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Effects of Supplemental Dietary Starch on Production and Reproductive Characteristics in Postpartum Dairy Cows

By

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DEDICATION

This thesis is dedicated to my husband whose amazing support I cannot do without.

ABSTRACT

This study evaluated the effects of dietary starch on productive and reproduction parameters of postpartum dairy cows. Three diets were fed, with increasing starch levels from calving until 70 days in milk. Treatment had no effect on dry matter intake, energy balance, specific metabolic hormones and metabolites, milk yield, or milk components with the exception that cows fed the low starch diet had higher levels of milk urea nitrogen. Cows fed the high starch tended to lose less body condition, had a shorter interval from calving to first ovulation, and a higher incidence of double first ovulations. There were no treatment effects on ovarian dynamics, luteinizing hormone, progesterone or estradiol concentrations. Number of cows confirmed pregnant 30 d after first insemination did not differ between treatments. Increasing dietary starch decreased the interval from calving to first ovulation, but had no impact on productivity and metabolic status of the postpartum cow.

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LIST OF ABBREVIATIONS

ADF	acid detergent fiber
BCS	body condition score
BHBA	β-hydroxybutryate
CL	corpus luteum
CP	crude protein
CV	coefficient of variation
DDF	days to reach a dominant follicle
DIM	days in milk
DM	dry matter
DMI	dry matter intake
EB	energy balance
FSH	follicle stimulating hormone
GH	growth hormone
GnRH	gonadotropin releasing hormone
HSD	high starch diet
IGFBP	insulin-like growth factor binding proteins
IGF-I	insulin-like growth factor I
LH	luteinizing hormone
LSD	low starch diet
mRNA	messenger ribonucleic acid
MUN	milk urea nitrogen
MSD	medium starch diet
NDF	neutral detergent fiber
NEB	negative energy balance
NEFA	non-esterified fatty acids
NE_L	net energy for lactation
OM	organic matter
PGF2α	prostaglandin F2α
SCC	somatic cell count
SEM	standard error of the mean
TMR	total mixed ration

CHAPTER 1.

REVIEW OF THE LITERATURE

1.1. Introduction

Reports of declining reproductive performance in dairy cows have been surfacing since the mid 1970's (Butler and Smith, 1989). As genetic selection has focused on improving milk yield and productivity of cows, an inverse relationship with milk production and reproductive performance has been observed. Butler and Smith reported in 1989 that a decline in conception rates from 66% in 1951 to 50% in 1975 was also associated with a 30% increase in milk production. Lucy (2001) found many conflicting reports, but stated that an overall trend of reduced reproductive performance in relation to rising levels of milk production has been observed from 1970 to 2000. Researchers have suggested that increased milk production with its associated high energy demand is an important factor affecting fertility. Infertility has been shown to be prevalent in cows with the greatest milk production (Lucy, 2001) and thus likely the cows with the greatest negative energy balance (NEB). Nebel and McGilliard (1993) suggest that high-yielding cows, who cannot consume enough energy to meet their production demands, must mobilize fat reserves and as a result are in a greater state of NEB. However, they also stated that some herds with high milk production actually have decreased days open due to superior management practices. Other factors such as poor nutritional management and larger herd size are also associated with reduced reproductive performance, but these issues are inherently management related and are not the true source of declining fertility (Butler, 2000).

While poor reproductive performance in relation to increased milk production is well documented (Lucy, 2001; Nebel and McGilliard, 1993), the physiological causes of this phenomenon are not as clear. Reduced concentrations of hormones involved in both lactation and reproductive function is observed when milk production is high (Butler and Smith, 1989) and milk synthesis takes priority in early lactation due to nutrient partitioning (Lucy, 2000). An increase in blood metabolites such as non-esterified fatty acids (NEFA) in early lactation due to fat mobilization may interfere with hormone availability (Bossaert et al., 2008). Specifically, insulin and insulin like growth factor-I (IGF-I), which play an important role in follicular development and resumption of cyclicity post calving (Thatcher et al., 1996; Butler et al., 2004; Butler, 2003), have been shown to be significantly decreased in early lactation (Bossaert et al., 2008). While lowered conception rates are a well documented indicator of reduced fertility, it may be that delayed initiation of cyclicity in early lactation is the most important factor affecting reproductive efficiency in the breeding period. Butler (2000) demonstrated that a delay in first ovulation postpartum is associated with poor conception rates. Negative energy balance is highly correlated with increased interval from calving to first ovulation and is also strongly associated with reduced dry matter intake (DMI) (Butler, 2003). Therefore, in order to improve reproductive efficiency it is logical to increase energy intake by increasing the energy content of the diet and by trying to promote DMI.

This review will focus on production and reproductive performance of the early lactation dairy cow. It will specifically examine the role of hormones and

metabolites that are important to follicular development and known to be influenced by milk synthesis.

1.2. The Postpartum Cow

1.2.1. Energy requirements and energy balance

Mature lactating cows require energy for maintenance and lactation while first parity cows also require energy for growth (NRC, 2001). In early lactation it is unlikely that the cow will be able to consume enough energy to meet all of these demands. De Vries et al. (1999) reported that first lactation cows have an energy requirement of approximately 31.1 Mcal NE_L/d on d 10 of lactation but have an intake of approximately 25.1 Mcal NE_L/d. Second and third lactation cows were reported to have a more severe energy deficit with energy requirements being approximately 44.2 Mcal NE_L/d and intake being approximately 32.3 Mcal NE_L/d. Butler and Smith (1989) reported that nearly all cows are in a state of NEB starting approximately 2 days after calving and reaching nadir, the most severe point of NEB, 14 days later. This is also in agreement with Nebel and McGilliard (1993) who state that the nadir occurs 15 days postpartum, however, more recent data reported cows reaching the nadir at 1 week post-calving (Doepel et al., 2002). A publication by De Vries et al. (1999) also showed an NEB nadir in the first week of lactation but reported that multiparous cows were in a state of NEB until 80 DIM but that primiparous cows recovered from NEB at approximately 55 DIM. Patton et al. (2007) reported that cows are genetically predisposed to be in severe NEB in early lactation due to selection for milk production.

1.2.2. Changes in metabolic profile

The profile of metabolic hormones and blood metabolites undergoes changes soon after calving in both primiparous and multiparous cows. These changes occur as a result of the drastic increase in milk synthesis, change in energy level of the diet and overall EB of the cow. The hormones and metabolites most affected are glucose, insulin, IGF-I, NEFA and β-hydroxybutryate (BHBA).

Glucose, the main energy metabolite for the cow, plays a large role in milk lactose production with the mammary gland utilizing between 60-85% of all available plasma glucose (Knowlton et al., 1998). Doepel et al. (2002) reported glucose concentrations ranging from 60-65 mg/dl in the week prior to calving to 45-55 mg/dl in the week after calving and associates the drop in glucose to reductions in DMI and increased demand from the mammary gland. Since the demand for glucose is so great following calving, a drop in circulating glucose is seen for the first 2 weeks postpartum (Wathes et al., 2007a). Insulin concentrations are also reduced post-calving, but the mechanism that explains why this occurs is still somewhat unclear. Different independent studies (Gutierrez, 2006; Ingvartsen and Friggens, 2005) have shown a negative correlation between milk yield and blood insulin concentrations. Lower circulating insulin concentrations means less glucose is taken up by insulin sensitive tissues such as muscle and more is available for non-insulin sensitive tissues such as the mammary gland (Zhao et al., 1996). Insulin has been shown to be a key factor in repartitioning nutrients away from the body tissues towards milk production in early lactation (van Knegsel et al., 2007). Butler (2000) and

Lucy (2000) both reported that circulating insulin and IGF-I decreased after calving and then slowly rose again. Doepel et al. (2002) reported plasma insulin concentrations to range from 0.5-0.9 ng/ml in the week prior to calving and from 0.3-0.4 ng/ml in the week post-calving. A similar trend was also reported by Doepel et al. (2002) for IGF-I with plasma concentrations being 150 ng/ml in the week prior to and 50 ng/ml in the week following calving. Interestingly, McGuire et al. (1995) found that increased insulin levels have been shown to increase IGF-I concentrations in blood plasma. IGF-I is involved in growth hormone (GH) regulation as increased GH in early lactation has been linked to increased NEFA concentrations and lipolysis (Lucy et al., 2001). Finally, and most important to reproductive function, IGF-I plays a large role in the ovary (Beam and Butler, 1999).

Glucose, insulin and IGF-I all have a positive relationship with EB (Butler, 2000; Grummer et al., 2004); a lower EB results in lower insulin, IGF-I and plasma glucose concentrations, and the rate at which they fall and rise post-calving is affected by the degree of NEB. Beta-hydroxybutyrate and NEFA are also highly impacted by the initiation of lactation following calving. Non-esterified fatty acids are a direct product of lipid metabolism and become more abundant in the circulation as EB declines (Grummer et al., 2004). Non-esterified fatty acids are taken up by the liver and either fully oxidized or partially oxidized to form ketone bodies, predominately in the form of BHBA (Wathes et al., 2007a). High BHBA levels, indicative of subclinical ketosis, are associated with declining EB (Gummer et al., 2004) and are an adaptive strategy for maintaining

the glucose concentrations needed for the mammary gland (Wathes et al., 2007a). Wathes et al. (2007a) also found a positive relationship between NEFA and BHBA from the week prior to calving until 4 weeks post-calving. After week 4, NEFA tended to return to pre-calving levels while BHBA continued to rise until week 6 (Wathes et al., 2007a). This same publication also reported a negative correlation between NEFA and IGF-I from week -1 to +4 postpartum. Wathes et al. (2007a) found that primiparous cows had reduced BHBA and increased IGF-I concentration earlier post-calving than multiparous cows and that the peak in NEFA concentration occurred sooner than in multiparous cows. Grummer et al. (2004) found that higher plasma concentrations of NEFA and BHBA and lower levels of glucose, insulin and IGF-I were associated with cows in NEB. Therefore, increased concentrations of BHBA and NEFA are good indicators of nutritional status of the postpartum dairy cow. Canfield and Butler (1990) proposed that plasma NEFA concentrations may actually relay EB signals to the central nervous system.

1.2.3. Changes in DMI, milk yield and composition, body weight and body condition score

Many changes occur in the cow postpartum, such as a dramatic decrease in body weight and an increase in milk production. An increase in DMI is needed in order for the cow to maintain a good energy status. However, due to the overall stress of calving and the time needed for ruminal adaptation to the high concentrate diet, DMI may not meet required levels and low intake post-calving predisposes the cow to metabolic disorders and NEB. Dry matter intake is also

strongly related to body condition at calving (Butler, 2000). Cows that lose excessive weight and are over conditioned (above 3.5) at calving generally tend to have reduced DMI and lose more body condition score (BCS) during lactation than those calving at the recommended BCS (Roche, 2006). Dry matter intake postpartum is also highly correlated with NEB (Butler, 2000). Previous thinking was that increased milk production was the main cause of reduced EB but researchers have now focused their attention on the inability of the animal to consume enough dry matter to meet energy requirements needed for high milk production. Villa-Godoy et al. (1988) and Liefers et al. (2003) both stated that NEB is directly related to insufficient DMI. Research into increasing energy content in the diet to maximize energy intake is discussed later in this chapter.

Peak milk yield occurs between 5-7 weeks postpartum (Wathes et al., 2007a) and yield is lower in primiparous cows than multiparous cows. Insufficient DMI in relation to high milk yield has been associated with increased NEB and decreased levels of insulin and IGF-I (Gong et al., 2002). Although both Villa-Godoy et al. (1988) and Liefers et al. (2003) stated that cows with high milk yield might actually be in a more favorable state of EB compared to cows with low milk yield, Gong et al. (2002) found that all cows were in NEB for the first 5-6 weeks postpartum. Doepel et al. (2002) also reported a NEB for the duration of that 6 week study. As well, Butler and Smith (1989) found a negative correlation between EB and milk production. Milk composition may also be related to EB in early lactation. De Vries and Veerkamp (2000) used a database of 470 early lactation, first parity cows and found that reductions in milk fat

percentage were highly correlated a more severe NEB nadir. Grieve et al. (1986) also found that NEB was correlated to a decrease in milk fat percentage, an increase in protein percentage and a decrease in the fat to protein ratio. As well, milk urea nitrogen (MUN) levels may be associated with milk yield. Oltner et al. (1985) found a positive correlation between milk yield and MUN levels where as Ropstad et al. (1989) found no such correlation. While it is known that high MUN levels are associated with increased dietary protein and reduced reproductive performance (Eicher et al., 1999), it is not clear as to whether these levels in the early lactating cow are impacted through milk production and EB.

Significant changes in body weight and BCS are also seen in cows in early lactation. As stated above, BCS at calving seems to play a very crucial role in determining DMI and in turn EB in early lactation. Butler (2000) stated that NEB is directly related to DMI and BCS at calving. Cows calving with either too high or too low a BCS are at increased risk for metabolic problems and energy deficits. Roche (2006) showed that cows calving with too high a body condition are likely to lose a large amount of body condition, have reduced DMI and an increased period of NEB. Chagas et al. (2007) reported that an ideal BCS at calving was between 3.0-3.5 but that cows should not lose more than 0.5 BCS throughout lactation. Parity also plays a role in body weight in the postpartum cow. Primiparous cows have been shown to weigh significantly less than multiparous cows but have similar BCS (Tanaka et al., 2008).

1.3. Follicular Dynamics and Hormone Concentrations Early Postpartum

1.3.1. Interval from calving to first ovulation and relation to energy balance in early lactation

The interval (days) from calving to first ovulation postpartum is highly important for fertility later in lactation. Butler (2000) reported that a decreased interval from calving to first ovulation is positively correlated with conception rate. As dairy cows are not typically bred until after 60 days postpartum it is common and desirable for the animal to have already completed several estrous cycles and thus have an improved likelihood of conception at breeding (Butler and Smith, 1989).

Resumption of follicular development occurs soon after calving, between days 5-7 with a dominant follicle developing by approximately day 16-20 postpartum (Butler, 2000). As reported by Beam and Butler (1999) and Silvia et al. (2002), there are 3 possible events associated with the formation of the first dominant follicle post calving. The first possibility is that the follicle will ovulate and the cow will enter into its first estrous cycle. This initial cycle is often associated with a short luteal phase and no estrus activity as the hypothalamus requires progesterone priming of the hypothalamus in order to display estrous behavior. Secondly, the follicle may undergo atresia and be followed by a new follicular wave. Thirdly, the follicle will become cystic and a lengthened anestrous period will follow. Roche et al. (2006) reported that between 30-80% of cows will ovulate the first dominant follicle, 15-60% will fail to ovulate the follicle and follicular turnover will occur while 1-5% will develop a cystic

follicle. Nebel and McGilliard (1993) reported that the average number of days to first ovulation was 29.

One of the most important factors influencing the fate of the first dominant follicle is the extent of NEB. Cows showing NEB in the first 3 to 4 weeks postpartum and those with an increase in the number of days to the NEB nadir are likely to have an increased period to first ovulation (Butler, 2000; Canfield and Butler, 1990). It is also likely that the animal will not ovulate until the NEB nadir has passed and the hypophyseal-ovarian axis has been reactivated (Nebel and McGilliard, 1993). This is in agreement with Beam and Butler (1999) who found that follicles developed after the NEB nadir are more likely to ovulate as a result of enhanced follicular growth and estradiol production. As stated previously, both BHBA and NEFA have been shown to be indicators of energy status and are correlated with NEB in the cow. Canfield and Butler (1990) suggested that plasma concentrations of these metabolites may signal energy status to the hypothalamus and thus influence gonadotropin secretion and ultimately ovulation. Wathes et al. (2007a) found that elevated concentrations of BHBA and NEFA were related to the number of days to first ovulation, however, Harrison et al. (1990) found no correlation between these metabolites and time to first ovulation.

Cows that experience greater losses in body condition and body weight in early lactation generally have more severe NEB postpartum (Roche, 2006; Patton et al., 2007). Many researchers have found a link between increased losses of BCS after calving and increased days to ovulation postpartum. Cows in poor BCS, or those losing considerable weight in early lactation are also likely to have

poor reproductive performance, as they tend to prioritize nutrients toward milk production (Lucy, 2003).

