The Effects of Elevated Subcutaneous Fat Stores on Serum and Cellular Fatty Acid Composition and Gene Expression of Pro-Inflammatory Markers in Periparturient Dairy Cows

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Authorization to Submit Thesis

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Abstract

Dairy cows with greater subcutaneous fat stores release greater concentrations of nonesterified fatty acids (NEFA) into the blood during the periparturient period. Large quantities of circulating NEFA alter phospholipids (PL) fatty acid (FA) profile. Modified cellular FA profile affects immune cell function. The objective of this study was to determine the effect of elevated lipid mobilization during the periparturient period on serum, peripheral blood mononuclear cells (PBMC), and polymorphonucleocytes (PMN) NEFA and PL fraction and milk fatty acid (FA) profile, PBMC and PMN gene expression of selected markers of inflammation and production measures. Thirty-four cows were blocked by parity; treatment group received a high-energy prepartum ration. Control group received a normal-energy ration and monensin. In summary, increased subcutaneous fat stores altered the FA profile of serum, PBMC and PMN NEFA and PL fractions and milk as well as gene expression of PBMC in periparturient dairy cows.

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Dedication

I would like to dedicate this thesis to my family.

Table of Contents

List of Figures

List of Tables

CHAPTER 1

Literature Review

Introduction

Often considered a resting period between lactation cycles, the dry period for cows is overlooked. In fact, cows experience fetal growth, mammary tissue remodeling and high nutritional demands. Dairy cows are at greatest risk for infectious disease and metabolic disorders during this transition period, typically defined as the three weeks pre- and postpartum (Drackley, 1999), which can affect subsequent lactation performance. As milk is the primary cash commodity, highly focused management is implemented at this time to ensure optimal productivity and performance while minimizing potential health problems.

The primary challenge faced by dairy cows during the transition period is a substantial increase in energy requirement when nutrient supply is inadequate from insufficient dry matter intake (DMI). Bell (1995) demonstrated that consumed net energy and metabolizable protein in healthy cows in 4 d postpartum only accounted for 65% and 75%, respectively, of the total requirements. Furthermore, calculated net energy and metabolizable protein utilization by the mammary gland accounted for 97% and 83%, respectively, of the amount consumed and therefore leaving minimal left to supply maintenance requirements. A cow's adaptive response to this negative nutrient balance is lipid and protein mobilization of adipose stores (Goff, 2006).

Lipids are stored in adipocytes in the form of triglycerides (TG) and released in the form of nonesterified fatty acids (NEFA) by the action of hormone sensitive lipases hydrolyzing the TG. The majority of NEFA are bound to albumin in circulation, but a small portion of NEFA travel through circulation as unbound monomers in aqueous solution

(Katoh, 2001; Richieri and Kleinfeld, 1995). The fatty acids (FA) travel to the heart, skeletal muscles, liver and other tissues for oxidation or conversion to other lipids (Douglas et al., 2007). The liver uptakes the circulating NEFA to either oxidize to form ketone bodies, completely oxidize for energy and carbon dioxide, or resterify for production of TG. Ruminant livers are slow to export resterified TG in very low-density lipoproteins (Grummer, 1993). Fatty liver occurs when TG synthesis and storage exceed the rate of TG hydrolysis and export. Slow accumulation of TG eventually disrupts liver function. During the transition period, high producing dairy cows experience excessive lipid mobilization and high circulating concentrations of NEFA that predispose them to fatty liver.

Fatty liver and ketosis are often considered to be related metabolic disorders. Kronfeld (1982) described four different types of ketosis in dairy cows: primary and secondary from underfeeding, alimentary and spontaneous. Primary ketosis occurs when a cow is not offered enough acceptable feed, whereas secondary ketosis is related to a decrease in DMI associated with another disease or disorder already present, such as a displaced abomasum. Alimentary ketosis results from feeding fermented products that contain great concentrations of ketogenic precursors, such as poorly fermented silage containing great amounts of butyric acids that are metabolized into beta-hydroxybutyric acid (BHB). Spontaneous ketosis occurs when there is elevated ketone concentrations even though the diet is nutritionally adequate. Susceptibility to this type of ketosis is greatest during periods of high milk production as circulating glucose and insulin decrease and plasma NEFA and BHB increase. Insufficient circulating glucose in a dairy cow during lactation results from the high glucose demand of the mammary gland, insufficient circulating concentrations, and suppressed intake leading to negative energy balance. This glucose deficiency in turn causes decreased concentrations of insulin released

into the blood. Low circulating insulin promote lipolysis in adipocytes and release of NEFA into circulation.

Zammit (1990) postulated that this period of ketosis can be separated into two phases. The first phase is related to a decrease in insulin concentration that causes an increase in malonyl-CoA and increased ketogenesis. The second phase is initiated by reduced feed intake and decreased malonyl-CoA formations. Malonyl-CoA is produced by acetyl-CoA carboxylase and acts as an intermediary for FA synthesis pathway. Concentrations of malonyl-CoA are high when rates of lipogenesis are elevated via hormonal control. High concentrations of malonyl-CoA inhibit carnintine palmitoyltransferase-1 (Bruss et al., 1986), an essential protein for translocating FA from the cytosol to mitochondria for oxidation. Decreased malonyl-CoA alleviates suppression of carnintine palmitoyltransferase-1 and FA oxidation. Malonyl-CoA concentrations appear to be the critical regulator for controlling FA partitioning to oxidation and esterification in ruminants (Grummer, 1993).

Mobilization of lipid stores into free FA decreases the quantity of the stores and results in less subcutaneous fat and body weight losses. The degree of change over time in both of these characteristics serve as a simple tool for herd management to assess the energy status of the animal.

Body Condition Score

The most common non-invasive tool to monitor subcutaneous adipose stores in dairy cows is a visually assessed body condition score (BCS), with high scores for over-conditioned cows and low scores for emaciated cows. The scale used in the United States for dairy cows scores body condition from 1 to 5 with 0.25 unit increments (Wildman et al., 1982). Scales and increments vary by country and regions from 5-units of the United Kingdom to 10-units

scales in New Zealand (Roche et al., 2009). Although body condition score is subject to observers' opinions, experience and training, it serves as a better assessment of adipose stores over body weight (BW) measurement alone as it does not account for parity, stage of lactation and gestation, frame size and breed (Berry et al., 2006). Changes in BW are further masked in early lactation by increasing dry matter intake (DMI), and therefore do not serve as reliable markers for changes in adipose stores (NRC, 2001).

Body condition score profiles typically resemble that of inverted milk lactation curves (Roche et al., 2009). Lowest BCS occurs shortly after peak milk production, 40 to 100 days in milk (DIM), and peak just before parturition (Roche et al., 2006). During the first 60 DIM, marked with increased lipid mobilization, typical changes in BCS range from 0.5 to 1.0 unit (Wildman et al., 1982). This can result in 385 Mcal of NE_L released from body energy stores if a cow weighing 600 kg lost one BCS unit, from 4 at parturition to a 3 (NRC, 2001).

Ideally, BCS during the dry period should range from 2.75 to 3.0 and between 3.0 and 3.25 at calving for near ideal lactation performance and not compromise health and welfare (Edmondson et al., 1989). When scores fall below these ranges, there is a significant decrease in milk production and increased risk to the welfare of the animal (Roche et al., 2009). Cows that go through parturition with BCS greater than the suggested range by Edmondson and colleagues (1982) will have compromised lactation performances in addition to decreased DMI and increased risk of disease and metabolic disorders. Cows fed to be over-conditioned at parturition had two- to threefold more liver lipid within 1 wk of parturition than those fed for normal or thin body condition (Fronk et al., 1980). Excess liver lipid predisposes these over-conditioned cows to fatty liver and subsequent diseases and metabolic disorders, such as

ketosis (Correa et al., 1990), displaced abomasum (Cameron et al., 1998), and hypocalcemia (Andrews et al., 1991).

Improved nutritional management and grouping strategies are often implemented by farmers to ensure optimal calving BCS with the most common strategy being the creation of two dry cow groups managed separately: far-off (60 to 21 d prepartum) and close-up (21 d prepartum to parturition). Manipulation of BCS in prepartum cow involves changes in DMI and NE_L intake with expected excess of estimated requirements increasing BCS and shortages resulting in loss of BCS during the far-off period. Interestingly, Douglas et al. (2006) found that excess and restricted intake prepartum diets resulted in similar postpartum BCS nadirs at 4 to 6 weeks after parturition. Janovick and associates (2010) reported similar results among primiparous cows; however, multiparous cows fed excess prepartum energy diets lost more BCS postpartum than cows fed normal or energy restricted diets. Therefore, initial prepartum BCS at dry-off has some role on postpartum BCS in regard to parity of the animal.

In summary, records of body condition and weight during the periparturient period can be useful for accessing overall energy status and disease-risk of an animal. Ideally, cows should be monitored throughout this critical period to ensure proper transition into lactation for maximum production and welfare of the animal. The significant factors that result in changes to BCS and BW are feed intake and energy density of the diet.

Dry Matter Intake

During the periparturient period, DMI is one of the critical factors that will determine the success of overall milk production and disease incidence. During the transition from non-lactating to lactating, cows undergo a brief period of decreased DMI. Feed intake is the most compromised during the days prior to and after calving. On average, DMI decreases by 30%

one to two days prior to calving and does not recover until one to two days after calving (Bertics et al., 1992). The decline in DMI begins long before this. Typically, observed declines occur in the last few weeks before parturition. Dry matter intake can begin to decline because of pregnancy as early as 16 weeks prior to parturition in both multiparous and primiparous cows with primiparous showing a steeper decline as compared with that of multiparous (Ingvartsen et al., 1997). Primiparous and multiparous cows consume approximately 1.7% and 2.0% of their BW in dry matter during the last 60 days before parturition. This declines to an average of 1.3% and 1.4% on the final days for primiparous and multiparous, respectively (Grummer et al. 2004). A decreased DMI as a percentage of BW for primiparous cows as compared with multiparous cows seems counterintuitive as primiparous have greater energy demands for growth in addition to sustaining pregnancy, maintenance, and initiation of lactation. Parity has a considerable effect on DMI as multiparous cows have substantially greater intake in contrast to primiparous cows. Two year old primiparous cows have roughly 80% the intake capacity of multiparous cows (Jarrige, 1986).

