The Effects of Differing Planes of Pre- and Post-Weaning Phase Nutrition on the Development of Holstein Heifer Calves

by

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Abstract

Pre- and post- weaning planes of nutrition offered to heifer calves raised on farm can significantly alter future profitability, both through reduced costs and increased animal performance. The objective of this research was to examine the effects of pre- and post-weaning diets, with differing levels of dietary energy, on intake, growth, select hormone and metabolite concentrations, and overall sexual development of Holstein heifer calves. In study 1, energy intake was greater for heifers offered the high plane of nutrition in both the pre- and post- weaning periods, while growth was greater in high plane heifers compared to low plane heifers in each phase, but not consistently. Concentrations of key hormones, including insulin, IGF-1 and leptin were affected by pre- and post- weaning diets, but no interactions between the two phases were detected. In study 2, LH pulse release parameters at 3 and 6 mo were not affected by pre- or post- weaning diets. The overall age and body weight at first ovulation, were also not affected by pre- or post- weaning diets, however, heifers offered the post-weaning high plane of nutrition were more likely to have attained puberty at 7 and 8 mo of age than low plane heifers. Also, heifers attaining puberty at ages less than 7 mo tended to have increased serum leptin concentrations in the first 6 mo of life. No interactions between pre- and post-weaning planes of nutrition were noted in study 2. Overall, these findings suggest that interactions between pre- and post- weaning planes of nutrition are limited before 6 mo of age and/or the attainment of puberty, given the parameters measured in this study. Additionally, this thesis provides information that can help enhance the understanding of relationships between levels of intake, growth, hormone and metabolite concentrations, and sexual

development in heifer calves. These results are important in designing, implementing, and optimizing nutritional planes for heifer rearing.

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ADG	Average daily gain
BCS	Body condition score
BHBA	β - hydroxybutyric acid
BW	Body weight
BBW	Birth body weight
CF	Crude fat
CP	Crude protein
CV	Coefficient of variance
DM	Dry matter
DMI	Dry matter intake
FE	Feed efficiency
GC	Gas chromatography
GnRH	Gonadotropin releasing hormone
IGF-1	Insulin-like growth factor 1
LH	Luteinizing hormone
ME	Metabolizable energy
MR	Milk replacer
NE	Net energy
SE	Standard error
TMR	Total mixed ration
VFA	Volatile fatty acid

1.0 Review of Literature

1.1 Importance and economics of rearing

In the dairy industry, the development of heifer calves from birth until breeding is an important task that can significantly impact the future efficiency, production, and profitability of a farm. Zanton and Heinrichs (2005) describe the development of dairy heifers as "a long-duration, high-cost period that creates a lag in capturing a return on investment". Programs that successfully grow and develop heifers dedicate time, facilities, and capital towards this critical enterprise. In these successful programs, the end result is a uniformly grown group of heifers with high production potential, capable of reproducing at approximately 2 years of age. Thereby the number of non-productive days on feed is reduced. Poor heifer development can result in heifers of various sizes at desirable times of breeding or calving, with reduced reproductive capability, which require additional feed while not producing. In addition, heifer development programs should take precautions to limit the high incidence of morbidity and mortality that is known to occur during rearing (NAHMS, 2011). Genetics, management, technology and nutrition all play a part in the successful development of replacement dairy heifers.

Of the total costs associated with heifer development from birth to first calving, the cost of feed can range from 50% (Zwald et al., 2007) to nearly 73% of the total heifer development budget (Heinrichs et al., 2013). On average, a producer spends $1,808.23 USD $\pm$ $338.62 to raise a heifer until first breeding, with pre-weaning feed costs averaging $217.49 ± $86.21, costs from weaning to 6 months averaging $247.38 ± $78.89, 6 months until breeding averaging

$607.02 ±$192.28, and breeding to calving averaging $736.33 ± $162.86 (Heinrichs et al., 2013). The knowledge of these costs, combined with the early realization that nutritional plane has an effect on the growth and production of heifers (Crichton et al., 1959), has led to many changes in the way heifer calves are now reared. Many of these changes are further supported by a greatly improved understanding of the long term impacts of early life nutrition. While many early studies were based off small-scale trials from repeatedly tested animals, (Matthews and Fohrman, 1954; Davis and Hathaway, 1956) more recent research has begun to address nutritional management in all phases of heifer development including the pre-weaning, pre-pubertal, and post- pubertal phases.

1.12 Planes of nutrition

At birth, the digestive system of a calf operates such of that as a monogastric. The esophageal groove guides the mostly liquid diet during the first 2-3 weeks of life directly into the abomasum, bypassing the rumen and reticulum. As the calf slowly begins to ingest solid feed, normally in the form of starter, the reticulorumen rapidly develops, allowing for the absorption of volatile fatty acids (VFA) produced from microbial fermentation (Drackley, 2008).

Conventional pre-weaning feeding regimes were developed where the volume of relatively expensive milk or milk replacer offered to calves was limited in order to encourage starter intake and stimulate early life rumen development. By reducing milk availability to calves, Jasper and Weary (2002) demonstrated that hay and starter intake were increased by 84% and 83% respectively when compared to calves with *ad libitum* milk access. This method of feeding, often referred to as low plane, limits milk intake to approximately 10% of birth body

weight (resulting in 4-5L of milk consumption per calf per day. Low plane feeding is still commonly implemented across much of North America, where rates of morbidity and mortality in pre-weaned calves remain quite high (USDA, 2007). Since environmental factors, disease agents, and nutritional status all interact to determine the disease status of an animal (Davis and Drackley, 1998), ensuring that sufficient nutrition is provided helps to reduce the occurrence of disease.

Feeding regimens offering increased volumes of milk in the pre-weaning phase (20% BBW, 8-10L/day), referred to as high plane, have been shown to improve growth, positively impact behaviour, and improve lifetime production (Soberon et al., 2012). The offering of a high plane of nutrition closely mimics natural *ad libitum* milk consumption, where calves have been shown to routinely consume 16-20% of BBW in milk daily (Hafez and Lineweaver, 1968). When high planes of nutrition are offered in the pre-weaning phase, starter intake is reduced compared to that of calves offered a low plane (Stamey et al., 2005). As weaning is approached and milk allocation is reduced, rates of starter intake between high plane and low plane calves become similar (Stamey et al., 2005). Offering increased volumes of milk can be successfully accomplished with many different feedings systems, including buckets, bottles, nipple pails, or automated systems (Davis and Drackley, 1998). Despite the many benefits, producers are still hesitant to implement high plane feeding regimens in the pre-weaning period, citing greater feed costs, weaning challenges and concern of increased fat deposition (Vasseur et al., 2010).

Following weaning, calves are fully functioning ruminants. During this time, many different feed types can provide sufficient nutrients for development. Depending on producer preference, commonly fed post-weaning diets include silages, dry hay, grain supplements, TMR,

and leftover lactating cow rations. While silage rations are often negatively associated with proper calf nutrition, Drackley (2008) noted successful heifer development can be accomplished with silage based rations, provided that calves are gradually transitioned to these feeds. For producers, the goal during the post-weaning period should be to provide a diet which allows fast growth until breeding age for the heifers, while avoiding fat deposition (Drackley, 2008). According to NRC recommendations (2001), heifers should be bred at 55% of mature body weight in order to maximize production and minimize non productive days on feed. While terminology such as high plane and low plane are not as frequently used in the post-weaning phase, varying levels of protein and energy in feed have led to a wide range of observed ADG from lows of 0.5kg/day to highs of 1.1kg/day (Zanton and Heinrichs, 2007). In an experimental setting, and using bull calves, Groen et al. (2015) were able to achieve ADG of 1.7kg/d while offering similar diets to those described in chapter 2.

1.13 Nutritional requirements and provision

While differences in pre-weaning feeding volume have been discussed previously, differences in the composition of milk fed on-farm also occur. In conventional milk replacer (MR) feeding protocols, crude protein (CP) generally ranges from 18-24 (%DM) while fat ranges between 20-22 (%DM) (Davis and Drackley, 1998). Manufacturers' recommended mixing rates may vary, but calves are generally offered between 450 and 550 grams of MR daily when fed at a rate of 10% of BBW per day (Raeth-Knight, 2009). When taking into account the nutrient requirements of pre-weaned calves with the nutrients provided through conventional milk feeding (450-550g of MR daily to a 50kg calf), the predicted rate of gain would be between 200 and 300 g/d (NRC, 2001). Therefore, it is evident starter consumption is necessary to support

increased growth. Many producers still implement this feeding technique, citing potentially reduced feed costs and a decreased age at weaning as benefits thought to occur. Although calves offered an elevated plane of nutrition require more feed, economic advantages in areas such as reduced morbidity, reduced mortality, reduced labor, and fewer non-productive days on feed lead to cost savings of approximately $35 USD per calf when compared to conventional feeding (Overton et al., 2013).

When offering an elevated plane of nutrition using MR, the amount of CP must be increased while the amount of fat must be decreased in order to promote lean tissue growth and reduce fat deposition (Drackley, 2008). Increased growth rates, seen as a result of elevated planes of nutrition, lead to an increase in a calf's protein requirements that conventional MR cannot provide (Brown et al., 2005a). The use of MR containing 26-28% CP and 15-20% fat coupled with increased milk consumption has resulted in increased lean tissue growth (Diaz et al., 2001). The effects of additional protein availability on growth are further demonstrated in a study by Margerison et al. (2013), where calves offered whole milk supplemented with synthetic amino acids and plant carbohydrates grew at a faster rate than calves offered solely whole milk or whole milk with plant carbohydrates.

Feeding of non-saleable whole milk has also become increasingly popular in recent years. Concerns of feeding non-saleable milk stem from the bacterial content or antibiotic residues that may be present (Selim and Cullor, 1997), but proper pasteurization can help mitigate the risk. Purchase and upkeep of equipment capable of properly pasteurizing milk requires significant investment, however these costs are offset through cost savings from feeding whole milk in operations where more than 23 calves are fed pasteurized milk daily

(Godden et al., 2005). Following pasteurization, whole milk provides high levels of energy and protein (27% CP and 26-28% fat, DM) to calves that can lead to increased growth (Jasper and Weary, 2002).

A large variety of calf starters are commercially available in the dairy industry. Many different feedstuffs are commonly used as ingredients in calf starters, however palatability and availability of ingredients are primary areas of focus. Maximizing intake, and subsequently improving growth, are two of the main goals when formulating a starter. According to Nutrient Requirements for Dairy Cattle (2001), Holstein calves require starter that contains 16-20% CP, 0.7% calcium, 0.45% phosphorous, and 0.65% potassium in addition to small amounts of minerals and vitamins including zinc, manganese, cobalt, selenium, and vitamins A, D, and E (as-fed basis). Various studies have examined the effects of differing CP content of calf starter on growth, though the results are not always consistent. Hill et al. (2007) determined that growth is maximized when CP content of starter is 18% DM, and found no benefit to increasing CP content to 20% DM. Stamey et al. (2012) determined the opposite to be true, as high CP starter (25.5% DM) increased growth and intake when compared to conventional CP starter (19.6% DM). In contrast to both previous studies, Daneshvar et al. (2017) found no effect of starter CP content on growth, or performance. Lower levels of CP in starter should be avoided, however, as 15% CP starter (as-fed basis) can limit growth (Hill et al., 2007). Ensuring that sufficient protein, trace minerals, and vitamins are present in the starter is essential for optimal growth and development of young calves.

Following weaning, heifer calves are often allowed access to the same pre-weaning starter for a period of time to enhance solid feed intake once liquid feed is removed from the

diet. To ensure continued health and growth at weaning, it is recommended that calves be consuming at least 0.68 kg of starter for 3 consecutive days before gradually reducing the volume of milk offered (NRC, 2001). Following successful weaning, there is little information regarding proper feeding practices for developing heifers. In order to meet growth targets for future breeding, the NRC (2001) recommends 430g CP and 8.22 MCal ME for 100kg heifers to maintain high (900g ADG) levels of growth. Dry matter intake (DMI) for these growing heifers can be predicted using the equation from NRC (2001):

$$DMI\ (kg/d) = (BW^{0.75} \times (0.2435 \times NE_M - 0.0466 \times NE_M^2 - 0.1128) / NE_M)$$

where BW equals bodyweight (kg) and NE_M is the net energy of the diet for maintenance. Several other factors, including temperature, housing, mud, and body condition of the animals may affect NE_M and therefore alter the equation (Hoffman et al., 1994). Diets for post-weaned heifers generally include a forage source as well as concentrate, which may be fed separately or in a TMR. Due to the relatively low requirements of calves during this stage, a wide range of feed ingredients and processing may be used to continue lean growth, however the combination of ingredients and processing may influence intake. Interestingly, in a study comparing a completely pelleted post- weaning diet and a texturized starter mixed with chopped straw, no differences in ADG were noted; however, calves receiving the chopped straw diet had improved rumen function as measured by pH and total VFA production (Pavlata et al., 2017). The scarcity of published data aligning intake, requirements, feed ingredients, and

feed intake of the post-weaned calf illustrate the opportunity that exists to optimize calf nutrition for improved efficiency of production during this period.

1.14 Factors influencing intake

Heifer calves are separated from their dams at birth and often raised individually in order to closely monitor intake and health. The high variance in the amount or makeup of milk provided to calves on farm after separation from the dam, lead to patterns of intake in dairy calves that are often greatly influenced by management (Miller-Cushon et al., 2013). When offered *ad libitum* access to milk, calves increase intake at dawn and dusk and ingest several small meals throughout the day (Senn et al., 2000). As it is still common for producers to supply 5L of milk daily to calves, milk consumption is restricted to only certain feeding times (Appleby et al., 2001) and at levels less than half of expected *ad libitum* intake (Khan et al., 2011). In a study examining feeding behavior of *ad libitum* fed calves vs. calves offered 5L/day, Miller-Cushon et al. (2013) reported more frequent feeding visits at 6 weeks of age (7.1 vs 2.0) but no difference in non-nutritive suckling. Further, management decisions that may impact feeding behaviors in calves and developing heifers include stocking density, feed mixing, and feed delivery timing (Keys et al., 1978; Quigley et al. 1992; DeVries and von Keyserlingk, 2009).

The chemical composition of the diet supplied can also affect patterns of starter intake. Elevated fat content of milk reduced starter consumption (Kertz and Lofton, 2013), while increasing milk feeding rates can also reduce starter consumption (Kristensen et al., 2007). When feeding different dilutions of milk replacer (12% vs. 18.5% DM), Terré et al. (2007) noted depressed starter intake but increased growth for animals receiving the higher DM milk. Altering the chemical composition of starter also affects intake, although the wide variety of

textures and ingredients used in starters make it difficult to draw clear conclusions. Adding fibrous ingredients such as cottonseed hulls or chopped roughage to calf starter has been shown to increase intake (Terré et al., 2013; Hill et al., 2009) as calves may ingest additional feed to combat sub-acute ruminal acidosis (Laarman and Oba, 2012). Increased roughage intake in calves can also help to stimulate rumen development and reduce the incidence of parakeratosis (McGavin and Morrill, 1976; Greenwood et al., 1997).

In the post-weaning phase, the chemical composition of the diet can also alter intake. Tomlinson et al. (1997) determined that intake was maximized when the diet was 40% NDF and heifers weighed less than 200kg, or when the diet was 70% TDN and heifers weighed more than 200kg. Dietary energy and carbohydrates appear to play a large role in post-weaning feed intake, and Quigley et al. (1986) reported that the proportion of ADF to NDF was positively correlated with DM intake so long as NDF was greater than 41% DM. Predictions of DMI in growing heifers should then include body weight, ADG, and ration energy in order to maintain accuracy (Quigley et al., 1986). In studies where the same post-weaning diet is fed to all treatment groups, but amount differs, changes in feeding behavior were not noted (Quintana et al., 2018).

1.2 Mammary Development

While much of this review has focussed on optimizing early-life growth in heifers, one aspect of development that must also be considered is the development of the mammary gland. The number of mammary epithelial cells after the allometric growth phase is a major factor in determining future milk production (Tucker, 1981), highlighting the importance of

early life development. Pre-pubertal mammary development is influenced by growth rate during this same period (Sejrsen et al., 1982). Specifically, Sinha and Tucker (1969) showed that mammary DNA content increased at a rate of 1.6 times body weight in the pre-weaning phase, and at a rate 3.5 times the rate of body weight from months 3 to 9 before decreasing until puberty was attained. This data is still commonly cited, however more recent studies have shown that allometric growth of mammary tissue occurs earlier than once thought (Capuco and Akers, 2010; Esselburn et al., 2015). Using a combination of ultrasonography and dissection, Esselburn et al. (2015) demonstrated that from birth to 2 months of age the parenchyma tissue in Holstein heifer calves can increase from an average of 6.6 mm^2 to 42.1 mm^2 per quarter regardless of milk energy content. This information is especially pertinent when combined with additional data from Soberon et al. (2012) stating that a 1 kg increase in pre-weaning ADG was correlated to an increase in milk production in excess of 1000kg, and Sejrsen et al. (1982) demonstrated a reduction in parenchymal growth and future milk production potential with excessive post-weaning ADG. Although the exact mechanisms behind these relationships are not known, there appears to be a significant difference between increased growth in the pre-weaning and post-weaning phases and the effect on mammary development. Multiple studies have now reported that elevating the plane of nutrition in the pre-weaning phase can help stimulate mammary development (Brown et al., 2005b; Meyer et al., 2006; Geiger et al., 2016), or have no negative effect on mammary development in the pre-weaning phase (Daniels et al. 2009a).

The same principle does not hold true in the post-weaning phase, as feeding for high (1.1 kg) and low (0.4kg) rates of gain result in similar parenchymal growth between treatments,

but increased fat deposition for high gain calves (Brown et al., 2005b). Offering diets with elevated protein levels, in order to promote lean muscle growth and reduce fat deposition, does not result in a change in the amount of mammary DNA content (Whitlock et al. 2002). When previously weaned heifers were offered high energy diets for varying periods of time and slaughtered at a common age, total mammary gland mass was increased linearly with increased time on the high energy diet (Davis Rincker et al., 2008). However, when adjusted for carcass weight as an indicator of maturity, a linear decrease in fat free parenchymal tissue was revealed for animals spending a greater number of weeks on the high energy diet (Davis Rinker et al., 2008). Had heifers been slaughtered and eveluated once puberty was confirmed, these results may have been different due to a shortening of the allometric growth phase and the stimulatory effects of estrogen on mammary growth (Davis Rinker et al., 2008; Geiger et al., 2016). Further research is required in order to fully understand the mechanisms underlying the relationships between nutrition, mammary development, and future milk production.

1.3 Significant periods of development

A heifer's developmental life is often categorized by 3 distinct periods of development; the pre-weaning, post-weaning, and post-pubertal phases. These distinctions by phase are made in large part due to mammary gland research where pre- and post-weaning growth rates have measurable impact on future milk production as previously discussed. As such, and due to the vast resources necessary to carry out a long-term heifer study, the majority of research has focussed on the pre- weaning phase. As studies have revealed the importance of calf nutrition, additional trials involving the weaning transition and post-weaning heifers have been

published, yet these phases remain relatively understudied. The focus of this review, and of the experiment to follow, will be the pre- and post- weaning phases.

1.31 Birth to weaning

Improved nutrition and increased growth in the pre-weaning phase has most notably been connected to mammary development and future milk production. A combination of studies and meta-analyses have demonstrated the significant increases in lactation performance that are associated with greater ADG during the pre- weaning phase (Bar-Peled et al., 1997; Shamay et al., 2005; Terré et al., 2009; Moallem et al., 2010; Soberon et al., 2012; Margerison et al., 2013; Soberon and Van Amburgh, 2013; Gelsinger et al., 2016). In order for these increases to be possible, some epigenetic event during the pre-weaning phase is thought to occur, although the exact mechanisms of action are not known. In addition, other studies have not found the same influence of ADG on future milk production (Morrison et al., 2009; Raeth-Knight et al., 2009, Kiezebrink et al., 2015).

Other benefits to elevated nutrition in the pre-weaning phase also exist, in addition to future changes in milk production. Diaz et al. (2001) and Khan et al. (2007a) both determined that feed efficiency was improved in calves offered increased volumes of milk when compared to conventional feeding. As feed energy is used to perform basic body maintenance, excess energy can be used for growth, leading to increased efficiencies of gain (NRC, 2001). When growth occurs at a greater rate, increases in plasma glucose, insulin, IGF-1 and immune function have also been noted (Smith et al., 2002), as the somatotropic axis is functionally coordinated with nutrient supply. However, Nonnecke et al. (2003) did not find any significant differences in immune system components between calves offered differing volumes of milk

replacer. The variation of responses to differing nutrition in the pre-weaning phase demonstrates the need for additional research in this period of critical development.

1.32 The weaning transition

Despite the increasing number of calf nutrition studies, a paucity of information still exists regarding the long-term impacts of the weaning process in dairy heifers. The weaning process plays an important role in development, as a smooth transition from liquid feed to solid feed is essential for maximizing growth and reducing stress (Weary et al., 2009). In most studies examining the weaning process, milk intake is restricted in order to promote early weaning and cost savings. While this method of weaning increases starter intake, growth rates often suffer compared to those from calves raised on cows naturally (Flower and Weary, 2001). However, increasing milk allotment in the pre-weaning phase does not simply optimize the weaning process. The abrupt weaning of calves receiving elevated volumes of milk in the pre-weaning period can reduce solid feed intake in the post-weaning phase (Terré et al., 2007; Weary et al., 2008). Since post-weaning growth depends on the capabilities of the calf to ingest and digest solid feed, the advantages in pre-weaning growth rate may quickly dissipate if the intake of solid feed is depressed following weaning (Khan et al., 2010). Reduced growth following the weaning of calves offered high planes of milk nutrition has been attributed to poor rumen development (Khan et al., 2007b; Sweeney et al., 2010).

To address the post-weaning reduction in growth that may occur in calves offered high levels of milk in the pre-weaning phase, recommendations for gradual weaning protocols have been made. Khan et al. (2007a) recommend a step down weaning process, where a 50% milk reduction occurs over 16 days, as calves were able to maintain their growth advantage over

conventionally raised calves in the post-weaning period. In addition, behavioral studies demonstrate gradual weaning processes reduce distress at weaning through decreased cross sucking and non-rewarded feeder visits (Nielsen et al., 2008) and reduced vocalization (Jasper et al., 2008). Notably, the benefits of a gradual weaning process are only manifested when calves are 3 weeks of age or older, as calves weaned before this time are unable to increase their energy intake through starter consumption (Sweeney et al., 2010). Eckert et al. (2015) noted advantages of later weaning, where calves receiving high planes of milk and stepped down at 8 weeks of age had increased starter intake and growth compared to those calves stepped down at week 6. Improving the growth and welfare of heifer calves throughout the weaning process offers immediate benefits, but in order to determine the potential long term effects of optimal weaning strategies more research is required.