1.3.2. Selection, recruitment and dominance of the ovulatory follicle

Follicle stimulating hormone (FSH) plays an important role in orchestrating the development of the first follicular wave that occurs between days 5 and 7 post-calving (Butler, 2000). However, FSH concentrations appear to not be affected by NEB nor be a causative factor of delayed ovulation as cows develop a follicular wave soon after calving regardless of energy status (Roche, 2006). During the emergence of small follicles, FSH release is highly important; it plays not only a role in follicle recruitment but also is important for initial increases in estradiol and inhibin. Glister et al. (2006) found that cultured granulosa cells produced increased plasma concentrations of estradiol and inhibin when cultured with FSH and IGF-I and a greater increase when the two were in combination. Both estradiol and inhibin work to suppress release of FSH from the anterior pituitary (Roche, 2006) in order to prevent growth and development of other follicles. Only the follicle selected for dominancy will be able to continue growing despite the reduced FSH (Roche, 2006). Selection is defined by Wiltbank et al. (2000) as the follicular diameter (8.5 ± 1.2 mm) at which the future dominant follicle expresses an abrupt deviation in growth rate from other follicles. This selection process is also characterized by increasing concentrations of luteinizing hormone (LH), production of estradiol and suppression of FSH through release of inhibin from granulosa cells (Wiltbank et al., 2000).

1.3.3. Role of NEB and LH pulsatility in the first ovulation

The concept that NEB and its associating factors have a negative impact on initiation of ovulation and cyclicity postpartum is well documented. Roche (2006) identified the factors affecting the ability of the dominant follicle to ovulate as 1) reduced size of the dominant follicle 2) low LH pulse frequency and 3) reduced concentrations of plasma IGF-I. The growth and ultimate ovulation of the dominant follicle is then dependent on the frequency and amplitude of LH pulses and the ability of the follicle to respond to LH. Roche (2006) reported that one pulse per hour is needed for ovulation. Negative energy balance has been shown to have a severe impact on LH pulsatility (Beam and Butler, 1999) and can actually decrease the follicle's responsiveness to LH (Butler, 2000). Roche (2006) also found that a significant loss in body condition during early lactation was directly related to reduced follicle size and lowered LH pulsatility. Lower LH pulsatility results in an insufficient rise in the estradiol needed to stimulate the LH surge to trigger ovulation. Interestingly, a positive correlation was also found between increased DMI postpartum and the size of the preovulatory follicle (Armstrong et al., 2003). Roche (2006) also reported that cows in poor BCS after calving had a reduction in the diameter of the dominant follicle.

Lucy (2003) reported that an increase in LH pulse frequency should begin 1-2 weeks postpartum, and that an LH surge, potentially leading to ovulation of the dominant follicle, should occur shortly thereafter. The lack of adequate gonadotropin releasing hormone (GnRH) release, resulting from poor production of estradiol from the dominant follicle is ultimately responsible for a poor LH

surge that is unable to induce ovulation (Lucy, 2003). Insulin and IGF-I also play a large role in potential ovulation of the follicle and the responsiveness of the follicle to FSH and LH. These factors are addressed in detail later on in this chapter.

Aside from EB, parity appears to impact follicle growth and potential for the first dominant follicle to ovulate postpartum. Wathes et al. (2007a) reported that a greater proportion of primiparous cows (21%) took longer than 45 days to ovulate when compared to multiparous cows (9%). This is in agreement with Santos et al. (2009) who found that a significant proportion of multiparous cows resumed estrous cyclicity before primiparous cows. Although it has been shown that primiparous cows are likely to have higher levels of NEFA postpartum, different findings on IGF-I and insulin concentrations have been reported. Meikle et al. (2004) found that primiparous cows had longer intervals from calving to first ovulation, had increased NEFA concentrations for a longer period of time and lower concentrations of IGF-I. Wathes et al. (2007a) reported an opposite trend in primiparous cows, with higher concentrations of IGF-I and lower concentrations of BHBA and NEFA, but found that primiparous cows still took longer to resume estrous cycles postpartum. While the findings of Meikle et al. (2004) were more in line with the accepted theory of EB and delay in ovulation, Wathes et al. (2007a) suggested that nutrient partitioning into milk and continued growth in the first parity cow is the reason for delayed ovulation.

1.3.4. The effects of hormone concentrations and energy balance on conception and pregnancy

Not only do hormone and metabolite concentrations, as well as NEB, affect the rate of ovulation of the first dominant follicle, but they have also been shown to affect conception rates and maintenance of pregnancy. Given that it takes approximately 2 months for a follicle to develop from the primordial stage to the ovulatory stage, it is likely that the NEB experienced during the initial stages of development has long lasting effects on the follicle and oocyte (Lucy, 2003). Butler (2000) stated that cows losing more than 1 unit of BCS are at increased risk for poor conception rates. Other studies have also shown that cows with low BCS between weeks 7 and 10 postpartum have increased calving to conception intervals (Wathes et al., 2007b). Another association has also been made between NEB and progesterone concentrations. A study by Villa-Godoy et al. (1988) found that cows in NEB during the first 9 days of lactation showed decreased plasma progesterone concentrations during their third estrous cycle. Since NEB has a negative effect on first ovulation, it is likely that a cow in serious NEB will be bred to her third estrous cycle (Villa-Godoy et al., 1988) and may have reduced CL function and lowered ability to maintain pregnancy. Wathes et al. (2007b) found multiparous cows were correlated with greater peak milk yield, low BCS by week 7 of calving and increased interval from calving to conception. Another finding from Wathes et al. (2007b) was that cows conceiving at less than 80 days postpartum produced 13 kg/day less milk than those taking more than 150 days to conceive (Wathes et al., 2007b). Other studies

have also shown that NEB causes irregular cycles postpartum that are associated with increased intervals to first service and conception (Wathes et al., 2003). As well, increased NEFA concentrations have been shown to negatively impact oocyte quality *in vitro* through delayed maturation and development of the oocyte (Leroy et al., 2005). These reports demonstrate that changes in hormones, metabolites and body condition in early lactation due to NEB not only affect the first ovulation, but also contribute to poor conception rates in dairy cattle.

1.4. IGF-I and Insulin: Effects on Reproduction

1.4.1. Initial discovery

Research into the effects of insulin, IGF-I and glucose on reproduction began in the mid 1980's. In 1987, Rutter and Manns found that high glucose clearance due to milk production was associated with decreased LH pulsatility in beef cows. Butler and Smith (1989) found that depressed LH pulsatility was more closely linked with low insulin concentrations compared to glucose after calving as LH pulsatility did not improve until insulin levels began to recover. They found that when insulin levels improved post-calving so did LH pulsatility. However, Canfield and Butler (1990) found no significant correlation between insulin and glucose and interval from calving to first ovulation or LH secretion early postpartum.

Adashi et al. (1985) found that *in vitro* follicular IGF-I acts on granulosa cells to stimulate cellular growth and steroidogenesis. Continued *in vitro* work on IGF-I and insulin with granulosa cells found that both hormones were involved in steroidogenic synthesis of estradiol and progesterone and increased granulosa cell

numbers (Spicer et al., 1993). Badinga et al. (1992) published research in support of the previous findings that IGF-I concentrations in follicular fluid were related to increased estradiol concentrations and size of the dominant follicle. Nebal and McGilliard (1993) reported that cows with greater NEB had lowered insulin and IGF-I concentrations, follicles with reduced ability to respond to gonadotropins, impaired ovarian function and poor conception rates. Continued research has shown that both insulin and IGF-I play important roles at the follicular level (Spicer et al., 1993) and that decreased concentrations of these hormones during NEB can have detrimental effects on reproduction (Butler, 2000).

1.4.2. Role in dominancy of the follicle, prevention of atresia and ovulation

Plasma concentrations of insulin and IGF-I have been shown to have a strong influence on development of the first dominant follicle and ovulation. Low concentrations of plasma insulin have been reported to impair follicular development and, therefore, cause atresia of the follicle (Vanholder et al., 2005). However, Gong et al. (2002) fed cows either a control diet or a diet to promote increased plasma insulin concentration, and found that most cows did not ovulate the first dominant follicle regardless of treatment. IGF-I, however, appears to be specifically involved in producing the first dominant follicle postpartum that is ultimately capable of ovulating (Vanholder et al., 2005). Beam and Butler (1997) showed that cows who ovulated the first dominant follicle post-calving had 40-50% higher concentrations of IGF-I in blood plasma. Thatcher et al. (1996) also found that cows initiating cyclicity sooner post-calving were generally those that had higher concentrations of IGF-I. Lucy (2003) reported that insulin and IGF-I

may act directly on the ovary to increase the sensitivity to FSH and LH and may be involved in providing the hypothalamus with nutritional cues influencing the release of GnRH. This is in agreement with the findings of Butler (2003) who showed that increased levels of IGF-I are associated with increased follicular estradiol production and results in more ovulating follicles.

As higher levels of IGF-I are positively associated with more ovulating follicles, it is no surprise that Echternkamp et al. (1990) suggested that increased levels of IGF-I may be associated with an increased number of double ovulations and thus with twinning rates in cattle. Butler et al. (2004) found that when animals were treated with a hyperinsulinemic-euglycemic clamp, insulin concentrations did not have any effect on LH pulsatility or the LH surge but did in fact increase estradiol and IGF-I production. The mechanisms by which IGF-I and insulin act to improve the capacity of the follicle to ovulate may occur at the level of the hypothalamus. However, research has primarily focused on the ability of these two hormones to impact the theca and granulosa cells of the follicle.

1.4.3. Impact on theca and granulosa cells of the follicle

Through their influence on theca and granulosa cells, both insulin and IGF-I affect the ovary by influencing development of the dominant follicle. The fate of the dominant follicle depends on its ability to produce enough estradiol to cause an LH surge capable of triggering ovulation. High quantities of estradiol are only produced by follicles that are large enough to respond to limited concentrations of FSH, have become more dependent on LH concentrations and have an increased number of granulosa cells. It is logical that IGF-I and insulin

play a major role in follicular growth through cell proliferation and steroidogenesis.

Most reports on the direct effects of insulin and IGF-I in follicular fluid have been from *in vitro* studies. Insulin and IGF-I have been found to increase steroidogenesis, specifically synthesis of estradiol, and are involved in granulosa cell proliferation (Spicer and Echternkamp, 1995; Gutierrez et al. 1997). Beam and Butler (1999) found that increased IGF-I levels were associated with increased estradiol concentrations in blood plasma. This parallels the findings of Lucy (2000) who reported that, although the IGF-I in follicular fluid is the factor that acts on the follicle, it is derived from plasma IGF-I with the remainder potentially produced by the granulosa cells of the follicle (Perks et al., 1999). A report by Gutierrez (1997) stated that while there is evidence of IGF-I production by granulosa cells in sheep there has been no proof in bovine follicles. Armstrong et al. (2003) also reported that insulin and IGF-I interact to increase estradiol production by granulosa cells and may act to increase the follicles' sensitivity to FSH. Early studies with beef cattle found that when insulin was injected into the animal the diameter of the dominant follicle increased, thereby supporting the findings of increased cell proliferation (Simpson et al., 1994).

Several studies have also examined how insulin and IGF-1 can affect the follicle during different stages of development. Spicer et al. (1993) conducted a trial to evaluate how insulin and IGF-I affect bovine follicles of different sizes cultured *in vitro* and found that granulosa cell numbers were increased when IGF-I and insulin were added into culture. Insulin, only in the presence of FSH, acted

to increase estradiol concentrations in both small and large follicles, while IGF-I only had a stimulatory affect on estradiol production in large follicles and actually had an inhibitory effect in small follicles. An *in vivo* study by Gutierrez et al. (1997) also showed that insulin increased the number of small follicles but that IGF-I did not have the same effect. These findings support the hypothesis of Gutierrez et al. (1997) that both hormones may have different roles during different stages of follicular development. Armstrong et al. (2002) examined how increased concentrations of plasma insulin and IGF-I induced by a high energy diet fed for a 17-day experimental period affected the granulosa cells of small and medium sized follicles collected at the end of treatment. They found that both hormones had a stimulatory effect on steroidogenic potential of small follicles, but no effect on medium follicles. These findings contradict those produced by Gutierrez et al. (1997); however, this study only evaluated follicular development by ultrasonography and did not measure steroid production from follicles. Other studies have also shown that a preantral follicle will show increased growth and a higher percentage of antrum formation *in vitro* when cultured with FSH and IGF-I (Gutierrez et al., 2000). It is known that insulin and IGF-I play an important role in follicular development and ovulation, however, there is no clear evidence yet on how these hormones affect the follicle during different stages of development.

1.4.4. Intra-ovarian IGF-I system

The intra-ovarian IGF-I system involves both IGF-I and IGF-II, hormone receptors, ligands and binding proteins, and all are involved in IGF-I's effect on granulosa and theca cells within the follicle (Lucy, 2000). Perks et al. (1999)

stated that IGF-I is produced by granulosa cells and that IGF-II is produced by theca cells, but that not all reports on the topic have produced the same results. This publication also showed that expression of mRNA encoding IGF-II was greater in small follicles than large follicles and healthy versus atretic follicles. The authors also concluded that IGF-I present within follicular fluid must be primarily derived from circulating plasma IGF-I as only 17% of all follicles tested showed IGF-I mRNA expression. Insulin-like growth factor binding proteins (IGFBP) are also found in follicular fluid, granulosa and theca cells (Lucy, 2000). It appears that both type 1 and type 2 IGF receptors are involved in mediating IGF-I with type 1 being present only in granulosa cells and type 2 receptors located in both granulosa and theca cells (Teissier et al., 1994). Type 2 IGF receptors are located on both cell types but both IGF-I and IGF-2 have a greater potency to the type 1 receptor (Lucy, 2000). Armstrong et al. (2001) also showed that IGF-II production occurred only in theca cells and that mRNA for both type 1 IGF receptor and insulin receptors was found in granulosa cells.

While much focus remains on IGF-I and its role in follicular development, researchers have also found evidence of IGF-II's importance (Spicer, 2004). However, results appear to indicate that IGF-I is more effective in stimulating steroidogenesis due to its greater ability to bind to type 1 receptors (Spicer and Echternkamp, 1995). Spicer and Echternkamp (1995) reported the ability of FSH and LH to increase expression of type 1 IGF receptors and potentially increase IGF-I synthesis within granulosa cells. Data also reveal changes in IGF-I and IGF-II mRNA expression during development of the follicle and that mRNA

expression for these hormones was greatest in dominant follicles (Yuan et al., 1998).

IGFBPs play an important role in regulating the availability and production of both IGF-I and IGF-II in follicular cells. Specific IGFBPs that have been shown to be important are IGFBP-2 and IGFBP-4 due to their synthesis within granulosa and theca cells of bovine follicles (Armstrong et al., 1998). IGFBPs are involved in inhibiting the ability of IGF to bind to its appropriate receptors within granulosa and theca cells and thus high levels may be associated with increased risk of atresia and prevention of dominancy (Lucy, 2000). In accordance with their function, IGFBPs are found in increased concentrations in smaller follicles and atretic follicles, but are in reduced concentration in large, estrogen-producing follicles (Nicholas et al., 2002). IGFBP-2 concentrations have been found to be nearly undetectable in dominant follicles compared to subordinate follicles (Yuan et al., 1998). Besnard et al. (1997) found that IGFBPs are not down-regulated during follicular growth, but are in fact degraded by IGFBP proteases in porcine follicles. It is apparent that it is not only the concentrations of IGF-I in plasma and follicular fluid that influence follicular dynamics, but in fact it is this complex system working as whole.

1.4.5. IGF-I and insulin: impact on conception rates and pregnancy

IGF-I and insulin in plasma and follicular fluid not only have an impact on development of the first dominant follicle and initiation of cyclicity postpartum, but also are involved in progesterone production during the luteal phase of the estrous cycle, maintenance of pregnancy and the quality of the oocyte. The

following section will focus on the positive and negative aspects of IGF-I and insulin in conception and pregnancy.

