAMP) are increased in pre-adipocytes and this is required to initiate the differentiation process. Then, pre-adipocyte differentiation initiates with sequential appearance of β and δ isoform of CAAT/enhancer binding protein (C/EBP) which seems to trigger the expression of transcription factors C/EPBα and peroxisome proliferator-activated receptor γ (PPARγ), involved in the late stages of differentiation. C/EPBα and PPARγ regulate each other ensuring the preservation of the differentiated stage of the adipocyte. PPARγ alone is enough to arouse adipocyte differentiation

indicating its critical role in adipogenesis, but this is not the case for C/EPBα (Rosen et al., 1999; Fernyhough et al., 2007; Hausman et al., 2009; Du et al., 2010c; Du et al., 2013).

2.6.3. Evidences of epigenetic systems in developmental programming

The effects of maternal nutrition status during gestation on performance of the offspring have been attributed to fetal programming and/or epigenetic effects (Wu et al., 2006; Gicquel et al., 2008; Du et al., 2010a; Ford and Long, 2012). Moreover, DNA methylation and histone modification may be altered by nutritional status, more specifically by overall availability of amino acids and micro-nutrients (Wu et al., 2004). The expression of genes associated with growth as well as muscle and adipose tissue development in the offspring has been affected by the nutrient status and nutrient supply to the dam during gestation in several mammalian species.

The timing of changes in nutrient intake of the dam during gestation has also been shown to affect the expression of different genes in the offspring. Brameld et al. (2000) evaluated the effects of restricting the energy intake (60 vs. 150% of energy requirements) of pregnant ewes from early- to mid- gestation (28 to 80 d) on the mRNA concentration of IGF1 and IGF2 genes of liver and skeletal muscle at both 80 and 140 d of pregnancy. It was found that mRNA of IGF1 was lower in liver of fetuses from energy-restricted ewes at 80 d but higher at 140 d of gestation, after 60 d of re-feeding, compared to fetuses from non-restricted ewes. No difference was found for IGF2 mRNA in liver at any age. On the other hand, no difference was observed for IGF1 mRNA in skeletal muscle of fetuses at any age due to diet, while IGF2 mRNA in skeletal muscle of nutrient restricted fetuses was higher at 80 d and lower at 140 d compared to those from well-fed ewes. Micke et al. (2011) evaluated the effect of high (240%) and low (70%) levels of recommended daily crude protein intake for beef heifers during the first and second trimesters of gestation on the expression of IGF1, IGF2, IGF1R, and IGF2R mRNA in perirenal, omental, and sub-cutaneous adipose tissue at 680 days of age of the progeny. It was found that high compared with low protein diet during the second trimester increased IGF1R mRNA in both perirenal and omental adipose tissue of all progeny, and IGF2 and IGF2R mRNA in omental adipose tissue of all progeny; contrary, IGF2 mRNA in perirenal fat of all progeny was decreased following exposure to high compared with low protein diets during the second trimester. These data clearly

show an interaction between level of nutrition and timing within gestation period on the expression of IGFs in progeny.

Myogenic and adipogenic genes of the offspring have also been affected by maternal nutrition during gestation. Tong et al. (2009) found that expression of MYOD, MYOG, and *β*-catenin signaling were downregulated in *semitendinosus* muscle of 75 d fetuses from ewes fed 150 vs. 100% of the requirements. *β*-Catenin is a protein which inhibits adipogenesis through the downregulation of PPAR*γ* and C/EPB*α* (Lee et al., 2010). Muhlhausler et al. (2007ab) measured the relative expression of PPAR*γ* and leptin mRNA in perirenal fat of 140 d fetuses, and in both perirenal and sub-cutaneous fat of 30 d lambs born to ewes fed either 100% or 155% of energy requirements during the last third of gestation. It was found that relative expression of PPAR*γ* and leptin mRNA in perirenal adipose tissue of fetuses, and expression of leptin mRNA in both sub-cutaneous and perirenal adipose tissue of 30 d lambs were greater in offspring from ewes fed above requirements. In another study, Zhu et al. (2008) measured the relative expression of PPAR*γ* mRNA in *semitendinosus* muscle of 75 d fetuses from ewes fed either 100 or 150% of requirements from 60 d before to 75 d after conception, and found that those fetuses from over fed dams had higher expression of PPAR*γ* mRNA in *semitendinosus* muscle. Also, maternal nutrition during gestation has also shown to affect factors acting in the metabolic regulation of muscle tissue. Du et al. (2004) found that the expression of both calpains and calpastatin, two proteins that regulate the degradation of myofibrillar proteins, were reduced in the *longissimus dorsi* muscle of fetuses born to cows restricted from early- to mid-gestation.

In addition, not only maternal diet during gestation but also the combination of periconceptual and gestation nutrition, or gestation and lactation diets may affect the gene expression of the progeny. Gallaher et al. (1998) report that nutrient restriction from 60 d previous to 30 d after conception has an effect on the regulation of IGF1 in the developing sheep fetuses. Bayol et al. (2005), compared the effects of feeding a balanced diet vs. a high-caloric/low-protein diet to rats during gestation and lactation, and observed that pups born and nursed by dams fed the high-caloric/low-protein diet showed greater expression of IGF1, IGF1R, and PPAR*γ* genes in muscle tissue whereas no change was observed in those only born to unbalanced diet group.

DNA methylation and, consequently, gene expression have been affected by maternal nutrition during gestation. Lillycrop et al. (2005) evaluated the effect of unbalanced maternal nutrition on the methylation status and expression of PPAR genes in the offspring after weaning by feeding rats throughout pregnancy with either a control (18% casein plus 1 mg/kg folic acid), a restricted (9% casein plus 1 mg/kg folic acid), or a restricted with supplementation (9% casein plus 5 mg/kg folic acid) diet. It was found that consumption of the restricted diet during pregnancy resulted in 21 and 17% lower CpG methylation in promoter region of the PPARα gene in the livers of the offspring compared with the control and restricted with supplementation groups respectively. Consequently, PPARα mRNA expression in livers of restricted group was 945 and 526% greater compared to pups born to control and restricted with supplementation dams respectively. Finally, no difference was observed in methylation status and expression of PPARα mRNA between control and restricted with supplementation groups. These data show that unbalanced nutrition during gestation induces epigenetic changes, such as DNA methylation, that alter gene expression.

2.7. Summary

The nutrient requirements of the beef cow need to be met in order to maximize her performance. However, these requirements will increase when the cow is facing stages other than maintenance such as growth, gestation, lactation and return to estrous. All nutrients are important for the proper performance of the beef cow, but the amount of energy required is relatively greater compared to other nutrients such as protein whose requirements are met in part by microbial synthesis. Therefore, providing the cow herd with diets that deliver the energy necessary in an efficient manner is crucial for the success of the cow-calf operation.

Among all sources of energy used for feeding beef cattle, fat sources provide more energy per unit of DM due to greater energetic density. However, factors such as the total amount of fat in the diet, the type of fatty acid in the diet (i.e. SFA vs. MUFA vs. PUFA) and the fate of these fatty acids in the animal's metabolism need to be considered when fat sources are used to boost the energy intake of cattle. Despite these limitations, research has shown that supplementing fat at proper levels pre- and postpartum can improve the reproductive performance in beef cows

without negatively impacting ruminal fermentation and/or DMI. Moreover, the improvement in reproductive performance depends on the type of fatty acid included in the diet with n-3 PUFA having the greater impact.

Meeting the nutrient requirements of the beef cow is not only beneficial for the performance of the dam. Proper nutrition of the dam during gestation has shown to positively impact the performance of the progeny later in life. However, factors such as the timing during gestation and the nutrient will have different effects on the development of the progeny. Moreover, nowadays there is clear scientific evidence that these changes/adjustments occurring during fetal development, and based on nutrient supply of the mother, are sometimes explained by epigenetic systems involved in the fetal development process.

Finally, it is the hypothesis of this thesis that supplementing fat in the pre- and postpartum diet of beef cows will result in an improvement in reproductive performance. Moreover, this improvement will depend on the type of fatty acid (i. e. MUFA vs. PUFA) being included in the diet, with a superior response (i.e. improved pregnancy rates, increase in number of calves born to cows exposed to breeding) for females receiving a high-fat diet enriched with PUFA. Further, including fat in the diet of gestating beef cows will induce fetal programing mechanisms that result in superior birth to slaughter performance of the progeny.

Three studies were carried out with the following objectives:

1. To evaluate the effects of postpartum fat supplementation and source of fat (PUFA vs. MUFA) on the reproductive performance of young lactating beef cows grazing mid-summer to early fall tame pastures.
2. To evaluate the effects of fat level and source of fat (PUFA vs. MUFA) in the gestating (2 and 3rd trimester of pregnancy) diet of mature beef cows on the prepartum performance of the dam and birth weight of the progeny.
3. To evaluate the effects of fat level and source of fat (PUFA vs. MUFA) in the gestating (2 and 3rd trimester of pregnancy) diet of mature beef cows on the postpartum and reproductive performance of the dam as well as the birth to slaughter performance of the progeny.

3. EFFECTS OF POSTPARTUM FAT SUPPLEMENTATION AND SOURCE ON PERFORMANCE OF LACTATING YOUNG BEEF COWS GRAZING COOL-SEASON GRASS PASTURES[1]

3.1. Abstract

A three-year study was conducted to evaluate the effects of fat supplementation and source on the reproductive performance of grazing lactating beef cows. Each year, 36 second- and third-calving lactating Angus cows were stratified by BW (554±15.5 kg) and days postpartum (38±1.5 d), and randomly assigned to 9 paddocks (4 cows/paddock) where cows grazed cool-season grass (CSG) pastures (12.5±2.5% CP and 56.5±2.9% TDN). Each paddock was randomly assigned to one of three replicated treatments: a non-supplemented control (CON), and two supplemented (SUP) treatments where cows were offered either a canola seed (CAN) or a flaxseed (FLX) based pellet targeting 300 g/cow/d of supplemental fat (EE) over 42 d. Data were analyzed as a RCBD with contrasts for the effect of fat supplementation (CON vs. SUP) and source (CAN vs. FLX). Results indicate that CON had greater (P=0.01) forage utilization and tended (P=0.08) to have greater estimated forage DMI compared to SUP, while no difference (P≥0.76) was observed between CAN and FLX treatments. At the end of trial, no difference (P≥0.20) was observed among treatments for BW, ADG, BCS, subcutaneous fat thickness (SCFT), proportion of cows cycling, and serum NEFA concentration. However, all treatments resulted in positive ADG, maintained or increased BCS and SCFT, and reduced serum NEFA concentration. No differences (P≥0.12) were observed among treatments for pregnancy rate, calving distribution and calving to calving interval likely as a result of sufficient nutrient availability and similar nutritional status across treatments. There was a trend (P=0.08) for heifer calves of FLX supplemented cows to have greater ADG than those of CAN cows. These results indicate that pre-breeding supplementation and source of fat had no beneficial effects on reproductive performance of lactating young beef cows grazing good quality pastures.

[1]The content of this chapter has been accepted for publication in Applied Animal Science journal.

3.2. Introduction

There is a significant cost associated to culling young beef cows. At 2.5 years of age, no returns have been generated from pregnant heifers despite their significant rearing costs, making them the most expensive animals in the herd (Larson, 2012). Also, two- and three-year old cows represent a major proportion of anestrous females at the onset of the breeding season and nearly one-third of open cows culled (Funston, 2014). Thus, getting first- and second-calf cows re-bred early in the season is of great importance for increasing their productive life (Waldner et al., 2009; Hughes, 2013; Hersom et al., 2015).

Nutritional programs that increase pregnancy rates early in the breeding season can potentially increase lifetime profitability of the cow (Patterson et al., 2003). Compared to other nutrients, energy deficiency has been shown to have the most significant impact on the postpartum anoestrus period of the beef cow (Richards et al., 1986; Sinclair et al., 2002; Hess et al., 2005). In ruminants, supplementing fat up to 6% of the total DMI increases the energy density of high-forage diets while avoiding negative effects associated with starch supplementation (Palmquist, 1994; Bowman and Sanson, 1996; Hess et al., 2008). Moreover, high-fat supplements have been shown to have a greater positive effect on reproductive performance of beef females compared to low-fat supplements with similar energy content (Lammoglia et al., 1997ab).

The inclusion of fat supplements providing poly-unsaturated fatty acids (PUFA) in the pre- and postpartum diets of cows has also been shown to improve the overall reproductive performance compared to other fat sources. For example, Petit and Berthiaume (2006) offered 600 g/cow/d of one of two supplements that differed in the source of supplemental fat (flaxseed vs. calcium salts of palm oil) to beef cows during late gestation. These authors found that cows supplemented with flaxseed had higher cumulative pregnancy rates compared to cows offered the supplement containing calcium salts of palm oil (92.9 vs. 76.7%, respectively). Lopes et al. (2009) supplemented *Bos indicus* beef cows with either a rumen protected PUFA or saturated fatty acid (SFA) source from estrus synchronization until 28 d after artificial insemination (AI),

and found that cows offered the PUFA source had greater pregnancy rates compared to SFA supplemented cows (47.9 vs. 35.5%, respectively).

Overall, fat supplementation has shown to benefit the reproductive performance of ruminants. However, the benefits of supplementing fat postpartum to grazing young beef cows in western Canada are unknown. A fat supplementation program prior to breeding could improve the reproductive performance of lactating young beef cows. Moreover, postpartum interval to conception could be reduced by supplementing fat high in PUFA compared to fat high in MUFA. The objective of this study was to evaluate the effects of supplementing fat (non-supplementation vs. supplementation), and source of fat (i.e. MUFA vs. PUFA) to lactating young beef cows grazing cool-season grass pastures prior to the breeding.

3.3. Materials & Methods

3.3.1. Location

A three-year study was conducted during the spring-summer of 2014, 2015, and 2016 at the Termuende Research Ranch of the Western Beef Development Centre located near Lanigan, Saskatchewan, Canada (lat. 51°51´N, long. 105°02´W). A total of 16.2 ha, divided into nine 1.8 ha paddocks of cool-season grass (CSG) pastures, were used for this study. Pastures consisted of long-established crested wheatgrass (*Agropyron cristatum* L.) with some smooth bromegrass (*Bromus inermis* L.) and Kentucky bluegrass (*Poa pratensis* L.) invasion in lower moist areas. Soils at the study site are Oxbow black soil association on a medium textured sandy loam soil (Wright, 1986).

Over the three years of the study, the average (mean ± SD) total precipitation at the Lanigan area was 35.2 ± 29.0, 77.1 ± 56.8, and 93.8 ± 86.5 mm for May, June and July, respectively. These values are similar to the 30-yr average total precipitation for May (39.6 ± 28.5 mm) and June (75.9 ± 30.9 mm), but greater for July (58.1 ± 33.7 mm). Mean monthly temperatures over the 3-yr of the study were 11.2 ± 1.2, 16.1 ± 1.5, and 18.1 ± 0.4ºC for May, June, and July, respectively. These levels are similar to the 30-yr average temperatures for the

same months (9.9 ± 4.5, 15.1 ± 3.6, and 17.6 ± 3.1ºC, respectively) for the Lanigan area (Environment Canada weather station in Watrous SK, http://www/weatheroffice.gc.ca).

3.3.2. Animals and Treatments

Animals for this study were obtained from the main herd of the WBDC research ranch. All animals were cared for in accordance with the Canadian Council of Animal Care guidelines (CCAC, 2009), and all experimental procedures were approved by University of Saskatchewan Animal Care Committee (Protocol No. 20090107).

Each year, a total of 36 second- and third-calf Angus cows (554 ± 15.5 kg and 38 ±7.8 d post-calving) were stratified by BW and days since calving and divided into 9 homogenous groups (4 cows/group). Subsequently, each group was randomly assigned to 1 of the 9 CSG pasture paddocks, and each paddock was then randomly assigned to 1 of 3 replicated (n=3) supplementation strategies which lasted for 42 d prior to the onset of the breeding season.

Supplementation strategies consisted of a non-supplemented control (CON) group, and two supplemented (SUP) groups. For the latter, two pelleted feed products were formulated to differ in the high-fat ingredient used for their formulation. These included canola seed (CAN) and flaxseed (FLX) which served as sources of mono- and poly-unsaturated fatty acids, respectively (Tables 3.1 and 3.2). The average amount (DM basis) of supplement offered was 3.5 and 3.6 kg/cow/d for CAN and FLX, respectively. These amounts were calculated to ensure ~300 g/cow/d of fat intake from supplement. Pellets were offered daily between 0800 and 0900 in feeder-troughs. All groups had *ad libitum* access to a 2:1 mineral supplement [15.5% Ca, 7% P, 30 ppm Se, 20 ppm Co, 200 ppm I, 1500 ppm Cu, 5000 ppm Mn, 5000 ppm Zn, 1000 ppm Fe, 1.0 ppm F (max), 500 000 IU/kg vitamin A (min), 50000 IU/kg vitamin D (min), 2500 IU/kg vitamin E (min); Cargill Animal Nutrition, MB, Canada] and cobalt-iodized salt [99.0% NaCl (min), 39.0% Na, 150 ppm I, 100 ppm Co; FeedRite Ltd., Humboldt, SK, Canada]. After the 42-d supplementation period, all 36 cows were managed as a single group and exposed to two bulls for a 63-d breeding season. Bulls were previously evaluated for breeding soundness. During the breeding season, cows, calves, and bulls were grazing CSG pastures (12.6 ± 4.7% CP, 39.3 ± 4.7% ADF, and 62.1 ± 4.2% NDF) similar to those grazed during the supplementation period.

Table 3.1. Ingredient and nutrient composition of pelleted supplements by year.

	CAN[1]				FLX[2]			
Year	**1**	**2**	**3**	**Avg ± SD**	**1**	**2**	**3**	**Avg ± SD**
Ingredient (% AF)								
DDGS[3]	4.0	4.0	4.0	-	4.0	4.0	4.0	-
Oat hulls	41.0	41.0	41.0	-	41.0	41.0	41.0	-
Wheat	40.0	40.0	40.0	-	40.0	40.0	40.0	-
Canola seed	15.0	15.0	15.0	-	-	-	-	-
Flaxseed	-	-		-	15.0	15.0	15.0	-
Nutrient (% DM)								
DM	90.2	90.1	89.9	90.1±0.2	89.4	90.3	90.0	89.9±0.5
CP	13.9	15.8	16.5	15.4±1.4	15.2	14.1	14.5	14.6±0.6
ADICP	0.86	1.70	1.30	1.29±0.40	0.81	1.13	0.96	0.97±0.16
NDICP	1.43	2.57	1.51	1.84±0.64	1.14	2.16	1.25	1.51±0.56
ADF	16.9	22.1	21.8	20.3±2.9	16.6	22.3	21.6	20.1±3.1
NDF	33.0	36.3	32.8	34.0±2.0	29.9	36.6	38.7	35.0±4.6
Lignin	4.81	4.89	4.56	4.75±0.17	3.63	4.57	4.39	4.20±0.50
Starch	33.3	24.2	28.4	28.6±4.6	35.3	25.4	24.5	28.4±6.0
Ash	4.35	8.09	6.53	6.32±1.88	4.62	7.15	6.25	6.00±1.28
Ca	0.18	0.39	0.44	0.34±0.14	0.23	0.20	0.34	0.25±0.07
P	0.34	0.40	0.48	0.40±0.07	0.39	0.36	0.37	0.37±0.02
EE	8.43	7.85	11.2	9.14±1.76	8.84	7.75	8.91	8.50±0.65
TDN[4]	80.4	73.9	80.8	78.4±3.7	82.1	75.0	76.5	77.8±3.7
DE (Mcal/kg)	3.51	3.25	3.56	3.44±0.16	3.61	3.30	3.36	3.42±0.16

[1]CAN: canola seed based pelleted supplement.
[2]FLX: flaxseed based pelleted supplement.
[3]DDGS: wheat dried distiller grains with solubles.
[4]Calculated using the Weiss equation (Weiss et al., 1992).

Table 3.2. Fatty acid composition of pelleted supplements by year.

	CAN[1]				FLX[2]			
Year	**1**	**2**	**3**	**Avg ± SD**	**1**	**2**	**3**	**Avg ± SD**
Total FA (mg/g of feed)								
	89.3	86.0	105.7	93.7±10.6	90.1	84.2	95.1	89.8±5.45
Fatty acid[3] (% of total)								
∑PUFA	33.1	32.2	32.3	32.5±0.49	67.6	68.0	65.8	67.2±1.16
∑n-3	8.66	7.75	7.94	8.11±0.48	43.1	43.4	41.9	42.8±0.84
18:3n-3	8.66	7.75	7.94	8.11±0.48	43.1	43.4	41.8	42.8±0.84
20:3n-3	-	-	-	-	0.06	0.04	0.05	0.05±0.01
∑n-6	24.4	24.4	24.4	24.4±0.04	24.5	24.6	24.0	24.4±0.33
18:2n-6	24.4	24.4	24.3	24.3±0.04	24.4	24.5	23.9	24.3±0.32
20:2n-6	0.06	0.07	0.07	0.07±0.01	0.06	0.04	0.05	0.05±0.01
∑MUFA	56.4	57.3	57.2	57.0±0.53	19.6	19.4	20.6	19.9±0.61
c 9-16:1	0.28	0.28	0.28	0.28±0.00	0.14	0.09	0.09	0.11±0.03
c 9-17:1	0.06	0.05	0.05	0.05±0.01	0.05	0.03	0.04	0.04±0.01
c 9-18:1	50.7	51.6	51.4	51.2±0.46	18.3	18.1	19.2	18.6±0.58
c 11-18:1	3.94	4.03	4.03	4.00±0.05	0.79	0.80	0.86	0.81±0.04
c 11-20:1	1.19	1.21	1.21	1.20±0.02	0.33	0.33	0.33	0.33±0.00
c 15-24:1	0.20	0.20	0.21	0.20±0.01	-	-	-	-
∑SFA	10.6	10.5	10.5	10.5±0.04	12.7	12.6	13.6	13.0±0.55
14:0	0.09	0.09	0.09	0.09±0.00	0.09	0.10	0.10	0.09±0.01
16:0	7.53	7.43	7.44	7.46±0.06	8.95	8.83	9.40	9.06±0.30
17:0	0.06	0.07	0.06	0.06±0.00	0.07	0.07	0.09	0.08±0.01
18:0	1.73	1.73	1.75	1.73±0.01	3.05	3.04	3.42	3.17±0.22
20:0	0.55	0.58	0.58	0.57±0.01	0.21	0.20	0.22	0.21±0.01
22:0	0.36	0.37	0.35	0.36±0.01	0.20	0.21	0.21	0.21±0.00
24:0	0.24	0.22	0.22	0.23±0.01	0.17	0.16	0.16	0.16±0.00

[1]CAN: canola seed based pelleted supplement.
[2]FLX: flaxseed based pelleted supplement.
[3]*c*= *cis*; ΣPUFA =sum of polyunsaturated fatty acids (Σn-6 +Σn-3); Σn-3 = 18:3n-3 + 20:3n-3; Σn-6 = 18:2n-6 + 20:2n-6; ΣMUFA= sum of monounsaturated fatty acids (Σ*c*-MUFA); ΣSFA =sum of saturated fatty acids (14:0 + 16:0 + 17:0 + 18:0 + 20:0 + 22:0 + 24:0).

3.3.3. Data collection

3.3.3.1. Forage utilization and DM intake.

Forage utilization was estimated using forage disappearance (difference between dried weights before and after grazing) according to the following equation (Jasmer and Holecheck, 1984):

$$Forage\ utilization\ (\%) = \frac{Available\ DM\ (g/m^2) - Residual\ DM\ (g/m^2)}{Available\ DM\ (g/m^2)} \times 100 \qquad \textbf{Equation 3.1}$$

Available and residual forage were estimated using the "weight before and after grazing" technique as described by Cook and Stubbendieck (1986). Briefly, for each paddock, 30 randomly distributed quadrats (0.25 m^2) were clipped to a 5-cm stubble height and composited at the start (available) and end (residual) of the 42-d supplementation period. Sub-samples were taken from each composite, placed in paper bags and dried in a forced-air oven at 55°C over 72 h for DM determination.

Forage disappearance was used to estimate forage DMI (consumption) according to the following equation (McCartney et al., 2004; Baron et al., 2006):

$$Forage\ DMI\ (kg/hd/d) = \frac{Available\ DM\ (kg) - Residual\ DM\ (kg)}{n \times d} \qquad \textbf{Equation 3.2}$$

where n is the number of cows per paddock and d is the number of days the paddock was grazed.

3.3.3.2. Forage and feed samples.

To estimate changes in forage quality throughout the course of the experimental period, five randomly distributed quadrats were clipped from each paddock every 2 wk during the experimental period and every 3 wk during the breeding season. Supplement samples were collected every 2 wk. All forage and supplement feed samples were dried in a forced-air oven at 55°C for 72 h. After drying, samples were ground to pass through a 1 mm screen (Thomas-Wiley Laboratory Mill Model 4; Thomas Scientific, Swedesboro, NJ). Ground forage samples collected during the supplementation period were composited (DM basis) by paddock and stored at -20ºC until analysis. Biweekly supplement samples for fatty acid analysis were ground without drying,

composited to obtain two samples per supplement corresponding to the first and last 21 d of the supplementation period and stored at -20ºC until analysis.

3.3.3.3. Animal measurements.

Data collected from animals included BW, BCS, and sub-cutaneous fat thickness (SCFT). In order to minimize variation due to rumen fill, each cow was weighed over two consecutive days at 0800 h at the start and end of the supplementation period as well as at the end of the breeding season. Throughout the experimental period, each cow was weighed every 2 wk with weights measured before offering the supplements. The BW of each calf was determined at the start and end of the experimental period. The BCS of each cow was determined by the same experienced technician at the start and end of the experimental period using the Scottish scale where 1=emaciated and 5=grossly fat (Lowman et al., 1976; Wildman et al., 1982). The SCFT of cows was measured at the start and end of the experimental period, as well as at the end of the breeding season for each cow by ultrasound over the third quarter of the rib eye (*Longissimus dorsi*) muscle, between the 12th and 13th rib, and at the thurl location on the rump area using an Aloka SSD-500V ultrasound machine and an Aloka UST-5044 probe (3.5 MHz; Aloka Inc., Wallingford, CT).

Resumption of cyclicity, pregnancy rate, and calving to calving interval were also evaluated. Cyclicity status (i.e. presence of a corpus luteum) of all cows was determined at the beginning and end of the experimental period, and pregnancy status was determined 45 d after the end of the breeding season. Both cyclicity and pregnancy status determination were carried out by an experienced veterinarian via transrectal ultrasound using an Easi-Scan Curve ultrasound machine (3.0 - 7.0 MHz; BCF Technology Ltd., Rochester, MN). The actual calving dates were recorded in order to correctly determine the beginning of pregnancy, assuming a gestation period of 283 days.

3.3.3.4. Blood serum collection.

Blood samples were collected from each cow via coccygeal venipuncture into 10-mL untreated vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) at the start and end of the experimental period. Samples were allowed to clot at room temperature, centrifuged at 1500 × g

for 15 min at 4ºC, and serum was harvested into 1.5 mL tubes (Eppendorf, GCS, New York, NY). Serum samples were stored at -20°C until analysis.

3.3.4. Laboratory analysis

3.3.4.1. Forage and supplement feeds analyses.

Forage and supplement feed samples were analyzed at Cumberland Valley Analytical Services (Hagerstown, MD, USA). Forage samples were analyzed through near infrared spectroscopy (NIRS) using a Foss NIRSystems 5000 (NIR Systems, Inc., Silver Spring, MD, USA) for determination of CP [standard error of calibration (SEC) = 0.51, regression coefficient (R^2) = 0.99], ether extract (EE; SEC = 0.32, R^2 = 0.87), acid detergent fiber (ADF; SEC = 1.24, R^2 = 0.95), neutral detergent fiber (NDF; SEC = 1.69, R^2 = 0.96), lignin (SEC = 0.41, R^2 = 0.92), ash (SEC = 0.84, R^2 = 0.85), acid detergent insoluble crude protein (ADICP; SEC = 0.27, R^2 = 0.59), neutral detergent insoluble crude protein (NDICP; SEC = 0.62, R^2 = 0.92), and soluble protein (SP; SEC = 0.57, R^2 = 0.91). Supplement feed samples were analyzed for DM by drying at 135°C for 2 h (method 930.15; AOAC, 2012). Crude protein was determined by nitrogen combustion (method 990.03; AOAC, 2012) using a Leco FP-528 Nitrogen Combustion Analyzer (LECO Corp., St. Joseph, MI). Neutral detergent fiber was determined as described in Van Soest et al. (1991) with the addition of heat stable α-amylase and sodium sulphite. Acid detergent fiber was determined according to method 973.18 (AOAC, 2012). Ether extract was determined according to method 920.39 (AOAC, 2012). Starch was determined using the method described by Hall (2009). Ash was determined by heating samples at 550°C for 4 h (method 942.05; AOAC, 2012). For all samples, Ca and P were analyzed using the dry ashing procedure (methods 927.02 and 965.17, respectively; AOAC, 2012), and Ca concentration was determined using an atomic absorption spectrophotometer (model 2380; Perkin-Elmer, Corp.; Norwalk, CT) whereas P concentration was read at 410 nm on a spectrometer (Ultospec III; Pharmacia LKB Biotechnology, Stockholm, Sweden). Total digestible nutrients (TDN; % DM) was calculated as per Weiss summative equation (Weiss et al., 1992).

3.3.4.2. Fatty acid analysis.

Supplement and CSG forage fatty acids were methylated using the method of Palmquist and Jenkins (2003). Briefly, 50 mg of supplement and 150 mg of CSG forage were methylated at 90ºC for 2 hr using 3 N methanolic HCl. An internal standard (*cis*10-17:1 methyl ester) in toluene, was added prior to addition of the methylating reagent. After methylation, samples were cooled, 6% K_2CO_3 added and fatty acid methyl esters (FAME) were extracted into hexane. Completeness of methylation was determined and FAME purified by thin layer chromatography (TLC) using silica gel G plates and hexane:diethyl ether:acetic acid (85:15:1) as a developing solvent. Fatty acid methyl esters were analyzed using a Varian CP-3800 gas chromatograph (Varian Inc., Walnut Creek, CA, USA) using the conditions described by Dugan et al. (2007). Fatty acids were identified using reference standard No. 603 from Nu-Check Prep Inc. (Elysian, MN, USA). The FAME were quantified using chromatographic peak area and internal standard based calculations.