1.33 Weaning to puberty

In the same manner as increased pre-weaning gains, increased post-weaning gains in heifer calves have also been associated with increased future milk yield (Stelwagen and Grieve, 1992; Choi et al., 1997; Zanton and Heinrichs, 2005; Krpálková et al., 2014). Not all studies found this association to be valid (Van Amburgh et al., 1998; Lammers et al., 1999; Abeni et al., 2000; Radcliff et al., 2000), and some research has even suggested negative effects on mammary development when ADG is in excess of 0.7kg/day in the post-weaning period (Sejrsen and Purup, 1997). There are additional factors to consider in addition to future milk production, as heifers with increased post-weaning growth rates (>0.8 kg/day) have reduced age at first calving to 22 months (Van Amburgh et al., 1998) which can greatly reduce non-productive days on feed. A met-analyses by Bach (2011) provides support for the benefits of

this decrease, finding that age at first calving as young as 21.7 months did not reduce future cow survival. The long-term effects of post-weaning nutrition, and development during this period, require additional study in order to gain a better understanding of the mechanisms at work in calves during this time. Some potentially significant benefits in future production have been discussed (Van Amburgh et al., 1998; Radcliff et al., 2000), and improved feeding strategies and nutritional supply during this timeframe may help animals reach that potential.

1.4 Important hormones and metabolites of development

Differing early life planes of nutrition can lead to differences in feed intake, growth, and future production, as outlined above. In addition to these large scale changes, differing planes of nutrition can elicit changes in concentrations of important metabolites and hormones circulating in the developing heifer. Understanding the interplay between these metabolites and hormones, and the role they play in the many developmental changes occurring within the heifer, helps to appreciate the span of influence that nutrition plays in the early life phase.

1.41 IGF-1

While many bovine proteins have been studied in relation to early life development, insulin-like growth factor 1 (IGF-1) has perhaps been researched most heavily in recent years. IGF-1, synthesized in the liver in response to the release of growth hormone (GH) by the hypothalamus, is responsible for much of the growth-promoting action in the body (Thissen et al., 1994). As a result, plasma IGF-1 levels are an area of research interest throughout a heifer's developmental life.

Circulating levels of IGF-1 are correlated with increased feeding frequency, protein intake, and energy intake (Brown et al., 2005a). The mechanism behind this correlation has been shown to be an increased concentration of insulin and glucose in the blood stream causing a maturation of the somatotropic axis, which has been shown in both *in-vivo* and *in-vitro* studies (Brameld et al., 1999; Butler et al., 2003). Neonate nutrition therefore plays a critical role in early life IGF-1 levels, as proper colostrum feeding increases the amounts of circulating insulin and glucose in the calf (Hadorn et al., 1997). Providing greater volumes of colostrum and milk in the neonatal period leads to increased growth (Schäff et al., 2016) and plasma IGF-1 concentrations have also been positively correlated with growth (Ronge and Blum 1989; Roberts et al., 1990).

Once passed the neonatal phase, IGF-1 is thought to continue to play a major role in mammary development. Although the mechanisms remain unclear, *in-vitro* studies have shown that IGF-1 helps to mediate mammary development (Berry et al., 2003; Weber et al., 1999). This data is far from conclusive, as other studies have not detected any difference in IGF-1 components, thought to affect mammary development, as a result of dietary treatment (Meyer et al., 2007; Daniels et al., 2009b). More research is needed to fully understand the interactions between IGF-1 and mammary development.

The onset of puberty may be another event in which IGF-1 plays a significant role, as reports of increased circulating levels of IGF-1 have been noted in heifers approaching puberty (Luna-Pinto and Cronjé, 2000; Jones et al., 1991). Again, nutritional plane should be considered, as the level of nutrients available in the lead up to sexual maturation can impact the concentration of IGF-1 detectable in blood (Yelich et al., 1996). While it is clear that IGF-1 is one

of several hormones that influences puberty (Rawlings et al., 2003), the mechanisms through which it influences sexual development remain unknown (Simpson et al., 1991). Reducing its expression through vaccination led to increased age at puberty, demonstrating that IGF-1 is one of the most important metabolic mediators in sexual development (Simpson et al., 1991). However, further research in heifer calves is necessary to fully understand the many roles that IGF-1 plays in development.

1.42 Plasma glucose

In the pre-weaning phase, where calves can be considered pseudo-ruminants, plasma glucose levels are relatively high as calves derive their main energy source, glucose, from lactose in milk replacer or whole milk (Drackley, 2008). Many factors may influence plasma glucose levels, including feed intake, endogenous glucose production from the liver, and the breakdown of glycogen stored in the liver, muscle, and adipose tissue (Aronoff et al., 2004). In several pre-weaning studies, increased plasma glucose concentrations have been associated with increased volumes of milk feeding, as milk bypasses the rumen via the esophageal groove, allowing absorption to occur in the small intestine (Smith et al., 2002; Bartlett et al., 2006; Kamiya et al., 2009).

During a calf's transition to become a fully functioning ruminant, increasing amounts of energy are supplied by volatile fatty acids (VFA) generated by rumen microbes (Drackley, 2008). When solid feed is fermented in the rumen, dietary carbohydrates are consumed by the rumen microbiota, and therefore, very few are available for use or absorption by the calf (Huntington et al., 2006). Once calves are completely weaned from milk, and that source of glucose is

removed from the diet, glucose levels are greatly decreased in comparison to the pre-weaning phase. The exception to this process occurs when high concentrate diets are fed, where starch escapes or is resistant to rumen fermentation, and absorption and fermentation occurs in the small intestine. In most common feeding regimens, VFA's propionate and lactate are absorbed in the rumen and transported to the liver where gluconeogenesis occurs (McDonald et al., 2011). Studies solely examining plasma glucose levels are limited, due to the great impact of feed type and feed intake. Instead, most research focuses on glucose levels through the scope of another important metabolite: insulin.

1.43 Insulin

Insulin, made in the β cells of the pancreas, is one of the key anabolic hormones responsible for lowering glucose levels in the blood (Aronoff et al., 2004). Insulin acts to reduce glucose levels in the blood in 3 distinct ways: first by promoting glucose uptake by peripheral tissues, second by promoting glycogenesis, and third by reducing gluconeogenesis or glycogenolysis (Holst., 1994). When high blood glucose levels are detected, insulin is rapidly released into the bloodstream. Insulin synthesis and release occurs at a more moderate level until desired glucose levels are reached (Aronoff et al., 2004). In the pre-weaned calf these insulin surges occur most frequently, as the digesta (containing mostly milk) bypasses the rumen and empties directly into the small intestine. The rate with which the digesta enters the small intestine (gastric emptying) has been shown to play a major role in plasma glucose, and therefore insulin, concentrations (Aronoff et al, 2004; Tong and D'Alessio, 2014). Decreased insulin sensitivity, caused by offering limited and large milk meals over a long period, is a major concern in the veal industry where diabetic-like symptoms have been noted (Doppenberg and

Palmquist, 1992; Hostettler-Allen et al., 1993; Vicari et al., 2008). However, recent dairy research has demonstrated that high plane milk feeding does not reduce insulin sensitivity, even when calves are fed only twice daily (MacPherson et al., 2016).

As discussed above, the transition to solid feed causes a shift away from glucose and towards VFA as the primary source of energy. Insulin and glucose levels have been shown to decrease with age, as the calf's ability to clear glucose is reduced (Colvin et al., 1967; Palmquist et al., 1992, Bunting et al., 2000; Benschop and Cant, 2009; Yunta et al., 2015). As a result, studies examining post-weaning insulin levels are limited. In one study examining the interaction between pre-weaning plane of feeding and post-weaning insulin levels, Yunta et al. (2015) demonstrated decreased insulin sensitivity in the pre-weaning phase did not persist later into life. In the study, calves offered 8L of milk daily required increased concentrations of insulin to control glycemia at day 42 of life when compared to those calves offered 4L and 6L daily. At day 300, once all treatment groups had received the same post-weaning diet for more than 200 days, the 8L calves actually required decreased concentrations of insulin to control glycemia when compared to the other treatment groups (Yunta et al., 2015). As the dairy industry transitions towards elevated planes of nutrition in the pre-weaning phase, additional research regarding long term insulin implications will be necessary.

1.44 β- hydroxybutyric acid

β- hydroxybutyric acid (BHBA) is one of the main ketones produced when the VFA butyrate is oxidized by the ruminal epithelium (Bergmand, 1971; Leighton et al., 1983). Production of BHBA may also be accomplished through incomplete oxidation of fatty acids in the liver, however this generally occurs in states of negative energy balance not seen in growing

calves. Therefore, as the growing calf develops into a fully functioning ruminant, dependent on solid feed and VFA for energy, the level of BHBA in the blood may be used as an indicator of rumen development (Quigley et al., 1991).

In the pre- weaning phase, when starter intake is limited, blood BHBA levels are relatively low and rarely exceed 0.1 mmol/L (Khan et al., 2007b; Eckert et al., 2015). During the weaning transition, BHBA concentrations have been shown to increase up to 6-fold (Baldwin and Jesse, 1992; Khan et al., 2007a; Eckert et al., 2015). For this reason, recent research has shown commercially available BHBA tests to be valuable in monitoring starter intake and evaluating weaning transitions (Deelen et al., 2016). Post- weaning blood BHBA levels remain relatively constant so long as calves do not enter states of negative energy balance.

In sheep, BHBA concentrations in blood have been shown to be affected more by animal age than dietary treatment (Lane et al., 2002). In calves, the weaning transition has been shown to be a crucial period where blood concentrations of BHBA increase rapidly regardless of age (Nemati et al., 2015; Eckert et al., 2015). However, some effect of age on BHBA production does exist. Calves weaned at 28 days and sampled at 30 days had 40% of the ketone producing capacity of a mature ruminant, but when sampled at 60 days had similar ketogenic capacities to mature animals (Bush, 1988). Further study is required to fully understand the many factors which may impact BHBA levels.

1.5 Important hormones of sexual development

For producers raising heifers, the age at which puberty is attained may carry significant economic importance. Sorenson et al., 1959, Lammers et al. (1999), and Chelikani et al. (2003) have all demonstrated that heifers with increased early life growth rates attain puberty sooner,

while Brickell et al (2009) and Moallem et al. (2010) noted similar decreases in age at first breeding for heifers with improved early life growth. As a result of earlier sexual maturity and breeding, age at first calving and non-productive days on feed can be reduced. In addition, Bach (2011) determined that increased pre-weaning growth can lead to cows remaining in the milking herd for an increased number of lactations, and that the likelihood of a heifer to complete her first lactation was increased when age at first calving was decreased. While mammary development and milk production must also be considered, improving early life growth rates in order to increase the rate at which puberty is attained appears to be beneficial to producers raising heifers for use in-herd (Overton et al., 2013). This knowledge has developed with additional research, as many of the initial studies in heifer development suggested that restricting nutrient intake allowed heifers to develop into cows with increased life expectancy (Reid et al., 1957, 1964).

The attainment of puberty, defined in heifers as ovulation and subsequent normal luteal function, is influenced by a multitude of endocrine factors. While nutrition, age, and genetics may in part regulate puberty, the maturation of the hypothalamic- pituitary- ovarian axis is essential for continued cyclicity (Perry, 2016). In short, estradiol from the ovarian dominant follicle acts to reduce the release of gonadotropin releasing hormone (GnRH) from the hypothalamus in a negative feedback loop. Although its role in prepubertal animals is not well understood, kisspeptin also plays a significant role in stimulating GnRH release from neurons alongside estradiol just prior to ovulation (Estrada et al., 2006; Colledge, 2008). GnRH then acts on the anterior pituitary to cause the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH), which in turn stimulate steroidogenesis in the ovaries. As puberty approaches,

the sensitivity of the loop is reduced through a decrease in the number of estradiol receptors in the hypothalamus (Day et al., 1987), and LH is released in greater amounts, causing stimulated follicular growth to the point where a LH surge is induced (Day and Anderson, 1998). The opposite effect is true in mature and cycling animals, where estrogen released from the ovaries acts in a positive feedback loop on the hypothalamus and anterior pituitary. Many of the individual hormones that play a role in this feedback loop are discussed in more detail below.

1.51 Kisspeptin

Kisspeptin is a peptide with the capacity to greatly increase the release of gonadotropins in bovines (Kadokowa et al., 2008). The potential importance of kisspeptin is a relatively new topic, and as such, studies in cattle are few in number. Although all the mechanisms of kisspeptin action are not yet fully understood, it appears that kisspeptin is capable of directly signalling for the release of GnRH (Colledge, 2008) through increased firing of GnRH neurons (Han et al., 2005). In cattle this has been shown to be true, as both beef and dairy heifers have responded to administration of kisspeptin with surges of LH (Kadokowa et al., 2008; Ezzat-Ahmed et al., 2009). The response to kisspeptin injection in pre-pubertal bovines appears to be influenced by several factors. Echeverría et al. (2014) noted repeated kisspeptin injection is capable of triggering ovulation in pre-pubertal heifers, although no normal estrous cycle followed. In the same study, heifers who ovulated had increased plasma IGF-1 concentrations, and decreased serum leptin concentrations compared to those who did not ovulate (Echeverria et al., 2014). Increased study of the effects of kisspeptin in cattle are expected, as studies in other species such as rats, mice, and humans continue to show evidence for the many ways kisspeptin may influence the onset of puberty.

1.52 Gonadotropin releasing hormone

As previously mentioned, GnRH is released from the hypothalamus in response to kisspeptin and estradiol, and travels via the bloodstream to the anterior pituitary. At the anterior pituitary, the presence of GnRH stimulates the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH). One of the major events that must occur in order for puberty to be attained in cattle is the increase in pulse frequency of LH (Schams et al., 1981), therefore the upstream release of GnRH plays a vital role in sexual maturation. The importance of GnRH in the attainment of puberty has been demonstrated in beef heifers, where immunization against GnRH has resulted in puberty being delayed 112 to 172 days, depending on the number of injections (Prendiville et al., 1995). In the same manner, injection of exogenous GnRH has been shown to stimulate LH release in pre-pubertal heifers (Barnes et al., 1980), post-partum transition cows, (Fernandes et al., 1978), and post-pubertal bulls (Thibier, 1976). In addition, superovulation of heifer calves at ages as young as one month is possible when GnRH is combined with LH, but not when GnRH is solely administered (Seidel et al., 1971).

The release of GnRH from the hypothalamus is influenced by several factors, including those from the metabolic, neuroendocrine, and gonadal systems. From a neuroendocrine perspective, the rate of neuron development affects the rate at which GnRH is released from the hypothalamus. Kisspeptin has also been discussed, but neuropeptide Y (NPY) and agouti-related protein (ARP) neurons are two of the most commonly examined due to their close proximity and direct actions upon GnRH neurons (Li et al., 1999; Allen et al., 2012). Ovariectomized cows treated with NPY have reduced amounts of circulating LH due to a

reduction in GnRH secretion (Gazal et al., 1998), and changes in NPY concentrations are thought to be mirrored by ARP expression (Allen et al.,2012). In addition, NPY and ARP neuron levels of expression are closely tied to nutritional status, where high planes of nutrition elicit decreased NPY and ARP neuron expression and an increased release of GnRH (Allen et al., 2012).

Both chronic and acute nutritional restriction are known to slow the rate at which puberty is attained in heifers (Day et al., 1984; McCann and Hansel, 1986; Chelikani et al., 2003), however these delays are thought to occur as a result of supressed LH secretion. A study by I'Anson et al. (2000) using sheep determined nutrient restriction to directly inhibit the release of GnRH. Interestingly, some research suggests that short term fasting in normally fed cattle can lead to increased release of GnRH through the actions of leptin (Zieba et al., 2004, Amstalden et al., 2014), though that will be discussed in greater detail is section 1.55. In the same manner that inadequate nutrition may hinder sexual development, proper nutrition can help to hasten sexual development (Chelikani et al., 2003). Hormones and metabolites such as insulin, leptin, IGF-1, glucose, and fatty acids are all thought to act as signalling molecules that are able to act on hypothalamic neurons to increase the secretion of GnRH (Crown et al., 2007).

Lastly, estradiol from the ovaries may also impact the release of GnRH. The action of gonadotropins (LH and FSH) support increased steroidogenesis in the growing follicles of the ovary, increasing the release of estradiol, and reducing the sensitivity of estradiol negative feedback at the hypothalamus. While the mechanism explaining the change from negative feedback to positive feedback are unclear, the importance of estradiol secreted by the ovary is apparent in ovariectomized heifers where removal of the gonads reduces LH release (Day et al.,

1984). Subsequent administration of estradiol to the ovariectomized heifers increased the release of LH to expected levels (Day et al., 1984). The impact of nutrition on estradiol release from the ovaries warrants further study, as low planes of nutrition have been reported to decrease LH and FSH release, regardless of plasma estradiol concentration (Mackey et al., 1999). Since estradiol from the ovary is required for GnRH release, these results suggest possible mediation by other factors such as leptin, or NPY when animals are undergoing a nutritional challenge.

1.53 Estradiol

The source of estradiol in the ovary are the follicles, the predominant source being the dominant follicle. Follicular development occurs in a wave-like manner and has been documented in animals as young as 2 weeks of age (Evans et al., 1994). As animals age and develop, selection of the dominant follicle from the recruited cohort occurs alongside changing concentrations of FSH, inhibin, and estradiol (Reviewed by Taya et al., 1996). In the peripubertal period, the maximum diameter of the dominant follicle is increased until puberty occurs (Bergfeld et al., 1994; Evans et al., 1994). As wave after wave of development repeats and the maximum diameter of the follicle increases, the concentration of estradiol also increases until puberty is attained (Melvin et al., 1999). The increased concentration of estradiol is the combination of multiple factors, but can partially be explained by a reduction in the negative feedback loop at the hypothalamus. Even when the ovaries are removed from a heifer, a decline in the negative feedback system still occurs if exogenous estradiol is supplied (Day et al., 1984). It is hypothesized that one of the main mechanisms involved in the decline of

negative feedback is the down-regulation of estradiol specific receptors in certain regions of the hypothalamus (Day et al., 1986; Day and Anderson, 1998).

The sensitivity of the negative feedback loop can also be effected by dietary restriction, as reduced energy intake can prolong this phase (Kurz et al., 1990). However, the opposite effect is also true, as a combination of early weaning and high concentrate feeding has been shown to decrease the age at which reduced estradiol sensitivity occurs in the bovine hypothalamus (Gasser et al., 2006a).

1.54 Luteinizing hormone

In the peripubertal period, coinciding with the decreased sensitivity of the negative feedback system involving estradiol and the hypothalamus, LH pulses are released with greater frequency from the anterior pituitary (Day et al., 1984). The increased frequency with which LH is released causes increased growth of the dominant follicle and increased estradiol production, which feeds back to the hypothalamus to stimulate the pre-ovulatory LH surge (Bergfeld et al., 1994). Due to the nature of its release, LH data is commonly analyzed as both frequency and amplitude of pulse.

In pre-pubertal heifers, circulating concentrations of LH reportedly increase until approximately 3 months of age, before declining slightly up until the peripubertal surge occurs (Evans et al., 1994). It is therefore logical that multiple studies have shown the removal of the ovaries, from which estradiol is supplied, to cause an immediate increase in the release of LH in pre-pubertal heifers (Beck and Convey, 1977; Kiser et al., 1981; Day et al., 1984). Pulse frequency, but not amplitude, is also increased in heifers ovariectomized once having already attained puberty (Enright et al., 1994).

As is the case with many of the other hormones affecting sexual development, nutrition can significantly impact LH release. Pre-pubertal heifers feed restricted for more than 100 days did not demonstrate the characteristic peri-pubertal surge in LH at any point during restriction (Kurz et al., 1990). In chronically feed restricted heifers, reduction in the frequency of release and mean concentration of LH have been noted (Imakawa et al., 1986), though this reduction is hypothesized to be as a result of limited GnRH release. Chronic restriction is not necessary for the reduction of LH secretion, as weaned heifers fed for growth rates of 0.5kg ADG have significantly fewer LH pulses than those fed for an ADG of 1.1kg when sampled at 10 months of age (Chelikani et al., 2003). The long term effects of feed restriction are limited, as restricted heifers allowed realimentation return to normal LH release patters in less that 14 days (Kurz et al., 1990). In studies using heifers already cycling, no differences in LH release between heifers fed a maintenance diet or restricted diet have been noted, despite restricted heifers losing in excess of 15% of body weight (Rhodes et al., 1995; Stagg, 2000).

1.55 Leptin

Leptin is thought to be the hormone primarily responsible for communicating the nutritional status of the body to the reproductive axis in mammals. As it is mainly secreted by adipose tissue, leptin concentrations are highly correlated with total body fat mass (Zieba et al., 2005). Models in mice and humans have shown that low leptin concentrations due to reduced fat reserves divert energy away from reproductive functions (Ahima and Flier, 2000). It appears likely that a certain level of body fat must be attained before puberty is attainable (Chelikani et al 2003), demonstrating the permissive role that leptin plays in the cascade of events necessary for puberty to occur (Chelikani et al., 2009).

While leptin alone has not been established as capable of inducing puberty in heifers (Maciel et al., 2004), mean serum concentrations of leptin have been shown to linearly increase as puberty is approached in beef and dairy heifers (Diaz-Torga et al., 2001; Garcia et al., 2002). The linear increase has not been noted in all studies, as Block et al. (2003) found that plasma leptin concentrations only increased steadily in heifers where puberty was delayed. Similarly, in another study examining Holstein heifers, plasma leptin levels only increased in advance of puberty in heifers grown at low (0.5kg ADG) or medium (0.8kg ADG) rates of gain, but not for heifers grown quickly (1.1kg ADG) (Chelikani et al., 2009). The lack of increase in pre- pubertal leptin concentrations for heifers grown quickly suggests there are multiple factors at play besides leptin. However, the linear increase of leptin concentrations, coupled with data showing that leptin infusions in fasted ovariectomized cows can cause a rapid increase in LH (Amstalden et al., 2002), provide support for the role of leptin in the process of sexual maturation.

Post-weaning changes in plasma leptin concentrations are relatively easily detected, as evidenced above. The same may not be true in the pre- weaning phase, as Block et al. (2003) noted varying levels of early life leptin in bull calves receiving low planes of nutrition. For bull calves receiving high planes of nutrition, an increase in plasma leptin was detectable during week 3 of life (Block et al., 2003). Consequently, week 3 is the time increases in fat deposition were noted (Diaz et al., 2001). These results are also thought to apply to heifer calves, as early life growth curves are quite similar regardless of sex (Kuehn et al., 1994). Currently, there appears to be a knowledge gap regarding the influence of early life nutrition on leptin

concentrations, and how these may change for animals as they age and experience stressors such as weaning or dietary change.