Wathes et al. (2007a) reported that a lowered circulating concentration of IGF-I postpartum is associated with an increased calving to first conception interval. This publication also reported that multiparous cows with low concentrations of IGF-I in week 2 postpartum onwards had an increased interval to conception. It has also been shown that elevated concentrations of IGF-I, which can stimulate follicle growth and steroid production *in vitro*, may be detrimental to oocyte quality. Armstrong et al. (2001) found that when cows were fed a high energy diet to promote increased insulin and IGF-I concentrations, oocyte quality derived from these animals was compromised. Gong et al. (2002) fed cows a diet high in energy to promote increased plasma insulin concentrations and observed a significant rise in insulin, but only a numerical decrease in days to conception. While some reports have shown a negative association between high insulin and IGF-I and oocyte quality, Echternkamp et al. (1990) found that cattle selected for twinning actually had higher concentrations of these 2 hormones in both blood and follicular fluid. It has been established that insulin and IGF-I may increase ovulation rates (Echternkamp et al., 1990), but this study would not suggest any negative effect of these hormones on oocyte quality.

Lucy (2000) also showed that insulin and IGF-I are important for progesterone production by luteal cells of the corpus luteum (CL) and, therefore, these hormones are also implicated in maintenance of pregnancy. An *in vitro* study by Spicer et al. (1993) found that both insulin and IGF-I in combination or

alone increased progesterone production by luteinized granulosa cells which may also indicate a positive relation between these hormones and pregnancy. In relation to this, Wathes et al. (2003) reported that cows may have an adequate supply of insulin to promote estradiol production but insufficient IGF-I concentrations for cell proliferation. This may allow for the ovulation of a small follicle and, therefore, development of a smaller, less effective CL. Cell proliferation is of key importance to CL function as greater numbers of luteal cells translate into more production of progesterone, important for maintaining pregnancy. This same publication also goes on to address the roles of IGF-I in maintenance of pregnancy, possibly through increased production of interferon-τ, embryonic growth and secretions of the reproductive tract (Wathes et al., 2003). The role of IGF-I in pregnancy is still unclear and needs to be more thoroughly researched.

1.5. The Estrous Cycle

1.5.1. Ovarian dynamics and estrous behavior

As stated above, the first ovulation in the postpartum period has been shown to occur approximately 29 days after calving (Nebel and McGilliard, 1993). It is not uncommon for the luteal phase of the first estrous cycle to be shorter in length than average. Roche (2006) stated that this short luteal phase is due to the premature release of $PGF_{2\alpha}$ from the uterus, resulting from high estradiol production from the first wave dominant follicle, approximately 5-8 days after ovulation. The estradiol concentrations remove the negative feedback of progesterone on the hypothalamus and increase GnRH release. This causes

premature release of $PGF_2\alpha$ from the uterus, initiating regression of the CL and thus causing ovulation of the first wave dominant follicle (Roche, 2006).

The first ovulation post-calving is also generally associated with an absence of estrus behavior due to a lack of progesterone priming on the hypothalamus (Roche, 2006). Estrous cycles following the first postpartum cycle usually have normal luteal length and cows express estrus behavior (Roche, 2006). It was hypothesized that treating cattle with progesterone after calving would induce estrus behavior at the time of first ovulation due to progesterone priming of the hypothalamus. However, early studies found no difference between estrus behavior associated with the first, second or third estrous cycle postpartum and between treatment and control groups (Kyle et al., 1992). Rhodes et al. (2003) did, however, demonstrate that cows treated with progesterone show estrus behavior to the first cycle and thus supported the hypothesis on the need for progesterone priming of the hypothalamus.

The number of follicular waves per estrous cycle in a cow is normally 2 or 3; however, greater than 3 follicular waves per cycle has also been observed (Wolfenson et al., 2004; Sartori et al., 2004). Sartori et al. (2004) reported that it is common for Holstein cows to have 2 follicular waves per estrous cycle. This is in agreement with Wolfenson et al. (2004) and Bleach et al. (2004) who found that 75-79% of cows had 2-wave cycles, compared to 21-22% of cows having 3-wave cycles. The results from Townson et al. (2002) indicated that 68% had 2-waves, 30% had 3-waves and 2% had more than 3-waves. Ginther et al. (1989) hypothesized that lactating cows would be more likely to have 2-wave cycles

because of high milk production but found that heifers were just as likely to have 2-wave cycles as cows. Bleach et al. (2004) also found no difference between parities for follicular wave number.

Cows having 2-wave cycles generally tend to have shorter estrous cycles than cows having 3- or 4-wave cycles due to the decreased luteal length (Townson et al., 2002). Adams et al. (2008) reported that cows having 2-wave cycles generally have estrous cycle lengths of 19-20 days, compared to 22-23 days in 3-wave cycles. Another publication showed that cycle length was 21.9 ± 0.2 for 2-waves and 23.3 ± 0.5 days for 3-waves (Ginther et al., 1989). This is in agreement with Townson et al. (2002) who showed that 2-wave cycles have an estrous length of 21.2 ±0.5 and 3-waves having an estrous length of 24.5 ± 0.7 days. Interestingly, Wolfenson et al. (2004) reported a longer average cycle length of 24.6 ± 0.6 days in cows and a shorter cycle length in heifers by 2.6 days even though follicular wave number did not differ between heifers and cows.

Aside from estrous cycle length, differences between 2- and 3-wave cycles are the days of emergence of each follicular wave, as well as the size and age of the preovulatory follicle. Cows that have 3-wave cycles generally produce smaller preovulatory follicles that are considered to be less mature (Ginther et al., 1989). For those with 2-wave cycles, follicles grow to a larger size and are more mature (Townson et al., 2002). Emergence of a follicular wave is considered to occur on day 0 and 10 of a 2-wave cycle and on day 0, 9 and 16 of a 3-wave cycle (Ginther et al., 1989). As well, the preovulatory follicle remains in dominance for 2.1 days longer in 2-wave estrous cycles (Wolfenson et al., 2004). When there is

development of a third follicular wave, the CL lifespan is extended by 2-3 days thus causing an increased luteal phase length and longer length of the estrous cycle (Townson et al., 2002). Townson et al. (2002) found that cows having 2 follicular waves per cycle grew preovulatory follicles 1.2 mm larger than preovulatory follicles of 3-wave cycles. They also reported the average age of the preovulatory follicle to be 6.7 ± 0.3 days in 2-wave cycles and 5.2 ± 0.4 days in 3-wave cycles. Reports have associated 2-wave cycles and, therefore, prolonged growth of the preovulatory follicles to decreased fertility in dairy cows (Mihm et al., 1994). Townson et al. (2002) found that cows having 3-wave cycles had higher pregnancy rates than those having 2-wave cycles perhaps because the oocyte hasn't been exposed to detrimental effects from prolonged follicular development. However, not all studies have been able to produce the same result, thus the question as to whether 3-waves is more desirable for fertility than 2 is still up for debate. Although Wolfenson et al. (2004) found no difference between heifers and cows in the number of follicular waves per cycle, the preovulatory follicle maintained dominancy for 4 days longer in cows than in heifers. Those authors also found that cows had a 2.6 day longer estrous cycle, thus supporting the findings of Mihm et al. (1994) that a correlation exists between prolonged follicular development and lowered fertility in cows.

1.5.2. Gonadotropin and steroid hormone concentrations

While it is clear that the number of follicular waves per estrous cycle is related to either prolonged dominance or early regression of the dominant follicle, assessment of the hormonal mechanism involved provides a much clearer picture

of the events. Sartori et al. (2004) found that progesterone concentration was lower for cows having 2-wave cycles compared to heifers having either 2- or 3-wave cycles. Other reports have also shown that low concentrations of progesterone during the luteal phase cause prolonged dominance of the first wave dominant follicle and thus a delay in the initiation of the second follicular wave (Adams et al., 1992). Adams et al. (1992) also found that high concentrations of progesterone caused earlier regression of the second wave dominant follicle, which may have implications for the association of low progesterone and 2-wave cycles. Analysis of progesterone profiles has shown that progesterone peaks between days 10 and 14 of the estrous cycle and begins to decline on day 16 (Adams et al., 2008). Townson et al. (2002) reported that cows with 3-wave cycles show a later peak in plasma progesterone and extended luteal function. However, Wolfenson et al. (2004) found no difference between progesterone levels of 2- and 3-wave cycles but did observe differences in progesterone concentrations of heifers and cows with cows having lower concentrations from day 3 to 16 of the cycle despite having greater CL sizes. Similar findings were also reported by Sartori et al. (2004). Wolfenson et al. (2004) also reported that heifers had higher concentrations of estradiol around the time of estrus. Sartori et al. (2004) reported that lactating cows require increased follicular size and estradiol production to initiate an LH surge needed for ovulation. Cows have a longer interval from luteolysis to ovulation than heifers, and, therefore, increased follicular development but both have a similar peak in estradiol concentration (Sartori et al., 2004). As well, lactating cows have similar progesterone

concentrations to dry cows but increased luteal tissue suggesting that an increased CL size is needed in lactating cows to maintain adequate progesterone concentrations (Sartori et al., 2002).

1.5.3. Irregular cycles and double ovulations

Lucy (2003) reported an increase in the number of abnormal estrous cycles in early lactating dairy cows and Opsomer et al. (2000) reported that NEB is one of the risk factors for the prolonged luteal phase characteristics of abnormal cycles. Sartori et al. (2004) found that 12 out of 31 cows displayed abnormal estrous cycles through either ovulation of more than one follicle or by increased number of follicle waves. As well, several cows became temporarily an-ovulatory and failed to ovulate the dominant follicle after luteolysis presumably due to an inadequate LH surge. They also reported that cows with atypical cycles showed a higher incidence of double ovulation during the ovulatory wave. Several publications have also reported an increased risk of double ovulations when progesterone concentrations are low (Savio et al., 1990). Wiltbank et al. (2000) reported that the risk of double ovulations may be due to follicles not producing the estradiol concentrations needed to suppress FSH and prevent co-dominancy. This publication also showed that double ovulations were common in approximately 20% of high producing dairy cows and only 7% in low producing cows, possibly due to increased clearance rate of estradiol and progesterone from increased DMI in the high producing cows. As the fertility of dairy cows has declined, it is not surprising to see that researchers are finding abnormalities in the

characteristically normal cycle and that these anomalies have some connection to milk production.

1.6. Non-Structural Carbohydrates

1.6.1. Barley silage vs. alfalfa silage

Dairy cows are often fed total mixed rations (TMR) containing alfalfa or barley silage, particularly in Western Canada. One of the main differences between alfalfa and barley silage is the content of neutral detergent fiber (NDF). National Research Council (2001) report that barley silage has an NDF content of 56% and alfalfa silage NDF content of 46%. Allen (2000) reported that an increased content of NDF was related to a decline in DMI due to filling constraints of the rumen and slower digestion. Okine et al. (1994) also reported that high NDF is associated with reduced DMI.

Alfalfa and barley differ significantly in their content of starch with alfalfa containing lower levels of starch than barley (Mustafa et al., 2000). Khorasani et al. (1993) reported that alfalfa silage contained 1.8% starch and barley contained 15.9% starch on a dry matter basis. A report from Mustafa et al. (2000) also supports Khorasani's findings as they found alfalfa to have only 0.6% starch compared to 14.5% for barley. However, in cows fed the two silages both DMI and milk yield were comparable between the two silages. Milk composition was the same between these silages except that cows fed alfalfa silage produced milk with more protein and lower concentrations of MUN. Khorasani et al. (1993) found that alfalfa and barley silage-fed cows had no significant differences in DMI, milk yield or milk composition with the exception that barley fed-cows had

higher milk lactose concentrations potentially due to increased starch concentration in barley. While it seems that performance is similar when cows are fed either barley or alfalfa silage, the starch content of barley is higher and the protein content is lower compared to alfalfa silage thereby creating the potential for differences in milk composition.

1.6.2. Effects of dietary starch on energy balance and metabolic hormones

In typical dairy diets starch values range between 25 to 35% of DM (Eastridge, 2007), and many benefits have been reported for feedings diets to postpartum cows in the higher end of the recommended range usually through increased energy density via increased non-structural carbohydrates. It is unclear, however, whether this increase in dietary starch results in the cow actually having more available energy to partition toward other body processes such as reproduction.

Knowlton et al. (1998) carried out a study in which partially hydrolyzed starch was infused into the rumen or abomasum of cows to determine differences in starch digestion and glucose metabolism. They found that there was a tendency for milk yield and milk lactose to increase independent of site of infusion compared to those cows not infused with starch. As well, DMI decreased, insulin tended to increase, NEFA concentrations tended to decrease and EB was improved when starch was infused. As well, Kolver et al. (2006) found that inclusion of grain at 3-6 kg DM/cow/day increased blood concentrations of insulin and IGF-I. Armstrong et al. (2001) fed diets containing either low or high metabolizable energy and also found an increase in plasma IGF-I and insulin for

cows fed high-energy, but as stated previously the high energy diet reduced oocyte quality. Gong et al. (2002) fed two diets to dairy cows; a low starch diet (100 g/kg of DM) and a high starch diet (260 g/kg of DM) and found that the high starch diet increased plasma insulin concentration. This study also found that a diet high in dietary starch decreased the interval from calving to first ovulation and improved other reproductive parameters such as the number of days to first service and days to conception. Reist et al. (2003) fed a diet with concentrate at either 30 or 50% of DM and found that increasing concentrate content in the diet improved EB, increased milk production, increased plasma concentrations of glucose, insulin and IGF-I and lowered NEFA and BHBA concentrations. While research in this area is not abundant it appears that alterations in the diet through increased inclusion of non-structural carbohydrates can act to improve the cow's energy status and improve desirable plasma hormone concentrations.

1.7. Gaps in the Knowledge

Although a substantial amount of research has been done on metabolic status and reproductive performance in the postpartum dairy cow there are still several gaps in the knowledge that need to be addressed. A clearer understanding of the impact of IGF-I and insulin on reproductive function and fertility post-calving through to insemination and pregnancy maintenance is required. More *in vitro* studies examining the effect of insulin and IGF-I on the function of granulosa and theca cells of the follicle still need to be conducted. As *in vitro* studies become more advanced it may become easier to fully study the sensitivity of the ovary to these hormones. More research into how insulin and IGF-I

receptors on granulosa cells change through development of the follicle needs to be done (Spicer and Echternkamp, 1995). There are reports showing that an increase in insulin and IGF-I can be detrimental to the oocyte (Armstrong et al., 2001), but that conception rates are improved when circulating concentrations of these hormones are high (Echternkamp et al., 1990). As well, research has shown that increased inclusion of non-structural carbohydrates can reduce the interval from calving to first ovulation postpartum; however, no studies have directly examined LH pulsatility in the first few weeks postpartum in relation to the onset of cyclicity. Limited research has been conducted on the effect of IGF-I and insulin on progesterone production during the luteal phase. A further examination of this might show an important role for IGF-I and insulin on CL maintenance. There are also conflicting reports on the number of follicular waves per estrous cycle and whether cows that display 3-waves actually have better conception rates than those with 2-waves. There is little evidence as to whether the number of follicular waves is at all influenced by the level of milk production and EB or if the number of waves can be influenced by dietary practices. However, there is a strong understanding of the role of different hormones and metabolites in energy status and correlations have been made between EB and reproductive performance in the postpartum cow. A further understanding of the role of hormones and metabolites at the ovarian level will provide a clearer understanding of exactly how nutritional status affects reproductive processes in lactating cows.

1.8. Summary

It is well documented that the postpartum cow is in NEB due to her inability to consume enough energy to meet all production demands. The consequences of NEB include that insulin, glucose and IGF-I levels are less than ideal and that blood metabolites such as NEFAs and BHBA are at elevated concentrations. This combination of low hormone and high metabolite concentrations are highly correlated with reduced reproductive performance seen as a delay in the cow's ability to ovulate the first dominant follicle post-calving. Nutrient signaling to the hypothalamus through concentrations of insulin and NEFAs have been shown to impact release of GnRH and thus gonadotropins from the pituitary to allow for proper growth and maturation of follicles and their ovulation.

