# RELATIONSHIPS AMONG NUTRITIONAL REGIMEN, METABOLIC DISORDERS, REPRODUCTION, AND PRODUCTION IN DAIRY COWS DURING THE TRANSITION PERIOD

BY

FELIPE CARDOSO

DISSERTATION

Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy in Animal Sciences
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2012

Urbana, Illinois

Doctoral Committee:

Professor James K. Drackley, Chair
Professor Emeritus Michael F. Hutjens
Professor Emeritus Michael R. Murphy
Professor Manabu T. Nakamura
Adjunct Associate Professor Richard L. Wallace

## ABSTRACT

Negative energy balance (**NEB**) during the first weeks postpartum is associated with infertility in dairy cows. A meta-analysis approach was used to investigate the association between prepartum energy feeding regimen, productive parameters, health, and reproductive performance. Days to conception (**DTC**) was used as the dependent variable to assess reproductive performance. The database was developed from 7 experiments completed in our group from 1993 to 2010. Individual data for 408 cows (354 multiparous and 54 primiparous) were included in the analysis. The net energy for lactation (**$NE_L$**) intake was determined from each cow's average dry matter intake (**DMI**) and calculated dietary $NE_L$ density. Treatments were applied prepartum and were classified as either controlled energy (**CE**) or high energy (**HE**) diets fed during the far-off (**FO**) or close-up (**CU**) dry periods. Cow was the experimental unit. All analyses were carried out using SAS 9.2 (SAS Institute, Inc.). The Cox proportional hazard model revealed a significant difference in DTC between HE and CE during the CU period (median = 167 and 157 d; hazard ratio (**HR**) = 0.696). Cows fed HE diets during the last 4 wk prepartum lost more body condition score (**BCS**) in the first 6 wk postpartum than those fed CE (−0.43 and −0.30, respectively). Cows with 3 or more lactations lost more BCS than cows with one or two lactations (−0.42 and −0.33, respectively). Cows fed CE during the FO period had lower nonesterified fatty acids (**NEFA**) concentrations in wk 1, 2, and 3 of lactation compared with cows fed HE. Higher NEFA concentration in wk 1 postpartum was associated with a greater probability of diseases (n = 251; OR = 1.176). Cows on the CE regimen during the FO period had greater plasma glucose concentrations during wk 1 and 3 after calving than cows fed the HE regimen. Higher plasma glucose (**HG**) concentration compared with lower glucose (**LG**) in wk 3 (HG, n = 154; LG, n = 206) and wk 4 (HG, n = 71; LG, n = 254) after calving was associated

with greater HR for DTC (wk 3: median = 151 and 171 d for HG and LG, HR = 1.334; wk 4: median = 148 and 167 d, HR = 1.394). In the first 2 wk after calving, cows that received HE in the FO period had higher concentrations of total lipids and triglyceride and greater ratio of triglyceride to glycogen in liver than did CE.

There was no statistical difference for milk production in the first 4 wk postpartum between cows fed CE or HE prepartum. Cows fed HE during CU had greater milk fat concentration than cows fed CE on wk 2, 3, and 4. Cows fed HE during CU had higher protein concentration during wk 3 and 4 then cows fed CE. Cows that were fed HE during CU lost more body weight (**BW**) either as absolute value (kg) or as percentage loss during the first 6 wk postpartum (38.5 vs 19.7 kg, SEM 8.9, $P = 0.01$ and 5.6 vs 2.9 %, SEM 1.2, respectively). In addition, cows that were fed HE during the dry period had more odds of experiencing displaced abomasum or ketosis when compared to cows that received CE.

Lastly, principal component (**PC**) analysis was conducted on 8 variables: glucose wk 3 (**GLU3**), glucose wk 4 (**GLU4**), β-hydroxybutyrate wk 1 (**BHBA1**), insulin wk 2 (**INS2**), nonesterified fatty acids wk −1 (**NEFA-1**), energy-corrected milk wk 4 (**ECM4**), fat corrected milk wk 4 (**FCM4**), and milk urea nitrogen wk 5 (**MUN5**). For linear regression analysis from PC, animals were classified in two groups based on first and fourth quartile values for DTC as high (slow; ≥ 174 d) or low (fast; < 87 d). Principal component scores were generated for each extracted PC. Four PC were extracted from the analysis, accounting for 79.63% of total variability. The PC loadings indicated that, for PC1, increased ECM4 and FCM4 were associated with decreased INS2, GLU3, and GLU4. Principal component 2 represented animals with higher NEFA-1 and BHBA1. Principal component C3 had higher values for ECM4, FCM4, GLU3, and GLU4; whereas, PC4 had higher values for MUN5. Regressing PCS of PC2 on PC1 indicated

that the relation between these PC differed between diets. For increased values of PC1, HE cows had increased values of PC2; whereas, those fed CE showed decreased values of PC2. Inclusion of PC in a logistic model revealed that cows with high values of PC2 were associated with greater odds of being classified as slow (greater DTC) when compared to cows classified as fast (smaller DTC) [odds ratio (**OR**) = 2.257, 95%CI = 0.979 to 7.763]. Overall, prepartum nutrition was shown to have great impact on metabolic, production, and reproductive parameters of dairy cattle.

Key Words: periparturient cow, energy intake, days to conception, reproductive performance, principal component

## ACKNOWLEDGMENTS

"Give me six hours to chop down a tree and I will spend the first four sharpening the axe." Reading what Abraham Lincoln once said makes me think how grateful I am to be given the opportunity to sharpen my axe (i.e. perform my PhD program). Throughout this "blacksmith" path I was fortunate to meet many important people that I am thankful for.

I thank Dr. Drackley for giving me the opportunity in his worldwide-recognized program. Not only for selecting me without any personal contact before but also to give me the freedom to pursue answers to my questions. To ensure one to do his job was never his aim; instead, he always gave space and freedom to seek questions and answers on one's own pace. Recognition can be obtained in many different ways. Slaves would recognize their leaders by necessity and pain. They follow their owners with no option. However, when individuals are stimulated to find freedom, by being stimulated in their own thinking, they then recognize a real leader and instead of just obeying him they follow him. I have never felt as a slave during my PhD studies; thank you Dr. Drackley.

The members of my committee were extremely important. I thank Dr. Wallace for always being an "anchor" and a reference here at the University of Illinois since my first visit in 2000. I will always appreciate his support and the opportunity to be around them: "the cows". I thank Dr. Hutjens not only for his worldwide recognition that made it possible to contact him and get in touch with Dr. Drackley but also for the inestimable experience I have acquired while helping him with lab in ANSC 100 and priceless conversations about the dairy industry. I thank Dr. Murphy for his contribution in all the chapters of this thesis and for "inducing me" to "think outside the box" with the Lunar Society meetings. I thank Dr. Nakamura for providing insight and opinions toward my work and always being so accessible.

Additionally, I thank Dr. Stephen LeBlanc from the University of Guelph for his contribution on all the chapters of this thesis. While in Guelph I had the opportunity to meet great graduate students that made my work there even more enjoyable; thank you Rafael Neves, Julie Wittrock, Jessica Gordon, Nuria Chapinal, Laura Pieper and Les Gonzalez.

I would like to thank the staff of the Dairy Cattle Research Unit that made the impossible possible sometimes. Thank you Travis, Linda, Josh, Jim, and Patty. Even with the burden of long hours of work every day they were willing to help me during my projects. I thank HiDee Ekstrom and Evonne Hausman for their constant help and support. I can really think of them as my angels on earth.

I thank my fellow graduate students here in Champaign-Urbana not only for helping me with the cargo of hard work but also for having lots of fun while doing it. Thank you my friends Arnulfo Pineda, Johan Osorio, Juan Castro, Luan Shaoyu, Dr. Peng Ji, Jackie Ploetz, Kate Cowles, Kaetlin Vazquez, Joel Vazquez, Dr. Bruce Richards, Dr. Guido Invernizzi, Dr. Daniel Graugnard, Sonia Moisa, Jawad Khan, Crystal Allen, Sergio Machado, Nicola and Mariana Serão, Jacob Segers, Lindsay Shoup, Ferdinando and Juliana Almeida.

Last but not least I thank my family for always trusting in me and allowing me to pursue my dreams. Thank you Mãe, Pai, Gui, Kim, Leo and Mari. Thank you for always bringing out the best in me. Thank you for always being there. Thank you for always being here. I feel very blessed to be part of this family. I cannot imagine being anywhere else besides always on your side. Carved in me will always be: "**O importante é ter alegria no coração**".

## TABLE OF CONTENTS

# INTRODUCTION

During the transition period from late gestation through early lactation, the dairy cow undergoes tremendous metabolic adaptations (Bell, 1995). The endocrine changes during the transition period are necessary to prepare the dairy cow for parturition and lactogenesis. As peak milk yield increases, the transition period for dairy cows becomes much more challenging with most infectious diseases and metabolic disorders occurring during this time (Drackley, 1999; Grummer, 1995). Decreased dry matter intake (**DMI**) during late gestation influences metabolism leading to fat mobilization from adipose tissue and glycogen from liver.

Nutrient demand for milk synthesis is increased in early lactation; if no compensatory intake of nutrients is achieved to cope with the requirement, reproductive functions (i.e., synthesis and secretion of hormones, follicle ovulation, and embryo development) may be depressed. Milk production increases faster than energy intake in the first 4 to 6 wk after calving, and thus high yielding cows will experience negative energy balance (**NEB**).

Therefore, strategies that stimulate DMI around parturition are of grant advantage for the dairy cow. Based on previous reports (Kunz et al., 1985) and on field observations from experienced professionals such as Dr. Gordie Jones, Drackley's group at the University of Illinois was motivated to better understand the possible effects of controlled energy feeding during the transition period.

Controlling energy with high fiber rations seems to improve DMI after parturition, thereby avoiding excessive adipose tissue lipid mobilization. One can hypothesize that the benefits of the controlled energy diet prepartum could have a positive effect on cows' fertility. In order to best evaluate pregnancy risk to assess fertility of dairy cows, statistical inferences should be based on survival analysis.

Therefore, the objectives of the research conducted for my dissertation were as follows (Figure 1):

1. Examine the associations between feeding controlled energy (**CE**) or high energy (**HE**) diets during the dry period and reproductive success, measured as days to conception (DTC) in dairy cows (Chapter 2).

2. The effects of prepartum dietary energy regimen on BCS, $NE_L$ intake ($NE_{LI}$), diseases, blood concentrations of glucose, insulin, and NEFA in blood, and liver concentrations of total lipids, triglyceride triglyceride, and glycogen (Chapter 3).

3. To use statistically significant variables associated with reproductive performance from a univariate analysis in one model to reveal novel relationships and to better understand risk factors for pregnancy success in dairy cows (Chapter 4).

# CHAPTER 1

# LITERATURE REVIEW

### *Transition Period*

The transition period is usually identified as the 3 wk prior to and the 3 weeks following parturition. As peak milk yield increases, the transition period for dairy cows becomes much more challenging with most infectious diseases and metabolic disorders occurring during this time (Drackley, 1999; Grummer, 1995). During the transition period from late gestation through early lactation, the dairy cow undergoes tremendous metabolic adaptations (Bell, 1995).

### *Physiology and Metabolism*

The endocrine changes during the transition period are necessary to prepare the dairy cow for parturition and lactogenesis. Plasma growth hormone increases and insulin decreases as cows advance from late gestation to early lactation, with acute surges in plasma concentrations of both hormones at parturition (Kunz et al., 1985). Progesterone concentrations during the dry period are elevated to maintain pregnancy but drops quickly, approximately 2 days before parturition (Chew et al., 1979). Estrogen, primarily estrone of placental origin, increases in plasma during late gestation but decreases at calving (Chew et al., 1979). High estrogen in the blood of cows is believed to be an important factor leading to decreased dry matter intake (**DMI**) around calving (Grummer, 1993). Prolactin and glucocorticoid concentrations increase on the day of calving and return to concentrations similar to prepartum the next day (Edgerton and Hafs, 1973).

Decreased DMI during late gestation influences metabolism leading to fat mobilization from adipose tissue and glycogen mobilization from liver. Plasma nonesterified fatty acids

(**NEFA**) increase two-fold or more between 2 to 3 weeks prepartum and 2 to 3 days prepartum, when concentration increases dramatically until completion of parturition (Bertics et al., 1992; Vazquez-Anon et al., 1994; Grum et al., 1996). The concentration of NEFA in blood reflects the degree of adipose tissue mobilization (Pullen et al., 1989). The NEFA concentration in blood increases as negative energy balance (**NEB**; i.e., energy demand for maintenance and lactation exceeds that of dietary energy intake – Bauman and Currie, 1980) increases.

During the transition period, adipose tissue in the cow is oriented toward mobilization of NEFA, rather than lipid deposition (McNamara, 1991). Underwood (2003) observed increased blood NEFA concentrations in response to an intravenous epinephrine challenge. Blood NEFA was greatly increased at 7 d after calving relative to 10 d before calving, and was greater at 7 d than at 14 d postpartum. Therefore, stressors causing low voluntary DMI may result in large increase in NEFA immediately after calving.

Increased blood concentrations of NEFA around parturition are associated with greater NEFA uptake by the liver (Emery et al., 1992). The NEFA that reach the liver are extracted in a concentration-dependent manner and converted to acyl-CoA by the enzyme acyl-CoA synthetase. Carnitine plays an important role in the transport of acyl-CoA into the mitochondrial matrix, where β-oxidation occurs (Zammit, 1984). In the mitochondria, acyl-CoA is oxidized through β-oxidation into acetyl-CoA, which can be further oxidized to $CO_2$ and $H_2O$ for energy in the citric acid (**TCA**) cycle. However, if the amounts of acetyl-CoA challenge the processing capacity of the TCA cycle or if its activity is low due to low amounts of intermediates such as oxaloacetate, acetyl-CoA is then used for biosynthesis of ketone bodies (Drackley, 1999). Acetyl-CoA is converted into the ketone bodies acetoacetate (ACAC), β-hydroxybutyrate (**BHBA**), and acetone, which are released from the liver into the blood (Bell, 1979). Ketones can be used as an alternate water-soluble fuel source by many tissues (e.g., heart and skeletal muscle) when glucose is limited (Leslie et al., 2000). However, if the rate of lipid mobilization exceeds the rate of ketone body utilization, then ketones accumulate and may adversely affect the health and productivity of the cow (Ingvartsen and Andersen, 2000).

Some degree of liver triglyceride (**TG**) accumulation in ruminants is expected due to its low hepatic capacity for synthesis and secretion of very-low density lipoproteins (VLDL) to export TG from the liver (Kleppe et al., 1988; Pullen et al., 1989) but similar capacity to reconvert NEFA back to TG (Kleppe et al., 1988; Graulet et al., 1998) relative to many species. Deposition of TG in liver would be favored since ad libitum feeding of high-energy diets during the dry period can increase esterification capacity and decrease oxidation capacity in liver at 1 d after calving (Litherland et al., 2011).

Normal function of the liver is affected negatively as the degree of fatty infiltration increases. An increased degree of fat infiltration decreases the ability of the liver to convert ammonia to urea (Strang et al., 1998). The capacity of the liver to convert propionate to glucose is impaired (Overton et al., 1999), therefore linking fat accumulation to impaired gluconeogenesis in liver (Drackley et al., 2001). Fat accumulation in the liver by itself does not lead to liver malfunction (Drackley, 1999) but may be a contributing factor.

At the onset of lactation there is a marked increase in mammary glucose utilization, primarily for milk lactose synthesis; total glucose turnover in a high producing cow can exceed 3 kg/d with up to 85% being used by the mammary gland (Bauman and Elliot, 1983). Plasma glucose concentrations remain stable just before calving, increase dramatically at calving, and then decrease immediately after calving (Kunz et al., 1985; Vazquez-Anon et al., 1994). The capacity of liver tissue to convert alanine, an important glucogenic amino acid, into glucose was

198% of prepartum (21 d before parturition) values on d 1 after calving; whereas, capacity for conversion of propionate to glucose was increased by only 119 % (Overton et al., 1998).

Blood calcium decreases during the last few days prior to calving due to loss of calcium for the formation of colostrum (Goff and Horst, 1997). Therefore, blood calcium can drop abruptly leading to a metabolic disorder called hypocalcemia (blood calcium concentration < 5 mg/dL; Radostitis et al., 2007). Until the ability of the digestive tract to absorb calcium can increase, calcium must be obtained from the bones. Plasma calcium concentrations are controlled by the coordinated actions of parathyroid hormone and 1, 25 – dihydroxyvitamin $D_3$. These hormones act on the intestine, kidney, and bone to increase blood calcium during the transition period (NRC, 2001). Horst et al. (1997) observed that negative dietary cation-anion difference (**DCAD**) causes metabolic acidosis that favors mobilization of calcium from bone; whereas, high dietary potassium concentrations and positive DCAD suppress this process.

The immunologic status of the cow is compromised during the transition period. Lymphocyte and neutrophil function is depressed (Goff and Horst, 1997). The same authors observed that the immunosuppressive agents estrogen and glucocorticoids are increased in plasma around parturition. In addition, because DMI is compromised around parturition, lower intake of immune-enhancing components such as vitamin A and E as well as some trace minerals (selenium, copper, zinc) may contribute to the immunosuppression (NRC, 2001).

***Metabolic Disorders***

As early lactation is a critical time to establish high milk production and good reproductive performance, the avoidance of metabolic anomalies is important (Drackley, 2006). Failure to provide a sound transition program can affect both production and health of dairy cows (Drackley et al., 2005). Metabolic disorders such as ketosis, milk fever, and displacement of the abomasum are also more prevalent with an inadequate transition program. These disorders are costly in terms of lost milk production, veterinarian services, drugs, and discarded milk.

Estimates in North America place the economic loss per cow and per case at $334 for milk fever, $285 for retained placentas, $280 for metritis, $340 for left displacement abomasum, and $145 for ketosis (Kelton et al., 1998). One report documented that the incidences of these diseases in the U.S. were 6.5%, 8.6%, 10.1%, 1.7%, and 4.8%, respectively (Kelton et al., 1998). In addition, problems during the transition period can result in the loss of 5 to 10 kg of peak milk, which is equivalent to an economic loss of from $300 to $600 per lactation (Wallace et al., 1996). With a population of 9 million lactating dairy cows in the U.S., the overall negative economic impact is nearly $697M yearly.

Fatty liver and ketosis are two common metabolic disorders during early lactation (Grummer, 1993). Triglyceride accumulates in the liver when its synthesis exceeds disappearance via hydrolysis and lipoprotein export (Drackley, 2003). Fat infiltrates the liver prior to or soon after parturition (Vazquez-Anon et al., 1994) and may precede ketosis (Veenhuizen et al., 1991).

In order to reduce the incidence of metabolic disorders during early lactation, research has generally focused on dividing the dry period into the early, or “far-off,” period (generally the first 4 to 6 weeks) and the “close-up” period (generally the last 3 weeks before expected parturition). Numerous studies have been conducted that provide some insight into how to feed dry cows, particularly during the close-up period, with the aim to prevent metabolic disorders (Dann et al., 2006; Drackley et al., 2007).

***Nutrition***

Common dietary recommendations for close-up cows following publication of the NRC (2001) include feeding diets containing relatively high (36 to 44%) concentrations of nonfiber carbohydrate (**NFC**) to promote DMI during the peripartum period in an attempt to increase dietary energy intake and thus decrease the cow's reliance on NEFA. However, compared with diets lower in NFC content, excessive concentrations of starch-based NFC (>40%) in the prepartum diet may result in a greater decrease in DMI immediately before calving (Rabelo et al., 2005) and could potentially be detrimental to postpartum health and performance.

In a review, Grummer (1995) suggested that prepartum DMI was positively correlated with postpartum DMI and that prepartum DMI should be maximized to improve postpartum performance and health. This author also suggested that increasing the nutrient density of the diet could increase DMI and thus nutrient intake.

Cows that were overfed during the entire dry period had higher serum insulin concentration and lower basal lipolytic rates in adipose tissue during the last week prepartum than cows that were feed restricted (Rukkwamsuk et al., 1998). Conversely, VandeHaar et al. (1999) increased dietary protein and energy density during the prepartum period and observed a reduction in liver TG concentration (2.4 vs. 1.5%, wet basis) on day 1.

Dann et al. (2006) concluded that during the first 10 days in milk (**DIM**), far-off treatments had significant carryover effects on DMI, energy balance, serum NEFA concentration, and serum BHBA concentration. Cows with the lower energy balance during the far-off period had higher DMI and energy balance and lower serum NEFA and BHBA during the first 10 DIM. There were no effects of close-up diet and no interactions of far-off and close-up treatments.

Drackley (1999) suggested that dietary fat or elevated NEFA from decreased nutrient intake in the studies of Grum et al. (1996) and Douglas et al. (1998) may have increased expression and action of peroxisome proliferator-activated receptor, leading to increased hepatic oxidation and decreased esterification of FA observed in liver tissue (Figure 2). Both starvation and increased dietary fat intake lead to increases of acyl-CoA concentrations in rat liver (Ney et al., 1989). Increases in NEFA during the last few days before calving may contribute to decreased DMI due to hepatic oxidation of NEFA and neural signaling from the liver via the vagus nerve (Allen et al., 2005).

Hayirli et al. (1998) observed that over-conditioned cows experienced a gradual decline in DMI during the transition period; whereas, thin cows maintained DMI longer prior to experiencing a more abrupt decrease in DMI shortly before parturition.

***Controlled Energy***

Feeding diets with 1.30 Mcal $NE_L$/kg of DM accommodates energy requirements for maintenance, pregnancy, and mammary growth in mature cows during the dry period (NRC, 2001). Usually at dry-off, cows are fed high forage diets containing high fiber compared to the lactation diet. The diet change affects bacterial population, the absorptive capacity and size of the rumen papillae, and therefore the capacity for absorption of volatile fatty acids in the rumen (Goff and Horst, 1997). During the close-up period (usually 3 wk before parturition) rations of higher energy and nutrient density are fed (“steam-up” diets) with the objective to adapt the rumen microbial population and papillae to the high-energy content of the lactation diet just after calving (Grummer, 1995). However, there is evidence that cows can over consume energy relative to their energy requirement independent of diet adjustments (Dann et al., 2006, Janovick and Drackley, 2010). Even though steam-up diets are frequently used in dairy farms, research had not demonstrated that they improved milk production, body condition, or the immune status of the cow during the transition period.

Based on previous reports (Kunz et al., 1985) and on field observations from experienced professionals such as Dr. Gordie Jones, Drackley's group at the University of Illinois was motivated to better understand the possible effects of controlled energy feeding during the transition period. The strategy developed was to formulate and feed rations with relatively low energy density (1.30 to 1.39 Mcal $NE_L$/kg of DM) during the entire dry period. The incorporation of low energy ingredients (straw or low quality grass hays) allows cows to consume *ad libitum* without exceeding their daily energy requirements (Janovick and Drackley, 2010). Controlling energy with high fiber rations seems to improve DMI after parturition, avoiding excessive adipose tissue mobilization (Douglas et al., 2006). Milk production appears to be similar when compared to higher energy close-up programs (Douglas et al., 2006, Janovick and Drackley, 2010).

Benefits of feeding controlled energy (**CE**) diets prepartum to dairy cows have been reported (Dann et al., 2006; Douglas et al., 2006; Janovick and Drackley, 2010). Recently, Janovick et al. (2011) suggested that cows fed CE during the dry period had fewer diseases and disorders than cows fed high-energy (**HE**) diets. Also, Beever (2006) stated that farmers have repeatedly observed easier calving and greater DMI around parturition when energy intake is controlled prepartum. Excess energy consumption prepartum also seems to result in a larger decline in DMI prepartum compared with cows having controlled intake prepartum (Janovick et al., 2011). Such steep changes in DMI prepartum have been associated with increased deposition of lipid in liver postpartum (Drackley et al., 2005).

From a practical standpoint, the CE approach may simplify dry cow management by avoiding social stress due to group changes (Cook and Nordlund, 2009) and allowing a single group feeding instead of the two-group approach (Dann et al., 2006).

### *Reproduction and Nutrition*

Nutrient demands for milk synthesis are increased in early lactation, and if no compensatory intake of nutrients is achieved to cope with requirements reproductive functions (i.e., synthesis and secretion of hormones, follicle ovulation, and embryo development) may be depressed. The incidence of diseases and disorders can be high during this time period and have a negative impact on reproductive performance. The risk of pregnancy was reduced if cows had retained placenta (**RP;** i.e., presence of fetal membranes 12 hours or more following parturition – Radostitis et al., 2007) or lost one body condition score (**BCS**) unit (Goshen and Shpigel, 2006; Santos et al., 2008).

Milk production increases faster than energy intake in the first 4 to 6 wk after calving. High yielding cows will experience NEB where blood concentrations of NEFA increase, while concentrations of insulin-like growth factor-I (**IGF-I**), glucose, and insulin are low. If extreme, these changes in blood metabolites and hormones may compromise ovarian function and fertility. In addition, energy balance and DMI can decrease plasma concentrations of progesterone (Vasconcelos et al., 2003; Villa-Godoy et al., 1988), possibly interfering with follicle development and pregnancy maintenance.

Improved management of herds and genetic selection during the last decades have increased milk production of dairy cows, at the same time that fertility has decreased (Butler, 2003). Selection for increased milk yield in the dairy cow has changed endocrine profiles of those animals, including increased blood concentrations of bovine somatotropin and prolactin and decreased insulin (Bonczeck et al., 1988). The difference in the metabolite and hormonal profile together with increased nutrient demands for milk production might have a negative impact on reproduction of the dairy cow. Nevertheless, good management and adequate nutrition have been shown to alleviate the depression of fertility on herds with average milk production exceeding 12,000 kg/cow annually (Nebel and MacGilliard, 1993; Jordan and Fourdraine, 1993).

Different nutritional strategies have been proposed to improve reproduction of the dairy cow with no detrimental effect on lactation performance. Feeding high quality forages, increasing the concentrate:forage ratio, or adding supplemental fat to diets are some of the most common ways to improve energy intake in cows. Reproduction of dairy cattle may be benefited by maximizing DMI during the transition period, minimizing the incidence of periparturient problems, and promoting increased concentrations of insulin in early lactation.

***Uterine Health and Fertility***

A large number of epidemiological studies have demonstrated strong relationships between diseases postpartum and subsequent reproductive performance in dairy cattle. Cows identified with clinical hypocalcemia were 3.2 times more likely to experience RP than cows that did not have clinical hypocalcemia (Curtis et al., 1983). Whiteford and Sheldon (2005) also found an association between hypocalcemia and occurrence of uterine disease in lactating dairy cows. Markusfeld (1985) suggested that 80% of cows with ketonuria developed metritis (i.e., cows with abnormally enlarged uterus and a purulent uterine discharge detectable in the vagina, within 21 d postpartum – Sheldon et al., 2006).

Usually, cows with RP have increased risk of developing metritis compared with cows without RP. Both metritis and RP double the risk of cows remaining with uterine inflammation at the time of first postpartum insemination (Rutigliano et al., 2008). A USDA study (NAHMS, 1996) showed that the incidence of RP in dairy cows was 7.8 ± 0.2 %. Goshen and Shpiegel (2006) in a study on 5 dairy farms in Israel observed that RP was diagnosed in 13.1 % (9.4 to 18.1 %) and 9.2 % (3.6 to 13.8 %) of multiparous and primiparous cows, respectively. In the same study, metritis affected 18.6 % (15.2 to 23.5 %) and 30 % (19.4 to 42.3 %) of the multiparous and primiparous cows, respectively. Both RP and metritis have a negative impact on reproductive success in lactating dairy cows, with reduced conception rates and extended intervals to pregnancy (Goshen and Shpiegel, 2006). Furthermore, not only does the clinical disease negatively affect fertility, but subclinical endometritis, a disease characterized by increased proportion of neutrophils in uterine cytology without the presence of clinical signs of inflammation of the uterus, has deleterious effects on conception rates of lactating dairy cows at first postpartum insemination (Sheldon et al., 2006).

The neutrophils are known as polymorphonuclear (**PMN**) leukocytes for their multi-lobed nucleus and granulated cytoplasm; these cells constitute up to 70% of the circulating white blood cells (Goldsby et al., 2000). During an infection, PMN migrate from blood to the tissue in response to chemo-attractants secreted from either macrophages or the epithelial cells within the infected tissue (Goldsby et al., 2000).

Feed intake and feeding behavior around parturition might mediate some of the increased risk for uterine diseases in dairy cattle (Hammon et al., 2006; Huzzey et al., 2007; Urton et al., 2005). Hammon et al. (2006) observed that cows developing uterine disease postpartum experienced reduced DMI beginning 1 wk before parturition. In concordance, cows diagnosed with severe metritis after calving were already consuming less DMI 2 wk prior to calving (Huzzey et al., 2007). In the same study, cows that subsequently developed mild metritis had reduced DMI 1 wk before calving compared with cows with healthy uterus. Urton et al. (2005) observed that cows subsequently developing metritis spent significantly less time eating before and after calving than cows that did not develop metritis. Therefore, diminished intake of nutrients or alterations in feeding behavior prior to calving may be major risk factors for development of metritis postpartum.

The immune status of the cow may be the link between nutrient intake and development of uterine diseases. Kimura et al. (2002) evaluated neutrophil function in periparturient dairy cows (n = 142) from 2 herds by evaluating chemotaxic and killing activity of those cells. Researchers observed that 14.1 % of the cows developed RP. Neutrophils isolated from blood of cows with RP had decreased ability to migrate to placental tissue and reduced myeloperoxidase activity, a marker for oxidative burst and killing activity of neutrophils. The reduced neutrophil function was observed between 1 and 2 wk before parturition, which suggests that the innate immune function may be part of the cause of RP rather than a consequence of the disease. These data strongly suggest that inadequate nutrient intake before calving might predispose cows to impaired immune function, and consequently, increased risk for uterine diseases that negatively affect reproduction.

Since intake of nutrients influences energy status and immune function of dairy cows, both being related to the risk of uterine diseases, one could suggest that nutritional and management strategies that optimize nutrient intake around parturition should improve uterine health and subsequent fertility of dairy cows.