3.3.4.3. Blood serum analyses.

Blood serum samples were analyzed to determine non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHBA), and trace mineral (Cu, Zn, Mn) concentrations. Serum BHBA concentration was measured according to Williamson et al. (1962) using the enzymatic oxidation of BHBA to acetoacetate with 3-hydroxybutratedehydrogenase (H6501; Roche, Mississauga, ON, Canada). The color change associated with the reduction of NAD to NADH was determined using a plate reader (340 nm; SpectraMax PLUS384; Molecular Devices Corp). A commercial kit was used to determine serum NEFA concentration (NEFA- HR 2; Wako Diagnostics, Richmond, VA). Trace minerals were determined by inductively coupled plasma mass spectrometry (ICP-MS) at Prairie Diagnostic Services Inc. (Saskatoon, SK, Canada).

3.3.5. Statistical analysis

Data were analyzed as a Randomized Complete Block design (RCBD) using the Mixed procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC). The statistical model included the fixed effect of treatment, and the random effect of year. The Glimmix procedure of SAS was used to analyze categorical data including BCS and pregnancy status. The effects of treatment,

time, and the treatment × time were evaluated for all forage quality variables and BW of cows using a RCBD accounting for repeated measures where eight covariance structures were tested, and the covariance structure with the lowest Akaike's and Bayesian information criterion (AIC and BIC) values was selected (Littell et al., 1998). In case of significant treatment × time effect, the repeated measures analysis was used to model dependant variables as a function of time to determine whether the relationship was linear or quadratic. Covariance analyses using the initial BW as covariate, were carried out to analyze the performance data of heifer and steer calves. The Kenward-Roger option was used to estimate denominator degrees of freedom. Pre-planned contrasts were used to determine the effects of: 1) fat supplementation (CON vs. SUP), and 2) source of supplemental fat (CAN vs. FLX). For all analysis, paddock represented the experimental unit. Significant differences were declared at $P < 0.05$, and trends at $P < 0.10$.

3.4. Results and Discussion

3.4.1. Supplement composition

The ingredient, nutrient and fatty acid composition of the high-fat pelleted supplements are shown in Tables 3.1 and 3.2. Supplements were formulated to differ only in the ingredient providing the supplemental fat content (canola seed vs. flaxseed). Since canola seed and flaxseed have similar CP (20.2 and 22.1%, respectively) and fat (44.4 and 45.5% of oil, respectively) content (Barthet, 2017; Siemens; 2017), the CAN and FLX supplements had similar energy (3.4 ± 0.2 Mcal/kg of DE), protein (CAN: 15.4 ± 1.4% and FLX: 14.6 ± 0.6% of CP), and fat (CAN: 9.1 ± 1.8 and FLX: 8.5 ± 0.7% of EE) content. The fatty acid content of the pelleted supplements varied according to the ingredient used for their formulation. The total fatty acid concentration of the CAN pellet averaged over the three years of the study was 93.7 ± 10.6 mg of FAME/g of DM. Oleic acid (*cis*-9 18:1) represented the largest proportion (51.2 ± 0. 46%) among all fatty acids in the CAN pellet; hence, the total proportion of MUFA (57.0 ± 0.53%) was larger compared to the SFA (10.5 ± 0.04%) and PUFA (32.5 ± 0.49%) fractions. The total three year average for the fatty acid concentration of FLX pellet was 89.8 ± 5.45 mg of FAME/g of DM. In contrast to the CAN pellet, both α-linolenic (18:3n-3) and linoleic acid (18:2n-6) represented the largest proportions (42.8 ± 0.84 and 24.3 ± 0.32%, respectively) among fatty acids in FLX pellet, for a total 67.2 ± 1.16% PUFA.

On average, offered amounts (as fed) of CAN and FLX supplements were 3.9 and 4.0 kg/cow/d, respectively; providing 0.54 and 0.52 kg/cow/d of CP, 12.1 and 12.2 Mcal/cow/d of DE, and 0.31 and 0.30 kg/cow/d of fat (EE), for CAN and FLX, respectively. The amounts of supplement offered were such that each cow received ~300 g/d of supplemental fat. Studies with dairy cows have shown that reproductive performance can be affected when receiving diets with fat levels like those offered in the present study, as well as when the type of fat differed. Bilby et al. (2006) fed diets containing 245 g/d of fat differing in fatty acid content (MUFA vs. PUFA) to Holstein cows; and found that cows receiving the PUFA diet had larger preovulatory follicle and greater volume of the corpus luteum compared to cows fed the MUFA diet.

3.4.2. Forage quality

Results for the nutrient composition of pastures are shown in Table 3.3. Over the 42 d of the trial, average nutrient composition of CSG pastures did not differ ($P \geq 0.10$) among treatments. The average CP and ADF content in CSG pastures were 12.5 ± 2.5 and 41.5 ± 4.7%, respectively. These values are similar to the 11 and 39% of CP and ADF content, respectively, reported for the same location during a similar time frame over the years 2007 to 2009 (Durunna et al., 2014). However, in the present study, time had an effect ($P \leq 0.04$) for almost all variables. Only lignin content tended ($P = 0.08$) to be affected while phosphorus and ADICP content were not affected ($P \geq 0.17$) by time. When modelling time (d) as a regression variable, it was found that it had a quadratic effect ($P < 0.01$) on ADF content of CSG pastures, while the quadratic effect of time on CP content tended to be significant ($P = 0.06$). Overall, quality of the CSG pastures increased over the first 14 d with CP content improving almost 1% (from 11.9 to 12.8%), and ADF content decreasing almost 2% (from 42.8 to 40.9%). Over the next 28 d, CP and ADF content remained constant until day 42 averaging 12.7 ± 2.9 and 41.1 ± 3.3%, respectively.

The fat content of the CSG pasture was not different ($P = 0.10$) among treatments averaging 2.4 ± 0.48% EE over the three years of trial. According to Boufaied et al. (2003) and Khan et al. (2012), the main factors affecting the fatty acid composition of forages are species, vegetation stage, conditions of conservation and N fertilization. Since in the present study all these factors were similar across treatments, CSG forage samples were composite by treatment and analyzed for fatty acid composition. The results showed that all treatments were exposed to pastures with similar fatty acid profiles. For example, total fatty acid content of the CSG forage averaged 7.3 ± 0.8, 7.1 ± 0.5, and 6.8 ± 0.6 mg of FAME/g of DM for CON, CAN, and FLX, respectively. Also, the three-year average content for palmitic (C16:0), linoleic (C18:2n-6), and linolenic (C18-3n-3) acid were 30.9 ± 0.42%, 14.0 ± 0.03%, and 31.2 ± 0.45%, respectively. These values, particularly for the PUFA, are somewhat lower than expected possibly due to the fact that the samples were oven dried prior to fatty acid analysis.

Table 3.3. Average nutrient composition of cool-season grass pastures over the 42 d of supplementation.

	Treatment[1] (trt)				Time (d)					*P*-value		
Item	**CON**	**CAN**	**FLX**	**SEM[2]**	**0**	**14**	**28**	**42**	**SEM[2]**	**trt**	**d**	**trt × d**
Nutrient (% DM)												
CP	12.7	12.5	12.4	0.25	11.9	12.8	12.8	12.6	0.45	0.75	0.04	0.64
ADICP	1.38	1.37	1.39	0.08	1.36	1.39	1.38	1.39	0.08	0.88	0.40	0.75
NDICP	3.55	3.43	3.45	0.26	3.41	3.71	3.47	3.30	0.29	0.56	0.02	0.60
ADF	41.5	41.5	41.5	0.78	42.8^{b}	40.9^{a}	40.5^{ab}	41.7^{ab}	0.91	0.99	< 0.01	0.50
NDF	65.0	65.3	65.3	0.49	65.0	66.4	65.0	64.3	0.79	0.86	0.02	0.74
Lignin	6.12	6.22	6.18	0.18	6.14	5.97	6.23	6.36	0.22	0.89	0.08	0.49
Ash	8.32	8.01	8.02	0.18	8.65^{b}	7.92^{a}	7.67^{a}	8.22^{ab}	0.21	0.41	< 0.01	0.44
Ca	0.40	0.39	0.39	0.02	0.42^{b}	0.39^{ab}	0.39^{ab}	0.37^{a}	0.02	0.83	< 0.01	0.52
P	0.16	0.17	0.16	0.01	0.15	0.16	0.16	0.18	0.01	0.56	0.17	0.64
EE	2.35	2.43	2.35	0.30	2.19^{a}	2.31^{b}	2.37^{b}	2.63^{c}	0.30	0.10	< 0.01	0.77
TDN[3]	56.4	56.5	56.5	0.55	55.8^{a}	56.7^{b}	57.1^{ab}	56.4^{ab}	0.54	0.99	< 0.01	0.19
DE, Mcal/kg	2.48	2.49	2.49	0.02	2.46^{a}	2.49^{b}	2.51^{ab}	2.48^{ab}	0.02	0.99	< 0.01	0.18

[1]CON: no supplement offered; CAN: cows supplemented with a canola seed-based pellet; FLX: cows supplemented with a flaxseed-based pellet.
[2]SEM: pooled standard error of mean.
[3]Calculated using the Weiss equation (Weiss et al., 1992).
[a,b,c]Least square means with different letters in the same row are different ($P < 0.05$) using Tukey-Kramer's method.

3.4.3. Forage utilization and DM intake

Results for available and residual forage, estimated forage utilization, and DMI are shown in Table 3.4. At the start of the trial, no difference ($P = 0.49$) was observed among treatments for available forage. At the end of the 42 d of supplementation, residual forage was lower ($P < 0.01$) and forage utilization was greater ($P = 0.01$) for CON (645 kg/ha and 63.8%) when compared with SUP treatments, while residual forage of CAN (779 kg/ha) tended ($P = 0.07$) to be lower than that of FLX (899 kg/ha) with no difference ($P = 0.76$) for forage utilization (50.5 vs. 48.8% for CAN and FLX respectively). Estimated forage DMI of CON (11.5 kg/cow/d) tended ($P = 0.08$) to be greater compared to SUP with no difference ($P = 0.88$) between CAN and FLX. It should be noted that the use of the pre and post grazing herbage disappearance technique is only an estimate of forage intake and does not account for trampling, regrowth and herbivory of other species. Steps were taken as per Pigden and Minton (1969) to minimize these sources of error by increasing the number of quadrats sampled (n = 30 per replicate paddock) pre and post grazing, and the use of a slightly higher than the recommended (Saskatchewan Ministry of Agriculture, 2008) stocking rate for similar pastures (3.8 vs. 3.5 AUM/ha).

The reduced forage utilization and intake for SUP treatments compared to CON is most likely due to a diet substitution effect as a result of supplementation. According to Moore et al. (1999), supplementation decreases forage DMI when the TDN to CP ratio of the forage is less than 7 or when forage DMI is more than 1.75% of BW when fed alone. In this study, the average TDN to CP ratio of the forage was 4.5, and the forage DMI of CON cows (11.5 kg/d) was 2.0% of the BW. While fat supplementation has been shown to decrease forage DMI, it is not likely that this was the case in the current study. For example, considering the fat content of the CSG pastures (Table 3.3), the estimated forage DMI of SUP treatments (10.6 and 11.7 kg/d for CAN and FLX, respectively), and the 0.31 and 0.30 kg/cow/d of supplemental fat offered to CAN and FLX cows (respectively), the dietary fat would represent 4.2 and 4.1% of the total DMI for CAN and FLX, respectively. Several studies have documented that fat supplementation decreases forage DMI in ruminants only when the level of dietary fat exceeds 5-6% of the total DMI (Jenkins, 1993; Palmquist, 1994; Hess et al., 2008).

Table 3.4. Effects of postpartum fat supplementation and source on available and residual forage, forage utilization, and DMI of young beef cows grazing cool-season grass pastures.

	Treatment[1]					Contrasts	
Item	**CON**	**CAN**	**FLX**	**SEM[2]**	***P*-value**	**CON vs. SUP[3]**	**CAN vs. FLX**
Forage (kg of DM/ha)							
Available	1723	1614	1734	298.7	0.49	0.62	0.28
Residual	645	779	899	230.1	< 0.01	< 0.01	0.07
Forage utilization (%)	63.8	50.5	48.8	6.40	0.03	0.01	0.76
DMI (kg/cow/d)							
Forage	11.5	8.9	8.9	1.20	0.21	0.08	0.88
Total	11.5	12.7	12.4	1.24	0.79	0.51	0.86

[1]CON: no supplement offered; CAN: cows supplemented with a canola seed-based pellet; FLX: cows supplemented with a flaxseed-based pellet.
[2]SEM: pooled standard error of mean.
[3]SUP: average of CAN and FLX.

3.4.4. Animal performance

Results for performance of cows are shown in Table 3.5. No differences ($P \geq 0.81$) were found among treatments for cow BW at the start and end of the supplementation period or at the end of the breeding season. Also, total BW gained and ADG of cows did not differ ($P \geq 0.69$) among treatments during the supplementation period and during the breeding season. Furthermore, when data were analyzed over time, no differences ($P \geq 0.80$) were found among treatments for the average BW of cows during both periods. However, the repeated measures analysis showed that time had a quadratic effect ($P < 0.01$) on BW of cows during the 42 d of supplementation (Figure 3.1), which is in agreement with the same effect that time had on CGS pasture quality during the same period. During the supplementation period, the average BW of all treatments increased 39.7 ± 4.3 kg over the first 28 d from 554 ± 4.9 to 593 ± 8.3 kg, and decreased 15.5 ± 2.9 kg over the last 14 d to a BW of 578 ± 8.4 kg. In contrast, during the 63 d of the breeding season, the average BW of all treatments increased constantly from 578 ± 13.1 to 607 ± 12.5 kg (Figure 3.2). The positive gain in cow BW was also reflected in their subcutaneous fat tissue accretion during the supplementation and breeding season periods. Although no differences ($P \geq 0.23$) were observed among treatments for BCS and SCFT from the start of trial to the end of the breeding season, all groups were able to at least maintain their BCS and to increase the SCFT at both the rib and rump location. These results are consistent with those reported by Thomas et al. (1997) who did not find differences among treatments in BW or BCS of mature beef cows fed a diet with no added fat, or 1 of 3 fat added diets where tallow, soybean oil, or fish oil were incorporated up to 4% of the total DMI.

Table 3.5. Effects of postpartum fat supplementation and source on performance of young beef cows grazing cool-season grass pastures.

Item	Treatment[1]			SEM[2]	*P*-value	Contrasts	
	CON	CAN	FLX			CON vs. SUP[3]	CAN vs. FLX
Start of supplementation							
Days post-calving	36	40	39	4.16	0.47	0.25	0.69
BW (kg)	555	553	553	9.85	0.81	0.55	0.81
BCS[4] (% of cows)							
Thin	2.1	2.1	6.7	4.22	0.44	0.62	0.33
Optimal	97.9	97.9	93.3	4.22	0.44	0.62	0.33
Over conditioned	0.0	0.0	0.0	-	-	-	-
SCFT (mm)							
Rib	2.8	2.6	2.6	0.22	0.48	0.23	0.99
Rump	3.7	3.6	3.4	0.36	0.54	0.38	0.50
End of supplementation/Start of breeding season							
BW (kg)	577	576	580	26.25	0.84	0.86	0.58
ADG (kg/d)	0.53	0.57	0.63	0.40	0.69	0.50	0.59
Average over 42 d							
BW (kg)	577	575	579	11.52	0.98	0.97	0.84
BCS[4] (% of cows)							
Thin	0.0	2.8	0.0	1.58	0.99	0.99	0.98
Optimal	100.0	97.2	100.0	1.58	0.99	0.99	0.98
Over conditioned	0.0	0.0	0.0	-	-	-	-
SCFT (mm)							
Rib	2.8	2.7	3.0	0.25	0.53	0.64	0.31
Rump	3.8	3.7	3.8	0.16	0.96	0.99	0.77
End of breeding season							
BW (kg)	608	607	608	11.42	0.96	0.85	0.83
ADG (kg/d)	0.51	0.51	0.52	0.05	0.94	0.79	0.81
Average over 63 d							
BW (kg)	590	590	593	12.95	0.80	0.79	0.56
BCS[4] (% of cows)							
Thin	0.0	0.0	0.0	-	-	-	-
Optimal	94.4	100.0	100.0	2.20	0.99	0.97	0.99
Over conditioned	5.6	0.0	0.0	2.20	0.99	0.97	0.99
SCFT (mm)							
Rib	3.5	3.4	3.3	0.30	0.84	0.56	0.96
Rump	4.6	4.4	4.1	0.50	0.23	0.20	0.25

[1]CON: no supplement offered; CAN: cows supplemented with a canola seed-based pellet; FLX: cows supplemented with a flaxseed-based pellet.
[2]SEM: pooled standard error of mean.
[3]SUP: average of CAN and FLX.
[4]Thin: cows with BCS ≤ 2; Optimal: cows with 2.5 ≤ BCS ≤ 3; Over conditioned: cows with BCS ≥ 3.5.

The reason for the lack of difference in performance between CON and SUP treatments is most likely due to CON cows being able to improve their nutrient intake through selective grazing. Such selective grazing has been demonstrated using grazing ruminants with esophageal cannulas. In a study conducted by Barth and Kazaal (1971) using beef steers with esophageal cannulas and grazing tall fescue and orchard grass, it was observed that steers selected forages with greater CP and energy content. In the present study, using the NRC model (NASEM, 2016) and entering data such as estimated forage DMI and quality, the ADG and average BW of CON cows over the 42 d of supplementation, the predicted ME and MP balance (supplied - required) were -3.32 Mcal/d and -48.9 g/d, respectively, for a predicted negative gain versus the actual positive gain for the CON group. Moreover, it is likely that selective grazing also influenced the performance of the SUP cows since actual daily gains for CAN and FLX cows were greater than the 0.1 kg/d of predicted gain for both treatments using the same model.

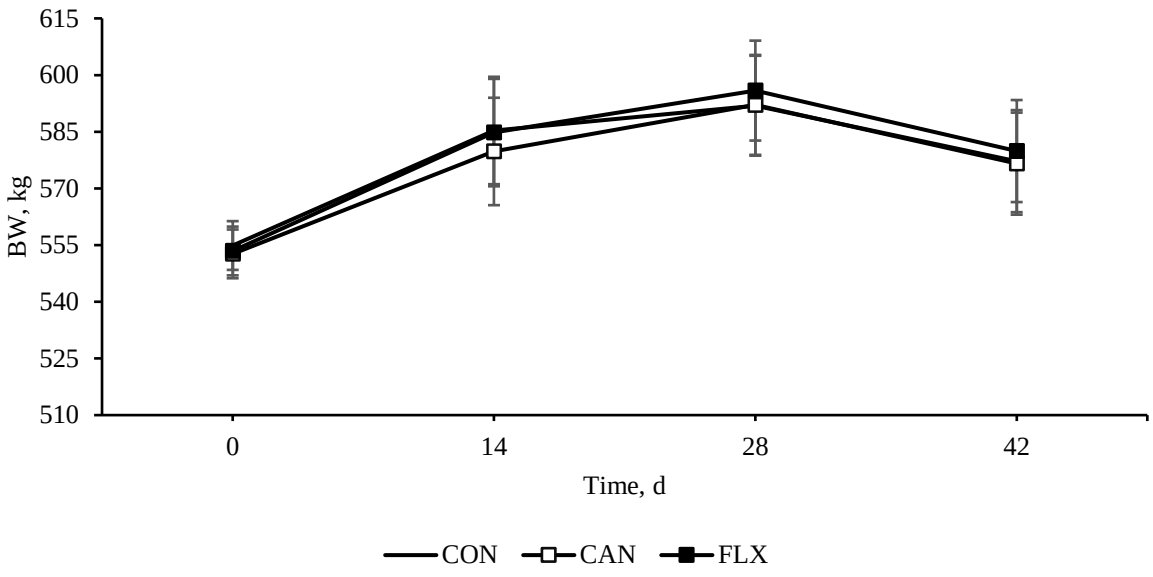

Figure 3.1. Body weight of cows over the 42 d of supplementation period. Least square means ± SE.

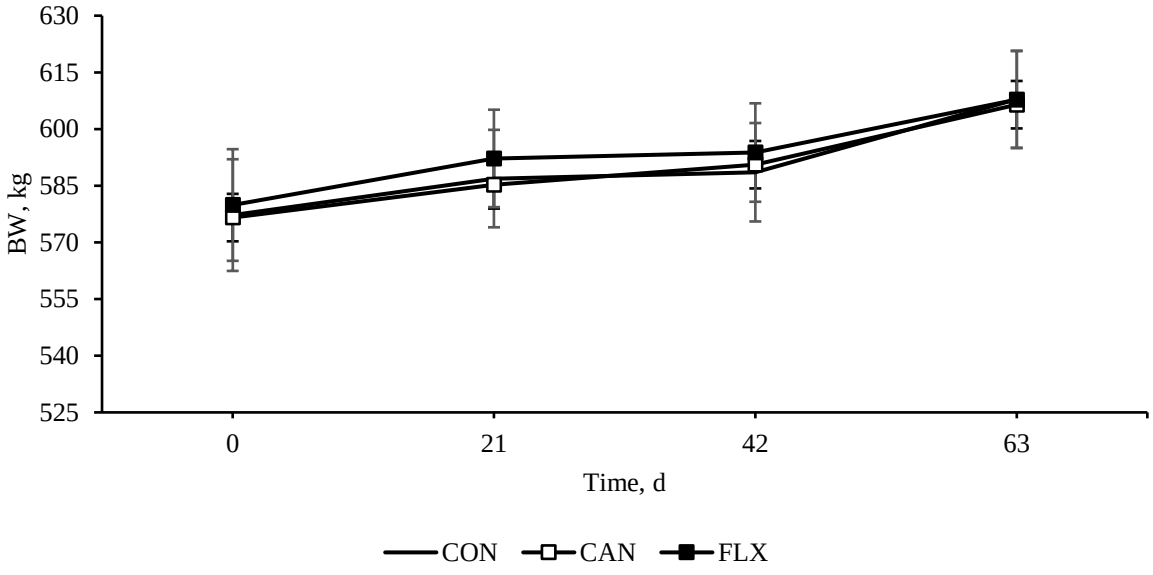

Figure 3.2. Body weight of cows over the 63 d of breeding season. Least square means ± SE.

3.4.5. Blood metabolites and trace minerals

The results for blood metabolite and trace mineral concentrations are shown in Table 3.6. No differences ($P \geq 0.22$) were observed among treatments on serum NEFA and BHBA concentrations at the start of the supplementation period. Serum NEFA concentration was also not affected ($P = 0.27$) by treatment following the supplementation period. This is consistent with findings by Bottger et al. (2002) where beef cows had similar serum NEFA concentrations after being supplemented over a 90-d postpartum period with safflower seeds high in linoleic or oleic acid. In the present study, serum NEFA concentrations across treatments decreased an average 359 ± 70 µEq/L throughout the 42 d of supplementation and were not different ($P = 0.27$) at the end of this period. The increase in serum NEFA concentration at the start relative to the end of the supplementation period indicates that cows were mobilizing more body fat at the start than at the end of the supplementation. The reason for this increase in fat mobilization at the start of the trial is most likely due to a greater energy demand since cows were approaching peak of lactation. In two studies where the lactation curves of beef cows were evaluated, it was reported that the peak of lactation occurred at 41 (Casebolt, 1984) and 46 d (Roca-Fraga, 2013). In the present study, the average time that cows started the trial was at 38 ± 1.5 d postpartum. In contrast, the decreased NEFA concentrations at the end of the supplementation period indicate that cows of all treatments were able to improve their energy balance throughout the supplementation period.

Table 3.6. Effects of postpartum fat supplementation and source on blood serum metabolites and trace minerals concentration of young beef cows grazing cool-season grass pastures.

	Treatments[1]					Contrasts	
Item	**CON**	**CAN**	**FLX**	**SEM[2]**	***P*-value**	**CON vs. SUP[3]**	**CAN vs. FLX**
Start of supplementation							
NEFA[4] (µEq/L)	925	903	816	127.7	0.22	0.25	0.19
BHBA[5] (mg/dL)	12.0	11.6	12.2	0.72	0.67	0.83	0.39
Copper (ppm)	0.682	0.649	0.699	0.018	0.15	0.72	0.06
Manganese (ppm)	0.005	0.007	0.007	0.001	0.09	0.03	0.86
Zinc (ppm)	0.932	1.137	1.109	0.116	0.15	0.06	0.80
End of supplementation/Start of breeding season							
NEFA[4] (µEq/L)	558	459	494	135.9	0.27	0.21	0.31
BHBA[5] (mg/dL)	12.0	12.7	11.3	0.46	0.07	0.96	0.02
Copper (ppm)	0.665	0.673	0.726	0.016	0.02	0.09	0.02
Manganese (ppm)	0.007	0.006	0.006	0.001	0.74	0.46	0.86
Zinc (ppm)	0.984	1.073	0.962	0.093	0.67	0.77	0.40

[1]CON: no supplement offered; CAN: cows supplemented with a canola seed-based pellet; FLX: cows supplemented with a flaxseed-based pellet.
[2]SEM: pooled standard error of mean.
[3]SUP: average of CAN and FLX.
[4]NEFA: non-esterified fatty acids.
[5]BHBA: β-hydroxy butyrate.

There was no difference among treatments in serum BHBA levels at the start of the supplementation period (Table 3.6). Similarly, at the end of the supplementation period serum BHBA concentration of CON cows (12.0 mg/dL) was not different ($P = 0.96$) from those of SUP. However, serum BHBA concentration of CAN cows (12.7 mg/dL) was greater ($P = 0.02$) than those from FLX cows (11.3 mg/dL). It is known that butyrate resulting from ruminal fermentation is largely metabolized by the rumen epithelium, and it has been shown (in-vitro) that approximately 94% is converted to ketone bodies (Bergman, 1990; Sehested et al., 1999). However, ruminal fermentation of fiber is reduced when fat is added to the diet, and this negative effect of dietary fat on rumen fermentation is positively correlated with the degree of unsaturation in the fatty acids (Jenkins, 1993). Therefore, the decreased serum BHBA concentration for FLX cows could be a consequence of less BHBA synthesis by the rumen epithelia due to a diminished butyrate production during ruminal fermentation.

Serum Cu levels of all treatments were marginal at the start and end of the trial according to the range (0.55 and 0.70 ppm) established by Smart et al. (1992). At the start of the trial, the serum Cu level in CON cows (0.682 ppm) was similar to SUP cows, while FLX cows tended ($P = 0.06$) to have greater serum Cu than CAN cows (0.699 vs. 0.649 ppm). At the end of the supplementation period, serum Cu level of CON cows (0.665 ppm) tended ($P = 0.09$) to be lower than SUP, and serum Cu level of FLX cows remained greater ($P = 0.02$) than that of CAN cows (0.726 vs. 0.673 ppm). The tendency to for lower serum Cu levels in the CON cows may be a reflection of the low Cu content of forages found in Saskatchewan (Saskatchewan Forage Council, 2014). However, all treatments showed adequate serum Cu levels at the start of the breeding season, and as a result serum Cu levels likely did not affect pregnancy rates. In a study involving 771 beef cows grazing rangelands, Van De Weyer et al. (2011) showed that the probability of non-pregnancy due to Cu deficiency was absent when the serum Cu levels of cows at the beginning of the breeding season was at least 0.6 ppm. At the end of the trial, no differences ($P \geq 0.40$) were observed among treatments for serum Mn and Zn levels, and all treatments showed levels for these two minerals that were adequate according to ranges established by Puls (1981).

3.4.6. Reproductive performance

Results of postpartum fat supplementation on reproductive performance are shown in Table 3.7. Proportions of cows cycling were not different ($P \geq 0.52$) among treatments at the start or at the end of the supplementation period. The lack of effect of level of fat supplementation on cycling activity is contrary to the findings reported by Wehrman et al. (1991), where the proportion of Brahman cows cycling at the start of the breeding season was greater for those that received a high-fat supplement (8% EE) over the previous 30 d compared to those fed a control diet (2.5% EE). However, the total fat fed in this study for the high-fat group was 5.5% of total DMI, while in the present study the average amount of fat in the diet of SUP cows was 4.0% of total DMI. After the onset of the breeding season, no differences ($P \geq 0.12$) were found among treatments on days to conception or on postpartum interval to conception. After the 63 d of the breeding season, no treatment differences ($P = 0.85$) were found on pregnancy rates. At calving, date of first calving of CAN cows tended ($P = 0.06$) to occur sooner compared to FLX cows (107 vs. 113 d), with no difference ($P = 0.16$) between CON and SUP. However, calving span, calving distribution, and calving to calving interval were not different ($P \geq 0.12$) among treatments.

Table 3.7. Effects of postpartum fat supplementation and source on reproductive performance of young beef cows grazing cool-season grass pastures.