1.56 Progesterone

Progesterone, often referred to as the hormone of pregnancy, also plays some role in the attainment of puberty in heifers. In order for producers to efficiently raise heifers and calve them at 2 years of age, normal estrous cycles are recommended to be occurring at 12 to 13 months of age (Heinrichs et al., 2013). In normally developing heifers, transient increases in progesterone occur before the initiation of puberty (Berardinelli et al., 1979). The exact mechanisms of this progesterone increase are not well known, although it has been hypothesized that the source of these progesterone surges are the ovary (Berardinelli et al., 1979). Progesterone can stimulate an increase in follicular growth in the ovary (Wetteman and Hafs,1972; Sheffel et al.,1982), which in turn increases the amount of estradiol secreted. Therefore, exogenous administration of progesterone, or similar progestogens, can induce puberty in pre-pubertal heifers when estradiol is also administered (in order to simulate natural hormone fluctuations) (Gonzalez-Padilla et al., 1975; Short et al., 1976; Berardinelli, 1979; Sheffel et al., 1982; Smith and Day, 1990).

1.57 IGF-1

Lastly, IGF-1 is also thought to play a significant role in the attainment of puberty in heifers. Radcliff et al. (2004) offered high and low planes of nutrition in the post-weaning phase and documented increased concentrations of IGF-1 in high plane heifers, and a reduction in age at first ovulation. Gasser et al. (2006b) fed diets for high rates of gain, hypothesized to increase

IGF-1 concentration, and found ovarian development was enhanced when compared to heifers fed for lower rates of gain. IGF-1 is thought to target ovarian follicles in prepubertal heifers, where it is involved in the process of regulating follicular growth and development (Webb et al, 1999; Renaville et al, 2002). Therefore, greater concentrations of IGF-1 may lead to increased rates of ovarian follicular development, increasing estradiol concentrations and hastening the onset of puberty through a combination of the previously described mechanisms (Marquivar and Day, 2009).

1.6 Summary and research objectives

The early life nutritional management of dairy heifers has become an area of increased focus due in large part to the significant impacts that this phase can bestow on future production. Although the cost of feeding replacement heifers until first lactation makes up a significant portion of the heifer rearing budget, limiting liquid feed intake to reduce feed costs is continually decreasing in popularity due to associations with reduced growth. Instead producers are beginning to offer increased milk volume at approximately 20% of BW, a level near what calves consume on cow, in order to capitalize on improved growth, health, and future production for these calves. The majority of research in calf nutrition has taken place during the pre-weaning phase, largely due to the previously listed benefits. The post-weaning and post-pubertal phases remain relatively understudied in comparison. Additionally, interactions between the pre- and post-weaning phases are rarely discussed, although weaning is recognized as a critical transition phase in development. Few recommendations for feeding strategies from birth to puberty are available, in large part due to many unknowns in terms of

growth, mammary development, and sexual development. Therefore, the objectives of my research were to:

1. Determine the effects of pre- and post-weaning diets, with differing energy levels, on the intake, growth, and metabolic development of Holstein heifer calves.

2. Monitor sexual development, including LH release, leptin concentration, and onset of puberty, as potentially affected by pre- and post- weaning diets with differing energy levels in Holstein heifer calves.

3. Identify potential interactions between pre- and post- weaning diets on overall heifer development.

2.0 Chapter 1: The effects of differing planes of pre- and post-weaning phase nutrition on intake, growth, and key hormone and metabolite concentrations in Holstein heifer calves.

2.1 Introduction

In recent years, research focus on the growth and development of replacement heifer calves on dairy operations has increased due to greater priority being placed on successful early life development of these animals due to short and long-term implications on health, welfare, and production. Improved early life nutrition has led to improved immune response (Ollivett et al., 2012), welfare (Thomas et al., 2001; Krachun et al., 2010), and growth rate similar to those that occur in suckling calves (Drackley, 2008). While technologies, management, and genetics all play a role in improving heifer development, enhancing nutritional provision may be one of the most cost-effective ways of doing so. The majority of the costs associated with rearing heifers from birth to first calving are incurred due to feed (Heinrichs et al., 2013), yet providing more high quality feed and improving early-life growth rates can reduce heifer development costs due to decreases in veterinary expenses, labor, and housing, and increases in future production (Overton et al., 2013). Therefore, altering early-life nutritional provision from conventional feeding schemes can potentially improve the efficiency of heifer rearing and profitability of operations.

The majority of the nutritional studies conducted with heifer calves focus on the pre-weaning phase of life. Improving nutritional provision in the pre-weaning phase, generally through increased volumes of milk offered or greater milk protein content, has led to increased intake (Khan et al., 2011), growth (Diaz et al., 2001), blood concentrations of IGF-1 and leptin

(Brown et al., 2005a), mammary development (Geiger et al, 2016), and lifetime production (Bach, 2011; Soberon et al., 2012). A limited number of studies have also focussed on increasing growth in the post-weaning or pre-pubertal phase, generally through the provision of high energy and protein content concentrates (2.8 Mcal/kg ME and 19.7% CP, Radcliff et al., 2000; 3.6 Mcal/kg and 20% CP, Brown et al., 2005). In these post-weaning studies, results indicate improved growth and gain:feed ratio (Brown et al., 2005a), reduced age at first breeding (Radcliff et al., 2000) and first calving (Heinrichs et al., 1993; Radcliff et al., 2000), and reduced feed costs through a reduction in non-productive days on feed (Overton et al., 2013). The primary concern with increased early life growth is that the growth of the mammary gland will be impaired, through increased fat deposition or a shortening of the allometric growth phase (Sejrsen and Purup, 1997). There is little understanding of the mechanisms that may be responsible for these potential production-reducing changes, and varied data regarding mammary development and the effects of feed energy and protein (Whitlock et al., 2002; Davis Rincker et al., 2008), rate of growth (Sejrsen and Purup, 1997; Brown et al., 2005a; Davis Rincker et al., 2008), age at the time of accelerated growth (Capuco and Akers, 2010; Geiger et al., 2015), and effect on lifetime production (Radcliff et al., 2000; Morrison et al., 2009; Soberon et al., 2012). The paucity of studies examining the effects of both pre- and post – weaning planes of nutrition further complicates attempts to rear heifer calves in the most optimal manner possible.

Further study regarding the overall effects of early-life nutrition in Holstein heifer calves is necessary in order to improve and optimize the rearing process, especially in the pre- and post- weaning phases. We hypothesized that when differing planes of nutrition are offered in

the pre-weaning period, the influence of post-weaning diets on overall development will be dependent on the diet offered pre- weaning.

The objectives of the present study were to determine the effects of pre- and post-weaning diets, with differing energy levels, on:

1) daily feed and ME intake for heifer calves from birth to 6 mo of age

2) the growth of heifers from birth to 6 mo, as measured by weekly BW gain and body measures

3) the concentrations of key metabolites and hormones, including glucose, insulin, IGF-1, leptin, BHBA, and rumen VFA.

2.2 Materials and Methods

2.21 Animals

Thirty-six Holstein heifer calves, born between February and August 2017 at the University of Alberta Dairy Research and Technology Centre (Edmonton, AB, Canada), were used in this study (Mean BW ± SD = 39.07 ± 3.50kg). Animals were randomly assigned to either a low (n = 18; 5 L/d) or high (n = 18; 10 L/d) pre-weaning diet of pasteurized whole milk, from wk 1 to 8.5, and to either a low (n = 18; 30% straw and 70% concentrate) or high (n = 18; 15% straw and 85% concentrate) ad-libitum post-weaning dry TMR diet (described in detail in 2.22), from wk 9 to 25 of age, in a split plot design. As a result of the dietary treatments, 4 treatment groups were assigned and included: high plane pre-weaning, high plane post-weaning (HH), high plane pre-weaning, low plane post-weaning (HL), low plane pre-weaning, high plane post-weaning (LH), and low plane pre-weaning, low plane post-weaning (LL). Free choice water was

available to all calves for the entire study, in which calves were enrolled from birth until 6 mo of age. All procedures were approved by the Animal Care and Use Committee at the University of Alberta (AUP 00001553) and conducted according to guidelines of the Canadian Council of Animal Care (Ottawa, ON, Canada).

2.22 Feeding and housing

A feeding and housing summary for the heifers in the current study is available in Figure 2-1. Calves were allowed to be licked by the dam before being placed in individual stalls bedded with wood shavings and wheat straw. Within 2h of birth, all calves were offered 2L of powdered colostrum (minimum guaranteed analysis bovine IgG 60g/L; Headstart Bovine Dried Colostrum, The Saskatoon Colostrum Company, Saskatoon, SK, Canada) via bottle. All refusals from the bottle feeding were delivered to the calves via esophageal tube. After the first feeding, all calves were offered 3 additional 2L feedings of whole pooled pasteurized (heated to 60°C for 60 min) colostrum 8,16, and 24h after the first feeding. Only colostrum measuring >23% with a BRIX refractometer (Misco Refractometer, Solon, OH, USA) was used, and therefore minimum IgG concentration of the pooled colostrum was greater than 50g/L according to work by Bielmann et al. (2010). After the four colostrum feedings, calves were trained to use a Calf Rail system (Foerster-Technik, Engen, Germany) where 2.5L meals of whole milk were delivered four times daily to high plane (H) calves, and twice daily to low plane (L) calves. The portable calf rail system offered milk to calves based on sensors assigned to individual pens, and the system was calibrated weekly to ensure accurate measures. Feeding times were 0615, 1130, 1630, and 2200h for H calves, and 0615 and 1630 for L calves. The milk fed to calves was sampled daily throughout the experiment and averaged 24.8% ± 0.32 CP (DM), and 30.3% ±

0.68 CF (DM), while ME content was calculated to be 4.58 Mcal/kg. From d7 ± 1 to d21, calves had access to free choice textured starter (22.4% CP, 7.6% crude fibre, 2.9% CF, 22.3% starch, textured; Trouw Nutrition, Guelph, ON, Canada) from a bucket, which was emptied and weighed back twice weekly to monitor intake. *Ad libitum* access to water was also available to all calves in this period from an identical bucket. At d21 ± 1, calves were moved to a group pen bedded with wheat straw and offered milk through a stationary automated feeder (CF1000+, DeLaval Canada, ON Canada). The same starter continued to be offered free choice using BioControl bunks (BioControl, Rakkestad, Norway), which identify calves through radio frequency identification tags and monitor feed disappearance on a calf by calf as-fed basis. BioControl bunks were calibrated on a bi-weekly basis and debris removed from scale load cells daily to ensure accurate measurements. Free choice chopped straw (Skyline Harvest, Blumenort MB, Canada, 1-inch chop length, 4.6% CP, 71.6% NDF, 1.6 Mcal/kg ME) was made available to the all calves via Biocontrol bunks on d21, however intake was not monitored due to small amounts ingested and the confounding effect of straw bedding. All feed intake data were collected on an as-fed basis, and DMI was later calculated based on monthly DM analysis of feed.

A 10d weaning transition began at d50, where the total volume of milk was reduced by 10% each day, regardless of treatment group. All calves were completely weaned from milk following d60. Calves continued to have access to separate straw and starter for 5 days, however on d65 these components were combined to form a dry TMR (85% starter, 15% chopped straw by weight, Table 2-1) which all calves had access to until d80. Calves were transported to an outdoor group housed facility at day 70±1. Three paddocks bedded with

wheat straw, and equal shelter, were available to the calves within each paddock. Calves were fitted with a magnetic collar and assigned an individual automated feed bunk (American Calan, Northwood, NH, USA). Upon arrival, calves were individually penned until adjusted to the new feeding system. The maximal time necessary to completely train calves to use the feeding system was 60h. On d80 (after a 15d TMR acclimation period) the L calves were abruptly changed to the L post-weaning treatment feed containing 70% starter and 30% chopped straw by weight (Table 2-1) while the H calves continued to receive the H ration until day 180. All calves were offered feed at a rate of approximately 3.5% of BW immediately following weaning, and feed allocation was adjusted daily to allow for *ad libitum* intake and refusals less than 10%.

2.23 Sample and Data Collection

Feed intake was recorded on a daily basis throughout the study. Once calves were transitioned to the TMR, the amount of feed refused was weighed before feeding each day. Feed allocation was increased by 0.2kg when refusals were <10%, and decreased when refusals were >20% for 3 consecutive days. Feed was mixed once weekly for each dietary treatment.

Blood samples were collected once weekly 2.5h after the morning feeding. Calves were manually restrained and a total of 20mL of blood was collected from the jugular vein and placed into tubes containing silica (10mL) or lithium heparin (10mL) (BD Vacutainer, Franklin Lakes, NJ, USA) for harvest of serum and plasma, respectively. Serum tubes were rested at room temperature for 1h at a 45°angle before centrifugation. Immediately following collection, 5μl of aprotinin (100μg/mL) was pipetted into the plasma tubes, which were inverted 5 times, and placed on ice before centrifugation. Blood samples were centrifuged for 20 min at 4°C and 3000 *g* before the supernatant was aliquoted into four 1.5mL microcentrifuge tubes and stored

at -20°C until analysis for glucose, insulin, IGF-1, and BHBA. While samples were collected each week, sample analysis for parameters in the blood occurred on a bi-weekly basis starting at wk 1. Following blood sample collection, body measures were taken for all calves. Measures included weight, hip height, wither height, heart girth, and barrel girth. Average daily gains (ADG) were calculated on a 4-week basis in order to reduce variability due to calves being weighed once weekly.

Rumen fluid and BCS were collected at the end of wk 4,8,12,16,20, and 24. BCS was determined by a single trained observer, blind to the treatment assigned to the calf. Rumen fluid was collected using an adapted version of the process and equipment described by Geishauser (1993). In summary, a custom made bronze probe (Grayco Machine, Leduc, AB, Canada) was attached to a 4 foot reinforced vinyl pipe (Watts Canada, Burlington, ON, Canada) and introduced to the rumen via the esophagus. Once the probe was within the rumen, a 100mL syringe was attached to the end and drawn back to collect fluid. Approximately 20mL of rumen fluid was collected and filtered through cheese cloth (Uline Canada, Milton, ON, Canada), transferred to 2 sterile 10mL tubes, and frozen using liquid nitrogen before being stored at -20°C until analysis.

2.24 Sample Analysis

Concentrations of plasma glucose were determined by using a quantitative enzymatic assay combining capsules of PGO Enzyme Preparation (Sigma-Aldrich Canada Co., Oakville, ON, Canada) and dianisidine dihydrochloride (Sigma- Aldrich Canada Co., Oakville, ON, Canada). Standard curves from 0 – 100 mg glucose/mL were then prepared using distilled water and stock solution and ran in duplicate. Samples (10μl) and stock solution (300μl) were combined in

a plate with flat-bottom wells, shaken for 10 seconds, and allowed to sit at room temperature for 45 min while covered with foil. The absorbance of the samples was read at 450nm by a SpectraMax 190 plate reader (Molecular Devices Corp., Sunnyvale, CA, U.S.A) in duplicate. Inter and intra assay CV were 1.48% and 1.03%.

Plasma concentrations of insulin were analyzed using a competitive solid-phase immunoassay previously described by Takahashi et al. (2006). Plates were coated with anti-guinea pig gamma globulin antiserum (second antibody) before diluted antibody to human insulin (first antibody) was added and incubated overnight. Following incubation, the plates were washed and standards and plasma samples were added to the wells and again allowed overnight incubation. On the 3rd day, europium labelled insulin was added to each well and incubated at 6°C for 2h before the plate was washed, enhancement solution added to each well, and fluorescence measured. Inter and intra assay CV were 12.38% and 7.13% respectively.

Plasma BHBA concentrations were determined using the enzymatic oxidation of BHBA to acetoacetate with 3-hydroxybutrate dehydrogenase. Briefly, 4.884g of Tris (hydroxymethyl) aminomethane was diluted to a pH of 9.0 using distilled water. Plasma samples and standards were added to the plates and shaken for 15 seconds before being placed in the reader at 340 nm. Initial readings were recorded before 10µl 3-Hydroxybutyrate dehydrogenase (Hoffman- La Roche LTD, High River, AB, Canada) was added to each well and again agitated for 15 seconds. Plates were covered with a plate lid before being incubated at 38.5°C for 1h, and once again read at 340nm. The increase in absorbance is directly proportional to the amount of BHBA in the sample, and inter and intra CV were 4.50% and 2.78%, respectively.

Serum IGF-1 concentrations were quantified using an IMMULITE IGF-1 kit, which is an established solid-phase, enzyme-labeled chemiluminescent immunometric assay (Siemens Healthcare Limited, Oakville, ON, Canada). Sample analysis was performed at the Endocrine Service Lab, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada. The overall inter and intra assay CV were 5.1% and 4.5%, respectively.

Leptin concentrations were determined by competitive enzyme immunoassay as described by Sauerwein et al. (2004). Briefly, samples were analyzed using specific polyclonal antisera against leptin from rabbits and biotinylated recombinant ovine leptin as tracer. Samples were loaded in duplicate to pre-incubated microtitre plates containing sheep anti-rabbit-Fc fragment antibodies and assay buffer, and antiserum was also added. After incubation at room temperature for 16 h, the biotinylated tracer was added and the amount of tracer bound was quantified by streptavidin-peroxidase as described by Hennies et al. (2001). The inter and intra assay CVs were 9.1% and 8.8%, respectively.

Total VFA concentration of ruminal fluid was determined by gas chromatography (GC) as described by Schlau et al. (2012). In brief, 25% phosphoric acid was added to thawed rumen fluid samples at a volume rate of 1:4 (phosphoric acid: rumen fluid sample) before centrifugation. Gas chromatography vials were loaded with 1mL sample solution and 200µl internal standard solution and assayed in duplicate on a Varian gas chromatograph (Model 3400). Inter and intra CV for VFA concentrations were 0.65% and 0.93%, respectively.

2.25 Statistical analysis

To determine the effect of dietary treatments, all data were analyzed using the Statistical Analysis System 9.4 (Studio 3.7 platform, SAS Institute, Cary, NC). For repeated measurements obtained daily and weekly, including intake, BW, body measures, blood metabolites and hormones, the GLIMMIX procedure was used. Fixed effects of the model included pre-weaning treatment, post- weaning treatment, day or wk, and interactions, while birth BW was included as a covariate. Calf was considered the repeated measures subject, and the covariance structure with the lowest Bayesian information criterion (BIC) was chosen. Interactions were always initially included in the model; however they were removed from the final model if their effects showed $P > 0.10$. Post-hoc tests were performed using Bonferroni adjustment and least squares means (LSM) by week assessed using the SLICE command, which allows for a partitioned analysis of the LSM for an interaction. Normality and homoscedasticity of the residuals were assessed graphically using standardized residuals, and one extreme value each for insulin and BHBA were identified as outliers and removed. All values reported are LSM ± standard error with significance declared at $P \leq 0.05$ and tendencies at $P > 0.05$ and ≤ 0.10.

2.3 Results

2.31 Feed and energy intake

Offering increased volumes of milk in the pre-weaning phase allowed for greater average daily milk intake for high plane calves (7.67L/d vs. 4.47L/d, SE= 0.09, $P< 0.0001$) compared to low plane calves (Figure 2-1 A). No differences in daily starter intake between treatment groups in the pre-weaning phase were noted until wk 7 where on average low plane calves ingested more starter daily until the end of the pre-weaning phase compared to high plane calves (wk 7: 225.28 g/d vs. 847.95 g/d, SE = 100.51, $P = 0.002$, wk 8: 475.21 g/d vs.

1,293.43 g/d, SE = 100.51, *P* < 0.0001, wk 9: 1,467.42 g/d vs 2,193.14 g/d, SE 100.51, *P* < 0.001, Figure 2-2 A). Pre-weaning ME intake was greater in high plane calves in wk 2 through 7, however low plane calves had greater ME intake during wk 9 (Figure 2-2 C).

In the post-weaning phase, DMI was not affected by pre-weaning plane of nutrition (overall *P* = 0.42), and therefore only the post-weaning treatments are shown in figures. Heifers that were offered the high plane diet post-weaning had greater DMI than their low- plane counterparts in all wk but 10 and 11 (Figure 2-2 B). Likewise, ME intake in the post-weaning phase was greater for high plane calves in wk 10 through 25 when compared to low plane post-weaning calves, regardless of pre-weaning dietary treatment (Figure 2-2 D).

2.32 Growth and feed efficiency

Heifers offered the high plane diet in the pre-weaning phase weighed more than low plane heifers in wk 2 through 8 (Figure 2-2 E), and also tended to weigh more in wk 9 (87.66kg vs. 80.76kg, SE = 0.72, *P* = 0.054) and 10 (96.57 vs. 90.56, SE = 0.72, *P* = 0.09). The ADG of high plane heifers was greater in wk 1 through 4, but did not differ from low plane heifers in wk 4 through 8 (Figure 2-3 A). Pre-weaning feed efficiency, measured as average weekly ME intake per weekly gain tended to be influenced by diet (*P* = 0.052), but differences between treatments were only noted during wk 3 (Figure 2-3 C).

No interactions between pre- and post- weaning planes of nutrition on BW or ADG were detected, and therefore data from the pre- and post-weaning phases are presented separately. In the post-weaning phase, plane of nutrition did not have an effect on BW until wk 16, where heifers offered the high plane feed in the post-weaning phase weighed more than their counterparts for the remainder of the trial (Figure 2-1 F). Post- weaning ADG, though not

differing in wk 8-12, 16-20, or 20-24, were greater for high plane heifers than low plane heifers in wk 12-16 (Figure 2-3 B). Overall post-weaning feed efficiency was not effected by diet (P = 0.13), however high plane post-weaning heifers were numerically less efficient than low plane heifers from wk 20 – 25, with a weekly difference noted at wk 21 (Figure 2-3 D). Body condition scores, taken every 4 weeks, tended to be greater in high plane pre-weaning calves at wk 4, and greater in high plane post-weaning calves at wk 12, 16, 20, and 24 (Figure 2-2 E and F).

Influences of diet on body measures were evident and are summarized in Figure 2-4. Heart girth was equal at birth, but increased in high plane pre-weaning calves from wk 2 – 9. Pre-weaning withers height and hip height tended to be greater or were greater in high plane heifers in wk 6,7,8, and 9. Again, no interactions between the pre- and post- weaning phases were noted, and data is displayed as such. Post-weaning plane of nutrition affected hearth girth (P = 0.02), where high plane heifers had increased girth from wk 16-25, excluding wk 17. Pre-weaning high plane heifers also had increased girth in wk 10 (105.73 cm vs. 103.41 cm, SE = 0.81, $P < 0.05$) ,11 (108.01 vs 104.88, SE = 0.81, $P < 0.01$) and tended to have increased grith in wk 12 (109.58 vs. 108.97, SE = 0.81, P = 0.07) of the post-weaning phase, regardless of post-weaning diet. There was no overall effect of post-weaning diet on hip (P = 0.26) of withers height (P = 0.23), however high plane heifers tended to be taller at the shoulder in wk 23, 24, and 25 and taller at the hip at wk 16.

2.33 Hormones and metabolites

Concentrations of plasma glucose did not differ between pre-weaning treatments in wk 1 or 9, however high plane heifers had or tended to have greater concentrations during sampling at wk 3, 5, and 7 compared to low plane heifers (Figure 2-5 A). No interactions

between the pre- and post- weaning phases were noted, and post-weaning concentrations of glucose did not differ between treatments (Figure 2-5 B).