***Postpartum Cyclicity***

The NEB experienced by dairy cows antagonizes the resumption of ovulatory cycles. During early postpartum, reproduction is deferred in favor of individual survival. In the modern dairy cow, lactation becomes the priority, in detriment to reproductive functions.

During periods of energy restriction, oxidizable fuels consumed in the diet are prioritized toward essential processes such as cell maintenance, circulation, and neural activity (Wade and Jones, 2004). Homeorhetic controls in early lactation assure that body tissue, primarily adipose stores, will be mobilized in support of milk production. That is why the modern cow will sustain high yields of milk and milk components at the expense of body tissues. Delayed ovulation has been linked repeatedly with energy status (Butler, 2003). Energy shortage reduces the frequency of pulses of luteinizing hormone, therefore impairing follicle maturation and ovulation. In addition, undernutrition inhibits estrous behavior by reducing responsiveness of the central nervous system to estradiol by reducing the estrogen receptor-α content in the brain (Hileman et al., 1999).

Usually, the first ovulation after calving in dairy cattle occurs 10 to 14 d after the nadir of NEB (Butler, 2003). Drastic weight and BCS losses caused by inadequate feeding or disease are associated with anovulation and anestrus in dairy cattle. Cows with low BCS at 65 d postpartum are more likely to be anovular (Santos et al., 2008), which compromises reproductive success at first postpartum insemination. Extended postpartum anovulation or anestrus extends the period from calving to first artificial insemination (AI) and reduces fertility during the first postpartum service (Santos et al., 2008). Anovular cows not only have reduced estrus detection and conception rates, but also have compromised embryo survival (Santos et al., 2004a). The timing of the first postpartum ovulation determines and limits the number of estrous cycles occurring before the beginning of the insemination period. In most dairy herds, fewer than 20 % of cows should be anovulatory by 60 d after calving (Santos et al., 2008). Estrus expression, conception rate, and embryo survival improved when cows were cycling prior to an estrous synchronization program for first postpartum insemination (Santos et al., 2004 a,b).

Feeding management that minimizes loss of BCS during the early postpartum period and incidence of metabolic diseases during early lactation should increase the number of cows experiencing first ovulation during the first 4 to 6 wk after calving.

***Protein***

Diets with limited crude protein content can compromise microbial growth and rumen fermentation, resulting in a decline in milk production and feed intake. In contrast, feeding protein in excess of what is needed by the cows is associated with increased ammonia and urea concentrations in blood and milk, which have been used as markers for reduced fertility (Butler, 1998). The author suggested that the decline in fertility of cattle fed excess protein is caused by alterations in uterine physiology with a decline in uterine pH during the early luteal phase of the estrous cycle (Butler, 1998). The more acidic uterine environment is less conductive to maintenance of pregnancy in cattle (Ocon and Hansen, 2003). This effect is suggested to be restricted to the early stages of embryo development (Rhoads et al., 2006). Cows are more efficient in utilizing protein sources when diets are moderate in crude protein and are balanced for the predicted supplies of metabolizable protein and limiting amino acids (Noftsger and St. Pierre et al., 2003). It is not economically justified to feed diets with protein concentrations that will increase urea N and harm fertility.

***Energy***

The early-lactation NEB and a subsequent failure to replenish body energy stores have been associated with reduced reproductive performance (Butler and Smith, 1989; Buckley et al., 2003; Roche et al., 2007). Villa-Godoy et al. (1998) reported that variation in energy balance in postpartum Holstein cows was influenced most strongly by DMI (r = 0.73) and less by milk yield (r = -0.25). Therefore, differences in NEB between cows are more related to how much energy they consume than with how much milk they produce. Plasma progesterone concentrations are affected by the energy balance of dairy cows. Glucose, insulin, and IGF-I, which are low during periods of NEB, together with progesterone have been shown to affect folliculogenesis, ovulation, and steroid production *in vitro* and *in vivo* (Thatcher et al., 2011). Even though the exact mechanism by which energy affects secretion of releasing hormones is not well defined, it is clear that lower levels of blood glucose, IGF-I, and insulin may mediate this process.

Studies have demonstrated the importance of insulin as a signal mediating the effects of acute changes in nutrient intake on reproductive parameters in dairy cattle. In early postpartum dairy cows under NEB, reduced expression of hepatic growth hormone receptor 1A (GHR-1A) is thought to be responsible for the lower concentrations of IGF-I in plasma of cows (Radcliff et al., 2003). Since IGF-I is an important hormonal signal that influences reproductive events such as stimulation of cell mitogenesis, hormonal production, and embryo development, among other functions, increasing concentrations of IGF-I early postpartum are important for early resumption of cyclicity and establishment of pregnancy.

Insulin mediates the expression of GHR-1A in dairy cows (Butler et al., 2003; Rhoads et al., 2004), resulting in increased concentrations of IGF-I in plasma. IGF-I and insulin are important for reproduction in cattle; for instance feeding diets that promote greater insulin concentrations should benefit fertility. Gong et al. (2002) fed cows of low- and high-genetic merit isocaloric diets, which differed in the capacity to induce high or low insulin concentrations in plasma. The diets that induced high insulin reduced the interval to first postpartum ovulation and increased the proportion of cows ovulating in the first 50 d postpartum.

Due to ethical considerations and law in most countries, and where it is permitted research on human embryos is restricted; only surplus (often poor quality) in vitro-produced embryos can be used. Unfortunately, in vitro culture is known to affect the morphological and biochemical characteristics of mammalian embryos in a significant manner (Thompson et al., 1998). Kues et al. (2008) concluded that the bovine embryo is an alternative to the mouse for the analysis of mammalian pre-implantation development and it is important for understanding the causes of aberrations in embryonic and fetal development in humans.

Jousan et al. (2008) found that one of the best characterized regulatory molecules for the preimplantation bovine embryo is IGF-I, which in the cow is secreted by the oviduct (Pushpakumara et al., 2002), endometrium (Geisert et al., 1991), and embryo (Lonergan et al., 2000). One of the functions of IGF-I is to act as a cytoprotective molecule for the embryo. Thus, IGF-I reduced effects of hydrogen peroxide on the development of mouse embryos (Kurzawa et al., 2002), blocked apoptosis in rabbit embryos exposed to UV radiation (Herrler et al., 1998) and blocked apoptosis in mouse embryos treated with tumor necrosis factor-$\alpha$, campothecin, and actinomycin D (Kurzawa et al., 2001; Byrne et al., 2002; Fabian et al., 2004). In the preimplantation bovine embryo, continuous culture with IGF-I beginning after fertilization blocked the detrimental effects of heat shock at day 5 after fertilization on induction of apoptosis and inhibition of development (Hansen and Jousan, 2007; Hansen et al., 2004). The cytoprotective actions of IGF-I may be beneficial to the bovine embryo during early development (Jousan et al., 2008).

The addition of IGF-I to cultured embryos alters expression of several transcripts, which may be important for embryo development and survival following transfer (inovulation) (Block et al., 2008). Addition of IGF-I increased amounts of mRNA for IGF binding protein-3 and desmocollin II. Moreover, IGF-I treatment decreased steady state amounts of transcripts for heat shock protein 70 and tended to reduce amounts of IGF-I receptor mRNA. Increased survival of embryos treated with IGF-I does not appear due to effects on cell number, percent apoptosis, or cell allocation (Block et al., 2008).

Kerestes et al. (2009) showed that severe inflammatory diseases like puerperal metritis with intensive release of pro-inflammatory cytokines potentially depress insulin secretion of pancreatic β-cells and whole-body insulin responsiveness in dairy cows, with long-term effects on metabolism and reproduction. Also, pancreatic β-cell function and the biological potency of insulin is impaired in cows with long-term hyperketonemia.

Kendrick et al. (1999) randomly assigned 20 dairy cows to 1 of 2 treatments formulated so that cows consumed either 3.6 % (high energy) or 3.2 (low energy) of their body weight. Follicles were transvaginally aspirated twice weekly and oocytes were graded based on cumulus density and ooplasm homogeneity. Cows in better energy balance (high energy) had greater intrafollicular IGF-I and plasma progesterone levels and tended to produce more oocytes graded as good. Thus, NEB not only delays ovulatory cycles after calving, but might influence the quality of oocytes once cows are inseminated.

***Fat***

Risk factors for pregnancy have been improved by feeding fat to dairy cattle, but responses have not been consistent (Santos et al., 2009). In one study fat feeding improved production and increased body weight loss whereas primiparous cows experienced reduced pregnancy success at first AI (Sklan et al., 1994). In contrast, Ferguson et al. (1990) observed a 2.2 fold increased risk of pregnancy at first AI and all AI in lactating cows fed 0.5 kg/d of fat, which tended to enhance the proportion of pregnant cows at the end of the study (93 vs 86.2 %).

Feeding calcium salts of long chain fatty acids (Ca-LCFA) of palm oil increased pregnancy of dairy cows (Schneider et al. 1988), although the authors did not report statistical significance. Others did not observe improved fertility of dairy cows supplemented with Ca-LCFA (Scott et al., 1995; Sklan et al., 1991) or oilseeds (Schingoethe and Casper, 1991), which might be attributed to increased milk yield and body weight losses (Sklan et al., 1991; Sklan et al., 1994).

The beneficial effects of feeding fat may originate from specific fatty acids (**FA**) (Staples et al., 1998; Staples and Thatcher, 2005). Researchers have evaluated whether feeding FA differing in the degree of saturation might influence fertility of cows. Essential FA of the n-6 and n-3 families are available in smaller supply to ruminants than nonruminants due to microbial biohydrogenation of FA in the rumen (Juchem et al., 2010), suggesting that their supplementation may increase fertility of the dairy cow (Staples and Thatcher, 2005; Santos et al., 2009).

In cows fed 0.75 kg of fat from flaxseed, a source rich in C18:3 n-3, or sunflower seed, a source rich in C18:2 n-6, conception rate tended to be greater for cows fed n-3 FA (Ambrose et al., 2006). However, similar responses were not observed by others when cows where fed flaxseed as the source of n-3 FA (Fuentes et al., 2008; Petit and Twagiramungu, 2006). In accordance, feeding n-3 FA from fish oil as Ca-LCFA did not improve fertility in high producing dairy cows when compared with a source rich in saturated FA (Juchem et al., 2010) or with Ca-LCFA of palm oil (Silvestre et al., 2008).

Feeding cows before and after calving with Ca-LCFA of either mostly saturated and monounsaturated FA or a blend of C18:2 n-6 and *trans*-octadecenoic FA impacted fertility of dairy cattle (Juchem et al., 2010). In that study cows fed unsaturated FA had 1.5 times greater risk of pregnancy at either 27 or 41 d after AI compared with cows fed mostly saturated FA. Increased pregnancy risk was found when cows were fed C18:2 n-6 and *trans*-octadecenoic FA due to improved fertilization and embryo quality in non-superovulated lactating dairy cows (Cerri et al., 2004).

One of the proposed mechanisms for the effects of n-3 FA on fertility of dairy cows is that it can suppress uterine secretion of PGF2 (Mattos et al., 2002, 2003, 2004), which is hypothesized to improve embryonic survival in cattle (Mattos et al., 2000). Silvestre et al. (2008) fed n-3 FA as fish oil rich in eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) and observed a reduction in pregnancy losses in lactating dairy cows after the first postpartum AI.

***Survival Analysis***

Survival analysis is a statistical methodology to model the probability of occurrence of an event at a specific time or hazard and the association of the explanatory variables with the hazard. The time-to-event response variable can include censored records. Censoring takes place when there is incomplete information of an event. In this manner, if the event is not observed within a specified time frame, it means that the event could have occurred before, after, or even during this period of time. These are respectively known as left, right, and interval censoring (Allison, 2010). Death will be used as an example of the event to facilitate the description of the model. For this example, right censoring is applicable because not all subjects studied will be dead by the end of the period considered. In this manner, the consideration of censored data is an important characteristic of survival analysis, requiring the application of special methods that account for this binary variable.

Survival data are generally described and modeled in terms of survival and hazard functions (Clark et al., 2003). Given that $T$ denotes the survival time, where $T \geq 0$, and $t$ is a specific value for $T$, the probability that a person survives longer than a specific time $t$, i.e., $P\ (T > t)$, is defined as the survival function $S(t)$ (Kleinbaum, 1996):

$$S(t) = P(T > t)$$

In contrast to $S(t)$, which represents the probability of non-occurrence of death at a particular time, the hazard function $\lambda(t)$ describes the event (death) rate given that the individual survived to a particular time and is expressed as the following (Kleinbaum, 1996):

$$\lambda(t) = \lim_{\Delta t \to \infty} \frac{P(t \leq T < t + \Delta t | T \geq t)}{\Delta t}$$

where $\Delta t$ is a small time interval. This equation gives the instant potential per unit of time for the event to occur. The hazard function is graphed with values ranging from 0 to ∞, but in contrast to the survival function, the plotted hazard function exhibits several profiles.

A semiparametric procedure uses the Cox proportional hazards model to describe the hazard (Cox and Oakes, 1984; Bradburn et al., 2003):

$$\lambda(t) = \lambda_0(t) exp\left[\sum_{i=1}^{p} X_i \beta_i\right]$$

where $\lambda(t)$ is the hazard function on time $t$; $\lambda_0(t)$ is the baseline hazard function on time; $exp$ is the exponential function; and $X_i$ is the vector of explanatory variables (e.g., sex, treatment, and others), with $i = 1, 2, \dots, p$ and $\beta_i$ is the vector of coefficients associated with $X_i$. In this manner, the Cox model accounts for two different portions. The first is the nonparametric baseline hazard function $\lambda_0(t)$, which is time-dependent, and the second is the exponential expression of the time-independent explanatory variables $e^{\sum_{i=1}^{p} X_i \beta_i}$. The relation $\lambda(t) = \lambda_0(t)$ occurs if $X_1 = X_1 = \dots = X_p = 0$ or if there are no explanatory variables (i.e., no $X's$) in the model. This property indicates that the Cox model is a semi-parametric method, although it yields results approximating parametric models such as the Weibull and exponential models (Kleinbaum, 1996).

In the Cox model, an unknown parameter is the hazard ratio $(HR)$ that compares the hazard function of two explanatory variable levels. The estimated hazard ratio $(HR)$ of the i$^{th}$ explanatory variable is:

$$\widehat{HR}_\iota = exp\left[\widehat{\beta}_\iota (X_i' - X_i)\right]$$

where $X_i'$ is the vector of the explanatory variable being tested against $X_\iota$ and $\widehat{\beta}_\iota$ is the vector of estimated coefficients associated with $(X_i' - X_i)$. The hazard ratio estimates describe the association between the explanatory variable (e.g., treatments; controlled energy against high energy) and the event hazard (days to conception, DTC; i.e., time in days from calving until the last breeding in which the cow became pregnant). The values of $\widehat{HR}$ range from 0 to infinity (∞) because this estimate is the result of the exponentiation of a number ranging from –∞ to +∞. Estimates below 1 indicate that a particular factor level (e.g., CE), decreases the hazard of pregnancy success (DTC), and thus increases the survival time when compared to the other level of the factor (e.g., HE). Conversely, estimates higher than 1 indicate that the hazard associated with one factor level increased when contrasted with another factor.

The Cox proportional hazard model assumes that the HR comparing any two explanatory variables levels is constant over time (Bradburn et al., 2003). The hazard functions for the explanatory factor levels or groups are proportional and are parallel or maintain the same distance (i.e., group effect) across the time points (Bradburn et al., 2003). The proportional assumptions are met when the survival curves for $X_i'$ and $X_i$are parallel across time. The assumption of proportional hazard must be verified in order to use estimated hazard ratios to explain how some factors act on survival.

### ***Conclusion***

As cows have become more specialized in producing milk, an increased likelihood for health disorders and reproductive failure has been observed. Studies have shown that nutritional management during the transition period can minimize metabolic distress in the postpartum period and subsequently improve uterine health.

Managing NEB by increasing energy intake during the early postpartum and breeding period may improve resumption of cyclicity and reproductive outcomes. Supplementation with unsaturated fat may improve fertility, as long as it is protected from rumen biohydrogenation of fatty acids. Nutritional management during the late lactation period must meet nutritional requirements for production and fetal development without allowing cows to become obese, which negatively impacts animal performance in the following lactation.

Controlling energy prepartum with high fiber rations seems to improve DMI after parturition, thereby avoiding excessive adipose tissue lipid mobilization. Milk production appears to be similar to the yield obtained with higher energy close-up programs. The utilization of low energy ingredients (straw or low quality grass hay) is pivotal in the formulation of dry period rations since it allows the cows to consume rations at *ad libitum* DMI without exceeding their daily energy requirements. One could hypothesize that the benefits of the controlled energy diet prepartum could have a positive effect on cow's fertility. In order to best evaluate pregnancy risk to access fertility of dairy cows, statistical inferences should be done based on survival analysis.

## REFERENCES


Allen, M. S., B. J. Bradford, and K. J. Harvatine. 2005. The cow as a model to study food intake regulation. Annu. Rev. Nutr. 25:523-547.

Allison, P. D. 2010. Survival Analysis using SAS®: A Practical Guide. Second Edition, Cary, North Carolina, USA.

Ambrose, D. K., J. P. Kastelic, R. Corbett, P. A. Pitney, H. V. Petit, J. A. Small, and P. Zalkovic. 2006. Lower pregnancy losses in lactating dairy cows fed a diet enriched in $\alpha$-linolenic acid. J. Dairy Sci. 89:3066-3074.

Bauman, D. E., and J. M. Elliot. 1983. Control of nutrient partitioning in lactating ruminants. Biochemestry of Lactation. T.B. Mepham, ed. Elsevier Science Publishers B.W., Amserdam, The Netherlands. Pages 437-468.

Bauman, D. E., and W. B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: A review of mechanisms involving homeostasis and homeorhesis. J. Dairy Sci. 63:1514-1529.

Beever, D. E. 2006. The impact of controlled nutrition during the dry period on dairy cow health, fertility and performance. Anim. Reprod. Sci. 96:212-226.

Bell, A. W. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. J. Anim. Sci. 73:2804-2819.

Bell, A. W. 1979. Lipid metabolism in liver and selected tissues and in the whole body of ruminant animals. Progr. Lipid Res. 18:117-164.

Bertics, S. J., R. R. Grummer, C. Cadorniga-Valino, and E. E. Stoddard. 1992. Effect of prepartum dry matter intake on liver triglyceride concentration and early lactation. J. Dairy Sci. 75:1914-1922.

Block, J., C. Wrenzycki, H. Niemann, D. Herrmann, and P.J. Hansen. 2008. Effects of insulin-like growth factor-1 on cellular and molecular characteristics of bovine blastocysts produced in vitro. Mol. Repro. Dev. 75:895-903.

Bonczeck, R. R., C. W. Young, J. E. Wheaton, and K. P. Miller. 1988. Responses of somatotropin, insulin, prolactin, and thyroxine to selection for milk yield in Holsteins. J. Dairy Sci. 71:2470-2478.

Bradburn, M. J., T. G. Clark, S. B. Love, and D. G. Altman. 2003. Survival analysis part II: Multivariate data analysis-an introduction to concepts and methods. Br. J. Cancer 89:431-436.

Buckley, F., K. O'Sullivan, J. F. Mee, R. D. Evans and P. Dillon. 2003. Relationships among milk yield, body condition, cow weight, and reproduction in spring-calved Holstein-Friesians. J. Dairy Sci. 86:2308-2319.

Butler, W. R. 2003. Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows. Livest. Prod. Sci. 83: 211-218.

Butler, S. T., A. L. Marr, S. H. Pelton, R. P. Radcliff, M. C. Butler, and W. R. Butler. 2003. Insulin restores GH responsiveness during lactation-induced negative energy balance in dairy cattle: Effects on expression of IGF-I and GH receptor 1A. J. Endocrinol. 176:205-217.

Butler, W. R. 1998. Review: Effect of protein nutrition on ovarian and uterine physiology in dairy cattle. J. Dairy Sci. 81:2533-2539.

Butler, W. R., and R. D. Smith. 1989. Interrelationships between energy balance and postpartum reproductive function in dairy cattle. J. Dairy Sci. 72:767-783.

Byrne, A. T., J. Southgate, D. R. Brison, and H. J. Leese. 2002. Effects of insulin like growth factors I and II on tumor-necrosis-factor-alpha-induced apoptosis in early murine embryos. Reprod. Fertil. Dev. 14:79-83.

Cerri, R. L. A., R. G. S. Bruno, R. C. Chebel, K. N. Galvão, H. Rutgliano, S. O. Juchem, W. W. Thatcher, D. Luchini, and J. E. P. Santos. 2004. Effect of fat sources differing in fatty acid profile on fertilization rate and embryo quality in lactating dairy cows. J. Dairy Sci. 87(Suppl. 1):297 (Abstr.).

Chew, B. P., R. E. Erb, J. F. Fessler, C. J. Callahan, and P. V. Malven. 1979. Effects of ovariectomy during pregnancy and of prematurely induced parturition on progesterone, estrogens, and calving traits. J. Dairy Sci. 62:557-566.

Clark, T. G., M. J. Bradburn, S. B. Love, and D. G. Altman. 2003. Survival analysis part I: Basic concepts and first analyses. Br. J. Cancer 89:232-238.

Cook, N. B., and K. V. Nordlund. 2009. The influence of the environment on dairy cow behavior, claw health and herd lameness dynamics. Vet. J. 179:360-369.

Cox, D. R., and D. Oakes. 1984. Analysis of survival data: 1st ed. London: Chapman and Hall.

Curtis, C. R., H. N. Erb, C. J. Sniffen, R. D. Smith, P. A. Powers, M. C. Smith, M. W. White, R. B. Hillman, and E. J. Pearson. 1983. Association of parturient hypocalcemia with eight periparturient disorders in Holstein cows. J. Am. Vet. Med. Assoc. 183:559-561.

Dann, H. M.; N. B. Litherland, J. P. Underwood, M. Bionaz, A. D’Angelo, J. W. McFadden, and J. K. Drackley. 2006. Diets during far-off and close-up dry periods affect periparturient metabolism and lactation in multiparous cows. J. Dairy Sci. 89:3563-3577.

Douglas, G. N., T. R. Overton, H. G. Bateman, H. M. Dann, and J. K. Drackley. 2006. Prepartal plane of nutrition, regardless of dietary energy source, affects periparturient metabolism and dry matter intake in Holstein cows. J. Dairy Sci. 89:2141-2157.

Douglas, G. N., J. K. Drackley, T. R. Overton, and H. G. Bateman. 1998. Lipid metabolism and production by Holstein cows fed control or high fat diets at restricted or ad libitum intakes during the dry period. J. Dairy Sci. 81(Suppl. 1):295. (Abstr.).

Drackley, J. K., H. M. Dann, and N. A. Janovick. 2007. New concepts for feeding dry cows. In Proceedings of the Tri-State Dairy Nutrition Conference, Fort Wayne, IN, USA. Pages 17-28. Edited by M. Eastridge, Ohio State University, Columbus.

Drackley, J. K. 2006. Advances in transition cow biology. New frontiers in production diseases. In Production Diseases in Farm Animals. Proceedings 12$^{th}$ International Conference, pp. 24-34. Edited by N. Joshi and T. H. Herdt, Wageningen Academic Publishers, Waggeningen, The Netherlands.

Drackley, J. K., H. M. Dann, N.A. Janovick, N. B. Litherland, J. P. Underwood, and J. J. Loor. 2005. Physiological and pathological adaptations in dairy cows that may increase susceptibility to periparturient diseases and disorders. Ital. J. Anim. Sci. 4:323-344.

Drackley, J. K. 2003. Interrelantionships of prepartum dry matter intake with postpartum intake and hepatic lipid accumulation. J. Dairy Sci. 86(suppl.1):104-105. (Abstr.).

Drackley, J. K., T. R. Overton, and G. N. Douglas. 2001. Adaptations of glucose and long chain fatty acid metabolism in liver of dairy cows during the perparturient period. J. Dairy Sci. 84(E Suppl):E100-E112.

Drackley, J. K. 1999. Biology of dairy cows during the transition period: the final frontier? J. Dairy Sci. 82:2259-2273.

Edgerton, L. A. and H. D. Hafs. 1973. Serum luteinizing hormone, prolactin, glucocorticoid, and progestin in dairy cows from calving to gestation. J. Dairy Sci. 56:451-458.

Emery, R. S., J. S. Liesman, and T. H. Herdt. 1992. Metabolism of long chain fatty acids by ruminant liver. J. Nutr. 122:832-837.

Fabian, D., G. Il'kova, P. Rehak, S. Czikkova, V. Baran, and J. Koppel. 2004. Inhibitory effect of IGF-I on induced apoptosis in mouse preimplantation embryos cultured in vitro. Theriogenology 61:745-755.

Ferguson, J. D., D. Sklan, W. V. Chalupa, and D. S. Kronfeld. 1990. Effects of hard fats on in vitro and in vivo rumen fermentation, milk production, and reproduction in dairy cows. J. Dairy Sci. 73:2864-2879.

Fuentes, M. C., S. S. Calsamiglia, C. Sánchez, A. González, J. Newbold, J. E. P. Santos, L. M. Rodríguez-Alcalá, J. Fontecha. 2008. Effect of extruded linseed on productive and reproductive performance of lactating dairy cows. Livest. Sci. 113: 144-154.

Geisert, R. D., C. Y. Lee, F. A. Simmen, M. T. Zavy, A. E. Fliss, F. W. Bazer, and R. C. Simmen. 1991. Expression of messenger RNAs encoding insulinlike growth factor-I, -II, and insulin-like growth factor binding protein-2 in bovine endometrium during the estrous cycle and early pregnancy. Biol. Reprod. 45:975-983.

Goff, J. P., and R. L. Horst. 1997. Physiological changes at parturition and their relationship to metabolic disorders. J. Dairy Sci. 80:1260-1268.

Goldsby, R. A., R. J. Kindt, and B. A. Osborne. 2000. Kuby Immunology. W.H. Freeman and Co. 4th ed.

Gong, J. G., W. J. Lee, P. C. Garnsworthy, and R. Webb. 2002. Effect of dietary-induced increases in circulating insulin concentrations during the early postpartum period on reproductive function in dairy cows. Reproduction 123:419-427.

Goshen, T., and N. Y. Shpigel. 2006. Evaluation of intrauterine antibiotic treatment of clinical metritis and retained fetal membranes in dairy cows. Theriogenology 66:2210-2218.

Graulet, B., D. Gruffat, D. Durand, and D. Bauchart. 1998. Fatty acid metabolism and very low density lipoprotein secretion in liver slices from rats and preruminant calves. J. Biochem. 124:1212-1219.

Grum, D. E., J. K. Drackley, R. S. Younker, D. W. LaCount, and J. J. Veenhuizen. 1996. Nutrition during the dry period and hepatic lipid metabolism of periparturient dairy cows. J. Dairy Sci. 79:1850-1864.

Grummer, R. R. 1995. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. J. Anim. Sci. 73:2820-2833.

Grummer, R. R. 1993. Etiology of lipid-related metabolic disorders in periparturient dairy cows. J. Dairy Sci. 76:3882-3896.

Hammon, D. S., I. M. Evjen, T. R. Dhiman, J. P. Goff, and J. L. Walters. 2006. Neutrophil function and energy status in Holstein cows with uterine health disorders. Vet. Immun. Immunopathol. 113:21-29.

Hansen, C., A. Fu, C. Li, W. T. Dixon, R. Christopherson, and S. S. Moore. 2004. Global gene expression patterns spanning 3T3-L1 preadipocyte differentiation. Can. J. Anim. Sci. 84:367-76.

Hansen, P. J., and F. D. Jousan. 2007. Insulin-like growth factor-i promotes resistance of bovine preimplantation embryos to heat shock through actions independent of its anti-apoptotic actions requiring pi3k signaling. Mol. Repro. Dev. 74:189-196.

Hayirli, A., R. R. Grummer, E. Nordheim, P. Crump, D. K. Beede, M. J. VandeHaar, and L. H. Kilmer. 1998. A mathematical model for describing dry matter intake of transition cows. J. Dairy Sci. 81(Suppl. 1):296 (Abstr.).

Herrler, A., C. A. Krusche, and H. M. Beier. 1998. Insulin and insulin-like growth factor-1 promote rabbit blastocyst development and prevent apoptosis. Biol. Reprod. 59:1302-1310.

Hileman S. M., L. S. Lubbers, H. T. Jansen, and M. N. Lehman. 1999. Changes in hypothalamic estrogen receptor-containing cell numbers in response to feed restriction in the female lamb. Neuroendocrinology 69:430-437.

Horst, R. L., J. P. Goff, T. A. Reinhardt, and D. R. Buxton. 1997. Strategies for preventing milk fever in dairy cattle. J. Dairy Sci. 80:1269-1280.

Huzzey, J. M., D. M. Veira, D. M. Weary, and M. A. von Keyserlingk. 2007. Prepartum behavior and dry matter intake identify dairy cows at risk for metritis. J. Dairy Sci. 90:3220-3233.

Ingvartsen, K. L., and J. B. Andersen. 2000. Integration of metabolism and intake regulation: A review focusing on periparturient animals. J. Dairy Sci. 83:1573-1597.

Janovick, N. A., Y. R. Boisclair and J. K. Drackley. 2011. Prepartum dietary energy intake affects metabolism and health during the periparturient period in primiparous and multiparous Holstein cows. J. Dairy Sci. 94:1385-1400.

Janovick, N. A., and J. K. Drackley. 2010. Prepartum dietary management of energy intake affects postpartum intake and lactation performance by primiparous and multiparous Holstein cows. J. Dairy Sci. 93:3086-3102.

Jordan, E. R., and R. H. Fourdraine. 1993. Characterization of the management practices of the top milk producing herds in the country. J. Dairy Sci. 76:3247-3256.