	Treatments[1]					Contrasts	
Item	**CON**	**CAN**	**FLX**	**SEM[2]**	***P*-value**	**CON vs. SUP[3]**	**CAN vs. FLX**
Start of supplementation							
Cycling (% cows)	37.2	43.9	30.6	18.17	0.55	0.99	0.30
End of supplementation/Start of breeding season							
Cycling (% cows)	91.7	91.7	80.6	5.35	0.52	0.61	0.41
Days to conception	11	15	11	3.33	0.34	0.46	0.20
PPI[4] (d)	90	98	92	6.34	0.12	0.17	0.12
Conception at 21 d (%)	84.8	91.2	85.7	5.70	0.77	0.68	0.61
Conception at 42 d (%)	97.0	94.1	97.1	3.32	0.82	0.83	0.65
End of breeding season							
Pregnancy rate (% cows)	94.4	100.0	97.2	2.71	0.85	0.97	0.97
At calving							
Calving span (d)	27	23	24	4.19	0.76	0.51	0.74
First calving (Julian d)	106	113	107	2.68	0.07	0.16	0.06
Last calving (Julian d)	132	135	130	5.21	0.79	0.91	0.50
C-C[5] (d)	373	381	375	6.34	0.12	0.17	0.12

[1]CON: no supplement offered; CAN: cows supplemented with a canola seed-based pellet; FLX: cows supplemented with a flaxseed-based pellet.
[2]SEM: pooled standard error of mean.
[3]SUP: average of CAN and FLX.
[4]PPI: postpartum interval.
[5]C-C: calving to calving interval.

Although the statistical power (i.e. number of replicates) of the present study was somewhat limited as compared to that suggested by Engstrom et al. (2010), the lack of significant response in reproductive performance to postpartum fat supplementation was most likely due to CON cows improving their nutrient intake and having similar nutritional status to those of SUP cows. Hess et al. (2005) demonstrated that BCS change and energy balance from calving to breeding were correlated to postpartum interval to first estrous, concluding that reproductive performance of the beef cow declines when facing a negative energy balance during lactation. As discussed previously, in the present study cows in all treatments decreased and had similar serum NEFA concentrations after the 42 d of supplementation. In addition, all treatments were able to maintain or increase their subcutaneous fat depots and end the supplementation period with similar BCS and subcutaneous fat thickness. All these parameters indicate that CON, CAN, and FLX cows were able to equally improve their nutritional status throughout the 42 d of supplementation; thus, showing similar reproductive performance during the breeding season. The reason for this effect of positive nutritional status on reproduction is possibly due to an increase of circulating leptin as a result of adipose tissue accretion. Leon et al. (2004) observed that plasma leptin concentration was positively correlated with BCS of cross-bred beef cows, and the degree of the correlation was almost doubled when cows gained vs. lost weight. Further, infusion of leptin has been reported to prevent the fasting induced reduction in LH pulse frequency in female ovine and to increase the LH secretion in mature beef cows (Henry et al., 1999; Zieba et al., 2002).

Also, despite the lack of a statistical difference on reproductive performance between CAN and FLX supplemented cows, the estimated values for days to conception, postpartum interval, date of first calving, and calving to calving interval were numerically greater for the CAN group suggesting a longer period to achieve conception for these cows compared to those from the FLX group. Since the estimated forage intake was the same for both CAN and FLX group, and CSG forage across treatments had similar fatty acid profiles, the only difference in fatty acid intake between these treatments was from the supplement. The difference in fatty acid profiles between CAN and FLX supplements resulted in the CAN cows having a greater n-6:n-3 ratio in their fatty acid intake. *In-vitro* studies, using oocytes collected from follicles produced in ovaries of dairy cows, have shown that the n-6 concentration in fluid from both the external and internal environment of the oocyte can inhibit its maturation (Homa and Brown, 1992; Kim et al.,

2001). In contrast, including n-3 fatty acids in the diet of ruminant females, thus reducing the dietary n-6:n-3 ratio, has improved the oocyte maturation both *in-vitro* (Fouladi-Nashta et al., 2009) and *in-vivo* (Zeron et al., 2002).

The performance of heifer and steer calves are shown in Table 3.8. Supplementing fat to lactating young beef cows did not affect ($P = 0.27$) ADG or BW of heifer calves. However, ADG and BW of heifer calves from FLX (1.40 kg/d and 139 kg) supplemented cows tended ($P = 0.08$) to be greater than those from CAN (1.27 kg/d and 133 kg) supplemented cows. In a study where milk replacers differing in their fatty acid content were included in the diet of Holstein calves, Hill et al. (2007) concluded that increasing the essential fatty acid content (including C18:3n-3) in milk replacers improved the ADG in calves. However, in the present study it is unknown if the source of supplemental fat had any effect on the fatty acid profile of milk. Further research about the possible effect of source of supplemental fat to lactating beef cows on the performance of heifer calves is required. No differences ($P \geq 0.57$) were observed among treatments on performance of steer calves.

Table 3.8. Effects of postpartum fat supplementation and source to young beef cows grazing cool-season grass pastures on performance of their calves.

	Treatments[1]					Contrasts	
Item	**CON**	**CAN**	**FLX**	**SEM[2]**	***P*-value**	**CON vs. SUP[3]**	**CAN vs. FLX**
Heifer calves							
Start of trial							
BW (kg)	81.4	78.5	81.7	6.50	0.82	0.80	0.58
End of trial							
BW (kg)	133	133	139	2.31	0.12	0.27	0.08
ADG (kg/d)	1.26	1.27	1.40	0.06	0.13	0.27	0.08
Steer calves							
Start of trial							
BW (kg)	77.7	77.9	75.7	6.77	0.91	0.86	0.69
End of trial							
BW (kg)	131	132	133	2.42	0.73	0.57	0.59
ADG (kg/d)	1.29	1.31	1.33	0.06	0.74	0.57	0.60

[1]CON: no supplement offered; CAN: cows supplemented with a canola seed-based pellet; FLX: cows supplemented with a flaxseed-based pellet.
[2]SEM: pooled standard error of mean.
[3]SUP: average of CAN and FLX.

3.5. Conclusions

Postpartum fat supplementation and source of fat had no effect on performance of lactating young beef cows grazing good quality cool-season grass pastures. However, non-supplemented cows had greater estimated forage utilization and forage intake than supplemented cows suggesting a diet substitution effect due to supplementation. Sufficient forage availability with adequate levels of energy and protein, resulted in non-supplemented cows being able to improve their nutritional status; thus, not allowing for a response to fat supplementation. Also, more research is needed to determine the effect of fat supplementation and source to lactating young beef cows on performance of the calves.

These results suggest that under sufficient and good quality pasture scenarios, supplementation using high-fat pelleted feeds either high in MUFA or PUFA does not benefit the reproductive performance of lactating young beef cows. However, more research is needed in order to evaluate the effects of fat supplementation and source of fat to lactating young beef cows under limited forage and/or nutrient scenarios.

4. LEVEL AND SOURCE OF FAT IN THE DIET OF GESTATING BEEF COWS ON THE PREPARTUM PERFORMNACE OF THE DAM AND BIRTH WEIGHT OF THE PROGENY

4.1. Abstract

A two-year study was conducted to evaluate the effects of level and source of fat in the diet of gestating beef cows on their pre-partum performance and birth weight of progeny. Each year, 75 multiparous (≥3 calving) pregnant Angus cows were stratified by BW (663±21.5 kg) and BCS (2.6±0.12), and randomly assigned to 15 outdoor pens. Subsequently, each pen was randomly assigned to one of three (n=5) treatments: a low-fat diet (LF; 1.4±0.12% EE) consisting of grass-legume hay, barley straw, and barley grain; and two high-fat diets (HF; 3.3±0.20% EE) including a canola seed (CAN) or a flaxseed (FLX) based pelleted feed. Diets were formulated to meet the requirements of pregnant beef cows during the last two trimesters of gestation (183±4.8 d), adjusted for changes in environmental conditions, and offered such that each pen on average received similar amounts of DE (31.2±2.8 Mcal/cow/d), CP (1.36±0.13 kg/cow/d), and DM (12.9±1.0 kg/cow/d). Data were analyzed as a randomized complete block design with contrasts to separate the effects of level (LF vs. HF) and source (CAN vs. FLX) of fat. After 160 d on trial, conceptus corrected-BW (CC-BW) of LF cows (708 kg) and the proportion of over conditioned cows (13.2%) were greater ($P \leq 0.04$) than those of HF, with no difference ($P \geq 0.84$) between CAN and FLX for CC-BW (697 kg) and proportion of over conditioned cows (3.6 vs. 2.9%). Feeding FLX diet during gestation resulted in subcutaneous adipose tissue (SCAT) with greater ($P \leq 0.01$) concentration of CLnA (0.12 vs. 0.05%) and n-3 (0.58 vs. 0.37%) fatty acids, and a tendency ($P=0.09$) for CLA concentration (1.05 vs. 0.88%) to be greater when compared to CAN diet. By the end of gestation, serum NEFA concentration of LF cows (592 µEq/L) was lower ($P<0.01$) than that of HF cows, and FLX cows had greater ($P<0.01$) serum NEFA concentration than CAN cows (636 vs. 961 µEq/L). Cows receiving the LF diet over gestation gave birth to lighter

($P \leq 0.01$) calves compared to those receiving the HF diets (40.2 vs. 42.9 kg), with no difference ($P=0.24$) between calves born to CAN (42.4 kg) and FLX (43.3 kg) cows. In conclusion, these results suggest a partitioning of the ME in pregnant beef cows that is dependant on the type of dietary energy, resulting in heavier calves at birth for cows fed high-fat diets. Also, the type of fatty acid in the diet of gestating beef cows affected the fatty acid profile in SCAT and serum NEFA concentration.

4.2. Introduction

In the northern Great Plains of North America, pregnant beef cows can be exposed to extreme low-temperatures during winter which often overlaps with the last two trimesters of gestation. This is a critical time since fetal secondary myogenesis, muscle fibre hypertrophy, and adipogenesis occur during the last two trimesters of gestation (Du et al., 2010a). As a result, during mid- and late gestation, beef cows experience an increase in their energy requirements for maintenance and pregnancy to maintain body temperature and to ensure proper fetal growth (NRC, 2000). Therefore, diets that provide energy in an effective manner is a major goal to increase performance of both cows and calves.

Fat inclusion at levels up to 6% of the total DMI of ruminants increases the energy density of high-forage diets without detrimental effects on animal performance, and avoids the negative effects associated with starch inclusion (Palmquist, 1994; Bowman and Sanson, 1996; Hess et al., 2008). Also, compared to low-fat diets with similar energy content, high-fat diets have been shown to have a positive effect on reproductive performance of beef cows (Bellows, 1999; Bellows et al., 2001; Graham et al., 2001), and a cost-effective benefit in backgrounding beef steers (Zenobi et al., 2014).

Adequate nutrient supply during gestation is not only necessary to meet the nutrient requirements of the dam but can also benefit the performance of the offspring of many mammalian species, including cattle (Wu et al., 2004; Du et al., 2010a). The effects of under or over feeding beef cows during gestation on performance of the offspring have been extensively documented (Funston et al., 2010), but few studies have looked at the effects of nutrient source

(i.e. starch vs. fat). Radunz et al. (2010) fed multiparous beef cows from mid-gestation until calving with similar amounts of NEm using a grass-hay based diet (244 g/d of crude fat), or two diets supplemented with corn or corn DDGS (270 and 455 g/d of crude fat, respectively). It was found that calves born to the cows fed the corn and corn DDGS diets were heavier at birth than those born to cows fed grass-hay. However, because cows fed corn DDGS consumed greater amounts of CP than those fed corn (1.1 vs. 1.6 kg/d of CP), it was not clear if the improvement in birth weight for corn DDGS calves was a result of the source of energy or level of CP intake of the dam over gestation.

Compared to low-fat diets, feeding high-fat diets over gestation has been shown to improve placental nutrient transport to the fetus in mice, hence increasing the fetal weight of the progeny (Jones et al., 2009). Moreover, placental and fetal tissues from humans and rodents have been reported to have a preference for absorption of long-chained polyunsaturated fatty acids, especially during the late stages of gestation (Herrera, 2002; Jones et al., 2007). This has important implications as low maternal intake of n-6 and n-3 fatty acids during gestation has resulted in reduced neonatal growth in humans (Jumpsen et al., 1997). However, such effects of level and source of fat in the diet of the dam over gestation on performance of the progeny have not been studied in cattle.

In western Canada and the north-west of the United States there are a number of by-product feeds that vary in oil and fatty acid content. Examples include oilseeds such as off grade canola and flaxseed which are high in MUFA and PUFA, respectively. Research has shown that relative to conventional feed sources, inclusion of these by-products in the form of blended pelleted feeds has resulted in equal or superior performance of growing cattle (Añez-Osuna et al., 2015; Zenobi et al., 2014). Such high-fat by-product feeds may also be viable supplements for gestating beef cows to meet pregnancy requirements and could potentially contribute to improve the pre- and postnatal growth of progeny through developmental programming mechanisms.

The objective of this study was to evaluate the effects of feeding beef cows over the last two trimesters of gestation with a low-fat or two high-fat diets based on by-product feeds that differed in their fatty acid composition (MUFA vs. PUFA) on pre-partum performance of the dam and birth weight of the progeny.

4.3. Materials & Methods

4.3.1. Location

A two-year study (2014-2015 and 2015-2016 for year 1 and 2, respectively) was conducted from late October to late April (on average) at the Termuende Research Ranch of the Western Beef Development Centre (WBDC) near Lanigan (51°51'N, 105°02'W), Saskatchewan, Canada.

Over the two years of the study, the monthly average of the mean daily temperatures reported for the Lanigan area (51°40'N, 105°24'W) were 6.4 ± 0.5, -6.2 ± 5.0, -10.0 ± 0.4, -12.4 ± 0.6, -12.7 ± 6.8, -2.0 ± 0.2, and 4.5 ± 0.7ºC from October to April, respectively (Government of Canada, Environment and Natural Resources, http://weather.gc.ca). These values were similar for October to February (4.0 ± 2.3, -5.6 ± 4.7, -14.2 ± 4.4, -13.2 ± 2.5 and -13.2 ± 5.5 ºC, respectively) and slightly warmer for March and April (-5.6 ± 5.7, and 1.9 ± 3.6 ºC, respectively) to the five-year (2012-2016) average reported at the same location.

4.3.2. Animals and Housing

All animals were cared for in accordance with the Canadian Council of Animal Care guidelines (CCAC, 2009), and all experimental procedures were approved by University of Saskatchewan Animal Care Committee (Protocol No. 20090107).

Animals were obtained from the main herd of the WBDC's research ranch. Each year, 75 multiparous (≥3 calving) pregnant Angus cows were housed in 15 outdoor research pens (7.4 × 24.5 m) separated by metal rail fences and equipped with feed bunks, water bowls, and 20% porosity windbreaks. Wood-chips were used for bedding and provided twice per week. The same animals were used for each year of the study unless culled for injury or failure to conceive, in which case, similar replacements were obtained from the same herd. Prior to the start of the trial, all cows were managed together and exposed to a 63-d breeding season during the summer starting on July 2nd and July 5th for year 1 and 2, respectively. In both years, four half-sibling, registered Angus bulls were used as sires (25:1 cow to bull) after passing a breeding soundness evaluation. Forty-five days after ending the breeding season, all cows were pregnancy checked by a veterinarian using an Easi-Scan Curve ultrasound machine (3.0 - 7.0 MHz; BCF Technology

Ltd., Rochester, MN). At this time, a vitamin ADE (Bimeda-MTC, Cambridge ON) injection (5 mL) was administered to each cow.

4.3.3. Treatments, Feeding and Handling

Each year (Oct 24th and Oct 23rd for year 1 and 2, respectively), cows were stratified by IBW (662 ± 52.4 kg) and BCS and divided into 15 homogenous groups (5 cows/group). Subsequently, each group was randomly assigned to 1 of the 15 outdoor research pens and each pen was then randomly assigned to 1 of 3 replicated (n=5) dietary treatments which consisted of: a low-fat (**LF**) diet (1.4 ± 0.13% EE), and two high-fat (**HF**) diets (3.3 ± 0.10% EE). Hay consisting of bromegrass (*Bromus sp.*) and alfalfa (*Medicago sativa* L.), barley straw, rolled barley grain, and two high-fat pelleted feeds were used as ingredients to formulate the diets (Table 4.1). The two high-fat pellets were formulated using canola seed (**CAN**) as a source of mono-unsaturated fatty acids (**MUFA**), or flaxseed (**FLX**) as a source of poly-unsaturated fatty acids (**PUFA**). High-fat diets (CAN and FLX) were formulated to provide each cow with 300 g of fat from pelleted feeds daily. Feeding amounts were such that each pen received equal amounts of DE, CP, and total DM. Diets were formulated to meet the DE and CP intake requirements of pregnant beef cows over the second and third trimester of gestation according to NRC (2000). The goal was to have the cows maintain BW over the course of the trial while accounting for the estimated increase due to uterine and fetal tissue growth corresponding to a projected 40-kg calf at birth (NRC, 2000). The amounts fed were adjusted every two weeks according to estimated day of gestation, gained weight, and actual changes in weather conditions. Diets were offered daily as total mixed rations (TMR) using a mixer wagon with feeding starting at 0800 h, and bunks were cleaned every two weeks due to accumulation of orts if needed. Each year, the trial lasted from the start of the second trimester of gestation until calving (183 ± 14 d). Prior to estimated calving date (23 ± 14 d before calving), cows were moved from replicate pen and relocated according to treatment into one of three common calving pens (76 × 73 m) equipped with feed bunks and water bowls. Cows continued receiving their treatment diets in their respective group pen until calving. All animals had *ad-libitum* access to a 2:1 mineral [15.5% Ca, 7% P, 30 ppm Se, 20 ppm Co, 200 ppm I, 1500 ppm Cu, 5000 ppm Mn, 5000 ppm Zn, 1000 ppm Fe, 1.0 ppm F (max), 500 000 IU/kg vitamin A (min), 50000 IU/kg vitamin D (min), 2500 IU/kg vitamin E (min); Cargill Animal Nutrition, MB, Canada] and cobalt-iodized salt (FeedRite Ltd., Humboldt, SK, Canada).

Table 4.1. Nutrient and fatty acid composition (average ± SD) of feed ingredients by year.

Feeds[1]	**Grass hay**		**Barley straw**		**Barley grain**		**CAN pellet**		**FLX pellet**	
Year	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
Ingredients[2] ***(% AF)***										
Oat hulls	-	-	-	-	-	-	40.0	40.0	40.0	40.0
Wheat	-	-	-	-	-	-	41.0	41.0	41.0	41.0
DDGS	-	-	-	-	-	-	2.0	2.0	2.0	2.0
Feed binder	-	-	-	-	-	-	2.0	2.0	2.0	2.0
Canola seed	-	-	-	-	-	-	15.0	15.0	-	-
Flaxseed	-	-	-	-	-	-	-	-	15.0	15.0
Nutrients[3] ***(% DM)***										
DM (% As fed)	75.9±5.61	76.0±7.62	71.7±8.34	69.3±6.82	87.4±3.01	84.0±2.21	90.1±1.87	88.9±1.60	88.9±2.68	88.8±1.76
CP	10.7±0.15	12.5±0.19	6.65±0.17	8.09±0.17	12.2±0.05	13.0±0.21	12.4±0.25	13.4±0.30	13.0±0.92	13.1±0.22
ADF	46.2±0.75	48.8±0.52	51.7±0.14	52.5±0.33	14.3±0.19	8.84±0.45	20.8±0.38	26.2±0.29	18.8±1.15	24.3±0.39
NDF	65.5±0.92	66.1±1.00	75.0±0.11	75.2±0.22	31.0±0.63	21.7±1.10	35.7±0.41	44.4±0.75	29.9±1.09	42.5±1.97
EE	1.38±0.02	1.55±0.04	1.16±0.01	0.87±0.07	1.95±0.15	1.73±0.33	7.51±0.10	9.28±0.17	6.36±0.18	8.05±0.25
Ca	0.68±0.03	0.96±0.05	0.38±0.02	0.35±0.02	0.09±0.00	0.07±0.00	0.83±0.04	0.23±0.00	0.13±0.00	0.49±0.01
P	0.22±0.01	0.21±0.01	0.16±0.01	0.16±0.01	0.43±0.00	0.37±0.02	0.30±0.00	0.38±0.00	0.31±0.01	0.37±0.01
TDN	49.9±0.85	46.9±0.59	43.7±0.16	42.7±0.38	74.5±0.09	77.2±0.22	71.4±0.18	68.8±0.14	72.4±0.55	69.7±0.19
Fatty acid[4] ***(% of total)***										
16:0	33.4±0.95	30.5±0.84	31.0±0.28	28.1±1.81	28.3±0.51	27.6±0.71	10.1±0.62	8.13±0.01	10.4±0.06	9.57±0.15
18:0	4.00±0.48	4.93±0.26	3.72±0.09	4.27±0.25	1.63±0.05	1.67±0.05	2.17±0.08	1.84±0.01	3.01±0.04	3.34±0.10
*c*9-18:1	8.98±2.32	9.06±0.77	11.1±0.53	13.0±1.84	18.1±1.29	17.0±0.21	53.5±0.75	53.2±0.14	19.9±0.09	19.4±0.29
*c*11-18:1	1.27±0.15	1.58±0.11	1.48±0.07	1.90±0.18	1.18±0.09	1.08±0.03	3.86±0.06	4.14±0.03	0.88±0.01	0.88±0.00
18:2n-6	13.6±0.50	15.3±0.59	16.6±0.40	17.3±2.52	39.8±1.01	41.3±0.79	20.2±0.94	21.1±0.04	23.6±0.14	20.7±0.23
18:3n-3	22.7±2.63	22.4±1.77	11.6±0.61	11.5±2.22	6.95±0.34	7.39±0.32	6.57±0.58	8.24±0.16	40.4±0.25	44.6±0.34
∑SFA	49.5±0.45	48.7±0.95	55.8±0.73	52.3±3.60	31.9±0.57	31.3±0.85	13.9±0.79	11.4±0.03	14.4±0.13	13.8±0.25
∑MUFA	14.0±2.23	13.4±0.90	15.7±0.21	18.7±1.62	21.3±1.46	19.9±0.26	59.3±0.83	59.2±0.18	21.6±0.09	21.0±0.29
∑PUFA	36.5±2.15	37.8±1.32	28.5±0.92	29.0±4.48	46.8±1.34	48.8±1.07	26.8±1.50	29.4±0.18	64.0±0.21	65.3±0.54

[1]**CAN pellet**: pelleted feed formulated using whole canola seed; **FLX pellet**: pelleted feed formulated using whole flaxseed.
[2]**DDGS**: dry distillers grains with solubles; **Feed binder**: sodium bentonite (Canapell®; Canadian Clay Products, Inc. Wilcox, SK, Canada).
[3]**TDN**: calculated using the Pennsylvania-State equations (Adams, 1980).
[4]∑**SFA**: sum of saturated fatty acids; ∑**MUFA**: sum of monounsaturated fatty acids; ∑**PUFA**: sum of polyunsaturated fatty acids.

4.3.4. Data collection

4.3.4.1. Feeds and DM intake

Grass-legume hay, barley straw, and barley grain were collected weekly, placed in a forced air oven at 55º C for 48 h for DM determination. Pelleted feed samples were also collected weekly. All feed samples were ground to pass a 1-mm screen using a Thomas-Wiley Laboratory Mill (Model 4, Thomas Scientific, Swedesboro, NJ) and stored at -20º C. Ground feed samples were composited (DM basis) after every 5 or 6 weeks of collection in order to obtain two composite samples per trimester corresponding to the first and second half of the second and third trimester of gestation. Composite samples were stored at -20º C until analysis.

The total amount of TMR fed to each treatment was recorded daily and distributed equally across pens. Thus, within treatment, all pens received the same daily amount of feed. Total amounts of orts were collected every two weeks, recorded, and a representative sample was placed in a forced air oven at 55ºC for 48 h to determine DM. Dry matter intake was calculated using the difference between the quantity of DM offered and the quantity of DM refused.

4.3.4.2. Body weight

In order to minimize variation due to rumen fill, each cow was weighed over two consecutive days at the start of the trial and at the time of relocation in the common calving pens. Throughout the 160 d of the winter feeding, all cows were weighed once every two weeks with weights measured before feeding. Throughout the calving season, all cows were checked twice daily for signs of parturition, and birth weight of each calf was determined within the first 24 h after birth. Calving date and birth weight of each calf were used to estimate fetal and associated uterine tissue growth (assuming a gestation of 283 d) using the NRC (2000) model. The conceptus corrected-BW (**CC-BW**) of each cow was calculated by subtracting the fetal and gravid uterine weights from the pregnant BW.

4.3.4.3. Body Condition Scoring and Subcutaneous Fat Thickness

At the start of the trial and at relocation into common calving pens, the BCS of each cow was determined by the same experienced technician using the Scottish scale where 1 = emaciated and 5 = grossly fat (Lowman et al., 1976; Wildman et al., 1982). Ultrasound measurements of subcutaneous fat thickness (**SCFT**) over the third quarter of the rib eye muscle, between the 12th and 13th rib, and at the thurl location on the rump area were determined on each cow at the start and at end of the feeding period, and at calving using an Aloka SSD-500V ultrasound machine and an Aloka UST-5044 probe (3.5 MHz-17 cm; Aloka Inc., Wallingford, CT).

4.3.4.4. Blood Serum and Subcutaneous Adipose Tissue

Each year at the start of the trial, a representative sample of 15 cows were randomly selected (n=1 cow/pen) and used for blood and adipose tissue collection. At the time of relocation in common calving pens, 30 cows were randomly selected (n=2 cows/pen) and used for blood and adipose tissue collection. Blood samples were collected from each cow via coccygeal venipuncture into 10-mL untreated vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ). Blood samples were allowed to clot at room temperature for 30 min, centrifuged (2000 × g at 4°C for 15 min), and serum was harvested into 1.5-mL tubes (Eppendorf, GCS, New York, NY) and refrigerated at -20°C until analysis. Sub-cutaneous adipose tissue biopsies (5 g) were taken from the caudal portion of the tail-head of each cow under local anaesthesia using 4-mL of lidocaine HCl 2% (Zoetis Canada Inc., Kirkland, QC). Adipose tissue biopsies were placed into 60-mL sterile polyethylene bags (Fisher Scientific, Ottawa, ON), and stored at -20°C until analysis.

4.3.5. Laboratory analysis

4.3.5.1. Feeds

All feed samples were analyzed in duplicate for nutrient composition by Cumberland Valley Analytical Services Inc. (Hagerstown, MD). Grass-legume hay and barley straw samples were analyzed by near infrared spectroscopy (NIRS) using a Foss NIRSystems 5000 (NIR Systems, Inc., Silver Spring, MD, USA) for determination of DM [standard error of calibration (SEC) = 0.31, regression coefficient (R^2) = 0.93], CP (SEC = 0.51, R^2 = 0.99), ADF (SEC = 1.24, R^2 =

0.95), NDF (SEC = 1.69, R^2 = 0.96), EE (SEC = 0.32, R^2 = 0.87), ash (SEC = 0.84, R^2 = 0.85), Ca (SEC = 0.07, R^2 = 0.80), and P (SEC = 0.04, R^2 = 0.80). Barley grain and pelleted feed samples were analyzed for DM by drying at 135°C for 2 h (method 930.15; AOAC, 2012), CP (method 990.03; AOAC, 2012) using a Leco FP 528 Nitrogen Combustion Analyzer (Leco, St. Joseph, MI, USA), EE using a tecator extraction unit (method 2003.05; AOAC, 2012), ADF (method 973.18; AOAC, 2012), ash (method 942.05; AOAC, 2012), and Ca and P (method 985.01; AOAC, 2012). The method of Van Soest et al. (1991) with the addition of amylase and sodium sulfite was used to determine NDF content. The Pennsylvania-State equations based on ADF were used to calculate the total digestible nutrient (TDN) values for all feeds (Adams, 1980). Digestible energy (DE), net energy of maintenance (NEm) and gain (NEg) were calculated according to NRC (2000).

4.3.5.2. Blood metabolites

Blood serum samples were used for determination of non-esterified fatty acid (**NEFA**) and β-hydroxybutyrate (**BHBA**) concentrations. Serum NEFA concentration was determined using the NEFA-HR (2) kit (Wako Diagnostics Corp., Richmond, VA). Serum BHBA concentration was determined through the enzymatic oxidation of BHBA to acetoacetate caused by incubation in 3-hydroxybutrate dehydrogenase (Williamson et al., 1962). The associated reduction of NAD to NADH was determined through photometric methods at a wavelength of 340 nm using a microplate spectrophotometer (Epoch 2, Biotek Instruments Inc., Winooski, VT, USA).

4.3.5.3. Fatty Acids Extraction and Gas Chromatography

Fatty acid methyl esters (FAME) were obtained from feeds and SCAT samples. Fatty acids of feed samples were methylated using the method of Palmquist and Jenkins (2003) and heptadecenoic acid (standard no. U-42M from Nu-Chek Prep Inc., Elysian, MN, USA) as internal standard. Briefly, 150 mg of forage and barley grain samples, and 50 mg of pelleted feed samples were methylated at 90ºC for 2 hr using 3 N methanolic HCl. Internal standard (4 mg) in toluene was added prior to addition of the methylating reagent. After methylation, samples were cooled, 10-mL of 6% K_2CO_3 added and fatty acid methyl esters (FAME) were extracted into hexane. Completeness of methylation was determined and FAME purified by thin layer chromatography (TLC) using silica gel G plates and hexane:diethyl ether:acetic acid (85:15:1) as a developing

solvent. For adipose tissue samples, 40 ± 5 mg of thin shavings were weighed into a culture tube with teflon lined cap, and freeze dried overnight to constant weight. Subsequently, samples were methylated using 0.5 N sodium methoxide. Internal standard (4 mg) was added prior to addition of the methylating reagent. Fatty acid methyl esters obtained from feeds and adipose tissue samples were analyzed using a Varian CP-3800 gas chromatograph (Varian Inc., Walnut Creek, CA, USA) using the conditions described by Dugan et al. (2007). Fatty acids were identified using reference standard No. 603 from Nu-Chek Prep Inc. (Elysian, MN, USA). Branched-chain FAME were identified using a GLC reference standard BC-Mix1 from Applied Science (State College, PA, USA). The UC-59M standard from Nu-Chek Prep, which contains all four positional CLA isomers, was used for conjugated linoleic acid (CLA) isomers. Biohydrogenation intermediates, such as *trans*-18:1 CLA isomers, not included in the standard mixtures were identified by their retention times and elution orders as reported in literature (Cruz-Hernandez et al., 2004; Gomez-Cortes et al., 2009; Kramer et al., 2008) and this included recently identified Δ-9 desaturation products of trans-18:1 isomers (Vahmani et al., 2016a) The FAME were quantified using chromatographic peak area and internal standard as detailed in Vahmani et al. (2017).