Plasma concentrations of insulin in the pre-weaning phase were greater in wk 1 and and lower in wk 9 for calves offered the high plane compared to those offered the low plane (Figure 2-6 A). No interactions between pre- and post- weaning diets on insulin concentrations were noted, and so post-weaning results are presented regardless of pre-weaning dietary plane. In the post-weaning phase, insulin concentrations were elevated in wk 19, 23, and 25 in heifers offered the high plane TMR in comparison with those offered the low plane TMR post-weaning (Figure 2-6 B).

Serum IGF-1 concentrations were affected by dietary treatment in the pre-weaning phase, where heifers offered the high plane of nutrition had increased concentrations at wk 3, 5, and 7 (Figure 2-6 C). No interaction between pre- and post-weaning planes of nutrition was detected. Post-weaning diet influenced serum IGF-1 concentrations as heifers offered the high plane TMR in the post-weaning phase had greater concentrations than low plane heifers in all measured weeks save for wk 11 (Figure 2-6 D).

Pre-weaning serum leptin concentrations were greater for high plane than low plane heifers in wk 1, 3 and 5, but not affected by diet in wk 7 or 9 (Figure 2-6 E). There were no interactions between pre- and post-weaning planes of nutrition and therefore data is presented based only on pre- or post-weaning plane of nutrition, however calves receiving the high plane pre-weaning treatment had greater leptin concentrations at wk 11 (2.72 ng/mL vs. 2.19 ng/mL, SE = 0.17, $P = 0.03$), 13 (2.76 vs. 2.00, SE = 0.23, $P = 0.02$), 15 (3.20 vs. 2.23, SE = 0.28, $P = 0.03$) and tended to have greater concentrations at wk 17 (3.04 vs. 2.51, SE = 0.22, $P = 0.09$) than

those offered the low plane in the pre-weaning phase, regardless of post-weaning plane of nutrition. In all treatment groups, leptin concentrations decreased from birth to wk 13, before increasing until wk 25. In the post-weaning phase, serum leptin concentrations were reduced in low plane heifers, compared to high plane heifers, from wk 17 onward with the exception of wk 23 (Figure 2-6 F).

Plasma BHBA levels were reduced in pre-weaning high plane in wk 7 and 9 (Figure 2 -5 C). In the post-weaning period, post-weaning diet increased BHBA concentrations in high plane calves, regardless of pre-weaning diet, in wk 17, 19, and 21 (Figure 2-5 D). Overall, there was a tendency for an interaction between pre- and post-weaning planes of nutrition (P = 0.054), where low pre-weaning high post-weaning heifers had increased BHBA concentrations through much of the post-weaning process, and most notably at wk 17 (HL = 10.09 mg/dL LL= 6.23 mg/dL, SE = 0.92, P = 0.04).

At the wk 8 rumen fluid sampling, calves offered the low plane of nutrition in the pre-weaning phase had increased concentrations of propionic acid compared to those offered the high plane of nutrition, however none of the other VFA measured in the pre-weaning phase differed in concentrations between treatments (Appendix Table A1). Again, no interaction between pre- and post- planes of nutrition and VFA concentrations were detected. In the post-weaning phase, where samples were taken at wk 12, 16, 20, and 24 there was an effect of diet on total VFA production as well as on several of the specific VFA analyzed (Appendix Table A1).

2.4 Discussion

The novelty of the present study is largely based in the examination of both the pre- and post- weaning phases as well as monitoring of overall heifer development from birth to 6 mo of

age. Few studies have taken into account both the pre- and post- weaning phases, although the important role nutrition plays in each phase is evident (Radcliff et al., 2000; Diaz et al., 2001; Brown et al., 2005a; Soberon et al., 2012). We hypothesized that when differing planes of nutrition are offered in the pre-weaning period, the influence of post-weaning diets on overall development will be dependent on the previously offered diet. Contrary to our hypothesis, minimal interactions between the pre- and post-weaning phases were detected for measured parameters in this study. Rather, differences in parameters measured were explained solely by differences in pre-weaning plane of nutrition or post-weaning plane of nutrition, regardless of the diet offered in the opposite phase.

It is plausible that very little interaction exists between pre- and post-weaning nutritional planes. Reports of early life nutrition influencing future production (Van Amburgh et al., 1998; Radcliff et al., 2000; Soberon et al., 2012) have, in general, not examined both the pre- and post- weaning phases. Even with these changes in production as a result of early life nutrition, it is possible that the parameters measured in the current study are not those responsible for the nutritional programming thought to take place.

Nevertheless, several factors may have contributed to the lack of interaction between the two phases in the present study. Firstly, the weaning transition experienced by the calves was over a relatively long period of time which likely allowed for sufficient adaptation to the reduction in milk, even for heifers accustomed to receiving 10L/day. Sweeney et al. (2010) noted increased overall gains in high plane heifers weaned over 10 days, compared to those weaned abruptly, over 4 days, or over 22 days. While the present study and the study by Sweeney et al. (2010) both found success with a 10d weaning protocol, the 10d reduction in

milk availability is greater than what is often implemented, or practical, on farms. Studies offering an elevated plane of nutrition in the pre-weaning phase have often reported a decrease in post-weaning intake (Strzetelski et al., 2001; Hill et al., 2006 a,b; Hill et al., 2010) and growth rate (Bar-Peled et al., 1997; Jasper and Weary, 2002; Cowles et al., 2006). In the present study, the lack of change in intake or BW through the weaning phase as effected by pre- weaning plane of nutrition suggests that the 10d step down weaning was sufficient to allow heifers to avoid depressed post-weaning performance.

A second factor that may be responsible for the lack of interaction between pre- and post- weaning phases in the present study is the lack of difference in energy content between both pre- and post- weaning diets. In the pre-weaning phase, the allowance of *ad libitum* starter access for both treatment groups led to high rates of growth and similar ME intakes, especially as weaning approached. As a result, pre-weaning low plane heifer growth rates in the current study were greater than those typically noted in studies where milk intake is limited (Bartlett, 2001; Brown et al., 2005a). Both post-weaning diets were also relatively high in both protein and energy, allowing for high ADG in both post-weaning treatment groups. Growth rates of weaned heifers offered elevated planes of nutrition in studies by Chelikani et al. (2003) and Gasser et al. (2006) are similar to the growth rates of the low plane heifers in the current study, demonstrating the high rates of growth permissible with both post-weaning ration. It is possible that with a more conventional weaning transition, or a greater difference in energy content of the post-weaning TMR, interactions between the pre- and post- weaning phases may have been noted. As a result of the lack of interaction between the two phases in the

current study, discussion from this point forward will focus on the pre- and post- weaning periods independent of each other.

2.41 Feed and energy intake

Offering a high plane of nutrition in the pre- weaning phase led to increases in both volume of milk ingested and total ME intake. The increase in volume of milk ingested was expected, as calves offered *ad libitum* access to milk have been noted to reach intake levels equal to approximately 20% of BW daily (Jasper and Weary, 2002; Khan et al., 2007a; Sweeney et al., 2010). Milk intake for high plane pre-weaning calves was below the targeted intake, but was influenced by feeding time restrictions from the automated feeding system (Haisan et al., in press), and the inclusion of the 10d step-down weaning phase. Likewise, the increased intake of starter by low-plane heifers in the pre-weaning phase was an expected result, especially as BW and energy requirements increased with size. Further factors associated with the digestive tract, including glucose and insulin levels, rate of abomasal emptying, digestive capacity, and action of mechanoreceptors also play a role in regulating both liquid and solid feed intake (Reviewed by Khan et al., 2011). As a result of these, an increase in solid feed intake by calves offered limited volumes of milk is consistently noted across many studies (Kertz et al., 1979; Jasper and Weary et al., 2002; Terré et al., 2007; Raeth-Knight et al., 2009). A calf's transition from a functional monogastric, dependent on a liquid diet, to functioning ruminant, dependent on solid feed, requires significant rumen development. Quigley et al. (1991) suggested that BHBA can be used as an indicator of rumen development, closely related to starter intake, as it is a ketone produced by the rumen epithelium in response to absorption of the VFA butyrate. In the current study, concentrations of butyrate were numerically greater in pre-weaning low

plane heifers than high plane heifers, in agreement with both the starter intake and BHBA data. Rumen fluid samples were collected once every 4 wk, and as a result it is possible that weekly variations in butyrate concentrations as a result of starter intake were not detected. The use of blood BHBA as a tool to evaluate starter consumption in calves has been confirmed in additional studies (Khan et al., 2011; Omidi-Mirzaei et al., 2015; Overvest et al., 2016, Deelen et al., 2016), and therefore the elevated concentrations of BHBA in pre-weaning low plane heifers during wk 7 and 9 in the current study are with precedent.

Combining daily milk and starter intake data allowed for the calculation of total ME intake data, where in the current study similarities in wk 8 (during weaning) and the increased ME intake for low plane calves in wk 9 (immediately following weaning) were noted. The ability of all calves to thrive through the weaning transition and into the post-weaning phase in the current study illustrate the importance of ME intake as an indicator for successful weaning transition, as opposed to commonly used measures such as raw starter intake.

In the post-weaning phase, DMI was affected by both type of feed offered and week of life. The *ad libitum* feeding strategy used led to post-weaning levels of intake not commonly reported for heifers, with low plane heifers reaching a maximum average intake of 6.63kg/d and high plane heifers reaching maximal levels at 7.79kg/d in the final week of the trial. Using the NRC dairy (2001) predictive equation for DMI of growing heifers, low plane heifers in the current study were predicted to ingest 6.00 kg/d and high plane heifers 6.04 kg/d during the final week. Early in the post-weaning phase, heifers exceeded wk 9 and 10 DMI reported by Geiger et al. (2016, 1273.25g/d) greatly, though this may have also been a function of increased BW in the present study so comparisons are limited. Heifers in the current study also had

greater DMI in wk 13,14, and 15 than heifers weaned early and offered TMR and hay (4.44 kg/d) or weaned late and offered TMR and hay (4.06 kg/d, Van Ackeran et al., 2010). Similar levels of intake for both treatment groups using identical post-weaning diets were noted by Groen et al. (2015), however bull calves with a 5 wk adaptation period were used in their study, where both factors likely increased DMI. The increased intake in the current study, specifically for high plane post- weaning heifers, caused greater concentrations of butyric acid in the rumen fluid at all post-weaning samplings. The increased BHBA concentrations at wk 17,19, and 21 are likely the result of the increased butyric acid production, which is converted to BHBA by the rumen epithelium. It appears that greater amounts of carbohydrates are being fermented in the rumen and therefore increasing the production of BHBA in post-weaning high plane heifers. Comparisons to the increased BHBA concentrations in high plane post-weaning heifers at wk 17, 19, and 21 in literature are difficult to come by, as long term heifer studies do not appear to examine BHBA levels. One similar plane of nutrition study did not document any influence of diet on BHBA concentrations (Anderson et al., 2015), however differing levels of fat from distillers dry grains were used and were not likely to influence BHBA concentrations in the same manner that differing concentrate levels would. Perhaps the most interesting result is the interaction between pre- and post-weaning planes of nutrition and BHBA concentrations. Heifers with access to the low plane diet in the pre-weaning phase and high plane diet in the post-weaning phase have consistently elevated BHBA concentrations throughout the majority of the post-weaning phase. The elevated BHBA concentrations are likely the result of a combination of effects, where a low plane of nutrition in the pre-weaning phase has allowed for sufficient development of the digestive tract and increased digestive capacity, and a high plane

of nutrition in the post-weaning phase has allowed for a high amount of carbohydrate digestion. It is also interesting to note that the opposite is not true, as high pre-weaning low post-weaning heifers did not exhibit consistently decreased BHBA concentrations throughout the post-weaning phase. To date, we are not aware of published BHBA data for a study conducted over a similar length of time to ours. The weekly data from the current study may be useful in understanding patterns of intake or overall feed consumption in settings where monitoring individual feed intake is not possible.

High plane heifers not only exceeded their predicted DMI by a greater amount, but also ingested more of an energy dense feed, leading to significantly greater energy intake over the low plane heifers throughout the entire post-weaning phase. High levels of energy intake, and consequently high rates of gain, have led to excess fat deposition in Holstein heifers (Swanson, 1960; Petitclerc et al., 1984; Stelwagen and Grieve, 1990; Radcliff et al., 2000), although the overall effects of this fat deposition are not fully understood, and will be discussed later on. There is a paucity of information regarding post-weaning ME intake for *ad libitum* fed heifers and we believe the data reported in the current study is therefore novel. Further research is required to improve the understanding of allowing *ad libitum* access to post-weaning feeds, regardless of energy content, and the impact this may have on heifer development.

2.42 Growth and feed efficiency

High plane heifers in the pre-weaning phase had greater BW than low plane heifers in wk 2 through 8, likely as a result of the increased ME intake noted in wk 2 through 7. These results are consistent with those of Diaz et al., (2001), Jasper and Weary (2002), and Brown et al., (2005a), who improved early life growth through increasing whole or powdered milk

provision. The increased ADG of high plane calves during the 4 wk of life are likely explained by their increased milk allowance and similar starter intake to low plane calves, as was also described by Jasper and Weary (2002). Similar ADG in wk 4 to 8 are then likely explained by significantly greater starter intake for low plane calves as weaning approached, allowing for similar energy intakes. Previous studies have indicated increased feed efficiencies for heifers offered elevated planes of nutrition in the pre-weaning phase (Brown et al., 2005a; Bartlett et al., 2006), but in our study pre-weaning high plane heifers had no efficiency advantage and were less efficient during wk 3. These lack of difference in pre-weaning feed efficiency is likely the result of the high rates of gain that were possible, even for calves offered the low plane of nutrition, where differences in growth were not great enough to offset the extent to which ME intake was increased.

Pre-weaning effects on BW dissipated by wk 11, and all calves were of similar weight until wk 16, which was likely the result of post-weaning planes of nutrition that allowed for high rates of growth regardless of treatment group. It is interesting to note that the lack of interactions between ADG and pre- and post-weaning planes of nutrition, as post-weaning growth was not limited in high plane pre-weaning heifers as seen by Davis Rincker et al., (2011), and compensatory growth was not demonstrated by pre- weaning low plane heifers. High-energy post-weaning diets have previously been used to elicit increased growth in post-weaned heifers (Radcliff et al., 2000; Chelikani et al., 2003), and so the effect of post-weaning diet on BW from wk 16 forward as well as on ADG from wk 12 to 16 were expected. The tendency for high plane heifers to be less efficient in the post-weaning phase, notably towards the

conclusion of the study, are logical as high energy diets during this time likely favor the deposition of nutritionally demanding fat, and not lean tissue.

2.43 Hormones and metabolites

The elevated concentrations of glucose in the pre-weaning phase of high plane heifers in the current study were in agreement with values reported previously across similar studies (Hugi et al., 1997; Bach et al., 2013; Yunta et al., 2015; Macpherson et al., 2016). Because the pre-weaned ruminant operates essentially as a monogastric, glucose is the main energy source for young heifers (Reviewed by Drackely, 2008). Factors influencing plasma glucose concentrations include feed intake, gluconeogenesis in the liver, and catabolism of glycogen stores (Aronoff et al., 2004). High plane heifers had elevated milk intakes throughout the entire pre-weaning phase and likely were able to store excess amounts as glycogen, explaining the differences detected at wk 3, 5, and 7. The lack of difference in glucose concentrations between treatments in wk 1 and 9 may therefore be explained by a lack of reserves or a reduced magnitude of difference in volume of milk intake as calves were increasing intake daily in the first wk or were weaned in wk 9. Post-weaning concentrations of plasma glucose were similar between treatment groups at all times and lower than in the pre-weaning phase, as post-weaned calves derive energy from VFA produced from dietary carbohydrates in the rumen and a reduced amount of glucose molecules reach the small intestine (Baldwin et al., 2004; Huntington et al., 2006).

Differences in plasma insulin concentrations did not follow the detected differences in plasma glucose mentioned above. This lack of association is in opposition to what is commonly reported (Hugi et al., 1997; Bach et al., 2013; Yunta et al., 2015; Macpherson et al., 2016), as

insulin concentrations are primarily regulated by the rate at which glucose rich nutrients enter the small intestine, feed composition, and neuroendocrine signals (Aronoff et al., 2004; Stahel et al., 2017). The lack of difference in insulin concentration at pre-weaning samplings where differences in glucose concentrations were noted can likely be explained by the time of sampling. All blood samples in the present study were taken approximately 3h following the morning feeding, where each treatment group received 2.5L of whole milk. Had the samples been taken following the next feeding, where high plane heifers received 2.5L and low plane heifers did not receive any milk, differences in insulin concentration would likely have been noted. Differences in post-weaning plasma insulin concentrations are less frequently discussed, due to the majority of dietary carbohydrates being digested in the rumen. However, da Silva et al. (2017) reported increased insulin concentrations in post-weaning growing Nelore heifers receiving supplement to improve energy intake compared to those who received no supplement, citing increased glucose concentrations as justification. The lack of differences in post-weaning glucose concentrations in the current study suggest research on additional relationships between post-weaning feed, glucose, and insulin are necessary.

Insulin-like growth factor 1 has previously been correlated with increased feeding frequency, growth rate, and protein intake (Bartlett, 2001). In addition, correlations between IGF-1 concentrations, plasma glucose concentrations, and plasma insulin concentrations have also been reported (Butler et al., 2003). The differences in IGF-1 concentrations between treatment groups in the pre-weaning phase of the present study, noticed at wk 3, 5, and 7, can likely be attributed to differences in the aforementioned parameters correlated with IGF-1 that were also noted in our high plane heifers. Smith et al. (2002) fed and slaughtered bull calves at

three different weights, and found that plasma IGF-1 concentrations increased as BW increased, despite similar ADG. Petitclerc et al. (1999) raised heifers to 4 mo of age on differing planes of nutrition and found that *ad libitum* intake in the post-weaning phase caused significant increases in IGF-1 concentrations, although different planes were not offered but growth was restricted to 700g/d. It is likely that a combination of these factors were at work in our study, where post-weaning IGF-1 concentrations were increased throughout the majority of the post-weaning phase for high plane heifers. Heifers offered the low plane TMR, while being offered *ad libitum* access, may have had intake limited through gut fill or physically effective neutral detergent fiber due to the high straw inclusion rate in the diet (Allen, 2000).

The serum leptin concentrations in our study decreased from wk 1 until wk 13, with the most noticeable decrease occurring from wk 1 to wk 7. Kesser et al. (2016) noted a similar decrease in leptin concentrations in German Holstein calves, and further suggested the high levels of leptin in the early life period to be the result of colostrum intake. The transfer of leptin from maternal colostrum to the bloodstream of the offspring has previously been documented in rats (Casabiell et al. 1997) and piglets (Woliński et al., 2003), and likely explains the relatively high concentrations of leptin reported in our study, as wk 1 samples were taken at least 3 days after colostrum feeding was completed. The tendency for high plane calves to have greater leptin concentrations than low plane calves in wk 1 is therefore difficult to explain, as equal volumes of pooled colostrum were provided to each calf regardless of treatment. It is possible that the early effects of increased milk intake were detected in wk 1 leptin levels, however Block et al. (2003) failed to detect differences in leptin concentrations for high plane calves until wk 3. Differences between pre-weaning treatment groups at wk 3 and 5 are in agreement with

data from Block et al. (2003) and Brown et al. (2005a) who used bull and heifer calves, respectively, to demonstrate that early life nutritional provision influences early life leptin concentrations. The lack of difference between treatment groups from wk 7 to wk 11 in the current study may be the result minimal differences in adipose tissue between calves, as during this time period ME intake were similar. When compared to data from Brown et al. (2005a), the post-weaning leptin concentrations the present study are lower than those for heifers limit fed for an ADG of 0.4kg/d. However, the limit fed heifers were offered diets consisting of 30% rolled corn and 70% standard calf starter, with no forage component. It is then very interesting that the effect of pre-weaning diet is seen on post-weaning leptin concentrations for multiple weeks, regardless of post-weaning diet. To our knowledge, this pattern of leptin concentration has not yet been described in literature. We hypothesize that since leptin acts as a satiety signal (Henry et al., 1999; Farooqi et al., 1999; Ahima and Flier, 2000), reduced post-weaning leptin concentrations in calves offered the pre-weaning low plane are indicative of the failure of both high and low plane TMR to adequately fulfill the heifers hunger during this time. The increased leptin concentrations in pre-weaning high plane calves could then have been the result of decreased digestive tract size, where less feed is required for satiety, or increased energy stores that were not detected in wk 7-11 as a result of increased starter intake in pre-weaning low plane calves. The effects of post-weaning diet on serum leptin concentrations are first noted in wk 17, where high plane calves have increased concentrations which can likely be attributed to increased adipose tissue deposition. However, in terms of excess adipose tissue deposition associated with high energy feeds, the concentrations of leptin for heifers in the current study appear similar to those documented by Chelikani et al. (2003) for heifers with similar ages and

slower rates of gain. Chelikani et al. (2003) determined that DMI (18%), BW (17%), and back fat thickness (5%) combined to explain approximately 40% of the variation in leptin concentrations in growing heifers. When comparing leptin concentrations at similar BW and levels of DMI, the present study and the study by Chelikani et al. (2003) appear to report similar values. High plane heifers from Chelikani had increased back fat at 30 wk of age, suggesting that some degree of fat deposition may have been occurring in our heifers, although this can only be hypothesized as measures of fat deposition were not implemented in the current study.

2.5 Conclusions

In conclusion, planes of nutrition with differing energy levels in the pre- and post-weaning phases affected several parameters associated with overall heifer development. Support for our hypothesis of pre- weaning nutrition governing the effects of post-weaning parameters was not provided, as interactions between pre- and post-weaning planes of nutrition were not found in the majority of the parameters measured. Pre-weaning growth and energy intake were increased through the provision of high planes of nutrition, but these effects dissipated in the post-weaning phase under the influence of post-weaning diet. In the same manner, post-weaning growth and intake was increased through the provision of high planes of nutrition, but these increases occurred independent of pre-weaning planes. Increased concentrations of glucose, insulin, IGF-1, and leptin were noted for high plane calves in various weeks throughout the study, but were again the result of either pre- or post- weaning diets and not a combination of both. Overall, the results from this study indicate that the differences in intake, growth, and select metabolite and hormone concentrations, seen as a result of differing

pre-weaning planes of nutrition, may have little influence in the post-weaning phase when high and low planes of nutrition are offered.

Table 2-1. Ingredients and nutrient composition of post-weaning experimental diets.