Several publications have shown that dietary manipulation through inclusion of more non-structural carbohydrates causes an increase in the levels of circulating insulin and IGF-I and a drop in NEFA concentrations. This is a desirable combination for the cow in terms of reproductive performance and improvement of EB.

1.9. Research Objectives

The main objectives of the research described in the following chapter are to determine if supplementing the diet of postpartum dairy cows with corn starch will improve reproductive processes and production parameters. While previous works by Armstrong et al. (2001) and Gong et al. (2002) have shown that increased inclusion of non-structural carbohydrates can improve the energy status

of the cow and increase insulin and IGF-I concentrations, neither paper thoroughly examined both production and reproduction aspects of the postpartum cow. This project examines not only reproductive parameters, but also production qualities such as dry matter intake, body condition, body weight, milk production and milk composition.

Specific reproduction traits examined are: interval (days) from calving to first ovulation, LH pulsatility within 2 weeks post-calving and ovarian dynamics observed through one whole estrous cycle starting approximately 45 d postpartum.

Finally, as stated previously, cows will be observed for production traits as increasing the energy content in the diet could in fact improve body condition, milk yield and milk composition. However, it is likely that an observed improvement in both reproductive and productive components will not be seen in combination due to nutrient partitioning. The following chapter will include the results as well as a thorough discussion of the outcomes of the research project.

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CHAPTER 2.

SUPPLEMENTAL STARCH IN POSTPARTUM DAIRY COW DIETS: EFFECTS ON PRODUCTIVITY AND REPRODUCTIVE PROCESSES

2.1. Introduction

Declining reproductive performance in postpartum dairy cows has been reported since the mid 1970's (Butler and Smith, 1989). While genetic selection has focused on improving milk yield and productivity, an inverse relationship with these traits and reproductive performance postpartum has been observed (Lucy, 2001). Doepel et al. (2002) found that cows were in a state of negative energy balance (NEB) immediately following calving and maintained NEB for the 6 wk postpartum experimental period. Negative EB has been identified as a risk factor for delay in first ovulation postpartum (Butler and Smith, 1989) and this delay has been negatively correlated with conception rate (Butler, 2000).

The hormones, insulin and IGF-I, have been shown to play a critical role in follicular cell proliferation and steroidogenesis *in vitro* (Spicer and Echternkamp, 1995) and increased circulating concentrations have been positively associated with ovulation rates and follicular growth *in vivo* (Armstrong et al., 2001; Armstrong et al., 2003). The circulatory levels of both insulin and IGF-I generally decline post-calving (Beam and Butler, 1999) and this decline is associated with a more severe NEB and an increase in the number of days to first ovulation (Gong et al., 2002).

Previous research has identified that plasma concentrations of insulin and IGF-I can be augmented by increasing the inclusion of nonstructural

carbohydrates in the diet. Armstrong et al. (2001) fed diets containing either low or high metabolizable energy and found an increase in plasma IGF-I and insulin concentrations for those cows fed the high energy diet. As well, Kolver et al. (2006) found that inclusion of grain at 3-6 kg dry matter (DM)/cow/day compared to no grain inclusion also increased concentrations of plasma insulin and IGF-I.

We hypothesized that increasing dietary starch would improve energy balance (EB), lower the concentration of NEFA and BHBA, raise concentrations of IGF-I, insulin and glucose and decrease days to first ovulation postpartum. Thus, the objective of this study was to examine the effects of feeding increased levels of starch on metabolites and hormones mentioned above, progesterone and estradiol concentrations, and follicular development. In addition, production parameters such as milk yield and composition, dry matter intake (DMI) and body condition score (BCS) were also evaluated.

2.2. Materials and Methods

2.2.1. Animals and treatment

Forty Holstein cows (16 primiparous, 24 multiparous) were selected for this study. Cows were housed in a tie-stall barn at the University of Alberta, Dairy Research and Technology Centre in Edmonton, Alberta. Animals were cared for according to the guidelines of the Canadian Council on Animal Care (1993) and all procedures conducted on animals were approved by the University of Alberta's Livestock Animal Care and Use Committee. Cows were let out for exercise daily for approximately 2 hours every morning. Cows were blocked by parity and milk yield in the previous lactation and randomly assigned within block

to one of three dietary treatments. The three dietary treatments were formulated to contain low (23.5%, LSD), medium (25.3%; MSD), or high (26.9%; HSD) starch. All diets contained ~46% barley-based concentrate and ~10% alfalfa hay. In addition, the LSD (n=14) contained 43.8% barley silage, the MSD (n=13) contained 44.5% alfalfa silage, and the HSD (n=13) contained 39.9% barley silage and 3.8% corn starch (Melojel, Quadra Chemicals, Edmonton, AB). Cows were given unlimited access to water. Ingredient and nutrient composition of the diets is shown in Table 2.1. Cows were assigned to the experimental diet immediately after calving and were off trial at approximately 70 days in milk (DIM). Cows on this study calved between the months of December to April.

Cows were fed the diets as total mixed rations (TMR) once daily at 0800 h *ad libitum* allowing for 10% refusals with orts removed at 0600 h. Cows were body condition scored (BCS) at calving and then biweekly at 0700 h until 70 DIM. Body condition score was determined by one technician using the scale of 1 (emaciated) to 5 (overconditioned; Edmonson et al. 1989). Cows were milked twice daily at 0400 h and 1500 h and yields recorded daily. Milk samples (am and pm) were collected weekly from 7 d after calving until 70 DIM for composition analysis.

2.2.2. Feed sampling

To determine individual dry matter intake (DMI), the amount of feed offered and orts collected were weighed and recorded daily during the trial. Silage DM was determined weekly and the formulated DM composition of the diet was maintained by adjusting the forage to concentrate ratio. Samples of hay,

silages and concentrates were collected weekly and composited monthly for analysis of DM, organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin and starch. A sample of orts from each cow was collected weekly for calculation of DMI.

2.2.3. Blood sampling

20 ml of blood was collected from all cows within 24 h of calving and on d 7, 14, 28, 42, 56 and 70 postpartum from the coccygeal vein or artery using evacuated Vacutainer® tubes containing sodium heparin (Beckton Dickinson and Co., Franklin Lakes, NJ, USA). Blood was collected 2 h after feeding to avoid the effects of feeding on plasma hormone and metabolite concentrations. A subset of cows (n=15) was also used to determine luteinizing hormone (LH) pulsatility at approximately 15 d post-calving. Five cows per treatment were catheterized in the jugular vein (Zalkovic et al., 2001) on the day of collection and 10 ml of blood was collected every 15 min for 8 h from 0900 to 17:00. All blood samples were immediately placed on ice and centrifuged at 3000 *g* for 25 min at 4˚C within one h of collection and plasma was harvested and frozen at -20˚C until further analysis. Number of LH peaks was determined by taking the overall mean of LH concentration and then adding the standard deviation. Any value above this was determined a peak. As well, LH amplitude was determined by taking the concentration of LH from the baseline to the top of the peak. Blood samples were also collected every second day throughout the observed estrous cycle, explained under *Ultrasonography,* to determine estradiol and progesterone concentrations. Blood samples were collected every second day so that progesterone and estradiol

concentrations could be determined during the dominancy of the first follicular wave and the preovulatory follicle of the last follicular wave.

2.2.4. Ultrasonography

Transrectal ultrasonography (Aloka-500V scanner equipped with a 7.5 MHz linear transducer, Aloka Co., Tokyo, Japan) was performed to observe ovarian dynamics twice weekly from 7 ± 1 d after calving until the first ovulation or 62 DIM. The diameter and location of follicles and corpus luteum (CL) were recorded as previously described by Pierson and Ginther (1984). An ovulation was presumed if the dominant follicle observed in the previous scanning was replaced by a CL. In some cases two dominant follicles ovulated and two CLs were present. Size of ovarian structures was determined by measuring diameter.

One complete estrous cycle was monitored for ovarian dynamics in 8 LSD, 7 MSD and 9 HSD cows that had ovulated before 45 DIM. Prior to monitoring the cycle the cows were given 500 μg cloprostenol (PGF; Estrumate, Schering-Plough Animal Health, Pointe-Claire, QC) to regress the CL and were then monitored once daily by ultrasonography to determine ovulation and thus the beginning of a new estrous cycle. After ovulation, ovarian follicular and CL dynamics were monitored by ultrasonography every second day throughout the cycle until the subsequent ovulation.

The Ovsynch protocol was initiated by the barn staff to synchronize ovulation and insemination after cows had completed the 70 d trial. Pregnancy was diagnosed in cows 30 days after artificial insemination (AI) by transrectal ultrasonography. Those not confirmed pregnant to the first insemination were

resynchronized and pregnancy was again determined. This process continued until the third insemination.

2.2.5. Laboratory analyses

Milk samples were sent to Canwest Central Milk Testing Laboratory (Edmonton, AB, Canada) after being preserved with 1.5 mg of 2-bromo-2-nitropropane-1, 3-diol and 0.7 mg of Pimaricin (Brotab 10®, Systems Plus, Baden, ON) for analysis of fat, protein, lactose, milk urea nitrogen (MUN) and somatic cell count (SCC) by infrared spectroscopy (Milk-O-Scan 605; Foss Electric, Hillerød, Denmark, AOAC, 1996).

Moisture content of feed ingredients and orts were determined by drying samples at 55°C in a forced air oven for 48 h. Samples of silages, hay and concentrates were ground in a Wiley mill (Thomas Co., Philadelphia, PA) to pass through a 1 mm screen. Neutral detergent fiber, ADF and lignin were determined using a Filter Bag Technique (ANKOM^{2000} fiber analyzer, ANKOM Technology) previously reported by Van Soest et al. (1991) using heat-stable alpha amylase. Organic matter was determined by placing samples in a 550°C oven for 5 h and ash concentration determined as the remaining weight. Nitrogen concentration was determined on a Leco carbon/nitrogen determinator (TruSpec CN, St. Joseph, MI) and then CP was calculated as N x 6.25. Starch content was measured using an enzymatic method with samples being gelatinized with sodium hydroxide and resulting glucose measured with a glucose oxidase/peroxidase enzyme (Sigma No. P7119) and dihydrochloride (Sigma No. F5803) (Silveira et al., 2007a).

Plasma glucose was analyzed using a glucose oxidase/peroxidase enzyme and dianisidine dihydrochloride system (Sigma Cal. No. P7119 and No. F5803) (Silveira et al., 2007b). A commercial kit was used to analyze NEFA (NEFA-HR(2) Procedure, Wako Chemicals, USA, Richmond, VA) and BHBA was measured as per Williamson and Mellanby (1974) using a 96-well micro plate. A solid-phase radioimmunoassay kit was used to determine concentrations of plasma insulin (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA). Intra- and inter-assay coefficients of variation (CV) were 8.0% and 19.9% and the assay sensitivity was 1.3 IU/ml. IGF-I was determined as described by Novak et al. (2002) with the following modification: IGF-I used for iodination and reference standards was GroPep Receptor Grade Human IGF-I (GroPep # CU100, GroPep Ltd., 28 Dalgleish Street, Thebarton SA 5031, PO Box 10065 BC, Adelaide, SA, Australia). The intra-assay CV was 5.9%, the inter-assay CV was 26.0% and the average assay sensitivity was 5.2 ng/ml. Luteinizing hormone concentrations were determined by a previously validated, double-antibody radioimmunoassay (Rawlings and Evans, 1995). Samples were analyzed in one assay with the intra-assay CVs being 4.1% and 6.6% for a reference sera with a sensitivity of 0.1 ng/ml. A solid-phase radioimmunoassay kit was used for progesterone determination (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA). The intra- and inter-assay CV were 2.9 and 7.4%, respectively and the average sensitivity was 0.1 ng/ml. For estradiol determination a double-antibody radioimmunoassay kit was used (Coat-A-Count, Diagnostics Products Corporation, Los Angeles, CA) (Malhi et al., 2006). Kit standards were not used

and instead made from charcoal-stripped bovine serum, to remove endogenous hormone, from castrated animals, with known amounts of estradiol added back in. Samples were analyzed in two assays with intra-assay CVs being 10.2% and 1.5% for low reference sera and 5.9% and 0.6% for high reference sera. The inter-assay CV was 8.2% for low reference sera and 5.5% for high reference sera with a sensitivity of 1.0 ng/ml.

2.2.6. Energy balance

Energy balance was calculated for each cow on a weekly basis for overall energy balance and daily for days to reach the energy balance nadir using energy consumed minus energy required, with energy required equaling maintenance plus milk production requirements (Rabelo et al., 2003). Net energy for maintenance was calculated using the formula Mcal/d = 0.08 x kg of $BW^{0.75}$ and net energy for milk was calculated as Mcal/d = milk yield x [(0.0929 x Fat%) + (0.0547 x Protein%) + (0.0395 x Lactose%)] (NRC, 2001). Net energy for growth, taken from NRC (2001) was also part of energy required for first lactation cows.

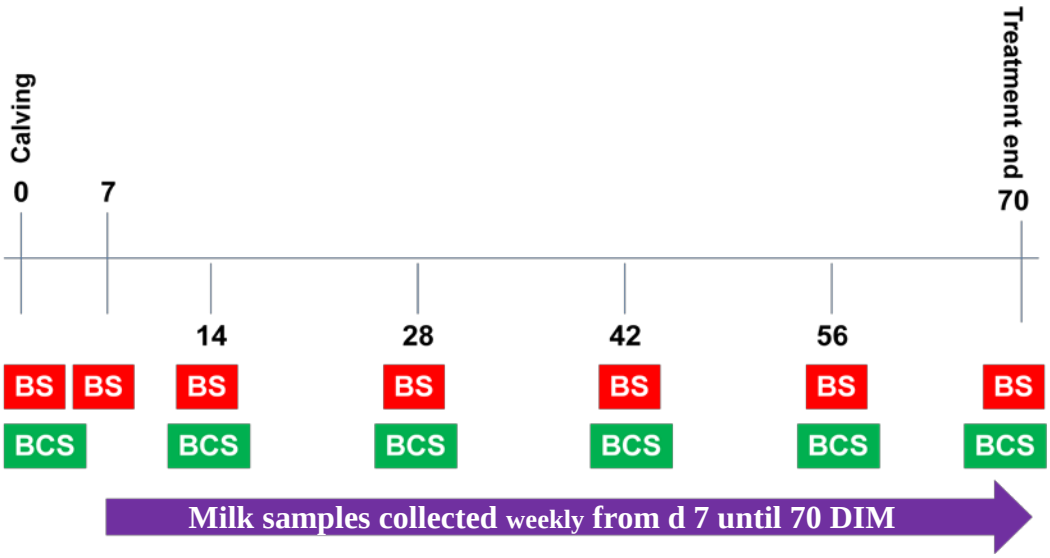
2.2.7. Statistical analyses

One cow from the HSD group that was selected for LH determination was removed from the trial on d 20 due to bad temperament. Energy balance, milk yield and composition, and DMI values were not used for the first two weeks postpartum for one LSD and one HSD animal due to early lactation metabolic conditions. Prior to statistical analysis, DMI, milk yield and composition were reduced to weekly means. Repeated measures in the MIXED procedure of SAS

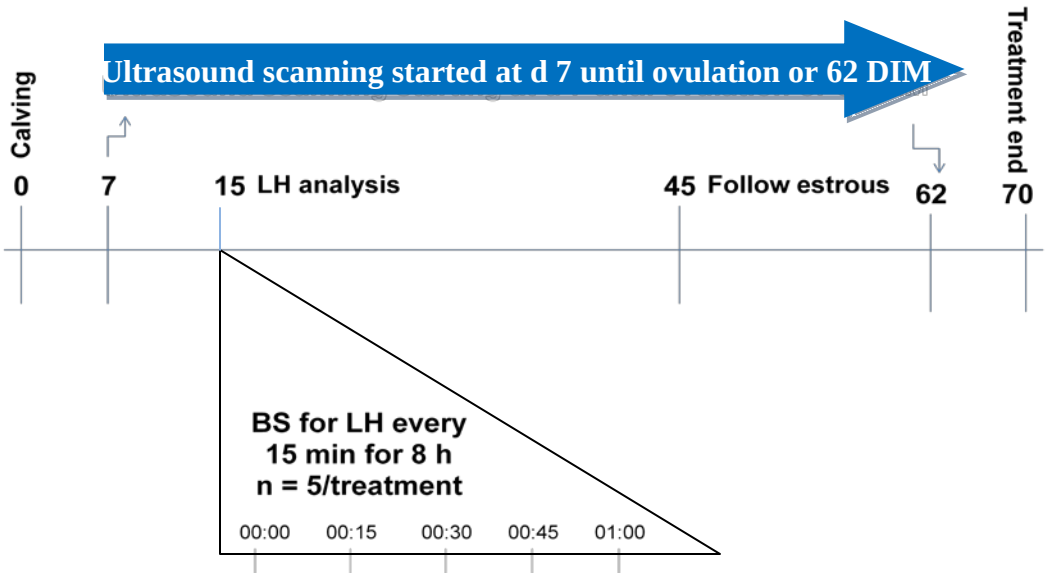
(version 9.1; SAS Institute Inc., Cary, NC) were used for DMI, milk yield, milk composition, BCS, insulin, IGF-I, glucose, BHBA and NEFA. The statistical model included treatment, parity, week (or DIM), treatment x parity interaction, treatment x week interaction and treatment x parity x week interaction. Block was considered a random effect and cow, block, week, parity and treatment considered fixed effects. The repeated measure was week or DIM. A covariance structure resulting in the lowest Swantzs' Bayesian Information Criterion (BIC) indicated best fit. Repeated measures in the MIXED procedure of SAS were also used for progesterone and estradiol. Progesterone concentrations were statistically analyzed from day 0-14 of the cycle and estradiol analyzed on d 4, 6 and 8 of the cycle and then the last five samples prior to ovulation. For example, if ovulation happened on d 24 then estradiol was measured on d 16, 18, 20, 22 and 24. All single measurement data (e.g., length of estrous cycle, size of preovulatory follicle) were analyzed using PROC MIXED in SAS with treatment, parity and treatment x parity interactions in the model. A Survival Analyses in SAS was performed for interval (days) from calving to first ovulation postpartum data. Pregnancy to first, second and third AI, number of double first ovulations and number of cows not ovulating before 62 DIM were all analyzed using chi-square analysis. Differences were considered significant if the *P* value was ≤ 0.05 and *P* values was between 0.06 and 0.10 were considered trends.