4.3.6. Statistical analysis

Of the 75 cows used in each year of the study, three cows (one from each treatment) died during the winter-feeding period from causes (hardware in the TMR) unrelated to treatment. Data from these animals were removed from the analysis. As well, data from cows carrying more than one fetus (2 FLX, 1 CAN and 1 CON), were removed before analysis. All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC). The average of each research pen where cows received their treatment diets during gestation represented the experimental unit. Data were analyzed as a randomized complete block design using the Mixed procedure. The statistical model included the fixed effect of treatment and the random effect of year. Covariance analyses were performed using the number of days cows were exposed to treatment diets, as well as the proportion of cows carrying heifer calves within research pen, as covariate. The Glimmix procedure was used to analyze BCS using the same model. The Kenward-Roger option was used to estimate denominator degrees of freedom. Pre-planned contrasts were used for the effects of level (LF vs. HF) and source (CAN vs. FLX) of fat. Significant difference was declared at $P < 0.05$, and trend at $P < 0.10$.

4.4. Results & Discussion

4.4.1. Feed and diet compositions

The nutrient composition of the hay, straw, barley grain, and high-fat pelleted feeds are shown in Table 4.1. The high-fat pellets were formulated using by-product feeds derived from local processing of cereal grains in western Canada and proven to meet the nutrient requirements of beef cattle (Zenobi et al., 2014; Añez-Osuna et al., 2015). Formulation of high-fat pelleted feeds was such that the only difference among their ingredients was the major source of fat (canola seed vs. flaxseed). As a result, the level of fat and the fatty acid profiles reflected the source of fat. For both years, CAN pellets had greater total MUFA content (59.3 and 59.2%) than FLX pellets (21.6 and 21.0%). The major fatty acid responsible for the difference in MUFA content was oleic acid (*cis*9-18:1) averaging 53.4 ± 0.53 and 19.6 ± 0.35% for CAN and FLX (respectively) over both years. In contrast, total PUFA content for FLX pellets (64.0 and 65.3%) was greater than that of CAN pellet (26.8 and 29.4%) in both years. The main fatty acid responsible for the difference in PUFA was α-linolenic acid (18:3n-3) with a two-year average of 7.41 ± 0.97 and 42.5 ± 2.27% for CAN and FLX, respectively. Within each year of this study, there was little variation in the fatty acid content of the two pelleted feeds. In year one, the 18:3n-3 content of CAN pellet had the largest coefficient of variation (CV = 8.8%) while the *cis*9-18:1 content of FLX pellet had the smallest CV (0.5%). For year two, stearic acid (18:0) content in FLX pellet had the largest CV (3.0%), while the palmitic (16:0) and linoleic (18:2n-6) content of CAN pellet had the smallest CV value (0.2%). This consistent fatty acid profile in the high-fat pellets in each year indicates a relatively high natural antioxidant activity in both canola seed and flaxseed (Siger et al., 2008).

The ingredient and nutrient composition of dietary treatments are shown in Table 4.2. On average, over the 2nd and 3rd trimester of gestation as well as over the entire feeding period, dietary treatments were similar in energy (2.38 ± 0.10, 2.43 ± 0.13, and 2.44 ± 0.09 Mcal/kg of DE for LF, CAN, and FLX respectively) and crude protein (10.3 ± 0.5, 10.6 ± 0.4, and 10.7 ± 0.4% for LF, CAN, and FLX respectively) content. As expected, the average dietary fat (EE) content by trimester of gestation and over the entire feeding period was different between LF and HF diets. The average fat (EE) content was 1.40 ± 0.12% for LF diet, and 3.31 ± 0.16 and 3.27 ±

0.23% for CAN and FLX treatments, respectively. Also, the fatty acid profile of treatment diets differed across treatment diets. Over the two years, the average total saturated fatty acid content of the LF diet (34.8%) was twice that of the CAN (17.0%) and FLX (17.1%) diets. The average MUFA content of CAN diet was 2.5 times greater than that of the FLX diet (52.7 vs. 21.0%), while the average PUFA content of FLX diet was twice that of the CAN diet (61.9 vs. 30.3%).

Table 4.2. Ingredient, nutrient, and fatty acid composition (average ± SD) of treatment diets by trimester of gestation.

***Treatment*[1]**	**LF**			**CAN**			**FLX**		
Trimester	**2nd**	**3rd**	**Avg ± SD**	**2nd**	**3rd**	**Avg ± SD**	**2nd**	**3rd**	**Avg ± SD**
Ingredient (% DM)									
Grass hay	33.7±2.56	36.4±2.44	**35.0±2.84**	27.2±1.82	29.0±2.27	**28.1±2.22**	28.2±2.82	29.9±2.30	**29.0±2.73**
Barley straw	38.3±6.87	36.1±2.33	**37.3±5.34**	35.9±8.38	33.7±5.30	**34.9±7.17**	33.4±6.48	33.1±1.74	**33.3±4.86**
Barley grain	28.0±7.48	27.5±3.50	**27.8±5.94**	7.45±5.10	9.11±1.77	**8.23±3.98**	1.93±2.29	4.38±3.55	**3.08±3.19**
CAN pellet	-	-	-	29.4±4.18	28.2±2.98	**28.8±3.71**	-	-	-
FLX pellet	-	-	-	-	-	-	36.4±7.43	32.6±3.29	**34.6±6.15**
Nutrient*[2] *(% DM)									
CP	10.3±0.44	10.3±0.56	**10.3±0.50**	10.5±0.32	10.7±0.40	**10.6±0.37**	10.6±0.42	10.9±0.37	**10.7±0.42**
ADF	39.4±3.01	39.4±1.07	**39.4±2.31**	39.5±4.15	38.9±2.40	**39.2±3.44**	38.9±3.22	38.7±1.14	**38.8±2.46**
NDF	58.5±2.60	58.5±0.57	**58.5±1.93**	58.6±4.73	58.1±2.62	**58.4±3.88**	56.9±5.12	57.1±1.42	**57.0±3.85**
EE	1.35±0.14	1.45 ±0.07	**1.40±0.12**	3.30±0.20	3.32±0.08	**3.31±0.16**	3.29±0.29	3.24±0.15	**3.27±0.23**
Ca	0.44±0.07	0.45±0.05	**0.45±0.06**	0.53±0.05	0.51±0.06	**0.52±0.06**	0.46±0.10	0.46±0.07	**0.46±0.09**
P	0.25±0.03	0.24±0.02	**0.25±0.02**	0.25±0.01	0.25±0.00	**0.25±0.01**	0.24±0.01	0.24±0.01	**0.24±0.01**
TDN	54.0±2.87	54.0±1.27	**54.0±2.26**	54.9±3.50	55.3±2.04	**55.1±2.90**	55.4±2.80	55.4±0.90	**55.4±2.13**
NEm (Mcal/kg)	1.09±0.10	1.09±0.04	**1.09±0.08**	1.12±0.12	1.14±0.07	**1.13±0.10**	1.14±0.10	1.14±0.03	**1.14±0.07**
NEg (Mcal/kg)	0.52±0.09	0.53±0.04	**0.53±0.07**	0.56±0.11	0.57±0.06	**0.56±0.09**	0.57±0.09	0.57±0.03	**0.57±0.07**
Fatty acid*[3] *(% of total)									
16:0	28.2±0.74	28.2±0.66	**28.2±0.70**	11.8±1.36	12.7±1.20	**12.3±1.36**	11.5±0.52	12.3±0.46	**11.9±0.63**
18:0	2.01±0.19	2.06±0.10	**2.03±0.16**	2.07±0.05	2.12±0.13	**2.09±0.10**	3.14±0.15	3.18±0.11	**3.16±0.13**
*c*9-18:1	17.3±0.90	16.1±0.33	**16.7±0.91**	47.6±1.38	46.8±0.83	**47.2±1.21**	19.0±0.42	19.1±0.31	**19.1±0.37**
*c*11-18:1	1.24±0.05	1.20±0.05	**1.22±0.05**	3.59±0.26	3.55±0.13	**3.57±0.19**	0.93±0.03	0.94±0.03	**0.93±0.03**
18:2n-6	36.6±0.51	37.2±0.73	**36.9±0.70**	22.4±1.00	22.5±0.65	**22.5±0.85**	22.4±1.20	22.9±0.53	**22.7±0.98**
18:3n-3	7.94±0.54	8.35±0.35	**8.13±0.50**	7.93±0.61	7.56±0.99	**7.76±0.83**	40.0±1.33	38.3±0.67	**39.2±1.34**
∑SFA	34.7±0.92	34.9±0.70	**34.8±0.81**	16.3±1.36	17.5±1.31	**17.0±1.34**	16.4±0.71	17.6±0.52	**17.1±0.84**
∑MUFA	20.7±1.14	19.4±0.33	**20.1±0.99**	53.4±1.55	52.3±0.79	**52.7±1.32**	20.9±0.43	21.0±0.27	**21.0±0.33**
∑PUFA	44.6±0.97	45.7±0.78	**45.1±1.02**	30.4±0.67	30.2±1.59	**30.3±1.20**	62.4±0.60	61.3±0.32	**61.9±0.74**

[1]**LF:** low-fat diet; **CAN:** high-fat diet including canola seed based pelleted feed; **FLX:** high-fat diet including flaxseed based pelleted feed.
[2]**NEm and NEg**: calculated using the NRC (2000) summative equation.
[3]∑**SFA**: sum of saturated fatty acids; ∑**MUFA**: sum of monounsaturated fatty acids; ∑**PUFA**: sum of polyunsaturated fatty acids.

4.4.2. Dry matter and nutrient intake

The dry matter and nutrient intake of dietary treatments are shown in Table 4.3. As per the experimental design, the average DMI over the entire feeding period was similar across treatments (12.9 ± 0.9, 12.9 ± 0.9, and 12.8 ± 1.0 kg/cow/d for LF, CAN, and FLX, respectively). Also, when expressed relative to BW, the DMI was similar across treatment with averages of 1.81 ± 0.14, 1.82 ± 0.14, and 1.80 ± 0.17 as percent of BW over the entire feeding period for LF, CAN, and FLX treatments, respectively. In the same way, the average energy and protein intakes over the entire feeding period were similar across treatments. On average, estimated consumption of ME and MP were 25.2 ± 2.2, 25.7 ± 2.3, and 25.7 ± 2.4 Mcal/d and 0.70 ± 0.04, 0.74 ± 0.04, and 0.75 ± 0.05 kg/d for LF, CAN, and FLX treatments, respectively. According to NASEM (2016), a mature (655 kg of BW) and pregnant (42 kg of calf birth weight) beef cow under similar environmental conditions requires on average 20.5 Mcal/d of ME and 0.57 kg/d of MP during the second and third trimesters of gestation. In the present study, it was observed that the average ME and MP consumption of all treatment groups exceeded requirements by 24.7 and 26.2%, respectively.

The average fat (EE) consumption over the last two trimesters of gestation in the LF diet was 181 ± 23 g/cow/d, while HF diets had a fat consumption of 427 ± 34 and 419 ± 39 g/cow/d for CAN and FLX treatments, respectively. The average amount of fat (EE) provided by pelleted feed in HF diets were similar to the target of 300 g/d (309 ± 25 and 314 ± 32 g/d for CAN and FLX, respectively). Other authors, that have targeted level of fat intake from both MUFA and PUFA similar to those offered in the present study, have shown superior reproductive performance for cows receiving high PUFA levels as indicated by larger preovulatory follicles at insemination and subsequently a larger corpus luteum (Bilby et al., 2006).

Table 4.3. Dry matter and nutrient intake of pregnant beef cows fed their treatment diets during the last two trimesters of gestation.

***Treatment*[1]**	**LF**			**CAN**			**FLX**		
Trimester	**2nd**	**3rd**	**Avg ± SD**	**2nd**	**3rd**	**Avg ± SD**	**2nd**	**3rd**	**Avg ± SD**
Ration DMI									
Kg/cow/d	12.4±1.18	13.5±0.47	**12.9±1.05**	12.5±1.12	13.4±0.36	**12.9±0.98**	12.4±1.31	13.4±0.40	**12.8±1.11**
% of BW	1.79±0.17	1.82±0.08	**1.81±0.14**	1.81±0.18	1.84±0.06	**1.82±0.14**	1.79±0.22	1.82±0.06	**1.80±0.17**
Nutrient*[2] *(kg/d)									
CP	1.28±0.13	1.39±0.12	**1.33±0.14**	1.31±0.13	1.44±0.08	**1.37±0.12**	1.31±0.15	1.45±0.08	**1.38±0.14**
ADF	4.90±0.59	5.31±0.31	**5.09±0.52**	4.92±0.65	5.24±0.42	**5.07±0.58**	4.81±0.61	5.17±0.28	**4.98±0.51**
NDF	7.28±0.74	7.88±0.31	**7.56±0.65**	7.31±0.84	7.82±0.51	**7.55±0.75**	7.05±0.94	7.64±0.38	**7.33±0.79**
EE	0.17±0.03	0.20±0.01	**0.18±0.02**	0.41±0.04	0.45±0.02	**0.43±0.03**	0.41±0.05	0.43±0.03	**0.42±0.04**
Ca	0.06±0.01	0.06±0.01	**0.06±0.01**	0.07±0.01	0.07±0.01	**0.07±0.01**	0.06±0.01	0.06±0.01	**0.06±0.01**
P	0.03±0.00	0.03±0.00	**0.03±0.00**	0.03±0.00	0.03±0.00	**0.03±0.00**	0.03±0.00	0.03±0.00	**0.03±0.00**
TDN	6.71±0.72	7.27±0.15	**6.97±0.60**	6.84±0.76	7.43±0.22	**7.12±0.64**	6.86±0.81	7.40±0.16	**7.12±0.66**
NEm (Mcal/d)	13.5±1.73	14.6±0.34	**14.1±1.40**	14.0±1.94	15.2±0.76	**14.6±1.63**	14.1±1.90	15.2±0.35	**14.6±1.51**
NEg (Mcal/d)	6.52±1.24	7.07±0.39	**6.78±0.98**	6.95±1.47	7.64±0.75	**7.27±1.24**	7.11±1.28	7.65±0.29	**7.37±0.99**
ME (Mcal/d)	24.2±2.59	26.3±0.54	**25.2±2.17**	24.7±2.75	26.8±0.79	**25.7±2.32**	24.8±2.94	26.8±0.59	**25.7±2.38**
MP (kg/d)	0.68±0.05	0.72±0.02	**0.70±0.04**	0.71±0.06	0.76±0.01	**0.74±0.05**	0.72±0.07	0.77±0.02	**0.75±0.06**
Fatty acid*[3] *(g/d)									
Total	156±23.5	164±16.5	**160±20.9**	361±57.6	389±28.4	**374±48.2**	367±68.4	369±12.9	**368±50.5**
16:0	43.9±6.89	46.4±5.12	**45.1±6.24**	43.3±11.2	49.7±7.76	**46.3±10.3**	42.4±8.17	45.6±2.49	**43.9±6.38**
18:0	3.09±0.30	3.36±0.19	**3.21±0.29**	7.47±1.26	8.26±1.05	**7.84±1.23**	11.4±1.75	11.8±0.64	**11.6±1.35**
*c*9-18:1	27.0±5.11	26.4±2.91	**26.7±4.22**	171±24.0	182±15.6	**176±21.2**	70.1±14.1	70.6±2.67	**70.3±10.4**
*c*11-18:1	1.93±0.35	1.96±0.14	**1.94±0.27**	12.8±1.50	13.8±0.70	**13.3±1.28**	3.39±0.57	3.49±0.17	**3.43±0.43**
18:2n-6	56.9±8.69	61.2±6.82	**58.9±8.13**	81.2±15.7	87.4±4.57	**84.1±12.2**	83.0±18.8	84.7±3.37	**84.0±13.9**
18:3n-3	12.3±1.83	13.7±0.96	**13.0±1.63**	28.3±3.18	29.1±2.24	**28.7±2.80**	146±24.6	142±5.88	**144±18.4**
∑SFA	53.8±7.32	57.4±5.62	**55.5±6.80**	60.0±13.2	68.3±9.37	**63.9±12.2**	60.7±10.4	65.1±3.03	**62.8±8.14**
∑MUFA	32.3±6.04	31.8±3.35	**32.1±4.95**	191±26.6	201±16.9	**197±23.3**	77.0±15.4	77.7±2.77	**77.3±11.3**
∑PUFA	69.4±10.4	75.1±7.78	**72.1±9.68**	110±18.6	117±4.19	**113±14.2**	229±43.0	227±8.11	**228±31.8**

[1]**LF:** cows fed a low-fat diet; **CAN:** cows fed a high-fat diet including canola seed based pelleted feed; **FLX:** cows fed a high-fat diet including flaxseed based pelleted feed.
[2]**ME** = kg/d of TDN×4.409×0.82 (NRC, 2000). **MP:** calculated using the NASEM (2016) summative equation.
[3]∑**SFA**: sum of saturated fatty acids; ∑**MUFA**: sum of monounsaturated fatty acids; ∑**PUFA**: sum of polyunsaturated fatty acids.

4.4.3. Animal performance

Performance parameters are shown in Table 4.4. At the start of the trial, no difference ($P \geq 0.52$) was observed among treatments for remaining days until calving, with an average of 183 d for all treatments. Also, pregnant- and CC-IBW (accounting for fetal and gravid uterine weights) were not different ($P \geq 0.67$) among treatments. At the end of the second trimester, no effects of level ($P \geq 0.15$) and source ($P \geq 0.41$) of dietary fat were observed on pregnant-BW, CC-BW, ADG and CC-ADG of cows over the second trimester of gestation. However, by the end of the third trimester of gestation, LF cows tended ($P = 0.09$) to have greater cumulative ADG (0.59 vs. 0.55 kg/d) and greater ($P < 0.01$) cumulative CC-ADG (0.31 vs. 0.25 kg/d) than those of HF cows. Therefore, the CC-BW of cows fed the LF diet (708 kg) was 11 kg greater ($P = 0.04$) than that of HF (697 kg) cows. No differences ($P \geq 0.76$) were observed between CAN and FLX cows on cumulative ADG, cumulative CC-ADG, pregnant-BW and CC-BW at the end of the third trimester of gestation.

Table 4.4. Effects of level and source of fat in the diet of gestating beef cows on their prepartum performance and birth weight of the progeny.

	Treatments[1]				Contrasts[2]	
Item[3]	**LF**	**CAN**	**FLX**	**SEM**	**LF vs HF**	**CAN vs. FLX**
Start of trial						
Days until calving (d)	183	183	184	2.02	0.87	0.52
IBW (kg)	664	662	664	19.1	0.79	0.67
CC-IBW (kg)	659	657	658	19.0	0.72	0.69
BCS[4]	2.65	2.59	2.63	0.04	0.37	0.46
Thin (% of cows)	0.0	0.0	0.0	-	-	-
Optimal (% of cows)	98.2	97.8	93.3	2.87	0.44	0.10
Over conditioned (% of cows)	1.8	2.2	6.7	2.73	0.50	0.28
SCFT[5]						
Rib (mm)	4.8	4.1	4.4	0.20	0.03	0.34
Rump (mm)	4.7	4.9	4.5	0.30	0.99	0.32
Second trimester of gestation						
Days until calving (d)	99	99	100	2.02	0.87	0.52
FBW (kg)	711	707	706	5.90	0.37	0.84
ADG (kg/d)	0.57	0.55	0.51	0.17	0.27	0.45
CC-FBW (kg)	693	688	687	5.45	0.25	0.78
CC-ADG (kg/d)	0.41	0.38	0.34	0.18	0.15	0.41
Third trimester of gestation						
Days until calving (d)	23	23	24	1.49	0.87	0.51
FBW (kg)	758	751	752	4.44	0.19	0.87
Cumulative ADG (kg/d)	0.59	0.55	0.55	0.11	0.09	0.88
CC-FBW (kg)	708	697	697	4.07	0.04	0.98
Cumulative CC-ADG (kg/d)	0.31	0.25	0.24	0.12	< 0.01	0.76
BCS[4]	2.80	2.64	2.68	0.12	0.01	0.52
Change	0.13	0.05	0.04	0.12	0.12	0.96
Thin (% of cows)	0.0	0.0	0.0	-	-	-
Optimal (% of cows)	86.8	96.4	97.1	7.55	0.03	0.84
Over conditioned (% of cows)	13.2	3.6	2.9	7.55	0.03	0.84
SCFT[5]						
Rib (mm)	5.5	4.2	4.8	0.36	< 0.01	0.14
Change (mm)	0.7	0.1	0.4	0.39	0.15	0.48
Rump (mm)	5.7	5.0	5.5	0.32	0.25	0.26
Change (mm)	1.0	0.0	1.0	0.35	0.25	0.06
Birth weight						
All calves (kg)	40.2	42.4	43.3	1.08	< 0.01	0.24
Bull calves (kg)	41.4	44.6	45.2	1.08	< 0.01	0.68
Heifer calves (kg)	39.0	40.5	41.0	1.65	0.20	0.73

[1]**LF:** low-fat diet; **CAN:** high-fat diet including canola seed based pelleted feed; **FLX:** high-fat diet including flaxseed based pelleted feed; [2]**HF:** average of CAN and FLX.
[3]**CC-:** conceptus corrected (NRC, 2000).
[4]**Thin:** cows with BCS ≤ 2; **Optimal:** cows with 2.5 ≤ BCS ≤ 3; **Over conditioned:** cows with BCS ≥ 3.5.
[5]**SCFT:** subcutaneous fat thickness.

The increase in CC-BW of cows fed the LF diet was reflected in their SCAT accretion during the feeding period. Despite no differences ($P \geq 0.44$) between LF and HF cows at the start of the trial, the proportion of cows classified as over conditioned at the end of the trial was greater ($P = 0.03$) for those fed the LF diet (13.2%) with no difference ($P = 0.84$) between CAN (3.6%) and FLX (2.9%). This is consistent with findings reported by Alexander et al. (2002) who observed that beef cows offered a low-fat supplement during 59 d prepartum, had greater BCS at parturition compared to those offered high-fat supplements. Moreover, in the present study, the increased accretion of SCAT in LF cows was confirmed by ultrasound. At the start of the trial, the SCFT at the rib location of LF cows was 14% greater ($P = 0.02$) than that of HF cows (4.8 vs. 4.2 mm); and by the end of the trial, that difference increased to 21% with SCFT of cows fed LF diet remaining greater ($P < 0.01$) than that of HF cows (5.5 vs. 4.5 mm). This lower accumulation of SCAT contributed to the lighter empty BW of HF cows by the end of pregnancy; and was likely a result of differential partitioning of ME as influenced by the dietary source of energy. Although all treatments showed similar ME intakes over the course of the second and third trimesters of gestation, the portion of daily ME intake that was derived from fat was greater for the HF cows. This greater caloric intake derived from fat may have led to an increase in placental nutrient uptake which in turn could influence fetal growth. In a study using rodents, Jones et al. (2009) fed female mice either a high- or a low-fat (32 vs.11% fat) diet over gestation and collected the placental and fetal tissues 18 d after mating. The authors reported that the high-fat diet increased the transplacental transport of glucose and neutral amino acids, and this effect was associated with an increase in protein expression of glucose transporter 1 (GLUT1) and sodium-coupled neutral amino acid transporter 2 (SNAT2) in microvillous plasma membranes of isolated placentas from high-fat fed dams.

The birth weights of the progeny are shown in Table 4.4. Calves born to cows fed HF diets during gestation were 2.6 kg heavier ($P < 0.01$) at birth than those born to cows fed the LF diet (42.9 vs. 40.2 kg), with no difference ($P = 0.24$) between calves born to cows fed CAN (42.4 kg) and FLX (43.3 kg) diets. This difference in birth weight by feeding the dam different levels of fat over gestation has been reported in lambs (Radunz et al., 2011) and beef calves (Lammoglia et al., 1999b; Radunz et al., 2010). Feeding a high-fat diet over gestation has also resulted in heavier progeny at birth in rodents (Jones et al., 2009; Strakovsky et al., 2011). In the study by Jones et al. (2009), they reported a 43% increase in the weight of mice fetuses from

high-fat fed dams at day 18 of gestation. This increase in fetal weight was attributed to the increased placental uptake of nutrients due to feeding the high-fat diet as discussed previously. In the current study, it is likely that HF diets prepartum increased placental nutrient uptake which resulted in heavier calves at birth.

When birth weight data were analyzed separately for bull and heifer calves, sex-specific effects were observed. Bull calves born to cows fed HF diets were 3.4 kg heavier ($P < 0.01$) at birth than those from cows fed LF diet (44.9 vs. 41.4 kg), with no difference ($P = 0.68$) observed between bull calves born to cows fed CAN (44.6 kg) and FLX (45.2 kg) diets. On the other hand, no difference ($P \geq 0.20$) was observed among treatments on birth weight of heifer calves, with an average of 40.2 kg across treatments. Similar sex-specific effects on BW of the progeny have been reported by Micke et al. (2010) after beef heifers were fed a low or a high CP diet during early and mid-gestation. However, the reason for this sex-specific effect of feeding HF diets during gestation on fetal growth is not clear. Although the present study was not a nutrient restriction model, the possible lower amount of nutrients to which fetuses of LF cows were exposed due to restricted placental upake of nutrients (as explained previously), could help to explain this sex-specific effect on birth weight. Studies using rodents suggest that the placenta of female fetuses is capable of adjusting and become more efficient in nutrient transport in the presence of dietary changes (Penaloza et al., 2009; Rosenfeld, 2015). This diet dependent adaptation capacity of the placenta of female fetuses has been attributed to sexual dimorphism in placental DNA methylation (Gallou-Kabani et al., 2010; Mao et al., 2010; Gabory et al., 2012). After feeding pregnant mice with a high- or a low-fat (60 vs. 10% fat) diet over gestation and collecting the placental and fetal tissue at 15 d of gestation, Gallou-Kabani et al. (2010) found that the placenta from female fetuses were more efficient in terms of the fetal to placental weight ratio than those from male fetuses when the low-fat diet was fed. Such increased placenta efficiency could help explain the differential response of male and female calves in the present study to prepartum fat supplementation.

4.4.4. Fatty acid profile of subcutaneous adipose tissue

The fatty acid profiles of the SCAT are shown in Tables 4.5 and 4.6. At the start of the feeding period, no major differences were found with the only difference being the lower ($P = 0.04$) total

PUFA level of FLX (1.14 vs. 1.29%) compared to CAN and LF cows. However, at the end of the feeding period, the total PUFA proportion of FLX cows (2.23%) tended ($P = 0.09$) to be greater than those of CAN (1.76%) and LF (1.58%) cows. Also, the total proportion of n-3 in SCAT of FLX cows (0.58%) was greater ($P \leq 0.01$) than that of LF and CAN cows (0.38 and 0.37%, respectively). This is consistent with findings reported by He et al. (2012) where, compared to no flaxseed inclusion, a 15% (DM basis) inclusion of ground flaxseed in the diet of beef cows increased their total PUFA and n-3 fatty acid concentrations in subcutaneous adipose tissue. According to Kouba and Mourot (2011), including flaxseed in the diet of ruminants results in an increase in the n-3 fatty acid content in the animal product. The proportion of α-linolenic acid in SCAT of FLX cows (0.52%) was greater ($P < 0.01$) than that of LF and CAN cows (0.33 and 0.32%, respectively). This can be explained by a greater amount of α-linolenic acid by-passing the rumen in FLX cows, since it has been suggested that increasing the ruminal concentration of this fatty acid reduces its rate of biohydrogenation in the rumen (Beam et al., 2000; Vahmani et al., 2017). No differences ($P \geq 0.11$) were observed among treatments in the proportion of total and individual n-6 fatty acids. Also, feeding the FLX diet over gestation resulted in a greater ($P > 0.01$) total proportion of biohydrogenation intermediates such as conjugated linolenic acid (CLnA), conjugated linoleic acid (CLA), and atypical dienes (AD) in the SCAT of FLX cows. This greater proportion of CLnA, CLA, and AD in SCAT of FLX cows is most likely the result of these intermediates leaving the rumen before complete biohydrogenation of substrates such as α-linolenic acid (Shingfield et al., 2013; Vahmani et al., 2016b). Among the CLA isomers, *cis*9,*trans*11-/*trans7*,*cis9*-18:2 represented 90.0, 90.9, and 87.6% of the total CLA in SCAT of LF, CAN, and FLX, respectively. This is consistent with *cis*9,*trans*11-18:2 being the major isomer found in ruminant fat (Bauman et al., 2000).

The total proportions of MUFA, branched chain fatty acids (BCFA), and SFA in SCAT were not different ($P \geq 0.30$) among treatments at the start of the trial (Table 4.6). However, by the end of the third trimester of gestation, the total proportion of MUFA was greater ($P = 0.03$) in LF (57.5%) cows than in HF cows, and greater ($P < 0.01$) in CAN (56.7%) cows than that in FLX (53.5%) cows. When analyzing the MUFA fractions (*cis* and *trans*) separately, it was found that the proportions of all *trans*-MUFA isomers in SCAT of LF cows were lower ($P < 0.01$) than those of HF cows, with vaccenic acid (*trans11*-18:1) being the most abundant among all *t*-MUFA isomers representing 47.1, 41.9 and 49.1% for LF, CAN and FLX, respectively. It is known that

when ruminants are fed diets with a low concentrate to forage ratio, *trans11*-18:1 is the major *t*-MUFA isomer (Madron et al., 2002; Dugan et al., 2007). Conversely, the total proportion of *c*-MUFA and all respective isomers in SCAT of LF cows were greater ($P < 0.01$) or tended ($P \leq 0.09$) to be greater than those of HF cows. Oleic acid (*cis9*-18:1) proportions were 73.9, 75.7 and 77.8% for LF, CAN and FLX, respectively, and was the most abundant among all *c*-MUFA isomers. This is consistent with findings reported by Dugan et al. (2007) for SCAT of finished beef cattle with a 73% barley grain diet. Also, the total proportion of *c*-MUFA in SCAT of FLX cows (50.4%) was lower ($P < 0.01$) than that of CAN cows (54.0%). This lower proportion of *c*-MUFA in SCAT of FLX cows is most likely due to the increase in dietary PUFAs leading to a decrease in the rate of biohydrogenation or a decrease in Δ-9 desaturase activity in adipose tissue (Shingfield et al., 2013; Mapiye et al., 2014).