The physiological mechanisms behind the severe decline in DMI immediately before parturition are not fully understood. One suggestion is physical compression of the rumen by the uterus as the fetus grows in addition to increasing abdominal fat (Lagerlof, 1929). Coppock and colleagues (1974) observed that DMI decline in late pregnancy is further accentuated by including a high proportion of concentrate as compared to low proportions of concentrate in the diet, suggesting that physical compression is not the only known factor limiting intake in prepartum dairy cows. After parturition, the abdominal cavity loses mass and gains volume once more from the expulsion of fetus, fetal membranes, and amniotic fluid.

This in turn should theoretically allow for a rapid increase in DMI; however, this is not what is typically observed as increases in DMI are relatively slow in comparison to rising milk production (Ingvartsen et al., 1999). This suggests that physical compression plays a smaller role in late pregnancy DMI decline in comparison to endocrine and metabolic changes.

Another suggestion involves the changes in blood concentration of steroid reproductive hormones, specifically estrogen, during the prepartal period. Injecting estrogen has shown to depress DMI in lactating dairy cows (Grummer et al., 1990). However, initiation of DMI decline occurs before the peak in circulating estrogen preceding parturition. It is likely that there are multiple factors playing a role in DMI decline that require further research, such as fat mass, blood metabolites, corticosteroids, leptin, insulin, cytokines and neuropeptides (Ingvartsen et al., 2000)

After calving, DMI begins to increase in response to increased energy and protein requirements from milk production. The rate of incline and overall quantity of DMI peaks vary considerably between cows. Typically, DMI will peak between one and three weeks after peak milk production. However, this can vary with peak DMI not occurring until 22 weeks postpartum (Ingvartsen et al., 2000). These differences can be attributed to parity, lactation diet, prepartum diet, diet energy density and degree of over-conditioning of the cow during the periparturient period.

Prepartal intake heavily influences postpartum lactation performance and DMI in dairy cows. Nutritional strategies attempt to promote increasing appetites after parturition that result in rapid increases in DMI to modulate lipid mobilization and hepatic TG accumulation. Cows with a restricted prepartum intake (80% of NE_L requirement) had greater DMI and NE_L intake postpartum than cows fed ad libitum (160% of NE_L requirement) prepartum (Douglas

et al., 2006). Grummer et al. (2004) showed similar results with diets high in energy having an increased effect on the degree of prepartal DMI decline as compared with diets low in energy density. Primiparous cows fed high and low energy density rations experience similar though significantly steeper declines in DMI as compared to multiparous cows (Ingvartsen et al., 1997). The physiological mechanisms are not well understood, but do point to the effects of slightly lower BCS or chronic loss of BCS as potential effectors. The best suggested predictor for postpartum DMI may be changes in prepartum DMI instead of absolute prepartum DMI.

Interestingly, Minor et al. (1998) reported that cows fed a prepartum diet high (44%) in non-fiber carbohydrates (NFC) had significantly greater DMI and NE_L compared to cows fed a typical diet of 24% NFC. The higher NFC diet resulted in positive energy balance during the last few weeks of gestation. This was reflected in lower prepartum serum NEFA and BHB concentrations as well as decreased postpartum hepatic TG concentrations. Postpartum NEFA concentrations did not differ and BHB concentrations were lower in cows fed the high NFC diet. This suggests that cows fed a typical, prepartum diet accumulated TG before parturition, consequently creating fatty liver disease and predisposing them to ketosis.

In contrast, restricted energy intake in prepartum cows led to decreased postpartum lipid mobilization and almost half the total liver lipid and TG concentrations as compared to cows fed excessive energy diets during the prepartum period (Douglas et al., 2006). Although the physiological mechanisms are not well understood, Rukkwamsuk et al. (1998) and Drackley (1999) suggested that alterations in hepatic metabolism from constant high concentrations of blood NEFA adapted the liver to better contend with increasing postpartum blood NEFA concentrations and therefore allowed cows with the restricted energy, prepartum

diet to not accumulate as high hepatic lipid concentrations as cows fed excessive energy, prepartum diets.

Dry matter intake patterns are influenced by health status. Once in a diseased state, whether clinical or subclinical, DMI will decline. This appears counterintuitive as the nutrient needs of the animal increase to fuel increased body temperature, metabolism and repair tissue breakdown, yet DMI decreases, especially in periparturient cows. Increased incidences of metabolic disorders, such as fatty liver and ketosis soon follow as the cow enters into negative energy balance and subsequent lipid mobilization that overwhelm the liver's metabolic activities and cause hepatic TG accumulation.

Further acerbation of fatty liver and ketosis can create greater susceptibility to other diseases and metabolic disorders. Retrospective analysis of prepartum DMI in cows experiencing one or more metabolic disorders found that DMI during the final 27-days prior to parturition decline from 1.8% to 0.9% of body weight as compared to a decline of 1.8% to 1.2% for cows that did not experience any postpartum metabolic disorders (Zamet et al., 1979). This indicates that cows with a greater prepartal DMI decline are more predisposed to contracting postpartum metabolic disorders.

Ionophores

Several factors alter prepartum and postpartum DMI that can ultimately lead to negative energy balance and increased disease incidence. Ionophoric agents, such as monensin and lasolacid, are antibiotics widely used in commercial animal feed operations to alter rumen microflora to prevent disease and improve feed and ultimately production efficiency. Monensin can form complexes with monovalent cations, such as sodium and potassium, and transport them across cellular lipid membranes, causing disruption in sodium-potassium balance and pH within the cell. This leads to disruption of critical cellular

processes and cellular death (Russell, 1987). Gram-positive bacteria have less complex outer cell membranes than gram-negative bacteria. Ionophores, therefore, selectively inhibit Gram-positive bacteria (Bergen and Bates, 1984).

Monensin exerts its effects in three major areas of metabolism: energy, nitrogen and general digestive effects, including reduction in bloat and lactic acidosis. Shifting rumen microflora results in altered volatile fatty acid production with increased propionic acid and reduced acetic and butyric acids (Richardson et al., 1976). Increased propionic acid improves gluconenogenesis (Schelling, 1984) and is beneficial to lactating cows as inclusion of monensin in peripartal diets is reported to improve energy balance by reducing lipid mobilization and subsequent NEFA concentrations as well as BHB synthesis. This decreases the overall incidence of subclinical ketosis (Duffield et al., 2008a).

In addition to impacting overall health, monensin affects overall milk production and efficiency. In a meta-analysis of the impact of monensin on production outcomes in dairy cows, Duffield and associates (2008b) reported that monensin increased milk yield by 0.7 kg and decreased milk fat and protein percentage by 0.13% and 0.03%, respectively. In addition, monensin significantly decreased DMI by 0.3 kg, therefore improved milk efficiency by approximately 2.5%. These effects on milk production and efficiency were not consistent among studies. The authors suggested that dietary factors, monensin dose and genetic merit may have contributed to the high variability observed.

In summary, ionophores play an important role in altering rumen microflora and metabolism for increased milk production efficiency and favorable health benefits, such as decreased incidence of ketosis, bloat and lactic acidosis. Although monensin is classified as

an antibiotic, its primary effects do not extend beyond the rumen and do little to aid the immune response of the cow against foreign pathogens.

Immune Response

Improved nutrition and management can reduce the incidence of disease and metabolic disorders in the periparturient dairy cow. However, susceptibility to infectious disease is dependent upon the integrity of the immune system. During the periparturient period, it is well documented that several components of the immune system are altered, including neutrophil function, lymphocyte responsiveness to mitogen stimulation, distribution of cell populations, and cytokine production by immune cells.

Polymorphonucleocytes

Polymorphonucleocytes (PMN) are circulating blood cells with multiple nuclei, such as neutrophils and eosinophils. Lactating Holstein cows have a large pool of over 100 billion circulating, mature PMN present (Paape et al., 1979). In comparison to other animal species where PMN comprise the majority of leuckocytes in the blood, bovine PMN account for only 25% of the total circulating leukocytes (Jain, 1986) and have a relatively short half-life of approximately 9 hours (Carlson and Kaneko, 1975).

Concentrations of PMN in total circulating leukocytes are not static. During an inflammatory challenge, PMN account for up to 60% of leukocytes in circulation (Paape et al., 1974). Kimura and associates (1999) reported a similar increase in PMN numbers shortly before parturition that decreased soon thereafter. It is well documented that dairy cows experience PMN dysfunction during the periparturient period (Paape et al., 2003). The mechanisms behind the immunosuppression remain however debatable with metabolites and hormones influencing the inflammatory reaction. The pattern of change in PMN profile

around calving suggests that a compensatory mechanism may exist because of lower phagocytic capacity of PMN during this period (Ingvartsen et al., 2003). Sander et al. (2011) however provided evidence that PMN phagocytic activity after parturition is not always lower than that prepartum.

Migration of PMN into infected tissue serves as a very important and primary line of defense against invading pathogens. Potent toxins released from bacteria activate nearby macrophages and endothelial cells near the infection site to secrete cytokines, such as interleukin (IL)-8, that recruit PMN to serve as phagocytes (Kettritz et al., 1998). Upon activation, various inhibitory factors begin to alter PMN morphology that ultimately decrease their effectiveness to battle the infection. *In vitro* exposure to high concentrations of NEFA similar to those observed in dairy cows experiencing negative energy balance shortly after parturition significantly decreased PMN viability and phagocytosis while increasing necrosis (Scalia et al., 2006). Milk PMN are less effective phagocytes than blood PMN from exposure to milk fat globules and casein micelles as these must be phagocytized to prevent PMN damage (Paape et al., 2003). Large quantities of globules stored in phagosomes reduce the quantity of plasma membrane available for formation of pseudopodia necessary for trapping and engulfing bacteria as well as available lysosomes fuse with phagosomes containing fat and casein globules instead of phagosomes containing bacteria (Paape and Guidry, 1977).