Jousan, F. D., L. J. Oliveira, and P. J. Hansen. 2008. Short-term culture of in vitro produced bovine preimplantation embryos with insulin-like growth factor-i prevents heat shock-induced apoptosis through activation of the phosphatidylinositol 3-kinase/akt pathway. Mol. Repro. Dev. 75:681-688.

Juchem, S. O., R. L. A. Cerri, M. Villaseñor, K. N. Galvão, R. G. S. Bruno, H. M. Rutigliano, E. J. DePeters, F. T. Silvestre, W. W. Thatcher, and J. E. P. Santos. 2010. Supplementation with calcium salts of linoleic and trans-octadecenoic acids improves fertility of lactating dairy cows. Reprod. Dom. Anim. 45:55-62.

Kelton, D. F., K. D. Lissemore, and R. E. Martin. 1998. Recommendations for recording and calculating the incidence of selected clinical diseases of dairy cattle. J. Dairy Sci. 81:2502-2509.

Kendrick, K. W., T. L. Bailey, A. S. Garst, A. W. Pryor, A. Ahmadzadeh, R. M. Akers, W. E. Eyestone, R. E. Pearson, and F. C. Gwazdauskas. 1999. Effects of energy balance on hormones, ovarian activity, and recovered oocytes in lactating Holstein cows using transvaginal follicular aspiration. J. Dairy Sci. 82:1731-1740.

Kerestes , M., V. Faigl , M. Kulcsár , O. Balogh , J. Földi, H. Fébel , Y. Chilliard , G. Huszenicza. 2009. Periparturient insulin secretion and whole-body insulin responsiveness in dairy cows showing various forms of ketone pattern with or without puerperal metritis. Domest. Anim. Endocrinol. 37:250-61.

Kimura, K., J. P. Goff, M. E. Kehrli, Jr., and T. A. Reinhardt. 2002. Decreased neutrophil function as a cause of retained placenta in dairy cattle. J. Dairy Sci. 85: 544-550.

Kleinbaum, D.G. 1996. Survival analysis: A self-learning text: 1st ed. New York, NY: Springer-Verlag.

Kleppe, A. B., R. J. Aiello, R. R. Grummer, and L. E. Armentano. 1988. Triglyceride accumulation and very low density lipoprotein secretion by rat and goat hepatocytes in vitro. J. Dairy. Sci. 71:1813-1822.

Kues , W. A., ; S. Sudheerb, D. Herrmanna, J. W. Carnwatha, V. Havlicekb, U. Besenfelderb, H. Lehrachc, J. Adjayec, and H. Niemanna. 2008. Genome-wide expression profiling reveals distinct clusters of transcriptional regulation during bovine preimplantation development in vivo. Proc. Natl. Acad. Sci. 105:19768-19773.

Kunz, P. L., J. W. Blum, I. C. Hart, H. Bickel, and J. Landis. 1985. Effects of different energy intakes before and after calving on food-intake, performance and blood hormones and metabolites in dairy-cows. Anim. Prod. 40:219-231.

Kurzawa, R., W. Glabowski, T. Baczkowski, and P. Brelik. 2002. Evaluation of mouse preimplantation embryos exposed to oxidative stress cultured with insulin-like growth factor I and II, epidermal growth factor, insulin, transferrin and selenium. Reprod. Biol. 2:143-162.

Kurzawa, R., W. Glabowski, and L. Wenda-Rozewicka. 2001. Evaluation of mouse preimplantation embryos cultured in media enriched with insulin-like growth factors I and II, epidermal growth factor and tumor necrosis factor alpha. Folia Histochem. Cytobiol. 39:245-251.

Leslie, A., A. J. Jones, and P. R. Goddard. 2000. The influence of clinical information on the reporting of CT by radiologists. Br. J. Radiol. 73:1052-1055.

Litherland, N. B., H. M. Dann and J. K. Drackley. 2011. Prepartum nutrient intake alters palmitate metabolism by liver slices from peripartal dairy cows. J. Dairy Sci. 94:1928-1940.

Lonergan, P., A. Gutierrez-Adan, B. Pintado, T. Fair, F. Ward, J. D. Fuente, and M. Boland. 2000. Relationship between time of first cleavage and the expression of IGF-I growth factor, its receptor, and two housekeeping genes in bovine two-cell embryos and blastocysts produced in vitro. Mol. Reprod. Dev. 57:146-152.

Markusfeld, O. 1985. Relationship between overfeeding, metritis and ketosis in high yielding dairy cows. Vet Rec. 116:489-491.

Mattos, R., C. R. Staples, A. Arteche, M. C. Wiltbank, F.J. Diaz, T. C. Jenkins, and W. W. Thatcher. 2004. The effects of feeding fish oil on uterine secretion of PGF2α, milk composition, and metabolic status of periparturient Holstein cows. J. Dairy Sci. 87:921-932.

Mattos, R., A. Guzeloglu, L. Badinga, C. R. Staples, and W. W. Thatcher. 2003. Polyunsaturated fatty acids and bovine interferon- modify phorbol ester-induced secretion of prostaglandin F2α and expression of prostaglandin endoperoxide synthase-2 and phospholipase-A2 in bovine endometrial cells. Biol. Reprod. 69:780-787.

Mattos, R., C. R. Staples, J. Williams, A. Amorocho, M. A. McGuire, and W. W. Thatcher. 2002. Uterine, ovarian, and production responses of lactating dairy cows to increasing dietary concentrations of menhaden fish meal. J. Dairy Sci. 85:755-764.

Mattos, R., C. R. Staples, and W. W. Thatcher. 2000. Effects of dietary fatty acids on reproduction in ruminants. Rev. Reprod. 5:38-45.

McNamara, J. P. 1991. Regulation of adipose tissue metabolism in support of lactation. J. Dairy Sci. 74:706-719.

Ney, D. M., J. B. Lasekan, T. Spennetta, M. Grahn, and E. Shrago. 1989. Effect of dietary fat on individual long-chain fatty acyl-CoA esters in rat liver and skeletal muscle. Lipids 24:233-235.

NAHMS. 1996. Dairy 1996 Part III: Reference of 1996 dairy health and health management. Online. Available: http://nahms.aphis.usda.gov/dairy/dairy96/DR96Pt3.pdf.

Nebel, R. L., and M. L. McGilliard. 1993. Interactions of high milk yield and reproductive performance in dairy cows. J. Dairy Sci. 76:3257-3268.

Noftsger, S., and N. R. St-Pierre. 2003. Supplementation of methionine and selection of highly digestible rumen undegradable protein to improve nitrogen efficiency for milk production. J. Dairy Sci. 86:958-969.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. National Academy Press, Washington, DC.

Ocon, O. M., and P. J. Hansen. 2003. Disruption of bovine oocytes and preimplantation embryos by urea and acidic pH. J. Dairy Sci. 86:1194-1200.

Overton, T. R., J. K. Drackley, C. J. Otteman-Abbamonte, A. D. Beaulieu, L. S. Emmert, and J. H. Clark. 1999. Substrate utilization for hepatic gluconeogenesis is altered by increased glucose demand in ruminants. J. Anim. Sci. 77:1940-1951.

Overton, T. R., J. K. Drackley, G. N. Douglas, L. S. Emmert, and J. H. Clark. 1998. Hepatic gluconeogenesis and whole-body protein metabolism of periparturient dairy cows as affected by source of energy and intake of the prepartum diet. J. Dairy Sci. 81(Suppl 1):295. (Abstr).

Petit, H. V., and H. Twagiramungu. 2006. Conception rate and reproductive function of dairy cows fed different fat sources. Theriogenology 66:1316-1324.

Pullen, D. L, D. L. Palmquist, and R. S. Emery. 1989. Effect on days of lactation and methionine hydroxyl analog on incorporation of plasma fatty acids into plasma triglycerides. J. Dairy Sci. 72:49-58.

Pushpakumara, P. G., R. S. Robinson, K. J. Demmers, G. E. Mann, K. D. Sinclair, R. Webb, and D. C. Wathes. 2002. Expression of the insulin-like growth factor (IGF) system in the bovine oviduct at oestrus and during early pregnancy. Reproduction 123:859-868.

Rabelo, E., R. L. Rezende, S. J. Bertics, and R. R. Grummer. 2005. Effects of pre- and postfresh transition diets varying in dietary energy density on metabolic status of periparturient dairy cows. J. Dairy Sci. 88:4375-4383.

Radcliff, R. P., B. L. McCormack, B. A. Crooker, and M. C. Lucy. 2003. Plasma hormones and expression of growth hormone receptor and insulin-like growth factor-I mRNA in hepatic tissue of periparturient dairy cows. J. Dairy Sci. 86:920-3926.

Radostits, O. M, C. C. Gay, D. C. Blood, K. W. Hinchcliff, and P. D. Constable. 2007. Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses, 10th ed. London: Saunders Elsevier.

Rhoads, M. L., R. P. Rhoads, R. O. Gilbert, R. Toole, and W. R. Butler. 2006. Detrimental effects of high plasma urea nitrogen levels on viability of embryos from lactating dairy cows. Anim. Reprod. Sci. 91:1-10.

Rhoads, R. P., J. W. Kim, B. J. Leury, L. H. Baumgard, N. Segoale, S. J. Frank, D. E. Bauman, and Y. R. Boisclair. 2004. Insulin increases the abundance of the growth hormone receptor in liver and adipose tissue of periparturient dairy cows. J. Nutr. 134:1020-1027.

Risco, C. A., C. A. Holmberg, and A. Kutches. 1992. Effect of graded concentrations of gossypol on calf performance: Toxicological and pathological considerations. J. Dairy Sci. 75:2787-2798.

Roche, J. R., K. A. Macdonald, C. R. Burke, J. M. Lee, and D. P. Berry. 2007. Associations among body condition score, body weight and reproductive performance in seasonal-calving dairy cattle. J. Dairy Sci. 90:376-391.

Rukkwamsuk, T., T. Wensing, and M. J. Geelen. 1998. Effect of overfeeding during the dry period on regulation of adipose tissue metabolism in dairy cows during the periparturient period. J. Dairy Sci. 81:2904-2911.

Rutigliano, H. M., F. S. Lima, R. L. A. Cerri, L. F. Greco, J. M. Villela, V. Magalhães, F. T. Silvestre, W. W. Thatcher, and J. E. P. Santos. 2008. Effects of method of presynchronization and source of selenium on uterine health and reproduction in dairy cows. J. Dairy Sci. 91:3323-3336.

Santos, J. E. P., H. M. Rutigliano, and M. F. Sá Filho. 2009. Risk factors for resumption of postpartum cyclicity and embryonic survival in lactating dairy cows. Anim. Reprod. Sci. 110:207-221.

Santos, J. E. P., T. R. Bilby, W. W. Thatcher, C. R. Staples, and F. T. Silvestre. 2008. Long chain fatty acids of diet as factors influencing reproduction in cattle. Reprod. Dom. Anim. 43 (Supp. 2):23-30.

Santos, J. E. P., W. W. Thatcher, R. C. Chebel, R. L. A. Cerri, and K. N. Galvão. 2004a. The effect of embryonic death rates in cattle on the efficacy of estrous synchronization programs. Anim. Reprod. Sci. 82-83:513-535.

Santos, J. E. P., S. O. Juchem, R. L. A. Cerri, K. N. Galvão, R. C. Chebel, W. W. Thatcher, C. Dei, and C. Bilby. 2004b. Effect of bST and reproductive management on reproductive and lactational performance of Holstein dairy cows. J. Dairy Sci. 87:68-881.

Sheldon, M., G. S. Lewis, S. LeBlanc, and R. O. Gilbert. 2006. Defining postpartum uterine disease in cattle. Theriogenology 65:1516-1530.

Schneider, P., D. Sklan, W. Chalupa, and D. S. Kronfeld. 1988. Feeding calcium salts of fatty acids to lactating cows. J. Dairy Sci. 71:2143-2150.

Schingoethe, D. J., and D. P. Casper. 1991. Total lactational response to added fat during early lactation. J. Dairy Sci. 74:2617-2622.

Scott, T. A., R. D. Shaver, L. Zepeda, B. Yandell, and T. R. Smith. 1995. Effects of rumen-inert fat on lactation, reproduction, and health of high producing Holstein herds. J. Dairy Sci. 78:2435-2451.

Silvestre, F. T., T. S. M. Carvalho, N. Francisco, J. E. P. Santos, C. R. Staples, and W.W.Thatcher. 2008. Effects of differential supplementation of calcium salts of fatty acids (csfas) on dairy cows. J. Dairy Sci. 91(Supp. 1):76.

Sklan, D., M. Kaim, U. Moallem, and Y. Folman. 1994. Effect of dietary calcium soaps on milk yield, body weight, reproductive hormones, and fertility in first parity and older cows. J. Dairy Sci. 77:1652-1660.

Sklan, D., U. Moallem, and Y. Folman. 1991. Effect of feeding calcium soaps of fatty acids on production and reproductive responses in high producing lactating cows. J. Dairy Sci. 74:510-517.

Staples, C. R., J. M. Burke, and W. W. Thatcher. 1998. Influence of supplemental fats on reproductive tissues and performance of lactating cows. J. Dairy Sci. 81:856-871.

Staples, C. R., and W. W. Thatcher. 2005. Effects of fatty acids on reproduction of dairy cows. Pages 229-256 in Recent Advances in Animal Nutrition, ed. P. C. Garnsworthy and J. Wiseman, Nottingham University Press, England.

Strang, B. D., S. J. Bertics, R. R. Grummer, and L. E. Armentano. 1998. Effect of long-chain fatty acids on triglyceride accumulation, gluconeogenesis, and ureagenesis in bovine hepatocytes. J. Dairy. Sci. 81:728-739.

Thatcher, W. W., J. E. P. Santos, and C. R. Staples. 2011. Dietary manipulations to improve embryonic survival in cattle. Theriogenology 76:1619-1631.

Thompson, E. M., E. Legouy, and J. P. Renard. 1998. Mouse embryos do not wait for the MBT: Chromatin and RNA polymerase remodeling in genome activation at the onset of development. Dev. Genet. 22:31-42.

Underwood, J. P. 2003. Protein nutrition of dairy cows during the transition period. Ph.D. Dissertation, Univ. Illinois, Urbana.

Urton, G., M. A. von Keyserlingk, and D. M. Weary. 2005. Feeding behavior identifies dairy cows at risk for metritis. J. Dairy Sci. 88: 2843-2849.

VandeHaar, M. J., G. Yousif, B. K. Sharma, T. H. Herdt, R. S. Emery, M. S. Allen, and J. S. Liesman. 1999. Effect of energy and protein density of prepartum diets on fat and protein metabolism of dairy cattle in the preiparturient period. J. Dairy Sci. 82:1282-1295.

Vasconcelos, J. L. M., S. Sangsritavong, S. J. Tsai, and M. C. Wiltbank. 2003. Acute reduction in serum progesterone concentrations after feed intake in dairy cows. Theriogenology 60:795-807.

Vazquez-Anon, M. S., S. J. Bertics, M. Luck, and R. R. Grummer. 1994. Peripartum liver triglyceride and plasma metabolites. J. Dairy Sci. 77:1521-1528.

Veenhuizen, J. J., J. K. Drackley, M. J. Richard, T. P. Sanderson, L. D. Miller, and J. W. Young. 1991. Metabolic changes in blood and liver during development and early treatment of experimental fatty liver and ketosis in cows. J. Dairy Sci. 74:4238-4253.

Villa-Godoy, A., T. L. Hughes, R. S. Emery, T. L. Chaplin, and R. L. Fogwell. 1988. Association between energy balance and luteal function in lactating dairy cows. J. Dairy Sci. 71:1063-1069.

Wade, G. N., and J. J. Jones. 2004. Neuroendocrinology of nutritional infertility. Am. J. Physiol. Regul. Integr. Comp. Physiol. 287:1277-1296.

Wallace, R. L., G. C. McCoy, T. R. Overton, and J. H. Clark. 1996. Effect of adverse health events on dry matter consumption, milk production, and body weight loss of dairy cows during early lactation. J. Dairy Sci. 79(Suppl. 1):205.

Whiteford, L. C., and I. M. Sheldon. 2005. Association between clinical hypocalcaemia and postpartum endometritis. Vet. Rec. 157:202-203.

Zammit,V. A. 1984. Mechanisms of regulation of the partition of fatty acids between oxidation and esterification in the liver. Prog. Lipid Res. 23:39-67.

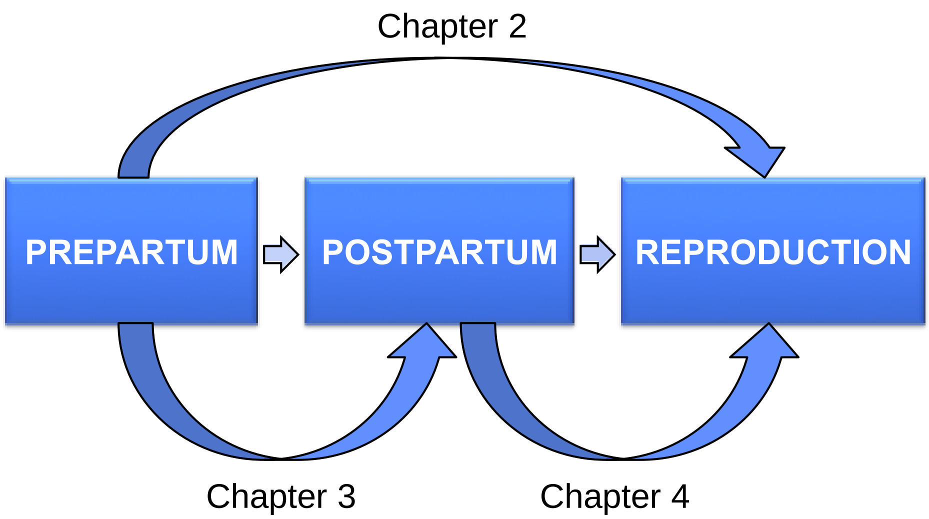


**Figure 1.** Scheme of thesis analysis and objectives divided by chapters.

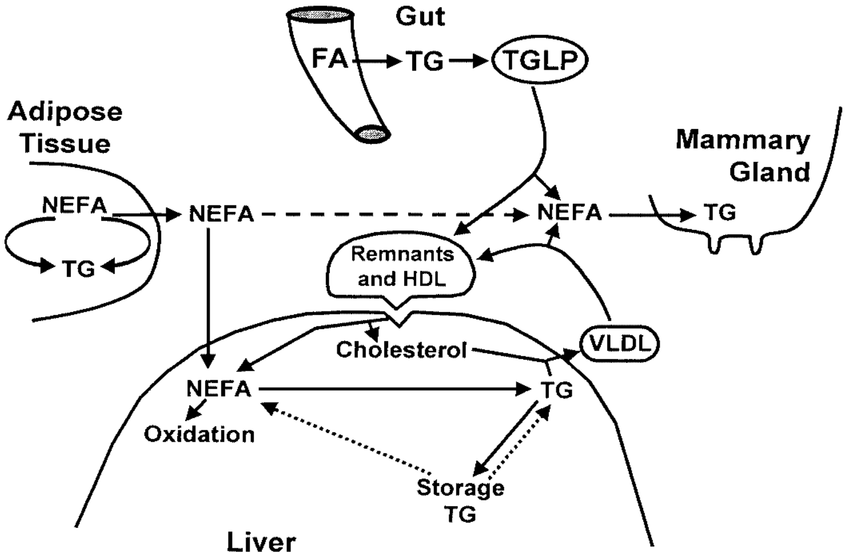


**Figure 2.** Schematic representation of metabolism of nonesterified fatty acids (NEFA) mobilized from adipose tissue and dietary fatty acids (FA) absorbed from the gut. After absorption, dietary FA are packaged into triglyceride (TG)-rich lipoproteins (TGLP), which deliver dietary FA to peripheral tissues. Dietary FA result in elevation of the concentration of NEFA as a consequence of lipoprotein lipase action in peripheral tissues. Remnant lipoproteins and high-density lipoproteins (HDL) are cleared by the liver, thereby delivering FA to the liver (Drackley, 1999).

## CHAPTER 2

## PREPARTUM NUTRITIONAL STRATEGY AFFECTS REPRODUCTIVE PERFORMANCE IN DAIRY COWS


### ABSTRACT

Negative energy balance (NEB) during the first weeks postpartum is associated with reduced reproductive performance in dairy cows. A meta-analysis approach was conducted to investigate the association between prepartum energy feeding regimen and reproductive performance. Days to conception (DTC) was used as the dependent variable to assess reproductive performance. The database was developed from 7 experiments completed in our group from 1993 to 2010. Individual data for 408 cows (354 multiparous and 54 primiparous) were included in the analysis. The net energy for lactation ($NE_L$) intake was determined from each cow's average DMI and calculated dietary $NE_L$ density. Treatments were applied prepartum and were classified as either controlled energy (CE) or high energy (HE) diets fed during the far-off (FO) or close-up (CU) dry periods. Cow was the experimental unit. The Cox proportional hazard model revealed a significant difference in DTC between HE and CE during the CU period (median = 167 and 157 d; hazard ratio (HR) = 0.696). Cows fed HE diets during the last 4 wk prepartum lost more BCS in the first 6 wk postpartum than those fed CE (−0.43 and −0.30, respectively). Cows with 3 or more lactations lost more BCS than cows with one or two lactations (−0.42 and −0.33, respectively). Cows fed CE during the FO period had lower nonesterified fatty acids (NEFA) concentrations in wk 1, 2, and 3 of lactation compared with cows fed HE. Higher NEFA concentration in wk 1 postpartum was associated with a greater probability of diseases (n = 251; OR = 1.176). Cows on the CE regimen during the FO period

had greater plasma glucose concentrations during wk 1 and 3 after calving than cows fed the HE regimen. Higher plasma glucose (HG) concentration compared with lower glucose (LG) in wk 3 (HG n = 154; LG n = 206) and wk 4 (HG n = 71; LG n = 254) after calving was associated with greater HR for DTC (wk 3: median = 151 and 171 d for HG and LG, HR = 1.334; wk 4: median = 148 and 167 d, HR = 1.394). In the first 2 wk after calving, cows that received HE in the FO period had higher concentrations of total lipids and triglyceride and greater ratio of triglyceride to glycogen in liver than did CE. In conclusion, cows fed CE during the CU period had greater HR for DTC, meaning a shorter interval between parturition and conception. The positive effect of CE may be explained by increased $NE_L$ intake during the first 4 wk postpartum and lower incidence of peripartal diseases. In addition, lower BCS loss during the first 6 wk postpartum and slightly higher glucose concentration at wk 3 likely contributed to improved reproductive performance.
Key Words: periparturient cow, energy intake, days to conception, reproductive performance

## INTRODUCTION

Infertility is a major reason for premature culling of dairy cows, having a great effect on lifetime milk production of individual cows (Beever, 2006). In the majority of herds in the USA, typically 30 to 35% of the cows are culled each year (Appleman, 1978; Quaiffe, 2002). Reproductive inefficiency also reduces the number of calves born, which decreases the number of replacements available (Grohn and Rajala-Schultz, 2000) and further increases the economic losses caused by infertility. Decreasing reproductive efficiency in dairy cattle has been documented not only in the USA but also in Ireland, United Kingdom, and Australia (Lucy, 2001). Butler (1998) showed a decline in first-service conception rate from approximately 65% in 1951 to 40% in 1996.

An index used to measure infertility in the dairy herd is days to conception (**DTC**), defined as the time in days from calving until the last breeding in which the cow became pregnant. Since conception does not necessarily occur at the first breeding, cows may have to be inseminated more than once. A cow that does not conceive or has low milk yield for an extended time is more likely to be culled (Grohn and Rajala-Schultz, 2000; Beever, 2006). Butler (2000) and Jorritsma et al. (2003) suggested that negative energy balance (**NEB**) is associated with infertility in dairy cows.

Negative energy balance suppresses pulsatile LH secretion and reduces ovarian responsiveness to LH stimulation, both of which result in reduced fertility in dairy cows (Butler, 2000). Such NEB results from the rapid increase in energy requirements at the onset of lactation due to milk production (Butler, 2000). Negative energy balance may begin just before calving and reaches its nadir about 2 wk after calving (Butler and Smith, 1989; Bell, 1995). Cows are unable to achieve the necessary DMI to maintain energy balance early postpartum (Bauman, 2000). Feeding strategies have attempted to maximize DMI by the cow, especially during early lactation.

Benefits of feeding controlled energy (**CE**) diets prepartum to dairy cows have been reported (Beever, 2006; Dann et al., 2006; Douglas et al., 2006; Janovick and Drackley, 2010). Recently, Janovick et al. (2011) suggested that cows fed CE during the dry period had fewer diseases and disorders than cows fed high-energy (**HE**) diets. Also, Beever (2006) stated that farmers have repeatedly observed easier calving and greater DMI around parturition when energy intake is controlled prepartum. Excess energy consumption prepartum also seems to result in a larger decline in DMI prepartum compared with cows having controlled intake prepartum (Janovick et al., 2011). Such steep changes in DMI prepartum have been associated with increased deposition of lipid in liver postpartum (Drackley et al., 2005). Nevertheless, the effect of energy intake prepartum on reproductive performance in dairy cows is still to be assessed. Previous experiments did not have the statistical power necessary to explore such relationships.

The objectives of this study were to examine the associations between feeding CE or HE diets during the dry period and reproductive success, measured as DTC in dairy cows. The effects of prepartum dietary energy regimen on BCS, $NE_L$ intake (**$NE_{LI}$**), diseases, concentrations of glucose, insulin, and NEFA in blood, and concentrations of total lipids, triglyceride (**TG**), and glycogen also were determined. Our hypothesis was that cows fed the CE regimen would have a more favorable transition period and consequently a shorter DTC. A meta-analysis approach was used to investigate these associations.

## MATERIALS AND METHODS

### *Database Construction and Data Collection*

The database was developed from 7 experiments completed in our group from 1993 to 2010 (Table 1). Individual cow experimental data were obtained from Microsoft Excel (Microsoft Corp., Redmond,WA) files from each experiment. Individual cow data for management, health, and reproduction were obtained from PCDART (Dairy Records Management Services, Raleigh, NC) herd management software, or individual cow record cards. A total of 408 cows (354 multiparous and 54 primiparous) were included in the analyses.

Prepartum treatments were defined as: 1) HE, where cows were allowed ad libitum access to moderate-energy diets that would allow cows to exceed NRC requirements (NRC, 2001) for $NE_L$; and 2) CE, where cows either were fed restricted amounts of moderate-energy diets to target $NE_L$ intakes of 80% of NRC requirements or were allowed ad libitum access to high-fiber low-energy diets to limit $NE_L$ intake to ~100% of NRC requirements.

Parity was dichotomized as cows with one or two lactations in one group (**LAG1**) and cows with three or more lactations in a second group (**LAG2**). In addition, calving season (winter: December to February; spring: March to May; summer: June to August; autumn: September to November), and peripartal events such as twin births, dystocia, and disease occurrence (**DISE**) were included in the database. Diseases and disorders included were retained placenta, ketosis, uterine prolapse, digestive problems, displaced abomasum, ovarian cyst, and metritis. Diseases and disorders were diagnosed by the research staff and responsible veterinarian. If cows had more than one occurrence of DISE they were classified as having multiple diseases (**MDISE**). At least one occurrence of retained placenta, metritis, or cystic ovaries was grouped as the explanatory variable reproductive pathology (**RPAT**).

Body condition score was assigned independently by more than 1 individual weekly using a 5-point scale (Ferguson et al., 1994) and the median score was used for each cow. Weekly BCS was used either as a continuous variable or was classified as thin (BCS $\leq$ 2.75), moderate (BCS $\geq$ 3 and $\leq$ 3.75), and fat (BCS > 3.75).

Dry matter intake was recorded daily for individual cows and weekly means by cow were used in the database. Dietary $NE_L$ density varied from 1.21 to 1.73 Mcal/kg of DM across experiments. The $NE_{LI}$ was calculated from the average DMI and the respective dietary

$NE_L$density. The close-up (**CU**) period included a negative DCAD diet that was fed during the last 3 wk prepartum (Table 1).

Blood was sampled at different times prepartum and postpartum among experiments. Weekly means were established for the blood metabolites and its sample size is indicated further in the respective analysis. The blood metabolites analyzed were NEFA (Johnson and Peters, 1993) in all experiments; glucose (Trinder, 1969; kit no 315, Sigma) in experiments 1 and 4 or by glucose/HK Kit (Roche Diagnostic Corp.) using the glucose-6-phosphate dehydrogenase reaction (Peterson and Young, 1968) in experiments 2, 3, 5, 6, and 7; and insulin by RIA (Coat-a-Count- insulin kit, Diagnostic Products Inc., Los Angeles, CA) as modified by Studer et al. (1993) in experiments 1, 2, 3, 4, 6, and 7. When of interest, the variable NEFA was dichotomized as high or low using a cut-off value of 700 μEq/L as defined by Ospina et al. (2010) and glucose concentration was categorized at the median concentration values of 60 mg/dL at wk 3 and 65 mg/dL at wk 4 as high (**HG**) or low (**LG**).

Puncture biopsy of liver was performed under local anesthesia to obtain approximately 3 to 5 g of liver tissue (Hughes, 1962; Drackley et al., 1991; Douglas et al., 2004) at d −65 (experiments 1, 2, 3, and 6), −30 (experiments 2 and 3), −21 (experiments 1, 4, and 5), −14 (experiments 2, 3, 6, and 7) relative to expected parturition and at d 1 (experiment 7), 10 (experiments 5 and 7), 14 (experiments 2, 3, and 6), 21 (experiments 1 and 4), 28 (experiments 2, 3, 5, and 6) 42 (experiments 2, 6, and 7), 49 (experiment 3), 60 (experiment 4), and 65 (experiment 1) after calving. Tissue was frozen immediately in liquid $N_2$ until analysis for contents of total lipid (Drackley et al., 1991) and glycogen (Lo et al., 1970). Total lipids were extracted from liver tissue by using a hexane and isopropanol (3:2) mixture as described by (Hara and Radin, 1978). Triglyceride (**TG**) was analyzed in the lipid fraction according to established methods (Fletcher, 1968; Foster and Dunn, 1973). Total lipids and TG were kept as continuous variables after calving due to lack of a considerable range in the values between cows. The ratio between liver TG and glycogen concentrations was named TG:GLY.