2.2.8. Sampling Timelines

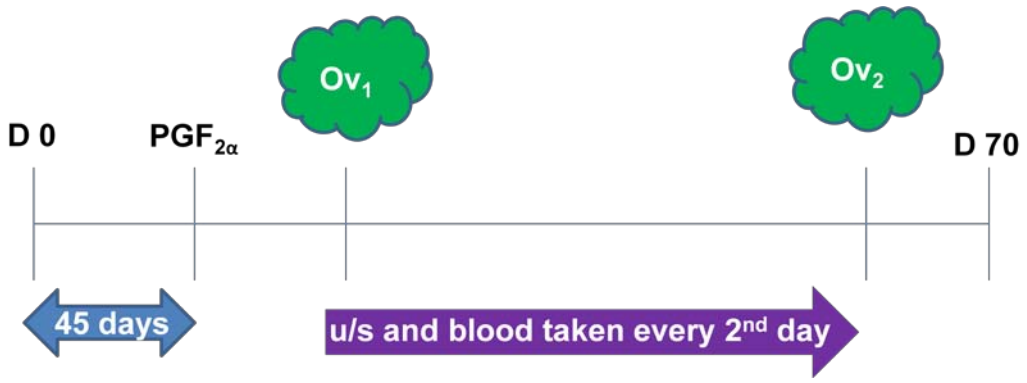
2.2.8.1. Sampling for blood, BCS and Milk



2.2.8.2. Sampling LH analysis and first ovulation postpartum



2.2.8.3. Sampling throughout the estrous cycle



2.3. Results

2.3.1. Production parameters

Dietary treatment had no effect on DMI with results being 18.0, 19.3 and 18.5 kg/d for LSD, MSD and HSD, respectively (P = 0.37; Table 2.2., Figure 2.1); however, primiparous cows had significantly lower DMI (16.7 kg/d) than multiparous cows (20.5 kg/d; P = 0.002, Table 2.3.). The overall BCS was not affected by treatment or parity, but a treatment by parity interaction (P = 0.02) existed with HSD primiparous cows having higher BCS than both MSD and LSD primiparous cows while HSD multiparous cows had lower BCS than the MSD and LSD multiparous cows. There was a difference in the change of BCS over the 70 d treatment period as the HSD cows tended to lose less body condition compared to the other two treatments (P = 0.06; Table 2.2.). The overall change was -0.57, -0.6 and -0.4 in BCS for the LSD, MSD and HSD respectively.

Treatment had no effect on milk yield which averaged 34.9 ± 1.6 kg/d across treatments (P = 0.89; Table 2.2., Figure 2.2.). There was a parity effect with primiparous cows producing less milk than multiparous cows (28.3 vs. 41.6 kg/d, P < 0.001; Table 2.3.). Milk composition data is displayed in Table 2.2.

Neither milk fat nor protein content was different among treatments or parity. Milk fat (%) averaged 3.71 ± 0.14 across treatments and was 3.70 for both primiparous and multiparous cows while milk protein (%) averaged 3.03 ± 0.07 among treatments and was 3.08 for primiparous cows and 2.97 for multiparous cows. Lactose concentration tended (P = 0.07) to be lower for HSD cows (4.53%) than that for LSD (4.66%) or MSD (4.67%) cows but no parity differences were observed. Somatic cell count was also not different among treatments and averaged 122 $x10^3$ cells/ml ± 35 $x10^3$. Milk urea nitrogen was significantly higher (P = 0.001) in LSD cows (16.3 mg/dl) compared to that in MSD (13.6 mg/dl) and HSD (13.4 mg/dl). Milk fat, protein and lactose yields were not different among treatments (Table 2.2.). Primiparous cows had lower yields (P < 0.001) than multiparous cows for all three components. Fat, protein and lactose yields for primiparous cows were 1083, 903, and 1389 g/d, respectively, while for multiparous cows they were 1576, 1271, and1965 g/d.

Net EB was not affected by treatment with values being -3.91, -1.18 and -2.77 Mcal/d for the LSD, MSD and HSD treatments (P = 0.16; Table 2.2., Figure 2.4.) but primiparous cows were in a less negative state of EB than multiparous cows (-1.65 vs. -3.98 Mcal/d, P = 0.04; Table 2.3.). Days to reach the NEB nadir was also not different between treatments or parity and were 7.4, 9.8 and 8.7 d for LSD, MSD and HSD respectively (P = 0.63; Table 2.2.). This parameter was 8.1 d for primiparous cows and 9.2 d for multiparous cows and did not differ (P = 0.59; Table 2.3.). Cows remained in a state of NEB for the first 7 wk post-calving with cows on the MSD treatment being the first to reach positive EB.

2.3.2. Blood metabolites

No treatment effects were observed for glucose, insulin, IGF-I, NEFA or BHBA plasma concentrations (Table 2.4.). For all treatments, glucose and insulin levels declined by d 7 postpartum and then slowly began to increase after 7 DIM. Primiparous cows had significantly higher glucose concentrations overall than multiparous cows (64.2 vs. 55.5 mg/dl, $P < 0.001$; Table 2.5.). No treatment or parity effects or interactions were seen for NEFA, however, the general pattern for all treatments is a high concentration on d 0 post-calving followed by a decline that levels out at approximately 42 DIM (Table 2.4.; Figure 2.5.). β-hydroxybutryate was not affected by treatment but was affected by parity with primiparous cows having lower levels than multiparous cows (7.4 vs. 9.1 mg/dl, $P = 0.003$, Table 2.5.).

2.3.3. Reproductive parameters

2.3.3.1. Ultrasonography to first ovulation. At the first ultrasound scanning of the ovaries at approximately 7 d postpartum the size (diameter) of the largest follicle observed on either ovary was 6.7 ± 1.1 mm ($P = 0.82$; Table 2.6.). As well, the days required for the development of a dominant follicle (DDF) of ≥ 10 mm was 11.9 ± 1.4 d ($P = 0.80$; Table 2.6.). Neither trait was affected by treatment or parity. A negative correlation was observed between the follicle size at first ultrasonography and DDF of ≥ 10 mm ($r = -0.76$, $P < 0.001$). The interval from calving to first ovulation postpartum was affected by treatment. Cows fed the HSD had a shorter interval from calving to first ovulation than cows fed the MSD or LSD ($P = 0.02$; Table 2.6.). Interval from calving to first ovulation was

positively correlated with TDF ≥ 1 0 mm (r = 0.39, *P* = 0.01) and negatively correlated with BCS (r = -0.37, *P* = 0.02). There was also a significant treatment and parity effect for those cows having a double first postpartum ovulation. None of the LSD cows had a double ovulation compared to 4 on the MSD and 6 on the HSD (*P* = 0.03; Table 2.6.). As well, 10 multiparous cows had double first ovulations compared to none of the primiparous cows (*P* = 0.004). Treatment (*P* = 0.09) and parity (*P* < 0.001) effects were also evident for cows not ovulating before 62 DIM. All of the HSD cows ovulated before 62 DIM while 3 LSD and 2 MSD cows did not (Table 2.6.). A greater number of primiparous cows (P < 0.0001; Table 2.7.) did not ovulate (4 out of 16) while only 1 out of 23 multiparous cows did not ovulate before 62 DIM.

2.3.3.2. Pulsatile LH analysis. No treatment or parity effects were observed for mean LH concentration, number of LH peaks or the amplitude of peaks (Table 2.8.). LH peaks were determined by any peak that was equal or greater to the baseline plus one standard deviation. A positive correlation existed between follicle size at first ultrasonography and number of LH peaks (r = 0.45, *P* = 0.09) and a negative correlation between DDF of ≥ 10 mm and number of LH peaks (r = -0.67, *P* = 0.006). Interval from calving to first ovulation tended to be negatively correlated with the number of LH peaks (r = -0.52, *P* = 0.06) and with mean LH concentration (r = -0.46, *P* = 0.09)

2.3.3.3. Monitoring of estrous cycle. Both the MSD and the HSD cows averaged 2.4 follicular waves per cycle compared to 1.7 waves in LSD cows, but neither was different (*P* = 0.36). Also, estrous cycle length was not different

between treatments (P = 0.63) or parity (P = 0.73; Table 2.10.). Diameter of the largest follicle observed during the first follicular wave was also not different among treatments (P = 0.40; Table 2.10.) with sizes being 18.2 mm and 17.7 mm for primiparous cows and multiparous cows, respectively (P = 0.63, SEM = 0.90; Table 2.11.). Estradiol concentrations during the first follicular wave of the estrous cycle also did not differ among treatments (P = 0.62; Table 2.10.). Estradiol concentrations were 5.7 pg/ml for primiparous cows and 6.1 pg/ml for multiparous cows (P = 0.16, SEM = 0.82; Table 2.11.). A parity by treatment interaction occurred for size of the first dominant follicle. Primiparous cows on the LSD had a larger (P = 0.01) first dominant follicle size than all other treatments and parities, while LSD multiparous cows had similar follicle sizes to MSD and HSD primiparous and multiparous cows. The pre-ovulatory follicle size averaged 17.0 ± 0.98 mm for treatment (P = 0.14; Table 2.10.) and 17.0 ± 0.8 mm for parity (P = 0.42, SEM = 0.77). Estradiol concentrations (pg/ml) during the final follicular wave were also not different among treatments (P = 0.34; Table 2.10.) or parity (5.0 and 5.6 for primiparous and multiparous cows, respectively; P = 0.45, SEM = 0.78; Table 2.11.). A treatment by parity effect occurred with LSD primiparous cows tending to have higher estradiol concentrations during the dominancy of the pre-ovulatory follicle than all other primiparous cows and LSD and MSD multiparous cows (P = 0.06). No treatment or parity effect was observed for progesterone concentration of the first 14 d of the cycle (P = 0.93 for treatment; Table 2.10. and P = 0.37 for parity; Table 2.11.).

2.3.3.4. Pregnancy data. No treatment or parity effect was observed for % pregnant to first, second or third AI (P = 0.78, 0.22, 0.40 for treatment; Table 2.10. and P = 0.23, 0.41, 0.96 for parity; Table 2.11.).

2.4. Discussion

The hypothesis of this study was that increased inclusion of dietary starch would improve production and reproductive parameters in postpartum dairy cows. While increased starch did result in lower body condition loss and reduced the interval from calving to first ovulation, no other measured parameters were affected by treatment. Therefore, the hypothesis was only partially supported by the results.

2.4.1. Production and metabolic data

Milk yield and DMI were not affected by dietary treatment in the present study. Gong et al. (2002) reported no change in milk yield after feeding a high starch diet but they did not report DMI. On the contrary, when Knowlton et al. (1998) infused starch into the rumen or abomasum, milk yield increased while DMI decreased compared to that of cows fed a basal control diet and no starch infusion. Patton et al. (2006) similarly found that a high energy diet with a higher level of concentrate than the control diet resulted in increased milk production. Knowlton et al. (1998) also found an increase in lactose yield with starch infusion; however, our results indicate that HSD cows tended to have lower lactose content in milk. Amaral et al. (1990) infused glucose intravenously and found no change in lactose percentage. Amaral's study has relevance to the current study as part of the supplemental starch in the current study may have

escaped ruminal degradation and been digested and absorbed through the small intestine as glucose. Furthermore, infusion of glucose into the abomasum during wk 3 to 6 of lactation had no significant impact on milk lactose percent (Dhiman et al., 1993). Patton et al. (2006) also found no differences in milk lactose when cows were fed either a control diet or a high energy diet. Sutten (1989) stated that milk lactose cannot be altered by dietary treatment and that any statistical differences observed are simply due to low coefficients of variation. Therefore, the tendency for lower milk lactose percent in the HSD cows cannot be explained.

No treatment differences were seen for milk fat and protein percentage even though NDF content was different between treatments; this is consistent with results published by Oba and Allen (1999) who found no differences in milk composition with varying dietary NDF levels. As well Briceno et al. (1987) found no differences in milk fat percentage when different levels of NDF were fed. However, Beauchemin and Buchanan-Smith (1989) found that milk fat percentage increased as NDF content in the diet increased from 26 to 30 to 34% but protein did not differ. The significantly lower MUN with both the MSD and HSD treatments is indicative of synchrony between energy and protein in the rumen which is important for maximum production of microbial protein (Huntington, 1997). Moharrery (2004) also stated that an improper balance between rumen degradable protein and fermentable carbohydrates results in lowered production of microbial protein and absorption of unused ammonia into the blood stream where it then travels to the liver to be converted to urea and is deposited into milk. This is likely the case in the current study as an increase in

the level of highly fermentable non-structural carbohydrates resulted in a decrease in the concentrations of MUN. However, Lykos et al. (1997) fed a diet with increased levels of rumen degradable starch and found no significant decreases in MUN as degradability went up. Decreased MUN levels are desirable as high levels, usually due to a diet improperly balanced in protein can be detrimental to oocyte quality (Eicher *et al.*, 1999).

Overall, BCS was not affected by treatment; this result is supported by Gong et al. (2002) who found no treatment differences in BCS after feeding a high starch diet for 50 d. Patton et al. (2006) also found no difference in loss of body condition after feeding a high energy diet for the entire lactation. However, in the present study, HSD cows tended to have lower BCS loss than the other treatments throughout the study. Similarly, a study using cows at 55 DIM, and diets of either 21% or 32% starch for 21 d found that high starch resulted in increased BCS gain over the low starch diet (Oba and Allen, 2003). While overall BCS was the same between treatments, a tendency for a less severe reduction in BCS for the HSD suggests that the extra energy consumed by the HSD cows allowed them to maintain body weight to a better degree than cows on the other treatments. As well, primiparous HSD cows in the current study had higher BCS than primiparous MSD and LSD cows, indicating a positive effect of HSD in younger cows. As the feeding trials discussed have only used multiparous cows, it is only possible to speculate on the reasons why increased dietary starch has an effect on primiparous cows and not multiparous cows in terms of BCS. As milk production is lower in primiparous cows compared to multiparous it may be that

increasing dietary starch levels allowed them to partition more energy to growth, instead of lactation, thus allowing for higher BCS.