As PMN phagocytose and destroy pathogens, PMN release chemicals that not only kill the invading pathogens, but also damage the surrounding tissues. To minimize damage, PMN undergo apoptosis, programmed cellular death. The life span of PMN is ultimately determined by the onset of apoptosis. Some cytokines, such as IL-8 and tumor necrosis factor-α (TNFα), increase PMN life span by delaying apoptosis onset (Kettritz et al., 1998). Nearby

macrophages will engulf apoptotic PMN to minimize the release of PMN granular contents that could damage nearby tissues (Cox et al., 1995).

Interleukin-8 receptor (IL-8R) is a G-protein coupled receptor found on the PMN cellular surface that binds free IL-8. There are two types of IL-8R: CXCR1 and CXCR2. Both are expressed on PMN and monocytes (Thomas et al., 1991). Endothelial cells and macrophages near sites of infection release IL-8 to function as a chemotactic factor to recruit circulating PMN to the infected site as well as induce production of reactive oxidative species (Bergin et al., 2010). Expression of IL-8R decreases soon after parturition possibly impairing chemotaxis of PMN to inflammatory sites (Seo et al., 2013). In contrast, IL-8 cytokine expression in bovine PMN increased at parturition (Madesen et al., 2004), suggesting that upregulation of IL-8 may not be associated with upregulation of its functional receptor in PMN.

In order to gain access to infected tissue and communicate with other cells in the body, PMN possess a diverse array of cell surface receptors. L-selectin (SELL) is a cell adhesion molecule on the surface of bovine PMN that binds to ligands present on endothelial cells to slow PMN trafficking and facilitate entry into the infected tissue (Wang et al., 1997). McClenahan and associates (2000) found PMN exposed to IL-8 caused proteolytic shedding of SELL, thus decreasing PMN adhesion to inflamed tissue and diapedesis. Similarly around the time of parturition, SELL is shed from the surface of bovine PMN (Lee and Kehrli, 1998), indicating that PMN function during this period is compromised and could lead to immunosuppression. Cows treated with dexamethasone and cortisol had decreased SELL expression in milk PMN and increased susceptibility to bacterial infection following experimental mastitis challenge (Burton and Kehrli, 1995). Similar decreases in SELL

expression in circulating PMN have been reported in cows in the first few days following parturition (Berning et al., 1993; Seo et al., 2013). This indicates that bovine PMN have the capacity to alter SELL expression in response to changes in plasma glucocorticoid concentrations.

Caspase-1 (CASP1) is a rate-limiting, IL converting enzyme that cleaves precursor proteins from the pro-inflammatory cytokine IL-1β to make it an active peptide. Activation of CASP1 depends on inflammasomes that are members of the NOD-like receptor family, intracellular sensors associated with cell stress (Martinon and Tschopp, 2004). During periods of inflammation and exposure to infectious pathogens, IL-1β production by neutrophils, macrophages, monocytes and other immune cells activates other nearby phagocytes, increases diapedesis of effector cells into the site of infection, and causes release of toxic oxygen and nitrogen radicals as well as pro-inflammatory cytokines (Netea et al., 2010). Similar to IL-8, IL-1β production by PMN decreases with exposure to glucocorticoids (Burton and Erskine, 2003). Supplementation with OmniGen-AF in sheep immunosuppressed by glucocorticoids resulted in restoration of IL-1β concentrations (Wang et al., 2007) and increased CASP1 gene expression (Wang et al., 2008) suggesting that increased CASP1 expression is associated with increased IL-1β production in PMN.

In summary, PMN function as the primary defense against infection by foreign pathogens. During the periparturient period, PMN chemotaxis, diapedesis, phagocytosis and cytokine production is altered, leading to the possibility of an immunosuppressed state. However, PMN are not the only defense against invading pathogens as other immune cells present, such as macrophages and lymphocytes, provide a more specialized and longer lasting defense.

Peripheral Blood Mononuclear Cells

Peripheral blood mononuclear cells (PMBC) are blood cells that have a round nucleus. This includes macrophages, dendritic cells and lymphocytes (T cells, B cells, and natural killer cells). Macrophages represent the dominant cell type in milk and mammary glands in healthy, lactating cows (Sordillo et al., 2002). During bacterial infections, macrophages can serve as a part of either the innate or acquired immune responses. Similar to PMN, a function of macrophages is to destroy pathogens through phagocytosis, proteases and reactive oxygen species. Phagocytic rate in macrophages can be increased in the presence of opsonic antibodies (Miller et al., 1988). In the mammary gland, macrophage numbers tend to decrease during periods of inflammation and possess less phagocytic abilities in comparison to neutrophils (Niemialtowski et al., 1988).

A large, horseshoe-shaped nucleus in macrophages makes migration between endothelial cells difficult in comparison to PMN migration. Thus, PMN are the first, newly migrated phagocytic cells to arrive at the site of infection (Paape et al., 2003). This suggests that the ability of macrophages to secrete cytokines, prostaglandins and leukotrienes to facilitate migration and bactericidal activities of neutrophils as well as augment local inflammatory processes are of greater importance compared with macrophage nonspecific defense as a phagocyte (Kehrli et al., 1999). Macrophages also play a role in the development of specific immune response through antigen processing and presentation via MHC II complex to lymphocytes (Fitzpatrick et al., 1992). During the periparturient period, serum macrophage numbers tend to increase prepartum and peak around parturition (Contreras et al., 2010). Phagocytic capacity of these cells is however significantly decreased, possibly because of a lower opsonic activity (Sordillo et al., 2002). Expression of MHC II in macrophages is

decreased during this period, which could contribute to poor antigen presentation and a weaker response from lymphocytes (Fitzpatrick et al., 1992).

In comparison to macrophages, lymphocytes deliver a much slower, yet highly specialized attack against invading pathogens. Lymphocytes are able to recognize pathogens through membrane receptors that communicate with antigen presenting cells to define the specificity, diversity, memory and recognition of self versus non-self. Lymphocytes can be divided into two subsets: T and B lymphocytes. The T lymphocytes are classified further to CD4+ and CD8+ T cells and T helper cells. The CD4+ T cells, also known as T helper cells, aid in B cell maturation and activation of macrophages and CD8+ T cells through cytokine production. The CD8+ T cells (T cytotoxic cells) target and destroy virus-infected cells.

Predominant T cell subsets vary by tissue, stage of lactation, disease state, and species. In healthy bovine, human and porcine mammary glands, CD8+ T lymphocytes are the primary phenotype in contrast to peripheral blood where CD4+ is the most prominent cell type (Asai et al., 1998; Wagstrom et al., 2000). Caprine mammary glands have primarily the CD4+ phenotype throughout lactation (Ismail et al., 1996). Coinciding with parturition and initiation of lactation, peripheral T cell populations, relative to total PBMC, begin to decrease approximately one week before parturition and recover to prepartal levels shortly thereafter (Contreras et al., 2010). During mastitis, CD4+ T lymphocytes prevail and are activated by antigen presenting cells with the MHC class II complex, such as macrophages and B cells. Cytokines released by these cells mediate whether the T helper cell response will be cell-mediated or humoral. Interleukin-2 and interferon-γ are major cytokines produced during a cell-mediated response, whereas IL-4, IL-5 and IL-10 dominate a humoral response (Brown et al., 1998).

The immunological role of B lymphocytes is very different from macrophages, neutrophils and T lymphocytes. During an immune response, B lymphocytes produce massive amounts of cytokines against invading pathogens. Unlike macrophages and neutrophils, B cell rely on specialized cell surface receptors to recognize specific pathogens. Similar to macrophages, B lymphocytes function as antigen presenting cells to present antigen properties via MHC class II molecules to T helper lymphocytes. After presentation, T lymphocytes produce IL-2 to induce B lymphocyte proliferation and differentiation into plasma cells for more antibody production or memory cells. Unlike macrophage and T lymphocytes, the percentage of B cells remains fairly constant during lactation stages in cattle (Shafer-Weaver et al., 1996).

Of the many cytokines produced by the various cell subsets of PBMC, IL-1β, IL-6, intracellular adhesion molecule-1 (ICAM) and tumor necrosis factor-α (TNF-α) serve important roles. Pro-inflammatory cytokines IL-1β, IL-6 and TNF-α have been linked to the pathology of acute mastitis during early lactation (Oviedo-Boyso et al., 2007). Bacteria are recognized by specific pattern recognition receptors on the cell surface of neutrophils, monocytes and macrophages. This initiates signaling pathways that lead to the release of pro-inflammatory cytokines TNF-α and IL-1β. Stimulation of toll-like receptors activates the NFκB pathway to stimulate and secrete TNF-α (Bryant et al., 2010). Interleukin-1β also requires stimulation via toll-like receptors of immune cells to secrete the IL-1β procytokine that requires cleavage by CASP1 (Bryant and Fitzgerald, 2009). Both TNF-α and IL-1β stimulate macrophage and neutrophil phagocytosis of bacteria and production of toxic oxygen and nitrogen intermediates (Butterfield et al., 2006). Expression of TNF-α from isolated PBMC is greatest during the periparturient period compared with mid and late lactation

(Sordillo et al., 1995). During the peripartal period, bovine mammary endothelial cells had significantly increased expression of TNF-α approximately one week before calving as compared to 4-5 weeks pre- and postpartum (Aitken et al., 2009). Increased circulating TNF-α and IL-1β concentrations during this period may be a contributing factor to suppressed DMI as injections of TNF-α (Bernstein et al., 1991) and IL-1β (Langhans et al., 1993) in rodents reduced intake. In cattle, TNF-α injections had little impact on DMI, but did affect eating behavior (Bielefeldt et al., 1989). Authors speculate these changes in ruminants may in part be because of TNF-α mediated inhibition of rumen motility (Van-Miert et al., 1992).