### *Animals and Housing*

All experimental procedures were conducted according to protocols approved by the University of Illinois Institutional Animal Care and Use Committee. Before calving, cows were housed either in free stalls with individual Calan feed gates (American Calan, Northwood, NH, USA) or in tie stalls. Before expected parturition, cows were moved to individual maternity pens until parturition. After parturition, cows were returned to a tie stall. When housed in tie stalls, cows were allowed to exercise daily for 2 to 3 h in an outside lot. Cows were milked twice daily. During lactation all cows received the same diet within experiment (except experiment 5). All cows were bred through artificial insemination.

### *Statistical Analysis*

A final dataset including all the variables was constructed in SAS (SAS v9.2 Institute Inc., Cary, NC). Statistical analysis was performed using the GLIMMIX, MIXED, and PHREG procedures of SAS (SAS v9.2 Institute Inc., Cary, NC) considering cow as the experimental unit. The relationship between prepartum dietary energy and DTC was the outcome of most interest. The second outcome of interest was the relationship between prepartum dietary regimen and variables reflecting the physiological status of the animal (e.g., blood metabolites, liver composition, and disease occurrence). The statistical analysis was performed in a hypothesis-driven segmented scheme.

First, a Cox proportional hazard model (PHREG procedure) was used to assess DTC in a survival analysis where experiments were treated as strata to adjust for the random effect of experiment (Grohn et al., 1998; St-Pierre, 2001; Allison, 2010). Treatment effects, i.e., CE and HE during FO or CU periods were forced into the model and the interaction was included when statistically significant ($P < 0.1$). When statistically significant, the covariates parity, RPAT, and calving season were included in the model. A manual backward stepwise removal of these variables and interactions was used when $P \leq 0.05$. Parity, RPAT, and calving season were retained as covariates in the model for the variables glucose, insulin, liver total lipid, and liver TG. The variables RPAT and calving season remained in the model for liver total lipid in wk −3 and for TG in wk −3. The variable RPAT was used in the model for liver lipid in wk 4, TG in wk 2 and 4, and TG:GLY throughout.

The model considered reproductive data from 10 to 400 d postpartum. The assumption of the proportionality of hazard of the model was assessed graphically by plotting the logarithm of the hazard function by the logarithm of time. Residuals were evaluated for homogeneous distribution.

Second, once associations were established between treatments and DTC a linear mixed model (MIXED procedure) was constructed to explore associations between FO and CU feed regimens and BCS, DMI, NEFA, insulin, glucose, total lipid, TG, glycogen, and TG:GLY. In this case treatments were forced into the model. The covariates parity and calving season, and their interaction were left in the model when $P \leq 0.05$. Parity was used as a covariate in the model for the variables NEFA (wk −1 and 1), glucose, insulin (wk 1 and 2), total lipid (wk 1 and 2), and TG (wk 1 and 2). Experiment was considered a random effect (St-Pierre, 2001). Degrees of freedom were estimated by using the Kenward-Roger method in the model statement.

Residual distribution was evaluated for normality and homoscedasticity and variables were transformed if necessary. A log transformation was used for the variables NEFA, insulin, total lipid, TG, and glycogen for better homogeneity of the residuals distribution. Data shown in tables for these variables are back transformed.

Third, variables cited above as associated with treatments in the MIXED models were further investigated in the Cox proportional hazard model in the first phase. Variables were forced in the model with the outcome DTC.

Finally, multivariable logistic mixed models (GLIMMIX procedure) considering the binary outcome variables twins, dystocia, DISE, and MDISE were constructed. Treatments were forced into the model. Experiment was considered as a random effect (St-Pierre, 2001). Parity and calving season were included as covariates in the model when $P \leq 0.05$. Parity and calving season were used as covariates for DISE, MDISE, and twins; whereas, only calving season was used for dystocia.

## RESULTS

Descriptive statistics for the characterization of treatments CE and HE in the FO and CU periods are shown in Table 2. In addition, to characterize the herd, mean (± SD) values of milk production, 3.5% FCM, and BW at wk 4 postpartum were 34.9 ± 7.6 kg, 35.9 ± 9.0 kg and 607 ± 75 kg, respectively.

The Cox proportional hazard model (Table 3) stratified by experiment revealed no significant difference in DTC between cows (n = 332) fed HE vs. CE diets during the FO period (median = 164 and 165 d; HR = 1.229, $P$ = 0.29). In contrast, cows fed HE rather than CE during the CU period had a significant difference in the hazard ratio of DTC (median = 167 and 157 d, Figure 3; HR = 0.696, *P* = 0.04, Figure 4), indicating a shortened DTC for cows fed CE.

Cows (n= 296) fed the HE diet during the CU period lost more BCS in the first 6 wk postpartum (*P* = 0.04) than cows fed CE. Parity had a significant effect; cows with 3 or more lactations (LAG2) lost more BCS (*P* = 0.04) than cows with one or two lactations (LAG1; Figure 5). In addition, when evaluating the BCS change in the 4 wk postpartum (n = 262), the same effect was observed (*P* = 0.01).

In trying to relate physiological state with reproductive performance, cows classified as thin (n = 43) versus those that were fat (n = 26) at wk −4 prepartum had lower hazard ratios for difference in DTC (median = 207 and 116 days; HR = 0.509, *P* = 0.02; i.e., longer time to become pregnant). Accordingly, at wk 1 postpartum cows classified as thin (n = 154) had lower hazard ratio for difference in DTC when comparing to moderate cows (n = 160) since the category fat was absent at this period of time (median = 170 and 148 days; HR = 0.760, *P* = 0.05).

Treatment CE versus HE during CU tended (*P* = 0.10) to have a positive effect on DMI in the first 4 wk postpartum (least squares means and SEM = 16.5 ± 0.98 kg and 15.4 ± 0.93 kg), with calving season explaining part of the variability in the model (*P* < 0.01). Cows calving during the summer had lower DMI in the first 4 wk postpartum than cows calving in the other seasons of the year (*P* < 0.01). Additionally, CE during FO had a positive association (*P* = 0.01) with mean $NE_{LI}$ in the first 6 wk postpartum compared with HE during FO (Figure 6).

Neither dietary energy regimen was associated with a greater probability of cows having twins or dystocia (*P* > 0.4; Table 4). Not surprisingly, cows with twins (n = 36) or DYS (n = 41) had lower hazard ratio for difference in DTC when compared to single calving cows (n = 253;

median = 229 and 174 d; HR = 0.650, *P* = 0.02; Figure 7) and non-dystocia cows (n = 163; median = 217 and 170 days; HR = 0.662, *P* = 0.05; Figure 8), respectively. Dietary energy intake was not associated with DISE (*P* > 0.8) or MDISE (*P* > 0.2). As expected, DISE and MDISE were associated with lower hazard ratio for difference in DTC (*P* = 0.01; Figure 9 and *P* = 0.02; Figure 10). An interaction between DISE and MDISE with parity was present (*P* < 0.05) as cows in LAG2 were more likely to undergo disease (*P* < 0.01).

Cows fed CE during FO showed lower NEFA concentrations during wk 1, 2, and 3 (*P* < 0.01). However, cows fed CE during CU had higher (*P* < 0.01) NEFA concentrations at wk −2 and −1 before calving (Table 5). At wk −1 and wk 1 postpartum, cows in LAG2 had higher concentration of NEFA than those in LAG1. There was an association (*P* < 0.05) between higher NEFA concentration at wk 1 and a greater probability of DISE (n = 251; OR= 1.176).

Cows fed HE during CU had greater insulin concentrations at wk −2 and −1 before calving (*P* < 0.01) and wk 1 and 2 after calving (*P* < 0.05) compared to cows receiving CE during the same period (Table 5). In contrast, cows fed CE during FO had higher insulin concentrations at wk 1 and 2 (*P* < 0.01) when compared to HE. Parity remained in the model (*P* = 0.07) showing that cows in LAG1 had higher insulin concentrations when compared to LAG2. Higher concentrations of insulin at wk 2 after calving was associated with lower hazard ratio difference in DTC (*P* = 0.01) (Table 6).

Cows fed HE during CU had higher glucose concentrations at wk −1 and −2 before calving than cows fed CE (*P* < 0.01). Cows fed CE during the FO period had higher glucose concentration when compared to HE at wk 1 (*P* = 0.07) and wk 3 (*P* = 0.02) after calving (Table 5). Both prepartum and postpartum LAG1 had higher blood glucose concentration than LAG2 (*P* < 0.05). Higher glucose versus LG in wk 3 (HG n = 154; LG n = 206) and 4 (HG n = 71; LG n =

254) after calving was associated with greater hazard ratio for difference in DTC (wk 3: median = 151 and 171 d; HR = 1.334, *P* = 0.04; wk 4, median = 148 and 167 d; HR = 1.394, *P* = 0.04) (Table 6).

Liver total lipids were higher in cows on the HE regimen when compared to those on CE in either period (Table 5). In the first 2 wk after calving, cows receiving HE in the FO period had greater concentrations of total lipid and TG and a greater TG:GLY in liver compared to CE (Table 5). At wk 1, parity was included in the model ($P \leq 0.1$), where cows in LAG2 had higher total lipid, TG, and TG:GLY than cows in LAG1. At wk −3 before calving, higher concentrations compared with lower concentrations of total lipid and TG were associated with a lower hazard ratio for difference in DTC (total lipid: median = 230 and 132 d, *P* = 0.02; TG %, median = 208 and 147 d; *P* = 0.11) (Table 6); however, after calving total lipid, TG, and TG:GLY were associated with higher hazard ratio for difference in DTC (Table 6). Cows fed CE during the CU period had a greater concentration of glycogen in liver postpartum than cows fed HE (*P* = 0.03; Table 5).

## DISCUSSION

Cows receiving CE in the CU period had fewer DTC (Table 4). A HR >1 indicated that cows fed CE had a higher rate of pregnancy (i.e., the DTC when 50% of the cows were pregnant was lower) than cows fed HE. Parr et al. (1993) found that sheep fed high dietary energy had increased metabolic clearance of progesterone from blood by the liver. Plasma progesterone concentrations were about 25% lower in heifers fed a high energy diet as compared to those fed a low energy diet, perhaps because of greater progesterone clearance (Nolan et al., 1998). Butler

(2000) observed increased clearance of progesterone and a carry-over effect of NEB that resulted in lower plasma progesterone concentration that led to reduced fertility.

Previous research, besides the experiments included in this study, has found positive associations between the CE strategy and a smoother transition period. Such improvement would be reflected in better DMI postpartum (Drackley et al., 2005; Beever, 2006) and better health status of cows (Litherland et al., 2011; Drackley et al., 2005; Drackley et al., 2007). An important aspect is to successfully implement the CE diets. The descriptive statistics for HE and CE diets (Table 2) showed that, when measured, CE had greater particle size as demonstrated by the Pen State particle separator (PSPS) results. Larger particle size reflects the application of the high-bulk strategy. In diets with NDF of >50% of total DM, excessive particle size can lead to intake depression or to sorting of longer forage particles (NRC, 2001), which could further aggravate dietary nutrient status during the transition period. Good management strategies, such as including water in the diet mixture, were used in the different experiments to achieve adequate DMI.

Perhaps the most important reason that feeding CE during the FO enhanced subsequent reproductive performance is that cows in that dietary regimen had greater $NE_{LI}$ in the first 4 wk postpartum (24.1 versus 21.1 Mcal/d). Holcomb et al. (2001) studied different amount of forages in ad libitum and restricted prepartum regimens for Holstein cows and found that restricted feeding resulted in greater DMI postpartum compared with free-choice feeding prepartum. Higher energy intake in the first 4 wk postpartum may be related to a smaller NEB since the relationship between milk production and $NE_{LI}$ is diminished in this time period (Gerloff, 2000; Hayirli et al., 2002). Time to NEB nadir has been positively associated with time to first ovulation (Beam and Butler, 1999; Butler, 2000). A shorter delay to first ovulation may be positively related to higher conception rates (Butler, 2000). In addition, cows fed CE during the FO period had less BCS loss in the first 6 wk postpartum (Figure 5). Villa-Godoy et al. (1988) suggested that energy intake is the main factor reflecting the degree of body energy loss in early lactation. Butler and Smith (1989) showed that cows losing <1 BCS unit between calving and first service had a mean pregnancy rate of 53% compared with 17% for cows losing >1 BCS unit (on a 1 = thin to 5 = obese scale). In Ireland, a pasture-based system with a fixed breeding calendar, it was found that BCS loss should be limited to 0.5 units to avoid detrimental effects on reproductive performance (Buckley et al., 2003). Furthermore, other physiological variables in the present study were associated with reproduction and affected by prepartum dietary energy concentration.

Cows consuming HE diets had greater concentrations of NEFA in wk 1 postpartum (Table 5). Higher NEFA postpartum were associated with increased risk of cows becoming ill (Cameron et al., 1998; LeBlanc et al., 2005; Ospina et al., 2010). Not surprisingly, diseased cows were associated with poorer reproductive success in our study and in research by others (Curtis et al., 1985; Halpern et al., 1985; Sheldon et al., 2006). Cows receiving CE during CU had higher NEFA concentration than those receiving HE. However, those cows could be better adapted to use NEFA as a metabolic fuel after parturition (Friggens et al., 2004; Janovick et al., 2011).

Higher concentrations of glucose at wk 3 for the group fed CE during the FO period, although modest, were correlated with decreased DTC (Table 5). Higher blood glucose has been associated with improved fertility in Holstein cows (Plym Forshell et al., 1991). Higher blood glucose concentration may reflect greater energy intake by cows previously fed CE during the FO period (Figure 6). Concordantly, cows that received HE during CU had higher blood glucose concentrations, which was a response to the HE regimen (Table 5). Cows in LAG1 were able to maintain higher concentrations of glucose and insulin than those in LAG2. Younger cows are still growing and do not allocate so much energy to milk production, and so have lower milk production when compared to cows with more lactations (Hansen et al., 2006).

Modestly higher insulin concentrations postpartum for cows receiving CE during the FO period may be associated with better glucose clearance and, again, a reflection of higher $NE_{LI}$ postpartum. Increased insulin concentration at wk 2 was slightly associated with higher DTC. Other studies have shown that greater NEB balance during early lactation is related to lower concentrations of plasma insulin in early lactation, and prolonged intervals from calving to first ovulation (Beam and Butler, 1997; Beam and Butler, 1999). Gong et al. (2002) showed that dietary induction of increased insulin concentration reduced time to first ovulation. Nevertheless subsequent fertility parameters, including conception rate to first service and number of services required per conception, were not affected by diet; further work was suggested to determine the effects of the dietary treatment on fertility. Whether the large increase of plasma NEFA concentrations in HE cows around calving was simply a consequence of greater body fat storage or an effect of different insulin sensitivity and responsiveness in muscular and adipose tissues between cows with different BCS cannot be answered by the present work and requires further investigation.

Despite a reduced blood glucose concentration, Radcliff et al. (2006) observed that cows feed-restricted during the early postpartum period did not have reduced blood insulin concentration. This profile can be compared to that of type 2 diabetes in humans where there is no absolute insulin deficiency. Instead, impaired insulin action is thought to be the primary event. Schoenberg et al. (2012) found similar results when comparing dry cows fed at high or low energy intakes. After glucose tolerance tests (GTT) and hyperinsulinemic-euglycemic clamps, cows fed below energy requirements had greater reduction in plasma NEFA during GTT, greater NEFA clearance rate during GTT, and greater area under the curve indicating that those cows had lower insulin resistance related to lipid metabolism. In addition, Kerestes et al. (2009) showed that severe inflammatory diseases such as puerperal metritis with intensive release of pro-inflammatory cytokines potentially depress insulin secretion and decrease whole-body insulin responsiveness in dairy cows, with long-term effects on metabolism and reproduction. Whether cows receiving HE would have greater insulin resistance during the transition period is yet to be determined.

Higher hepatic total lipid concentration at wk −3 was associated with a lesser chance of becoming pregnant (Table 6). Cows receiving HE during FO and CU versus CE for the same periods had higher total lipid concentrations. Drackley et al. (1991) suggested that an excess of energy consumption could lead to increased lipid accumulation in the liver as a result of incapability either to oxidize or export the increased circulating NEFA. The association of liver lipid accumulation and infertility was explored before (Wensing et al., 1997). An in-vitro approach showed that oocytes harvested between 80 and 140 d postpartum had a decreased development capacity in cows with induced hepatic lipidosis postpartum.

Interestingly, cows with higher total lipid and TG:GLY after calving were weakly associated with better reproductive performance. One reasonable explanation could be that, in the present study, a maximum total liver lipid concentration of 12% during the transition period was found. According to Gaal et al. (1983) cows could be biochemically classified as mild fatty liver with total lipid concentrations from 8 to 13%, and more than 13% as moderate; the latter representing damage to the liver cells. In our data, cows classified as having “high” values for liver lipid might reflect cows with greater intake and greater capacity to metabolize circulating fuels because the hepatic lipid infiltration was mild. Epidemiological research, using different locations (herds), with a larger variation in values for liver lipid and TG [such as the one performed by Jorritsma et al. (2000)] would be better suited to rationalize the associations between reproductive success and liver lipid infiltration.

Another factor to be considered is the fact that cows have an individual variation in liver size that can be misleading when liver biopsy is used to determine the physiological capacity of the liver (Haudum et al., 2011). Rukkwamsuk et al. (1999) was unable to show statistical difference between days to first ovulation after calving and cows fed either a high-energy diet or a control diet according to energy requirements. However, when the data were pooled and separated by TG content there was a positive correlation between days to first ovulation and TG. In conclusion, the mechanism of how TG could have affected reproduction remained unclear. Cows that maintained low TG concentrations (between 6 to 17 d postpartum) and produced more milk had better or equal fertility results than cows with comparable levels of TG producing less milk (Jorritsma et al., 2000). Those results could suggest that individual cow variation regarding liver oxidative, storage, and export capacity has an important role in liver metabolism reflecting cow's adaptation to the onset of lactation.

Controlled energy cows had greater hepatic glycogen concentrations at wk 2, similar to findings by Van den Top et al. (1996). In the present study we did not find any association between glycogen and DTC. Harrison et al. (1990) found that high producing cows (10,814 kg, 305-d mature equivalent), in contrast to average producing cows (6,912 kg) had lower hepatic glycogen content and suggested it was involved in regulating days to first visual estrus and DTC, which both occurred much later in the high production group. Higher liver glycogen possibly was associated with better DMI (Grum et al., 2002) and a more available source of energy for reproductive function (Harrison et al., 1990).

## CONCLUSION

Cows that received CE diets during the last 4 wk prepartum had higher hazard ratio for difference in DTC. Such a finding may be explained by the increased $NE_{LI}$ in the first 4 wk postpartum, lower incidence of DISE, and lower incidence of MDISE. In addition, lower BCS loss in the first 6 wk and slightly greater glucose concentrations at wk 3 may have contributed to improved reproductive performance. Energy-limited cows had lower TG concentrations at wk −2, which led to fewer DTC. A strategy of CE prepartum may have a favorable impact on both health and reproductive performance. Research evaluating the impact of CE prepartum on more specific reproduction variables, such as progesterone concentrations, ovarian function, time to first ovulation, and embryonic death is needed.

## REFERENCES


Allison, P. D. 2010. Survival Analysis using SAS®: A Practical Guide. Second Edition, Cary, North Carolina, USA.

Appleman, R. D. 1978. Replacement economics. Page 512 in Large Dairy Herd Management. J. Wilcox, H. H. Van Horn, B. Harris Jr., H. H. Head, S. P. Marshall, W. W. Thatcher, D. W. Webb and J. M. Ming eds. Univ. Presses Florida, Gainesville.

Bauman, D. E. 2000. Regulation of nutrient partitioning during lactation: Homeostasis and homeorhesis revisited. Pages 311-328 in Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction. P. B. Cronje, ed.

Beam, S. W., and W. R. Butler. 1997. Energy balance and ovarian follicle development prior to the first ovulation postpartum in dairy cows receiving three levels of dietary fat. Biol. Reprod. 56:133-142.

Beam, S. W., and W. R. Butler. 1999. Effects of energy balance on follicular development and first ovulation in postpartum dairy cows. J. Reprod. Fertil. Suppl. 54:411-424.

Beever, D. E. 2006. The impact of controlled nutrition during the dry period on dairy cow health, fertility and performance. Anim. Reprod. Sci. 96:212-226.

Bell, A. W. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. J. Anim. Sci. 73:2804-2819.

Buckley, F., K. O'Sullivan, J. F. Mee, R. D. Evans, and P. Dillon. 2003. Relationships among milk yield, body condition, cow weight, and reproduction in spring-calved Holstein-Friesians. J. Dairy Sci. 86:2308-2319.

Butler, W. R. 1998. Review: Effect of protein nutrition on ovarian and uterine physiology in dairy cattle. J. Dairy Sci. 81:2533-2539.

Butler, W. R. 2000. Nutritional interactions with reproductive performance in dairy cattle. Anim. Reprod. Sci. 60-61:449-457.

Butler, W. R., and R. D. Smith. 1989. Interrelationships between energy balance and postpartum reproductive function in dairy cattle. J. Dairy Sci. 72:767-783.

Cameron, R. E., P. B. Dyk, T. H. Herdt, J. B. Kaneene, R. Miller, H. F. Bucholtz, J. S. Liesman, M. J. VandeHaar, and R. S. Emery. 1998. Dry cow diet, management, and energy balance as risk factors for displaced abomasum in high producing dairy herds. J. Dairy Sci. 81:132-139.

Carlson, D. B., J. W. McFadden, A. D'Angelo, J. C. Woodworth, and J. K. Drackley. 2007. Dietary L-carnitine affects periparturient nutrient metabolism and lactation in multiparous cows. J. Dairy Sci. 90:3422-3441.

Curtis, C. R., H. N. Erb, C. J. Sniffen, R. D. Smith, and D. S. Kronfeld. 1985. Path analysis of dry period nutrition, postpartum metabolic and reproductive disorders, and mastitis in Holstein cows. J. Dairy Sci. 68:2347-2360.

Dann, H. M., N. B. Litherland, J. P. Underwood, M. Bionaz, A. D'Angelo, J. W. McFadden, and J. K. Drackley. 2006. Diets during far-off and close-up dry periods affect periparturient metabolism and lactation in multiparous cows. J. Dairy Sci. 89:3563-3577.

Dann, H. M., D. E. Morin, G. A. Bollero, M. R. Murphy, and J. K. Drackley. 2005. Prepartum intake, postpartum induction of ketosis, and periparturient disorders affect the metabolic status of dairy cows. J. Dairy Sci. 88:3249-3264.

Douglas, G. N., T. R. Overton, H. G. Bateman 2nd, H. M. Dann, and J. K. Drackley. 2006. Prepartal plane of nutrition, regardless of dietary energy source, affects periparturient metabolism and dry matter intake in Holstein cows. J. Dairy Sci. 89:2141-2157.

Douglas, G. N., T. R. Overton, H. G. Bateman, and J. K. Drackley. 2004. Peripartal metabolism and production of Holstein cows fed diets supplemented with fat during the dry period. J. Dairy Sci. 87:4210-4220.

Drackley, J. K., H. M. Dann, and N. A. Janovick Guretzky. 2007. New concepts for feeding dry cows. In Proceedings of the Tri-State Dairy Nutrition Conference, Fort Wayne, IN, USA. Pages 17-28. Edited by M. Eastridge, Ohio State University, Columbus.

Drackley, J. K., H. M. Dann, N. A. Janovick, N. B. Litherland, J. P. Underwood and J. J. Loor. 2005. Physiological and pathological adaptations in dairy cows that may increase susceptibility to periparturient diseases and disorders. Ital. J. Anim. Sci. 4:323-344.

Drackley, J. K., J. J. Veenhuizen, M. J. Richard, and J. W. Young. 1991. Metabolic changes in blood and liver of dairy cows during either feed restriction or administration of 1,3-butanediol. J. Dairy Sci. 74:4254-4264.

Ferguson, J. D., D. T. Galligan, and N. Thomsen. 1994. Principal descriptors of body condition score in Holstein cows. J. Dairy Sci. 77:2695-2703.

Fletcher, M. J. 1968. A colorimetric method for estimating serum triglycerides. Clin. Chim. Acta 22:393-397.

Foster, L. B., and R. T. Dunn. 1973. Stable reagents for determination of serum triglycerides by a colorimetric hantzsch condensation method. Clin. Chem. 19:338-340.

Friggens, N. C., J. B. Andersen, T. Larsen, O. Aaes, and R. J. Dewhurst. 2004. Priming the dairy cow for lactation: A review of dry cow feeding strategies. Anim. Res. 53:453-473.

Gaal, T., I. M. Reid, R. A. Collins, C. J. Roberts, and B. V. Pike. 1983. Comparison of biochemical and histological methods of estimating fat content of liver of dairy cows. Res. Vet. Sci. 34:245-248.

Gerloff, B. J. 2000. Dry cow management for the prevention of ketosis and fatty liver in dairy cows. Vet. Clin. North Am. Food Anim. Pract. 16:283-292.

Gong, J. G., W. J. Lee, P. C. Garnsworthy, and R. Webb. 2002. Effect of dietary-induced increases in circulating insulin concentrations during the early postpartum period on reproductive function in dairy cows. Reproduction 123:419-427.

Grohn, Y. T., S. W. Eicker, V. Ducrocq, and J. A. Hertl. 1998. Effect of diseases on the culling of Holstein dairy cows in New York State. J. Dairy Sci. 81:966-978.

Grohn, Y. T., and P. J. Rajala-Schultz. 2000. Epidemiology of reproductive performance in dairy cows. Anim. Reprod. Sci. 60-61:605-614.

Grum, D. E., J. K. Drackley, and J. H. Clark. 2002. Fatty acid metabolism in liver of dairy cows fed supplemental fat and nicotinic acid during an entire lactation. J. Dairy Sci. 85:3026-3034.

Grum, D. E., J. K. Drackley, R. S. Younker, D. W. LaCount, and J. J. Veenhuizen. 1996. Nutrition during the dry period and hepatic lipid metabolism of periparturient dairy cows. J. Dairy Sci. 79:1850-1864.

Halpern, N. E., H. N. Erb, and R. D. Smith. 1985. Duration of retained placenta and subsequent fertility in dairy cows. Theriogenology 23:807-813.

Hansen, J. V., N. C. Friggens, and S. Højsgaard. 2006. The influence of breed and parity on milk yield, and milk yield acceleration curves. Livest. Sci. 104:53-62.

Hara, A., and N. S. Radin. 1978. Lipid extraction of tissues with a low-toxicity solvent. Anal. Biochem. 90:420-426.

Harrison, R. O., S. P. Ford, J. W. Young, A. J. Conley, and A. E. Freeman. 1990. Increased milk production versus reproductive and energy status of high producing dairy cows. J. Dairy Sci. 73:2749-2758.

Haudum, A., A. Starke, M. Beyerbach, P. Wohlsein, and J. Rehage. 2011. Ultrasonographic assessment of liver dimensions in dairy cows with different hepatic triglyceride content. J. Anim. Sci. 89:1392-1400.

Hayirli, A., R. R. Grummer, E. V. Nordheim, and P. M. Crump. 2002. Animal and dietary factors affecting feed intake during the prefresh transition period in Holsteins. J. Dairy Sci. 85:3430-3443.

Holcomb, C. S., H. H. Van Horn, H. H. Head, M. B. Hall, and C. J. Wilcox. 2001. Effects of prepartum dry matter intake and forage percentage on postpartum performance of lactating dairy cows. J. Dairy Sci. 84:2051-2058.

Hughes, J. P. 1962. A simplified instrument for obtaining liver biopsies in cattle. Am. J. Vet. Res. 23:1111-1113.

Janovick, N. A., Y. R. Boisclair, and J. K. Drackley. 2011. Prepartum dietary energy intake affects metabolism and health during the periparturient period in primiparous and multiparous Holstein cows. J. Dairy Sci. 94:1385-1400.

Janovick, N. A., and J. K. Drackley. 2010. Prepartum dietary management of energy intake affects postpartum intake and lactation performance by primiparous and multiparous Holstein cows. J. Dairy Sci. 93:3086-3102.

Johnson, M. M., and J. P. Peters. 1993. Technical note: An improved method to quantify nonesterified fatty acids in bovine plasma. J. Anim. Sci. 71:753-756.

Jorritsma, R., H. Jorritsma, Y. H. Schukken, and G. H. Wentink. 2000. Relationships between fatty liver and fertility and some periparturient diseases in commercial Dutch dairy herds. Theriogenology 54:1065-1074.

Jorritsma, R., T. Wensing, T. A. Kruip, P. L. Vos, and J. P. Noordhuizen. 2003. Metabolic changes in early lactation and impaired reproductive performance in dairy cows. Vet. Res. 34:11-26.

Kerestes, M., V. Faigl, M. Kulcsar, O. Balogh, J. Foldi, H. Febel, Y. Chilliard, and G. Huszenicza. 2009. Periparturient insulin secretion and whole-body insulin responsiveness in dairy cows showing various forms of ketone pattern with or without puerperal metritis. Domest. Anim. Endocrinol. 37:250-261.

LeBlanc, S. J., K. E. Leslie, and T. F. Duffield. 2005. Metabolic predictors of displaced abomasum in dairy cattle. J. Dairy Sci. 88:159-170.

Litherland, N. B., H. M. Dann, and J. K. Drackley. 2011. Prepartum nutrient intake alters palmitate metabolism by liver slices from peripartal dairy cows. J. Dairy Sci. 94:1928-1940.

Lo, S., J. C. Russell, and A. W. Taylor. 1970. Determination of glycogen in small tissue samples. J. Appl. Physiol. 28:234-236.

Lucy, M. C. 2001. Reproductive loss in high-producing dairy cattle: Where will it end? J. Dairy Sci. 84:1277-1293.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. National Academy Press, Washington, DC.