	Treatment[1]	
Ingredient	High	Low
Rumimax pellets, %DM	56.51	46.54
Beet pulp pellets, %DM	13.10	10.79
Rolled corn, %DM	12.75	10.5
CFS Mill Mix, %DM	2.55	2.10
Flavoring agent, %DM	0.09	0.07
Wheat straw, %DM	15.00	30.00
	Treatment	
Chemical composition	High	Low
CP, %	25.02	21.12
ME, Mcal/kg	2.94	2.49
NDF, %	30.05	45.96

[1]Treatments: High = high plane of post-weaning nutrition (85% concentrate as-fed dry TMR) and Low= low plane of post-weaning nutrition (70% concentrate as-fed dry TMR)

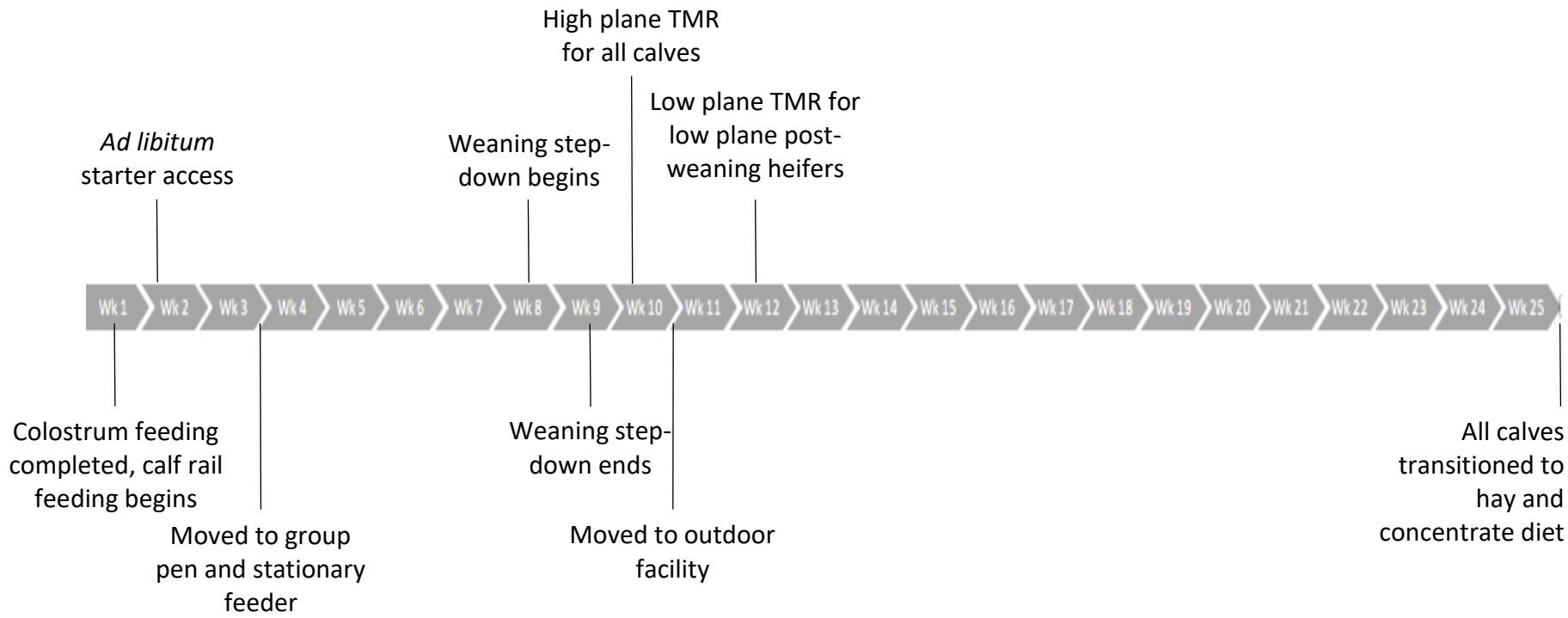


Figure 2-1: Schematic of animal feeding and movement throughout the study.

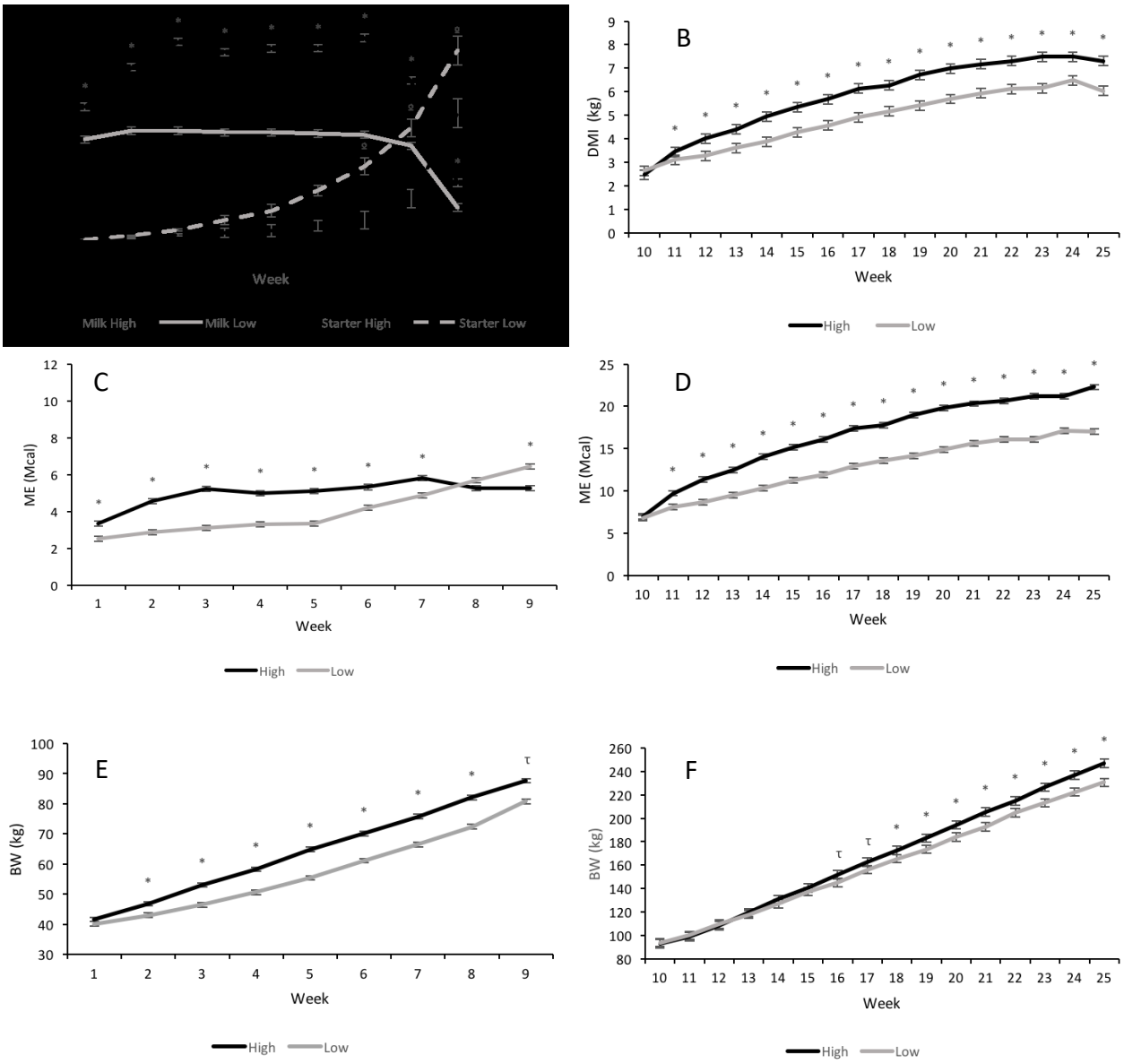


Figure 2-2: Effects of offering pre-weaning (A) and post-weaning (B) high and low planes of nutrition on daily average milk and starter intake and daily dry matter intake (DMI), pre-weaning (C) and post-weaning (D) high and low planes of nutrition on metabolizeable energy intake (ME), and pre-weaning (E) and post-weaning (F) high or low planes of nutrition on weekly bodyweight. No interactions were noted between pre- and post-weaning phases, and therefore graphs from each phase are presented separately. * indicates $P \leq 0.05$, t indicates $P > 0.05$ and ≤ 0.10.

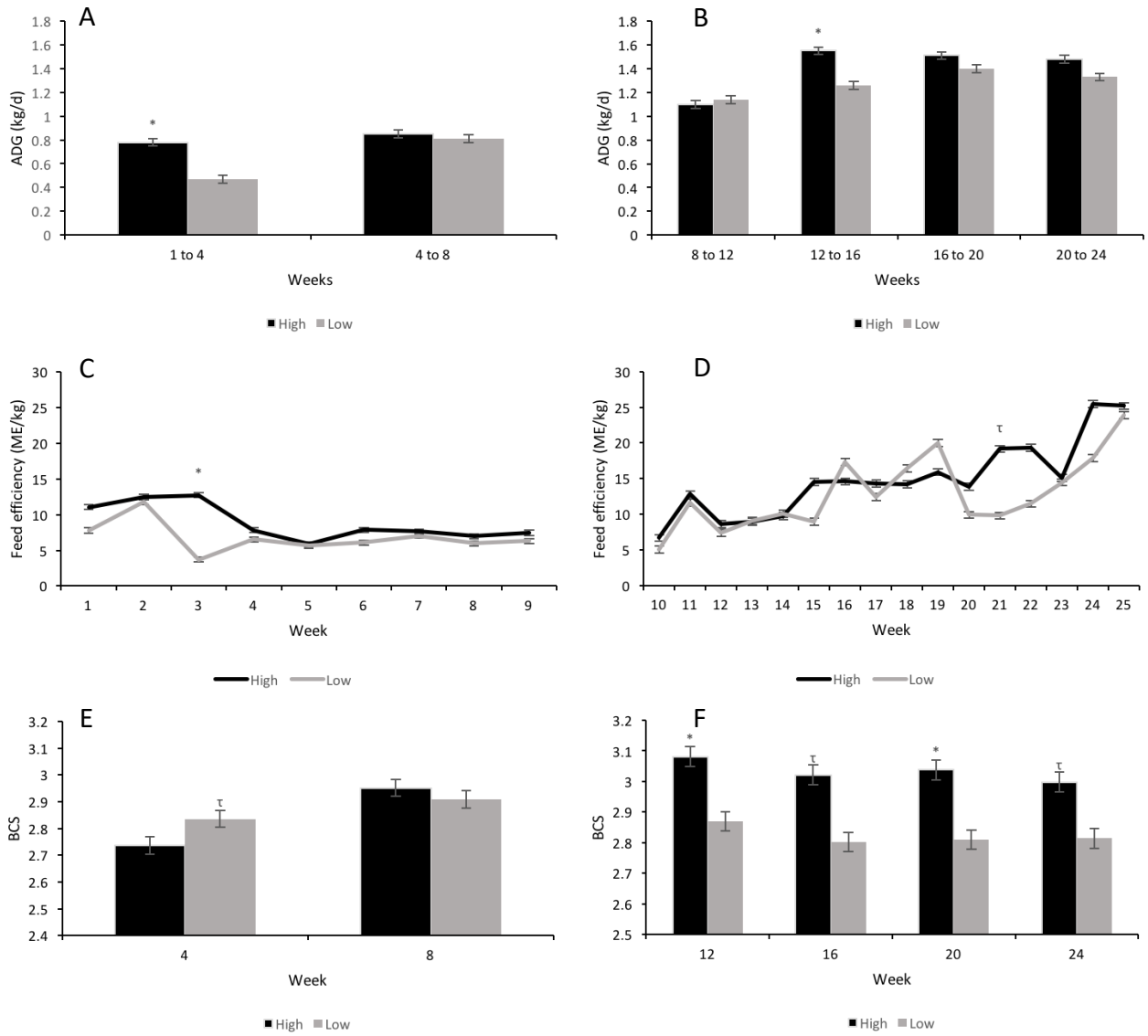


Figure 2-3: Effects of offering pre-weaning (A) and post-weaning (B) high and low planes of nutrition on average daily gain (ADG, grouped by 4 week increments), pre-weaning (C) and post-weaning (D) high and low planes of nutrition on feed efficiency (FE, ME/kg gain), and pre-weaning (E) and post-weaning (F) high and low planes of nutrition on body condition score (BCS, grouped by 4 week increments). No interactions were noted between pre- and post-weaning phases, and therefore graphs from each phase are presented separately. * indicates $P \leq 0.05$, t indicates $P > 0.05$ and ≤ 0.10 within week.

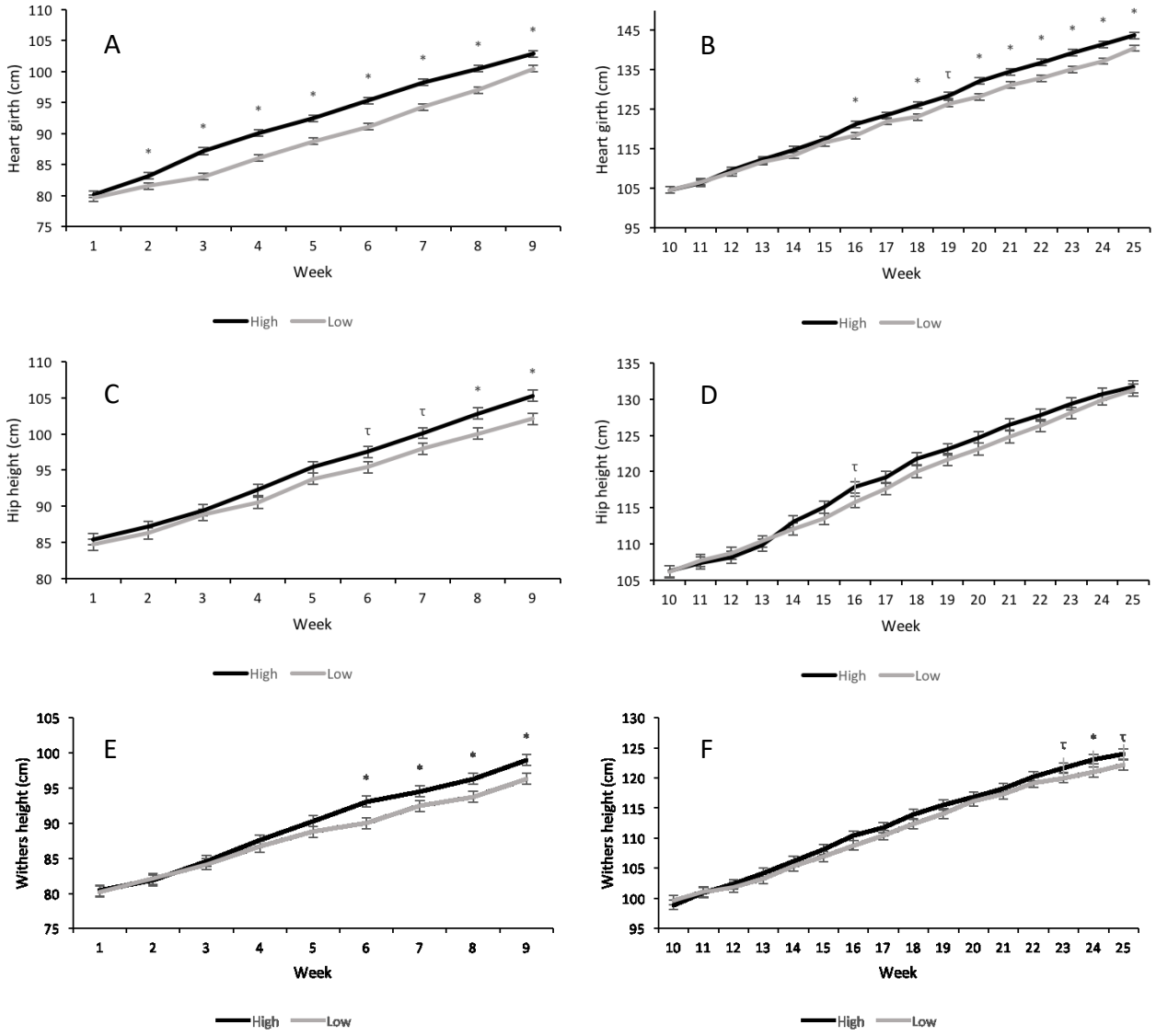


Figure 2-4: Effects of offering pre-weaning (A) and post-weaning (B) high and low planes of nutrition on heart girth, pre-weaning (C) and post-weaning (D) high and low planes of nutrition on hip height, and pre-weaning (E) and post-weaning (F) high and low planes of nutrition on withers height. No interactions were noted between pre- and post-weaning phases, and therefore graphs from each phase are presented separately. * indicates $P \leq 0.05$, t indicates $P > 0.05$ and ≤ 0.10.

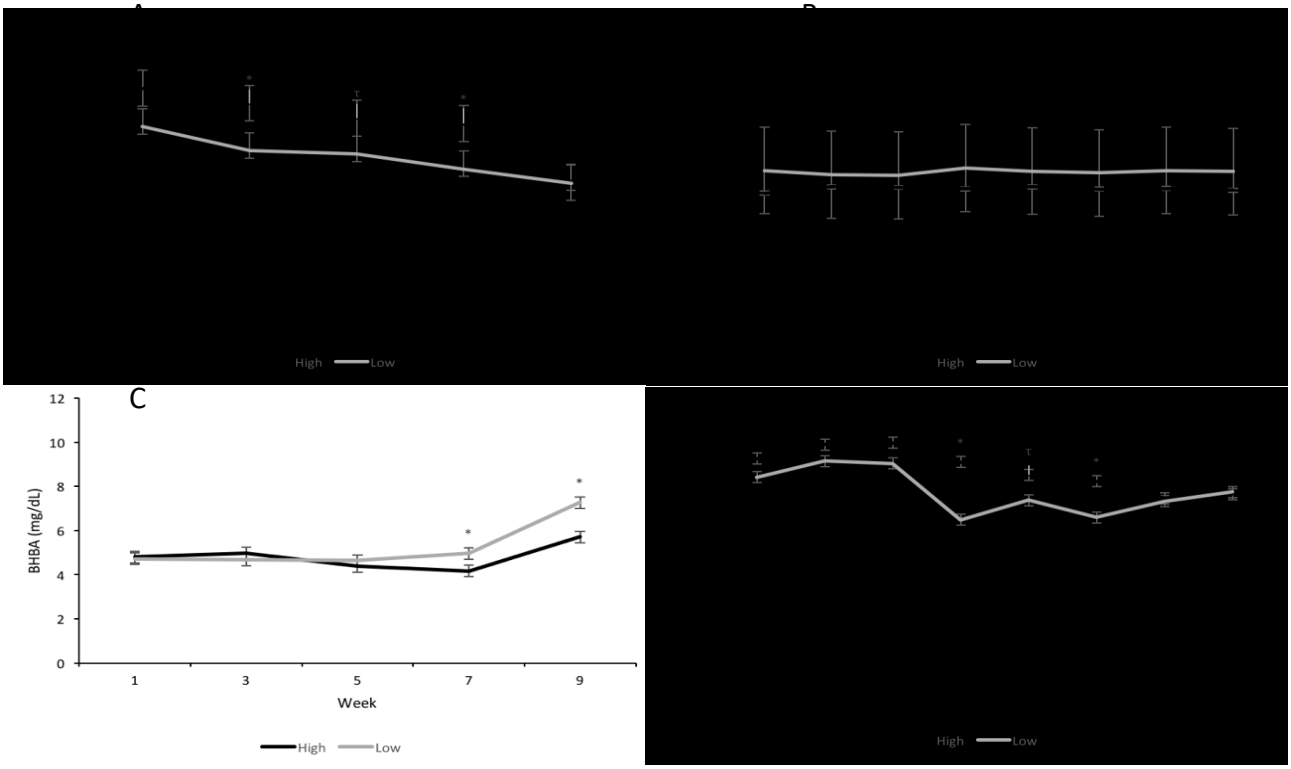


Figure 2-5: Effects of offering pre-weaning (A) and post-weaning (B) high and low planes of nutrition on plasma glucose, and pre-weaning (C) and post-weaning (D) high and low planes of nutrition on plasma β-Hydroxybutyric acid (BHBA). No interactions were noted between pre- and post-weaning phases for glucose, and therefore graphs from each phase are presented separately. Overall tendency for interaction pre- weaning planes of nutrition and post- weaning planes of nutrition on BHBA concentration ($P = 0.054$) not shown. * indicates $P \leq 0.05$, t indicates $P > 0.05$ and ≤ 0.10.

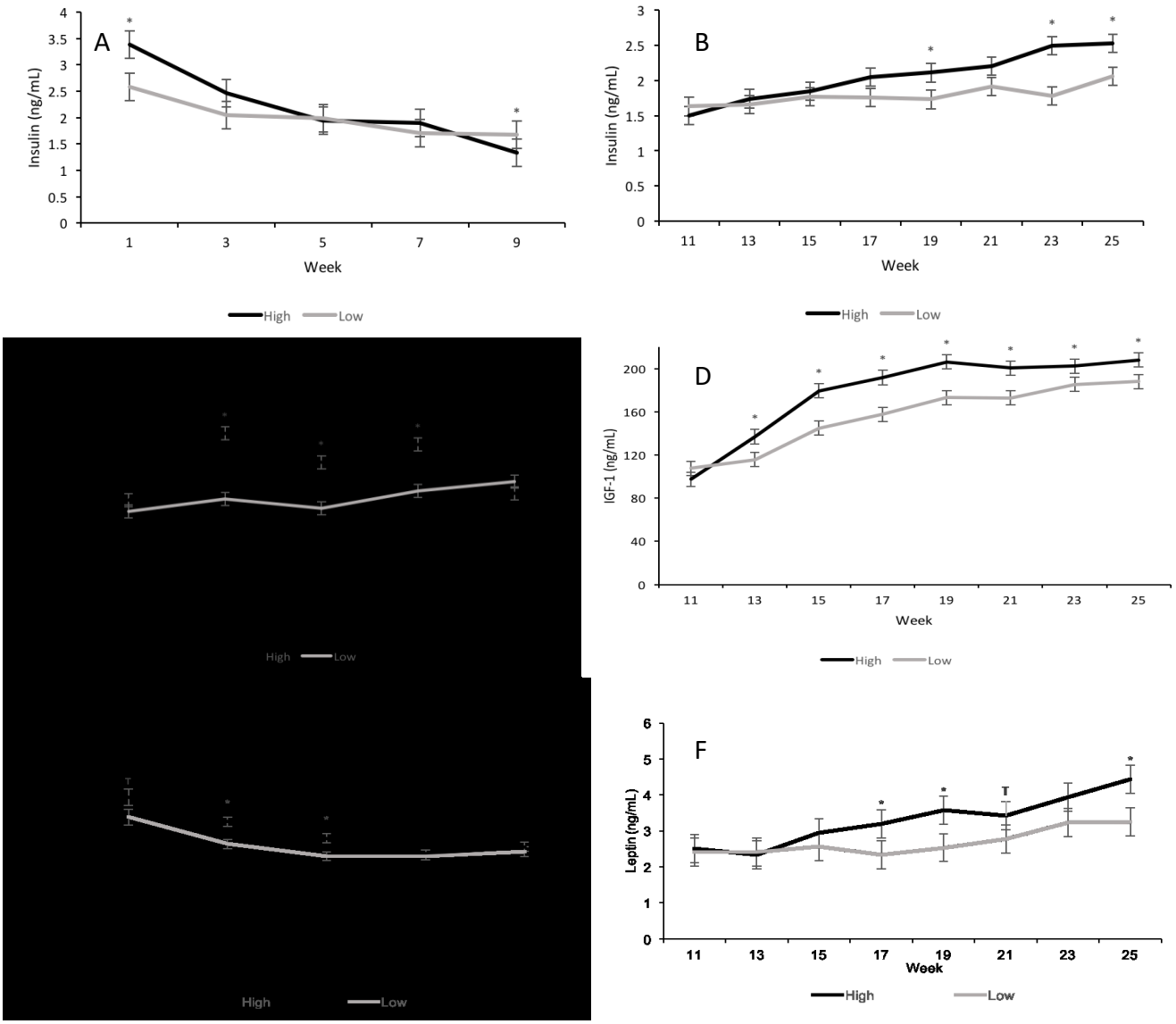


Figure 2-6: Effects of offering pre-weaning (A) and post-weaning (B) high and low planes of nutrition on plasma insulin concentrations, pre-weaning (C) and post weaning (D) high and low planes of nutrition on serum insulin-like growth factor 1 (IGF-1) concentrations, and pre-weaning (E) and post-weaning (F) high and low planes of serum leptin concentrations. No interactions were noted between pre- and post-weaning phases, and therefore graphs from each phase are presented separately. * indicates $P \leq 0.05$.