Interleukin 6 is a cytokine that influences several tissues and PBMC in the early stages of inflammation and immune responses and is produced by various cells of different origin, such as monocytes-macrophages, epithelial cells, fibroblasts, T and B lymphocytes, and endothelial cells (Ruef and Coleman, 1990). Secretions of IL-6 can have either pro- and anti-inflammatory effects that enhance or limit the immune response (Jones, 2005). Initially following IL-1β and TNF-α, IL-6 secretions attract neutrophils to the site of infection. Proteolytic processing of IL-6 receptor from invading neutrophils drives IL-6 trans-signaling in the surrounding tissue, leading to a switch from neutrophil to monocyte recruitment by suppressing neutrophil-attracting and enhancing monocyte-attracting chemokines (Scheller et al., 2011). Moreover, adhesion molecules, such as ICAM, on endothelial cells are upregulated by IL-6 trans-signaling and therefore enhance leukocyte transmigration (Kaplanski et al., 2003).

Besides its role in leukocytes, IL-6 plays a crucial role in influencing T and B cell lymphocytes. Interleukin-6 is necessary for T cell recruitment and has been shown to rescue T cells from entering apoptosis (McLoughlin et al., 2005; Jones, 2005). After T cell activation

by antigen presenting cells, the T-helper cell precursor is heavily influenced by IL-6 and leads to increased differentiation into T-helper cell type 2 and 17 (Diehl and Rincon, 2002). With B lymphocytes, IL-6 plays an important role in supporting B cell differentiation and maturation (Clogsten et al., 1989).

Large concentrations of IL-6 in circulation can arise from several sources of inflammation. Following exposure to an *in vivo* inflammatory challenge, similar to those found in the periparturient period, bovine and human subcutaneous adipose tissue produced a significant IL-6 response (Fried et al., 1998; Mukesh et al., 2010). Another possible source is the placenta, which has the ability to produce cytokines throughout its lifespan (Hauguel-de Mouzon and Guerre-Millo, 2006). Bovine with Johne's Disease, a chronic inflammatory bowel disease, have macrophages that upregulate expression of the IL-6 gene (Coussens et al., 2004).

Interleukin-6 plays a crucial homeostatic role in hepatocytes during inflammation and ketosis by exerting important effects on metabolic and energy production pathways. Induced ketosis in postpartum cows resulted in increased expression of IL-6 (Loor et al., 2007). This appears to play a central role in the impairment of normal liver function in transition cows. The extent of inflammatory response can be characterized by the liver functionality index, which takes into consideration the concentrations of circulating albumin, cholesterol and bilirubin (negative acute phase response proteins; Bertoni et al., 2006). Early lactation cows with low liver index are considered in a state of high inflammatory response and cows with high liver index having a low inflammatory response. Circulating IL-6 concentrations were greater in cows with increased inflammation, as determined by liver index, at d 3 and 21 postpartum with the greatest difference reached at d 28. Lowest concentrations of IL-6 were

present in cows with low and high inflammation around time of parturition (Trevisi et al., 2012).

The ICAM is an immunoglobulin- like cell adhesion molecule that is expressed by several cell types including leukocytes, hepatocytes, and epithelial and endothelial cells. The binding and cell-to-cell interaction of endothelial ICAM to leukocyte lymphocyte function-associated antigen-1 is essential for migration of leukocytes into pathogen-infected tissues (van de Stolpe and van der Saag, 1996). In T lymphocytes, ICAM serves as a co-stimulatory molecule on antigen presenting cells to activate MHC class II T cells (Zuckerman et al., 1998). Pro-inflammatory cytokines IL-1β and TNF-α along with other inflammatory mediators, such as oxidant stresses, retinoic acid and viral infection, upregulate ICAM expression (Shrikant et al., 1994; van de Stolpe and van der Saag, 1996). Over expression of ICAM can however lead to pathologic, pro-inflammatory circumstances (Sordillo et al., 2008). During the periparturient period, bovine mammary endothelial cells had a significantly increased expression of ICAM approximately one week before calving as compared to 2-3 weeks pre- and postpartum (Aitken et al., 2009).

In summary, PBMC include several cell types with diverse functions and responses to immunological challenges. Macrophages serve primarily as intermediates between innate and adaptive immune responses by functioning as antigen-presenting cells and cytokine producers that mediate lymphocyte cell activation, differentiation and maturation. The T and B lymphocytes operate as highly specialized and pathogen-specific immune effector cells. Cytokines and cellular surface molecules produced by immune cells and surrounding tissue are essential for intra- and intercellular communication. Many require FA as a part of their

activated structure. Changes to quantity and types of available FA in circulation are reported to alter cytokine production and ultimately affect cellular communication and function.

Fatty Acids

Fatty acids are long hydrocarbon chains with a carboxyl group at one end and a methyl group at the other. The most abundant FA have an even number of carbons and can vary from 2 to 30 or more carbons. Saturated fatty acids (SFA) are straight chains and do not contain carbon-carbon double bonds. Unsaturated fatty acids (UFA) contain one or more double bonds, giving the overall structure of the FA a kinked-appearance. A FA containing two or more double bonds is called a polyunsaturated fatty acid (PUFA).

Saturated fatty acids typically have names based upon the source from which they were originally isolated. Unsaturated fatty acids are named by identifying the number of double bonds and the position of the first double bond from the methyl-terminus. For instance, linoleic acid (C18:2) is the simplest member of omega-6 family (n6). After desaturation by Δ6-desaturase, C18:2 yields α-linolenic acid (C18:3n3), the simplest member of omega-3 (n3) family. Mammals lack the desaturase enzymes necessary to synthesize C18:2n6 and C18:3n3 acids (Calder, 2005). Further desaturation and elongation of C18:2n6 can yield γ-linolenic acid (C18:3n6), dihomo-γ-linolenic acid (C20:3n6) and arachidonic acid (C20:4n6). Similar enzymes used to metabolize the n6 family can convert C18:3n3 into eicosapentaenoic acid (C20:5n3), docosapentaenoic acid (C22:5n3) and docosahexaenoic acid (C22:6n3). Metabolic enzymes are shared by both n6 and n3 metabolic pathways, therefore leading to competition between families for synthesis of products. In mammals, the desaturation and elongation occur primarily in the liver (Calder, 2005).

Plant seed oils, such as canola, sunflower, cottonseed and soybean, have FA profiles rich in UFA, especially oleic acid (C18:1) and C18:2. Sunflower oil typically is >70% of C18:2, whereas canola oil is approximately 60% C18:1 (McKevith, 2005). Saturated fatty acids are found in high concentrations in animal products, such as tallow or lard, with palmitic acid (C16:0) and stearic acid (C18:0) having the greatest concentrations (Mitchaothai et al., 2007). Supplementing these different types of FA does not necessarily guarantee that they will be absorbed and available for metabolism as rumen microbes can reduce (biohydrogenate) UFA into SFA. The main FA substrates for biohydrogenation in dairy cows is C18:3n3 from grasses and other forages, and C18:2 from dietary lipid supplements (Lourenco et al., 2010). End-products from biohydrogenation include unsaturated intermediates conjugated linoleic acid (*cis*-9, *trans*-11 C18:2) and C18:1, and fully-saturated C18:0 (Chilliard et al., 2007).

Status of FA in ruminants varies because of dietary supply and FA profile, and degree of rumen biohydrogenation for absorption post ruminally (Glasser et al., 2008b). Given the altering effects of biohydrogenation on FA composition of lipid feed supplements, one would assume the only method of enriching UFA content would require the FA to be encapsulated or ruminally protected for adequate absorption. Simply increasing the dietary intake of UFA, especially PUFA however enhances PUFA content in milk and tissue (Dewhurst et al., 2006). Milk and tissue respond to PUFA supplementation with enriched n3 and n6 concentrations similar to that in nonruminant PUFA supplementation (Bilby et al., 2006). In fact, dietary strategies that involve altering the FA composition of the diet often combine bypass, excess FA quantities and manipulation of biohydrogenating bacteria (Lourenco et al., 2010). These

dietary and metabolized FA serve as the primary substrates in NEFA and phospholipids (PL) structure.

Nonesterified Fatty Acids

As previously mentioned, NEFA are free fatty acids released from hydrolyzed triglycerides stored in adipocytes during periods of energy shortage. Normal NEFA concentrations for cows in positive energy balance are less than 0.2 mEq / L. During the close-up period, concentrations typically range between 0.5 and 1.0 mEq / L until the week prior to parturition. Two to three days prior to parturition, concentrations will begin to increase and peak at 0.8 to 1.2 mEq / L after parturition (Drackley, 1999). These high concentrations, greater than 0.5 mEq / L, are detected for weeks after parturition and are indicative that the cows are experiencing intense lipid mobilization to compensate for negative energy balance and may be suffering from subclinical and clinical ketosis (Veenhuizen et al., 1991, Busato et al., 2002). By six weeks after parturition, plasma NEFA concentrations return to normal levels below 0.2 mEq / L (Drackley, 1999).

Dairy cows fed an energy-restricted, prepartum diet had greater concentrations of circulating NEFA prepartum until one week before parturition as compared with cows fed ad-libitum during the same period (Douglas et al., 2006). This is to be expected as greater rates of lipid mobilization were required to support energy needs and resulted in a sustained loss of BCS throughout the prepartum period. After parturition, cows fed ad-libitum had greater sustained NEFA concentrations, reflecting the decreased DMI for cows fed an energy dense diet in addition to decreasing BCS and excess lipid mobilization (Grummer et al., 2004). Concentrations of plasma NEFA were greater in the 2 weeks prior to and post-calving in Holstein cows with high inflammatory response compared with cows with low inflammatory

response, as determined by liver function index. Peak concentrations of 1.1 mmol/L was observed around d 3 postpartum (Trevisi et al., 2012). These higher concentrations of circulating NEFA have been associated with increased risk for development of metabolic disorders such as fatty liver and ketosis (Bobe et al., 2004).