Nolan, R., D. O'Callaghan, R. T. Duby, P. Lonergan, and M. P. Boland. 1998. The influence of short-term nutrient changes on follicle growth and embryo production following superovulation in beef heifers. Theriogenology 50:1263-1274.

Ospina, P. A., D. V. Nydam, T. Stokol, and T. R. Overton. 2010. Evaluation of nonesterified fatty acids and beta-hydroxybutyrate in transition dairy cattle in the northeastern United States: Critical thresholds for prediction of clinical diseases. J. Dairy Sci. 93:546-554.

Parr, R. A., I. F. Davis, M. A. Miles, and T. J. Squires. 1993. Feed intake affects metabolic clearance rate of progesterone in sheep. Res. Vet. Sci. 55:306-310.

Peterson, J. I., and D. S. Young. 1968. Evaluation of the hexokinase/glucose-6-phosphate dehydrogenase method of determination of glucose in urine. Anal. Biochem. 23:301-316.

Plym Forshell, K., L. Andersson, and B. Pehrson. 1991. The relationships between the fertility of dairy cows and clinical and biochemical measurements, with special reference to plasma glucose and milk acetone. Zentralbl. Veterinarmed. A 38:608-616.

Quaiffe, T. 2002. Don't blame the big guys for higher heifer prices. Pages 26-28 in Dairy Herd Management Magazine. January. Vance Publishing Corporation.

Radcliff, R. P., B. L. McCormack, D. H. Keisler, B. A. Crooker, and M. C. Lucy. 2006. Partial feed restriction decreases growth hormone receptor 1A mRNA expression in postpartum dairy cows. J. Dairy Sci. 89:611-619.

Richards, B. F. 2011. Strategies to Decrease Incidence of Fatty Liver in Dairy Cows. PhD Dissertation, University of Illinois, Urbana.

Rukkwamsuk, T., T. Wensing, and T. A. M. Kruip. 1999. Relationship between triglyceride concentration in the liver and first ovulation in postpartum dairy cows. Theriogenology 51:1133-1142.

Schoenberg, K. M., R. M. Ehrhardt, and T. R. Overton. 2012. Effect of plane of nutrition and feed deprivation on insulin responses in dairy cattle during late gestation. J. Dairy Sci. 95:670-682.

Sheldon, I. M., G. S. Lewis, S. LeBlanc, and R. O. Gilbert. 2006. Defining postpartum uterine disease in cattle. Theriogenology 65:1516-1530.

St-Pierre, N. R. 2001. Invited review: Integrating quantitative findings from multiple studies using mixed model methodology. J. Dairy Sci. 84:741-755.

Studer, V. A., R. R. Grummer, S. J. Bertics, and C. K. Reynolds. 1993. Effect of prepartum propylene glycol administration on periparturient fatty liver in dairy cows. J. Dairy Sci. 76:2931-2939.

Trinder, P. 1969. Determination of blood glucose using 4-amino phenazone as oxygen acceptor. J. Clin. Pathol. 22:246.

Van den Top, A. M., M. J. Geelen, T. Wensing, G. H. Wentink, A. T. Van't Klooster, and A. C. Beynen. 1996. Higher postpartum hepatic triglyceride concentrations in dairy cows with free rather than restricted access to feed during the dry period are associated with lower activities of hepatic glycerolphosphate acyltransferase. J. Nutr. 126:76-85.

Villa-Godoy, A., T. L. Hughes, R. S. Emery, L. T. Chapin, and R. L. Fogwell. 1988. Association between energy balance and luteal function in lactating dairy cows. J. Dairy Sci. 71:1063-1072.

Wensing, T., T. Kruip, M. J. H. Geelen, G. H. Wentink, and Van den Top, A. M. 1997. Postpartum fatty liver in high-producing dairy cows in practice and in animal studies. the connection with health, production and reproduction problems. Comp. Haematol. Intl. 7:167-171.

**Table 1.** Experiments used to develop the meta-analysis database

| EXP | Description | Reference |
|---|---|---|
| 1 | 59 multiparous Holstein cows receiving control diet, moderately high diet in nonfiber carbohydrates and isocaloric fat-supplemented, low NFC diet prepartum. FO from -60 d to until -14 d before calving. CU from -14 d to calving. | Douglas et al., (2006) |
| 2 | 34 multiparous Holstein cows receiving restricted (80%$NE_L$ reqt.) and ad libitum dietary treatments prepartum. FO from -60 d to until -21 d before calving. CU from -21 d to calving. | Dann et al., (2005) |
| 3 | 82 multiparous Holstein cows receiving restricted (80%$NE_L$ reqt.), control (100% $NE_L$ reqt.) and ad libitum(150%$NE_L$ reqt.) dietary treatments prepartum. FO from dry-off until -25 d before calving. CU from -25 d to calving. | Dann et al., (2006) |
| 4 | 29 multiparous Holstein cows receiving high forage, high forage plus fat and high grain diet dietary treatments prepartum. FO from -60 d until -7 d before calving. CU from -7 d to calving. | Grum et al., (1996) |
| 5 | 52 multiparous Holstein cows receiving 4 amounts of supplemental carnitine from -25 d prepartum trough 56 d in milk. FO dry-off until -25 d before calving. CU from -25 d to calving. | Carlson et al., (2007) |
| 6 | 25 multiparous and 23 primiparous Holstein cows receiving different levels (80%$NE_L$ reqt.), control (100% $NE_L$ reqt.) and ad libitum (150%$NE_L$ reqt.) dietary treatments prepartum. FO from -65 d until -21 d before calving. CU from -21 d to calving. | Janovick and Drackley, (2010) |
| 7 | 73 multiparous and 31 primiparous Holstein cows receiving controlled-energy high-fiber diet (CEHF, 1.34 Mcal $NE_L$ /kg DM ad libitum), overfed diet (OVERFED, 1.61 Mcal $NE_L$ /kg DM ad libitum) and 2-stage treatment (2-stage, CEHF from dry-off until 21 d prepartum followed by OVERFED until parturition). FO from -60 d until -21 d before calving. CU from -21 d to calving. | Richards, (2011) |

EXP: Experiment.
FO: Far-off.
CU: Close-up.

**Table 2.** Descriptive statistics from treatment diets for physical characteristics and its impact on $NE_{LI}$[1] analyzed from a database with 408 cows

| | $NE_{LI}$ (Mcal/d and SD)[1] | PSPS (% and SD)[2] | | |
|---|---|---|---|---|
| | | >19 mm | 8-19 mm | <8 mm |
| FO | | | | |
| HE | 20.2 ± 6.8 | | | |
| CE | 12.1 ± 4.2 | | | |
| CU | | | | |
| HE | 19.8 ± 6.8 | 6.44 ± 1.3 | 43.08 ± 1.3 | 50.48 ± 3.1 |
| CE | 12.1 ± 4.7 | 21.8 ± 2.6 | 35.2 ± 1.7 | 43 ± 1.4 |

[1] Net energy of lactation intake (Diet $NE_L$ × DMI) median during FO and CU.
[2] Mean and standard deviation (FO and CU) for offered diet as determined by Penn State particle separator from experiments 3, 6, and 7.
FO: Far-off.
CU: Close-up.
HE: High energy.
CE: Controlled energy.

**Table 3.** Final Cox proportional hazard model of time to conception (DTC) affected by different dietary treatments prepartum in 332 Holstein cows, accounting for experiment (n = 7) as a cluster effect

| Variable | Level | Coefficient | SEM | Hazard Ratio | 95% CI | *P*-value |
|---|---|---|---|---|---|---|
| FO | HE | 0.2061 | 0.19 | 1.229 | 0.839 - 1.801 | 0.29 |
| CU | HE | −0.3626 | 0.18 | 0.696 | 0.489 - 0.990 | 0.04 |
| CAVS | 2 | 0.1414 | 0.28 | 1.152 | 0.666 - 1.991 | 0.61 |
| | 3 | 0.6807 | 0.26 | 1.975 | 1.178 - 3.314 | <0.01 |
| | 4 | 0.5582 | 0.28 | 1.748 | 1.010 - 3.024 | 0.04 |
| RPAT | yes | −0.4411 | 0.16 | 0.643 | 0.467 - 0.887 | <0.01 |

FO: Far-off.
CU: Close-up.
CAVS: Calving season; 1 (winter, referent), 2 (spring), 3 (summer) and 4 (autumn).
RPAT: reproduction pathology (if any metritis, ovarian cyst, retained placenta)
HE: High energy diet compared to controlled energy (CE) diet.

**Table 4.** Logistic regression model of twins and dystocia affected by different dietary treatments prepartum in Holstein cows, accounting for experiment (n = 7) as a cluster effect

| Variable | Level | Odds Ratio | 95% CI | *P*-value |
|---|---|---|---|---|
| Twin[1] | | | | |
| FO | HE | 0.717 | 0.287 - 1.794 | 0.47 |
| CU | HE | 0.684 | 0.275 - 1.707 | 0.41 |
| | | | | |
| Dystocia[2] | | | | |
| FO | HE | 0.945 | 0.410 - 2.178 | 0.89 |
| CU | HE | 0.779 | 0.339 - 1.792 | 0.55 |

FO: Far-off.
CU: Close-up.
HE: High energy diet compared to controlled energy (CE) diet.
[1] 36 cows had twins and 252 had single calving (n = 289)
[2] 41 cows had dystocia and 163 had single calving (n = 204)

**Table 5.** Least squares means for blood metabolites and liver biopsies from Holstein cows fed different dietary treatments prepartum

| Variable | Wk | N | FO[1] | | | CU[2] | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | HE[3] | CE[4] | *P*-value | HE | CE | SEM[5] | *P*-value |
| Blood | | | | | | | | | |
| NEFA, μEq/L | -2 | 351 | 251.74 | 224.30 | 0.15 | 181.09 | 311.81 | 1.12 | <0.01 |
| | -1 | 349 | 456.05 | 408.10 | 0.14 | 362.60 | 513.27 | 1.12 | <0.01 |
| | 1 | 346 | 816.56 | 667.14 | <0.01 | 739.96 | 736.20 | 1.17 | 0.93 |
| | 2 | 309 | 617.95 | 508.11 | 0.01 | 597.05 | 525.89 | 1.21 | 0.09 |
| | 3 | 331 | 345.64 | 271.84 | <0.01 | 330.56 | 284.21 | 1.20 | 0.05 |
| Glucose, mg/dL | -2 | 349 | 62.35 | 61.15 | 0.22 | 63.15 | 60.35 | 2.37 | <0.01 |
| | -1 | 337 | 59.67 | 59.13 | 0.61 | 61.85 | 56.95 | 1.66 | <0.01 |
| | 1 | 343 | 55.12 | 57.35 | 0.08 | 57.06 | 55.41 | 1.90 | 0.19 |
| | 3 | 331 | 58.17 | 60.44 | 0.03 | 59.54 | 59.07 | 2.00 | 0.64 |
| Insulin, μIU/mL | -2 | 312 | 4.43 | 5.15 | 0.15 | 6.92 | 3.30 | 1.25 | <0.01 |
| | -1 | 217 | 2.85 | 3.30 | 0.21 | 3.96 | 2.37 | 1.33 | <0.01[6] |
| | 1 | 315 | 2.79 | 4.10 | <0.01 | 3.94 | 2.90 | 1.25 | <0.01 |
| | 2 | 293 | 2.73 | 3.89 | <0.01 | 3.70 | 2.87 | 1.29 | 0.02 |
| Liver | | | | | | | | | |
| Lipids, % wet wt | -2 | 180 | 4.47 | 4.29 | 0.20 | 4.24 | 4.52 | 1.02 | 0.04[7] |
| | 1 | 181 | 6.48 | 5.43 | <0.01 | 6.17 | 5.70 | 1.07 | 0.17 |
| | 2 | 195 | 8.22 | 6.51 | 0.01 | 7.63 | 7.00 | 1.12 | 0.32 |
| TG, % wet wt | -2 | 175 | 0.34 | 0.31 | 0.18 | 0.27 | 0.39 | 1.08 | <0.01[8] |
| | 1 | 181 | 1.90 | 1.26 | 0.02 | 1.66 | 1.44 | 1.31 | 0.38 |
| | 2 | 196 | 2.45 | 1.42 | <0.01 | 1.93 | 1.80 | 1.20 | 0.70 |
| Glycogen, % wet wt | -2 | 159 | 12.91 | 15.52 | 0.38 | 17.04 | 11.77 | 2.74 | 0.06 |
| | 2 | 179 | 7.96 | 9.60 | 0.24 | 7.36 | 10.39 | 1.66 | 0.03 |
| TG:GLY | -2 | 150 | 0.16 | 0.14 | 0.34 | 0.12 | 0.20 | 1.73 | <0.01 |
| | 1 | 125 | 1.20 | 0.61 | 0.03 | 0.85 | 0.86 | 1.46 | 0.98 |
| | 2 | 176 | 1.45 | 0.82 | 0.02 | 1.16 | 1.02 | 1.58 | 0.56 |

[1] Far-off.
[2] Close-up.
[3] High energy diet.
[4] Controlled energy diet.
[5] Standard error of the mean.
[6] Interaction (*P* =0.07) cows fed CE during FO and CU against cows fed HE during FO and CU.
[7] Interaction (*P* < 0.01) cows fed CE during FO and CU against cows fed HE during FO and fed CE during CU.
[8] Interaction (*P* = 0.04) cows fed HE during FO and CU against cows fed CE during FO and fed CE during CU.
Least squares means and SEM back transformed from natural log for variables NEFA, insulin, lipids, TG, glycogen and TG:GLY.

**Table 6.** Final Cox proportional hazard model of time to conception (DTC) affected by blood metabolites and liver contents in Holstein cows, accounting for experiment (n = 7) as a cluster effect

| Variable | Wk | N | Level | Coefficient | SEM[1] | Hazard Ratio | 95% CI[2] | *P*-value |
|---|---|---|---|---|---|---|---|---|
| Blood | | | | | | | | |
| Glucose, mg/dL | 3 | 360 | H | 0.2884 | 0.14 | 1.334 | 1.005 - 1.771 | 0.05 |
| | 4 | 325 | H | 0.3323 | 0.16 | 1.394 | 1.011 - 1.923 | 0.04 |
| Insulin, µIU/mL | 2 | 323 | | −0.0453 | 0.02 | 0.956 | 0.923 - 0.999 | 0.01 |
| | | | | | | | | |
| Liver | | | | | | | | |
| Lipids, % wet wt | -3 | 131 | H | -0.7603 | 0.33 | 0.468 | 0.242 - 0.902 | 0.02 |
| | | | M | −0.1560 | 0.22 | 0.855 | 0.548 - 1.336 | 0.49 |
| | 4 | 166 | - | 0.0891 | 0.03 | 1.093 | 1.030 - 1.160 | <0.01 |
| TG, % wet wt | -3 | 131 | H | −0.7244 | 0.45 | 0.485 | 0.199 - 1.179 | 0.11 |
| | | | M | 0.4504 | 0.36 | 1.569 | 0.775 - 3.177 | 0.21 |
| | 2 | 226 | - | 0.0645 | 0.02 | 1.067 | 1.013 - 1.123 | 0.01 |
| | 4 | 167 | - | 0.1300 | 0.03 | 1.139 | 1.064 - 1.220 | <0.01 |
| TG:GLY | 2 | 206 | H | 0.4642 | 0.22 | 1.591 | 1.032 - 2.452 | 0.04 |
| | | | M | 0.5053 | 0.18 | 1.657 | 1.152 - 2.385 | 0.01 |
| | 4 | 167 | H | 0.4665 | 0.23 | 1.594 | 1.015 - 2.504 | 0.04 |
| | | | M | −0.0232 | 0.21 | 0.977 | 0.637 - 1.499 | 0.92 |

[1] Standard error of the mean.

[2] 95 % confidence interval.

Lipids wk -3 quartiles: low <4% (L; referent) ; moderate 4 to 5% (M) and high > 5% (H). Lipids wk 4; continuous variable. Triglyceride (TG) at wk -3 low <0.5% (L, referent); moderate 0.5 to 1% (M); high .1% (H). TG at wk 2 and wk 4; continuous variable. TG:GLY at wk 2, quartiles low < 0.8 (L, referent); moderate 0.8 to 2 (M); high >2 (H). TG:GLY at wk 4, quartiles low < 0.1 (L, referent); moderate 0.1 to 0.6 (M); high >0.6 (H). Glucose quartiles wk 3, low < 60mg.dL (L, referent); high > 60mg/dL (H). Glucose quartiles wk 4, low < 65mg.dL (L, referent); high > 65mg/dL (H).

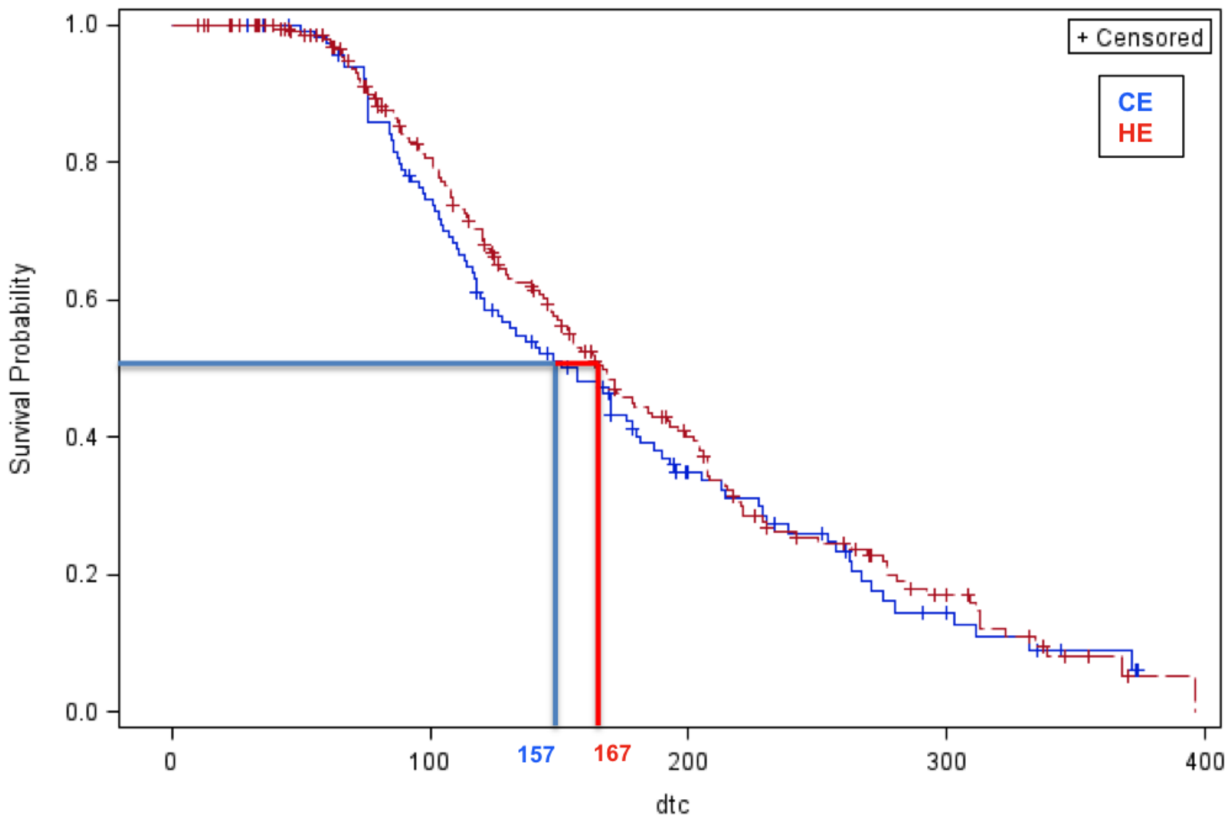


**Figure 3.** Survival function curves for days to conception (DTC) for 332 Holstein cows fed either controlled energy (CE = blue) or high energy (HE = red) during the last four weeks before calving. Blue and red lines represent median values for DTC when 50% of the cows were pregnant.

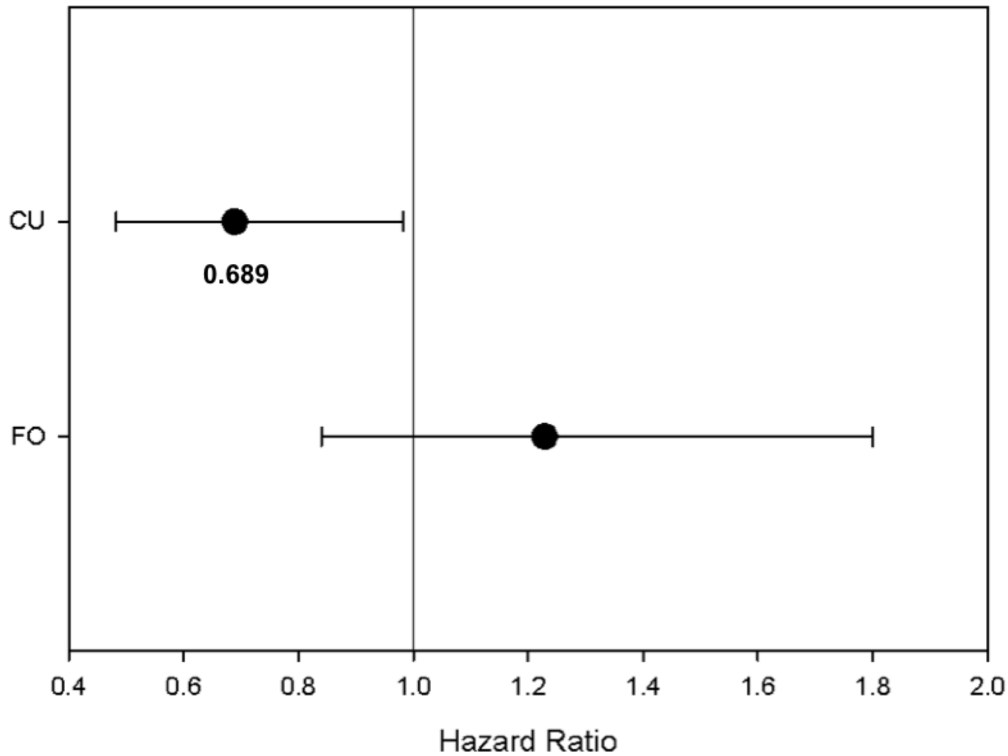


**Figure 4.** Hazard ratios and 95% confidence intervals for the association of high energy diet (HE) and control energy diet (CE, referent = line) with days to conception (DTC) ($P$ = 0.04). CU: Close-up. FO: Far-off.

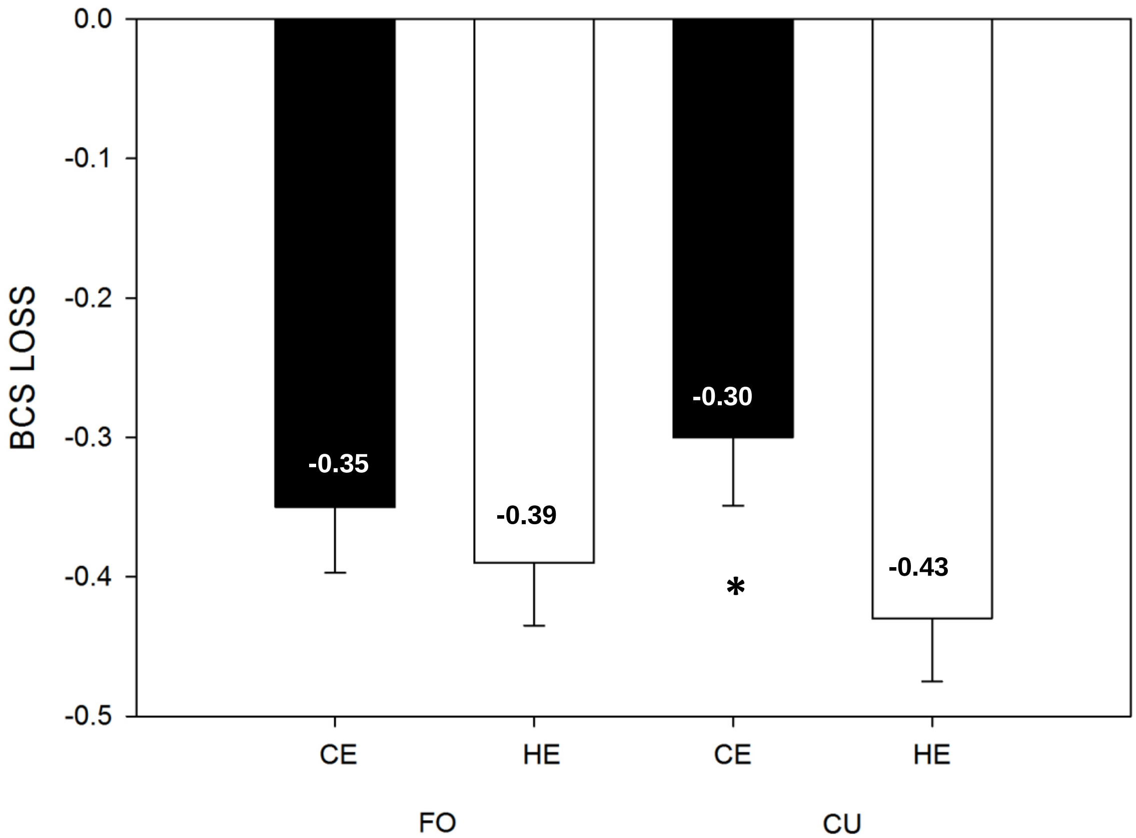


**Figure 5.** Body condition score (BCS) loss from wk 6 to wk 1 postpartum from cows receiving different dietary treatments prepartum. BCS (1-5) least squares means and standard errors. * $P$ = 0.04, CU: Close-up, FO: Far-off, HE: High energy, and CE: Controlled energy.

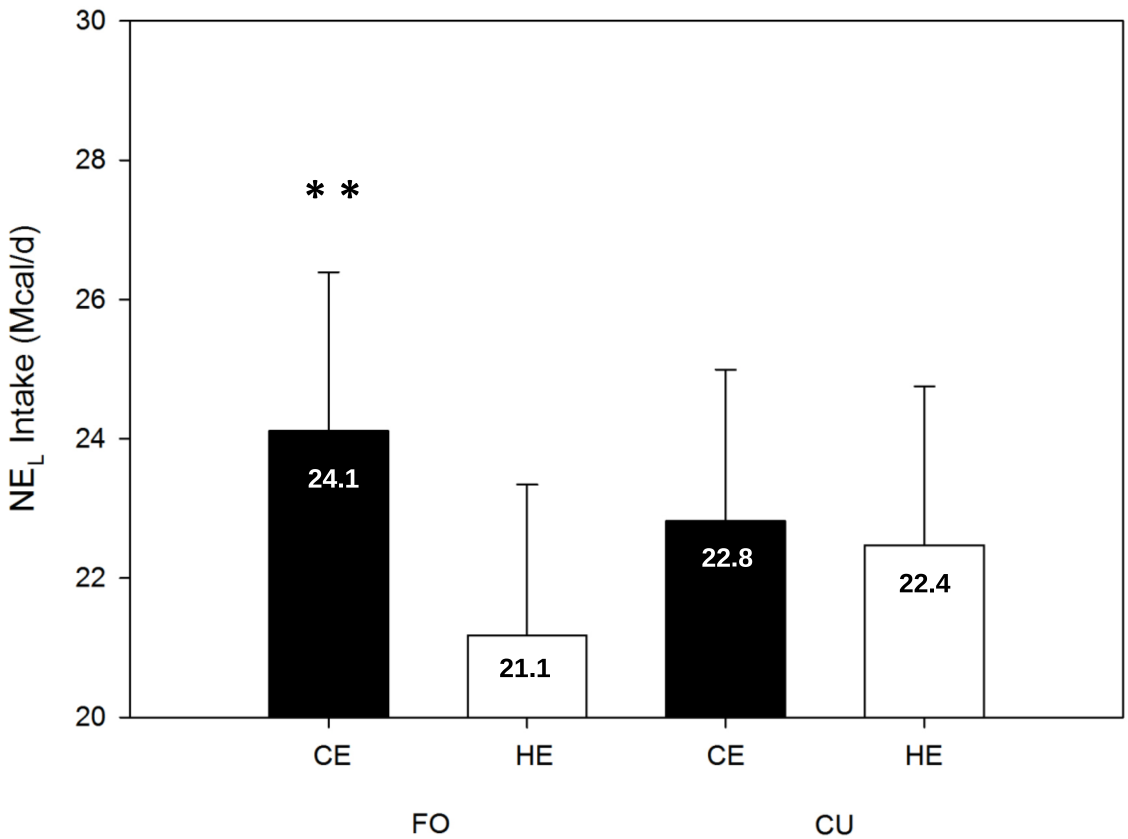


**Figure 6.** $NE_L$ intake (Mcal/d) least squares means and standard errors for the first 4 wk after calving, from different dietary treatments prepartum. ** *P* = 0.01, CU: Close-up, FO: Far-off, HE: High energy, and CE: Controlled energy.