Hormones and metabolites analyzed in this study all followed similar patterns postpartum to those reported by Doepel et al. (2002), Gong et al. (2002) and Van Knegsel et al. (2007). Doepel et al. (2002) observed a fall in concentrations of glucose, insulin and IGF-I and a rise in NEFA and BHBA following calving which coincides with the results observed in this study. Gong et al. (2002) also observed a drop in insulin levels postpartum and Van Knegsel et al. (2007) reported rises in NEFA and BHBA. However, no treatment differences were observed for glucose, IGF-I, insulin, BHBA and NEFA and this further translated into non-significant differences between treatments for EB. Doepel et al. (2002) also found that EB was negative at calving and didn't recover to positive levels until 6 wk into lactation. In the present study, NEB was observed until 7 wk into lactation.

Primiparous cows had higher levels of glucose, lower levels of BHBA and a more positive EB than multiparous cows in the present study. As well, no differences were seen for IGF-I and NEFA levels between primiparous and multiparous cows in the current study, however, Meikle et al. (2004) found that primiparous cows had prolonged increases in NEFA concentrations and lower concentrations of IGF-I relative to multiparous cows; however, they did not report EB. This contrasts with the findings of Wathes et al. (2007) who reported higher concentrations of IGF-I and lower concentrations of BHBA and NEFA in primiparous cows compared to multiparous cows thus partially supporting the

findings of this study. In the present study both glucose and insulin concentrations declined and reached a nadir between 7 and 14 DIM which coincides with the results of Bossaert et al. (2008) who also reported a glucose nadir on d 8 and insulin nadir on d 12. Leroy et al. (2008) attributed the decline in glucose concentrations to increased demand and uptake of glucose by the mammary gland for lactose synthesis. Consistent reports of declining insulin levels postpartum have been attributed to nutrient prioritization, specifically to balance glucose supply to tissues of the body during early lactation (Bauman, 2000). Lucy (2000) also reported a decline in IGF-I concentrations at the time of calving. In the current study, circulating IGF-I was low at calving but began to recover by d 7. IGF-I levels are also reduced post-calving due to reduced insulin levels as low insulin causes a reduction in growth hormone (GH) receptors in the liver which in turn cause IGF-I concentrations to be lowered (Butler et al. 2003). Non-esterified fatty acid concentrations were high during the first wk of lactation and slowly began to decline. This pattern was also reported by Doepel et al. (2002), and is attributed to drastic increases in lipolysis due to increased diversion of nutrients to the udder as NEFA acts as an alternative energy source when glucose needs to be reserved (Leroy et al., 2008). However, BHBA concentrations showed no clear rise and fall post-calving for all treatments in the current study. An increase in BHBA with a continued rise until 6 wk into lactation was reported by Wathes et al. (2007), and is a result of partial oxidation of NEFA into ketone bodies, primarily in the form of BHBA; however, this observation could not be seen in our results possibly due to reduced lipid

oxidation in the liver. In the present study NEFA and BHBA concentrations peaked at lower levels than those reported by Doepel et al. (2002) and Skaar et al. (1989) and milk production peaked at lower levels than reported by Doepel et al. (2002) and van Knegsel et al. (2007). As well, the cows in the present study had an average NEB nadir value of -12 Mcal/d compared to an approximate -14 Mcal/d reported by Doepel et al. (2002). This suggests that the cows used in the present study may have had reduced lipid mobilization and oxidation due to a less severe EB. Bossaert et al. (2008) also showed a rise of NEFA concentrations, and similar with our results, levels peaked at 4 DIM. Unlike the above results, Knowlton et al. (1998) found that NEFA concentrations were lowered when starch was added to the diet, however, in that study starch was directly infused into either the rumen or abomasum. Starch infusion also increased insulin, but neither BHBA nor glucose was altered. In contrast, Gong et al. (2002) fed a diet high in starch that increased plasma insulin; however, no other hormones or metabolites were measured. Given that treatment failed to increase circulating glucose levels, no glucose-induced increase in insulin was observed for the current study. Bossaert et al. (2008) also hypothesized that heightened NEFA concentrations postpartum could actually hinder the glucose-induced insulin response. Pires et al. (2007) induced a state of hyperlipidemia and found that increased NEFA concentrations impaired glucose clearance by causing insulin resistance. This may be a metabolic response from the cow to allow glucose to be carried to the mammary gland for milk lactose synthesis (Leroy et al., 2008). As feeding a HSD did not increase glucose levels we cannot speculate on the

hypothesis that a rise in glucose might not cause a rise in insulin and that glucose is simply redirected to non-insulin sensitive tissues such as the mammary gland. It appears that further investigation is needed to truly identify the roles of insulin and glucose when non-structural carbohydrates are added at increasing amounts to the diet.

2.4.2. Reproduction

Dietary supplementation of starch was found to affect reproductive characteristics of the cows in the present study. In particular, it was demonstrated that increasing the amount of starch in the diet reduced the interval from calving to first ovulation and resumption of estrous cyclicity earlier in the postpartum period. However, this was only evident in the HSD treatment as both the LSD and MSD cows had a longer interval to first ovulation than HSD cows. This is in agreement with a study from New Zealand that found cows ovulated sooner post-calving if they were given additional non-structural carbohydrates (Burke et al., 2006). Gong et al. (2002) fed lactating dairy cows diets containing 100 g or 260 g starch per kg of DM and found that significantly more cows ovulated before 50 DIM when fed increased levels of starch. It is desirable to have cows ovulate early postpartum as this allows them to complete one or more estrous cycles before they are due to be bred (Butler and Smith, 1989). The first estrous cycle postpartum is generally of shorter length due to premature $PGF_{2\alpha}$ release from the uterus causing CL regression, and estrous behavior is generally not observed (Roche, 2006). By the second cycle, estrous behavior is usually displayed and cycle lengths return to normal (Roche, 2006). As well, the cows ovulating

sooner postpartum are those with higher conception rates during the breeding period (Butler, 2000a; Ambrose and Colazo, 2007) as these cows have been able to complete several cycles before breeding and are more likely to have normal cycle lengths and increased estrous behavior.

In the present study, primiparous cows ovulated at 45 d while multiparous cows ovulated at 32 d postpartum. Santos et al. (2009) and Wathes et al. (2007) also found that primiparous cows took longer to ovulate postpartum than multiparous cows. This is also in agreement with Tanaka et al. (2008) who reported that primiparous cows took 31.8 d and multiparous cows took 17.3 d to ovulate after calving. Santos et al. (2009) suggested that this delay in ovulation in primiparous cows may be due to an increased incidence of uterine infections and metabolic disorders, while Tanaka et al. (2008) reported insufficient estradiol concentrations from the first dominant pre-ovulatory follicle.

While many factors have been shown to be correlated with the interval from calving to first ovulation, the fate of the first dominant follicle postpartum appears to regulate this event. The ability of the first dominant follicle to ovulate is related to follicle size, LH pulsatility and IGF-I concentrations (Roche, 2006). In the present study, the interval from calving to first ovulation was negatively correlated with LH pulsatility determined approximately 14 d post-calving. As well, the length of time to attain a follicle diameter of 10 mm or greater was positively correlated with the ovulation interval. However, IGF-I concentration was not correlated with the interval from calving to first ovulation, thus differing from results published by Roche (2006) and Beam and Butler (1998) who

reported that IGF-I was positively correlated with estradiol concentrations during the growth of the first dominant follicle. In the present study a decrease in the interval from calving to first ovulation cannot be attributed to increased IGF-I and insulin concentrations and is discussed later in this section.

Negative energy balance has also been found to be associated with an increased interval from calving to first ovulation. Beam and Butler (1999) found that an improvement in EB and fewer days to NEB nadir corresponded with fewer days to ovulation postpartum. More specifically, Butler (2000) found that NEB in the first 3-4 wk post-calving was highly correlated with an increased interval from calving to first ovulation. The present study found no correlation between NEB and days to ovulation and also found no improvement in EB by increasing the starch level of the diet. Harrison et al. (1990) compared cows of high or low milk production and found no association between EB in the first 2 wks of lactation and days to first ovulation. As well, Canfield and Butler (1990) found that average daily EB was not correlated with days to first ovulation but that cumulative EB and EB nadir were. IGF-I and insulin appear to be key factors in follicular development, steroidogenesis and length of the anovulatory period. Plasma concentrations of both of these hormones are normally low post-calving (Lucy, 2000) and when EB is negative (Lucy, 2003). In the present study, insulin concentrations showed a greater decline post-calving than IGF-I, however, similar to EB, increasing levels of dietary starch had no impact on these hormone concentrations. As well, no correlation was found between interval from calving to first ovulation and plasma insulin or IGF-I concentrations. This is in agreement

with Canfield and Butler (1990) who also found no correlation between insulin concentrations in the first 14 d postpartum and interval from calving to first ovulation but did find a strong correlation between days to EB nadir and first ovulation. In the current study the interval from calving to first ovulation was significantly shorter in cows fed the HSD compared to the LSD or MSD but cannot be attributed to an improved hormone profile or increases in EB or BCS. However, it may be that the HSD cows ovulated within a normal range postpartum and that the other treatments showed a delay in the first ovulation due to lower starch levels. Nebel and McGilliard (1993) stated that the average interval from calving to first ovulation was 30 d. The HSD cows ovulated on average 31 DIM compared to 41 d for the other two treatments. Another possible explanation is an increased incidence of uterine infections postpartum as this has been associated with an increased anovulatory interval (Peter et al. 2009). LeBlanc (2005) also reports that an increased anestrous period may be associated with uterine infections postpartum. The incidence of uterine infections postpartum was not recorded in this study so we can only speculate that this may have attributed to the increased interval from calving to first ovulation in LSD and MSD cows. As well, concentrations of leptin, which were also not analyzed in this study are decreased post-calving but have also been shown to be important in regulating the interval from calving to first ovulation (Kadokawa and Martin, 2006). Reist et al. (2003) also reported that cows fed 50% concentrate had higher concentrations of leptin postpartum than cows fed only 30% concentrate. While level of concentrate was not the dietary treatment in this study, it is possible that

the HSD treatment allowed cows to maintain a higher concentration of leptin which may have aided in reducing the interval from calving to first ovulation postpartum.

While circulating concentrations of these hormones *in vivo* appeared to have no impact on follicular growth or ovulation rates in the present study, several *in vitro* studies have identified roles for IGF-I and insulin in granulosa cell proliferation and estradiol production (Lucy, 2000). Armstrong et al. (2003) reported that insulin and IGF-I stimulated the granulosa cells of the follicle to increase estradiol production and increase the sensitivity of follicles to gonadotropins. Armstrong et al. (2001) reported that increased intra-follicular IGF-I, due to increased circulating IGF-I, increased the size of the follicle and its receptiveness to FSH. Gutiérrez et al. (1997) found that insulin was positively associated with an increased number of small follicles and Gong et al. (2002) showed that a rise in circulating insulin concentrations was associated with an increase in the proportion of cows ovulating before 50 DIM. In this study the cows fed the HSD, which was predicted to increase circulating concentrations of insulin and IGF-I, ovulated sooner post-calving. However, no change in IGF-I and insulin concentrations were observed and no correlations could be made with the improved reproductive performance early postpartum.

Other blood metabolites such as NEFA and BHBA have all been found to be negatively correlated with the interval from calving to first ovulation, but once again this was not the case in the current study. A negative correlation between IGF-I and NEFA was reported by Wathes et al. (2007) from wks -1 to +4

postpartum. These authors also found that the primiparous cows with high NEFA and BHBA concentrations were those taking longer to ovulate postpartum. None of these correlations could be made in our study; this agrees with Harrison et al. (1990) and Canfield and Butler (1990) who also found no correlation between glucose, NEFA or BHBA concentrations and interval from calving to first ovulation. Bossaert et al. (2008) found that NEFA levels post-calving did not affect the interval from calving to first ovulation but suggested that an inadequate sample size may have interfered with the analysis. As well, Nebel and McGilliard (1993) found no association between BHBA and NEFA levels and the anovulatory interval.

Other parameters investigated in this study that contribute to reduced EB are reduced DMI, high milk yield, low and high BCS and all of these parameters have been associated with an increased interval from calving to first ovulation. As there were no treatment differences in this study for DMI and milk yield, increased dietary starch did not impact these parameters. Only BCS was negatively correlated with the anovulatory interval in that the lower the BCS within the first month postpartum the longer it took for the cow to ovulate. This is also supported in that both the MSD and LSD cows tended to have a larger decrease in BCS over the treatment period and took longer to ovulate postpartum than the HSD cows. Lucy (2003) also reported that cows losing excessive BCS have poor reproductive performance and speculated that it may be due to nutrient partitioning towards milk production and growth in primiparous cows. This is also in agreement with Beam and Butler (1999) who found that cows losing more

BCS had an increased interval from calving to first ovulation. While studies have often proposed that high milk yield is primarily responsible for the decline in reproductive performance, Santos et al. (2009) actually found that cows producing the lowest amounts of milk were those most likely to have a delay in first ovulation postpartum and indicated that cows producing more milk might actually be those cows with greater DMI and thus be in a better state of EB. To the contrary, Wathes et al. (2007) reported that the interval from calving to first ovulation was correlated with peak milk yield in multiparous cows. While increased milk production may or may not be associated with an increased interval from calving to first ovulation, LeBlanc (2005) reported that high producing herds are often well managed and may in fact have higher pregnancy rates than lower producing herds.

Studies examining the impact of increased concentrations of circulating insulin and IGF-I through diet manipulation have often looked at the effects on the first dominant follicle postpartum and the period to regain cyclicity. As well, *in vitro* studies have closely examined the follicle, but shed little light on the effects of IGF-I and insulin on the varying cycles postpartum. In our study, we examined not only the first ovulation but also examined an estrous cycle in cows that were at least 45 DIM to determine the effects of IGF-I and insulin on ovarian dynamics and steroid hormone production later in lactation. However, no treatment effects could be detected for any of these factors thus indicating that increased inclusion of dietary starch may have less effect on cows that have completed at least 2 estrous cycles postpartum.

As stated above, Armstrong et al. (2001) reported that IGF-I is responsible for increasing the diameter of the dominant follicle. In our study we hypothesized that feeding a diet to promote increased insulin and IGF-I would increase the size of the ovulatory follicle and result in a larger CL with greater potential to produce progesterone during the luteal phase. However, the pre-ovulatory follicle size observed during the estrous cycle was not different between treatments and averaged 17.0 mm in diameter. This is consistent with Peter et al. (2009) who reported that a follicle will ovulate when it has developed to 10-20 mm in diameter. Lucy (2000) also reported that IGF-I works with gonadotropins to increase steroidogenesis and growth of follicular cells. This is also important for CL function and progesterone production as the number of pre-ovulatory granulosa cells is related to subsequent luteal cell numbers after luteinization of the follicle (Senger, 2003). Other *in vitro* work by Spicer et al. (1993) found that cultured granulosa cells produced increased concentrations of progesterone when in culture with insulin or IGF-I. This would suggest that insulin and IGF-I play a role in progesterone production during the luteal phase and higher levels of progesterone may be responsible for development of a third follicular wave. Sartori et al. (2004) reported that cows exhibiting low circulating progesterone concentrations had pre-ovulatory follicles that grew for longer periods. Extended periods of follicular growth are usually indicative of 2-wave vs. 3-wave cycles and some researchers have alluded that fertility is increased in cows having 3-wave cycles due to ovulation of a younger oocyte from an actively growing follicle. Mihm et al. (1994) found that when follicles remained in dominancy for

> 10 d, fertility was reduced and that highest fertility was achieved when the ovulatory follicle remained dominant for only 1 to 4 d. Townson et al. (2002) found that lactating cows having 3-wave cycles had higher pregnancy rates than cows having 2-wave cycles. However, Wolfenson et al. (2004) found no relationship between progesterone concentrations and number of follicular waves per cycle, although no pregnancy data were recorded in their study. Given that IGF-I and insulin were not different between treatments and no differences were observed in the length of the estrous cycle, a connection between plasma concentrations of these hormones and the estrous cycle cannot be made for the current study.