3.0 Chapter 2: The effects of differing planes of pre- and post-weaning phase nutrition on serum leptin concentrations from birth to 6 months of age, luteinizing hormone release at 3 and 6 months, and age at first ovulation.

3.1 Introduction

The optimal age for calving in Holstein heifers is between 22 and 24 mo (Gardner et al., 1988; Hoffman and Funk, 1992). In order to take advantage of the reduction in feed costs (Heinrichs et al., 2013) and increased overall economic returns (Heinrichs, 1993; Ettema and Santos, 2004) that have been associated with calving at this age, successful conception must occur between 12.5 and 14.5 mo. In Canada, however, the age of Holstein heifers at first calving averages 26.5 mo (Pietersma et al., 2006). Calving at an age exceeding recommendations by more than 2.5 mo, reduces farm profitability, as the period from birth to calving requires investment in feed, housing, and labour, with no economic returns (Radcliff et al., 1997). It has also been shown that heifers attaining puberty later in life are slower to breed and conceive than their counterparts who were cycling prior to the breeding season (Short and Bellows, 1971; Bagley, 1993). However, Pietersma et al. (2006) found that neither rate of growth nor BW was a limiting factor in attempts to lower the age at first calving for Canadian Holstein heifers. The lack of limitation from BW may indicate that a conscious decision to delay insemination by producers, for fear of increased rates of dystocia or reduction of growth due to conception occurring at too young of an age, exists. Reducing age at first ovulation, while maintaining recommended breeding weights at approximately 55% of mature BW (NRC, 2001), likely offers producers an opportunity to improve the economic efficiency of their operations.

Nutrition, among other factors, has been explored as one of the major factors able to affect the age at which puberty is attained in cattle. Many studies have demonstrated that increasing post-weaning growth rates causes a decrease in the age at puberty for heifers (Sejrsen et al., 1982; Schillo et al., 1992; Adam et al., 1994; Chelikani et al., 2003). Far fewer studies have examined the effect of pre-weaning growth rate on the attainment of puberty; however, Shamay et al. (2005) showed that calves fed whole milk grew faster in the pre-weaning phase and attained puberty at a younger age than those fed milk replacer. Additionally, providing supplemental energy to beef heifers in the pre-weaning stage increased weaning weight, and increased the likelihood of the early attainment of puberty in two separate trials by Buskirk et al. (1996). When raising dairy heifers, in addition to breeding and calving age, future production must also be considered. Various feeding strategies have been shown to increase fat deposition and reduce the allometric growth phase of the mammary gland, which may negatively affect future milk production (Sejrsen et al., 1982; Davis Rincker et al., 2008).

In recent years, a greater understanding of the mechanisms controlling the onset of puberty in heifers has been achieved. Studies involving differential nutrition provision have led to the discovery that the onset of puberty occurs when a certain percentage of mature BW is attained, across multiple breeds (Freetly et al., 2011). The composition of the body at pubertal onset is also important, as adipose tissue secretes leptin, the hormone responsible for communicating the nutritional status of the body to the hypothalamic – pituitary – ovarian axis. Leptin itself is not capable of inducing puberty, but plays a permissive role, and must be present for energy to be partitioned towards reproduction in both mice and humans (Ahima and Flier,

2000). Aside from physical development, the hypothalamic-pituitary-ovarian axis must also mature in order for puberty to come about (Perry, 2016). Estradiol, mainly from the dominant ovarian follicle, acts as an inhibitor on GnRH release in the prepubertal phase before the number of estradiol receptors in the hypothalamus decrease in the peripubertal phase (Day et al., 1987) and the sensitivity of this negative feedback loop is also decreased. Increased amounts of GnRH then travel to the anterior pituitary via the hypothalamohypophyseal portal system and cause an increase in the pulsatile release of LH. The increase in LH pulse frequency leads to greater overall concentrations of LH, but reduced amplitude of the pulses. Nonetheless, this loop continues to work until a LH surge is induced, and ovulation occurs. Monitoring the dynamics of LH release during the prepubertal phase is then a useful indicator of sexual development in heifer calves.

Previous studies (Reid et al., 1957; Sorenson et al., 1959, Reid et al., 1964, Short and Bellows, 1971; Radcliff et al., 1997; Chelikani et al., 2003; Shamay et al., 2005; Gasser et al, 2006a,b,c) have examined the relationship between early life nutrition and sexual development, however, further studies are required in order to better understand this relationship. In addition, the possible interactions between pre- and post- weaning diets and their effects on sexual development warrant further study as research has historically focused on one phase or the other. It was hypothesized that when adequate nutrition for maintenance and growth is provided in the pre-weaning phase, increasing post-weaning dietary energy content will enhance LH secretion and increase the rate at which puberty is attained. The objectives of this study were to determine the effects of pre- and post- weaning diets with

differing energy levels, on serum leptin concentrations from birth to 6 mo, LH pulsatility at 3 and 6 mo, and age at first ovulation in Holstein heifer calves.

3.2 Materials and Methods

3.21 Animals

The same 36 animals described in section 2.21 were enrolled in this study. All heifers were born from the same herd, starting in February and finishing in August 2017. Protocols approved by the Animal Care and Use Committee for Livestock at the University of Alberta were followed (AUP 00001553) and heifers were cared for according to standards from the Canadian Council of Animal Care (Ottawa, ON, Canada). Dietary treatments, animal movement, and animal handling were the same as described in chapter 2.

3.22 Environmental data

Raw environmental data, including daily temperature and day length for Edmonton, AB were obtained from timeanddate.com (Time and Date AS, Stavanger, Norway) and uploaded to a personal computer for analysis. Data were then matched to dates of heifer housing changes and first ovulation on a day by day basis to examine the effects of environment on development. Day length was considered to be time from sunrise to sunset, and daily temperature was considered to be the mean of hourly recorded temperature for 24 h each day.

3.23 Feeding and housing

Dietary treatments remained as described in section 2.22. Briefly, pre-weaning high plane treatments included 10 L/d whole milk per calf and *ad libitum* access to starter, while low

plane pre-weaning treatment included 5 L/d whole milk per calf and *ad libitum* access to starter. Weaning occurred at d 65, and calves were moved to outdoor facilities at d 75 ± 2. Post-weaning diets included a high plane dry TMR (85% concentrate, 15% chopped straw by weight) and a low plane dry TMR (70% concentrate, 30% chopped straw by weight). At 180±2 d, calves were abruptly transitioned to a diet of free choice alfalfa blend hay and 2 kg/calf per day developer ration (Trouw Nutrition, Sherwood Park, AB. 15.38 % CP, 38.20% Starch, 1.74 Mcal/kg NE). Animal housing was also consistent with the description in section 2.22, however at 6 mo of age heifers were moved to an adjacent paddock of identical size to minimize competition during the transition to the hay based diet.

3.24 Sample and data collection

Weekly blood samples were collected as described in section 2.24. In brief, calves were manually restrained and samples were collected once each week via jugular venipuncture until 6 mo of age. Blood samples were collected into lithium heparin tubes for plasma, and silicon coated tubes for serum (Vacutainer™, Becton Dickinson, Franklin Lakes, NJ, USA). Approximately 20 mL of blood was collected, and 10 mL of both serum and plasma samples were harvested. Plasma was mixed with 5μl aprotinin from bovine lung (100 μg/mL, Sigma-Aldrich Canada Co., Oakville, ON, Canada) before freezing to inhibit protease activity. Sample handling and storage occurred in the same manner as previously described in section 2.24. Analysis for weekly collected samples occurred on a biweekly basis beginning at wk 1 and concluding on wk 25.

Jugular catheters were placed at approximately 3 mo (105 ± 6.4 d) and 6 mo (177 ± 6.4 d) of age and remained installed for 26 h. Calves were brought indoors and restrained during

catheter installation before spending the night individually penned in a stable bedded with wood shavings. Feeding occurred as normally scheduled on sampling days, and free choice water was also available to all calves. Samples were taken every 12 min for 10 h, beginning 3 h before morning feeding. Between samples, the catheter was flushed with 1.5 mL of heparinized saline solution (2%) in order to keep the catheter patent. Each sample (4 mL) was collected using a 5 mL plastic syringe and transferred to a lithium heparin Vacutainer^{TM}, mixed with 0.2 μl aprotinin from bovine lung, inverted five times, and placed on ice immediately. An equal volume (4 mL) of saline solution was infused through the catheter to maintain hydration. All samples were centrifuged for 20 min at 4°C and 3000 *g*, and 2 mL of plasma was pipetted into two microcentrifuge tubes and stored at -20°C until assayed for LH.

Beginning at 6 mo of age, reproductive tracts of all heifers (n=36) were scanned weekly via transrectal ultrasonography (Aloka 500 with 7.5 MHz linear-array, transrectal transducer; ISM Inc, Edmonton, AB) to monitor ovarian changes and confirm puberty. Puberty was declared once a corpus luteum was first detected, and therefore from hereon in the terms puberty and first ovulation will be used interchangeably. Animals were restrained in a chute, and weight and hip height were also collected at the time of weekly scanning. At the conclusion of one estrous cycle, determined based on regression of the first corpus luteum, heifers were removed from the study.

3.25 Hormone assays

At the conclusion of the collections, frozen weekly serum samples were shipped on dry ice to Universität Bonn for analysis of leptin (North Rhine-Westphalia, Bonne, Germany). Leptin concentrations were determined by competitive enzyme immunoassay as described by

Sauerwein et al. (2004). Briefly, samples were analyzed using specific polyclonal antisera against leptin from rabbits and biotinylated recombinant ovine leptin as tracer. Samples were loaded in duplicate to pre-incubated microtitre plates containing sheep anti-rabbit-Fc fragment antibodies and assay buffer, and antiserum was also added. After incubation at room temperature for 16 h, the biotinylated tracer was added and the amount of tracer bound was quantified by streptavidin-peroxidase as described by Hennies et al. (2001). The inter and intra assay CV were 9.1% and 8.8%, respectively.

Luteinizing hormone concentrations in sequential plasma samples were quantified by an established radioimmunoassay (Endocrine Lab Services, University of Saskatchewan, Saskatoon, Sask., Canada). Rawlings and Evans (1995) describe the assay in its entirety. The standard used was AFP11118B, the standard curve used extended from 0.0625 ng/mL to 8 ng/mL, and the interassay and intrassay CV were 6.8% and 6.2% respectively. Briefly, LH antibodies from rabbit were used as the primary antibody, while the secondary antibody was raised in sheep against rabbit- γ-globulins and added with polyethylene glycol solution. Iodinated tracer was also added before analysis, and samples were allowed to incubate overnight before concentrations of LH were calculated using an in-house RIA program after samples were counted for 1 min on a γ-counter.

3.26 Statistical analysis

To determine the effect of dietary treatments, all reproductive data were analyzed using the Statistical Analysis System 9.4 (Studio 3.7 platform, SAS Institute, Cary, NC). Leptin concentrations over time were analyzed using the GLIMMIX procedure, and included the fixed effects of pre-weaning treatment, post- weaning treatment, season at birth, and interactions,

with birth weight as a covariate. A similar repeated measures model was used to examine the relationship of leptin levels and age at first ovulation, where calves were grouped by age at the attainment of puberty (FAST: puberty attained at ≤7 mo of age, n=11, MED: puberty attained between 7 and 9 mo, n= 12, and SLOW: puberty attained at age > 9 mo, n=13) and these groups were considered fixed effects. For all models, calf was considered the repeated measures subject, and the covariance structure with the lowest Bayesian information criterion (BIC) was chosen. Post-hoc tests were performed using Bonferroni adjustment and least squares means (LSM) by mo assessed using the SLICE command, which provides a general mechanism for performing a partitioned analysis of the LSM for an interaction.

For LH release data, pulse threshold was determined as the overall mean (ng/mL) plus one standard deviation according to (Dyck et al., 2011). Pulse frequency was calculated based on the number of pulses that occurred within the 10-h sampling window of a sampling period. Pulse amplitude was determined by calculating the difference between peak LH concentration in a pulse and nadir LH concentration immediately preceding the pulse, while pulse duration was estimated as the number of consecutive samples within a pulse and measured in min. The effects of dietary treatment on LH pulse release were analyzed using the GLIMMIX procedure, including the fixed effects of pre-weaning treatment, post-weaning treatment, and interactions, with birth weight as a covariate. Calf was considered the subject of the random statement. Each sampling period (3M and 6M) was analyzed separately for LH release. When limited effects of pre- and post- weaning planes of nutrition on LH release were detected, the CORR procedure was used to examine Pearson correlation coefficients among LH variables, birth weight, weight

at sampling, ADG during sampling mo, age at sampling, d to first ovulation, and d from sampling to first ovulation.

For days to first ovulation, the GLIMMIX procedure was used including the fixed effects of pre-weaning treatment, post- weaning treatment, and interactions, with birth weight as a covariate. Season of birth was added to the model following initial analysis, where limited effects of dietary planes were found. Because of the wide range of birth dates for heifers enrolled in the study, calves were considered to be winter born if birthed in January, February or March, spring born if birthed in April, May, or June, and summer born if birthed in July, or August. Since no calf used in this study was born in the month of September, October, November, or December, no autumn born group was assigned. Variables were removed from the final model if their effects showed $P > 0.10$, and post-hoc comparisons were performed using Tukey adjustment. Survival and hazard ratio analyses for interval to first ovulation were analyzed using the LIFETEST and PHREG procedures, with either pre- or post-weaning treatments as fixed effects. The CORR procedure was again used to obtain Pearson correlation coefficients between days to first ovulation and environmental factors, including temperature and day length. All values reported are LSM ± standard error with significance declared at $P \leq 0.05$ and tendencies at $P < 0.05$ and $\leqq 0.10$.

3.3 Results

3.31 Serum leptin concentrations

As reported in section 2.33, the effects of pre- and post- weaning diets on serum leptin concentrations were evident at multiple weeks during the pre- and post-weaning phases. No

interactions between pre- and post- weaning planes were detected. The effect of time, in this case the biweekly samples, on leptin concentration was also significant and leptin increased with age ($P < 0.0001$). Overall mean (± SE) leptin concentrations in heifers of the FAST (puberty ≤ 7 mo of age), MED (puberty > 7 and ≤ 9 mo of age) and SLOW (puberty > 9 mo of age) group were 3.37 ± 0.18 ng/mL, 2.92 ± 0.16 ng/mL, and 2.80 ± 0.16 ng/mL, respectively. At wk 17, FAST heifers tended to have greater concentrations of leptin than MED (3.44 ng/mL vs. 2.53 ng/mL, SE = 0.42, $P = 0.09$) and SLOW (2.51 ng/mL, SE = 0.42, $P = 0.08$). Heifers in the FAST group also had greater leptin concentrations than SLOW heifers at wk 19 (3.89 ng/mL vs. 2.52 ng/mL, SE = 0.50, $P = 0.02$) (Figure 3-1).

3.32 Luteinizing hormone release

Dietary treatment did not influence mean LH concentration at either sampling period (Table 3-1); however, during the 3M sampling, heifers offered the high plane of nutrition in the pre-weaning phase had increased duration of LH release compared to calves offered the pre-weaning low plane (H = 39.96 min ± 21.60, L = 27.24 ± 21.60, $P < 0.05$). No interactions between pre- and post-weaning diet on the release of LH were detected. Mean LH concentration increased with time but independent of diet from 3M sampling to 6M sampling (0.25ng/mL ± 0.02, 6M = to 0.33 ng/mL ± 0.02; $P < 0.01$). No significant differences were detected in LH pulse frequency, amplitude or duration, were noted between the 3M and 6M samplings (Table 3-2). A negative correlation existed between ADG during wk 20 - 24 and LH amplitude during the 6M sampling (r = -0.34, $P < 0.01$) and a positive correlation between ADG during wk 20 - 24 and LH pulse frequency during the 6M sampling (r = 0.32, $P < 0.01$) (Figure 3-2 A and B). In addition, no correlations were found between the number of days from the 6M sampling to the day of first

ovulation and frequency (r = -0.061, *P* = 0.61) or duration (r = 0.097, *P* = 0.42), but a weak positive correlation was found between the number of days from the 6M sampling to the day of first ovulation and amplitude (r = 0.32, *P* < 0.04) (Figure 3-2 C).

3.33 Age at first ovulation

Mean age at first ovulation was not affected by pre-weaning planes of nutrition (H = 257.1 d, L = 249.9 d, SE = 10.8, *P* = 0.62). The mean age at puberty did not differ (*P* = 0.14) between heifers that received the high plane post-weaning diet and those that received the low plane of post-weaning nutrition (Table 3-3). No interactions between the pre- and post-weaning treatments were noted to have an effect on the attainment of puberty, and therefore only results from the pre- and post-weaning phases will be presented. Post-weaning diet had an overall effect (*P* = 0.002) on ADG in the post-weaning period (d 65 - 180), however ADG was only different between post- weaning treatment groups from wk 12 - 16 (H= 1.51 kg/d ± 0.06, L = 1.26 kg/d ± 0.06, *P* < 0.001). Weight at first ovulation and hip height at first ovulation were also not different between treatment groups (Table 3-3).

Based on the LIFETEST and PHREG procedures, heifers offered the high plane diet in the post- weaning phase were 3.81 times more likely (hazard ratio; 3.81, 95% CI = 1.01 to 14.44, *P* = 0.049) to attain puberty by 7 mo of age than heifers offered the low plane diet post-weaning, regardless of pre-weaning diet. Likewise, at 8 mo of age, heifers offered the high plane post-weaning diet were 3.05 times more likely (hazard ratio; 3.05, 95% CI = 1.06 to 8.79, *P* = 0.04) to attain puberty. However, no effect of post-weaning diet on the likelihood of puberty at 10 mo of age was detected (Figure 3-3).

Season of birth influenced ($P < 0.05$) the age at puberty, as calves born in the winter months attained puberty earlier than those born in the summer months (Figure 3-4). Likewise, spring born heifers attained puberty at a younger age than summer born heifers. The age at which winter and spring born heifers attained puberty did not differ (mean of both groups = 230.3 ± 22.8). Moderate negative correlations were found between both day length (r = -0.51, $P < 0.01$; Figure 3-5 A) and average daily temperature from d 80 until first ovulation (r = -0.66, $P < 0.01$; Figure 3-5 B).

3.4 Discussion

The present study is novel in that it determined the effects of both pre- and post-weaning planes of nutrition on endocrine profiles, reproductive development and age at puberty of Holstein heifer calves. Several studies have examined the effects of differing planes of nutrition in either the pre- or post-weaning phases, but few have taken both into consideration. We hypothesized that the post-weaning diet would influence leptin concentrations, LH pulsatility, and age at puberty regardless of the pre-weaning plane of nutrition, provided sufficient nutrients for maintenance and minimal growth were supplied in the pre-weaning phase.

3.41 Serum leptin concentrations

The effect of dietary treatment on leptin concentrations in both the pre- and post-weaning phases are discussed in detail in section 2.43. In brief, high plane diets caused increases in serum leptin concentrations early in the pre-weaning phase, and late in the post-weaning phase. Relatively high concentrations of leptin noted during wk 1 are likely explained

by the offering of ample amounts of whole colostrum to all calves, which has been noted to be rich in transferable leptin in both cattle (Kesser et al., 2016) and other species (Casabiell et al. 1997; Woliński et al., 2003).

The overall tendency for relative age at first ovulation to be associated with serum leptin concentrations is in agreement with previous literature, where leptin appears to play a significant role in sexual development (Reviewed by Zieba et al., 2005). In the current study, serum leptin concentrations were similar between FAST, MED, and SLOW groups until approximately wk 13, at which point concentrations in FAST heifers appear to have increased at a greater rate than MED or SLOW heifers. Linear increases in leptin concentrations for heifers in the peripubertal period have been noted in some studies (Diaz-Torga et al., 2001; Garcia et al., 2002) but not others (Block et al., 2003; Chelikani et al., 2009). In our study, we were unable to determine linear increases due to the conclusion of leptin analysis well in advance of SLOW heifers attaining puberty, however, the overall tendency for the relationship between relative age at first ovulation and leptin concentrations provides support for the permissive role that leptin plays in the cascade of events necessary for puberty to be attained. The tendency for increased concentrations of serum leptin in FAST heifers is also interesting from the perspective of growth rate and age at puberty. Chelikani et al. (2009) found no evidence of a peripubertal increase in leptin concentrations for heifers grown for a targeted ADG of 1.1kg/d, but did find an increase for groups grown at 0.5kg/d and 0.8kg/d. Similarly, Block et al. (2003) only noted a peripubertal increase in leptin for heifers attaining puberty later in life (414 d ± 22) and not those attaining puberty at younger ages (286 ± 27). In the current study, differences in serum leptin in the peripubertal stage were noted for heifers that had attained puberty while

experiencing greater ADG, and at younger ages than in the previous studies. While this information appears interesting, comparisons between studies is limited due to the cessation of leptin analysis at wk 25 of the current study. Future research should focus on determining which factors are at play for heifers that do or do not display peripubertal increases in leptin concentrations, and the influence of planes of nutrition on these factors.