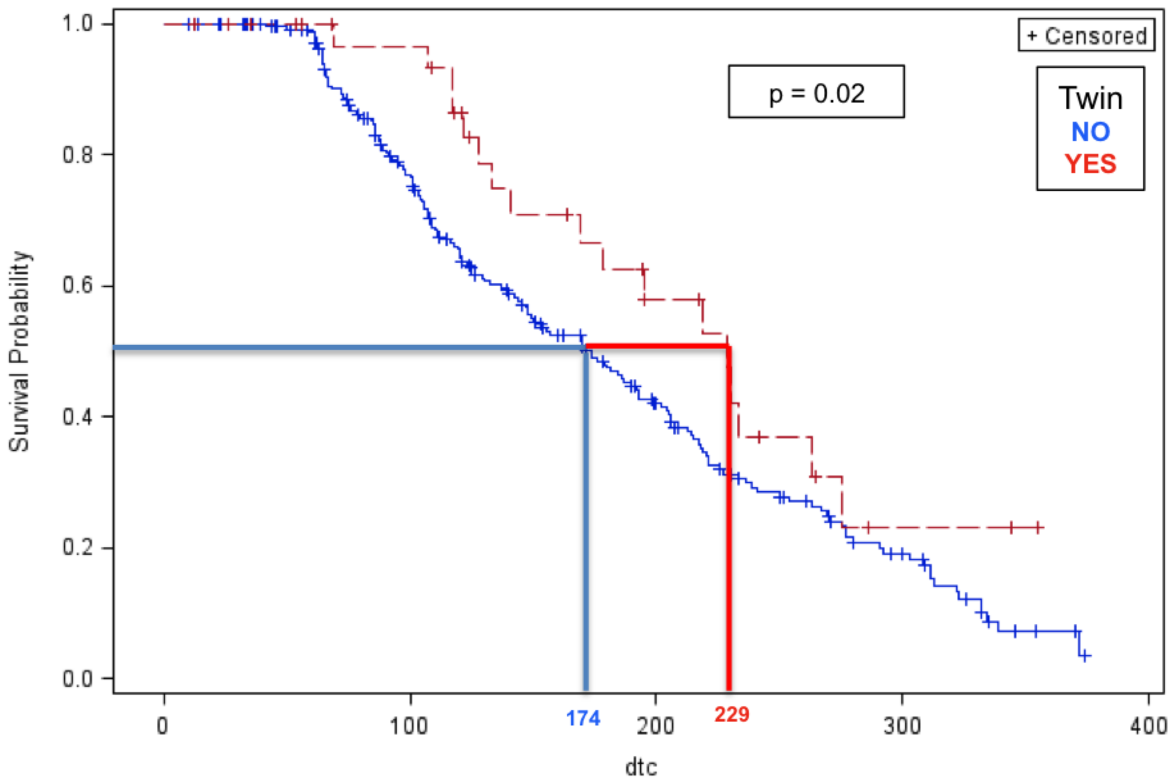


**Figure 7**. Survival function curves for days to conception (DTC) for 289 Holstein cows having twins [Twin = yes (red)] or not [Twin = no (blue)]. Blue and red lines represent median values for DTC when 50% of the cows were pregnant.

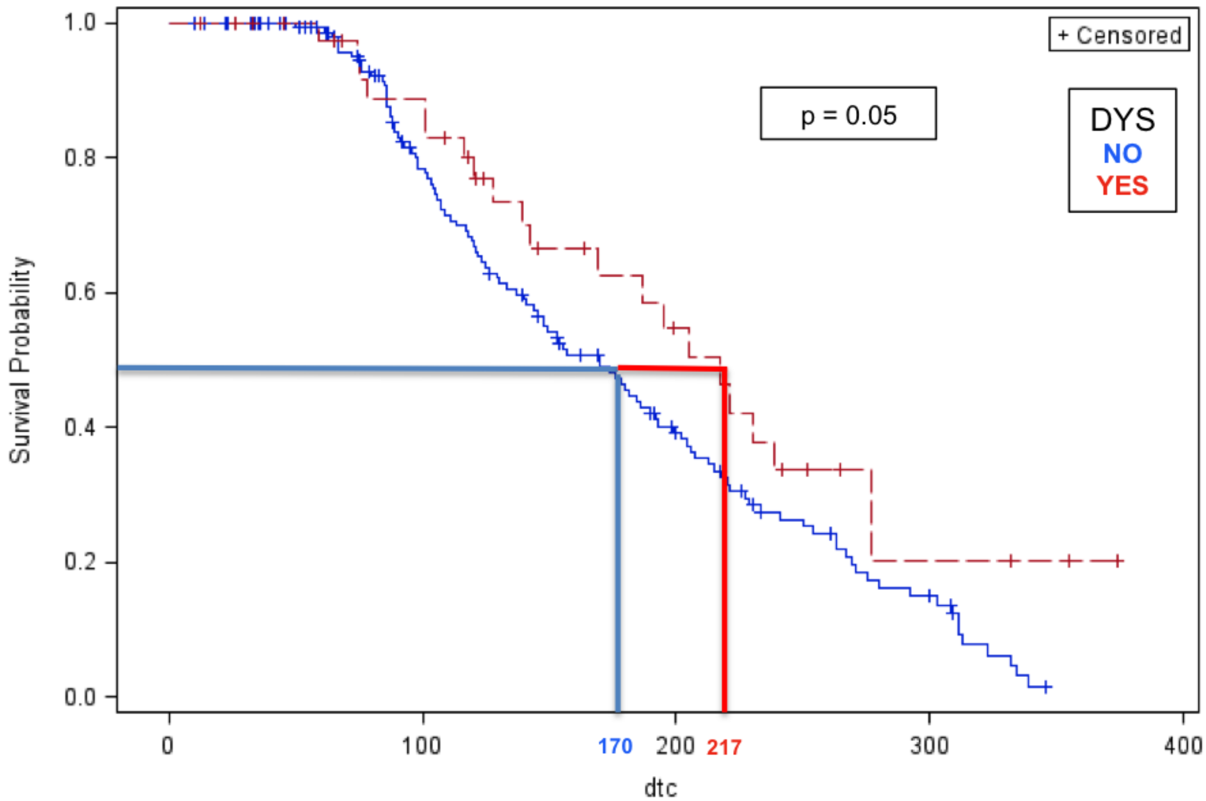


**Figure 8**. Survival function curves for days to conception (DTC) for 204 Holstein cows with dystocia [DYS = yes (red)] or not [DYS = no (blue]. Blue and red lines represent median values for DTC when 50% of the cows were pregnant.

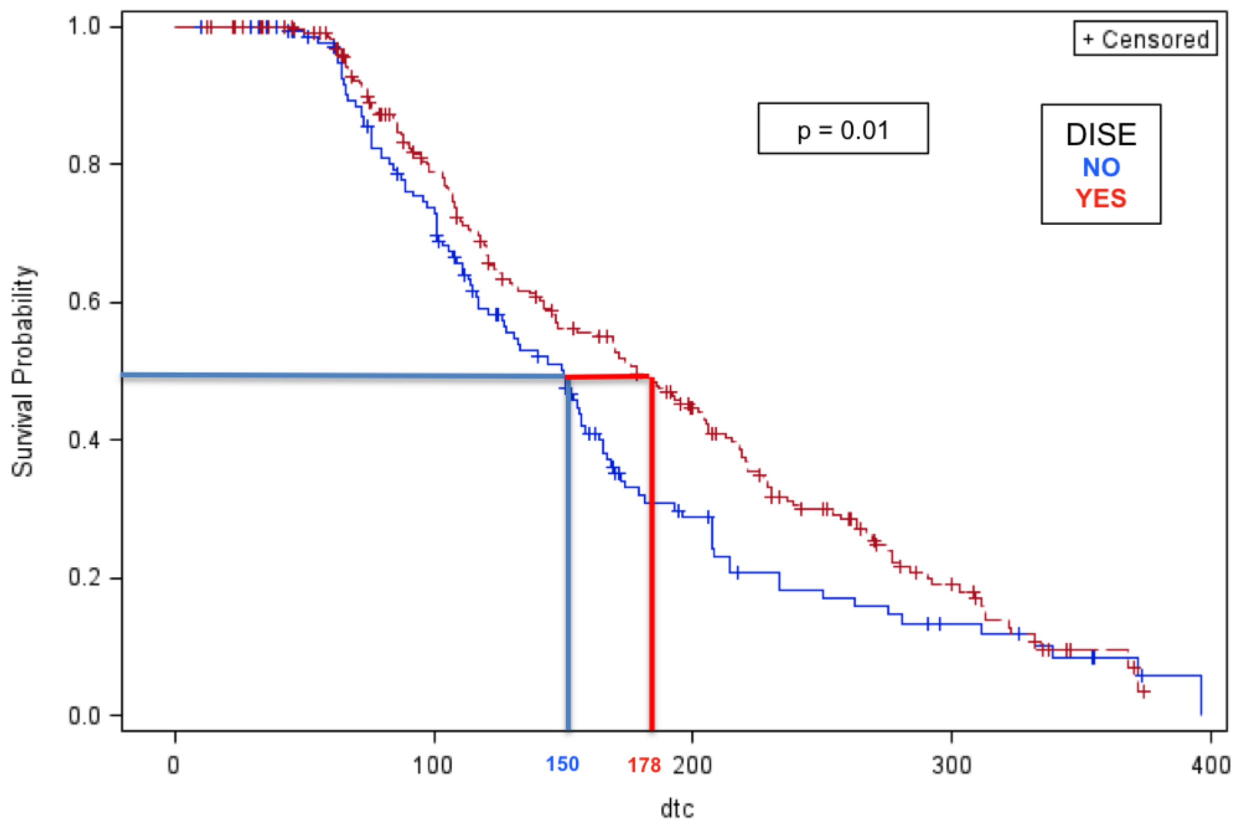


**Figure 9**. Survival function curves for days to conception (DTC) for 380 Holstein cows with disease [DISE = yes (red)] or not [DISE = no (blue)]. Blue and red lines represent median values for DTC when 50% of the cows were pregnant.

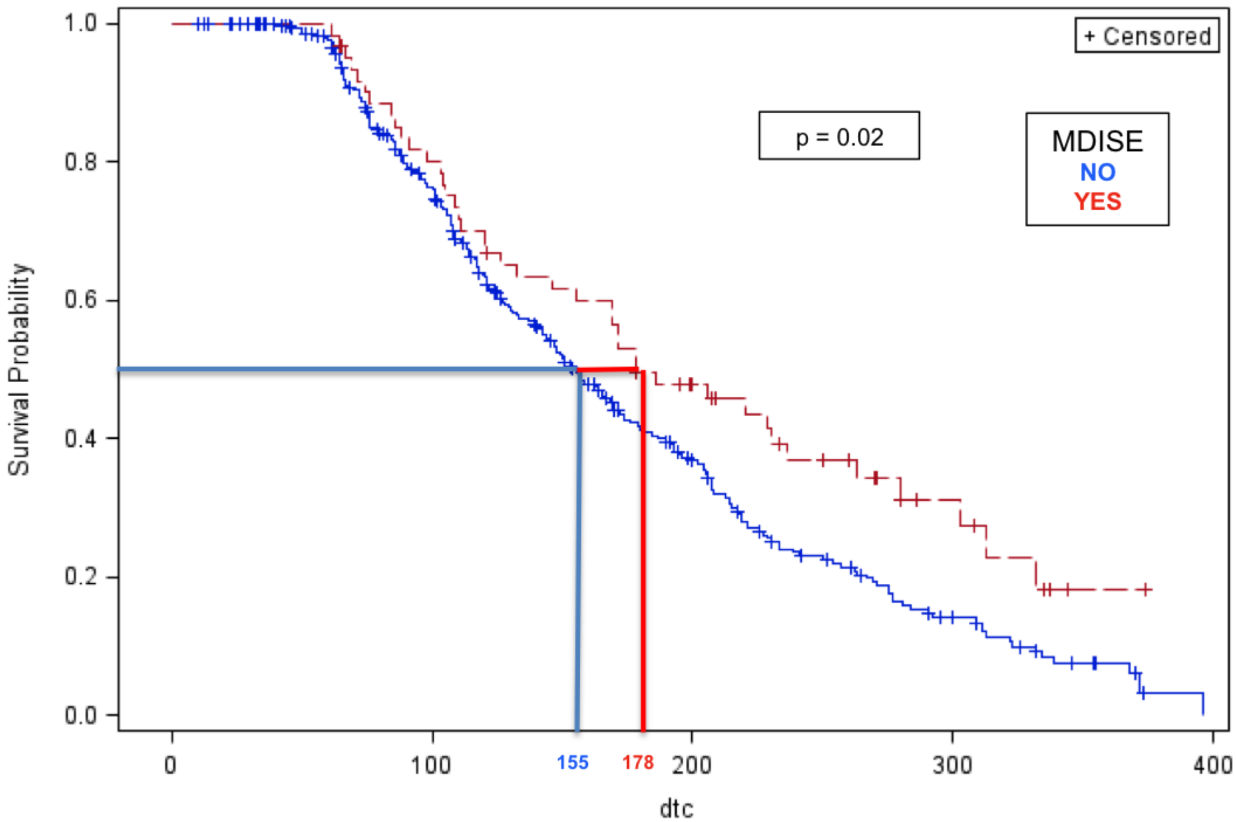

**Figure 10**. Survival function curves for days to conception (DTC) for 380 Holstein cows with multiple diseases [MDISE = yes (red)] or not [MDISE = no (blue)]. Blue and red lines represent median values for DTC when 50% of the cows were pregnant.

**CHAPTER 3**

# EFFECTS OF PREPARTUM FEEDING STRATEGY ON HEALTH AND PRODUCTIVE PARAMETERS IN DAIRY COWS

## ABSTRACT

To investigate the association between prepartum energy feeding regimen and productive performance and health, cow-level data from 7 different experiments completed in our group from 1993 to 2010 were analyzed. Milk production, milk components, BW, cholesterol, displacement of abomasum (DA), and ketosis (KET) were the variables used to assess productive performance and health status. A total of 408 cows (354 multiparous and 54 primiparous) were included. The net energy for lactation ($NE_L$) intake ($NE_{LI}$) was calculated from the cow's respective dietary $NE_L$ density and average DMI. Treatments consisted of controlled energy (CE; median $NE_{LI}$ = 13.7 Mcal/d) or high energy (HE; median $NE_{LI}$ = 22.1 Mcal/d) diets fed during the far-off (FO) or close-up (CU) dry periods. There was no statistical difference for milk production in the first 4 wk postpartum between cows fed CE or HE prepartum ($P > 0.14$). Cows fed HE during CU had greater milk fat concentration in wk 2, 3 and 4 than cows fed CE ($P < 0.05$). Cows fed HE during CU had higher protein concentration during wk 3 and 4 then cows fed CE ($P \leq 0.05$). Cows that were fed HE during CU lost more BW either as absolute amounts (kilograms) or as percentage loss during the first 6 wk postpartum (38.5 vs 19.7 kg, SEM 8.9, $P = 0.01$ and 5.6 vs 2.9 %, SEM = 1.2, $P = 0.02$, respectively). In addition, cows that were fed HE during the dry period had greater odds of experiencing DA ($P = 0.01$) or KET ($P = 0.04$) when compared to cows that received CE.

Key Words: Energy intake, transition period, health, lactation performance

## INTRODUCTION

Analysis of previous experiments from our group (Chapter 2) has provided conclusive evidence that prepartum controlled energy diets may enhance transition period success and reproductive performance in dairy cows when compared to high energy diets. The previous analysis showed that a controlled energy dietary approach during the dry period leads to less negative energy balance (NEB) and lower concentrations of non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHBA), and liver triglyceride (TG) in dairy cows after calving.

In a large study involving 100 herds and approximately 1,400 cows (Ospina et al., 2010b) concluded that elevated blood concentrations of NEFA and BHBA in the transition period predicted clinical disease (i.e., displaced abomasum [DA], ketosis [KET], metritis [MET], or retained placenta [RP]) in cows from TMR-fed northeastern US free-stall dairies. In another study, the same group observed that cows with elevated NEFA precalving and elevated NEFA and/or BHBA postcalving were 15 to 20% less likely to become pregnant within 70 d post-voluntary waiting period than cows with lower concentrations of these metabolites (Ospina et al., 2010a).

Our group and others have explored the effects of prepartum feed regimen on health of dairy cows (Janovick et al., 2011, Richards, 2011, Vickers et al., 2010). However, studies consistently lack statistical power to determine a strong association between treatments and classificatory outcome variables such as diseased or non-diseased cows. Trying to explore a specific disease (e.g. DA, KET, RP) becomes even harder due to the low occurrence of those pathologies in experiments.

Dohoo et al. (2009) suggested that in a logistic regression model (used to analyze binomial distributions; e.g., diseased or non-diseased), as a "thumb rule" at least ten observations for the lowest occurrence outcome should be present for each predictor variable in the model. For example, in order to analyze the effect of prepartum dietary regimen (treatment) we should have at least 10 cows with DA if we were to have only treatment as a predictor variable. If we were to include more predictor variables, such as parity, another 10 cows with DA would be needed. Since DA has a prevalence of 2 to 5%, experiments should consider enrolling approximately 200 cows to make statistical inferences with enough power.

To avoid intense health problems during the transition period cows should be prepared to use body reserves during early lactation. There is not extensive literature on the impact of dry period nutrition strategy on milk composition. Nevertheless, higher milk fat concentration during the first week after calving has been reported as an effect of either ad libitum feeding strategy prepartum (Holcomb et al., 2001) or high BCS at calving (Chilliard, 1992).

The transition management "challenge" is to coordinate systems (nutritional strategies, facilities, grouping management) that decrease individual variability (high risk for disease). The construction and analysis of large datasets providing statistical power may provide new insights in how decreased cow variability results in greater profit to the dairy farmer. The aim of this study was to investigate the associations between prepartum energy feeding regimen and variables associated with health and productive performance in dairy cows.

## MATERIALS AND METHODS

Database construction and data collection, animals and housing, and statistical analysis were the same as described in Chapter 2. Cows were milked twice daily and milk yields were recorded in and obtained from PCDART (Dairy Records Management Services, Raleigh, NC). Consecutive evening and morning milk samples were taken weekly in all experiments. Composite milk samples were prepared in proportion to milk yield at each milking, preserved (800 Broad Spectrum Microtabs II; D & F Control Systems, Inc., San Ramon, CA), and analyzed for contents of fat, protein, lactose, solids-not-fat (SNF), urea N, and somatic cell count (Dairy Lab Services, Dubuque, IA). Fat-corrected milk (**FCM**) and energy-corrected milk (**ECM**) were obtained using the respective formulas: FCM (3.5%) = ((0.4255 * Milk (kg) ) + (16.425 * ((Milk Fat (%) / 100) * Milk (kg)))) and ECM = ((12.82 * ((Milk Fat (%) / 100) * Milk (kg))) + (7.13 * ((Milk Protein (%) / 100) * Milk (kg) ) + (0.323 * Milk (kg))).

Body weight (**BW**) was measured weekly for each cow at the same time after the morning milking and before feeding. The blood metabolite concentration analyzed was cholesterol (determined using cholesterol esterase and cholesterol oxidase (Allain et al., 1974) coupled to the Trinder color development reaction (Trinder, 1969) cholesterol/HP kit, Roche Diagnostics) in an autoanalyzer) in experiments 1, 2, 5, and 7. Cholesterol was categorized at the median concentration value of 90 mg/dL as high (**HC**) or low (**LC**) during wk 2. Weekly means were established for the aforementioned variables and their sample size is indicated further in the respective analysis.

## RESULTS

The prepartum feeding strategies studied, either high energy (**HE**) or controlled energy (**CE**) during far-off (**FO**) or close-up (**CU**) periods, did not result in different milk yield during the first 4 wk after calving (n = 135, $P > 0.14$; Figure 11). The total milk produced in the first 4 wk postpartum did not differ ($P = 0.26$) between treatments (Table 7). To evaluate the postpartum acceleration in milk yield, the slope (i.e., percentage of milk yield increased from wk 1 relative to wk 3) for milk increase from wk 1 to wk 3 was determined, but no statistical difference between treatments was found (*P* = 0.62; Table 7).

Cows fed HE during CU had greater (*P* < 0.05) milk fat concentration in wk 2, 3 and 4 than cows fed CE (Figure 12). Cows fed HE during FO tended (*P* = 0.082) to yield greater amounts of milk fat in wk 4 than cows fed CE (1.30 vs. 1.19 kg, respectively; SEM = 0.07). No statistical difference was found in wk 1 to 3.

Cows (n = 270) fed HE during FO tended (*P* = 0.07) to have lower milk protein concentration in wk 4 then cows fed CE (2.77 vs 2.84 %, respectively; SEM = 0.06). Cows fed HE during CU had higher protein concentration during wk 3 and 4 then cows fed CE (*P* ≤ 0.05, Figure 13). There was no statistical difference in milk protein yield (kg) during the first 4 wk postpartum.

During wk 3 cows (n = 192) that were fed HE during FO had a higher (*P* = 0.03) concentration of urea N in milk (MUN) than cows fed CE (14.60 vs 13.34 mg/dL, respectively; SEM = 1.0). Cows (n = 282) that were fed HE during CU lost more body weight (**BW**) either as absolute amounts (kg) or as percentage loss during the first 6 wk (38.5 vs. 19.7 kg, SEM = 8.9, *P* = 0.01; and 5.6 vs. 2.9%, SEM = 1.2, *P* = 0.02, respectively). Lactation group was significant; cows with 2 lactations or more lost more BW both as absolute amount (kg) and percentage lost (*P* < 0.01).

As a possible indicator of the aforementioned alterations, cows (n = 230) with higher plasma concentrations of cholesterol (HC) during wk 2 had lower odds (*P* = < 0.001) of being classified as diseased (DISE; OR = 0.308; 95% confidence interval = 0.165 to 0.577). In addition, cows that were fed HE during the dry period had greater odds of having DA or KET when compared to cows that received CE (Figure 14).

## DISCUSSION

There was no statistical difference in milk production due to dietary treatments prepartum (Table 7 and Figure 11). In agreement with our results, Agenäs et al. (2003) showed that cows fed a high energy diet (42.3 Mcal/d of ME) for 2 mo prepartum did not yield different amounts of ECM than cows fed medium (25.3 Mcal/d of ME ) or lower (17 Mcal/d of ME ) energy diets. However, the authors found statistical differences in (DMI) between treatments in the first week after calving. Although milk production did not differ, cows fed the low energy diet were the first to reach positive energy balance due to increased DMI. One could hypothesize that cows fed the lower energy diets were metabolically driven to recover BW instead of increasing milk production.

Dairy cows need sufficient nutrients for synthesis of protein, lactose, and TG in the mammary glands (Bell et al., 1995). Klop et al. (1998) and Holcomb et al. (2001) reported 0.2 to 0.8 percentage units higher milk fat content during the first 5 wk of lactation for cows fed ad libitum compared to those on a restricted diet prepartum. Even though Agenäs et al. (2003) found that milk fat production was not significantly affected by high (H) or low (L) energy diets prepartum, they proposed that a high feeding intensity during the dry period may increase milk fat content in early lactation. The authors suggested that because the least squares means were about 0.3 percentage units higher in the H group than in the other groups in lactation wk 1 to 4. In the present study cows fed HE diets prepartum had higher milk fat percentage and milk fat yield (wk 4) in early lactation.

In our experiment we did not have data on fatty acids composition of milk fat for all studies. The results found by Agenäs et al. (2003) can serve as an excellent hypothesis. The authors suggested that the differences in milk fat content found between dietary energy levels prepartum were due to a greater supply of long chain fatty acids (LCFA) originating from NEFA mobilized from adipose tissue in the high energy diet group when compared to lower and medium energy intakes. The high energy group showed lower content of $C_{16:0}$ in milk fat along with reciprocally higher $C_{18:0}$ at the beginning of lactation. The $C_4$ to $C_{14}$ and part of the $C_{16}$ fatty acids in milk fat are known to be synthesized de novo in the mammary gland (Agenäs et al., 2003). The uptake of large quantities of LCFA inhibits de novo synthesis of short-chain fatty acids by the mammary gland (Palmquist et al., 1993). Therefore, changes in milk fat composition can reflect differences in energy balance resulting from prepartum nutrition.

Agenäs et al. (2003) and Chilliard (1992) did not find overall effects of plane of nutrition prepartum on milk protein content. However, least squares means were higher in the high energy diet group (Agenäs et al., 2003). In contrast, Klop et al. (1998) reported lower milk protein content in lactation wk 1 to 5 in cows that received ad libitum compared to restricted feeding during prepartum. In the present study cows receiving HE during CU had higher milk protein content during early lactation but cows fed the same diet during FO had lower milk protein content during wk 4. The contradictory findings from the studies of the previous authors may be explained by our results where an effect of FO and CU on milk protein concentration can be contrasted. The FO period seems to be of vital importance on subsequent lactation performance (Dann et al., 2006).

In the present study cows fed HE during CU lost more BW when compared to cows fed CE. Previous research has indicated that most of the body energy changes in early lactation are associated with adipose tissue TG mobilization (Andrew et al., 1994). In addition, cows fed a higher energy diet during the dry period were in greater negative energy balance for the first 14 d after calving compared with control cows (Guo et al., 2007). In contrast, Rabelo et al. (2003) and VandeHaar et al. (1999) did not find an effect of prepartum energy intake on postpartum energy balance over the first 70 DIM. The higher concentration of MUN during wk 3 for cows fed CE might be a response to the lower BW lost. Hojman et al. (2005) looked at field data from 1,996 cows (25,485 records) and found a negative linear association between BW and MUN concentration.

Cows attempt to fulfill energy demand through the process of TG mobilization from adipose tissues, resulting in high circulating concentrations of NEFA (Drackley et al., 2001). The NEB is to some extent a normal physiological process experienced by cows during early lactation; nevertheless, its severity can negatively affect production and health of dairy cows (Koltes and Spurlock, 2011) including greater incidence of metabolic disorders such as KET and fatty liver (Drackley, 1999). Collard et al. (2000) reported higher digestive and locomotive problems correlated with NEB. In our previous study (Chapter 2) higher concentrations of NEFA during wk 1 after parturition were associated with greater odds of cows being diseased.

Bjerre-Harpøth et al. (2012) defined physiological imbalance (PI) as a situation in which physiological parameters deviate from the normal, and cows consequently have an increased risk of developing production diseases and reduced production or reproduction. The authors found that the metabolites that better predicted degree of PI were glucose, NEFA, BHBA, and cholesterol. Plasma cholesterol increased during restriction in that study and was partly attributed to the hepatic re-esterification of NEFA as TG and its exportation into circulation within very low density lipoproteins (Bjerre-Harpøth et al., 2012). The authors speculated that it could be useful in an index for PI throughout lactation. Elevated plasma concentrations of cholesterol and NEFA before calving were associated with a greater risk of RP (Chapinal et al., 2011). In the present study high concentrations of cholesterol at wk 2 were associated with healthier cows. One possible explanation for this finding could be that the higher concentrations of cholesterol would be reflecting a better capacity of the cows to export TG from the liver.

Feeding low energy (high fiber) diets has been suggested to alleviate some of the burdens of a turbulent transition period such as reducing the incidence of DA, improvements in BCS, and better foot heath (Janovick and Drackley, 2010). The association of DA with high concentrations of NEFA during the transition period has been identified by previous studies (Chapinal et al., 2011, LeBlanc et al., 2005, Van Winden et al., 2003). Duffield et al. (2009) concluded that an improvement of energy balance before calving would be important for the prevention of energy associated metabolic diseases, such as RP, KET, and DA, which might occur immediately postcalving.

In the present study cows fed CE during the dry period had lower odds of developing DA and KET (Figure 14). The association of cows fed CE diets with less intense NEB (i.e., lower BW loss, lower milk fat concentration, and lower MUN in the present study) may represent lower circulating NEFA levels and less liver "exhaustion" (Chapter 2).

## CONCLUSION

Cows that received CE diets during the dry period yielded the same amount of milk as cows that received HE. Nevertheless, cows fed HE during the CU period had higher milk fat and milk protein during early lactation. In addition, cows fed HE during the FO period lost more BW in the first 6 wk after calving, which might be related to higher MUN concentration in wk 3 for those cows. Cows fed CE during the dry period had less odds for developing DA or KET. These data indicate that cows would be more likely to experience a smooth transition period when fed CE diets prepartum.

## REFERENCES


Agenäs, S., E. Burstedt, and K. Holtenius. 2003. Effects of feeding intensity during the dry period. 1. Feed intake, body weight, and milk production. J. Dairy Sci. 86:870-882.

Allain, C. C., L. S. Poon, C. S. Chan, W. Richmond, and P. C. Fu. 1974. Enzymatic determination of total serum cholesterol. Clinical Chemistry 20:470-475.

Andrew, S. M., D. R. Waldo, and R. A. Erdman. 1994. Direct analysis of body composition of dairy cows at three physiological stages. J. Dairy Sci. 77:3022-3033.

Bell, A. W., R. Slepetis, and R. A. Ehrhardt. 1995. Growth and accretion of energy and protein in the gravid uterus during late pregnancy in Holstein cows. J. Dairy Sci. 78:1954-1961.

Bjerre-Harpøth, V., N. C. Friggens, V. M. Thorup, T. Larsen, B. M. Damgaard, K. L. Ingvartsen, and K. M. Moyes. 2012. Metabolic and production profiles of dairy cows in response to decreased nutrient density to increase physiological imbalance at different stages of lactation. J. Dairy Sci. 95:2362-2380.

Chapinal, N., M. Carson, T. F. Duffield, M. Capel, S. Godden, M. Overton, J. E. P. Santos, and S. J. LeBlanc. 2011. The association of serum metabolites with clinical disease during the transition period. J. Dairy Sci. 94:4897-4903.

Chilliard, Y. 1992. Physiological constraints to milk production: factorswhich determine nutrient partitioning, lactation persistency and mobilization of body reserves. World Rev. Anim. Prod:19-26.

Collard, B. L., P. J. Boettcher, J. C. Dekkers, D. Petitclerc, and L. R. Schaeffer. 2000. Relationships between energy balance and health traits of dairy cattle in early lactation. J. Dairy Sci. 83:2683-2690.

Dann, H. M., N. B. Litherland, J. P. Underwood, M. Bionaz, A. D'Angelo, J. W. McFadden, and J. K. Drackley. 2006. Diets during far-off and close-up dry periods affect periparturient metabolism and lactation in multiparous cows. J. Dairy Sci. 89:3563-3577.

Dohoo, I., W. Martin, and H. Stryhn. 2009. Veterinary Epidemiologic Research. 2nd ed. VER Inc, Charlottetown, Prince Edward Island, Canada.

Drackley, J. K. 1999. ADSA Foundation Scholar Award. Biology of dairy cows during the transition period: The final frontier? J. Dairy Sci. 82:2259-2273.