At the end of the third trimester of gestation, the total proportion of SFA in SCAT tended ($P = 0.05$) to be greater for FLX compared to CAN cows, while no difference ($P = 0.20$) was observed between LF and HF cows. Among the SFA, the proportion of palmitic acid (16:0) was greater ($P < 0.01$) for LF cows than HF cows. This could be attributed to the fact that proportion of 16:0 was greater in the LF diet, as well as to greater *de novo* fatty acid synthesis since 16:0 is the final product of this process (Shingfield et al., 2013). However, the proportion of stearic acid (18:0) was lower ($P < 0.01$) and those of myristic (14:0) and pentadecanoic (15:0) acids tended ($P = 0.08$) to be lower for LF cows compared to HF cows. The lower proportion of 18:0 in SCAT of LF could be attributed to two reasons. First, fewer amounts of dietary *cis9*-18:1 and 18:3n-3 going through complete biohydrogenation since the proportions of these fatty acids were lower in LF diet compared to HF diets. Also, the lower 18:0 and greater of *cis9*-18:1 proportion in SCAT of LF cows could be the result of a greater Δ-9 desaturase activity at the tissue level as suggested by Mapiye et al. (2014). Also, the fact that *de novo* fatty acid synthesis was probably greater in LF cows (as discussed previously) supports the hypothesis of a greater Δ-9 desaturase activity in adipose tissue of this group of cows. According to Smith et al. (2006), desaturase gene expression is highly expressed during *de novo* fatty acid synthesis.

Table 4.5. Effects of level and source of fat in the diet of gestating beef cows on polyunsaturated fatty acid profiles in subcutaneous adipose tissue.

	Treatments[1]				Contrasts[2]	
Fatty acid (% of total)	**LF**	**CAN**	**FLX**	**SEM**	**LF vs. HF**	**CAN vs. FLX**
Start of trial						
∑PUFA	1.29	1.29	1.14	0.08	0.16	0.04
∑n-3	0.50	0.49	0.44	0.07	0.39	0.16
18:3n-3	0.44	0.43	0.39	0.06	0.37	0.34
22:5n-3	0.04	0.04	0.03	0.01	0.81	0.07
∑n-6	0.80	0.79	0.70	0.04	0.26	0.09
18:2n-6	0.73	0.72	0.63	0.04	0.19	0.08
20:4n-6	0.03	0.03	0.03	0.01	0.87	0.93
∑CLnA	0.06	0.08	0.06	0.01	0.16	0.16
*c*9,*t*11,*t*15-18:3	0.05	0.06	0.05	0.01	0.29	0.45
*c*9,*t*11,*c*15-18:3	0.01	0.02	0.01	0.00	0.21	0.07
∑CLA	0.94	0.99	0.89	0.16	0.99	0.46
*c*9,*t*11- + *t*7,*c*9-18:2	0.84	0.88	0.79	0.15	0.91	0.47
*t*11,*c*13-18:2	0.04	0.04	0.04	0.01	0.63	0.78
t,*t*-CLA	0.06	0.06	0.06	0.01	0.64	0.23
∑AD	0.55	0.61	0.55	0.09	0.76	0.52
*c*9,*t*14- + *c*9,*t*13-18:2	0.21	0.24	0.20	0.05	0.84	0.40
*c*9,*t*15-18:2	0.07	0.09	0.07	0.03	0.68	0.30
*t*11,*c*15-18:2	0.22	0.23	0.23	0.03	0.82	0.91
End of 3rd trimester of gestation						
∑PUFA	1.58	1.76	2.23	0.63	0.09	< 0.10
∑n-3	0.38	0.37	0.58	0.03	0.01	< 0.01
18:3n-3	0.33	0.32	0.52	0.02	< 0.01	< 0.01
22:5n-3	0.05	0.05	0.06	0.01	0.35	0.13
∑n-6	1.20	1.39	1.65	0.60	0.13	0.28
18:2n-6	1.10	1.29	1.53	0.56	0.11	0.26
20:4n-6	0.05	0.07	0.08	0.04	0.68	0.69
∑CLnA	0.05	0.05	0.12	0.02	< 0.01	< 0.01
*c*9,*t*11,*t*15-18:3	0.03	0.03	0.06	0.01	< 0.01	< 0.01
*c*9,*t*11,*c*15-18:3	0.02	0.02	0.06	0.01	< 0.01	< 0.01
∑CLA	0.70	0.88	1.05	0.22	< 0.01	0.09
*c*9,*t*11- + *t*7,*c*9-18:2	0.63	0.80	0.92	0.19	< 0.01	0.20
*t*11,*c*13-18:2	0.03	0.03	0.07	0.01	0.01	< 0.01
t,*t*-CLA	0.04	0.05	0.07	0.02	< 0.01	0.01
∑AD	0.40	0.57	0.90	0.22	< 0.01	< 0.01
*c*9,*t*14- + *c*9,*t*13-18:2	0.16	0.25	0.34	0.08	< 0.01	0.05
*c*9,*t*15-18:2	0.08	0.11	0.15	0.04	< 0.01	< 0.01
*t*11,*c*15-18:2	0.12	0.12	0.32	0.08	< 0.01	< 0.01

[1]**LF:** low-fat diet; **CAN:** high-fat diet including canola seed based pelleted feed; **FLX:** high-fat diet including flaxseed based pelleted feed; [2]**HF:** average of CAN and FLX.

c = cis; ***t*** = trans; **ΣPUFA** = sum of polyunsaturated fatty acids (Σn-6 +Σn-3); **Σn-3** = sum of n-3 fatty acids; **Σn-6** = sum of n-6 fatty acids; **ΣCLnA** = sum of conjugated linolenic acids; **ΣAD** = sum of atypical dienes; **ΣCLA** = sum of conjugated linoleic acids.

Table 4.6. Effects of level and source of fat in the diet of gestating beef cows on the monounsaturated and saturated fatty acid profiles in subcutaneous adipose tissue.

	Treatments[1]				Contrasts[2]	
Fatty acid (% of total)	**LF**	**CAN**	**FLX**	**SEM**	**LF vs. HF**	**CAN vs. FLX**
Start of trial						
∑MUFA	53.9	52.7	52.0	2.63	0.30	0.68
∑*t*-MUFA	2.37	2.73	2.64	0.27	0.31	0.79
*t*9-18:1	0.26	0.27	0.25	0.05	0.97	0.55
*t*10-18:1	0.20	0.21	0.19	0.03	0.96	0.48
*t*11-18:1	1.24	1.46	1.45	0.14	0.23	0.96
*t*13- + *t*14-18:1	0.28	0.36	0.33	0.04	0.14	0.57
∑*c*-MUFA	51.6	50.0	49.3	2.50	0.25	0.74
*c*9-14:1	1.23	1.10	1.42	0.10	0.81	0.05
*c*9-16:1	5.84	4.99	4.91	0.46	0.10	0.88
*c*9-17:1	1.13	1.03	1.07	0.09	0.24	0.61
*c*9-18:1	40.0	39.7	39.1	1.79	0.58	0.60
*c*11-18:1	1.58	1.39	1.24	0.12	0.06	0.34
∑BCFA	3.18	3.27	3.25	0.15	0.63	0.94
∑SFA	40.0	41.1	42.1	2.84	0.32	0.56
14:0	3.27	2.96	3.36	0.39	0.64	0.15
15:0	0.58	0.56	0.61	0.03	0.82	0.21
16:0	24.8	24.9	25.1	1.27	0.85	0.78
17:0	1.11	1.15	1.25	0.09	0.21	0.19
18:0	9.91	11.2	11.5	1.19	0.15	0.77
End of 3rd trimester of gestation						
∑MUFA	57.5	56.7	53.5	1.59	0.03	< 0.01
∑*t*-MUFA	1.53	2.72	3.16	1.03	< 0.01	0.14
*t*9-18:1	0.22	0.36	0.29	0.06	< 0.01	0.01
*t*10-18:1	0.14	0.28	0.25	0.07	< 0.01	0.36
*t*11-18:1	0.72	1.14	1.55	0.49	< 0.01	< 0.01
*t*13- + *t*14-18:1	0.20	0.34	0.45	0.17	< 0.01	0.22
∑*c*-MUFA	55.9	54.0	50.4	2.56	< 0.01	< 0.01
*c*9-14:1	1.66	1.58	1.22	0.12	0.08	0.04
*c*9-16:1	7.79	6.89	5.63	1.13	< 0.01	0.03
*c*9-17:1	1.04	0.90	0.86	0.18	< 0.01	0.39
*c*9-18:1	41.3	40.9	39.2	1.04	0.09	0.05
*c*11-18:1	2.30	1.95	1.70	0.58	< 0.01	0.03
∑BCFA	2.58	2.66	2.82	0.89	0.16	0.22
∑SFA	37.2	37.3	39.4	0.92	0.20	0.05
14:0	2.74	3.10	3.37	0.21	0.06	0.39
15:0	0.48	0.52	0.55	0.17	0.04	0.37
16:0	26.0	24.0	23.9	1.54	< 0.01	0.77
17:0	0.83	0.80	0.87	0.23	0.95	0.03
18:0	6.97	8.61	9.93	1.97	< 0.01	0.07

[1]**LF:** low-fat diet; **CAN:** high-fat diet including canola seed based pelleted feed; **FLX:** high-fat diet including flaxseed based pelleted feed. [2]**HF:** average of CAN and FLX.

c = cis; ***t*** = trans; **ΣMUFA** = sum of monounsaturated fatty acids; **Σ*c*-MUFA** = sum of *cis*-monounsaturated fatty acids; **Σ*t*-MUFA** = sum of *trans*-18:1 isomers; **ΣBCFA** = sum of branched chain fatty acids; **ΣSFA** =sum of saturated fatty acids.

4.4.5. Blood metabolites

The serum NEFA and BHBA concentrations of cows are shown in Table 4.7. No differences ($P \geq 0.35$) were observed among treatments for serum NEFA or BHBA concentrations of cows at the start of the trial. By the end of the third trimester of gestation, the serum NEFA concentration of cows fed HF diets was 206 µEq/L greater ($P < 0.01$) compared to those fed LF (592 µEq/L). This greater serum NEFA concentration in HF cows can be attributed to a greater adipose tissue mobilization at the time of sampling as evidenced by their lower SCAT accretion compared to LF cows (as discussed previously), and their change in CC-BW during the previous 14-d. Conceptus-corrected BW of cows at the time of blood sample collection (23 d prepartum) and those recorded 14-d previously (data not shown) indicated a loss in CC-BW across all treatments. Statistical comparison of the change in CC-BW occurring during this period showed that HF cows had greater ($P < 0.01$; SEM = 1.67) loss in CC-BW than LF cows (-7.0 vs. -2.1 kg), and the loss in CC-BW of FLX cows tended ($P = 0.07$; SEM = 1.93) to be greater than that of CAN cows (-8.8 vs. -5.2 kg). Also, the fact that HF cows were gestating heavier calves also helps to explain their increased serum NEFA levels compared to LF cows. Reid and Hinks (1962) found that plasma NEFA concentration of late pregnancy ewes was positively and highly correlated with total fetal weight. Finally, the greater fat content in HF diet could have increased the serum NEFA concentration in these cows. Fat inclusion in the diet of dry dairy cows during prepartum has been shown to increase their serum NEFA concentration (Leroy et al., 2014). The reason for this increase in NEFA due to dietary fat inclusion has been attributed to an incomplete uptake of free fatty acids by adipose tissue (Grummer and Carroll, 1991; Chilliard, 1993).

Serum NEFA concentration of cows fed FLX (961 µEq/L) was greater ($P < 0.01$) than those fed CAN (636 µEq/L). In general, it has been suggested that high PUFA diets increase blood NEFA concentration in ruminants (Bowden, 1971; Chilliard, 1993). However, in the present study, the greater serum NEFA concentration of FLX compared to CAN cows was most likely due to a greater adipose tissue mobilization as evidenced by the greater loss in CC-BW previously mentioned. The greater BW loss of FLX compared to CAN cows can be attributed to a reduced short-chain fatty acid production as a result of a decrease in ruminal fermentation. It is well documented that the addition of fat to the diets of ruminants negatively affects the fermentation of structural carbohydrates causing a reduction in short-chain fatty acid production

(Jenkins, 1993). This negative effect on fibre fermentation increases with the degree of fatty acid unsaturation (Jenkins et al., 2008; Buccioni et al., 2012). Also, a greater placental uptake of PUFA could have increased the demand for adipose tissue mobilization; hence, increasing NEFA circulation in FLX cows. Along with triglycerides, circulating NEFA are the main source of fatty acid uptake by the placenta (Lager and Powell, 2012). Moreover, fetal requirements for linoleic and α-linolenic acid and their associated long-chained PUFA increase by the end of gestation, and both placental and fetal tissue have been shown to have a preferential uptake of these fatty acids by the end of gestation (Herrera, 2002; Jones et al., 2007). Therefore, the fatty acids mobilized by FLX cows in the form of NEFA by the end of gestation were most probably PUFA and α-linolenic acid, since the total proportion of these were greater in the SCAT of FLX. No differences ($P \geq 0.13$) were observed among treatments on serum BHBA concentration by the end of the third trimester of gestation.

Table 4.7. Effects of level and source of fat in the diet of gestating beef cows on concentration of blood serum metabolites.

	Treatment[1]				Contrasts[2]	
Item	**LF**	**CAN**	**FLX**	**SEM**	**LF vs HF**	**CAN vs FLX**
At start of trial						
Days until calving (d)	183	182	192	4.67	0.47	0.15
NEFA (µEq/L)	581	601	574	157	0.92	0.72
BHBA (mg/dL)	13.7	12.6	11.4	1.78	0.35	0.55
At end of trial						
Days until calving (d)	22	22	23	2.80	0.78	0.94
NEFA (µEq/L)	592	636	961	56.9	< 0.01	< 0.01
BHBA (mg/dL)	9.75	10.2	8.96	0.96	0.78	0.13

[1]**LF:** low-fat diet; **CAN:** high-fat diet including canola seed based pelleted feed; **FLX:** high-fat diet including flaxseed based pelleted feed: [2]**HF:** average of CAN and FLX.

4.5. Conclusions

Feeding a low-fat diet to beef cows during gestation resulted in an increase in BCS and subcutaneous fat thickness. Moreover, when the BW of cows was corrected for fetal and gravid uterine weight, cows fed the low-fat diet were heavier than those fed the high-fat diets, and the extra weight gain was in part a result of subcutaneous fat accretion. In contrast, feeding high-fat diets during gestation resulted in leaner cows and heavier calves at birth. Also, the type of dietary fatty acid during gestation influenced the fatty acid profile in subcutaneous adipose tissue and NEFA concentration in blood serum of cows. Dams receiving a diet high in PUFAs during gestation, showed a greater proportion of CLA, CLnA, and 18:3n-3 fatty acids in their subcutaneous adipose tissue, and greater level of serum NEFAs by the end of gestation.

In conclusion, these results suggest that ME partitioning in gestating beef cows is influenced by level and source of dietary fat. Also, these results suggest that a high-fat diet over gestation increases the placental nutrient uptake, resulting in heavier calves at birth.

5. EFFECTS OF LEVEL AND SOURCE OF FAT IN THE DIET OF GESTATING BEEF COWS ON THE POSTPARTUM PERFORMANCE OF THE DAM AND THE PROGENY

5.1. Abstract

A two-year study was conducted to evaluate the effects of level and source of fat in the diet of gestating beef cows on the postpartum performance of the dam and the progeny, as well as the relative expression of growth, myogenic, and adipogenic genes in the *longissimus dorsi* (LD) muscle of male calves at birth and weaning. Each year, 75 mature pregnant (183±4.8 d until calving) Angus cows with similar BW (663±21.5 kg) and BCS (2.6±0.12) were randomly assigned to one of 15 outdoor pens, and each pen was then randomly assigned to receive one of three iso-caloric and iso-nitrogenous diets: a low-fat diet (LF; 1.4±0.12% EE), and two high-fat diets (HF; 3.3±0.20% EE) including a canola seed (CAN) or a flaxseed (FLX) based pelleted feed. Diets were formulated to meet the requirements of pregnant beef cows and fed until calving. Data were analyzed as a randomized complete block design with contrasts for the effects of level (LF vs. HF) and source (CAN vs. FLX) of fat. No differences ($P \geq 0.21$) were found for BW or calving to weaning ADG of cows. The average BCS during the first 42 d of lactation was greater ($P < 0.01$) for LF compared to HF (2.63 vs. 2.51) with no difference ($P = 0.35$) for CAN vs. FLX cows. Subcutaneous fat thickness over the ribs was greater ($P \leq 0.01$) for LF compared to that of HF cows at calving (5.7 vs. 4.3 mm) and at weaning (4.3 vs. 3.7 mm) with no difference ($P \geq 0.11$) for CAN vs. FLX cows. Over the first 42 d of lactation, no difference ($P \geq 0.23$) was observed for 12-h milk yield. However, milk protein concentration was greater ($P = 0.03$) for CAN compared to FLX (3.11 vs. 3.01%) cows while no difference ($P \geq 0.28$) was observed for any other milk component. Milk fat from FLX cows had greater ($P < 0.01$) CLA and CLnA concentrations than that of CAN cows during the first 42 d of lactation. Pregnancy rate, but not calving rate, of HF cows (95.4%) tended ($P = 0.07$) to be greater than that of LF cows with no difference ($P = 0.77$) for

CAN vs. FLX cows. Calves from HF cows were heavier ($P \leq 0.01$) at birth (42.9 vs. 40.2 kg) than those from LF cows. From calving to weaning, ADG of calves born to CAN cows was greater ($P=0.03$) that that of calves born to FLX cows (1.19 vs. 1.13 kg/d) with no difference ($P=0.18$) for calves born to LF vs. HF cows. At slaughter, progeny of HF cows had greater ($P \leq 0.03$) shrunk BW (605 vs. 579 kg) and HCW (355 vs. 339 kg) compared to those from LF cows with no difference ($P \geq 0.16$) for progeny of CAN vs. FLX cows. There was no effect ($P > 0.05$) of level or source of fat during gestation on the relative expression of the targeted genes in the LD muscle of male calves. These results show that feeding a HF diet over gestation results in heavier calves at birth and at slaughter, and superior calf gains from birth to slaughter as well as heavier carcasses, possibly due to a developmental programming effect.

5.2. Introduction

Reproductive performance of cows and birth to weaning performance of their progeny have been shown to be influenced by nutrition of the dam during gestation (Hess et al., 2005; Funston et al., 2010). In the Great Plains of North America, the energy requirement for maintenance of gestating beef cows increases as they are often exposed to temperatures below their thermo-neutral zone (NRC, 2000). Therefore, providing dietary energy to pregnant beef cows in an efficient manner is a major nutritional goal in cow-calf operations to increase performance of both the dam and progeny.

In ruminants, fat inclusion up to 6% of the total DMI increases the energy density of high-forage diets without negative effects on animal performance (Palmquist, 1994; Hess et al., 2008). Also, compared to diets with similar energy content, feeding high-fat diets during late gestation has been shown to improve pregnancy rates in beef cows (Bellows et al., 2001; Graham et al., 2001). Moreover, feeding a high-fat diet during gestation not only has a positive effect on performance of beef cows, but can also improve the performance of the offspring including heavier calves at birth (Chapter 4), reduced calf mortality at birth (Lammoglia et al., 1999b), and from birth to weaning (Petit and Berthiaume, 2006).

Recent research has shown that the degree of improvement observed by increasing the level of fat in the diet of beef cows may depend on the type of fatty acids in the diet. For example, relative to diets high in saturated fatty acids (SFA), feeding diets high in polyunsaturated fatty acids (PUFA) to beef cows has resulted in greater pregnancy rates (Petit and Berthiaume, 2006; Lopes et al., 2009). Birth to weaning performance of the calf has also been improved by feeding diets high in PUFA to the dam over gestation, and to the calf during early life. For example, Garcia et al. (2014 and 2015) increased the concentration of linoleic (LA) and α-linolenic (ALA) acid in the diet of dairy cows over late gestation and in milk replacers fed to their calves for 30 d after calving. These authors reported improvements in calves' birth to weaning ADG, immune response, and overall health by increasing the LA and ALA content in the diets of both the gestating cows and young calves.

Overall, feeding high-fat diets during gestation has been shown to benefit the reproductive performance of beef cows and the birth to weaning performance of the progeny. Moreover, the magnitude of improvement appears to depend on the degree of saturation of fatty acids included in the diet. However, few research studies have been conducted to evaluate the effects of level and source of fat fed to beef cows during gestation on the weaning to slaughter performance of their progeny. Therefore, the objective of this study was to evaluate the effects of level and source (monounsaturated vs. PUFA) of fat in the diet of gestating beef cows on their postpartum performance and on the birth to slaughter performance of their progeny.

5.3. Materials & Methods

All animals were obtained from the main herd of the Western Beef Development Centre's research ranch and cared for in accordance with the Canadian Council of Animal Care guidelines (CCAC, 2009). All experimental procedures were approved by University of Saskatchewan Animal Care Committee (Protocol No. 20090107).

5.3.1. Location

A two-year study (2015 to2016 and 2016 to2017 for year 1 and 2, respectively) was conducted at the Termuende Research Ranch of the Western Beef Development Centre (WBDC) near Lanigan (51°51'N, 105°02'W), Saskatchewan, Canada, and at the University of Saskatchewan Beef Cattle Research and Teaching Unit (BCRTU) located in Saskatoon (52°09'N, 106°36'W), Saskatchewan, Canada.

5.3.2. Treatments

Treatments consisted of three diets differing in level and source of fat that cows received during the last two trimesters of gestation. Details have been presented in Chapter 4. Briefly, on October 24th 2014 and October 23rd 2015 (for year 1 and 2, respectively), 75 multiparous (≥3 calving) pregnant Angus cows were stratified by initial BW (662±52.4 kg), and BCS and divided into 15 homogenous groups (5 cows/group). Each group was randomly assigned to 1 of 15 outdoor research pens. Subsequently, each pen was randomly assigned to 1 of 3 replicated (n=5) dietary treatments which consisted of: a low-fat (LF) diet (1.4 ± 0.12% EE), and two high-fat (HF) diets (3.3 ± 0.20% EE). Mixed hay consisting of bromegrass (*Bromus* sp.) and alfalfa (*Medicago sativa* L.), barley straw, rolled barley grain, and two high-fat pelleted feeds were used as ingredients to formulate the treatment diets (Table 5.1). The two high-fat pellets were formulated using canola seed (CAN) as an enriched source of mono-unsaturated fatty acids (MUFA), or flaxseed (FLX) as an enriched source of PUFA. High-fat diets (CAN and FLX) were formulated to provide 300 g of fat/cow/d from pelleted feeds. Amounts fed were such that each pen received equal amounts of DE (2.42 ± 0.11 Mcal/kg), CP (10.5 ± 0.4%), and total DM (12.9 ± 1.0

kg/cow/d). Diets were formulated to meet the DE and CP intake requirements of pregnant beef cows over the second and third trimesters of gestation for a projected 40 kg calf at birth according to NRC (2000). The amount fed was adjusted every two weeks according to estimated day of gestation, weight gain, and changes in weather conditions. Diets were offered once daily as total mixed rations (TMR) using a mixer wagon with feeding starting at 0800 h. Bunks were cleaned every two weeks due to accumulation of orts if needed. The same cows were used each year unless culled for injury or failure to conceive, in which case, similar replacements were obtained from the same herd. Each year, treatment diets were fed from the start of the second trimester of gestation until calving (183 ± 4.8 d).

Table 5.1. Dry matter intake and composition of treatment diets fed to beef cows over gestation.

	Treatments[1]		
Item	**LF**	**CAN**	**FLX**
DMI (kg/cow/d)			
Total	12.9±1.05	12.9±0.98	12.8±1.11
Ingredient (% DM)			
Hay	35.0±2.84	28.1±2.22	29.0±2.73
Barley straw	37.3±5.34	34.9±7.17	33.3±4.86
Barley grain	27.8±5.94	8.23±3.98	3.08±3.19
CAN pellet	-	28.8±3.71	-
FLX pellet	-	-	34.6±6.15
Nutrient[2] (% DM)			
CP	10.3±0.50	10.6±0.37	10.7±0.42
ADF	39.4±2.31	39.2±3.44	38.8±2.46
NDF	58.5±1.93	58.4±3.88	57.0±3.85
EE	1.40±0.12	3.31±0.16	3.27±0.23
Ca	0.45±0.06	0.52±0.06	0.46±0.09
P	0.25±0.02	0.25±0.01	0.24±0.01
TDN	54.0±2.26	55.1±2.90	55.4±2.13
NEm (Mcal/d)	1.09±0.08	1.13±0.10	1.14±0.07
NEg (Mcal/d)	0.53±0.07	0.56±0.09	0.57±0.07
Fatty acid[3] (% of total)			
16:0	28.2±0.70	12.3±1.36	11.9±0.63
18:0	2.03±0.16	2.09±0.10	3.16±0.13
*c*9-18:1	16.7±0.91	47.2±1.21	19.1±0.37
*c*11-18:1	1.22±0.05	3.57±0.19	0.93±0.03
18:2n-6	36.9±0.70	22.5±0.85	22.7±0.98
18:3n-3	8.13±0.50	7.76±0.83	39.2±1.34
∑SFA	34.8±0.81	17.0±1.34	17.1±0.84
∑MUFA	20.1±0.99	52.7±1.32	21.0±0.33
∑PUFA	45.1±1.02	30.3±1.20	61.9±0.74

[1]**LF:** cows fed a low-fat diet; **CAN:** cows fed a high-fat diet including canola seed based pelleted feed; **FLX:** cows fed a high-fat diet including flaxseed based pelleted feed.
[2]**TDN:** calculated using the Pennsylvania-State equations (Adams, 1980). **NEm and NEg**: calculated using the NRC (2000) summative equation.
[3]∑**SFA**: sum of saturated fatty acids; ∑**MUFA**: sum of monounsaturated fatty acids; ∑**PUFA**: sum of polyunsaturated fatty acids.

5.3.3. Housing, Handling and Feeding

All cow-calf pairs were managed in a single group and received equal management from calving until weaning. Within the first 48 h after birth, all calves were ear tagged, received injections (0.5 cc) of vitamin AD (Vitamin AD3 Forte®, Rafter 8 Products, Calgary, AB, Canada) and vitamin E plus selenium (Selon E® Injection, Vetoquinol Canada Inc., Lavaltrie, QC, Canada). Bull-calves were castrated within 48 h after birth using rubber bands. As cows calved, cow-calf pairs were moved to a common pen (120 × 77 m) equipped with water bowls and portable feed bunks. During the time spent in the common pen (53 ± 14 d postpartum), mixed hay (9.9 ± 0.4% CP, 51.1 ± 1.5% ADF and 69.1 ± 0.5% NDF) comprised of smooth bromegrass (*Bromus inermis* L.), hybrid bromegrass (*B. inermis* × *B. riparius*), and alfalfa (*Medicago sativa* L.) was offered free choice, and cows were supplemented with 1.8 kg/cow/d of rolled barley grain (12.9 ± 1.3% CP, 13.5 ± 2.0% ADF and 25.9 ± 3.3% NDF).

By mid June of each year, all cows and calves were vaccinated with Vista Once SQ® (Merck Animal Health, Kirkland, QC, Canada), Vision 7® with Spur (Merck Animal Health, Kirkland, QC, Canada), and Anthrax Spore Vaccine® (Colorado Serum Company, Denver, CO, USA). As well, all calves were implanted with Ralgro® (Merck Animal Health, Kirkland, QC, Canada). Subsequently, cow-calf pairs were moved to cool-season pastures until weaning where cows grazed a mixture of smooth bromegrass (*Bromus inermis* L.), red fescue (*Festuca rubra* L.), and Kentucky bluegrass (*Poa pratensis* L.) pastures until weaning. During this period, cows were exposed to a 63-d breeding season (25:1 cow to bull ratio) starting on July 2nd and July 5th for year 1 and 2, respectively. In both years, half sibling, registered Angus bulls were used as sires after passing a breeding soundness evaluation. All animals had *ad libitum* access to a 2:1 mineral [15.5% Ca, 7% P, 30 ppm Se, 20 ppm Co, 200 ppm I, 1500 ppm Cu, 5000 ppm Mn, 5000 ppm Zn, 1000 ppm Fe, 1.0 ppm F (max), 500 000 IU/kg vitamin A (min), 50000 IU/kg vitamin D (min), 2500 IU/kg vitamin E (min); Cargill Animal Nutrition, Manitoba, Canada] and cobalt-iodized salt [99.0% NaCl (min), 39.0% Na, 150 ppm I, 100 ppm Co; FeedRite Ltd., Humboldt, Saskatchewan, Canada] at all times.