3.42 Luteinizing hormone release

Contrary to our hypothesis, high planes of nutrition in the post-weaning phase did not cause an increase in mean LH concentrations, pulse frequency, or amplitude when sampling occurred at 3 and 6 mo of age. Examples of the pulsatile release of LH detected in our study are presented in Figure A1 of the Appendix. Gasser et al. (2006b) reported an increase in the frequency of LH pulses in heifers offered dietary treatments with greater dietary energy, however this difference was only apparent from 195d of age forward. This increase in LH frequency has previously been associated with the period immediately preceding puberty (Day et al., 1987; Schillo et al., 1992; Gasser et al., 2006b), although the specific factors at play that tie together high planes of nutrition and increased LH release not fully understood. A study using ovariectomized heifers has documented increased LH pulse frequency when high planes of nutrition are offered (Gasser et. al., 2006c) in comparison to lower plane offerings, indicating that planes of nutrition have some influence on the timing with which the negative feedback loop involving estradiol is reduced in sensitivity. It seems logical that increased planes of nutrition lead to a more rapid onset of puberty and therefore a detectable difference in the frequency of LH release as puberty approaches, however in the present study there was no notable correlation between frequency of LH release and the number of days until ovulation

occurred. In addition to an increase in pulse frequency in the peripubertal period, decreases in pulse amplitude have also been noted (Day et al., 1987). The weak positive correlation between pulse amplitude and the number of days from sampling until ovulation in our study is in agreement with the previous results, although Day et al. (1987) also found a strong positive correlation between LH pulse frequency and days to first ovulation (r=0.88). Long term studies with increased sampling periods have reported variability in LH dynamics from one time-point to the next, even as mean concentration increased (Dodson et al., 1988; Evans et al., 1994). Other studies have also indicated that an effect of time on overall LH concentration exists (Day et al., 1984; Day et al., 1987; Dodson et al., 1988; Gasser et al., 2006b). This increase is again likely to occur as a result of decreased estradiol negative feedback on the hypothalamus, as the hypothalamic- pituitary- ovarian axis matures alongside the body. Although heifers in the current study attained puberty at a relatively young age, the similarities in increasing mean LH concentrations between high and low plane post-weaning dietary treatments may indicate that similar mechanisms acting on the reproductive system are at play in both heifers that reach reproductive capacity early and those achieving reproductive capacity at more conventional ages, as discussed by Gasser et al. (2006b). A possible explanation for the relative lack of difference between pre- or post-weaning treatment groups may again be the relatively high energy content of both post-weaning diets, which allowed for high rates of growth in both post-weaning treatment groups. Dietary restriction has previously been shown to delay increases in mean LH concentration (Day et al., 1986), although the diets provided in the current study were not limiting in any way. It is more likely that interactions between nutrition and BW, leptin concentrations (Maciel et al., 2004), or IGF-1 concentrations (Yelich et al., 1996) work together

to complete the cascade of events necessary for puberty to occur. Therefore, continued sampling until puberty with more frequent sampling dates, or greater differences in energy content between post-weaning diets, may have allowed for detection of possible differences that may have been caused by dietary treatment.

The positive correlation detected between ADG during mo 6 and LH frequency warrants further discussion, as the relationship has been sparsely studied previously. Chelikani et al. (2003) noted increased ADG in heifers attaining puberty at a young age, while ADG was decreased in treatment groups attaining puberty at more advanced ages. Nutritionally induced early-life puberty has previously been reported to occur at relatively consistent BW, even when differing diets are fed (Kennedy and Mitra, 1963; Marston et al., 1995, Chelikani et al., 2003), although ages are decreased. A reduction in the age at the attainment of puberty combined with relatively consistent BW necessitate increased ADG, and so the correlation detected in this study is logical. It is plausible that the similar rates of growth in the treatment groups in the present study allowed for faster growing calves during wk 20 – 24 from both the low and high plane post-weaning treatments, explaining the lack of dietary effect and also the noted positive correlation. Further research in this area is needed in order to determine if increased ADG in the peripubertal period is responsible for increased LH frequency.

3.43 Age at first ovulation

No overall differences in the age at first ovulation as a result of diet were seen in this study. However, the high plane post-weaning diet did influence the proportion of heifers having ovulated at 7 and 8 mo of age (Figure 3-3), suggesting that as heifers age, additional factors outside of diet may play a role in the attainment of puberty (discussed below). These results

somewhat contradict what has been reported in literature, as multiple studies have demonstrated an overall reduction in age at puberty when higher plane diets are fed (Sorenson et al., 1959; Short and Bellows, 1971; Radcliff et al., 1997; Chelikani et al., 2003; Shamay et al., 2005; Gasser et al, 2006a). A possible explanation for the lack of difference in pubertal age in our study, compared to previous studies, is that the low plane diet used in our study was relatively high in both protein and energy (Table 2-1). As a result of this, the low-plane heifers in our study grew at a similar or greater rate than those receiving "high" or "elevated" planes of nutrition in previous studies (Short and Bellows, 1971; Radcliff et al., 1997; Chelikani et al., 2003; Gasser et al., 2006a). Chelikani et al. (2003) fed heifers for low (0.50 kg ADG), medium (0.83kg ADG) or high (1.04kg ADG) gains and found that age at puberty (9.27 vs 10.80 mo) differed, while no differences in weight at puberty were found. In the current study, ADG for heifers offered the low plane diet post-weaning was 1.20kg, demonstrating the high rate of growth allowed by the diet. Short and Bellows (1971) noted that differences in age at puberty caused by feed were largely explained by differences in BW, and when age at puberty was regressed by BW no significant differences were found between treatments. Because the growth rates between treatments in the current study were so similar, the lack of difference in weight and age at puberty is partially explained.

Seasonality also played a significant role in the age at which heifers attained puberty in the current study. Due to the relatively small size of the herd from which the heifer calves were collected, as well as the year round calving schedule, the 36 heifers in the current study were born from February to August. Pre-weaning environment was identical for all calves, as they were born and housed indoors in a temperature-controlled barn with common lighting patterns

year round. However, once weaned, calves were moved to outdoor paddocks where variations in photoperiod, humidity, and temperature occurred. Although cattle are not seasonal breeders, the effect of seasonality on the attainment of puberty has been previously noted (Arije and Wiltbank, 1971; Hansen et al., 1983; Schillo et al. 1983). In these studies, fall born heifers generally attained puberty at a younger age than those born in the spring. Arije and Wiltbank (1971) proposed this occurred as fall born heifers had access to freshly grown spring grass quickly following weaning, whereas spring born heifers were autumn weaned and grew slowly on middling quality winter feed in the post-weaning phase. While no fall born heifers were used in the current study, our findings are in agreement with data from Schillo et al. (1983) where heifers exposed to simulated Spring to Autumn weather attained puberty at a younger age than heifers exposed to simulated Autumn to Spring weather, regardless of mo of birth. Schillo et al. (1983) hypothesized that this increase in the rate at which puberty is attained was caused by increased photoperiod and temperature. Support for this hypothesis is provided by Hansen et al. (1983) and Small et al. (2003), where the onset of puberty was hastened through exposure to increased photoperiod. In the current study, heifers born in the winter and spring months were exposed to a greater average day length (Figure 3-3 A) during the outdoor-housed period than those born in the summer, which may have contributed to early onset of puberty. Because of the apparent random effects of weather at different geological locations, as well as year-to-year variation in the same geological location, the effect of additional weather factors such as temperature, day length, and humidity on puberty in heifers have been sparsely studied. The negative correlations between day length and ambient temperature in the current study suggest that increased d length and warmer temperatures are

associated with a reduction in age at first ovulation. These results are in agreement with those reported by Schillo et al. (1983) where increased day length and temperature led to a reduction in the age at puberty, regardless of season of birth. Comparisons between studies may be limited, as heifers from the study by Schillo et al. (1983) were exposed to natural weather for 6 mo after birth, before housing in environmental chambers began. The heifers also grew at a reduced rate and attained puberty at a greater age than heifers in the current study. Regardless of study symmetry, it is evident that the both day length and temperature influence the age at which puberty is attained, and therefore, may need to be considered when rearing replacement heifers outdoors.

In the current study, although the main effect of diet did not influence age at puberty, a greater proportion of heifers offered a high plane of nutrition in the post-weaning phase attained puberty at 7 and 8 mo of age than those offered the low plane. Interestingly, the first seven heifers who attained puberty had received the high plane post-weaning diet, and of those seven heifers, three attained puberty at an age less than 6 mo. Few published studies have documented the attainment of puberty in animals this young, as a result of diet; however, a series of studies by Gasser et al. (2006 a,b,c,) using early weaned beef heifers and high concentrate diets is useful for comparison. Gasser et al. (2006a) found that high energy diets from an age of 125 d to 195 d decreased age at first ovulation (262 d vs. 368 d, SE = 10, P <0.01) regardless of diet fed after 195 d. In the same study, a greater proportion (P < 0.05) of heifers receiving high energy diets from d 125 to d 300 attained puberty before 300 d of age than those offered lower energy diets in the same phase (67% vs. 20%, respectively). Puberty in the current study was attained at a much younger age, however the significant differences in the

proportion of pubertal heifers at 7 and 8 mo of age (Figure 3-1) in the current study are comparable to those at 10 mo of age reported by Gasser et al. (2006a). These similarities may be explained by the fact that high energy diets in the Gasser et al. (2006a) study were introduced later in life following weaning at approximately 125 d, while in the current study weaning and the subsequent introduction of the high energy diet occurred at approximately 65 d. In a similarly designed study, Gasser et al. (2006b) reported a greater proportion of heifers having attained puberty by 10 mo of age when a high energy diet was fed following weaning at approximately 70 d of age, compared to heifers offered a lower energy diet in the same timeframe. Although the difference in the number of pubertal heifers by post-weaning diet was no longer evident by mo 10 in the current study, it is important to note that differences in the breed of animals used and differences in energy intake between treatments likely played a role in the relative timing of puberty.

3.5 Conclusions

Overall, high plane diets in the pre- and post- weaning phases increased serum letpin concentrations, and there was a tendency for the relative age of heifers at first ovulation to be related to serum leptin concentrations, although concentrations were only measured until wk 25 when ovulation occurred in a range of wk 25 to wk 52. Calves offered the pre-weaning high plane of nutrition had increased duration of LH pulses at 3 mo of age compared to low plane pre-weaning calves, but there was no other effect of diet on LH release. A greater proportion of heifers placed on the high plane diet in the post-weaning phase attained puberty by both 7 and 8 mo of age than heifers placed on the low plane diet; however, no differences were observed on the overall age or weight at which first ovulation occurred. Growth rates between treatment

groups were similar, and the age at which puberty occurred was negatively correlated with both day length and mean daily temperature during the post-weaning phase of the experiment, which likely affected the onset of puberty for heifers in this study.

In conclusion, the results from the current study indicate there may be minimal interactions between pre- and post-weaning planes of nutrition on parameters of sexual development measured in this study. For producers and those rearing heifer calves from birth to breeding age, this data may suggest that so long as diets with energy levels sufficient for maintenance and growth are available in the pre- and post-weaning phases, overall sexual development occurs independent of dietary energy levels. It is recommended that future studies examine the effects of timing on nutritional provision, as early puberty (at 7 and 8 mo) was noted in a greater proportion of post-weaning high plane heifers than post-weaning low plane heifers, presenting a potential opportunity for accelerated heifer development.

Table 3-1: The effects of pre- and post- weaning diet on various luteinizing hormone parameters at 3-month (3M) sampling (average age ± SE = 105 ± 6.4d and at 6-month (6M) sampling (177 ± 6.4d). No interactions between pre- and post- weaning diets were noted and are therefore not presented.

LH pulse	3M sampling		*P* - value	6M sampling		*P* - value
	Pre-High (n=18)[1]	Pre-Low (n=18)[2]		Post-High (n=18)[3]	Post-Low (n=18)[4]	
Frequency[5] (pulses/10h)	1.47 ± 0.22	1.80 ± 0.22	0.29	2.19 ± 0.28	2.32 ± 0.28	0.73
Amplitude[6] (ng/mL)	2.06 ± 0.20	1.90 ± 0.20	0.58	1.84 ± 0.19	1.87 ± 0.19	0.91
Duration[7] (min)	39.96 ± 21.60	27.24 ± 21.60	<0.05	27.48 ± 2.52	31.44 ± 2.52	0.26
Mean concentration (ng/mL)	0.27 ± 0.017	0.26 ± 0.017	0.43	0.34 ± 0.017	0.33 ± 0.017	0.28

[1] High = high plane of pre-weaning nutrition (10L/d whole milk per calf and *ad libitum* access to starter)
[2] Low = low plane of pre-weaning nutrition (5L/d whole milk per calf and *ad libitum* access to starter)
[3] High = high plane of post-weaning nutrition (85% concentrate as-fed dry TMR)
[4] Low= low plane of post-weaning nutrition (70% concentrate as-fed dry TMR)
[5] Pulse frequency defined as number of pulses within the 10-hour sampling period where a pulse was defined as the overall mean LH concentration (ng/mL) plus 1 standard deviation.
[6] Pulse amplitude was determined as the difference between peak LH concentration in a pulse and nadir LH concentration immediately preceding the pulse.
[7] Pulse duration was measured in minutes based on the number of consecutive samples within a pulse.

Table 3-2. The effects of sampling period on various luteinizing hormone parameters regardless of plane of nutrition.

	Sampling period[1]		
LH pulse	3M (n = 36)	6M (n = 36)	*P* - value
Frequency[2] (pulses/10 h)	1.76 ± 0.19	2.09 ± 0.19	0.21
Amplitude[3] (ng/mL)	2.10 ± 0.14	2.14 ± 0.14	0.83
Duration[4] (min)	32.8 ± 0.14	30.6 ± 0.15	0.31
Mean concentration (ng/mL)	0.25 ± 0.02	0.33 ± 0.02	< 0.01

[1] Periods: 3M = average age of 105 ± 6.4d, 6M = average age of 177 ± 6.4d.
[2]Pulse frequency was defined as number of pulses within the 10-hour sampling period where a pulse was defined as the overall mean LH concentration (ng/mL) plus 1 standard deviation.
[3]Pulse amplitude was determined as the difference between peak LH concentration in a pulse and nadir LH concentration immediately preceding the pulse.
[4] Pulse duration was measured in minutes by calculating the number of consecutive samples within a pulse.

Table 3-3: The effects of feeding high and low planes of nutrition in the pre- and post-weaning phase on age, weight, hip height, and average daily gain (ADG) at the day of first ovulation.

	Pre- weaning treatment[1]			Post- weaning treatment[2]		
Variable	High (n = 18)	Low (n = 18)	*P* - value	High (n = 18)	Low (n = 18)	*P* - value
Age (d)	257 ± 11.1	250 ± 10.5	0.62	235 ± 10.6	253 ± 11.0	0.14
Weight (kg)	328.18 ± 11.47	326.99 ± 11.64	0.94	329.36 ± 11.44	325.81 ± 11.63	0.82
Hip height (cm)	131.32 ± 1.84	132.01 ± 1.84	0.71	130.96 ± 1.39	132.37 ± 1.31	0.45
ADG[3] (kg/d)	1.37 ± 0.067	1.33 ± 0.067	0.29	1.41 ± 0.06	1.28 ± 0.06	<0.01

[1] Pre- weaning treatment: High = high plane of pre- weaning nutrition (10L/d whole milk per calf and *ad libitum* access to starter) or Low = low plane of pre- weaning nutrition (5L/d whole milk per calf and *ad libitum* access to starter)

[2] Post- weaning treatment: High = high plane of post-weaning nutrition (85% concentrate as-fed dry TMR) or Low = low plane of post-weaning nutrition (70% concentrate as-fed dry TMR)

[3]ADG was calculated weekly during the post-weaning period from d65 to d180 and grouped in 4-week segments.

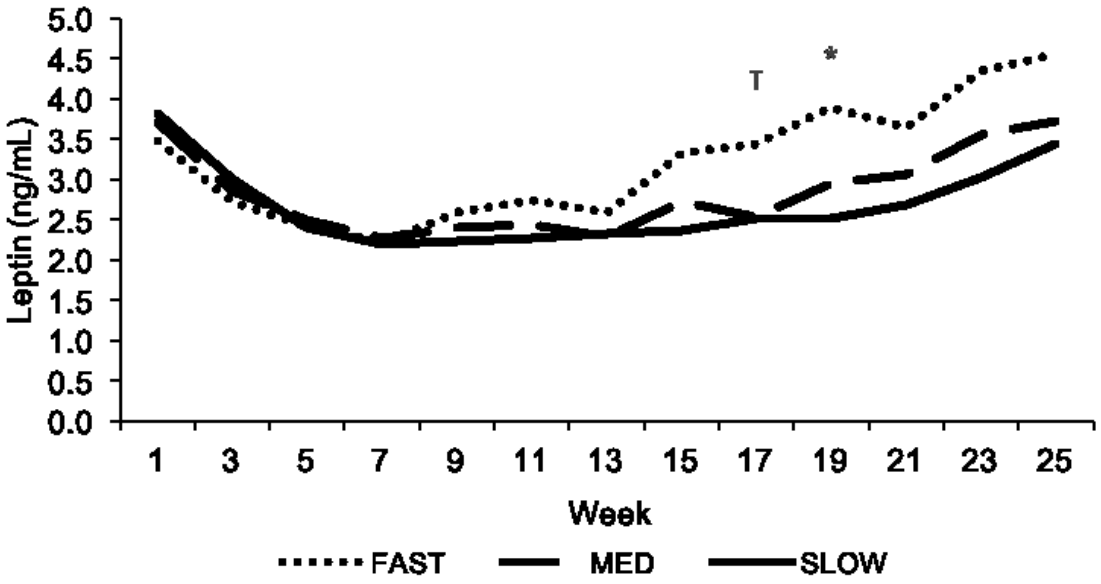


Figure 3-1. The effects of serum leptin concentrations by week on relative timing of first ovulation, where FAST = heifers (n=11) ovulating at 7 months of age or younger, MED = heifers (n=12) ovulating between 7 and 9 months of age, and SLOW = heifers (n=13) ovulating at an age greater than 9 months. Effect of relative age at first ovulation ($P = 0.059$), week ($P < 0.001$), and relative age x week interaction ($P = 0.96$).

[T] denotes tendency for FAST heifers to have greater concentrations (3.44ng/mL ± 0.42) of serum leptin than MED (2.53ng/mL ± 0.42, $P = 0.09$) and SLOW heifers (2.51 ng/mL ± 0.42, $P = 0.08$) at week 17.

* denotes greater ($P = 0.02$) serum leptin concentrations in FAST heifers (3.89 ng/mL ± 0.5) than SLOW heifers (2.52 ng/mL ± 0.5) at week 19.

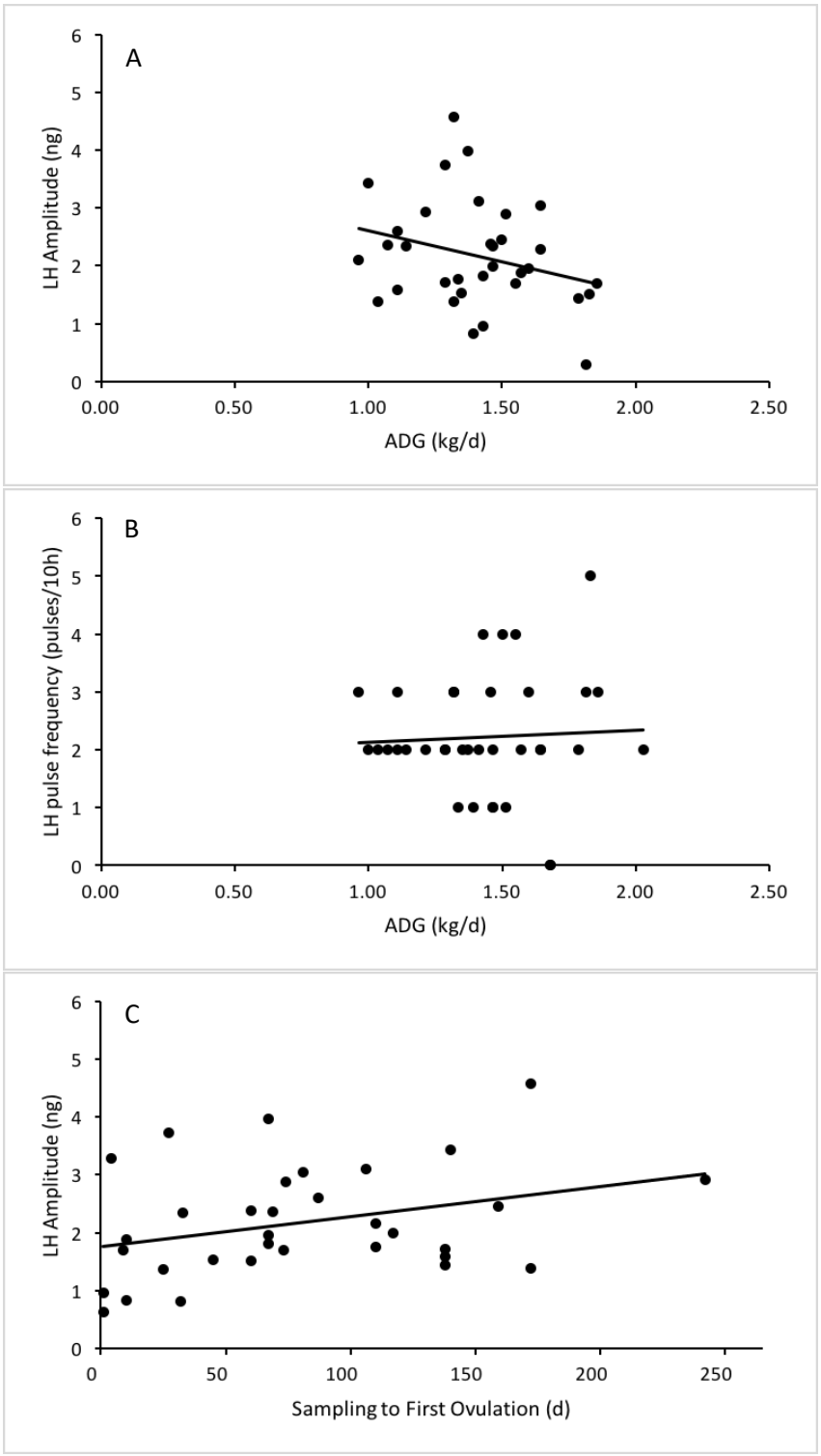


Figure 3-2. Correlations between A) ADG during from week 20 to 24 and LH pulse amplitude during the 6M sampling period, regardless of dietary treatment (r = -0.34, *P* < 0.01), and B) ADG from week 20 to 24 and LH pulse frequency during the 6M sampling period, regardless of dietary treatment (r = 0.32, *P* < 0.01) and C) Days from 6M sampling to first ovulation and LH pulse amplitude, regardless of dietary treatment (r = 0.32, *P* < 0.04).