Drackley, J. K., A. D. Beaulieu, and J. P. Elliott. 2001. Responses of milk fat composition to dietary fat or nonstructural carbohydrates in Holstein and Jersey cows. J. Dairy Sci. 84:1231-1237.

Duffield, T. F., K. D. Lissemore, B. W. McBride, and K. E. Leslie. 2009. Impact of hyperketonemia in early lactation dairy cows on health and production. J. Dairy Sci. 92:571-580.

Guo, J., R. R. Peters, and R. A. Kohn. 2007. Effect of a transition diet on production performance and metabolism in periparturient dairy cows. J. Dairy Sci. 90:5247-5258.

Hojman, D., M. Gips, and E. Ezra. 2005. Association between live body weight and milk urea concentration in holstein cows. J. Dairy Sci. 88:580-584.

Holcomb, C. S., H. H. Van Horn, H. H. Head, M. B. Hall, and C. J. Wilcox. 2001. Effects of prepartum dry matter intake and forage percentage on postpartum performance of lactating dairy cows. J. Dairy Sci. 84:2051-2058.

Janovick, N. A., Y. R. Boisclair, and J. K. Drackley. 2011. Prepartum dietary energy intake affects metabolism and health during the periparturient period in primiparous and multiparous Holstein cows. J. Dairy Sci. 94:1385-1400.

Janovick, N. A. and J. K. Drackley. 2010. Prepartum dietary management of energy intake affects postpartum intake and lactation performance by primiparous and multiparous Holstein cows. J. Dairy Sci. 93:3086-3102.

Klop, A., G. A. L. Meijer, and T. A. M. Kruip. 1998. Energy intake during the dry period and energy balance in dairy cows around calving. in 10th int. Conf. Prod. Diseases Farm Anim.

Koltes, D. A. and D. M. Spurlock. 2011. Coordination of lipid droplet-associated proteins during the transition period of Holstein dairy cows. J. Dairy Sci. 94:1839-1848.

LeBlanc, S. J., K. E. Leslie, and T. F. Duffield. 2005. Metabolic predictors of displaced abomasum in dairy cattle. J. Dairy Sci. 88:159-170.

Ospina, P. A., D. V. Nydam, T. Stokol, and T. R. Overton. 2010a. Associations of elevated nonesterified fatty acids and beta-hydroxybutyrate concentrations with early lactation reproductive performance and milk production in transition dairy cattle in the northeastern United States. J. Dairy Sci. 93:1596-1603.

Ospina, P. A., D. V. Nydam, T. Stokol, and T. R. Overton. 2010b. Evaluation of nonesterified fatty acids and beta-hydroxybutyrate in transition dairy cattle in the northeastern United States: Critical thresholds for prediction of clinical diseases. J. Dairy Sci. 93:546-554.

Palmquist, D. L., A. D. Beaulieu, and D. M. Barbano. 1993. Feed and animal factors influencing milk fat composition. J. Dairy Sci. 76:1753-1771.

Rabelo, E., R. L. Rezende, S. J. Bertics, and R. R. Grummer. 2003. Effects of transition diets varying in dietary energy density on lactation performance and ruminal parameters of dairy cows. J. Dairy Sci. 86:916-925.

Richards, B. F. 2011. Strategies to decrease incidence of fatty liver in dairy cows. PhD Dissertation, University of Illinois, Urbana.

Trinder, P. 1969. Determination of blood glucose using 4-amino phenazone as oxygen acceptor. J. Clinical Pat. 22:246.

Van Winden, S. C. L., R. Jorritsma, K. E. Müller, and J. P. T. M. Noordhuizen. 2003. Feed intake, milk yield, and metabolic parameters prior to left displaced abomasum in dairy cows. J. Dairy Sci. 86:1465-1471.

Vandehaar, M. J., G. Yousif, B. K. Sharma, T. H. Herdt, R. S. Emery, M. S. Allen, and J. S. Liesman. 1999. Effect of energy and protein density of prepartum diets on fat and protein metabolism of dairy cattle in the periparturient period. J. Dairy Sci. 82:1282-1295.

Vickers, L. A., D. M. Weary, D. M. Vieira, and M. A. G. Keyserlingk. 2010. Effects of a low energy diet prepartum on subclinical ketosis in dairy cows. J. Dairy Sci. E-Suppl. 1(93).

**Table 7.** Least squares means for milk production from Holstein cows fed different dietary treatments prepartum

| Variable | N | FO[1] | | | CU[2] | | | |
|---|---|---|---|---|---|---|---|---|
| | | HE[3] | CE[4] | *P* value | HE | CE | SEM[5] | *P* value |
| Milk | | | | | | | | |
| Sum 4 wk, kg | 177 | 115.5 | 117.3 | 0.78 | 120.2 | 112.7 | 3.2 | 0.26[6] |
| Slope wk 1 to 3, % | 168 | 45.4 | 42.3 | 0.66 | 42.1 | 45.6 | 4.1 | 0.62[6] |

[1] Far-off .
[2] Close-up.
[3] High energy diet.
[4] Controlled energy diet.
[5] Standard error of the mean.
[6] Lactation group statistically significant in the model ($P = 0.002$). Cows with 2 or more lactations had higher values for milk yield and slope than cows with 1 lactation.

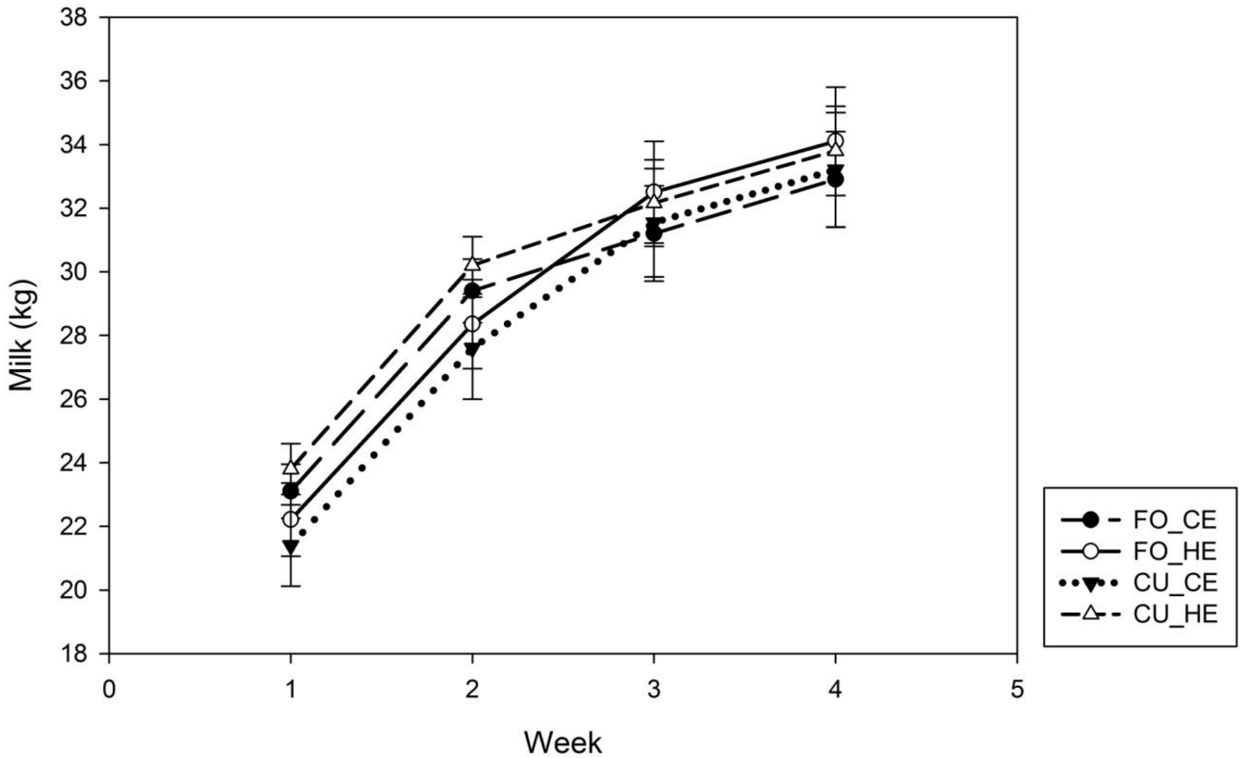


**Figure 11.** Least squares means and standard errors of the mean for milk production (kg/d). FO_CE: Far-off period receiving controlled energy diet. FO_HE: Far-off period receiving high energy diet. CU_CE: Close-up period and controlled energy diet. CU_HE: Close-up period and high energy diet.

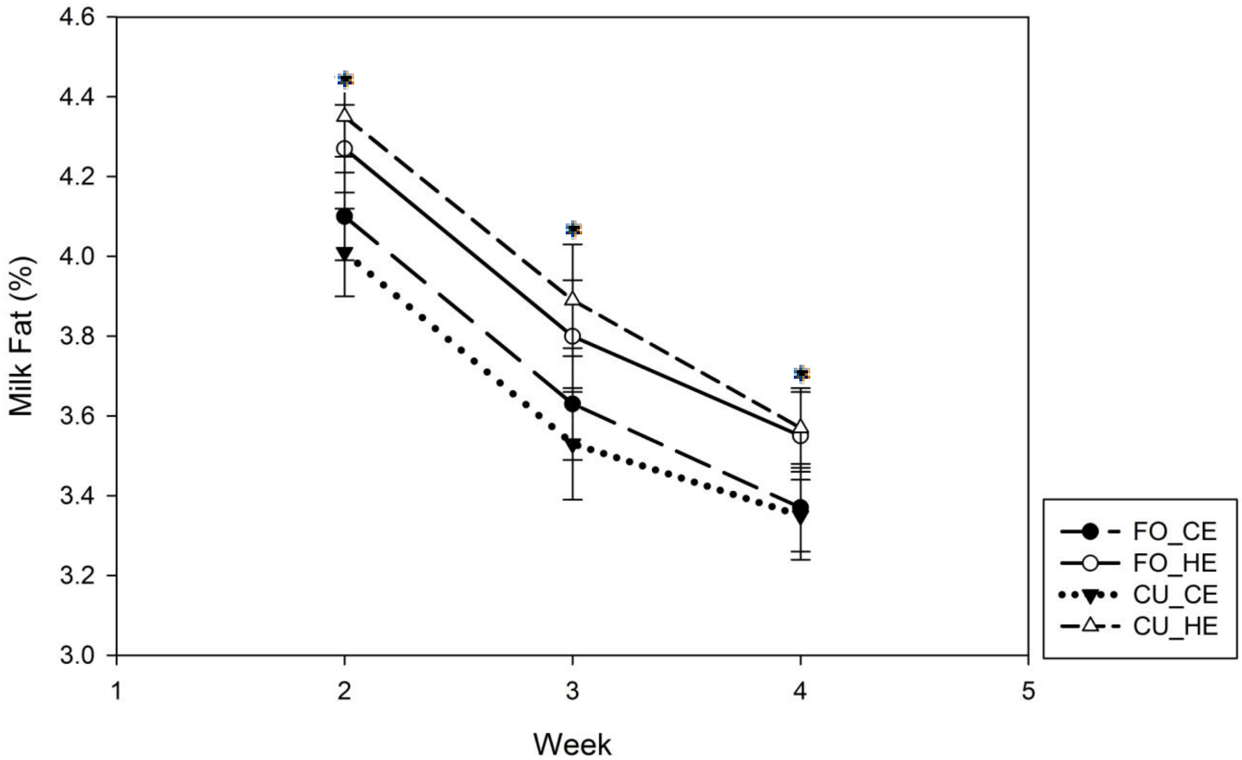


**Figure 12.** Least squares means and standard errors of the mean for milk fat percentage (%). FO_CE: Far-off period receiving controlled energy diet. FO_HE: Far-off period receiving high energy diet. CU_CE: Close-up period and controlled energy diet. CU_HE: Close-up period and high energy diet. * Statistically significant difference due to CU period diet for wk 2 (n = 299, $P = 0.03$), wk 3 (n = 270, $P = 0.008$) and wk 4 (n = 270, $P = 0.04$).

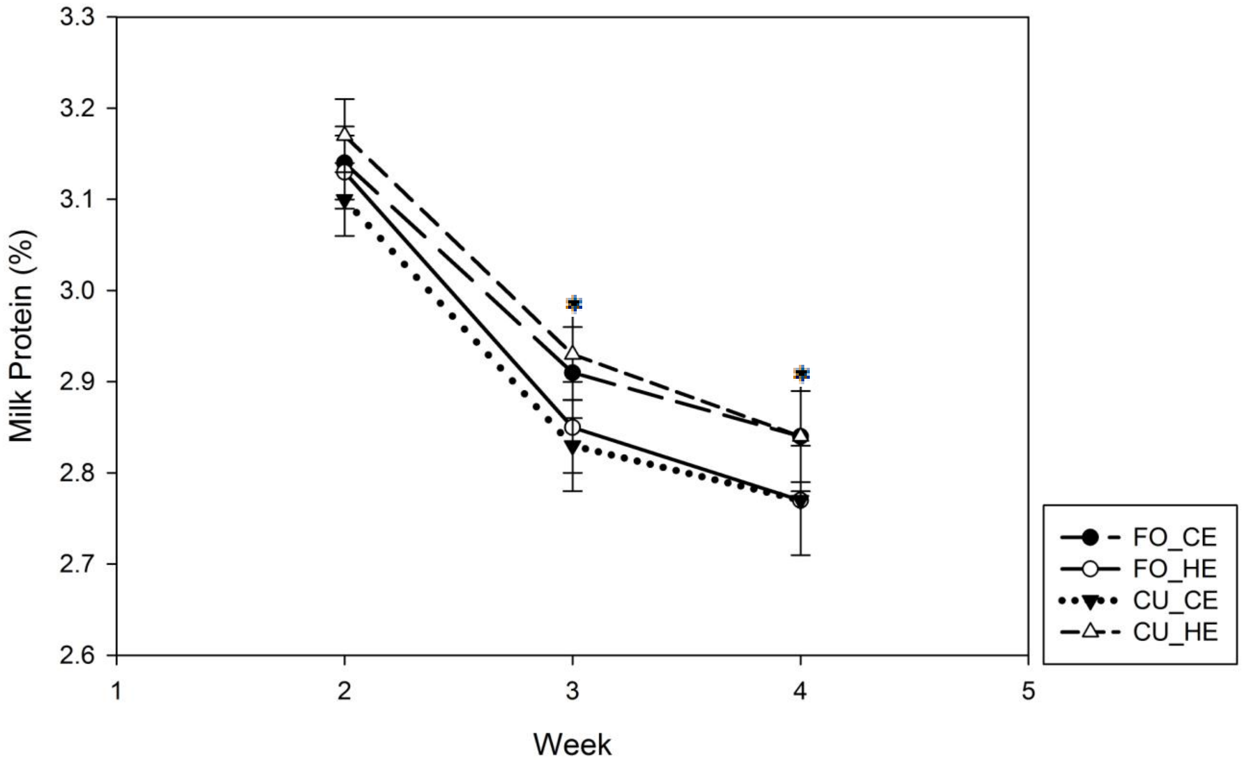


**Figure 13.** Least squares means and standard errors of the mean for milk protein percentage (%). FO_CE: Far-off period receiving controlled energy diet. FO_HE: Far-off period receiving high energy diet. CU_CE: Close-up period and controlled energy diet. CU_HE: Close-up period and high energy diet. * Statistically significant difference due to CU period diet for wk 3 (n = 270, $P$ = 0.02) and wk 4 (n = 270, $P$ = 0.05).

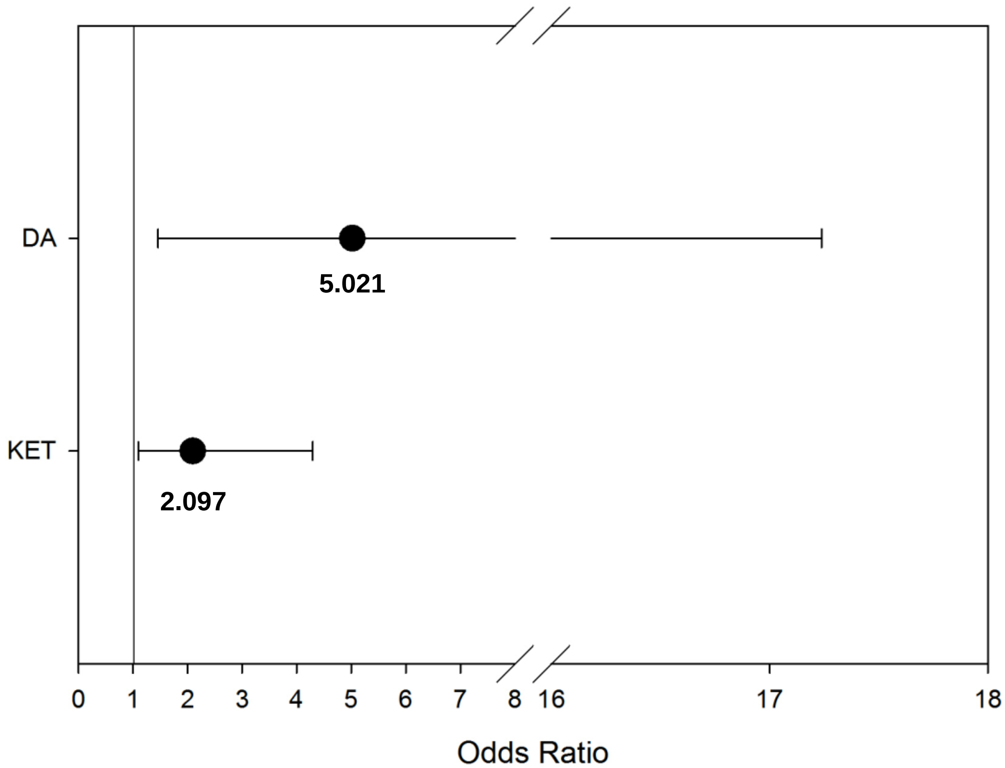


**Figure 14.** Odds ratios and 95% confidence intervals for the association of postpartum diseases with cows receiving high energy diets (HE; bullets) or controlled energy diets (CE; line) during far-off and close-up periods. DA: Displaced abomasum (n = 36/315, total n = 351, $P = 0.01$). KET: Ketosis (n = 61/289, total n = 350, $P = 0.04$).

## CHAPTER 4

# PHYSIOLOGICAL VARIABLES ASSOCIATED WITH REPRODUCTIVE SUCCESS IN DAIRY COWS WITH DIFFERENT PREPARTUM FEEDING STRATEGIES

## ABSTRACT

To investigate the association between physiological factors and reproductive performance (days to conception; DTC) in dairy cows fed different prepartal dietary energy regimens, cow-level data from 408 cows from 7 different experiments by our group from 1993 to 2010 were analyzed. Treatments were classified as controlled energy (CE; median $NE_L$ intake = 13.7 Mcal/d) or high energy (HE; median $NE_L$ intake = 22.1 Mcal/d) diets fed during far-off (FO) or close-up (CU) dry periods. Principal component (PC) analysis was conducted on 8 variables: glucose wk 3 (GLU3), glucose wk 4 (GLU4), β-hydroxybutyrate wk 1 (BHBA1), insulin wk 2 (INS2), nonesterified fatty acids wk −1 (NEFA-1), energy-corrected milk wk 4 (ECM4), fat corrected milk wk 4 (FCM4), and milk urea nitrogen wk 5 (MUN5). Prior to analyses, the multinormality of the variables was assessed. The effect of PC was investigated using linear and logistic (LR) regressions. For LR analysis, animals were classified in two groups based on first and fourth quartile values of DTC as high (S, slow; ≥ 174 d) or low (F, fast; < 87 d). All analyses were carried out using SAS 9.2 (SAS Institute, Inc.). All PC with eigenvalues (λ) greater than or equal to 0.9 were extracted, and only loadings greater than 0.42 were discussed. Principal component scores (PCS) were generated for each extracted PC. Four PC were extracted from the analysis, accounting for 79.6% of total variability. The PC loadings indicated that, for PC1, increased ECM4 and FCM4 were associated with decreased INS2, GLU3, and GLU4. The PC2 represented cows with higher NEFA-1 and BHBA1. The PC3 had higher values for ECM4,

FCM4, GLU3, and GLU4; whereas, PC4 had higher values for MUN5. Regressing PCS of PC2 on PC1 indicated that the relationship between these PC differed between diets (*P* = 0.07). For increased values of PC1, HE cows had increased values of PC2; whereas, those fed CE showed decreased values of PC2. Inclusion of PC in a logistic model revealed that cows with higher values of PC2 were associated with greater odds of being classified as S (greater DTC) when compared to cows classified as F (smaller DTC) [odds ratio (OR) = 2.257, 95%CI = 0.979 to 7.763; *P* = 0.054]. In conclusion, PC explained and predicted reproductive success in dairy cows of this dataset. These 8 variables might be used to predict reproductive success.

Key Words: risk factors, transition period, reproductive performance, principal component

## INTRODUCTION

Previous analysis of experimental data from our group (Chapters 2 and 3) has offered conclusive evidence that prepartum controlled energy (**CE**) diets may enhance transition period success and reproductive performance in dairy cows compared to prepartum high energy (**HE**) diets. The previous analyses showed that a controlled energy dietary approach during the dry period leads to improved energy balance and lower concentrations of non-esterified fatty acids (**NEFA**), β-hydroxybutyrate (**BHBA**), and liver triglyceride (**TG**) in dairy cows after calving.

The analysis of risk factors allows better understanding of the relationship of causation between the dependent variable (pregnancy) and its explanatory variables (e.g., NEFA, BHBA, BCS, dry matter intake (**DMI**), $NE_L$ intake, disease). The benefit of this methodology is that it helps us understand how the prevalence of a covariate can impact the strength of association between the explanatory variables and the dependent variable of interest (Dohoo et al., 2009). In other words, retained placenta can have an association with decreased reproductive performance;

however, when metritis is also included in the model, for example, that association can be even stronger. To better explain reproductive performance in dairy cattle, both variables should be considered, not just one.

However, variables can be correlated leading to multicollinearity that causes inflation of the standard errors and poor estimates (Dohoo et al., 2009). Principal components analysis (PCA) involves a mathematical procedure that transforms a set of correlated response variables into a smaller set of uncorrelated variables [i.e., principal components; Johnson (1998)]. Use of PCA has the capability to explain the variation in a dataset by reducing the number of variables necessary to do so (Johnson, 1998). As a second step, the principal components (**PC**) can be used in any model (e.g., logistic or linear regression, survival analysis) to further explore the underlying relationships.

Several studies have been performed in the attempt to establish the association of risk factors with reproductive success (Bruun et al., 2002, Opsomer et al., 2000, Santos et al., 2009). Even though previous research had been conducted with large number of experimental units (cows), factors (explanatory variables) involved in the analysis were not as detailed as the dataset constructed by our group. Obtaining daily DMI requires an experimental setting at facilities that are not conducive to large sample sizes. The data set constructed by our group may give new perspectives due to the uniqueness of its variables. The objectives of this study were to use statistically significant variables associated with reproductive performance from a previous univariate analysis in one model to reveal novel relationships and to better understand risk factors for pregnancy success in dairy cows. Such results may allow new insights on approaches to alleviate reproductive failure in dairy cattle.

## MATERIALS AND METHODS

The variable days to conception (**DTC**) was used as the dependent variable to assess reproductive performance of cows in a database developed from 7 different experiments completed in our group from 1993 to 2010 (Chapter 2). A total of 408 cows (354 multiparous and 54 primiparous) were included in the analysis (Chapter 2).

First, a Cox proportional hazard model (COX; PHREG procedure) was used to assess DTC in a survival analysis where experiments were treated as random effects (Allison, 2010, St-Pierre, 2001). Weekly variables were constructed from the dataset (prepartum and postpartum) and were tested using the COX model in a univariate approach. These variables were plasma BHBA, glucose, NEFA, cholesterol, insulin, and urea; liver composition (lipids, TG, and glycogen), body condition score (BCS), DMI, milk (yield and components), body weight (BW), rumen pH, and urine pH.

Milk urea N was categorized according to physiological significance as suggested by Rajala-Schultz et al. (2001) as low, < 10 mg/dL (L); moderate, ≥ 10 and ≤ 12 mg/dL (M, referent); or high, > 12 mg/dL (H). The variables NEFA wk – 1 and BHBA wk 1 were dichotomized as high (**H**) or low (**L**) using a cut-off value of 700 μEq/L and 10 mg/dL, respectively, as defined by Ospina et al. (2010b). Plasma glucose concentration was categorized at the median concentration values of 60 mg/dL at wk 3 and 65 mg/dL at wk 4 as high (**HG**) or low (**LG**). As a result of the univariate analysis eight variables (Table 8) were associated with fertility in dairy cattle and were considered as risk factors for pregnancy.

Secondly, principal component PCA analysis was conducted on the aforementioned eight continuous variables: glucose wk 3 (**GLU3**), glucose wk 4 (**GLU4**), BHBA wk 1 (**BHBA1**), insulin wk 2 (**INS2**), NEFA wk -1 (**NEFA-1**), energy-corrected milk wk 4 (**ECM4**), fat corrected milk wk 4 (**FCM4**), and milk urea nitrogen (**MUN**) wk 5 (**MUN5**). Prior to analyses, the multinormality of the variables was assessed. Insulin, NEFA, and BHBA were log transformed. The PC analysis was performed using the Factor procedure (method = prin) on the correlation matrix. All PC with eigenvalues (λ) greater or equal to 0.9 were extracted, and only loadings greater than 0.42 were discussed. Principal component scores (**PCS**) were generated for each extracted PC.

Finally, the effect of PC was investigated using linear (MIXED and GLM procedures) and logistic (**LR**) (LOGISTIC procedure) regressions models. For LR analysis, animals were classified in two groups based on the first and fourth quartile values of DTC as high (**S**, slow; ≥ 174 d) or low (**F**, fast; < 87 d). All analyses were carried out using SAS 9.2 (SAS v9.2 Institute Inc., Cary, NC)

## RESULTS

From the univariate analysis cows with higher concentrations of glucose and BHBA postpartum were associated with a statistical difference in the hazard ratio for DTC (Table 8), indicating a shortened DTC for cows in the HG and H groups, respectively. Accordingly, there was a positive association between milk production (FCM and ECM) and reproductive success (Table 8). In contrast, higher concentrations of NEFA prepartum, insulin at wk 2, and MUN at wk 5 were associated with greater DTC indicated by the lower (< 1) hazard ratio of having a successful pregnancy (Table 8).

In order to reduce correlation and better explain the risk factors associated with pregnancy (i.e., total variability) four PC were extracted from the PC analysis using the eight initial variables (Table 8). These four PC accounted for 79.6% of the total variability (Table 9).

The PC loadings (Table 10) indicated that, for PC1, increased ECM4 and FCM4 were associated with decreased INS2, GLU3, and GLU4. Principal component 2 represented animals with higher NEFA−1 and BHBA1. Principal component 3 had higher values for ECM4, FCM4, GLU3, and GLU4; whereas, PC4 had higher values for MUN5 and relatively small loading on INS2 (Table 10).

Regressing PCS of PC2 on PC1 showed a linear relationship between these PC explained by diet regimen prepartum. For increased values of PC1, HE cows had increased values of PC2; whereas, those fed CE showed decreased values of PC2 (Figure 15). The model revealed a tendency ($P$ = 0.07) for the interaction of PC2 and dietary treatment during the CU period. Interestingly, cows (n = 28) that received CE had an inverse linear association with PC2 (intercept = − 0.433 ± 0.131 and slope = − 0.291 ± 0.165) compared to cows (n = 61) receiving HE during the same period (intercept = − 0.242 ± 0.059 and slope = 0.296 ± 0.060) (Figure 15). Inclusion of PC in a logistic model revealed that cows with high values of PC2 were associated with greater odds of being classified as S (greater DTC) when compared to cows classified as F (smaller DTC) [odds ratio (OR) = 2.257, 95% CI = 0.979 to 7.763; $P$ = 0.054; n= 32].

## DISCUSSION

Physiological variables associated with fertility of dairy cows in our study can be seen to a great extent as a representation of the cows' energy metabolism during the transition period. Cows have an extreme ability to maintain blood glucose concentration due to their capacity to prioritize gluconeogenesis (Drackley, 1999). Nevertheless, higher plasma concentrations of glucose during wk 3 and 4 were associated with greater reproductive success (Table 8). Such association could be a direct reflection of cows experiencing a less severe (**NEB**) postpartum (e.g., by either consuming more energy or losing less BCS after calving) being able to reproduce better (Bell, 1995, Koltes and Spurlock, 2011). Induction of lipolysis in adipose tissue due to catabolic signals (McNamara, 1991) elevates the circulating concentrations of NEFA and increases the formation of ketone bodies (acetoacetate, β-hydroxybutyrate, and acetone) (Drackley et al., 2001).

In our study the second blood metabolite associated with improved fertility in the univariate analysis was BHBA. Several authors (Chapinal et al., 2011, Duffield et al., 2009, Ospina et al., 2010a) had identified the detrimental association between high concentrations of BHBA and cow health. At first glance it was somewhat unexpected to find better fertility (i.e., higher hazard ratio) for cows with higher blood concentration of BHBA at wk 1. Perhaps cows with the greater BHBA concentrations had a higher capacity for liver esterification of potentially detrimental higher NEFA concentrations during wk -1, which would be beneficial for the cow. Litherland et al. (2011) found a greater rate of hepatic fatty acid esterification but lower rate of β-oxidation in cows overfed with energy prepartum compared to cows that were energy restricted to 80% of NRC (2001). Chapinal et al. (2011) suggested that besides the higher weekly blood NEFA concentrations the period of time that cows experience these high levels may have greater impact on cow health. One could hypothesize that hepatic tissue from cows in our study classified as H for BHBA status at wk 1 were coping better with high NEFA concentration prepartum.