5.3.3.1. Backgrounding

On October 23rd 2015 and October 24th 2016 (for year 1 and 2, respectively), all calves were weaned, separated in two groups according to sex, and managed similarly until slaughter. At weaning, all calves were revaccinated with Vista Once SQ® (Merck Animal Health, Kirkland, QC, Canada) and Vision 7® (Merck Animal Health, Kirkland, QC, Canada). Immediately after weaning, calves were housed (according to sex) in two large outdoor pens (90 × 43 m) equipped with portable feeding troughs and water bowls; and backgrounded over the course of the fall-winter (143 ± 1.0 d) at the WBDC research ranch. During this period, calves had free choice access to mixed hay (12.3 ± 3.0% CP, 41.0 ± 1.9% ADF and 59.0 ± 1.7% NDF) comprised of smooth bromegrass (*Bromus inermis* L.), hybrid bromegrass (*B. inermis* × *B. riparius*), and alfalfa (*Medicago sativa* L.), and were supplemented with 1 kg/hd/d of a commercial (Blair's Crop & Livestock Solutions, Nokomis, SK, Canada) pelleted feed (15.7 ± 0.4% CP, 12.5 ± 1.3% ADF and 29.7 ± 0.9% NDF). Calves had *ad libitum* access to a 1:1 mineral [11.5% Ca, 10% P, 20 ppm Co, 200 ppm I, 2000 ppm Cu, 5000 ppm Mg, 5000 ppm Mn, 5000 ppm Zn, 4900 ppm Fe, 50 ppm F (max), 500 000 IU/kg vitamin A (min), 50000 IU/kg vitamin D (min), 2500 IU/kg vitamin E (min); Cargill Animal Nutrition, Manitoba, Canada] and cobalt-iodized salt [99.0% NaCl (min), 39.0% Na, 150 ppm I, 100 ppm Co; FeedRite Ltd., Humboldt, Saskatchewan, Canada] at all times. On March 14th 2016 and March 15th 2017 (for year 1 and 2, respectively), all calves were moved to the University of Saskatchewan BCRTU where they remained separated according to sex and housed in four outdoor pens (12 × 24 m) with 17 ± 2.3 hd/pen. Upon arrival to the BCRTU, all calves were vaccinated with Ultrabac 7/Somubac® (Zoetis Canada Inc., Kirkland, QC, Canada), Bovi-Shield GOLD® One Shot (Zoetis Canada Inc., Kirkland, QC, Canada), treated for external and internal parasites with Bimectin® Pour-On (Bimedia-MTC Animal Health Inc., Cambridge, ON, Canada), and implanted with Ralgro® (Merck Animal Health, Kirkland, QC, Canada). During the first 37 and 44 d (for year 1 and 2, respectively) at the BCRTU, calves continued to receive a high-forage backgrounding diet consisting of (DM basis) 52.2 ± 3.3% barley silage, 34.5 ± 3.6% rolled barley grain, 7.7 ± 0.7% canola meal, and 5.6 ± 0.4% mineral and vitamin supplement (9.0% CP, 9.2% Ca, 0.32% P, 1.6% Na, 0.28% Mg, 0.60% K, 0.12% S; 4.9 ppm Co, 185 ppm Cu, 16.6 ppm I, 84 ppm Fe, 500 mg Mn, 2.0 ppm Se, 558 ppm Zn, 550 ppm monensin; 40,000 IU vitamin A, 5,000 IU vitamin D, and 600 IU vitamin E per kg supplement), and formulated to provide (DM basis) 13.5 ± 0.5% CP, 1.56 ± 0.14 Mcal/kg NEm,

and 0.96 ± 0.13 Mcal/kg NEg. This high-forage diet was offered *ad libitum* (5% carry over) as a TMR with feeding occurring once daily during the morning. The targeted end point of the backgrounding program was 400 kg of average shrunk BW.

5.3.3.2. Finishing

Following the backgrounding phase, calves were transitioned over 16 d to a high-grain finishing diet using a five-step adaptation program. During the adaptation period, the diet composition was changed every 4 d in such a way that the barley silage and canola meal content in the diet were gradually decreased as barley grain was increased to formulated levels in the finishing diet. The finishing diet consisted of (DM basis) 10.8 ± 0.1% barley silage, 84.1 ± 0.1% rolled barley grain, and 5.1 ± 0.0% mineral and vitamin supplement (9.0% CP, 9.2% Ca, 0.32% P, 1.6% Na, 0.28% Mg, 0.60% K, 0.12% S; 4.9 ppm Co, 185 ppm Cu, 16.6 ppm I, 84 ppm Fe, 500 mg Mn, 2.0 ppm Se, 558 ppm Zn, 550 ppm monensin; 40,000 IU vitamin A, 5,000 IU vitamin D, and 600 IU vitamin E per kg supplement), and was formulated to provide 11.8 ± 0.4% CP, 1.85 ± 0.03 Mcal/kg NEm, and 1.22 ± 0.02 Mcal/kg NEg. This diet was fed over 76 and 87 d (for year 1 and 2, respectively) and offered *ad libitum* (5% carry over) as a TMR with feeding occurring once daily during the morning. Calves were re-implanted at 74 ± 5.7 d before slaughter with Revalor®-S (Merck Animal Health, Kirkland, QC, Canada) for steer calves and Revalor®-H (Merck Animal Health, Kirkland, QC, Canada) for heifer calves. The targeted end point of finishing was 595 kg of average shrunk BW.

5.3.4. Data collection

5.3.4.1. Feeds

All feed ingredients were sampled every two weeks. Hay, rolled barley grain and canola meal samples were dried in an air forced oven at 55°C for 48 h, whereas barley silage samples were dried for 72 h. Dried samples were ground to pass 1 mm screen (Thomas-Wiley Laboratory Mill Model 4; Thomas Scientific, Swedesboro, NJ) and stored at -20°C until analysis.

5.3.4.2. Body weight

Each cow was weighed over two consecutive days at calving (within 48 h after calving) and weaning. As well, cows used for partial milk yield estimation were weighed over 2 consecutive days at 21 and 42 d postpartum. Birth weight was recorded for all calves within the first 24 h after birth. Calves from cows used for partial milk yield estimation were weighed at 21 and 42 d of age after separation from the dam for 12 h. At weaning, as well as at the end of the backgrounding and finishing phases, all calves were weighed on two consecutive days. All calves were weighed once monthly throughout the backgrounding phase and every two weeks throughout the finishing phase. The BW of cows al calves at weaning (WW) were adjusted to 180 d, and the BW of calves at the end of backgrounding were adjusted to 365 d as follows:

$$\text{Cows' 180 d adjusted WW} = \text{Calving BW} + 180 \times (\text{calving to weaning ADG})$$

$$\text{Calves' 180 d adjusted WW} = \text{Birth weight} + 180 \times (\text{birth to weaning ADG})$$

$$\text{Calves' 365 d adjusted BW} = \text{Birth weight} + 365 \times (\text{birth to end of backgrounding ADG})$$

5.3.4.3. Body Condition Scoring and Subcutaneous Fat Thickness

Body condition score of each cow was determined by the same experienced technician at calving, 21 and 42 d postpartum, and at weaning using the Scottish scale where 1 = emaciated and 5 = grossly fat (Lowman et al., 1976; Wildman et al., 1982). Ultrasound measurements of subcutaneous fat thickness (SCFT) over the third quarter of the rib eye muscle, between the 12^{th} and 13^{th} rib, and at the thurl location on the rump area were determined on each cow at weaning using an Aloka SSD-500V ultrasound machine and an Aloka UST-5044 probe (3.5 MHz-17 cm; Aloka Inc., Wallingford, CT).

5.3.4.4. Milk Yield and Composition

On d 21 and 42 of lactation, partial milk yield was estimated from the first 3 to 4 cows calving from each pen. Briefly, on the day before sampling, cows were separated from their calves at 1300 h and then rejoined at 1900 h, calves were then allowed to suckle for 45 min to exhaust the milk from the mammary gland. Immediately after nursing, cows were separated from their

calves. On the next morning, starting at 0700 h, 30 IU of oxytocin (OXY-20 NW®, Rafter 8 Products, Calgary, AB, Canada) were administrated intravenously, and cows were milked from 2 diagonally-opposite quarters using a portable milking machine (Deluxe Portable Pump, E-Zee Milking Equipment, Gordonville, PA, USA). The total milk yield reached from both quarters was weighed and used as an estimator of the 12 h partial milk yield. Immediately after collection, two milk samples were obtained from the milk collected from each cow. One 20 mL sample containing a preservative was refrigerated at 4°C and sent for analysis within the next 72 h to the CanWest DHI Central Milk Testing Laboratory (Edmonton, AB, Canada). Another 40 mL sample was collected into a 50 mL sterile centrifuge tube (VWR International, Radnor, PA) and stored at -20º C until analysis.

5.3.4.5. Blood serum collection

Blood was collected from all cows at calving, and from cows used for milk yield estimation at 21 and 42 d of lactation. Blood samples were collected from each cow via jugular venipuncture into 10-mL untreated vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ). Blood samples were allowed to clot at room temperature for 30 min, centrifuged (2000 × g at 4°C for 15 min), and serum was harvested into 1.5 mL tubes (Eppendorf, GCS, New York, NY) and refrigerated at -20° C until analysis.

5.3.4.6. Muscle and Adipose Tissue Collection

At birth and weaning, biopsy samples of the *longissimus dorsi* (LD) muscle were collected between the 12^{th} and 13^{th} rib of the first two bull-calves born from each pen. Briefly, steers were restrained, hair was removed from the biopsy site, and 4 mL of a local anesthetic (lidocaine HCl 2%, Zoetis Canada Inc., Kirkland, QC) was administered. The biopsy site was then cleaned using 70% ethanol and a 2-cm incision was made using a sterile scalpel. Biopsy sample (approximately 1 g) was collected from the LD muscle using a 6 mm sterile biopsy punch (Integra Miltex, Integra LifeSciences Corp., Plainsboro NJ), washed with phosphate-buffered saline, placed into 1-mL sterile polypropylene cryogenic vial (Cryo.s, Greiner Bio-One North America, Inc., Monroe, NC), snap frozen in liquid N, and stored at -80°C until analysis. Samples were used for RNA extraction and evaluation of the relative expression of growth, myogenic, and adipogenic genes through real-time PCR.

Adipose tissue (AT) samples (approximately 5 g) were obtained from the brisket of all steers and heifers at slaughter. Samples were placed into 60 mL sterile polyethylene bags (Fisher Scientific, Ottawa, ON), and stored at -20°C until analysis. Samples were analyzed for fatty acid composition using gas chromatography.

5.3.4.7. Carcass Traits

Calves were slaughtered at a commercial processing plant (Cargill Foods, High River, AB, Canada) at the end of the finishing period at an average shrunk BW of 595 ± 58 kg. Hot carcass weight (HCW) was determined immediately, and the carcasses were chilled for 24 h and evaluated using the Computer Vision Grading System (VBG 2000 e + v Technology GmbH, Oranienburg, Germany) for yield grade and marbling score according to the Canadian Beef Grading Agency (CBGA, 2009). The yield grade (YG) is a measure of the overall lean yield calculated from the rib-eye area and fat depth and consists of Canada 1 = 59% or more; Canada 2 = 58%–54%; and Canada 3 = 53% or less. Marbling scores were A = trace; AA = slight; AAA = small to moderate; and prime = slightly abundant or greater (CBGA, 2009).

5.3.5. Laboratory analysis

5.3.5.1. Feeds

Chemical analyses of feeds were performed by Cumberland Valley Analytical Services Inc. (Hagerstown, MD), and analyzed in duplicate according to the AOAC International (AOAC, 2012). Hay and barley silage samples were analyzed by near infrared spectroscopy (NIRS) using a Foss NIRSystems 5000 (NIR Systems, Inc., Silver Spring, MD, USA) for determination of DM, CP, ADF, NDF, ash, Ca and P. Barley grain and canola meal samples were analyzed for DM by drying at 135°C for 2 h (method 930.15; AOAC, 2012), CP (method 990.03; AOAC, 2012) using a Leco FP 528 Nitrogen Combustion Analyzer (Leco, St. Joseph, MI, USA), ADF (method 973.18; AOAC, 2012), ash (method 942.05; AOAC, 2012), and Ca and P (method 985.01; AOAC, 2012). The method of Van Soest et al. (1991), with the addition of amylase and sodium sulfite, was used to determine NDF content. The Pennsylvania-State equations based on ADF were used to calculate the total digestible nutrient (TDN) values for all feeds (Adams, 1980).

Digestible energy (DE), net energy of maintenance (NEm) and gain (NEg) were calculated according to NRC (2000).

5.3.5.2. Blood metabolites

Serum samples were used to determine non-esterified fatty acids (**NEFA**) and β-hydroxybutyrate (**BHBA**) concentrations. Serum NEFA concentration was determined using the NEFA-HR (2) kit (Wako Diagnostics Corp., Richmond, VA) and absorbance was read on a spectrophotometer (Epoch 2, Biotek Instruments Inc., Winooski, VT, USA) at 550 nm. Serum BHBA concentration was determined through the enzymatic oxidation of BHBA to acetoacetate catalyzed by 3-hydroxybutrate dehydrogenase (Williamson et al., 1962). The associated reduction of NAD to NADH was determined photometrically at 340 nm using a microplate spectrophotometer (Epoch 2, Biotek Instruments Inc., Winooski, VT, USA).

5.3.5.3. Fatty Acid Extraction and Gas Chromatography

Fatty acid methyl esters (FAME) were prepared from milk fat and AT samples. Briefly, 25-mL of raw milk were centrifuged at 17,800 × g for 30 min at 4°C. Subsequently, 1 g of the resulting cream (top layer) was collected and transferred into a 2-mL microcentrifuge tube, centrifuged at 19,300 × g for 20 min at 20°C, and 40 mg of the top fat layer were weighed into a pyrex tube with a teflon lined screw cap. For AT samples, 40 mg were weighed into a pyrex tube with a teflon lined screw cap and freeze dried overnight to a constant weight. All milk fat and AT samples were methylated by base catalyzed methylation using 0.5 N sodium methoxide as detailed in Chapter 4. Fatty acid methyl esters obtained from milk fat and AT samples were analyzed using a Varian CP-3800 gas chromatograph (Varian Inc., Walnut Creek, CA, USA) using the conditions described by Vahmani et al. (2017) and Kramer et al. (2008), except only one GC analysis using the 175^{o}C plateau temperature program was used (i.e. further analyses to separate minor isomers was not conducted). Fatty acids were identified using reference standard No. 603 from Nu-Chek Prep Inc. (Elysian, MN, USA). Branched-chain FAME were identified using a GLC reference standard BC-Mix1 from Applied Science (State College, PA, USA). The UC-59M standard from Nu-Chek Prep was used for conjugated linoleic acid (CLA) isomers. Polyunsaturated fatty acid biohydrogenation intermediates not included in the standard mixtures were identified by their retention times and elution orders as reported in literature (Cruz-

Hernandez et al., 2004; Gomez-Cortes et al., 2009; Kramer et al., 2008) and this included recently identified Δ-9 desaturation products of trans-18:1 isomers (Vahmani et al., 2016a). The FAME were quantified using chromatographic peak area and internal standard based calculations.

5.3.5.4. RNA Extraction

Total RNA was extracted with Trizol reagent. *Longissimus dorsi* muscle tissue samples (100 mg) were homogenized in 1 mL of Trizol reagent (Life Technologies, Inc., Burlington, ON, Canada) using the Precellys 24 Tissue Homogenizer with the Cryolys accessory (Bertin Technologies, Montignyle-Bretonneux, France) and 2-mL bead tubes (Precellys Hard tissue grinding MK28, Bertin Technologies, Rockville, MD). Homogenized samples were incubated for 5 min at room temperature to allow complete dissociation of nucleoprotein complexes. Samples were then centrifuged at 12,000 × g for 10 min at 4°C and the supernatant was transferred to a 1.5-mL microcentrifuge tube. Chloroform was added (200 µL) and samples were shaken and incubated at room temperature for additional 2 to 3 min. Samples were centrifuged at 12,000 × g for 15 min at 4°C. After centrifugation, the dissolved RNA was pipetted to a new 1.5-mL tube, and the RNA was precipitated with 500 µL of isopropyl alcohol. Samples were then incubated at room temperature for 10 min and centrifuged at 12,000 × g for 10 min at 4°C. The supernatant was removed, and the RNA precipitate was washed with 75% ethanol. The RNA and ethanol were vortexed and centrifuged at 7,500 × g for 5 min at 4°C. The RNA was then dissolved in nuclease-free H_2O (Ambion, Foster City, CA, USA). All total RNA samples were quantified using a spectrophotometer (ND-1000, NanoDrop, Wilmington, DE, USA), were evaluated for RNA integrity (RIN) using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA), and stored at -80°C until cDNA synthesis. The RIN value of RNA isolated from all samples were >7.

5.3.5.5. Real Time-PCR

For gene expression analysis, total RNA (1.5 µg) from each sample was reverse transcribed with the High Capacity cDNA reverse transcription kit (Life Technologies Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. RNase inhibitor (Life Technologies Inc.) was also

added to the reaction at a concentration of 2 U/µl. After reverse transcription, the cDNA was diluted to 1 ng/µl with nuclease-free H2O (Ambion, Foster City, CA, USA). Real-Time PCR for gene expression analysis was performed in duplicate using 1 ng of cDNA in 96-well fast plates using the SYBR fast master mix ABI prism (D-Mark Biosciences) and the Step-One Plus Real-time PCR system (Life Technologies Inc.). A blank sample and a minus reverse transcriptase were added to control for nonspecific amplification. Relative standard curves, made from serial dilution of a pooled cDNA from all LD muscle samples and ranging from 20 to 0.02 ng, were used to determine the relative quantity of each sample. The Primer3 software and species-specific sequences found in GenBank were used for the design of primers (Table 5.2). Primers were designed to cover exon-exon junctions when possible, and ran with an annealing/extension temperature in the real time-PCR reaction of 60 °C (Paradis et al., 2017). The amplification efficiency for each gene was determined using serial dilution of tissue specific cDNA and was found to be 100 ± 10% for all genes (data not shown). Eukaryotic Translation Elongation Factor 1 Alpha 2 (EEF1A2), Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH), Hydroxymethylbilane Synthase (HMBS), Ribosomal Protein L19 (RPL19), and Tyrosine 3-Monooxygenase/Tryptophan 5-Monooxygenase Activation Protein, Zeta Polypeptide (YWHAZ) were tested as endogenous control genes, and the best individual or combination of endogenous control was chosen using NormFinder (Andersen *et al*., 2004). As a result, GAPDH and RPL19 were used as the endogenous controls to correct for RNA extraction and reverse transcription efficiency in LD muscle samples at birth and weaning, respectively. The endogenous control genes were tested for any treatment effect and were found to be stable confirming their usefulness as suitable endogenous controls.

Table 5.2. Primer sequences and amplification conditions for gene expression measured in *longissimus dorsi* muscle biopsy samples of male calves by real-time PCR.

Gene	Primer	Sequence	Product size (bp)
EEF1A2	Fwd	5’ - AGTTCACGTCCCAGGTCATC- 3’	149
	Rev	5’ - CTCCAACTTCTTGCCAGAGC - 3’	
GAPDH	Fwd	5’ - TGACCCCTTCATTGACCTTC - 3’	143
	Rev	5’ - GATCTCGCTCCTGGAAGATG - 3’	
HMBS	Fwd	5’ - CTACTTCGCTGCATTGCTGA - 3’	105
	Rev	5’ - CAGGTACAGTTGCCCATCCT - 3’	
IGF1	Fwd	5’ - GATGCTCTCCAGTTCGTGTG - 3’	141
	Rev	5’ - CTCCAGCCTCCTCAGATCAC - 3’	
IGF1R	Fwd	5’ - CAAAGGCAATCTGCTCATCA - 3’	139
	Rev	5’ - CAGGAAGGACAAGGAGACCA - 3’	
IGF2	Fwd	5’ - CCAGCGATTAGAAGTGAGCC - 3’	95
	Rev	5’ - AGACCTAGTGGGGCGGTC - 3’	
IGF2R	Fwd	5’ - GCAATGCTAAGCTTTCGTATTACG - 3’	188
	Rev	5’ - GGTGTACCACCGGAAGTTGTATG - 3’	
LPL	Fwd	5’ - GTGACCGAATCTGTGGCTAAC - 3’	251
	Rev	5’ - GGCACCCAACTCTCATACATT - 3’	
MYOD1	Fwd	5’ - GAACACTACAGCGGCGACTC - 3’	121
	Rev	5’ - AGTAAGTGCGGTCGTAGCAG - 3’	
MYOG	Fwd	5’ - CAGTGAATGCAGCTCCCATA - 3’	164
	Rev	5’ - CGACATCCTCCACTGTGATG - 3’	
PPARγ	Fwd	5’ - CGGTTTCAGAAGTGCCTT G - 3’	137
	Rev	5’ - GGTCAGCAGACTCTGGGTTC - 3’	
RPL19	Fwd	5’ - ACCCCAATGAGACCAATGAA - 3’	101
	Rev	5’ - ATGGACAGTCACAGGCTTCC - 3’	
SCD	Fwd	5’ - ACCTGGCTGGTGAATAGTGC - 3’	212
	Rev	5’ - AAGGTGTGGTGGTAGTTGTGG - 3’	
YWHAZ	Fwd	5’ -AGACGGAAGGTGCTGAGAAA - 3’	123
	Rev	5’ - CGTTGGGGATCAAGAACTTT - 3’	

GenBank accession numbers in Paradis et al. (2017).

5.3.6. Statistical analysis

Post-partum data on two cows and their calves from the FLX treatment (one in each year) was removed from the analysis due to death of the cow from natural causes unrelated to treatment during the breeding season. All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC). The average of each research pen where cows received their prepartum treatment diets represented the experimental unit. Data were analyzed as a Randomized Complete Block design (RCBD) using the Mixed procedure. The statistical model included the fixed effect of treatment and the random effect of year. Covariance analyses were performed using the number of days since calving, as well as the proportion of cows carrying heifer calves within research pen, as covariate. The effects of treatment, time, and their interaction were evaluated for milk parameters and animal BW measured at 21 and 42 d of lactation using a RCBD accounting for repeated measures where eight covariance structures were tested. The covariance structure with the lowest Akaike's and Bayesian information criterion (AIC and BIC) values was selected (Littell et al., 1998). The Glimmix procedure was used to analyze categorical data including BCS, pregnancy status, quality grade and yield grade score. The Kenward-Roger option was used to estimate denominator degrees of freedom. Pre-planned contrasts were used to determine the effects of level (LF vs. HF) and source (CAN vs. FLX) of fat. Significant differences were declared at $P < 0.05$, and trends at $P < 0.10$.

5.4. Results & Discussion

5.4.1. Postpartum Performance of Cows

Results on the performance of cows are presented in Tables 5.3 and 5.4. Level ($P \geq 0.72$) and source ($P \geq 0.28$) of dietary fat during gestation did not affect BW at calving (Table 5.3), or the average BW and ADG during the first 42 d of lactation (Table 5.4). While there was no interaction ($P = 0.18$) between treatment and time, BW of cows decreased linearly ($P < 0.01$) throughout the first 42 d of lactation (Table 5.4). At calving, BCS of cows tended ($P \leq 0.09$) to be greater for LF compared to HF cows (2.74 vs. 2.63) and for FLX compared to CAN (2.69 vs. 2.56) cows (Table 5.3). Greater BCS observed at calving in cows fed the LF diet over gestation is most likely a carryover effect of the greater BCS reported for the same group of cows at 23 ± 4.6 d prior to calving (Chapter 4). During the first 42 d of lactation, all treatments experienced a decrease ($P < 0.01$) in BCS from 2.7 to 2.4, and the average BCS of LF cows during the first 42 d of lactation was greater ($P < 0.01$) than that of HF cows (2.63 vs. 2.51) with no difference ($P = 0.32$) between CAN and FLX cows (Table 5.4). No effects ($P \geq 0.35$) of level or source of dietary fat fed over gestation were observed on BCS change during the first 42 d of lactation.

At weaning, the cumulative ADG, BW and BW adjusted to 180 d of the cows were not affected by level ($P \geq 0.54$) or source ($P \geq 0.21$) of dietary fat fed during gestation (Table 5.3). Also, BCS of cows at weaning and the change in BCS from calving to weaning were not affected ($P \geq 0.28$) by treatment. These results are consistent with those of Banta et al. (2011) who found no effect on BW, BW change, or BCS at weaning of multiparous beef cows receiving no supplement or fed either a high-linoleic or high-oleic supplement during mid to late gestation.

Table 5.3. Effects of level and source of fat in the diet of beef cows during gestation on their postpartum and reproductive performance.

	Treatments[1]				Contrasts[2]	
Item	**LF**	**CAN**	**FLX**	**SEM**	**LF vs. HF**	**CAN vs FLX**
At calving						
BW (kg)	704	703	704	7.51	0.95	0.81
BCS[3]	2.74	2.56	2.69	0.05	0.07	0.09
Thin (% of cows)	1.6	9.8	0.8	4.98	0.63	0.09
Optimal (% of cows)	95.8	86.9	98.4	4.29	0.90	0.08
Over conditioned (% of cows)	2.6	3.3	0.8	1.44	0.98	0.98
SCFT[4] (mm)						
Rib	5.7	3.9	4.6	0.30	< 0.01	0.11
Rump	6.4	4.9	5.7	0.40	0.02	0.12
At weaning						
Days postpartum (d)	183	184	182	1.51	0.81	0.34
BW (kg)	680	685	681	8.09	0.58	0.49
Cumulative ADG (kg/d)	-0.13	-0.10	-0.15	0.07	0.76	0.21
BW180 (kg)	680	684	682	7.82	0.54	0.69
BCS[3]	2.62	2.56	2.61	0.06	0.49	0.46
Thin (% of cows)	2.8	0.0	2.4	2.23	0.98	0.99
Optimal (% of cows)	96.2	100.0	95.4	2.63	0.97	0.97
Over conditioned (% of cows)	1.0	0.0	2.2	1.82	0.98	0.98
SCFT[4] (mm)						
Rib	4.3	3.4	3.9	0.43	0.01	0.11
Change	-1.2	-0.5	-0.8	0.29	0.16	0.54
Rump	4.5	4.4	4.4	0.52	0.84	0.90
Change	-2.0	-0.5	-1.5	0.42	0.05	0.14
Reproductive performance						
Pregnancy rate (% of cows)	85.3	96.0	94.7	4.01	0.07	0.77
Calving rate (% of cows)	83.3	90.1	89.0	5.23	0.33	0.89
First calving (Julian d)	106	104	105	2.62	0.33	0.68
Last calving (Julian d)	126	134	127	4.43	0.45	0.26
Calving span (d)	20	30	22	4.74	0.30	0.23
Calving distribution						
at 21 d (% of cows)	84.0	71.8	76.9	7.22	0.27	0.66
at 42 d (% of cows)	95.9	94.2	95.7	3.61	0.82	0.79
Average (Julian d)	14	16	14	2.00	0.55	0.43
C-C[5] (d)	366	369	367	2.28	0.56	0.50

[1]**LF:** cows fed a low-fat diet; **CAN:** cows fed a high-fat diet including canola seed based pelleted feed; **FLX:** cows fed a high-fat diet including flaxseed based pelleted feed: [2]**HF:** average of CAN and FLX.
[3]**Thin:** cows with BCS ≤ 2; **Optimal:** cows with 2.5 ≤ BCS ≤ 3; **Over conditioned:** cows with BCS ≥ 3.5.
[4]**SCFT:** subcutaneous fat thickness.
[5]**C-C:** calving to calving interval.

Table 5.4. Effects of level and source of fat in the diet beef cows during gestation, time and treatment × time on performance, partial milk yield and milk composition during the first 42 d of lactation.

	Treatment[1] (trt)				Days relative to calving (d)				*P* value[2]			
Item	**LF**	**CAN**	**FLX**	**SEM**	**0**	**21**	**42**	**SEM**	**LF vs. HF**	**CAN vs. FLX**	**d**	**trt × d**
Performance of cows												
BW (kg)	694	691	701	13.7	705	695	686	12.9	0.72	0.28	< 0.01	0.18
Cumulative ADG (kg/d)	-0.49	-0.53	-0.46	0.44	-	-0.54	-0.45	0.44	0.93	0.63	0.21	0.54
BCS[3]	2.63	2.49	2.53	0.07	2.66	2.56	2.44	0.07	< 0.01	0.32	< 0.01	0.75
Change	-0.20	-0.14	-0.15	0.09	-	-0.10	-0.22	0.08	0.35	0.92	0.04	0.68
Thin (% of cows)	3.7	16.7	6.7	8.34	2.9	6.2	21.6	9.51	0.04	0.09	< 0.01	0.84
Optimal (% of cows)	96.3	83.3	93.3	8.31	97.1	93.8	78.4	8.86	0.07	0.06	< 0.01	0.65
Over conditioned (% of cows)	0.0	0.0	0.0	-	0.0	0.0	0.0	-	-	-	-	-
NEFA (µEq/L)	1,043	1,032	1,014	92.0	1,049	1,038	1,002	91.9	0.73	0.78	0.75	0.28
BHBA (mg/dL)	11.3	10.8	11.4	0.64	10.7	11.3	11.4	0.59	0.75	0.31	0.24	0.39
Milk parameters[4]												
12-h milk yield (kg)	5.9	6.2	5.8	0.43	-	5.8	6.1	0.39	0.74	0.23	0.14	0.44
Fat (%)	3.50	3.51	3.66	0.17	-	3.24	3.87	0.12	0.70	0.54	< 0.01	0.97
Fat yield (g)	217	224	214	18.9	-	193	243	15.7	0.91	0.66	< 0.01	0.78
Protein (%)	3.07	3.11	3.01	0.07	-	3.22	2.90	0.07	0.82	0.03	< 0.01	0.81
Protein yield (g)	183	193	171	16.6	-	187	177	15.7	0.91	0.08	0.23	0.54
Lactose (%)	4.63	4.57	4.64	0.04	-	4.53	4.70	0.03	0.63	0.28	< 0.01	0.23
Lactose yield (g)	273	286	264	20.0	-	262	287	17.9	0.88	0.25	0.01	0.83
Total solids (%)	12.2	12.2	12.3	0.20	-	12.0	12.5	0.16	0.78	0.67	< 0.01	0.89
Total solids yield (g)	818	890	796	62.4	-	706	963	49.5	0.75	0.31	< 0.01	0.56
Milk energy (Mcal/kg)	0.68	0.68	0.69	0.02	-	0.66	0.70	0.01	0.72	0.57	< 0.01	0.97
Milk energy yield (Mcal)	4.09	4.26	3.97	0.29	-	3.85	4.37	0.24	0.94	0.37	< 0.01	0.69
MUN (mg/dL)	11.6	11.2	11.2	2.37	-	11.7	11.0	2.35	0.42	0.99	0.07	0.73

[1]**LF:** cows fed a low-fat diet; **CAN:** cows fed a high-fat diet including canola seed based pelleted feed; **FLX:** cows fed a high-fat diet including flaxseed based pelleted feed: [2]**HF:** average of CAN and FLX.
[3]**Thin:** cows with a BCS ≤ 2; **Optimal:** cows with 2.5 ≤ BCS ≤ 3; **Over conditioned:** cows with BCS ≥ 3.5.
[4]**Partial yield:** total milk collected from 2 quarters over 12 h. **MUN:** milk urea nitrogen.