The percentage of cows pregnant following the first, second or third AI was not significantly different between treatments in the current study. However, several publications have addressed correlations between pregnancy rates, EB status, BCS, insulin and IGF-I concentrations. Butler (2000) stated that cows losing more than 1 unit of BCS are at increased risk for poor conception rates. Other studies have also shown that NEB causes irregular cycles postpartum which are associated with increased intervals to first service and conception (Wathes et al. 2003). Wathes also showed a relationship between calving to conception, IGF-I levels and BCS. However, some publications have reported a detrimental effect of increased insulin and IGF-I on oocyte quality. Armstrong et al. (2001) found that when cows were fed a high energy diet to promote increased insulin and IGF-I concentrations that quality of oocytes derived from these animals was compromised.

Other studies have also shown correlations between IGF-I and insulin and the increased incidence of double ovulations and twinning rates. In the present study a significant percentage of MSD and HSD cows had double ovulations with the majority occurring on the first ovulatory event postpartum. Sartori et al. (2004) reported that 17.9% of multiparous cows in their study had multiple ovulations and only 1.9% of primiparous cows. This is also in agreement with our findings as 37% of multiparous cows had double ovulations, but none in primiparous cows. IGF-I and insulin have been found to be involved in increased ovulation rates. Butler (2003) showed that increased levels of IGF-I are associated with increased follicular estradiol production and Butler (2001) showed that ovulation of a dominant follicle is in part dependent on estradiol production. As well, cattle selected for natural twinning were shown to have increased levels of both circulating and follicular IGF-I (Echternkamp et al. 1990). As no significant treatment effects were observed for IGF-I and insulin it cannot be concluded from this study that increased circulating levels of these hormones did in fact increase the number of double ovulations. There is the potential that leptin, which was not analyzed in this study, may have been elevated when supplemental starch was added and may have aided in increasing the ovulation rate as Kadokawa and Martin (2006) have identified leptin in being important in ovulation.

2.5 Conclusion

In this study, increasing dietary starch decreased the interval from calving to first ovulation postpartum and tended to reduce BCS loss but was not

associated with an improvement in EB, metabolic profile or production parameters. The results also show that increased inclusion of dietary starch may provide primiparous cows with benefits such as improved EB but this did not translate into improved reproductive performance. Although increased starch inclusion reduced the interval from calving to first ovulation the benefits were not evident in subsequent estrous cycles or fertility. Therefore, starch inclusion may result in more favorable follicular development in the first few weeks post-calving. In conclusion, more *in vivo* studies need to be completed to define a clear role for increased non-structural carbohydrates in the diet of postpartum cows.

Table 2.1. Ingredient and nutrient composition of the dietary treatments.

	Treatments[1]		
	LSD	MSD	HSD
Ingredient composition (% DM)			
Alfalfa silage	…	44.5	…
Barley silage	43.8	…	39.9
Alfalfa hay	10.0	9.9	10.1
Corn starch[2]	…	…	3.8
Rolled barley	5.0	10.5	5.0
Corn gluten meal	3.0	4.0	3.0
Corn	21.2	25.4	21.2
Canola meal	6.9	…	6.9
Soy bean meal	6.9	2.5	6.9
Megalac[3]	0.5	0.5	0.5
Pork fat	1.0	1.0	1.0
Limestone	0.2	0.2	0.2
Magnesium oxide	0.1	0.1	0.1
Salt	0.2	0.2	0.2
Dicalcium phosphate	0.5	0.5	0.5
Vitamins/minerals[4]	1.6	1.7	1.6
Nutrient Composition (% DM)			
NE_L Mcal/kg DM[5]	1.68	1.64	1.72
DM%	65.8	66.5	67.9
OM%	93.2	92.4	89.7
NDF%	30.3	24.4	28.5
ADF%	17.4	15.8	16.4
Lignin%	2.8	3.9	2.7
CP%	18.9	17.3	18.3
Starch%	23.5	25.3	26.9

[1]LSD = low starch diet, MSD = medium starch diet, and HSD = high starch diet.
[2]Melojel, Quadra Chemicals, Edmonton, AB
[3] Church & Dwight Co., Inc., Princeton, NJ.
[4] Supplied the diet with 2.2 mg/kg Co, 80.1 mg/kg Cu, 3.3 mg/kg I, 142.1 mg/kg Mn, .65 mg/kg Se, 239.1 mg/kg Zn, 16.2 KIU/kg Vit A, 3.22 KIU/kg of Vit D3, 114.7 IU/kg Vit E, and 1.4 mg/kg Biotin.
[5] Calculated using NRC, 2001 assuming daily dry matter intake of 18.9kg/d

Table 2.2. Effect of dietary starch concentration on mean DMI, milk yield and composition, BCS and EB of treatments.

	Treatment[1]				
	LSD	MSD	HSD	SEM	P^2
DMI, kg/d	18.0	19.3	18.5	0.81	0.37
Milk yield, kg/d	34.9	34.4	35.5	1.6	0.89
Milk component yield, g/d					
Fat	1369	1276	1344	74.2	0.60
Protein	1100	1029	1132	47.4	0.24
Lactose	1700	1632	1699	82.0	0.77
Milk composition, %					
Fat	3.80	3.69	3.65	0.14	0.73
Protein	3.03	2.96	3.10	0.07	0.26
Lactose	4.66	4.67	4.53	0.05	0.07
SCC, $x10^3$ /ml	116	126	124	35	0.98
MUN, mg/dl	16.33^b	13.60^a	13.40^a	0.65	0.0012
BCS	3.01	2.94	3.08	0.06	0.15
Change in BCS from 0 to 70 DIM	-0.57	-0.60	-0.40	0.63	0.06
EB, Mcal/d	-3.91	-1.18	-2.77	0.90	0.16
Days to reach NEB nadir	7.4	9.8	8.7	2.05	0.63

[1]LSD = low starch diet, MSD = medium starch diet, and HSD = high starch diet.
[2]Different superscripts within a row represent significant differences among treatments ($P \leq 0.05$).

Table 2.3. Effect of dietary starch concentration on mean DMI, milk yield and composition, BCS and EB of parity.

	Parity			
	Primiparous	Multiparous	SEM	P^2
DMI, kg/d	16.7	20.5	0.84	0.0023
Milk yield, kg/d	28.3	41.6	1.41	0.00
Milk component yield, g/d				
Fat	1083	1576	66.3	0.0001
Protein	903	1271	43.9	0.0001
Lactose	1389	1965	69.6	0.0001
Milk composition, %				
Fat	3.69	3.73	0.12	0.80
Protein	3.08	2.97	0.07	0.23
Lactose	4.66	4.57	0.05	0.19
SCC, $x10^3$ ml	165.0	80.0	32.0	0.05
MUN, mg/dl	13.82	15.07	0.64	0.15
BCS	3.02	3.00	0.07	0.83
Change in BCS from 0 to 70 DIM	-0.47	-0.57	0.05	0.17
EB, Mcal/d	-1.65	-3.98	0.83	0.04
Days to reach NEB nadir	8.1	9.2	1.75	0.59

Table 2.4. Effect of dietary starch concentration on plasma hormone and metabolite concentrations of treatments.

	Treatment[1]				
	LSD	MSD	HSD	SEM	*P*
Glucose, mg/dl	59.4	60.0	60.2	1.07	0.86
Insulin, µIU/ml	6.3	6.6	7.8	0.72	0.33
IGF-I, ng/ml	55.7	52.1	56.0	3.23	0.62
NEFA, mEq/L	285.9	349.7	267.9	30.40	0.15
BHBA, mg/dl	8.4	7.8	8.5	0.34	0.19

[1]LSD = low starch diet, MSD = medium starch diet, and HSD = high starch diet.

Table 2.5. Effect of dietary starch concentration on plasma hormone and metabolite concentrations of parity.

	Parity			
	Primiparous	Multiparous	SEM	*P*
Glucose, mg/dl	64.3	55.5	0.96	0.00
Insulin µIu/ml	7.3	6.5	0.63	0.33
IGF-1, ng/ml	57.5	51.7	2.80	0.11
NEFA, mEq/L	292.8	309.6	27.14	0.63
BHBA, mg/dl	7.4	9.1	0.38	0.00

Table 2.6. Effect of dietary treatment on follicular parameters and ovulation of treatments.

	Treatment[1]				
	LSD	MSD	HSD	SEM	P^2
Follicle size at first scanning, mm	6.3	7.1	6.8	1.06	0.82
DDF[3] $\geq$ 10 mm in diameter	12.3	12.1	11.4	1.37	0.80
Interval from calving to first ovulation (days)[4]	38.1[b]	43.2[b]	30.6[a]	4.8	0.02
No. double first ovulations	0[a] (0%)	4[b] (31%)	6[b] (46%)		0.03
Cows not ovulating before 62 DIM	3 (21%)	2 (15%)	0 (0%)		0.09

[1]LSD = low starch diet, MSD = medium starch diet, and HSD = high starch diet.
[2]Different superscripts in a row represent significant differences among treatments ($P \leq 0.05$).
[3]Days to reach a dominant follicle of $\geq$ 10 mm in diameter.
[4]HSD tended to be different from LSD ($P = 0.07$)

Table 2.7. Effect of dietary treatment on follicular parameters and ovulation of parity.

	Parity			
	Primiparous	Multiparous	SEM	*P*
Follicle size at first scanning, mm	8.1	5.4	2.30	0.49
DDF[1] ≥ 10 mm in diameter	11.6	12.5	2.98	0.84
Interval from calving to first ovulation (days)	45.0	32.3	3.6	0.01
No. double first ovulations	0 (0%)	10 (43%)		0.00
Cow not ovulating before 62 DIM	4 (33%)	1 (4%)		0.05

[1]Days to reach a dominant follicle of ≥ 10 mm in diameter.

Table 2.8. Effect of dietary treatment on LH pulsatility of treatments. Blood samples taken at 15 min intervals over an 8 h period approximately 15 d postpartum for LH analysis.

	Treatment[1]				
	LSD	MSD	HSD	SEM	*P*
No. of plasma LH peaks	2.50	2.00	2.80	0.49	0.57
Mean plasma LH, ng/ml	0.49	0.43	0.45	0.16	0.95
Plasma LH amplitude, ng/ml	1.30	1.30	1.30	0.42	0.98

[1]LSD = low starch diet, MSD = medium starch diet, and HSD = high starch diet.

Table 2.9. Effect of dietary treatment on LH pulsatility of parity. Blood samples taken at 15 min intervals over an 8 h period approximately 15 d postpartum for LH analysis.

	Parity			
	Primiparous	Multiparous	SEM	*P*
No. of LH peaks	2.00	2.83	0.46	0.15
Mean plasma LH, ng/ml	0.40	0.51	0.14	0.54
LH amplitude, ng/ml	1.25	1.34	0.39	0.84

Table 2.10. Subset of cows evaluated by ultrasonography every second day for one estrous cycle, treatment.

	Treatment[1]				
	LSD	MSD	HSD	SEM	*P*
No. of follicular waves	1.7	2.4	2.4	0.43	0.36
Length of cycle (days)	20.7	24.4	24.4	3.68	0.63
Size of first dominant follicle (mm)	19.1	17.1	17.8	1.15	0.40
Preovulatory follicle size (mm)	18.5	16.0	16.4	0.98	0.14
Mean plasma estradiol during first follicular wave (pg/ml)[2]	5.5	6.7	5.4	1.48	0.62
Mean plasma estradiol during preovulatory follicle growth (pg/ml)[3]	7.6	5.5	6.5	1.17	0.34
Mean plasma progesterone from d 0 to 14 of the estrous cycle (ng/ml)	3.5	3.6	3.5	0.29	0.93
Pregnant to first AI %	43.0	31.0	33.0		0.78
Pregnant to second AI %	28.6	38.5	8.3		0.22
Pregnant to third AI %	7.1	23.1	8.3		0.40

[1]LSD = low starch diet, MSD = medium starch diet, and HSD = high starch diet.
[2]Estradiol concentration was determined by using the plasma samples collected on d 4, 6 and 8 of the estrous cycle.
[3]Estradiol concentration was determined by using the last 5 plasma samples collected before ovulation.

Table 2.11. Subset of cows evaluated by ultrasonography every second day for one estrous cycle, parity.

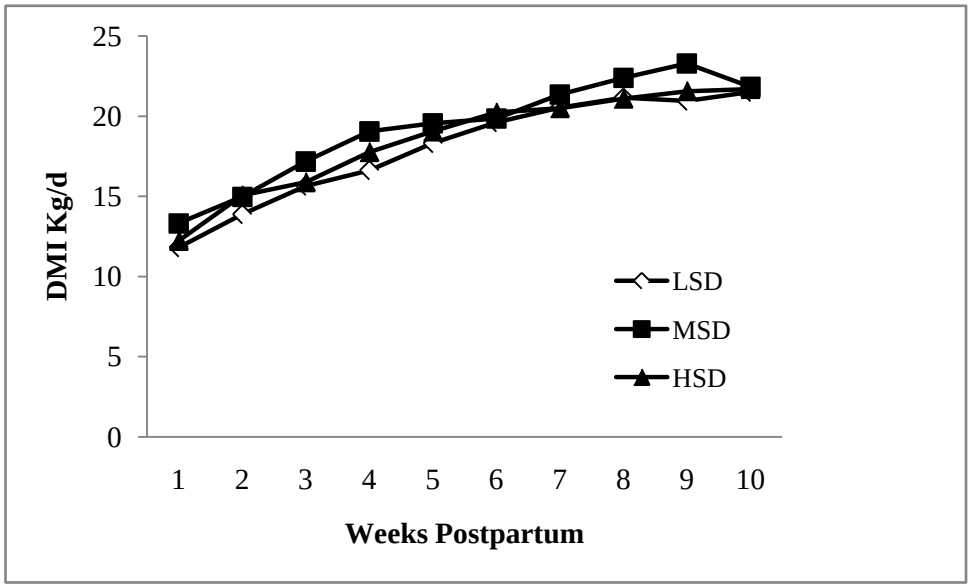
	Parity			
	Primiparous	Multiparous	SEM	*P*
No. of follicular waves	1.94	2.42	0.33	0.24
Length of cycle (days)	22.72	23.92	2.88	0.73
Size of first dominant follicle (mm)	18.24	17.70	0.90	0.63
Preovulatory follicle size (mm)	17.33	16.58	0.77	0.42
Mean plasma estradiol during the first follicular wave (pg/ml)[1]	5.70	6.10	0.82	0.16
mean plasma estradiol during preovulatory follicle growth (pg/ml)[2]	5.01	5.63	0.78	0.45
Mean plasma progesterone from d 0 to 14 of the estrous cycle (ng/ml)	3.55	3.54	0.17	0.37
Pregnant at first AI %	25.0	43.0		0.23
Pregnant at second AI %	18.8	30.4		0.41
Pregnant at third AI %	12.5	8.7		0.57

[1]Estradiol concentration was determined by using the plasma samples collected on d 4, 6 and 8 of the estrous cycle.

[2]Estradiol concentration was determined by using the last 5 plasma samples collected before ovulation.