Nonesterified fatty acids primarily consist of C16:0, C18:0 and C18:1 (Douglas et al., 2007). These FA remain the most prominent during the periparturient period (Contreras et al., 2010), early lactation (Watts et al., 2013), and late lactation (Tyburczy et al., 2008). Though used primarily for cellular energy synthesis through β-oxidation, they are also essential for milk fat synthesis and maintaining milk fluidity (Loften et al., 2014). Most of C16:0, C18:0 and C18:1 originate from dietary sources with an additional proportion coming from rumen microbial biohydrogenation of UFA into C18:0 primarily, C16:0 secondary and some intermediate UFA that exit the rumen before completion (Glasser et al., 2008a).

Phospholipids

Phospholipids (PL) are a class of lipid that generally consists of a hydrophobic tail and hydrophilic head that typically contain a diglyceride, glycerol molecule, and phosphate group. Given their structure, PL serve as the major component of cellular membranes contributing to the physical and functional properties of these membranes. Although essential for structure of the cell and organelles within the cell, PL constitute a relatively small fraction of total lipids within the cell and the overall distribution of lipid in circulation and tissue (Spector and Yorek, 1985). The FA composition of the PL fraction consist primarily of the common C16:0, C18:0 and C18:1, similar to NEFA. In contrast to the FA distribution in other fractions, there is a much greater percentage of UFA, particularly PUFA, relative to total lipid in PL with

greater concentrations of C20:4, C20:5 and C22:6 than those reported in NEFA or TG (Contreras et al., 2010; Watts et al., 2013).

Alterations to FA in PL can result in changes to the membrane and its many functions, such as physical properties (membrane fluidity and protein raft structure), cell signaling pathways (extra- and intracellular), and alteration in the pattern of lipid mediators (Calder, 2008). Changes to cellular signaling results in altered transcription factor activation and modified gene expression. As PL provide substrates for synthesis of potent pro- and anti-inflammatory mediators, such as eicosanoids, variation to PL results in significant changes to inflammation and the immune response.

Inflammation

Certain fatty acids are highly linked to inflammation because a family of inflammatory mediators, called eicosanoids, are synthesized by removing 20-carbon PUFA from PL in the cellular membrane. Typically, the FA profile of inflammatory cells producing eicosanoids contain a high proportion of n6, mostly C20:4, and low proportions of n3 PUFA (Calder, 2001). Eicosanoids include prostaglandins (PG), thromboxanes (TX), leukotrienes (LT) and hydroxyeicosatetraenoic (HETE) acids. Various phospholipase enzymes mobilize C20:4 from cell membranes to act as a substrate for the enzymes that synthesize eicosanoids. Cylco-oxygenase (COX) enzymes produce PG and TX. Metabolism of 5-lipoxygenase (LOX) enzymes synthesize HETE derivaties and 4-series LT. When inflammatory conditions are present, there are increased production rates of C20:4-derived eicosanoids (Calder, 2005). This leads to increased concentrations of eicosanoids in circulation and tissue.

Increased consumption of n3 PUFA results in increased proportion of these FA in circulation and in inflammatory cell phospholipids. Surprisingly, the proportion of C20:4

decreases when n3 increases in the PL fraction (Calder et al., 1990). Incorporation of increased supplemented amounts of n3 PUFA, specifically C20:5 and C22:6, into cellular membranes decrease the arachidonic acid content because of inhibition of arachidonic acid metabolism by n3 PUFAs and thus increasing available C18:2 (Peterson et al., 1998). As there is less C20:4 available, eicosanoid production, specifically derived from this FA, decreases in inflammatory cells (Calder et al., 2005). Instead, C20:5, acts as substrate for COX and LOX synthesis that result in the production of a different family of eicosanoids that are reported to be less potent pro-inflammatory mediators than those derived from C20:4 (Sperling et al., 1993; Lee et al., 1984). Anti-inflammatory mediators in the form of resolvins can also be synthesized from increased intake of C20:5 and C22:6 (Serhan et al., 2000; Hong et al., 2003).

Although incorporation of n3 PUFA into cells alters C20:4 metabolism and elicits anti-inflammatory effects, n3 have a number of other anti-inflammatory effects in modifying immune cell response. Contreras et al. (2010) and Watts et al. (2013) observed that increases in specific FA in plasma NEFA and PL fraction are reflected by changes in FA profile of PBMC. It is unclear to what extent immune cells synthesize these FA or uptake them from circulating lipoproteins to incorporate them into the membrane as immune cells likely obtain membrane PL from both sources (Calder, 2005). Cell culture studies with human macrophages demonstrated that additions of C20:5 and C22:6 inhibited IL-1β and TNF-α production in monocytes (Chu et al., 1999) and IL-6 and IL-8 production in endothelial cells (De Caterina et al., 1994). When supplemented in feed, rodent macrophage's production of IL-1β, IL-6 and TNF-α significantly decreased. Similar results were reported in healthy humans that supplemented their diet with fish oils (Wallace et al., 2003). In addition to altered

cytokine production, ICAM-1 surface expression on murine macrophages decreased because of C20:5 and C22:6 supplementation (Miles et al., 2000). Caprine PMN cultured in C20:5 and C22:6 showed increased phagocytosis and decreased production of reactive oxygen species (Pisani et al., 2009). Culturing cells in n3 PUFA is reported to exert anti-inflammatory effects through altered gene expression. Human chondrocytes cultured in C20:5 and C22:6 had decreased expression of TNF-α and IL-1β (46). Mice fed diets high in fish oil had decreased TNF-α, IL-1β and ICAM mRNA expression in macrophages (48 33 43).

In summary, FA serve as important components for energy, membrane structure and synthesis of pro- and anti-inflammatory mediators. Alteration to FA composition through diet supplementation and lipid mobilization has far reaching effects in overall FA composition of various fractions of tissue, circulation and cells as well as downstream cellular metabolism and gene expression. This is particularly important in immune cell response as altered FA profiles can disrupt cell responsiveness and overall effectiveness when fighting against an infection.

Hypothesis

Over-conditioned cows will have different serum FA profiles than under-conditioned cows. This altered profile will effect FA profiles of circulating PBMC and PMN as well as their gene expression of select markers of inflammation.

Objectives

The first objective of the present research study was to investigate the effects of subcutaneous fat stores, as assessed by BCS around parturition, and the subsequent lipid mobilization during early lactation on FA profile of serum NEFA and PL fractions and productive performance. The second objective was to determine the effects of elevated lipid mobilization from prepartum dietary manipulation on NEFA and PL fractions in serum, PBMC and PMN and milk FA profile as well as PBMC and PMN gene expression of pro-inflammatory mediators.

CHAPTER 2

Effects of Subcutaneous Fat Stores on Serum Phospholipids and Nonesterified Fatty Acid Lipid Fractions in Periparturient Dairy Cows

Abstract

Negative energy balance in early lactating dairy cows results in a massive release of fatty acids (FA) into the blood in nonesterified (NEFA) form. Large quantities of circulating NEFA may alter the serum FA profile of phospholipids (PL) fraction, which is responsible for cellular plasma membrane integrity and intercellular signaling. The objective of this study was to determine the effects of subcutaneous fat stores, as assessed by body condition score (BCS) on a scale of 1 to 5, around the time of calving and the subsequent lipomobilization on FA profile of serum NEFA and PL lipid fractions, and on productive performance. Based on BCS, cows were retrospectively dichotomized into two groups: over-conditioned (BCS $\geq$ 3.25) and control (BCS $\leq$ 3.0). Twenty-two cows had serum samples obtained at -28, -7, +8, +18, and +28 d relative to parturition and analyzed for the FA profile of the NEFA and PL fractions. As expected, over-conditioned cows had greater total plasma NEFA concentrations and decreased dry matter intake. Milk yield and composition did not differ between groups. More importantly however, several FA in the NEFA fraction of plasma lipids varied significantly, including C14:1, C16:0, C18:0 and C20:3n3. In the PL fraction, other FA varied significantly by BCS around time of parturition, including C16:0, C17:0, total C18:2 cis, and C20:2. In summary, BCS did affect FA profile of serum NEFA and PL lipid fractions. This may have drastic consequences for circulating immune cells and their ability to fight infection by altering their FA profile.

Introduction

Cows entering the periparturient period experience negative energy balance related to energy requirements associated with lactation. This results in mobilization of adipose tissues in the form of free fatty acids (FA). Fatty acids are transported through the blood in various lipid fractions including neutral lipids (NL), nonesterified fatty acids (NEFA) and phospholipids (PL). Circulating lipoproteins carry NL and PL and are made available to cells by lipoprotein lipases (Tall, 1995). The majority of NEFA are bound to albumin in circulation, but a small portion of NEFA travels through circulation as unbound monomers in aqueous solution (Richieri and Kleinfeld, 1995).

Lipomobilization is an adaptive response by cows to an energy shortage. This creates a large flux in the concentration of plasma lipids and corresponding fractions, leading to a shift in FA composition in various lipid fractions (Douglas et al., 2007). Changing FA profiles no longer reflect the shifts originating from diet. Moreover, total FA composition of plasma lipids does not reflect the FA composition of the fractions because the FA distribution among lipid fractions differs. Neutral lipids, such as triglycerides and cholesterol esters, provide substrates for lipid metabolism and energy. Phospholipids provide substrates for synthesis of potent pro- and anti-inflammatory mediators, such as eicosanoids and platelet-activating factors (Henneberry et al., 2002). Nonesterified fatty acids from lipomobilization is involved in modifying intracellular signaling processes and energy substrates.