Although no differences in BCS were observed at weaning, when measured using ultrasound at the rib and rump location, the level of dietary fat fed during gestation affected body fat reserves over the period from calving to weaning. At calving, the SCFT of LF cows was greater ($P \leq 0.02$) at both the rib (5.7 vs. 4.3 mm) and rump (6.4 vs. 5.3 mm) locations than those of HF cows, while the SCFT of CAN cows was not different ($P \geq 0.11$) from FLX cows at the rib (3.9 vs. 4.6 mm) or rump (4.9 vs. 5.7 mm) locations (Table 5.3). At weaning, the rib SCFT was still greater ($P = 0.01$) for LF compared to HF cows and still not different ($P = 0.11$) for CAN compared to FLX cows. However, cows fed the LF diet during gestation tended ($P = 0.05$) to have a greater reduction in the SCFT at the rump (-2.0 vs. -1.0 mm) location from calving to weaning than those fed the HF diets, while no difference ($P = 0.14$) was observed between cows fed the CAN and FLX diets (Table 5.4). The reason for the greater loss in subcutaneous fat of LF cows could be attributed to a possible reduction in energy intake through a negative feedback effect of leptin. According to Murdoch et al. (2005), cattle with greater adipose tissue deposits have a greater concentration of circulating leptin that might decrease the energy intake and the subsequent amount of body fat.

5.4.2. Blood Metabolites

Results for NEFA and BHBA concentrations in serum of cows during the first 42 d of lactation are shown in Table 5.4. No effects of treatment ($P \geq 0.31$) were observed with serum NEFA and BHBA concentrations averaging of 1029 ± 263 µEq/L and 11.2 ± 1.8 mg/dL (respectively) across treatments. Also, no effects of time or treatment × time interaction were observed for serum NEFA or BHBA concentrations from calving to 42 d of lactation. This lack of effect on serum NEFA and BHBA concentrations during the first 42 d of lactation is consistent with the fact that cows had similar ADG and average BW over the same period, as mentioned previously. Also, the lack of responses for serum NEFA or BHBA concentration during the first 42 d of lactation are likely due to the fact that all cows were fed the same diet after calving.

5.4.3. Milk Yield and Composition

The results for 12-h milk yield and milk composition during the first 42 d of lactation are shown in Table 5.4. The estimated 12-h milk yield during the first 42 d of lactation averaged 6.0 ± 1.0

kg across treatments and was not affected by either the level ($P \geq 0.74$) or source ($P \geq 0.23$) of dietary fat fed over gestation. This lack of response to prepartum fat supplementation is consistent with findings reported by Banta et al. (2011) who reported that supplementing beef cows during mid- to late-gestation with either a low- or two high-fat supplements had no effect on milk yield measured in early lactation.

The average across treatments for most milk components (3.6 ± 0.7% fat, 3.1 ± 0.2% protein, 4.6 ± 0.2% lactose and 12.3 ± 0.7% total solids) during the first 42 d of lactation were similar to those reported by Rodrigues et al. (2014) for Angus and Angus-cross beef cows at the beginning of lactation (18 to 58 d of lactation). Milk fat, lactose, total solids, milk energy, and milk urea nitrogen (MUN) concentrations were not affected by either the level ($P \geq 0.63$) or source ($P \geq 0.28$) of dietary fat during gestation. No effects ($P \geq 0.25$) were observed on milk fat, lactose, total solids and energy yield. These results are in agreement with Alexander et al. (2002) and Banta et al. (2011) who reported no effect on milk fat, urea N, or solids during early lactation after prepartum supplementation of beef cows with supplements high in oleic or linolenic acid. However, in the present study, milk protein concentration was greater ($P = 0.03$) for CAN compared to FLX cows (3.11 vs. 3.01%), but no difference ($P = 0.82$) was found between LF and HF cows. Similarly, the estimated 12-h milk protein yield of CAN cows (193 g) tended ($P = 0.08$) to be greater than that of FLX cows (171 g). The reason for this greater level of protein in milk from CAN cows might be due to an increased availability of essential amino acids (EAA) such as methionine and lysine. It is known that the first two limiting EAA for milk production are methionine and lysine; and contents of these two EAA are greater in canola seed compared to flaxseed (Sosulski and Sarwar, 1973; Lee et al., 1995; Schwab and Broderick, 2017). Moreover, studies conducted using humans have shown that albumin can capture the excess of dietary EAA and transport them to other tissues for protein synthesis (De Feo et al., 1992). Across treatments, the concentration of milk fat, lactose, total solids, energy, and MUN increased ($P \leq 0.01$) from 21 to 42 d of lactation while milk protein concentration was not affected ($P = 0.23$) by time. No treatment × time effect ($P \geq 0.23$) was observed for any of the milk parameters measured.

5.4.4. Milk Fatty Acid Profile

Results for milk fatty acid profile during the first 42 d of lactation are shown in Tables 5.5 and 5.6. Even though dietary treatments were ceased at calving and all cows received the same diet throughout the lactation period, differences were observed in milk fatty acid profile throughout the first 42 d of lactation. Cows fed the FLX diet during gestation had greater ($P < 0.01$) total PUFA concentrations in milk fat (40.6 mg/g), while the total MUFA, BCFA and SFA were not affected by either level ($P = 0.86$) or source ($P = 0.65$) of dietary fat fed over gestation. This is contrary to findings reported by Alexander et al. (2002) where supplementing fat prepartum to primiparous beef cows did not affect the fatty acid profile of milk collected at 30, 60, and 90 d of lactation. However, these authors only offered 115 g/d of supplemental fat in the form of high-fat range supplement over a 62-d prepartum period, while in the present study cows received 300 g/d of fat from a pelleted feed over 183 d prepartum.

Total concentration of PUFA (n-3 + n-6) in milk fat was not affected by either the level ($P = 0.88$) or source ($P = 0.65$) of dietary fat (Table 5.5). However, the n-6:n-3 ratio was lower ($P < 0.01$) for FLX compared to CAN (1.29 vs. 1.50) cows and lower ($P < 0.01$) for HF compared to LF cows (1.40 vs. 1.53). Total concentration of n-3 fatty acids in milk fat tended to be greater ($P = 0.09$) for FLX compared to CAN (1.30 vs. 1.20%) but not different ($P < 0.23$) between HF and LF (1.25 vs. 1.19%) cows; while the total concentration of n-6 fatty acids was not affected by the level ($P = 0.10$) or source ($P = 0.13$) of dietary fat fed over gestation. Among n-3 and n-6 fatty acids measured, the concentrations of ALA and LA in milk fat during the first 42 d of lactation were not affected by either level ($P \geq 0.39$) or source ($P \geq 0.22$) of dietary fat fed during gestation with an average across treatments of 0.84 and 1.24% for ALA and LA, respectively. Time had a significant effect on the concentration of most PUFA determined in milk fat during the first 42 d of lactation. The total concentration of PUFA and n-3 fatty acids decreased ($P < 0.01$) with time, while the total concentration of n-6 fatty acids remained without change ($P = 0.45$) through the first 42 d of lactation.

Table 5.5. Effects of level and source of fat in the diet of beef cows during gestation, time and treatment × time on milk fat polyunsaturated fatty acid profiles during the first 42 d of lactation.

	Treatment[1] (trt)				Days (d)			*P* value[2]			
Fatty acid (% of total)	**LF**	**CAN**	**FLX**	**SEM**	**21**	**42**	**SEM**	**LF vs. HF**	**CAN vs. FLX**	**d**	**trt × d**
∑PUFA	2.93	2.90	2.93	0.16	3.06	2.78	0.16	0.88	0.65	< 0.01	0.72
∑n-3	1.19	1.20	1.30	0.07	1.38	1.08	0.07	0.23	0.09	< 0.01	0.95
18:3n-3	0.82	0.82	0.89	0.06	0.94	0.74	0.05	0.39	0.22	< 0.01	0.94
20:3 + 20:4 + 20:5n-3	0.17	0.16	0.19	0.01	0.20	0.14	0.01	0.49	< 0.01	< 0.01	0.90
22:5 + 22:6n-3	0.20	0.22	0.23	0.02	0.23	0.20	0.02	< 0.01	0.07	< 0.01	0.16
∑n-6	1.74	1.71	1.63	0.11	1.68	1.70	0.11	0.10	0.13	0.45	0.82
18:2n-6	1.25	1.24	1.23	0.11	1.19	1.29	0.11	0.63	0.82	< 0.01	0.93
18:3n-6	0.04	0.04	0.03	0.00	0.04	0.04	0.00	< 0.01	0.01	0.17	0.24
20:2 + 20:3 + 20:4n-6	0.37	0.35	0.30	0.01	0.36	0.31	0.01	< 0.01	< 0.01	< 0.01	0.02
22:2 + 22:4n-6	0.08	0.08	0.08	0.00	0.09	0.07	0.00	0.85	0.03	< 0.01	0.22
∑CLnA	0.07	0.08	0.11	0.01	0.09	0.08	0.01	< 0.01	< 0.01	0.03	0.48
*c*9,*t*11,*t*15-18:3	0.04	0.03	0.05	0.00	0.04	0.04	0.00	< 0.01	< 0.01	0.68	0.70
*c*9,*t*11,*c*15-18:3	0.04	0.04	0.06	0.00	0.05	0.04	0.00	0.02	0.01	< 0.01	0.53
∑AD	0.37	0.40	0.58	0.09	0.43	0.47	0.09	< 0.01	< 0.01	< 0.01	0.07
*c*9,*t*14- + *c*9,*t*13-18:2	0.11	0.14	0.20	0.02	0.14	0.16	0.02	< 0.01	< 0.01	< 0.01	0.04
*c*9,*t*15-18:2	0.05	0.06	0.08	0.01	0.05	0.07	0.01	< 0.01	< 0.01	< 0.01	0.02
t11,c15-18:2	0.14	0.13	0.21	0.03	0.18	0.15	0.03	< 0.01	< 0.01	0.03	0.55
∑CLA	0.65	0.69	0.88	0.08	0.72	0.77	0.08	< 0.01	< 0.01	< 0.01	0.42
*c*9,*t*11- + *t*7,*c*9-18:2	0.55	0.60	0.76	0.07	0.61	0.67	0.07	< 0.01	< 0.01	< 0.01	0.62
*c*11,*t*13-18:2	0.04	0.04	0.05	0.00	0.04	0.04	0.00	0.89	0.15	0.32	0.43
*t*11,*c*13- + *c*11,*c*11-18:2	0.02	0.02	0.04	0.01	0.03	0.03	0.01	< 0.01	< 0.01	< 0.01	0.41
t,*t*-CLA	0.03	0.03	0.03	0.00	0.03	0.03	0.00	< 0.01	< 0.01	0.38	0.78
n-6:n-3	1.53	1.50	1.29	0.04	1.29	1.59	0.04	< 0.01	< 0.01	< 0.01	0.98
Total (mg/g)	35.9	36.5	40.6	2.74	38.9	36.4	2.72	< 0.01	< 0.01	< 0.01	0.43

[1]**LF:** cows fed a low-fat diet; **CAN:** cows fed a high-fat diet including canola seed based pelleted feed; **FLX:** cows fed a high-fat diet including flaxseed based pelleted feed: [2]**HF:** average of CAN and FLX.

c = cis; ***t*** = trans; **ΣPUFA** = sum of polyunsaturated fatty acids (Σn-6 +Σn-3); **Σn-3** = sum of n-3 fatty acids; **Σn-6** = sum of n-6 fatty acids; **ΣCLnA** = sum of conjugated linolenic acids; **ΣAD** = sum of atypical dienes; **ΣCLA** = sum of conjugated linoleic acids.

Table 5.6. Effects of level and source of fat in the diet of beef cows during gestation, time and treatment × time on milk fat monounsaturated, branched-chain and saturated fatty acid profiles during the first 42 d of lactation.

	Treatment[1] (trt)				Days (d)			*P* value[2]			
Fatty acid (% of total)	**LF**	**CAN**	**FLX**	**SEM**	**21**	**42**	**SEM**	**LF vs. HF**	**CAN vs. FLX**	**d**	**trt × d**
∑MUFA	31.9	31.4	31.7	3.03	29.0	34.3	3.02	0.68	0.75	< 0.01	0.96
∑*c*-MUFA	29.9	28.9	29.0	2.84	26.5	32.0	2.82	0.20	0.94	< 0.01	0.97
*c*9-14:1	0.75	0.69	0.72	0.08	0.68	0.76	0.08	0.22	0.38	< 0.01	0.65
*c*7- + *c*9- + *c*11-16:1	2.43	2.04	2.08	0.06	1.95	2.42	0.05	< 0.01	0.54	< 0.01	0.49
*c*5- + *c*7- + *c*9-17:1	0.69	0.59	0.61	0.06	0.57	0.69	0.06	< 0.01	0.54	< 0.01	0.82
*c*9- + *c*10-18:1	24.6	24.3	24.2	2.19	22.1	26.6	2.17	0.56	0.91	< 0.01	0.95
*c*11-18:1	0.85	0.78	0.74	0.07	0.71	0.87	0.07	< 0.01	0.17	< 0.01	0.75
*c*12- + *c*13- + *c*15-18:1	0.25	0.27	0.36	0.03	0.28	0.31	0.03	< 0.01	< 0.01	< 0.01	0.62
∑*t*-MUFA	2.00	2.53	2.73	0.32	2.49	2.34	0.32	< 0.01	0.03	< 0.01	0.56
*t*9- + *t*10- + *t*11- + *t*12-16:1	0.07	0.09	0.12	0.02	0.10	0.10	0.02	< 0.01	< 0.01	0.77	0.39
*t*6-18:1	0.09	0.13	0.16	0.01	0.12	0.13	0.01	< 0.01	< 0.01	0.01	0.04
*t*9-18:1	0.19	0.24	0.22	0.02	0.21	0.22	0.02	< 0.01	< 0.01	0.07	0.14
*t*10-18:1	0.12	0.17	0.16	0.01	0.15	0.15	0.01	< 0.01	0.11	0.56	0.53
*t*11-18:1	0.98	1.15	1.30	0.14	1.21	1.07	0.14	< 0.01	< 0.01	< 0.01	0.92
*t*12-18:1	0.10	0.16	0.17	0.02	0.14	0.15	0.02	< 0.01	0.08	0.01	0.16
∑BCFA	3.91	3.87	3.81	0.06	3.92	3.81	0.04	0.40	0.49	0.02	0.12
∑SFA	59.9	60.3	59.7	3.39	62.4	57.5	3.38	0.90	0.44	< 0.01	0.99
4:0 + 6:0 + 8:0	3.35	3.35	3.39	0.26	3.62	3.10	0.26	0.77	0.67	< 0.01	0.41
10:0	2.69	2.68	2.75	0.35	2.93	2.49	0.35	0.77	0.53	< 0.01	0.81
12:0	2.95	2.95	3.03	0.40	3.24	2.71	0.40	0.78	0.55	< 0.01	0.88
14:0	9.45	9.51	9.62	0.98	10.1	8.96	0.97	0.72	0.71	< 0.01	0.98
16:0	28.6	28.1	27.7	1.82	28.8	27.5	1.80	0.11	0.40	< 0.01	0.94
18:0	8.99	9.84	9.35	0.53	9.82	8.96	0.51	0.02	0.09	< 0.01	0.84
19:0 + 20:0 + 22:0 + 23:0 + 24:0	0.61	0.64	0.61	0.02	0.65	0.60	0.02	0.40	0.20	< 0.01	0.28
Total (mg/g)	857	856	860	20.1	865	851	20.0	0.86	0.65	0.05	0.82

[1]**LF:** cows fed a low-fat diet; **CAN:** cows fed a high-fat diet including canola seed based pelleted feed; **FLX:** cows fed a high-fat diet including flaxseed based pelleted feed: [2]**HF:** average of CAN and FLX.

c = cis; ***t*** = trans; **ΣMUFA** = sum of monounsaturated fatty acids; **Σ*c*-MUFA** = sum of *cis*-monounsaturated fatty acids; **Σ*t*-MUFA** = sum of *trans*-18:1 isomers; **ΣBCFA** = sum of branched chain fatty acids; **ΣSFA** =sum of saturated fatty acids.

Total concentration of conjugated linolenic (CLnA) and linoleic (CLA) acids, and atypical dienes (AD) were greater ($P < 0.01$) in milk fat of FLX cows than those of CAN cows, and greater ($P < 0.01$) in HF than in LF cows. The greater concentration of CLnA, AD, and CLA in milk fat of FLX cows is possibly due to mobilized adipose tissue with similar fatty acid composition. In our companion study (Chapter 4), the analysis of subcutaneous adipose tissue of cows at 23 d prior to calving showed that concentrations of CLnA, CLA and AD were greater in cows fed the FLX diet during gestation. Since all treatments had a loss in BCS and had high levels of serum NEFA concentration during the first 42 d of lactation as mentioned previously, the 18 carbon fatty acids present in milk fat most likely originated from circulating fatty acids mobilized from adipose tissue. This is consistent with the fact that all 18 carbon fatty acids in milk are derived from circulating plasma lipids (Shingfield et al., 2010). Time had a significant effect on the concentration of conjugated fatty acids determined in milk fat during the first 42 d of lactation. The total concentration of CLnA, AD and CLA increased ($P < 0.01$). Also, a tendency ($P = 0.07$) for a treatment × time effect was observed for total concentration of AD in milk fat. The total concentration of AD in milk fat of FLX cows tended ($P = 0.09$) to be greater at 21 d than those of CAN and LF cows and further increased by 42 d while remaining steady for CAN and LF cows.

Total concentration of SFA, BCFA, MUFA, and *cis*-MUFA isomers in milk fat during the first 42 d of lactation were not affected by level ($P \geq 0.40$) or source ($P \geq 0.44$) of dietary fat fed over gestation (Table 5.6). However, the total concentration of *trans*-MUFA was greater ($P < 0.01$) for HF compared to LF cows at 21 d (2.63 vs. 2.00%) and greater ($P = 0.03$) for FLX compared to CAN cows (2.73 vs. 2.53%). When looking at the SFA, only the concentration of stearic acid (18:0) in milk fat was affected by the level of dietary fat fed over gestation. The concentration of 18:0 in milk of HF cows was greater ($P = 0.02$) than that of LF cows (9.60 vs. 8.99%) and tended ($P = 0.09$) to be greater for CAN compared to FLX cows (9.84 vs. 9.35%), and likely relates to differences in complete biohydrogenation of 18 carbon PUFA. Among *cis*-MUFA isomers, the total concentrations of *cis*12-18:1, *cis*13-18:1 and *cis*15-18:1 were greater ($P < 0.01$) for HF cows compared to LF cows (0.32 vs. 0.25%) while *cis*11-18:1 was the only 18 carbon MUFA whose concentration in milk was greater ($P < 0.01$) in LF compare to HF cows (0.85 vs. 0.76%). This is also consistent with 18 carbon fatty acids in milk arising from circulating lipids as a result of adipose tissue mobilization. The concentration of *cis*11-18:1 was

greater in adipose tissue of LF compared to HF cows at the end of gestation (Chapter 4). However, the concentration of oleic acid (*cis*9-18:1) in milk fat was not affected by the level (P = 0.56) or source (P = 0.91) of fat. This lack of treatment effect on the concentration of *cis*9-18:1 in milk is most likely due to a greater rate of desaturation of 18:0 in LF compared to HF cows. Even though greater amounts of 18:0 likely reached the mammary gland of HF cows due to adipose tissue mobilization as mentioned previously, the activity of Δ9-desaturase was most likely decreased in HF cows. The inhibition of Δ9-desaturase activity due to greater PUFA concentration has been reported by Chilliard et al. (2000). Time had a significant effect on the concentration of most MUFA and SFA in milk fat. The total concentration of MUFA and *cis*-MUFA isomers in milk fat increased (P < 0.01) from 21 to 42 d of lactation, while the total concentration of *trans*-MUFA isomers, BCFA and SFA in milk fat decreased ($P \leq 0.02$) from 21 to 42 d of lactation.

5.4.5. Reproductive Performance

Reproductive performance parameters of cows are shown in Table 5.3. Cows fed the HF diets over gestation tended (P = 0.07) to have greater pregnancy rates than cows fed the LF diet (95.4 vs. 85.3%). This is consistent with other studies where beef cows supplemented with fat prepartum exhibited improved pregnancy rates (Lammoglia et al., 1997b; Bellows et al., 2001; Grings et al., 2001). According to Hess et al. (2005), feeding fat to beef cows at least 60 d before calving results in improved pregnancy rates. No difference (P = 0.77) was observed in pregnancy rates of cows fed the CAN and FLX diet (96.0 vs. 94.7%). Level or source of dietary fat over gestation had no effect on calving span ($P \geq 0.23$), calving distribution ($P \geq 0.27$), or calving to calving interval (P = 0.50). In contrast, although a numerical difference in calving rates (6 to 7%) was still observed in favor of cows fed the HF diets during gestation, no effect (P = 0.33) of level of dietary fat during gestation could be declared.

The lack of effect of the source of fat (CAN vs. FLX) fed over gestation on reproductive performance is likely the result of the rumen biohydrogenation of dietary n-6 and n-3 fatty acids. According to Santos et al. (2008), n-6 and n-3 PUFA have the greatest effect on improving the reproductive performance of cattle. However, the rumen biohydrogenation process changes the

configuration of n-6 and n-3 PUFA thus reducing their effectiveness in improving the reproductive performance (Staples et al., 2002; Santos et al., 2013).

5.4.6. Progeny Performance

Results for performance of the progeny are shown in Table 5.7. Cows fed HF diets during gestation had heavier ($P < 0.01$) calves (42.9 kg) at birth compared to those from LF (40.2 kg) cows, while no difference ($P = 0.24$) was found between calves from CAN (42.4 kg) and FLX (43.3 kg) cows. Despite being exposed to similar amounts of dietary energy and protein over the last two trimesters of gestation, calves born to cows fed HF diets most likely had greater fetal growth due to an increase in placental nutrient uptake as discussed in the companion study (Chapter 4). During the first 42 d after birth, no effects of either level ($P = 0.55$) or source ($P = 0.74$) of dietary fat fed over gestation were observed on the cumulative ADG of calves. This is consistent with Garcia et al. (2014) who reported no effects on ADG during the first 30 d for Holstein calves born to cows fed a low-fat or one of two high-fat diets over gestation. This lack of difference in ADG of calves during the first 42 d of lactation can be attributed to the fact that cows had similar ADG and milk yield during the same period, as mentioned previously. However, in the present study, the average BW during the first 42 d of calves from HF cows was greater ($P < 0.01$) than that of LF cows (65.9 vs. 61.8 kg), while no difference ($P = 0.55$) was observed between calves born from CAN and FLX cows (65.9 vs. 65.8 kg). This difference in average BW over the first 42 d between calves from HF and LF cows is most likely a reflection of the difference observed in birth weight between these two treatments.

At weaning, cumulative ADG of calves born to CAN cows was greater ($P = 0.03$) than that of calves from FLX cows (1.19 vs. 1.13 kg/d), with no difference ($P = 0.18$) between calves from LF and HF cows (1.13 vs. 1.16 kg/d). Consequently, the weaning BW of calves born to CAN cows tended ($P = 0.05$) to be greater than that of calves born to FLX cows (260 vs. 251 kg), and the weaning BW of calves from HF cows also tended ($P = 0.05$) to be greater than that of calves from LF cows (256 vs. 248 kg). These tendencies became significant ($P = 0.049$) when weaning BW was adjusted to 180 d.

Table 5.7. Effects of level and source of fat in the diet prepartum of beef cows on the performance of the progeny.

	Treatments[1]				Contrasts[2]	
Item[3]	**LF**	**CAN**	**FLX**	**SEM**	**LF vs. HF**	**CAN vs. FLX**
At birth						
Date (Julian d)	114	113	115	1.65	0.87	0.52
BW (kg)	40.2	42.4	43.3	1.08	< 0.01	0.24
First 42 d						
BW (kg)	61.8	65.9	65.8	1.22	< 0.01	0.94
Cumulative ADG (kg/d)	1.04	1.06	1.07	0.05	0.55	0.74
At weaning						
Age (d)	183	184	183	1.85	0.71	0.52
BW (kg)	248	260	251	8.39	0.05	0.05
Cumulative ADG (kg/d)	1.13	1.19	1.13	0.05	0.18	0.03
BW180 (kg)	244	256	247	9.19	< 0.05	< 0.05
Backgrounding period						
FBW (kg)	411	433	420	5.59	0.02	0.14
Backgrounding ADG (kg/d)	0.91	0.97	0.95	0.02	0.07	0.46
Cumulative ADG (kg/d)	1.02	1.08	1.04	0.02	0.07	0.14
BW365 (kg)	413	437	423	7.94	0.03	0.15
Finishing period						
FBW (kg)	603	639	620	9.88	0.03	0.21
Finishing ADG (kg/d)	1.90	2.04	1.97	0.06	0.16	0.43
Cumulative ADG (kg/d)	1.22	1.29	1.24	0.02	< 0.05	0.16
Shrunk BW (kg)	579	614	595	9.61	0.03	0.19
At slaughter						
Age (d)	463	465	463	10.4	0.55	0.30
HCW (kg)	339	361	349	5.51	0.02	0.16
Dressing (%)	58.5	58.8	58.7	0.27	0.48	0.77
Quality grade (%)						
AAA	78.6	78.7	84.2	12.9	0.68	0.54
AA	16.8	17.6	9.7	14.8	0.57	0.29
A	0.0	0.0	0.0	-	-	-
Yield grade (%)						
3	49.6	53.6	46.9	20.7	0.95	0.60
2	32.6	38.9	40.6	18.6	0.46	0.89
1	14.2	5.3	10.6	4.99	0.25	0.41
Ribeye area (cm)	13.1	13.2	12.9	0.19	0.86	0.23
Fat thickness (cm)	0.50	0.54	0.50	0.06	0.51	0.37
Marbling score	444	452	443	10.7	0.78	0.61

[1]**LF:** cows fed a low-fat diet; **CAN:** cows fed a high-fat diet including canola seed based pelleted feed; **FLX:** cows fed a high-fat diet including flaxseed based pelleted feed: [2]**HF:** average of CAN and FLX. [3]**Cumulative ADG:** average daily gain from birth; **BW180:** BW adjusted to 180 d. **BW365:** BW adjusted to 365 d.

The reason for the superior performance from birth to weaning of calves from CAN cows compared to those from FLX cows is not clear. A possible explanation could be the greater milk protein concentration and yield observed in CAN cows during the first 42 d of lactation. Daley et al. (1987) reported a significant positive correlation (r = 0.39) between milk protein yield of *B. taurus* and *B. Taurus* × *B. indicus* cows and the pre-weaning weight of their calves. However, whether this difference in milk protein was maintained after 42 d remains unknown. Another possible explanation could be a decrease in milk fat synthesis due to the greater concentration of CLA observed in milk fat from FLX cows. Milk fat concentration and yield have been decreased by including flaxseed in the diet of dairy cows (Cortes et al., 2010). This decrease in milk fat has been attributed to reductions in the expression of genes that encode for enzymes associated with lipid synthesis in the mammary gland tissue after being exposed to CLA isomers (Baumgard et al., 2002; Peterson et al., 2002). Whether the difference in CLA concentration in milk fat between FLX and CAN cows remained after 42 d of lactation is unknown. However, as mentioned previously, mobilized adipose tissue was likely the reason for the greater CLA concentration in milk fat of FLX cows; and this tissue mobilization was most likely increased after 42 d since cows were approaching peak milk yield. Peak of lactation has been established between 56 and 70 d of lactation for Angus and Angus-cross beef cows (Rodrigues et al., 2014).

At the end of the backgrounding phase, calves from HF cows tended ($P = 0.07$) to have greater cumulative (1.06 vs. 1.02 kg/d) and backgrounding (0.96 vs. 0.91 kg/d) ADG than calves from LF cows, while no difference ($P \geq 0.14$) was observed in cumulative (1.08 vs. 1.04 kg/d) or backgrounding (0.97 vs. 0.95 kg/d) ADG between calves born to CAN and FLX cows. As a result, the difference observed in weaning BW between calves from CAN and FLX cows disappeared after the backgrounding phase while calves from HF cows remained heavier than calves from LF cows. At the end of the backgrounding phase, calves born to HF cows had greater ($P \leq 0.03$) BW (427 vs. 411 kg) and 365-d adjusted BW (430 vs. 413 kg) compared to those from LF cows, while no difference ($P \geq 0.14$) was observed between calves from CAN and FLX cows. The differences observed in performance from birth to the end of backgrounding of calves from LF compared to those from HF cows suggest a developmental programing effect from the level of fat fed over gestation. The possible lower uptake and transport of nutrients by the placenta of cows fed the LF diet could not only have resulted in lighter calves at birth (as indicated previously), but also could have diminished the postnatal growth and development of the progeny

of LF cows compared to that of HF cows. According to Nathanielsz (2006), growth and development of fetal organs can be negatively affected when exposed to nutrient restricted environments. Consequently, the postnatal performance of the offspring may be reduced (Greenwood et al., 2017).