As expected, high NEFA concentration was associated with the worst reproductive peformance. Previous studies also found relationships between prepartum DMI and energy metabolism with reproductive failure in dairy cows (Hammon et al., 2006, Huzzey et al., 2007, Walsh et al., 2007). Chapinal et al. (2011) found an association of metritis with a significant increase in NEFA concentrations and reduction in neutrophil function 2 wk before parturition when compared to healthy cows (Hammon et al., 2006). The negative association between insulin and reproductive success (lower plasma concentrations associated with smaller hazard ratio for DTC; Table 8) could be a reflection of higher lipolysis rate and lower DMI. Insulin, as the major homeostatic hormone, functions primarily to stimulate lipogenesis and glucose utilization (which occurs at a low rate in ruminants) and to inhibit lipolysis in bovine adipose tissue. Plasma insulin concentration peaks at parturition, but is maintained at lower concentrations postpartum than prepartum (Grum et al., 1996). Thus, the increasing concentration of circulating NEFA during late gestation should be, at least somewhat, attributed to the decreased insulin.

In accordance with Rajala-Schultz et al. (2001) higher concentrations (> 12 mg/dL) of MUN were associated with reduced reproductive success (Table 8). The authors evaluated data from 24 herds in Ohio and suggested that increased MUN concentrations (mean of monthly MUN values of individual cows for the period from calving to conception or the end of the study) were negatively related to dairy cow fertility and were associated with a lower risk of detectable pregnancy at herd checks.

Greater yields of FCM or ECM were associated with better fertility (Table 8). Great discussions are held in the scientific community regarding the association between milk production and reproductive success. Throughout the years, milk yield per cow has increased dramatically and reproductive success has declined, which has led some authors to suggest a relationship of causation between these two events (Butler, 2003, Lopez et al., 2004, Walsh et al., 2011, Wiltbank et al., 2006).

However, LeBlanc (2011) questioned if these two events have a relationship of association or causation. Reporting unpublished data, the author concluded that a Canadian herd with average production of 1000 kg of mature equivalent milk higher than a similar herd was predicted to have a pregnancy rate 0.7% higher. Several factors may have contributed to these antagonist inferences about milk production and reproduction. These include: Confounding (failure to account for other variables that influence both production and reproduction), bias (selective decisions as a function of production), ecologic fallacy (erroneous inferences at the cow level from herd or population data; even if reproduction negatively correlated with production over time, it is not necessarily the cows with higher production within their cohort that have worse reproduction), and statistical analysis (pregnancy is a binary outcome that cannot be analyzed with linear regression at the cow level; survival analysis accounts for cows that do not become pregnant, therefore minimizing bias and using appropriate variance).

The association of higher milk production and better reproduction in our study most likely is a reflection of good nutrition, cow comfort, and alert management providing the conditions for both high production and good reproductive performance. However, a univariate analysis of the previous 8 variables (Table 8) may not result in construction of the true relationship among outcome (reproductive performance) and explanatory variables (risk factors) (Dohoo et al., 2009).

Surprisingly, but not unexpected, PC analysis associated variables in extremely meaningful groups. Principal component 1 explained 37.7 % of the total variation by itself (Table 9). Milk production (FCM4 and ECM4) was associated with INS2, GLU3, and GLU4. As the level of milk production increases it is expected that glucose would be directed to support milk production and consequently the life of the newborn since approximately 85% of total body glucose is partitioned to the mammary gland (Bickerstaffe et al., 1974). The lower levels of insulin concomitant with lower levels of glucose during early lactation can be indicative of insulin resistance (**IR**) [i.e., a state in which normal concentrations of insulin produce a less than normal biological response (Kahn, 1978)].

Lower circulating concentrations of insulin have been associated with reproductive pathologies and dysfunction in dairy cows (Vanholder et al., 2006). Insulin reduces gluconeogenesis; intravenous infusion of insulin into sheep decreased glucose synthesis rate (Brockman, 1990). Rukkwamsuka et al. (1999) found that both basal and stimulated (i.e., addition of glucose or glucose plus insulin) esterification rate of subcutaneous adipose tissue was markedly decreased postpartum (tested at 0.5, 1, and 3 wk post-calving) compared with that during the last week prepartum. In general, limitations prior to interaction of metabolite and receptor result in hypoinsulinemia, defects at the receptor level cause decreased insulin responsiveness, and compromised intracellular signal transduction results in low insulin sensitivity (Kahn, 1978).

Bjerre-Harpøth et al. (2012) observed increased insulin sensitivity during a dietary restriction challenge in cows during early lactation compared with cows in later lactation. During early lactation, the genetically driven NEB (Friggens et al., 2007) observed was related to an IR state in tissues such as adipose and skeletal muscle (Bauman, 2000; LeBlanc, 2010), which would ensure that increased lipolytic responsiveness favors body lipid mobilization to supply adequate nutrients to the mammary gland (Bauman, 2000). That such variables were grouped in the first PC can indicate the importance of IR on adaptation from the dry period to a secretory and reproductive state.

Variables with heavy positive loading in PC2 (which explained 16.3% of the variation, Table 9) were NEFA−1 and BHBA1. This PC represents the extent of NEB measured by 2 metabolites associated with a response to body tissue mobilization, specifically adipose tissue lipolysis. These metabolites have been extensively studied by the scientific community and have been associated with metabolic disorders and reproductive failure in dairy cows (Chapinal et al., 2011, Ospina et al., 2010a, b). Upon uptake into hepatocytes, NEFA can either be oxidized completely to $CO_2$ and provide energy or be oxidized incompletely to BHBA and exported for other organs and peripheral tissues, or NEFA can be esterified to TG and secreted in the form of very-low density lipoprotein (**VLDL**).

Synthesis of TG in excess of the rate of VLDL output leads to accumulation of TG in liver. Increased VLDL export or increased fatty acid oxidation may help to alleviate lipid accumulation in liver. Multiple factors have been demonstrated to influence the fate of NEFA metabolism in hepatocytes. Drackley et al. (1991) incubated liver slices *in vitro* and observed that the proportion of total palmitate uptake that was oxidized was inversely related with whole-animal energy balance and physiological state rather than with changes in substrate delivery, whereas the proportion of NEFA that was esterified decreased with NEB.

Variables positively loaded in PC3 (which explained 14.5% of the variation, Table 9) were FCM4, ECM4, GLU3, and GLU4. This PC represents the positive energy balance experienced by cows after calving, to a small extent. Cows that have a smooth transition are expected to have higher milk production and higher blood glucose concentration when compared to cows that experienced problems during the transition period (Drackley et al., 2006). The prioritization and regulation of glucose uptake is accomplished, in part, by changes in expression of cellular glucose transport molecules (**GLUT**) within the mammary gland (Mattmiller et al., 2011). The authors concluded that statistically significant increases in GLUT1 gene expression were observed during early lactation, whereas both GLUT3 and GLUT4 gene expression increased during late lactation. Therefore, there are clear physiological adaptations that stresses mediate the relationship between blood glucose and milk production (Mattmiller et al., 2011, Zhao and Keating, 2007).

Principal component 4 explained 11.2% of the total variation (Table 9). It was heavily and positively loaded by the variable MUN5 (Table 10). Failure to consume adequate protein to meet amino acid requirements for milk protein synthesis and hepatic gluconeogenesis during the first 2 wk postpartum leads to transition cows mobilizing tissue protein (Bell et al., 2000). However, body protein mobilization is more restricted and occurs for a shorter time than body fat mobilization (Tamminga et al., 1997).

Excessive mobilization of body protein leads to impaired reproductive performance (Canfield et al., 1990, Ferguson and Chalupa, 1989). Amino acids from mobilized skeletal muscle protein have to be deaminated or transaminated before their carbon skeletons enter the TCA where they ultimately yield energy (Champe et al., 2008). Deamination and transamination result in the production of ammonia ($NH_3$), which the body excretes as urea because it is less toxic than ammonia (Butler, 1998).

Nonetheless, urea production is an energy dependent process. Therefore, over-mobilization of body protein to provide amino acids for hepatic gluconeogenesis during early lactation also increases the energy required to excrete the $NH_3$ produced during transamination of amino acids, further exacerbating the energy deficit (NRC, 2001). The production of urea when skeletal muscle-derived amino acids are transaminated can lead to an increase in blood (BUN) and milk urea (MUN) concentration (Jorritsma et al., 2003).

Curiously, the linear relationship between PC1 (represented mainly by milk production) and PC2 (represented by severity of NEB) could be explained by diet regimen prepartum. The linear regression model revealed inverse slopes ($P$ = 0.07) for the dietary treatments HE and CE fed prepartum (Figure 15). Cows fed CE were able to better cope metabolically with higher milk production during early lactation as exemplified by decreased values of PC2 (NEB) as PC1 (milk production) increased when compared to HE (Figure 15). The positive association of CE diet prepartum on NEB alleviation compared to HE diets is discussed elsewhere (Chapters 2 and 3).

Not surprisingly, the logistic model revealed the association of PC2 (NEB) with pregnancy success evaluated as F or S DTC. However, it was not expected to be able to detect statistical differences with relatively small numbers of experimental units (cows = 32). Cows with higher levels of PC2 had greater odds of being classified as S (greater DTC) when compared to F. Other researchers also found relationships between NEB and its detrimental effects on dairy cattle fertility (Butler and Smith, 1989, Jorritsma et al., 2003, Santos et al., 2009).

## CONCLUSION

Eight variables from a univariate analysis (FCM4, ECM4, MUN5, INS2, NEFA−1, BHBA1, GLUW3 and GLU4) were used in a principal component analysis, which successfully explained a great part of the variation and predicted reproductive success in dairy cows. Those 8 variables might be used together to predict risk factors for reproductive success (i.e., DTC) in dairy cows. Cows fed CE prepartum had a more favorable transition period, represented in this study by its lower values of PC2 (NEB) as PC1 (milk production) increased when compared to cows fed HE. Utilization of these variables and methodology by other researchers with larger data sets and different sources of variation (e.g. herd effect) could reveal supplementary insights on dairy cow infertility.

## REFERENCES


Allison, P. D. 2010. Survival Analysis using SAS®: A Practical Guide. Second Edition, Cary, North Carolina, USA.

Bauman, D. E. 2000. Regulation of nutrient partitioning during lactation: Homeostasis and homeorhesis revisited. Pages 311-328 in Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction. P. B. Cronje, ed.

Bell, A. W. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. J. Anim. Sci. 73:2804-2819.

Bell, A. W., W. S. Burhans, and T. R. Overton. 2000. Protein nutrition in late pregnancy, maternal protein reserves and lactation performance in dairy cows. Proc. Nutr. Soc. 59:119-126.

Bickerstaffe, R., E. F. Annison, and J. L. Linzell. 1974. The metabolism of glucose, acetate, lipids and amino acids in lactating dairy cows. J. Agric. Sci. 82:71-85.

Bjerre-Harpøth, V., N. C. Friggens, V. M. Thorup, T. Larsen, B. M. Damgaard, K. L. Ingvartsen, and K. M. Moyes. 2012. Metabolic and production profiles of dairy cows in response to decreased nutrient density to increase physiological imbalance at different stages of lactation. J. Dairy Sci. 95:2362-2380.

Brockman, R. P. 1990. Effect of insulin on the utilization of propionate in gluconeogenesis in sheep. Br. J. Nutr. 64:95-101.

Bruun, J., A. K. Ersboll, and L. Alban. 2002. Risk factors for metritis in Danish dairy cows. Prev. Vet. Med. 54:179-190.

Butler, W. R. 1998. Review: effect of protein nutrition on ovarian and uterine physiology in dairy cattle. J. Dairy Sci. 81:2533-2539.

Butler, W. R. 2003. Energy balance relationships with follicular development, ovulation and ferility in postpartum dairy cows. Livest. Prod. Sci. 83:211-218.

Butler, W. R., and R. D. Smith. 1989. Interrelationships between energy balance and postpartum reproductive function in dairy cattle. J. Dairy Sci. 72:767-783.

Canfield, R. W., C. J. Sniffen, and W. R. Butler. 1990. Effects of excess degradable protein on postpartum reproduction and energy balance in dairy cattle. J. Dairy Sci. 73:2342-2349.

Champe, P. C., R. A. Harvey, and D. R. Ferrier. 2008. Lippincott's Illustrated Reviews: Biochemestry. 4th ed. Lippincott Williams & Wilkins.

Chapinal, N., M. Carson, T. F. Duffield, M. Capel, S. Godden, M. Overton, J. E. P. Santos, and S. J. LeBlanc. 2011. The association of serum metabolites with clinical disease during the transition period. J. Dairy Sci. 94:4897-4903.

Dohoo, I., W. Martin, and H. Stryhn. 2009. Veterinary Epidemiologic Research. 2nd ed. VER Inc, Charlottetown, Prince Edward Island, Canada.

Drackley, J. K. 1999. ADSA Foundation Scholar Award. Biology of dairy cows during the transition period: The final frontier? J. Dairy Sci. 82:2259-2273.

Drackley, J. K., A. D. Beaulieu, and J. P. Elliott. 2001. Responses of milk fat composition to dietary fat or nonstructural carbohydrates in Holstein and Jersey cows. J. Dairy Sci. 84:1231-1237.

Drackley, J. K., S. S. Donkin, and C. K. Reynolds. 2006. Major advances in fundamental dairy cattle nutrition. J. Dairy Sci. 89:1324-1336.

Drackley, J. K., J. J. Veenhuizen, M. J. Richard, and J. W. Young. 1991. Metabolic changes in blood and liver of dairy cows during either feed restriction or administration of 1,3-butanediol. J. Dairy Sci. 74:4254-4264.

Duffield, T. F., K. D. Lissemore, B. W. McBride, and K. E. Leslie. 2009. Impact of hyperketonemia in early lactation dairy cows on health and production. J. Dairy Sci. 92:571-580.

Ferguson, J. D., and W. Chalupa. 1989. Impact of protein nutrition on reproduction in dairy cows. J. Dairy Sci. 72:746-766.

Friggens, N. C., P. Berg, P. Theilgaard, I. R. Korsgaard, K. L. Ingvartsen, P. Lovendahl, and J. Jensen. 2007. Breed and parity effects on energy balance profiles through lactation: Evidence of genetically driven body energy change. J. Dairy Sci. 90:5291-5305.

Grum, D. E., J. K. Drackley, R. S. Younker, D. W. LaCount, and J. J. Veenhuizen. 1996. Nutrition during the dry period and hepatic lipid metabolism of periparturient dairy cows. J. Dairy Sci. 79:1850-1864.

Hammon, D. S., I. M. Evjen, T. R. Dhiman, J. P. Goff, and J. L. Walters. 2006. Neutrophil function and energy status in Holstein cows with uterine health disorders. Vet. Immunol. Immunopathol. 113:21-29.

Huzzey, J. M., D. M. Veira, D. M. Weary, and M. A. von Keyserlingk. 2007. Prepartum behavior and dry matter intake identify dairy cows at risk for metritis. J. Dairy Sci. 90:3220-3233.

Johnson, D. E. 1998. Applied multivariate methods for data analysts. Duxbury Press.

Jorritsma, R., T. Wensing, T. A. Kruip, P. L. Vos, and J. P. Noordhuizen. 2003. Metabolic changes in early lactation and impaired reproductive performance in dairy cows. Vet. Res. 34:11-26.

Kahn, C. R. 1978. Insulin resistance, insulin insensitivity, and insulin unresponsiveness: A necessary distinction. Metabolism 27(Suppl 2):1893-1902.

Koltes, D. A. and D. M. Spurlock. 2011. Coordination of lipid droplet-associated proteins during the transition period of Holstein dairy cows. J. Dairy Sci. 94:1839-1848.

LeBlanc, S. 2010. Monitoring metabolic health of dairy cattle in the transition period. J. Reprod. Dev. 56 Suppl:S29-35.

LeBlanc, S. J. 2011. Is high milk production compatible with good reproductive performance in dairy cattle? Page 14 in Proc. 21st ADSA Discover Conference: Improving reproductive efficiency of lactating dairy catttle, Itasca, IL.

Litherland, N. B., H. M. Dann, and J. K. Drackley. 2011. Prepartum nutrient intake alters palmitate metabolism by liver slices from peripartal dairy cows. J. Dairy Sci. 94:1928-1940.

Lopez, H., L. D. Satter, and M. C. Wiltbank. 2004. Relationship between level of milk production and estrous behavior of lactating dairy cows. Anim. Reprod. Sci. 81:209-223.

Mattmiller, S. A., C. M. Corl, J. C. Gandy, J. J. Loor, and L. M. Sordillo. 2011. Glucose transporter and hypoxia-associated gene expression in the mammary gland of transition dairy cattle. J. Dairy Sci. 94:2912-2922.

McNamara, J. P. 1991. Regulation of adipose tissue metabolism in support of lactation. J. Dairy Sci. 74:706-719.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. National Academy Press, Washington, DC.

Opsomer, G., Y. T. Grohn, J. Hertl, M. Coryn, H. Deluyker, and A. de Kruif. 2000. Risk factors for post partum ovarian dysfunction in high producing dairy cows in Belgium: A field study. Theriogenology 53:841-857.

Ospina, P. A., D. V. Nydam, T. Stokol, and T. R. Overton. 2010a. Associations of elevated nonesterified fatty acids and beta-hydroxybutyrate concentrations with early lactation reproductive performance and milk production in transition dairy cattle in the northeastern United States. J. Dairy Sci. 93:1596-1603.

Ospina, P. A., D. V. Nydam, T. Stokol, and T. R. Overton. 2010b. Evaluation of nonesterified fatty acids and beta-hydroxybutyrate in transition dairy cattle in the northeastern United States: Critical thresholds for prediction of clinical diseases. J. Dairy Sci. 93:546-554.

Rajala-Schultz, P. J., W. J. A. Saville, G. S. Frazer, and T. E. Wittum. 2001. Association between milk urea nitrogen and fertility in Ohio dairy cows. J. Dairy Sci. 84:482-489.

Rukkwamsuka, T., T. Wensinga, and T. A. M. Kruipb. 1999. Relationship between triglyceride concentration in the liver and first ovulation in postpartum dairy cows. Theriogenology 51:1133-1142.

Santos, J. E. P., H. M. Rutigliano, and M. F. Sa Filho. 2009. Risk factors for resumption of postpartum estrous cycles and embryonic survival in lactating dairy cows. Anim. Reprod. Sci. 110:207-221.

St-Pierre, N. R. 2001. Invited review: Integrating quantitative findings from multiple studies using mixed model methodology. J. Dairy Sci. 84:741-755.

Tamminga, S., P. A. Luteijn, and R. G. M. Meijer. 1997. Changes in composition and energy content of liveweight loss in dairy cows with time after parturition. Livest. Prod. Sci. 52:31-38.

Vanholder, T., G. Opsomer, and A. de Kruif. 2006. Aetiology and pathogenesis of cystic ovarian follicles in dairy cattle: A review. Reprod. Nutr. Dev. 46:105-119.

Walsh, R. B., J. S. Walton, D. F. Kelton, S. J. LeBlanc, K. E. Leslie, and T. F. Duffield. 2007. The effect of subclinical ketosis in early lactation on reproductive performance of postpartum dairy cows. J. Dairy Sci. 90:2788-2796.

Walsh, S. W., E. J. Williams, and A. C. Evans. 2011. A review of the causes of poor fertility in high milk producing dairy cows. Anim. Reprod. Sci. 123:127-138.

Wiltbank, M., H. Lopez, R. Sartori, S. Sangsritavong, and A. Gümen. 2006. Changes in reproductive physiology of lactating dairy cows due to elevated steroid metabolism. Theriogenology 65:17-29.

Zhao, F. Q. and A. F. Keating. 2007. Expression and regulation of glucose transporters in the bovine mammary gland. J. Dairy. Sci. 90(Suppl 1):76-86.

**Table 8.** Final Cox proportional hazard model of time to conception (DTC) using a univariate approach affected by blood metabolites and milk in Holstein cows, accounting for experiment (n = 7) as a cluster effect

| Variable | Week | N | Level | Coefficient | SEM[1] | Hazard Ratio | 95% CI[2] | *P*-value |
|---|---|---|---|---|---|---|---|---|
| Blood | | | | | | | | |
| Glucose, mg/dL | 3 | 360 | H | 0.2884 | 0.14 | 1.334 | 1.005 - 1.771 | 0.05 |
| | 4 | 325 | H | 0.3323 | 0.16 | 1.394 | 1.011 - 1.923 | 0.04 |
| Insulin, µIU/mL | 2 | 323 | | −0.0453 | 0.02 | 0.956 | 0.923 - 0.999 | 0.01 |
| BHBA, mg/dL | 1 | 375 | H | 0.2628 | 0.13 | 1.301 | 1.003 - 1.687 | 0.04 |
| NEFA, µEq/L | −1 | 374 | H | −0.3413 | 0.19 | 0.711 | 0.486 - 1.039 | 0.07 |
| Milk | | | | | | | | |
| FCM, kg/d | 4 | 181 | | 0.0230 | 0.01 | 1.023 | 0.999 - 1.048 | 0.05 |
| ECM, kg/d | 4 | 181 | | 0.0243 | 0.01 | 1.025 | 0.998 - 1.052 | 0.06 |
| MUN, mg/dL | 5 | 222 | H | −0.3827 | 0.18 | 0.682 | 0.476 - 0.977 | 0.03 |
| | 5 | 222 | M | −0.2458 | 0.26 | 0.782 | 0.464 - 1.317 | 0.35 |

[1] Standard error of the mean.
[2] 95 % confidence interval.
Glucose wk 3, low < 60 mg/dL (L, referent); high > 60 mg/dL (H). Glucose wk 4, low < 65 mg/dL (L, referent); high > 65 mg/dL (H). BHBA = β-hydroxybutyrate, low < 10 mg/dL (L, referent); high ≥ 10 mg/dL (H). NEFA = non-esterified fatty acids, low < 700 µEq/L (L, referent); high ≥ 700 µEq/L (H). FCM = 3.5% fat corrected milk. ECM = energy corrected milk. MUN = milk urea nitrogen, low < 10 mg/dL (L); moderate ≥ 10 and ≤ 12 mg/dL (M, referent); high > 12 mg/dL (H).

**Table 9.** Principal Components (PC), Eigenvalues ($\lambda$), Proportion (p), and Cumulative Proportion (Cp) of the Principal Component Analysis

| PC | $\lambda$ | p | Cp |
|---|---|---|---|
| PC1 | 3.013 | 0.3767 | 0.3767 |
| PC2 | 1.301 | 0.1626 | 0.5393 |
| PC3 | 1.156 | 0.1446 | 0.6839 |
| PC4 | 0.899 | 0.1124 | 0.7963 |
| PC5 | 0.762 | 0.0953 | 0.8917 |
| PC6 | 0.513 | 0.0641 | 0.9558 |
| PC7 | 0.348 | 0.0436 | 0.9994 |
| PC8 | 0.004 | 0.0006 | 1.0000 |

**Table 10.** Loadings of the extracted PCs

| Variable[1] | Loadings* | | | |
| --- | --- | --- | --- | --- |
| | PC1 | PC2 | PC3 | PC4 |
| FCM4 | 0.84586 | −0.06507 | 0.48878 | −0.10855 |
| MUN5 | 0.41889 | 0.22852 | 0.14527 | 0.77095 |
| INS2 | −0.57674 | 0.15068 | 0.33435 | 0.40443 |
| ECM4 | 0.84147 | −0.09724 | 0.48577 | −0.10659 |
| NEFA−1 | −0.21962 | 0.82556 | 0.02836 | −0.07317 |
| BHBA1 | 0.35493 | 0.72415 | 0.00129 | −0.27813 |
| GLU3 | −0.74230 | −0.07998 | 0.44787 | −0.11314 |
| GLU4 | −0.59727 | 0.01178 | 0.58932 | −0.15149 |

[1] FCM4 = 3.5% fat corrected milk at wk 4; MUN5 = milk urea nitrogen at wk 5; INS2 = plasma insulin at wk 2; ECM4 = energy corrected milk at wk 4; NEFA−1 = plasma non-esterified fatty acids wk −1; BHBA1 = plasma β-hydroxybutyrate at wk 1; GLU3 = plasma glucose at wk 3; GLU4 = plasma glucose at wk 4.
*Only loadings > 0.42 were considered.

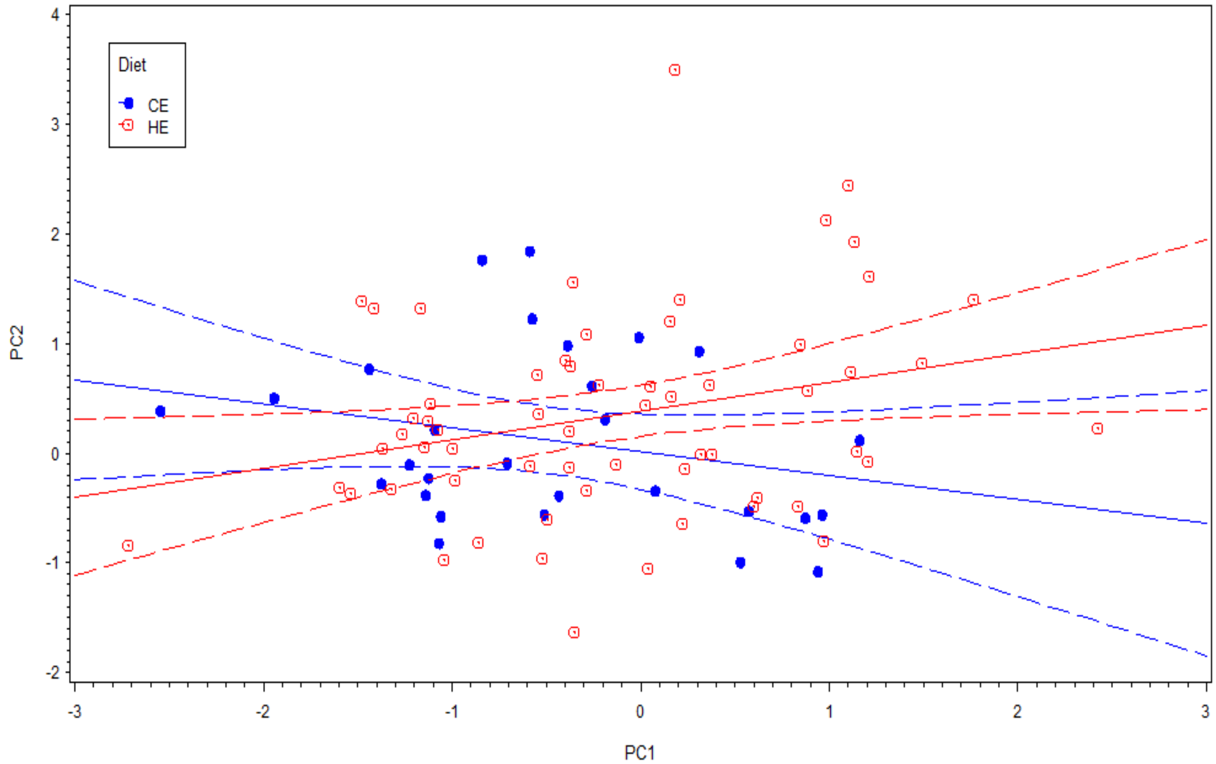


**Figure 15.** Cows (n = 89) fed two different diets during the close-up period. Diets plotted by controlled energy (CE; n= 28) or high energy (HE; n= 61) treatments on principal component analysis. Regression lines and 95% confidence interval represented by line and dashed line respectively.

# CHAPTER 5

## OVERALL SUMMARY, CONCLUSIONS, AND PERSPECTIVES

The overall objective of this thesis was to evaluate reproductive success, metabolic and physiological variables, and health of dairy cows affected by the plane of dietary energy prepartum. Our general hypothesis was that controlled dietary energy (**CE**) during the dry period, accompanied by the metabolic challenges associated with the onset of lactation would render better reproductive success to cows when compared to cows fed a high energy plane of nutrition (**HE**).

In Chapters 2, 3, and 4 we evaluated different aspects related to the transition period: Prepartum diet effect on reproductive success (Chapter 2), early lactation productive factors and health with prepartum diet effects (Chapter 3), and physiological variables (risk factors) during early lactation (first 5 wk) associated with reproductive efficiency in dairy cows.

In Chapter 2 we concluded that cows fed CE during the CU period had greater hazard ratio for days to conception (**DTC**), meaning a shorter interval between parturition and conception. The positive effect of CE may be explained by increased $NE_L$ intake during the first 4 wk postpartum and lower incidence of peripartal diseases. In addition, lower BCS loss during the first 6 wk postpartum and slightly higher glucose concentration at wk 3 likely contributed to improved reproductive performance.

Furthermore, in Chapter 3 we showed that there was no statistical difference for milk production in the first 4 wk postpartum between cows fed CE or HE prepartum. Cows fed HE during CU had greater milk fat concentration than cows fed CE on wk 2, 3 and 4. Moreover, cows that were fed HE during the dry period had more odds of experiencing displaced abomasum or ketosis when compared to cows that received CE.

Lastly, in Chapter 4 we concluded that the utilization of principal component analysis on eight physiological variables revealed the association between NEB with reproductive success in dairy cows. These eight variables might be used as risk factors to predict reproductive success and further research should be encouraged with different datasets.

In the present work CE and HE did not affect dystocia or twinning. However, these two variables are too short in duration in trying to explain the complex interaction between dam and fetus. In humans, mice, and sheep the field of epigenetics has been evolving trying to better explain the differences in gene expression that is not caused by any nucleotide change in the DNA. It studies the differences in gene expression due to the influence of external factors. Including offspring information from cows treated either with CE or HE during prepartum in our dataset could be extremely important. Milk production, health, and reproductive parameters are some of the parameters that could be evaluated for the offspring. Nutritional effects on the offspring, if any, could represent a greater economic impact at the farm.

Another area worth exploring would be a comprehensive model in measuring the economic impacts of CE and HE prepartum. In our studies we found no significant differences in milk production. However, most farmers are paid on a milk components basis and, in this case, HE cows would provide more income. However, looking only at milk production and components does not give the whole picture. Our study provides also the effects on health and reproduction of dairy cows. Exploring the economic impacts can be of great influence on farmers trying to better understand the feeding strategies prepartum.