Nonesterifed fatty acids consist primarily of palmitic, stearic and oleic acids. This may directly reflect the FA composition of stored adipose tissue. During the periparturient period, plasma lipids contain high concentrations of these saturated FA (Douglas et al., 2007). Previous studies involving periparturient dairy cows have shown that elevated NEFA from

lipomobilization alters immune cell function and population (Lacetera et al., 2004; Scalia et al., 2006). The structure of plasma lipids was also reflected in the FA profile of the PL fraction of peripheral mononuclear cells with increased concentrations of saturated FA and reduced concentration of polyunsaturated fatty acids (Contreras et al., 2010).

The most common non-invasive tool to monitor subcutaneous fat stores is body condition score (BCS), with high scores for over-conditioned cows and low scores for emaciated cows. During the first 60 days postpartum, marked with increased lipomobilization, typical observed changes in BCS range from 0.5 to 1.0 unit, based on the five-point scale (Wildman et al., 1982). This can result in 385 Mcal of net energy for lactation (NEL) released from body energy stores if a cow weighing 600 kg lost one BCS unit, from 4 at calving to 3 (National Research Council, 2001).

It is hypothesized that cows with a greater BCS around the time of calving would have a decreased dry matter intake (DMI) and different serum FA composition of NEFA and PL fractions as compared with those in cows with a lesser BCS. The objective of the study was to determine the effects of subcutaneous fat stores, as assessed by BCS around the time of calving, and the subsequent lipomobilization during early lactation on FA profile of serum NEFA and PL fractions and productive performance.

Materials and Methods

Animals, Treatments and Experimental Design

A total of 22 (10 primiparous and 12 multiparous) healthy, Holstein dairy cows were monitored from -28 d through +28 d, relative to parturition (0 d). Cows were housed in tie-stalls with free choice water and fed ad-libitum twice daily to ensure 5% to 10% refused feed. Cows were fed individually and intake was recorded daily. Prepartum, cows were fed a total mixed ration with the following ingredients: grass hay, alfalfa hay, canola meal, rolled corn,

rolled barley, dried distiller's grain and a mineral/vitamin pre-mix and contained 16.0% crude protein, 30.0% acid detergent fiber, 45.0% neutral detergent fiber, 3.1% crude fat, and 1.4 Mcal/kg NEL (on a dry matter basis). Postpartum cows were fed a total mixed ration consisting of triticale silage and sodium bicarbonate in addition to the ingredients of the prepartum ration, and contained 19.4% crude protein, 24.1% acid detergent fiber, 37.5% neutral detergent fiber, 4.0% crude fat, and 1.6 Mcal/kg NEL. Cows were milked twice daily at 06:00 h and 18:00 h in a double-4 herringbone parlor by a single milker with milk yields recorded daily. Cows were assessed for BCS on -28 d, between -5 d to -7 d, and then +8, +18, and +28 d relative to calving by two trained observers and averaged. The BCS recorded between -7 d to -5 d before calving was used to retrospectively dichotomize cows into two groups: HIGH with BCS $\geq$ 3.25 and LOW with BCS $\leq$ 3.0. All procedures involving animals were approved by the University of Idaho Animal Care and Use Committee.

Sample Collection

Blood samples were obtained on -28 d and -7 d relative to expected calving and +8 d, +18 d and +28 d postpartum. Approximately 7-10 mL of blood was collected via coccygeal venipuncture into serum BD Vacutainers (Becton, Dickson and Company, Franklin Lakes NJ). Blood was allowed to coagulate for 24 h before serum was obtained after centrifugation (1,500 × g at 4 °C for 15 min). Milk was collected on 7, 14, 21 and 28 d postpartum at both milkings on the sample day and pooled in proportion to the yield at each milking. Fifteen mL of milk was stored at -20 °C before being analyzed for fat, true protein, lactose, solids-not-fat, and somatic cell count by near infrared analysis (Washington DHIA, Burlington, WA).

Lipid Analysis

As previously described by Watts et al. (2013), the lipid fraction was extracted from the serum via a modified protocol (Clark et al., 1982) of the Folch method (Folch et al., 1957). The lipids were fractionated through silica-based, solid bonded phase Sep-Pak aminopropyl (NH2) cartridges (Waters Corporation, Milford, MA) to yield three fractions: NL, NEFA, and PL. The NL fraction was eluted via chloroform: 2-propanol (2:1), the NEFA fraction via 2% acetic acid in ethyl ether and the PL fraction via methanol (Kaluzny et al., 1985). The NEFA and PL fractions were methylated using a two-step procedure (Kramer et al., 1997), in which the lipid sample incubated in 0.5 M sodium methoxide for 10 min in a 50 °C water bath before adding 5% methanolic hydrochloric acid and incubating for 10 min in an 80 °C water bath. Potassium carbonate and hexane were added before centrifugation at 350 × g for 5 min. Fatty acid methyl esters were analyzed with an Agilent 7890A gas chromatograph equipped with an Agilent J&W HP-88 column (100 m × 0.250 mm × 0.20 μm film; Agilent Technologies, Santa Clara, CA) in a modified 45 min method [10]. Peaks were identified using a Supelco® 37 Component FAME Mix (Sigma-Aldrich, St. Louis, MO).

Serum Nonesterified Fatty Acids

Total serum NEFA concentrations were determined colorimetrically using the NEFA-HR-2 kit (Wako, Richmond, VA) following the manufacturer's protocol. Samples, standards and blanks were run in duplicate.

Data Analysis

Response variables were analyzed as repeated measures using the MIXED model procedure (Version 9.2, SAS Institute Inc., Cary, NC). Sources of variation in the model included effects of treatment, day, and treatment × time interaction. Cow was designated as a

random effect in the model. Significant effects were declared $P < 0.05$. Data are presented as least square means (LSM) ± standard errors of the mean (SEM).

Results and Discussion

Nonesterified Fatty Acid

Total serum NEFA concentrations varied by treatment (0.96 vs. 0.68 ± 0.06 mEq/L; $P = 0.005$) and time ($P < 0.0001$). Both treatments remained at similar concentrations through the prepartum period but varied significantly after parturition at 8 d and 18 d with the HIGH treatment having a greater concentration than the LOW treatment (Fig. 1.1). By 28 d postpartum, concentrations returned to those observed in the prepartum period for both treatments. These results are in agreement with concentrations observed in cows with high and low liver lipid concentrations observed by Weber et al. (2013). LOW group overall serum NEFA concentrations concurred with concentrations reported by others (Douglas et al., 2007; Contreras et al., 2010).

The FA composition of the NEFA fraction differed by treatment for C14:1 (0.48 vs. 0.39 ± 0.04 g/100 g; $P = 0.08$) and C18:0 (21.2 vs. 24.7 ± 0.09 g/100 g; $P = 0.004$) as C14:1 concentration tended to increase in LOW as compared with HIGH treatment, whereas C18:0 significantly increased in HIGH. The C14:1 is uncommon and present only in minute amounts of the overall FA profile of serum. The C18:0 mean values in the present study were greater than those reported by Douglas et al. (2007) and lower than those from Watts et al. (2013) and Contreras et al. (2010), likely due to diet influence on circulating FA and their effects on stored lipids. Overall higher C18:0 concentrations in LOW are likely from increased DMI as opposed to differences in lipid mobilization. The FA of greatest concentration for HIGH was total C18:1 cis (22.0 g/100 g), C18:0 (21.2 g/100 g), and C16:0 (19.6 g/100 g); whereas in

LOW, the same FA was observed at different concentrations: C18:0 (24.7 g/100 g), C16:0 (20.4 g/100 g) and total C18:1 cis (19.2 g/100 g).

Several FA varied significantly through the periparturient period as shown in Table 1.1. The primary FA of C16:0 and total C18:1 cis concentrations increased around time of parturition and remained at high concentrations through the postpartum period as compared with those observed prepartum. The C16:0 concentrations over the periparturient period followed a similar trend to those reported by Contreras et al. (Contreras et al., 2010). However, C18:1 trends varied as Contreras observed greatest C18:1 concentrations at parturition in contrast to the present study's peak occurring around d 18 postpartum. These differences may be attributed to the lipid profile of the diets.

Phospholipids

Phospholipids constitute a fraction of the serum lipids used primarily for cell-to-cell communications and lipid bi-layers, and function as mediators of inflammation. The serum PL fraction differed by treatment for several FA, where C16:0 (22.4 vs. 19.7 ± 1.0 g/100 g; P = 0.08) and sum of C18:2 cis (28.7 vs. 26.0 ± 1.1 g/100 g; P = 0.08) tended to increase in LOW as compared with HIGH cows. Conversely, C17:0 (0.51 vs. 0.81 ± 0.08 g/100 g; P = 0.02) decreased and C20:2n6 (0.38 vs. 1.2 ± 0.3 g/100 g; P = 0.06) tended to decrease in LOW as compared to HIGH treatments. Overall higher concentrations of C16:0 and total C18:2 cis in HIGH are likely from increased lipid mobilization and metabolism of NEFA from lipid body stores. This possibly created higher concentrations of the FA available in circulation and thus available for PL synthesis rather than using the FA from the diet and de novo synthesis. The C17:0 and C20:2n6 FA acted primarily as intermediates during elongation of long chain FA and were found here in similar concentration to those reported by

Watts et al. (2013). The FA C20:2n6 is a naturally occurring polyunsaturated fatty acid found typically in small concentrations in animal tissues that serves as an intermediate for C20:4n6 (arachidonic acid) and C18:2n6 (linoleic acid), known pro-inflammatory mediators. Huang et al. (2011) reported altered cellular macrophage phospholipids profiles in a dose-dependent manner from C20:2n6 supplementation that ultimately affected macrophage responsiveness in inflammatory stimulation. This suggests that circulating FA profiles can alter immune cell phospholipids FA profile and thus affect overall response to immune challenges.