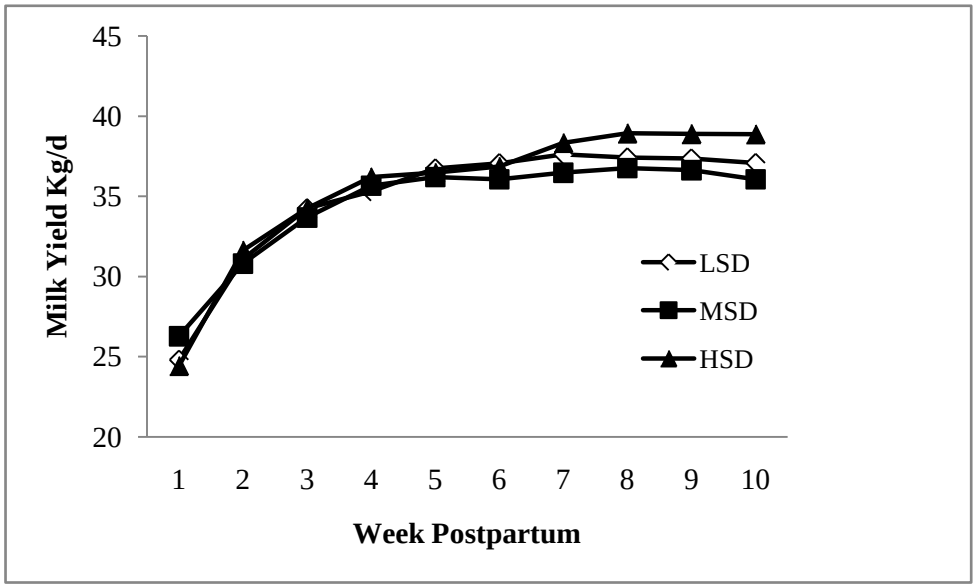
[3]Significantly more primiparous cows never conceived (P=0.03) than multiparous cows.

Figure 2.1. Weekly DMI until 70 DIM.



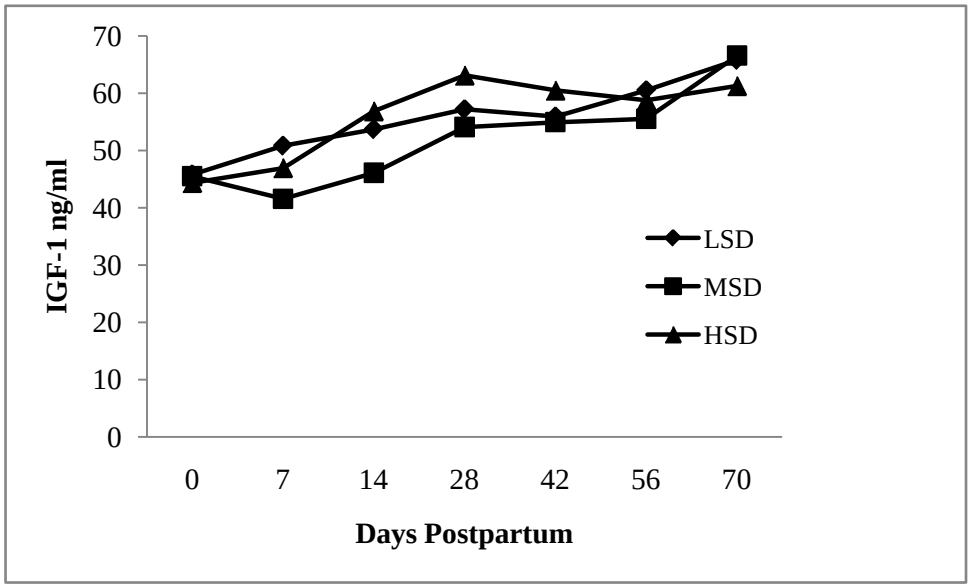
No treatment effects were observed (P = 0.37; pooled SEM = 0.81). Cows were kept on treatment for 70 d starting the day of calving and were fed either a low starch diet (LSD), medium starch diet (MSD) or high starch diet (HSD).

Figure 2.2. Weekly milk yield until 10 wk into lactation.



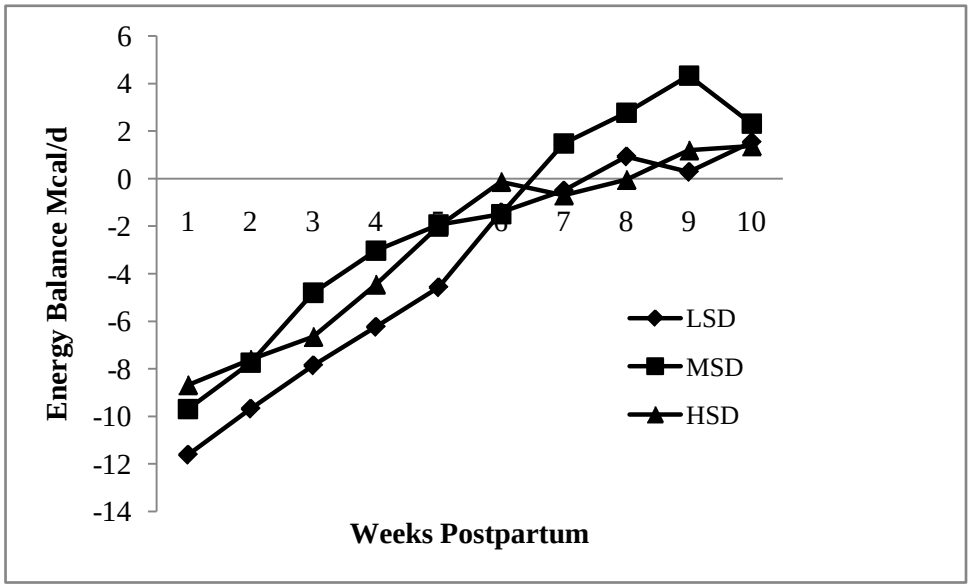
No treatment effects were observed (P = 0.89; pooled SEM = 1.6). Cows were kept on treatment for 70 d starting the day of calving and were fed either a low starch diet (LSD), medium starch diet (MSD) or high starch diet (HSD).

Figure 2.3. IGF-I concentrations until 70 DIM.



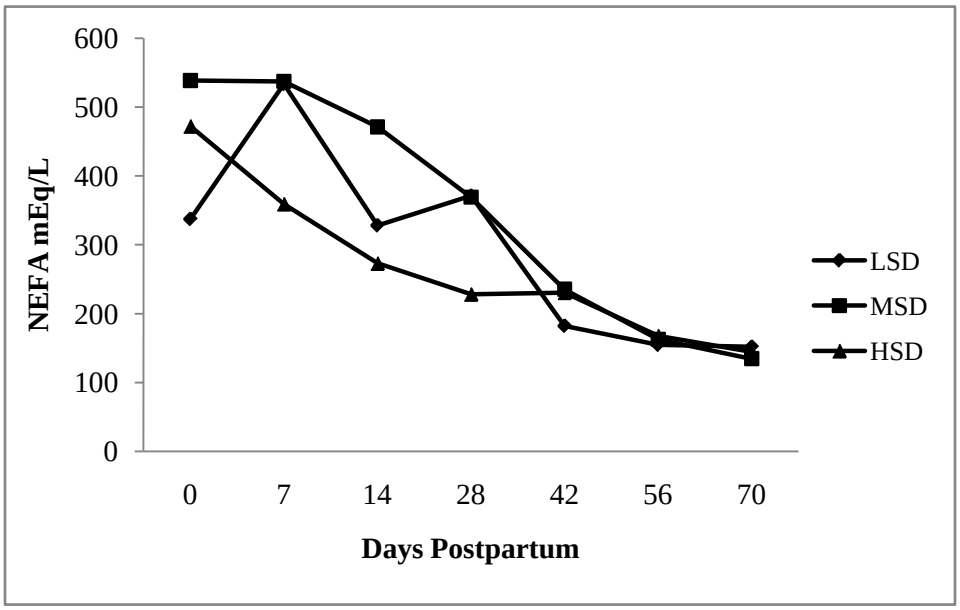
There were no treatment effects observed (P = 0.62; SEM = 0.72). Cows were kept on treatment for 70 d starting the day of calving and were fed either a low starch diet (LSD), medium starch diet (MSD) or high starch diet (HSD).

Figure 2.4. Weekly energy balance until 10 wk into lactation.



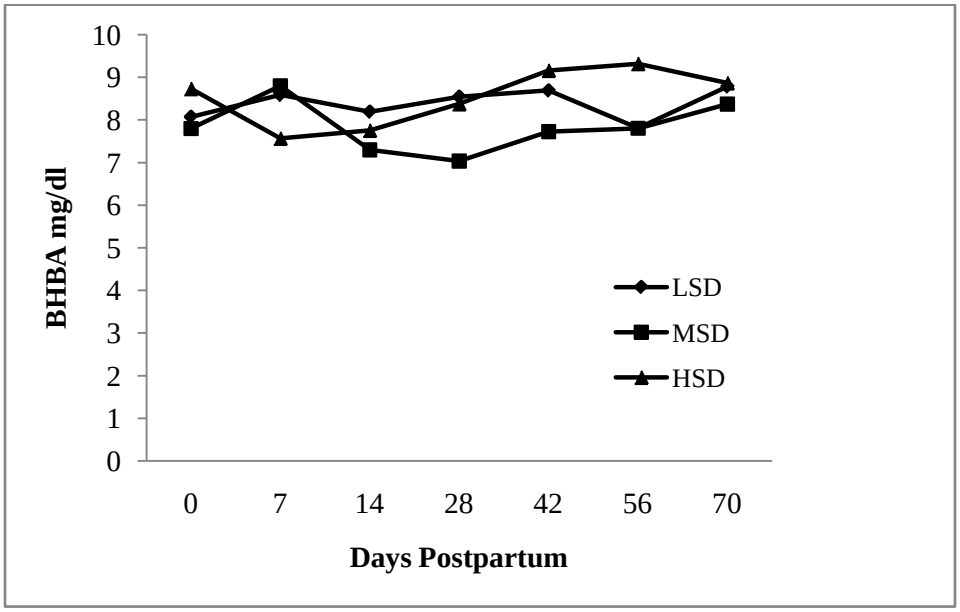
There were no treatment effects observed (P = 0.16; pooled SEM = 0.90). Cows were kept on treatment for 70 d starting the day of calving and were fed either a low starch diet (LSD), medium starch diet (MSD) or high starch diet (HSD).

Figure 2.5. Plasma NEFA concentrations until 70 DIM.



There were no treatment effects observed (P = 0.15; SEM = 30.40). Cows were kept on treatment for 70 d starting the day of calving and were fed either a low starch diet (LSD), medium starch diet (MSD) or high starch diet (HSD).

Figure 2.6. Plasma BHBA concentrations until 70 DIM.



There were no treatment effects observed (P = 0.19; SEM = 0.34). Cows were kept on treatment for 70 d starting the day of calving and were fed either a low starch diet (LSD), medium starch diet (MSD) or high starch diet (HSD).

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CHAPTER 3.

SUMMARY OF FINDINGS, FUTURE RESEARCH, INDUSTRY PERSPECTIVE AND FINAL CONCLUSION

3.1. Summary of Findings

- Supplementation of dietary starch had no impact on EB, DMI or milk yield.
- Increased dietary starch tended to reduce loss of body condition over the 70 d treatment period.
- No differences among treatments were observed for glucose, insulin, IGF-I, BHBA or NEFA concentrations.
- Cows fed the LSD had significantly higher levels of MUN compared to cows fed the MSD or the HSD.
- The interval from calving to first ovulation was significantly reduced for cows fed the HSD.
- Increased inclusion of dietary starch had no impact on LH pulsatility or ovarian dynamics in early lactation.
- The number of cows ovulating 2 follicles at the first ovulation postpartum was significantly higher in the HSD and MSD treatment groups.
- Primiparous cows had higher concentrations of glucose, lower concentrations of BHBA and greater EB than multiparous cows, but had an increased interval from calving to first ovulation.

3.2. Future Research

Several avenues of research are worth pursuing in the area of dietary energy allowance, non-structural carbohydrate availability and reproductive performance and are discussed below.

The limited positive effects observed on reproduction and production parameters may have been due to the fact that the dietary starch content was too low in this study to promote increases in circulating metabolites and hormones. Future research could examine the effects of increased concentrations of dietary starch at a level greater than that used in this study. Eastridge (2007) pooled together a database of studies and reported that minimum and maximum percentages of starch inclusion on a DM basis should be between 25 and 35% respectively, while Hutjens (2007) recommended that nutritionists formulate starch levels be 21-26% of DM but reported industry fed values to range from 18 to 32%. Silveria et al. (2007) fed four experimental diets ranging in starch content from 22% to 32% on a DM basis and found no differences in milk yield or DMI but the cows on the high starch treatment did have increased periods of low ruminal pH. These reports suggest that incorporation of starch at percentages higher than used in this study can be incorporated into dairy rations. However, care must be taken as high levels of non-structural carbohydrates decrease digestion of fibrous carbohydrates and can lower the pH of the rumen, leaving the animal susceptible to sub-acute ruminal acidosis.

It may also be beneficial to measure leptin concentrations in response to dietary manipulation in future studies as this hormone is also an indicator of

metabolic status. Chelikani et al. (2004) reported that early lactation cows, fasted for 48 h had a significant decrease in plasma leptin concentrations thus identifying a potential role for leptin in energy signaling to the hypothalamus. Reist et al. (2003) found that low leptin concentrations postpartum coincided with the highest levels of NEFA and were associated with fat mobilization. Leptin was also found to be positively correlated with EB, insulin, IGF-I and BCS and was considerably lower in cows fed only 30% concentrate compared to 50% concentrate over the first 20 wks postpartum (Reist et al., 2003). Kadokawa and Martin (2006) also suggested that leptin functions with IGF-I in regulating the event of the first ovulation postpartum and found that the delay in resumption of ovulation was correlated with the increased interval to leptin concentration nadir. However, Wathes et al. (2007) was unable to show a correlation between postpartum leptin concentrations and onset of cyclicity. Further research could help clarify the role of leptin in regulating reproductive function of postpartum cows.

Growth hormone is another hormone involved in regulating metabolic status that was not measured in this study but that may provide further insight into the effects of dietary starch postpartum. Reist et al. (2003) found GH levels to be elevated when low levels of concentrate were fed and stated that GH was likely involved in increased mobilization of fat stores. This is likely due to the fact that GH is involved in partitioning of nutrients away from adipose tissue and towards milk production in early lactation (Doepel et al., 2002; Baumen and Currie, 1980). Wathes et al. (2007) also reported that elevated GH postpartum, in association with low insulin, promotes gluconeogenesis and lipolysis. Examining this

hormone may give insight into the relationship between lipolysis and energy levels, manipulated via dietary starch, in early lactation.

As well, further research on inclusion of dietary starch postpartum should include primiparous and multiparous cows to further examine differences in metabolic status and reproductive performance. This was included in the study presented in this thesis but other researchers in this area often use only multiparous cows in feed trials. Wathes et al. (2007) reported that tissue mobilization and nutrient partitioning is different in primiparous and multiparous cows and, therefore it is not surprising that these two groups of cows responded differently to treatment. As differences were noted between the groups, further research is needed to fully understand and confirm the results produced above.

It could also be recommended to begin dietary starch treatment in the weeks prior to calving. This may allow for improvement in metabolic status in the first week post-calving that may not occur when treatment starts immediately after calving.

The incorporation of the above suggestions into future research with increased inclusion of dietary starch may provide further understanding and clarifications for its influence on metabolic status and potential reproductive improvement in the postpartum dairy cow.

3.3. Industry Perspective

Reduced conception rates mean increased days open after calving and fewer calves born during the lifetime of a dairy cow, resulting in decreased profits for the dairy farmer. The practical implications of this study include the prospect

of adding a low cost starch additive to the diet to reduce the interval from calving to first ovulation. The results of the study presented here show that increased dietary starch can decrease the days to first ovulation postpartum, but improvements in conception rates were not evident possibly due to low sample size. Larger scale studies are needed to determine fertility improvements of dairy cows when increased levels of starch are added to the diet.

3.4. Final Conclusion

While increasing levels of dietary starch improved reproduction in early lactation through a decreased interval from calving to first ovulation, no effects were observed in LH pulsatility or ovarian dynamics, estradiol and progesterone production or in any of the metabolic parameters mentioned above. It did appear that primiparous cows showed improvements in EB and overall metabolic status compared to multiparous cows when fed the HSD, but these improvements did not result in reproductive improvements and additional energy may have been diverted to milk production and growth. Further research in this area should be conducted to examine the effects of increased starch inclusion at dietary levels higher than those used in the present study as several researchers have identified benefits through this dietary manipulation. This area of research holds potential benefits to the producer in the areas of improved EB, and therefore, lowered risk of metabolic disorders and improved conception rates through earlier resumption of reproductive cycles postpartum.

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