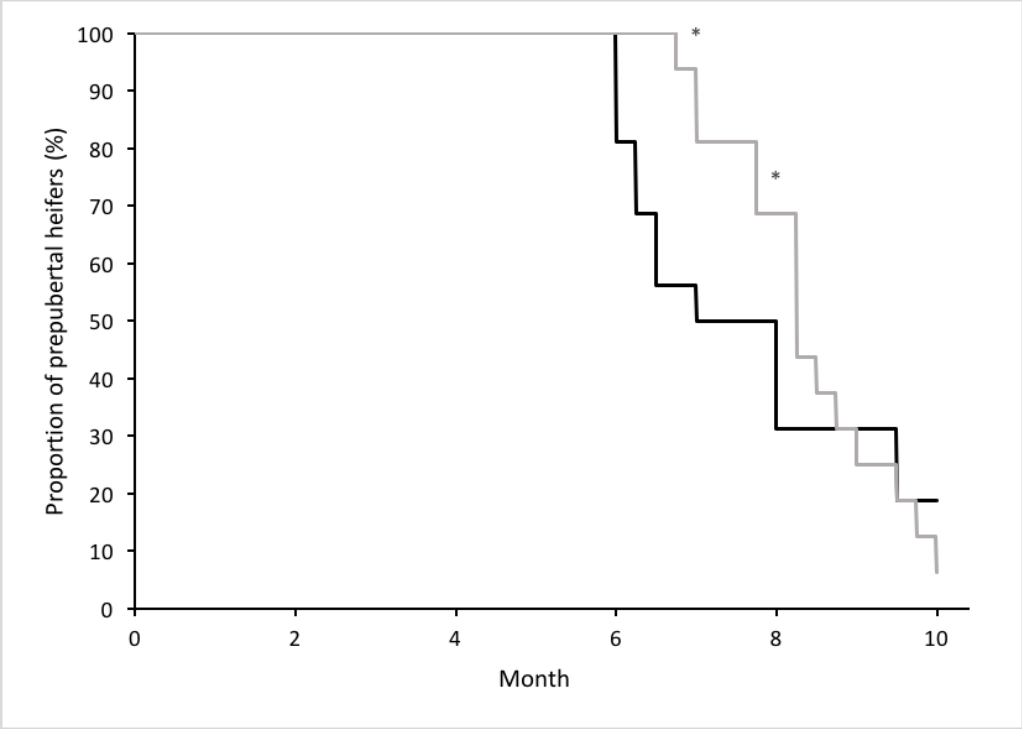


Figure 3-3. Survival plot of the effect of post-weaning diet on the proportion of prepubertal heifers by age in months. The black line represents heifers on the post-weaning high plane diet (n=18), while the grey line represents heifers on the post-weaning low plane diet (n=18, regardless of pre- weaning diet received). *$P < 0.05$. Differences denoted at 7 months of age where 50% of high plane heifers had ovulated and 18.75% of low plane heifers had ovulated, and 8 months of age where 68.5% of high plane heifers had ovulated and 31.25% of low plane had ovulated. Lines in the figure are overlapping from birth to approximately 6 months of age, as no heifers had yet ovulated.

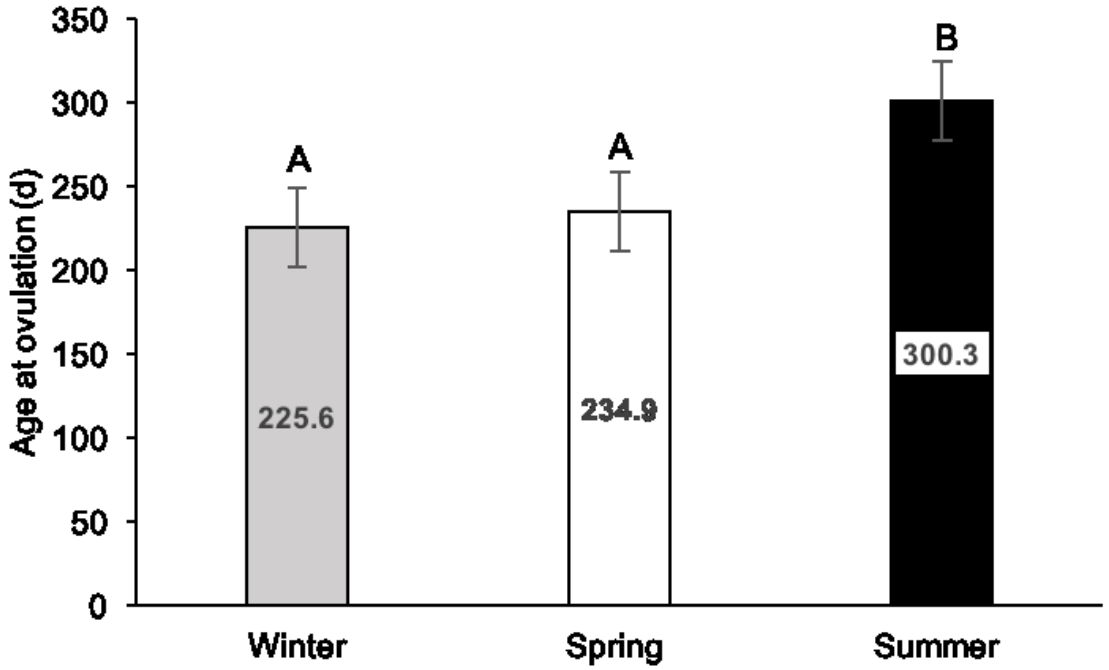
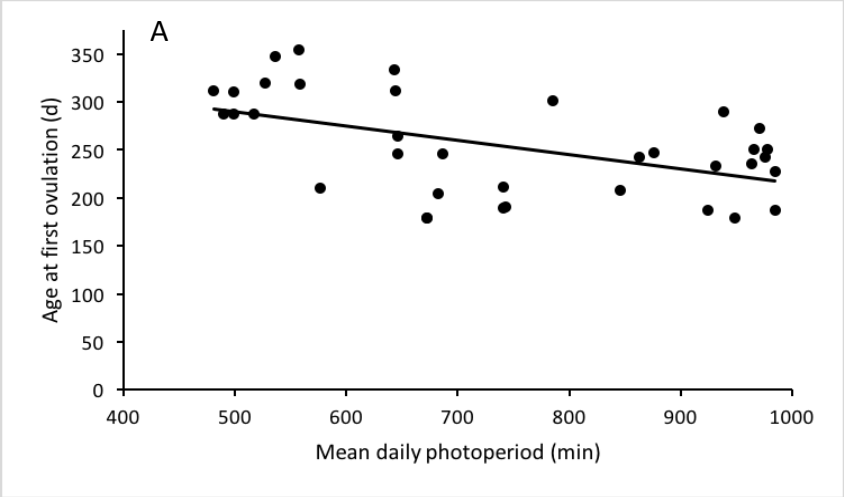


Figure 3-4. Effect of season of birth on age at first ovulation for experimental heifers regardless of plane of nutrition in the pre- or post-weaning phases. Heifers were considered winter born when born in January, February, and March (n=11), Spring born when born in April, May, and June (n=18), and Summer born in July and August (n=7). Differing superscripts denote $P < 0.05$.



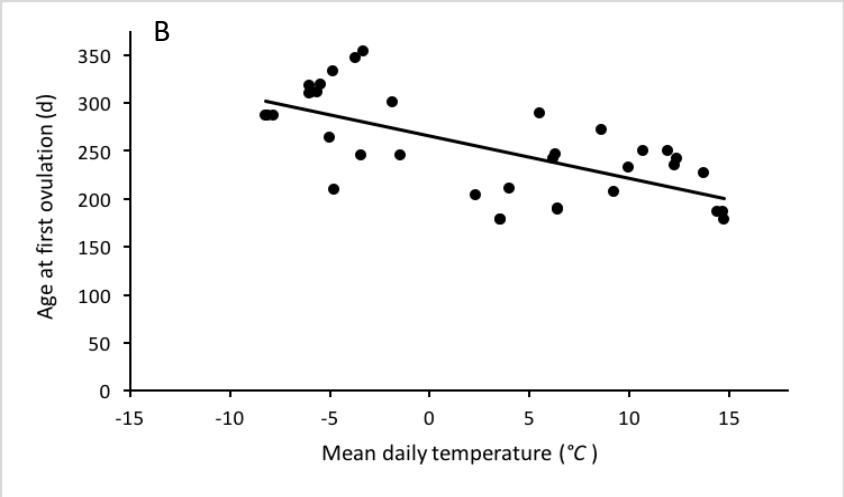


Figure 3-5. Correlations between A) days to first ovulation and mean daily photoperiod during the outdoor post-weaning phase of the study (d80-180, r=-0.51, $P < 0.01$) and B) days to first ovulation and mean daily temperature from d80 to date of first ovulation (r=-0.66, $P < 0.01$).

4.0 General Discussion

4.1 Importance of the current study

Previous studies have shown that elevated planes of nutrition in the pre-weaning phase have led to increased intake (Khan et al., 2011), growth (Diaz et al., 2001), IGF-1 and leptin concentrations (Brown et al., 2005a), rates of sexual development (Shamay et al., 2005), and future productivity (Soberon et al., 2012). Likewise, elevated planes of nutrition in the post-weaning phase have caused increased rates of intake and growth (Meyer et al., 2006; Gasser et al., 2006), decreased feed costs (Radcliff et al., 2000; Heinrichs et al., 2013), and a decreased age at first calving (Van Amburgh et al., 1998). However, very few studies have been conducted to date have examined the plane of nutrition in each phase, and the interactions that may occur between them. As a result, the effects of pre- weaning plane of nutrition, post-weaning plane of nutrition, and the interactions between these 2 phases were the focus of this thesis.

We found that when provided diets that allow for both maintenance and elevated growth, interactions between the pre- and post- weaning phases are minimal. In addition, we found that high planes of nutrition consistently increased feed and ME intake, though increased rates of growth did not always follow, indicating that energy was being partitioned elsewhere in the body. Feed intake data, recorded daily from birth to 25wk, indicated that *ad libitum* access in the post-weaning phase led to levels of DMI, greater than expected using NRC (2001) predictive equations. In both the pre- (high = 0.82 kg/d, low = 0.65 kg/d) and post- weaning phases (high -= 1.41, low = 1.28 kg/d), heifers grew at high rates, resulting in age and weight at first ovulation (high = 235d and 320.82kg, low = 253d and 304.88kg, by post-weaning diet) approaching BW recommendations for breeding (NRC, 2001) at a far younger age than heifers

conventionally reared in Canada (Pietersma et al. 2006). Concentrations of hormones and metabolites, including glucose, insulin, IGF-1, and leptin, were influenced by plane of nutrition, however the lack of interactions between pre- and post- weaning planes indicate that the influence of diet was the result of the diet currently being offered, and not previous nutritional plane. Effects of plane of nutrition on the attainment of puberty or on LH concentrations at 3 and 6 mo were not noted, though pulsatile release was detected at 3mo of age and mean LH concentration increased with time.

4.2 Limitations

In the past, studies offering differing planes of nutrition in either the pre- or post-weaning phases have successfully elicited significant differences in growth and development through dietary treatments (Brown et al., 2005a; Gasser et al., 2006). The lack of differences caused by plane of nutrition, specifically in growth and sexual development specifically in the post-weaning phase, was likely the result of post-weaning treatments that were too similar in energy and protein content to significantly elicit change. The high plane post-weaning diet may have supplied energy in excess of what the body could use for growth, allowing the low plane heifers to grow at a similar rate, and at a rate greater than heifers are conventionally raised on farm. Additionally, the *ad libitum* style of feed provision may have allowed for sorting behaviour to be rewarded with increased feed allocation, as low plane heifers could have selected for concentrate components and further reduced difference in energy contents between post-weaning diets (Engelking et al., 2018). The unintended similarities in post-

weaning diets, and the possibility of feed sorting in the post-weaning phase, should be considered limitations of this study.

As a result of the high energy diets, and high rates of growth in our study, questions regarding fat deposition are natural. The increased BCS and leptin concentrations found in high plane post- weaning heifers indicate some degree of increased fat deposition, and studies offering similar planes of nutrition have documented increased adipose content in the body (Van Amburgh et al., 1998; Chelikani et al., 2003) and mammary glands (Brown et al., 2005b) of heifers fed high energy diets. Since deposition of fat in the mammary gland of heifers is likely to reduce future milk production (Drackley, 2008), and the value of cows to a farm is closely related to their milk production, excess fat deposition for growing heifers should be avoided. Overall, our study was limited by a lack of measurement of fat deposition and body fat composition, however this could be corrected in future studies by the use of ultrasonography for back fat and mammary adipose measurement, or total body fat composition analysis.

4.3 Future research

To improve our understanding of pre- and post- weaning planes of nutrition, and possible interaction that may occur between them, future studies should focus on a few key knowledge gaps that still exist. Firstly, future studies should consider providing control diets in the pre- and post- weaning phases that may more accurately represent diets that are currently being fed on farm. While elevated planes of nutrition are gaining popularity on farm, the economic appeal of conventional milk feeding at approximately 10% of BBW (Vasseur et al., 2010) results in many calves still being fed at rates that only support growth at less than 0.5kg/d (Jasper and Weary, 2002). Although less attention is often given to heifers in the post-

weaning phase, ranges of growth from 0.5kg/d to 1.1 kg/d demonstrate the substantial variability that may occur from farm to farm (Reviewed by Zanton and Hienrichs, 2007). In the current study, growth rates for heifers offered the low plane diets in each phase exceeded levels of growth that would commonly be seen on farm, which may have reduced the likelihood of detecting differences on a number of parameters measured. In a similar manner, the 10d step-down milk weaning protocol and lengthy TMR transitions employed in our study are likely not representative of the current starter-based weaning strategies (Khan et al., 2011), nor are they practical for producers not using automated milking systems. Instead, future studies could offer a "conventional" plane of nutrition in each phase, with a conventional weaning transition, to potentially demonstrate the advantages thought to be associated with elevated planes of nutrition.

Secondly, future studies should make an effort to focus on measuring adipose tissue deposition and mammary fat pad deposition in relation to pre- and post- weaning planes of nutrition. Sejrsen and Purup (1997) reported ADG greater than 0.7kg/d favored fat pad deposition with the potential to reduce future milk yield. More recently, studies have shown that increased pre-weaning ADG can increase the rate of parenchyma tissue deposition in the mammary gland (Brown et al., 2005b; Geiger et al., 2016), and that increasing the protein to energy ratio in post-weaning diets supporting gains greater than 1.2kg/d did not impair mammary development (Davis - Rincker et al., 2008). Ultrasonography of the mammary gland has been shown to be an accurate, non-invasive method for measuring mammary gland composition (Esselburn et al., 2015), and may be an option for determining the effects of high

energy pre- and post-weaning diets, as well as their possible interactions, on the potential for future milk production in heifers.

In addition to the above mentioned knowledge gaps, an area of potential interest for future studies is the additional effects on sexual development that planes of nutrition may play. While no dietary differences in age or weight at first ovulation were noted between treatment groups in the current study, it is possible that postpubertal ovarian follicular dynamics, estrous behavior, and corpus luteum function may have been altered by the diets offered. Previously, differing planes of nutrition have been shown to impact follicular development (Murphy et al., 1991; Bergfeld et al., 1994). While age and weight at first ovulation were not affected by pre- or post- weaning diet, these additional parameters, which play important roles in fertility, moving forward, warrant consideration in future studies.

Lastly, while the considerations of health and immune function were not documented in the current study, future research may wish to document the potential health benefits that elevated planes are thought to elicit in heifer calves. Previous studies have documented improved responses to disease challenges as a result of increased planes of nutrition in the pre- and post- weaning phases (Ollivett et al., 2012; Ballou 2012). In the current study, the incidence of sickness was very low for both treatment groups, which was likely the result of sufficient dietary energy being provided for maintenance of body systems and growth. This topic warrants further study, as the cost savings and reduction of antibiotic use associated with healthier calves offered elevated planes of nutrition may improve the welfare of heifers, as well as overall farm economics.

4.4 Considerations

In moving forward with nutritional studies intended to improve the efficiency with heifers are raised, it will be important to consider the social license of dairy production. As an example from Google (2018), the term “veganism” was one of the most highly searched trends of 2017 in Canada, and input from the general public has led to proposed updates in Canada’s Food Guide promoting plant-based foods (Government of Canada, 2018). Increasingly, consumers appear to prefer food that, in their eyes, is raised more naturally or from animals with improved welfare. From this perspective, elevated planes of nutrition for dairy heifers (especially in the pre-weaning phase), may help to improve the public perception of dairy through improved health (Ollivett et al., 2012) and the potential reduction of antibiotic use, or through “biologically appropriate nutrition” which allows calves to grow at a similar rate as those who are raised by their dam (Drackley, 2008). In addition to these examples, the influence of early life nutrition on improved cow longevity (Bach, 2011) may help to alter the perception that sustainability is not often prioritized in the dairy industry. Therefore, further research demonstrating the effects of improved early life nutrition as a tool to improve welfare, sustainability, and efficiency of dairy production deserve strong consideration.

4.5 Conclusions

The objective of this thesis was to examine the effects of pre- and post- weaning diets with differing energy levels on intake, growth, hormone and metabolite concentrations, and sexual development of Holstein heifer calves. Throughout both studies, no interactions between pre- and post- weaning planes of nutrition on parameters measured, excluding VFA,

were noted. Overall, high planes of nutrition in the pre- and post- weaning phases increased feed intake and ME intake, although growth was not consistently increased as a result. High planes of nutrition also tended to increase concentrations of various hormones and metabolites in the pre- and post- weaning phases, including insulin, IGF-1, leptin, and BHBA. There was no effect of plane of nutrition on the age or weight of heifers when first ovulation occurred, although heifers offered the post-weaning high plane of nutrition were more likely to attain puberty at 7 and 8 mo of age than those offered the post-weaning low plane. In the same manner, the effects of plane of nutrition on pulsatile LH release at 3 and 6 mo of age were limited to a dietary effect on LH pulse duration at the 3M sampling. In summary, this thesis demonstrates so long as heifers are offered diets that allow for maintenance and growth in the pre-weaning phase, post-weaning dietary energy levels may be the primary factor regulating development in heifers up until 6 mo of age.

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Appendix

Table A1: Pre- and Post-weaning dietary effect on volatile fatty acid production

Week	Treatment[1]		SE	*P* - value
	High	Low		
Total VFA				
4	45.66	53.70	4.28	0.18
8	48.90	57.51	4.08	0.14
12	64.76	54.15	2.40	0.002
16	60.19	53.37	2.42	0.04
20	58.24	54.36	2.41	0.24
24	63.80	53.40	2.40	0.003
Propionic				
4	10.59	12.84	1.23	0.20
8	11.73	16.04	1.16	0.01
12	16.50	12.60	0.68	<0.0001
16	13.15	11.77	0.68	0.14
20	13.08	11.63	0.70	0.13
24	14.17	10.77	0.68	0.0005
Acetic				
4	28.88	33.30	2.48	0.21
8	30.51	33.70	2.37	0.34
12	38.86	33.26	1.55	0.02
16	37.44	33.51	1.55	0.07
20	35.07	35.36	1.60	0.89
24	39.37	34.04	1.55	0.02
Butyric				
4	5.02	5.26	0.84	0.84
8	5.28	5.78	0.80	0.65
12	8.61	6.84	0.35	0.0004
16	8.04	6.32	0.35	0.0006
20	7.68	6.06	0.36	0.0015
24	8.57	6.23	0.35	<0.0001

[1] Treatment: High = high planes of nutrition at weeks 4 and 8 during the pre-weaning phase (10L/d whole milk per calf and *ad libitum* access to starter) and high planes of nutrition during weeks 12,16,20, and 24 during the post-weaning phase (85% concentrate as-fed dry TMR) or Low = low plane of pre- weaning nutrition (5L/d whole milk per calf and *ad libitum* access to starter) and low plane of post-weaning nutrition (70% concentrate as-fed dry TMR). No interactions between the phases were detected.

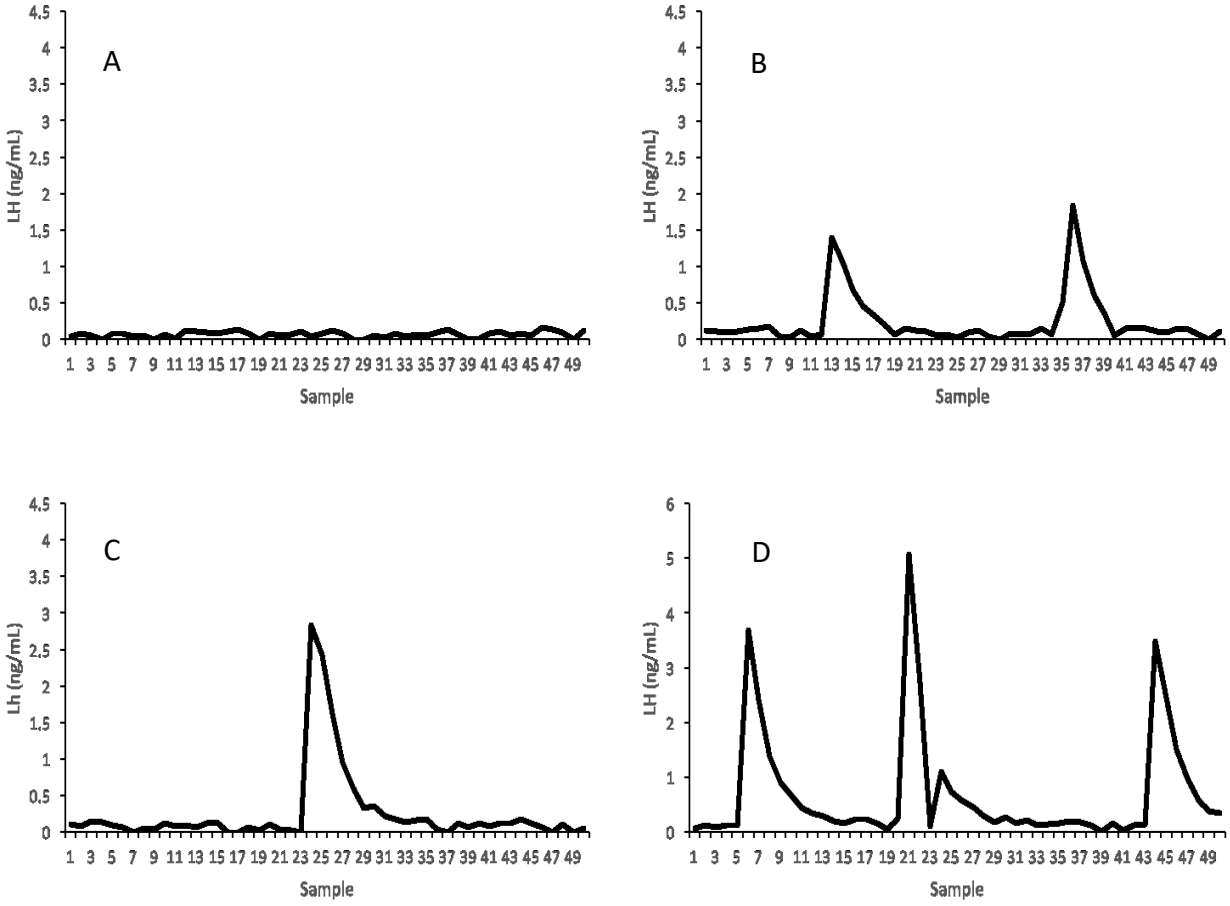

Figure A1: Representative figures of the pulsatile release of luteinizing hormone (LH) in Holstein heifers illustrating A) lack of LH pulsatile release B) relatively low pulse amplitude and a frequency of 2 pulses/ 10 h C) middling pulse amplitude and a frequency of 1 pulse / 10 h and D) relatively high amplitude and a frequency of 3 pulses / 10 h. Samples were taken at 12-min intervals over the course of 10 h.