Several FA of the serum PL fraction differed over time as reported in Table 1.2. The C16:0 increased around time of parturition and remained at higher concentrations postpartum than those observed prepartum. This trend was similar to the C16:0 concentrations examined in the serum NEFA fraction as observed by Contreras et al. (2010). Conversely, C18:0 decreased in concentration around time of parturition. This does not reflect the trend of serum NEFA fraction C18:0 concentrations. Therefore, the decrease in the serum C18:0 PL fraction is likely from decreased dietary intake of the FA. The sum of C18:1 cis increased in postpartum relative to prepartum concentrations ($P = 0.001$). The C20:3n3 FA decreased significantly until +8 d and then returned to similar concentrations observed prepartum.

Production

Prepartum DMI differed by treatment and tended to differ due to a treatment × time interaction ($P = 0.09$) with LOW consuming more than HIGH, as reported in Fig. 1.2. Postpartum DMI tended to differ with LOW again consuming more than HIGH ($P = 0.09$; Table 1.3). This concurs with the general understanding that cows with greater BCS around time of parturition will consume less dry matter than lower conditioned cows (Brooster et al., 1998; Roche et al., 2008). Average BCS between treatments was greater for HIGH than LOW

by design (3.17 vs. 3.02 ± 0.04; P = 0.006). Milk composition, somatic cell count, and yield did not differ by treatment (Table 1.3).

Conclusions

Lipid mobilization affects all lactating dairy cows as they go through the periparturient period. During this time period, cows with greater BCS mobilize more lipid than cows with lower BCS. Small changes in prepartum BCS altered the FA profile of serum NEFA and PL fractions during the periparturient period in the present study. Some FA profile changes are to be expected as predominant FA in the circulating NEFA fraction provide energy to meet the energy deficit caused by the onset of lactation and insufficient DMI. In the present study, C18:0 was greater in LOW as compared to HIGH, whereas C14:1 was greater in HIGH as compared to LOW. However, changes in the FA profile of the PL fraction could have greater consequences as PL provide substrates for synthesis of potent pro- and anti-inflammatory mediators and therefore can alter immune cell responsiveness. The C17:0 and C20:2n6 concentrations were greater in LOW with comparison to HIGH, whereas C16:0 and C18:2 cis were greater in HIGH as compared with LOW. Several of these FA in the NEFA and PL fractions changed through the periparturient period. These results support the hypothesis that cows with increased subcutaneous fat stores around time of parturition have different circulating NEFA and PL fatty acid profiles and consume less dry matter than cows with decreased subcutaneous fat. Further research is needed to understand the direct effects of these fat stores on the change in FA profile of circulating NEFA and PL fractions and consequently on immune cell FA profiles and their responsiveness to pathogens.

CHAPTER 3

The Effects of Elevated Subcutaneous Fat Stores on Fatty Acid Composition and Gene Expression of Pro-Inflammatory Markers in Periparturient Dairy Cows

Abstract

Periparturient dairy cows with increased subcutaneous lipid stores release greater concentrations of nonesterified fatty acids (NEFA) into circulation around parturition. Phospholipids (PL) are essential for maintaining cellular plasma membrane integrity, lipoprotein synthesis and intercellular signaling through lipid mediators. Large quantities of circulating NEFA are shown to alter circulating PL fatty acid (FA) profile. Modified cellular FA profile affects immune cell function. The objective of this study was to determine the effect of elevated lipid mobilization during the periparturient period on serum, peripheral blood mononuclear cells (PBMC), polymorphonucleocytes (PMN) NEFA and PL fraction and milk fatty acid (FA) profile, PBMC and PMN gene expression of selected markers of inflammation and production measures. Thirty-four cows were blocked by parity; treatment group received a dry cow ration with an additional 10 kg of corn / head per day starting -28 d relative to parturition. The control group received the dry cow ration (no additional corn) with 400 mg of monensin / head per day. Data were analyzed as repeated measures analysis of variance using mixed model procedures in SAS (9.3) and significance was declared at $P \leq 0.05$. Serum NEFA concentration of C20:4n6 tended to be greater in treatment compared with that of control (1.12 vs 0.86 ± 0.11%; $P = 0.06$). Within the serum PL fraction, C20:4, C20:5, total n3, and n6:n3 ratio varied significantly across treatment, parity, and time. Total n6:n3 was greater for treatment animals prepartum and multiparous cows postpartum. Concentrations of C20:4 and total n6 FA were greater in the PL fraction of PBMC from cows

that received the treatment, high-energy diet prepartum. However, gene expression for IL-1β in PBMC was greater for control, whereas ICAM-1, IL-1β, IL-6, and TNF-α were greater in primiparous than multiparous cows, without a detectable treatment effect. Also, no detectable treatment effect was observed in PMN gene expression for IL-8R, SELL, and CASP. In summary, high energy prepartal diets altered FA profile in serum, milk, PBMC and PMN lipids; however, there was little effect on gene expression of pro-inflammatory mediators. Further research is needed to understand the mechanisms of FA profile on immune cell function.

Introduction

The dry period for cows is often overlooked, when in fact it is a critical time for fetal growth, mammary tissue remodeling and high nutritional demands as animals' transition into lactation. The primary challenge faced by cows during this periparturient period is the substantial increase in nutrient requirements when supply is inadequate from insufficient dry matter intake (DMI). To compensate for this negative nutrient balance, cows will mobilize lipid and protein stores (Goff, 2006).

Lipids are transported and found in various fractions in blood and tissue including, neutral lipids, nonesterified fatty acids (NEFA) and phospholipids (PL). Neutral lipids, such as triglycerides and cholesterol esters, are the most abundant fraction of lipids. The majority of lipids are stored in adipocytes in the form of triglycerides and released into circulation as NEFA. These fatty acids (FA) travel through circulation bound to albumin to the heart, skeletal muscle, liver and other tissues for β-oxidation or conversion to other lipids (Richieri and Kleinfeld, 1995). During the transition period, high producing dairy cows experience lipid mobilization and elevated circulating concentrations of NEFA. This can overwhelm and

disrupt liver function, leading to fatty liver disorder, ketosis and other metabolic disorders (Rukkwamsuk et al., 1999).

Phospholipids are a small fraction of lipid that serve primarily as a component of cell membrane structure, lipoprotein structure and precursors for synthesis of bioactive pro- and anti-inflammatory mediators, such as eicosanoids and platelet-activating factors (Henneberry et al., 2002). Altering the FA profile of PL may alter eicosanoid metabolism and synthesis of inflammatory mediators. For example, supplementation of omega-3 fatty acids (n3) in the diet increases n3 concentrations in PL fraction and alters the eicosanoid metabolism to produce less potent pro-inflammatory mediators, more anti-inflammatory mediators, and reduce cytokine production in immune cells (Calder, 2005). Immune cell's function and population distributions are also altered by the elevated NEFA concentration from excessive lipid mobilization (Lacetera et al., 2004; Scalia et al., 2006).

The most common non-invasive tool to monitor mobilization of lipids from subcutaneous stores in dairy cows is a visually assessed body condition score (BCS). The scale used in the United States for dairy cows ranges from 1 (emaciated) to 5 (obese) with 0.25 unit increments (Wildman et al., 1982). In a previous study, Scholte et al. (2014) observed that cows with a BCS $\geq$ 3.25 at d -7 relative to parturition had significantly greater concentrations of circulating NEFA postpartum and altered FA profiles in circulating NEFA and PL fractions compared with cows with a BCS $\leq$ 3.0. This suggests that cows with greater prepartum BCS mobilize more lipid. These excessive NEFA concentrations could potentially put cows at increased risk for metabolic disorders and infectious diseases resulting from altered immune cells' functions and sub-populations.

The objective of this study was to determine the effects of prepartum dietary manipulation to increase subcutaneous lipid stores and elevate periparturient lipid mobilization on serum and peripheral blood mononuclear cells (PBMC) and polymorphonuclear (PMN) FA profile in NEFA and PL fractions as well as PBMC and PMN gene expression of pro-inflammatory cytokines and adhesion molecules.

Materials and Methods

Animals, Treatments and Experimental Design

A total of 34 (14 primiparous and 20 multiparous) healthy, Holstein dairy cows were included in the experiment from -28 d through +21 d, relative to parturition (0 d). Cows were blocked by parity and assigned to one of two groups administered prepartum: treatment and control. All multiparous cows received 0.23 kg / hd of SoyChlor (West Central, Ralston, IA) starting d -14 through parturition to help maintain desired dietary cation-anion balance. Control animals received the basal dry cow ration with 400 mg of Monensin / hd per day in 0.23 kg of corn to minimize lipid mobilization (Table 3.1). Treatment animals received the basal dry cow ration with an additional 10 kg of dry, cracked corn / hd per day and were fasted for 8 h on d +3 postpartum to increase lipid mobilization.

Cows were housed in open pens prepartum and tie-stalls postpartum. Free choice water was available and cows were fed ad-libitum twice daily to ensure 5% to 10% refusals. Cows were fed by pen prepartum and individually postpartum, and intake was recorded daily. Prepartum, cows were fed a total mixed ration with the following ingredients: grass, alfalfa hay, triticale silage, oat and pea silage, cracked corn, dry rolled barley, liquid mineral/vitamin pre-mix (Performix Nutrition Systems, Caldwell, ID), and salt (Table 2.1). Postpartum, cows were fed a total mixed ration consisting of dry distillers grains with solubles, calcium soaps of

fatty acids (EnerGII; Virtus Nutrition, Corcoran, CA), canola meal, sodium bicarbonate in addition to the ingredients of the prepartum ration. Nutrient analysis of the basal rations using near-infrared analysis is shown in Table 3.3. Cows were milked 3X daily at 0600, 1400 and 2200 h in a double-4 herringbone parlor by a single milker with milk yields recorded daily. Energy corrected milk was calculated using the following equation: 0.327 × milk yield kg + 12.95 × fat yield kg + 7.65 × protein yield kg (Dairy Record Management Systems, 2013). Cows were assessed for body condition score (BCS; Wildman et al., 1982) and body weights (BW) on d -28 and -7 relative to predicted parturition, and then +3, +12, and +21 d after parturition by two trained observers and averaged values were used. Change in BCS and BW between d -28 and +21 (relative to parturition) were calculated separately for prepartum (d -28 to -7) and postpartum (d +3 to +21) and recorded as ΔBCS and ΔBW. All procedures involving animals were approved by the University of Idaho Animal Care and Use Committee (Protocol 2011-24).