During the finishing phase, no effects of either level ($P = 0.16$) or source ($P = 0.43$) of dietary fat fed over gestation were observed for finishing ADG of calves. However, the cumulative ADG from birth to finishing was greater ($P = 0.045$) for calves born to cows fed the HF diets relative to those born to cows fed the LF diet (1.27 vs. 1.22 kg/d), while no difference ($P = 0.16$) was observed between calves from CAN and FLX cows (1.29 vs. 1.24 kg/d). Therefore, calves born to HF cows had greater ($P = 0.03$) final (630 vs. 603 kg) and shrunk (604 vs. 579 kg) BW at the end of the finishing phase than calves born to LF cows, while no difference ($P \geq 0.20$) was observed between calves from CAN and FLX cows. At slaughter, the HCW of calves born to HF cows was 16 kg greater ($P = 0.02$) than that of calves born to LF cows (355 vs. 339 kg), while no difference was observed between calves from CAN and FLX cows. No effects of level ($P \geq 0.25$) or source ($P \geq 0.16$) of dietary fat fed during gestation to pregnant cows was observed for slaughter dressing percent, quality and yield grade, ribeye area, fat thickness or marbling score of the progeny (Table 5.7). As well, no major effects were observed on fatty acid composition of subcutaneous adipose tissue of the progeny at slaughter (Table 5.8). Among all the evaluated fatty acids, only the concentrations of 20:3n-6 and 20:4n-6 in adipose tissue of calves from LF cows were greater ($P \leq 0.04$) than calves from HF cows. This greater concentration of 20:3n-6 and 20:4n-6 could be attributed to a carry-over effect from lactation since concentrations of these fatty acids were also greater ($P < 0.01$) in milk fat from LF cows compared to HF cows.

Table 5.8. Effects of level and source of fat in the diet prepartum of beef cows on the fatty acid profiles in subcutaneous adipose tissue of their progeny at slaughter.

	Treatments[1]				Contrasts[2]	
	LF	**CAN**	**FLX**	**SEM**	**LF vs. HF**	**CAN vs. FLX**
∑PUFA	1.11	1.09	1.11	0.08	0.84	0.74
∑n-3	0.26	0.27	0.27	0.02	0.75	0.86
18:3n-3	0.23	0.23	0.23	0.02	0.57	0.95
22:5n-3	0.04	0.03	0.04	0.00	0.32	0.51
∑n-6	0.85	0.82	0.84	0.08	0.66	0.71
18:2n-6	0.75	0.74	0.75	0.07	0.90	0.87
20:3n-6	0.05	0.04	0.05	0.00	0.04	0.07
20:4n-6	0.05	0.04	0.05	0.01	0.02	0.20
∑CLnA	0.04	0.04	0.04	0.00	0.90	0.16
*c*9,*t*11,*t*15-18:3	0.01	0.01	0.01	0.00	0.42	0.78
*c*9,*t*11,*c*15-18:3	0.03	0.02	0.03	0.00	0.50	0.08
∑AD	0.41	0.42	0.41	0.05	0.79	0.53
*c*9,*t*14- + *c*9,*t*13-18:2	0.18	0.18	0.18	0.02	0.91	0.83
*c*9,*t*15-18:2	0.09	0.09	0.09	0.01	0.95	0.73
∑CLA	0.48	0.51	0.51	0.06	0.34	0.96
*c*9,*t*11- + t7,c9-18:2	0.45	0.47	0.47	0.05	0.39	0.96
*t*11,*c*13-18:2	0.02	0.02	0.02	0.00	0.86	0.35
t,*t*-CLA	0.01	0.02	0.02	0.01	0.19	0.30
∑MUFA	59.8	59.9	60.3	0.49	0.58	0.60
∑*c*-MUFA	57.8	57.9	58.4	0.58	0.56	0.54
*c*9-14:1	1.31	1.24	1.21	0.10	0.35	0.75
*c*9-16:1	5.75	5.58	5.44	0.23	0.25	0.57
*c*9-17:1	1.82	1.75	1.75	0.06	0.20	0.96
*c*9-18:1	44.0	44.5	45.2	0.74	0.15	0.32
*c*11-18:1	2.83	2.78	2.78	0.08	0.54	0.95
∑*t*-MUFA	2.02	2.01	1.91	0.11	0.63	0.49
*t*6-18:1	0.12	0.12	0.11	0.01	0.40	0.50
*t*9-18:1	0.20	0.20	0.20	0.01	0.90	0.82
*t*10-18:1	1.01	0.98	0.88	0.08	0.44	0.40
*t*11-18:1	0.31	0.33	0.33	0.05	0.25	0.98
∑BCFA	1.15	1.18	1.17	0.04	0.65	0.79
∑SFA	37.0	36.8	36.5	0.44	0.51	0.59
14:0	2.83	2.72	2.61	0.11	0.08	0.32
16:0	24.1	24.0	23.6	0.53	0.39	0.29
17:0	1.50	1.47	1.47	0.04	0.65	0.98
18:0	7.80	7.88	8.03	0.37	0.64	0.69
20:0	0.04	0.05	0.05	0.00	0.69	0.90

[1]**LF:** cows fed a low-fat diet; **CAN:** cows fed a high-fat diet including canola seed based pelleted feed; **FLX:** cows fed a high-fat diet including flaxseed based pelleted feed; [2]**HF:** average of CAN and FLX.
c = cis; ***t*** = trans; **ΣPUFA** = sum of polyunsaturated fatty acids (Σn-6 +Σn-3); **Σn-3** = sum of n-3 fatty acids; **Σn-6** = sum of n-6 fatty acids; **ΣCLnA** = sum of conjugated linolenic acids; **ΣAD** = sum of atypical dienes; **ΣCLA** = sum of conjugated linoleic acids; ∑**MUFA** = sum of monounsaturated fatty acids; ∑***c*-MUFA** = sum of *cis*-MUFA fatty acids; ∑***t*-MUFA** = sum of *trans*-MUFA fatty acids; ∑**BCFA** = sum of branched chain fatty acids; ∑**SFA** = sum of monounsaturated fatty acids.

From weaning to slaughter, calves were separated in two large groups according to sex. Consequently, it was not possible to statistically analyze the DMI and G:F of calves during the post-weaning period. Therefore, the reason for the superior performance of calves born to cows fed the HF diets over gestation is unclear. A possible greater synthesis of adipose tissue related hormones due to a greater body fat mass of calves born to HF cows might help explain their increased performance. Although no statistical difference was observed between calves from HF and LF cows in subcutaneous or intramuscular fat at slaughter, the total amount of body fat including visceral fat may have been greater in calves born to HF cows. Studies using rodent (Guo and Jen, 1995) and swine (Quiniou et al., 2008) models have shown that feeding a high-fat diet during gestation increases the total body fat content of the progeny. Adipose tissue can act as a highly active endocrine organ where steroid hormones (including estrogen) are synthesized (Kershaw and Flier, 2004). Estrogen dosing of beef cattle has resulted in improved ADG and G:F (Beconi et al., 1995; Cleale et al., 2013). This improvement in performance has been attributed to an increase in the secretion frequency of pituitary GH and its concentration in serum (Grigsby and Trenkle, 1986; Plouzek and Trenkle, 1991), and to an increase in circulating and muscle expression of IGF1 (Dayton and White, 2014).

5.4.7. Gene Expression

At birth (Figure 5.1), the IGF2 mRNA abundance in LD muscle of male calves tended ($P = 0.07$) to be greater for those born to LF cows compared to those born to HF cows. Previous studies have found that nutrient restriction during gestation resulted in an increased mRNA expression of IGF2 in skeletal muscle of sheep (Brameld et al., 2000) and beef cattle (Paradis et al., 2017) fetuses. Although in the present study LF cows were not nutrient restricted during the last two trimesters of gestation, LF fetuses were most likely exposed to lower plane of nutrition compared to HF fetuses due to a reduced placental uptake of nutrients, as explained previously. This could help to explain the up regulated expression of IGF2 in LD muscle at birth of calves from LF cows compared to those from HF cows. Moreover, Brameld et al. (2000) and Maltin (2008) suggest that fetuses exposed to lower levels of nutrients would experience an early and accelerated increase in IGF2 expression in skeletal muscle, and this would lead to a greater fiber type specification rather than secondary myogenesis. This possible lower number of secondary muscle fibers could help to explain the diminished growth observed in calves from LF cows relative to

those from HF cows. However, this hypothesis needs to be substantiated and more research is required. No effects of level or source of dietary fat during gestation were observed in the expression of any other gene evaluated in LD muscle of calves at birth.

Contrary to findings at birth, the IGF2 mRNA abundance in LD muscle of male calves at weaning (Figure 5.2) tended ($P = 0.08$) to be lower for those born to LF cows compared to those born to HF cows. The reason for this trend for IGF2 expression in LD muscle of calves from HF cows at weaning is not clear. According to Schiaffino et al. (2013), hypertrophy and fusion of myosatellite cells are the most common mechanism for postnatal muscle growth, and both IGF1 and IGF2 are upregulated in skeletal muscle undergoing this process (Charge and Rudnicki, 2004). However, the role of IGF1 in muscle growth (hypertrophy) is significantly greater than that of IGF2 (Schiaffino et al., 2013). Further research is needed to establish the reason for the upregulation of IGF2 in LD muscle at weaning from calves born to HF cows.

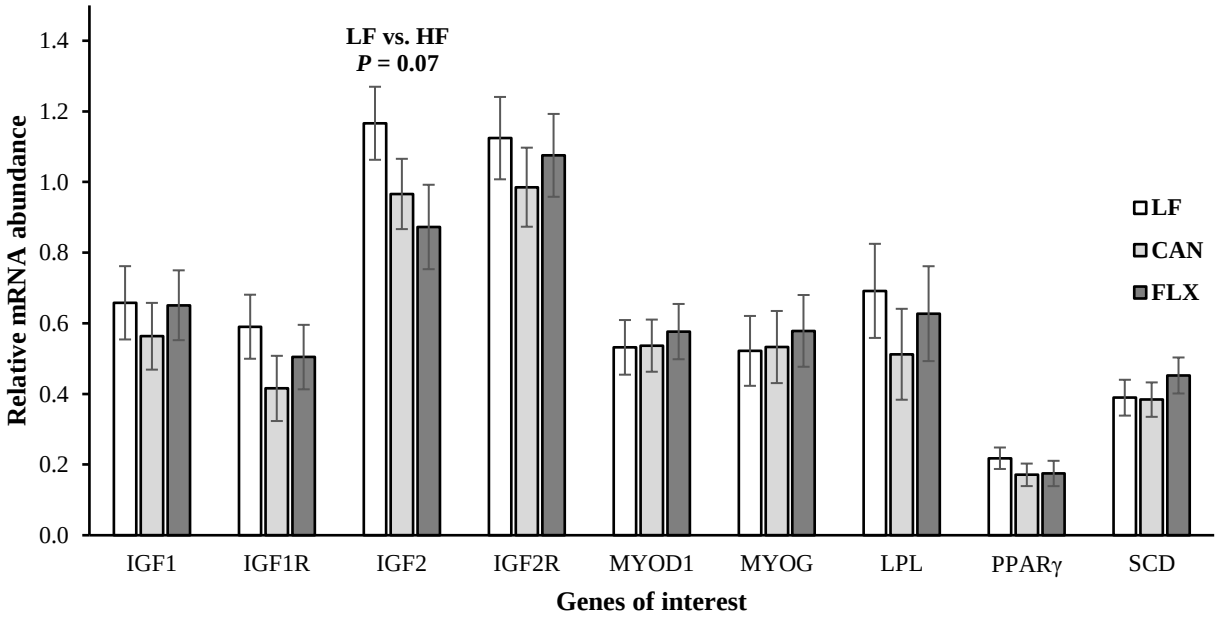

Figure 5.1. mRNA abundance for Insulin-like growth factors, and myogenesis and adipogenesis related genes, in *longissimus dorsi* muscle from calf at birth exposed to a low-fat or two high-fat diet in utero during the 2nd and 3rd trimester of gestation.

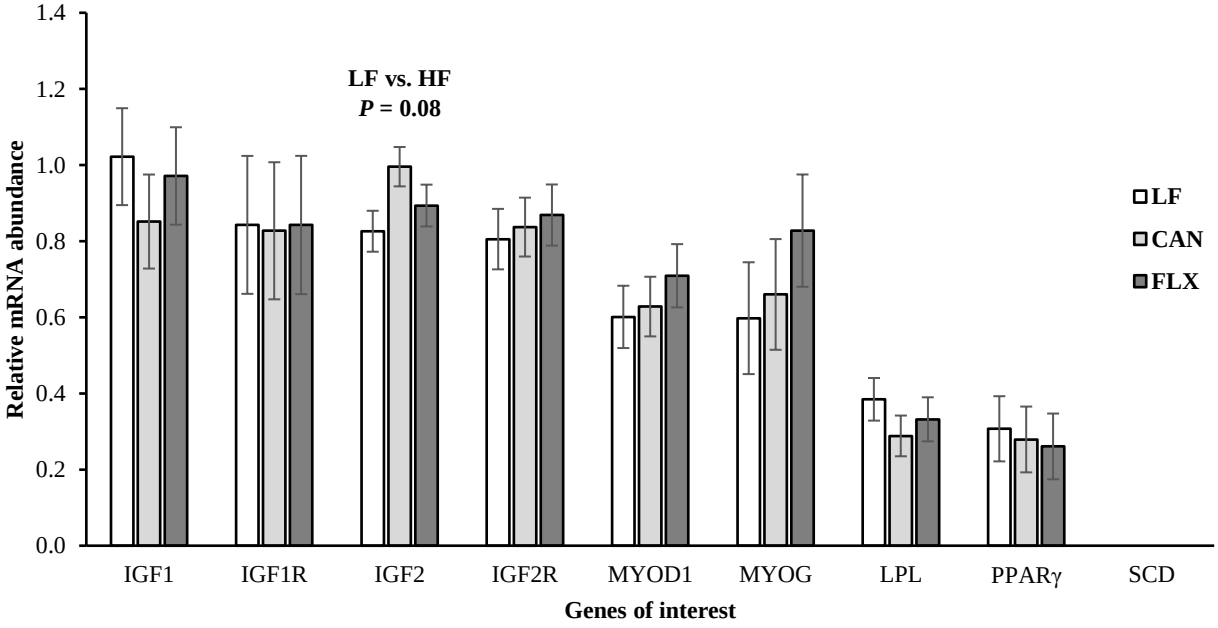

Figure 5.2. mRNA abundance for Insulin-like growth factors, and myogenesis and adipogenesis related genes, in *longissimus dorsi* muscle from calf at weaning exposed to a low-fat or two high-fat diet in utero during the 2nd and 3rd trimester of gestation.

5.5. Conclusions

The level and source of fat in the diet of gestating beef cows during the last two trimesters of gestation did not affect their BW or ADG from calving to weaning. However, cows fed the LF diet over gestation had greater BCS at calving and experienced a greater loss in adipose tissue from calving to weaning. Also, feeding the FLX diet over gestation increased the total concentration (mg/g) of PUFA, CLnA, CLA and AD during the first 42 d of lactation. Pregnancy rate of cows fed the HF diets over gestation tended to be greater at the end of the breeding season compared to those fed the LF diet.

Compared to the LF diet, feeding the HF diets over gestation resulted in heavier calves at birth, greater calf performance from birth to slaughter and superior shrunk-FBW and HCW of the progeny at slaughter. The reason for this difference in performance between the progeny of HF and LF cows is likely due to a developmental programming effect as the result of a possible greater placenta nutrient uptake and transport. However, feeding the FLX diet during gestation diminished the birth to weaning performance of the progeny possibly due to a negative effect of the increased levels of CLA isomers in milk fat on milk fat yield.

In conclusion, these data suggest that feeding beef cows a high-fat diet over gestation results in a heavier progeny, superior postnatal performance and greater HCW at slaughter, which indicates the possibility of improving the performance of beef cattle through a developmental programing effect. However, more research is needed in order to establish the physiological mechanisms involved.

6. GENERAL DISCUSSION AND CONCLUSSION

Formulating two high-fat pelleted feeds differing only in the ingredient providing the supplemental fat (i. e. canola seed vs. flaxseed) resulted in two supplements with similar energy, protein and fat content but with different fatty acid profiles. Using nutrient composition values for canola seed (CAN) and flaxseed (FLX) based pellets reported in study 1 and 2 (chapter 3 and 4), the energy, protein and fat content averaged 3.3 ± 0.1 and 3.3 ± 0.2 Mcal/kg of DE, 14.4 ± 1.7 and 14.0 ± 0.9% CP, and 8.9 ± 1.5 and 8.0 ± 1.0% EE for CAN and FLX pellets, respectively. However, the average MUFA content in the CAN pellet was larger than that of the FLX pellet (57.9 ± 1.3 vs. 20.4 ± 0.9%) with oleic acid representing the largest proportion among all fatty acids in the CAN pellet (52.1 ± 1.2%). On the other hand, the average PUFA content in FLX pellets was larger than that of CAN pellets (66.1 ± 1.7 vs. 30.8 ± 2.6%) with linolenic acid representing the largest proportion among all fatty acids in the FLX pellet (42.7 ± 1.6%). These nutritional parameters in both pellets reflect the similar protein and fat content but different fatty acid composition reported for canola seed and flaxseed (Barthet, 2017; Siemens, 2017).

Supplementing high-fat pellets prior to breeding to young beef cows grazing good quality cool-season grass pastures significantly reduced forage utilization (49.7 vs. 63.8%) compared to non-supplementation. As a result, a trend was observed for fat supplemented cows to reduce their estimated forage DMI (8.9 vs. 11.5 kg/cow/d) compared to those non-supplemented. The reduced forage utilization and intake observed in supplemented cows was likely due to a diet substitution effect rather than a negative effect of fat supplementation on forage intake. It has been documented that supplementation decreases forage DMI when the TDN to CP ratio of the forage is less than 7 or when forage DMI is more than 1.75% of BW (Moore et al., 1999). The average TDN to CP ratio of the cool-season grass forage was 4.5, and the forage DMI of non-supplemented cows represented 2.0% of the BW. Also, it has been documented that fat supplementation decreases DMI in ruminants only when the level of dietary fat exceeds 5-6% of the total DMI (Jenkins, 1993; Palmquist, 1994; Hess et al., 2008). However, supplemented cows

in chapter 3 had an estimated dietary fat of 4.2% of the total DMI which is below the value reported to affect forage DMI.

A greater than expected quality of the CSG forage during the supplementation period did not allow for a performance response of the young-cows due to fat supplementation prior to breeding. Over the 42 d of the trial, no significant difference was observed among treatments on nutrient composition of CSG pastures with an average across treatments of 12.5 ± 2.5 and 41.5 ± 4.7% for CP and ADF content, respectively. Consequently, no significant difference was observed on BW, ADG, BCS SCFT and blood metabolites of cows after the 42-d supplementation period, as well as there was no beneficial response in terms of reproductive performance of cows at the end of the breeding season. However, regardless of treatment, all cows were able to gain weight, to maintain their BCS and to increase their SCFT during the supplementation period. Moreover, the predicted ADG of CON cows was negative when using the NASEM model (NASEM, 2016) and estimated values such as forage DMI and quality. The gain in BW and SCFT, as well as the discrepancy between actual and predicted performance suggest that control cows were able to improve their nutrient intake through selective grazing as reported for beef cattle in previous studies (Barth and Kazaal, 1971).

Contrary to the lack of response to postpartum supplementation, including the high-fat pellets in the diet of beef cows during the last two trimesters of gestation resulted in differences in both cow and progeny performance. After receiving over the last two trimesters of gestation diets that were iso-caloric and iso-nitrogenous but differing in the amount of fat (1.4 ± 0.12 vs. 3.3 ± 0.20% EE), cows that were fed the low-fat (LF) diet had greater subcutaneous adipose tissue (SCAT) accretion. By the end of gestation (23 ± 4.6 d prior to calving), the conceptus-corrected BW of HF cows was lower which was reflected in less BCS and SCFT accretion compared to those receiving the LF diet. This lower accumulation of SCAT, contributing to lighter cows by the end of pregnancy, was likely a result of a differential partitioning of ME as influenced by the dietary source of energy. The greater caloric intake derived from fat in HF cows may have led to greater amounts of energy crossing the placenta rather than converted into fat depots. An increased placental uptake of glucose and amino acids due to feeding high-fat diets to the dam has been observed in studies using rodents (Jones et al., 2009).

Feeding the high-fat diet over gestation using either canola seed- or flaxseed-based pellet, resulted in changes in the SCAT composition by the end of gestation and changes in milk fatty acid composition during early lactation. The SCAT of HF cows had greater concentration of n-3 fatty acids, CLnA, CLA and AD than that of LF cows by the end of gestation, which reflected the greater concentration of these fatty acids in SCAT of FLX compared to CAN cows. Consequently, as a result of adipose tissue mobilization during early lactation, the milk fat from FLX cows had greater concentration of CLnA, CLA and AD. This increased concentration of conjugated fatty acids in SCAT and milk fat of FLX cows is the result of the greater amounts of dietary α-linolenic acid fed during gestation and partial ruminal biohydrogenation, resulting in intermediate isomers (Shingfield et al., 2013; Vahmani et al., 2016b). Also, due to the changes in configuration of dietary n-3 fatty acids after ruminal biohydrogenation, a beneficial response to the type of dietary fatty acid on reproductive performance of cows was not possible. This agrees with the fact that delivering n-3 and n-6 fatty acids to the lower gut in the form of protected fat is a more effective way to improve reproductive performance in cattle (Staples et al., 2002; Santos et al., 2013). However, regardless the degree of unsaturation, feeding the HF diets resulted in a tendency to greater pregnancy rate of cows at the end of the breeding season, which agrees with prepartum fat feeding improving reproductive performance of beef cows (Hess et al., 2005).

Feeding a high-fat diet over gestation resulted in increased birth weights and birth to slaughter performance of the progeny. Calves born to cows fed HF diets during gestation were 2.6 kg heavier at birth than those born to cows fed the LF diet. Greater birth weight due to high levels of dietary fat over gestation has previously been reported in lambs and beef calves (Lammoglia et al., 1999; Radunz et al., 2010; Radunz et al., 2011); and it is most likely due to a developmental programming effect as a result of the possible increased placental uptake of nutrients mentioned previously. From birth to weaning, calves born to cows fed the FLX diet over gestation had lower ADG (1.13 vs. 1.19 kg/d) compared to those from cows fed the CAN diet, while the level of dietary fat during gestation did not affect the birth to weaning performance of the progeny. The reason for the reduced performance observed in calves from FLX cows is not clear. The lower milk protein content and the greater CLA concentration in milk fat from FLX cows observed during the first 42 d of lactation, suggest that calves from FLX cows could have been exposed to less protein and energy content during part of the lactation period. However, whether the difference in milk protein was maintained and the total milk fat content was reduced

after 42 d of lactation, due to a milk fat depression caused by large amount of CLA isomers at the mammary gland tissue (Bauman and Griinari, 2001), needs to be studied with further research.

At the end of the backgrounding phase, calves born to HF cows had superior BW (427 vs. 411 kg) compared to that from LF cows due to a tendency in superior cumulative ADG (1.06 vs. 1.02 kg/d), while no difference was observed in performance between calves born to CAN and FLX cows. At the end of the finishing phase, calves born to HF cows had greater shrunk BW (604 vs. 579 kg) than calves born to LF cows, while no difference between calves from CAN and FLX cows. The greater BW of calves from HF cows was due to a superior cumulative ADG from birth to finishing (1.27 vs. 1.22 kg/d). As a result, the HCW of calves born to HF cows was 16 kg heavier than that of calves born to LF cows (355 vs. 339 kg). The differences observed in performance at the end of backgrounding and at the end of the finishing phase suggest a developmental programing effect from the level of fat fed over gestation. Feeding high-fat diets over gestation has resulted in greater uptake and transport of nutrients by the placenta, as indicated previously, and in total body fat content of the progeny (Guo and Jen, 1995; Quiniou et al., 2008). Therefore, a possible increase in growth and development of fetal organs including muscular tissue, as well as a possible greater synthesis of estrogen from adipose tissue, could help to explain the superior performance of calves born to HF cows (Beconi et al., 1995; Nathanielsz, 2006; Cleale et al., 2013).

At slaughter, carcass quality parameters of the progeny such as dressing percent, quality and yield grade, ribeye area, fat thickness and marbling score were not affected by either level or source of dietary fat fed during gestation. Also, fatty acid composition of subcutaneous adipose tissue of the progeny at slaughter did not show major effects due to level or source of dietary fat fed over gestation. Only the concentrations of 20:3n-6 and 20:4n-6 in adipose tissue of calves from LF cows were greater than those of calves from HF cows; and probably as due to a carry-over effect from lactation when these fatty acids were also greater in milk fat from LF cows.

The relative mRNA expression of myogenic and adipogenic genes in LD muscle of male calves at birth and weaning was not affected by level or source of dietary fat fed over gestation. Only the IGF2 mRNA expression in LD muscle of male calves seems to be affected by the level of dietary fat fed over gestation. At birth, the mRNA expression of IGF2 in LD muscle of male

calves tended to be greater for those born to LF cows compared to those born to HF cows. This could be attributed to the possible lower amounts of nutrients crossing the placenta of LF cows indicated previously. Similar increase in the mRNA expression of IGF2 in skeletal muscle has been reported for nutrient restricted fetuses from sheep and cattle (Brameld et al., 2000; Paradis et al., 2017). In contrast, the IGF2 mRNA abundance in LD muscle of male calves at weaning tended to be lower for those born to LF cows compared to those born to HF cows. Although, IGF1 and IGF2 are upregulated in skeletal muscle undergoing hypertrophy (Charge and Rudnicki, 2004), the role of IGF1 in muscle growth (hypertrophy) is significantly greater than that of IGF2 (Schiaffino et al., 2013). Therefore, the reason for the downregulation of IGF2 in LD muscle of male calves at weaning is not clear and further research is needed. Finally, due to the lack of significant evidence in the relative expression of the genes analyzed, it can not be confirmed whether epigenetic systems are involved in the possible changes in body composition occurring during gestation of HF calves.

Overall, the reproductive performance of lactating young beef cows grazing good-quality pastures was not affected by the level or source of fat in their diet. On the contrary, a prepartum high-fat diet tended to increase the pregnancy rate of beef cows at the end of the breeding season. Also, increasing the level of fat in the prepartum diet of gestating beef cows, resulted in a reduced amount of subcutaneous adipose tissue in the dam but heavier calves at birth which suggests a partitioning of the ME dependant on the type of dietary energy. Moreover, a high-fat diet during gestation resulted in improved birth to slaughter performance and superior HCW at slaughter of the progeny. However, the reasons for the superior performance of the progeny from high-fat fed dams are not clear and more research is further needed.

7. IMPLICATIONS

These results showed that applying a fat supplementation program prior to breeding to lactating young beef cows grazing good quality pastures did not improve their reproductive performance. However, under low quality forage scenarios such as mature pastures, where lactating young beef cows would not be able to meet their high demands for nutrients, a fat supplementation program might help to increase their energy intake and lead to an improvement in reproductive performance. Moreover, since our data suggest that fat supplementation resulted in a diet substitution effect by decreasing forage utilisation, a fat supplementation program could also help overcome the high nutrient demands of lactating young beef cows grazing limited pasture resourses or under conditions of high stocking rates.

These results also showed that fat inclusion in the diet of gestating beef cows has the potential to increase profitability of both cow-calf and feedlot operations. The cow-calf producer can benefit by having alternative sources of energy to feed their cows over the winter. Western Canada is caractherized by large extensions of land which every year are seeded with several crops that can be incorporated as sources of fat in the diet of beef cows. Crops like the ones used in this study (canola and flax) and others like soy, high-fat oats, and corn have the potential to increase the fat content when added to ruminant diets. Moreover, by-products that arise from the processing of these crops such as soy and oat hulls, canola and soy fines, and distillers' grains from the ethanol industry, can also be incorporated into the diet of ruminants as alternative sources of energy. Another benefitial aspect of feeding fat to gestating beef cows is the possibility of more kilograms sold on each calf-crop. The 10% increase in pregnancy rate of both high-fat treatments plus the 12 kg of extra BW at weaning observed in calves from cows fed the canola pellet would translate in greater income for cow-calf producers. From the perspective of the cattle feeder producer, a benefit can be obtained by more kilograms sold when sending the animals to slaughter.

The findings of this study indicate several new opportunities for research. Queries such as determining whether the level or source of dietary fat during gestation affected the postpartum DMI of cows remain unanswered. Also, it would be interesting to determine how much longer the difference in the protein and fatty acid content of milk observed during the first 42 d of the lactation period existed. Finally, the reasons behind the superior performance of calves born to cows fed high-fat diets during gestation remain unclear. Research conducted to measure the feedlot performance (i.e. DMI and feed efficiency) of the calves born to cows fed high-fat diets over gestation could help explan their superior FBW and HCW as well as further research into underlying biological mechanisms regulating pre- and post weaning growth.

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APPENDIX

A. Estimated costs of dietary treatments

Table A.1. Feed and total cost of feeding pregnant beef cows a low-fat diet and two high-fat diets based on canola or flaxseed during the last two trimesters of gestation.

	LF			**CAN**			**FLX**		
Year	1	2	**Avg.**	1	2	**Avg.**	1	2	**Avg.**
Feed costs ($/cow/d)									
Hay	0.51	0.96	**0.73**	0.43	0.73	**0.58**	0.45	0.73	**0.59**
Straw	0.17	0.29	**0.23**	0.15	0.29	**0.22**	0.15	0.26	**0.20**
Rolled barley	0.74	0.73	**0.73**	0.26	0.20	**0.23**	0.07	0.20	**0.13**
CAN pellet	-	-	**-**	0.99	0.86	**0.93**	-	-	**-**
FLX pellet	-	-	**-**	-	-	**-**	1.36	1.20	**1.28**
Total	1.41	1.98	**1.70**	1.83	2.08	**1.96**	2.03	2.38	**2.21**
Total cost of calf at birth ($/kg)	6.53	9.40	**7.97**	7.18	7.98	**7.58**	8.24	9.16	**8.70**