Sample Collection

Blood samples were obtained on d -28 and -7 relative to expected calving and d +1, +3, +6, +15 and +21 postpartum. Approximately 7-10 mL of blood was collected via coccygeal venipuncture into serum BD Vacutainers (Becton, Dickson and Company, Franklin Lakes, NJ). Blood was allowed to coagulate for 24 h before serum was obtained after centrifugation (1,500 × *g* at 4 °C for 15 min).

Additional blood samples were obtained on d -28, +3, +12, and +21, relative to calving for peripheral blood mononuclear cells (PBMC) and polymorphonucleocytes (PMN) isolation. Approximately 50 mL of blood was collected via jugular venipuncture and 100 μL of >180 USP units of sodium heparin (Sigma Aldrich, St. Louis, MO) were added. After

centrifugation (740 × *g* at 10°C for 10 min), plasma was removed and red blood cells were lysed with sterile water for 20 sec before adding 2.5 mL of sterile 10% NaCl solution to stop lysis. The PBMC and PMN were isolated by gradient centrifugation using Histopaque 1077 and 1119 (Sigma Aldrich, St. Louis, MO). Isolated PBMC and PMN were washed in Hank's Balanced Salt Solution (HBSS; Sigma Aldrich, St. Louis, MO) then re-suspended in HBSS. Cell suspensions were aliquoted into 2 tubes and stored at -80°C for RNA and fatty acid analysis, respectively.

Milk was collected on d +1, +3, +6, +15 and +21 postpartum at all three milkings on the sample day and pooled in proportion to the yield at each milking. Fifteen mL of milk was stored at -20 °C before being analyzed for milk urea nitrogen (Minnesota DHIA, Zumbrota, MN) and fat, true protein and lactose via infrared spectroscopy, and solids-not-fat, and somatic cell count by Fossomatic analysis (Washington DHIA, Burlington, WA).

Lipid Analysis

The lipid was extracted from the feed, serum, milk, PBMC and PMN via a modified protocol (Clark et al., 1982) of the Folch method (Folch et al., 1957). Briefly, 38 mL of chloroform: methanol (2:1) was added to the sample. The solution was vacuumed through #1 Whatman paper and 7.2 mL of 0.58% NaCl was added. After centrifugation and removal of the upper aqueous layer by aspiration, the chloroform extract was dried under light nitrogen stream to determine total lipid recovery. The serum, PBMC and PMN lipids were fractionated through silica-based, solid bonded phase Sep-Pak aminopropyl (NH2) cartridges (Waters Corporation, Milford, MA) to yield three fractions: neutral lipid (NL), nonesterified fatty acids (NEFA), and phospholipids (PL). The NL fraction was eluted via chloroform: 2-propanol (2:1), the NEFA fraction via 2% acetic acid in ethyl ether and the PL fraction via

methanol (Kalunzy et al., 1985). The NEFA and PL fractions of serum, PBMC and PMN along with extracted milk lipids were methylated using a two-step procedure (Kramer et al., 1997), in which the lipid sample was incubated in 0.5 M sodium methoxide for 10 min in a 50 °C water bath before adding 5% methanolic hydrochloric acid and incubating for 10 min in an 80 °C water bath. Potassium carbonate and hexane were added before centrifugation at 350 × *g* for 5 min. Fatty acid methyl esters were analyzed with an Agilent 7890A gas chromatograph equipped with an auto sampler, flame ionization detector and an Agilent J&W HP-88 column (100 m × 0.250 mm × 0.20 μm film; Agilent Technologies, Santa Clara, CA) in a modified 45 min method as described by Watts et al. (2013). Peaks were identified using a Supelco® 37 Component FAME Mix (Sigma-Aldrich, St. Louis, MO).

Gene Expression Analysis

Total RNA was collected from PBMC using the NucloSpin RNA kit (Machery-Nagel, Duren, Germany) according to the manufacturer's instructions. A NanoDrop ND-1000 (NanoDrop technologies, Rockland, DE) spectrophotometer was used to determine RNA concentration. One hundred ng of RNA was used for synthesis of single stranded complimentary DNA (cDNA) using the Applied Biosystems High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA). Single stranded cDNA was then used for gene expression analysis with a 7500 Fast Real-Time System (Applied Biosystems). Twenty μL PCR reactions were prepared in duplicate using Taqman Universal PCR Master Mix (Applied Biosystems) and Custom Taqman Gene Expression assays (Applied Biosystems). Expression of interleukin-1β (IL-1β), interleukin-6 (IL-6), intercellular adhesion molecule 1 (ICAM) and tumor necrosis factor-α (TNF-α) was measured in PBMC. Interleukin-8 receptor (IL-8R), L-selectin (SELL) and caspase-1 (interleukin converting

enzyme; CASP) expression was measured in PMN relative to endogenous controls, glyceraldehyde 3-phophate dehydrogenase (GAPDH) and ribosomal protein S9 (RPS9). Primer/probe set for IL-8R (Accession number DQ389113, Forward Primer ATGCGGGTCATCTTTGCTG, Reverse Primer ATGAGGGTGTCCGCGATC, Probe CTCGTCTTCCTGCTCTGCTGGCT) was designed using Primer Express® software v. 1.5 (Applied Biosystems). Caspase-1 (Accession Number XM_002692921) was designed by Custom Plus TaqMan RNA Assay (Applied Biosystems). All other gene primer/probe sets were developed by Applied Biosystems (Table 2.19).

Data Analysis

Response variables were analyzed as repeated measures using the MIXED model procedure (Version 9.3, SAS Institute Inc., Cary, NC). Sources of variation in the model included effects of treatment, time, and parity as well as 2- and 3-way interactions amongst the main effects. Cow nested within treatment × parity interaction was designated as a random effect in the model. Significance was declared at $P \leq 0.05$. Data are presented as least square means (LSM) ± standard errors of the mean (SEM). Gene expression data were analyzed using Ct values normalized to the average of the endogenous control genes, GAPDH and RPS9, and are presented graphically as fold change ($2^{-\Delta\Delta Ct}$) relative to the control treatment.

Results and Discussion

Production Measures

As expected, prepartum dry matter intake (DMI) differed between groups with the control group consuming more feed than the treatment group (8.9 vs. 6.3 ± 0.6 kg, $P < 0.01$). This was also reflected in prepartum DMI as a percentage of body weight (BW; 1.60 vs. 1.21 ± 0.04% for control and treatment, respectively, $P < 0.01$). Grummer and associates (2004)

found similar averages for DMI as primiparous and multiparous animals consumed 1.3% and 1.4%, respectively. Prepartum DMI also differed by treatment across time ($P = 0.01$; Figure 3.1). This corresponds with the treatment prepartum ration having a greater energy density from the addition of 10 kg of corn / hd per day over the control prepartum ration. Similar results were observed in a pooled study of prepartal cows consuming diets either high or low in NDF (Grummer et al., 2004) or diets with restricted intake (80% of NE_L requirement) or fed ad libitum (160% of NE_L requirement; Douglas et al., 2006). Cows consuming the diet with high NDF or restricted energy had greater DMI than cows fed the low NDF or excessive energy ration. Postpartum intake did not vary between treatment groups (Figure 3.1). Although it has been reported that cows fed restricted diets prepartum have greater DMI postpartum (Douglas et al., 2006; Holcomb et al., 2001), this was not observed in the current experiment, possibly because of the limited duration of the postpartum sampling period of only 3 weeks as compared to other studies that continued for at least 6 weeks.

Prepartal treatments did not alter the BCS of treatment groups. Body condition scores did not follow the typical decline after parturition as reported in some other studies (Roche et al., 2006, 2009; Edmondson, 1982). As shown in Figure 3.2, average postpartum BCS increased nearly 0.5 units following parturition instead of declining approximately 0.5 units. Although body condition scores were recorded by two trained observers and averaged, it appears that the observers over-compensated with their postpartum observations. Body weight of treatment groups varied with the treatment group weighing more than the control group throughout the sampling period (554 vs. 612 ± 16 kg, $P = 0.01$). Postpartum change of BW between d +3 and +21 tended to differ between treatment groups with control animals losing more BW than treatment animals (-150 vs. -114 ± 16 kg, $P = 0.10$).

Milk composition varied slightly between treatment groups. Protein, as a percent of volume over time, was greater for treatment cows ($P < 0.01$). Total milk fat yield varied by treatment × parity × time interaction ($P < 0.01$). Holcomb et al. (2001) reported similar results with cows fed a restricted prepartum diet producing significantly less milk fat, similar to multiparous control animals observed in the current study. Milk yield did not vary between treatments; however, a treatment effect across time and parity was detected for energy-corrected milk ($P = 0.04$; Figure 3.3). Energy corrected milk yield mirrored that of milk fat yield. This is not surprising, given the importance of milk fat yield in calculating energy corrected milk (Dairy Record Management Systems, 2013). Janovick et al. (2010) speculate that the increased lipid mobilization observed in cows fed an energy-dense prepartum diet is a potential mechanism for increased milk fat percentage and therefore increased energy-corrected milk. Significant differences are mostly influenced through effects of parity and time. Primiparous animals produce less quantities of milk than multiparous cows (Janovick et al., 2010), in addition to milk production increasing during early lactation (Miller et al., 2006).

Fatty Acid Analysis

The FA profile of the prepartum ration was primarily C18:3n3 (31.6 g / 100 g fatty acid methyl esters; FAME), C16:0 (24.8 g / 100 g FAME), total C18:2 *cis* (20.8 g / 100 g FAME) and total C18:1 *cis* (11.4 g / 100 g FAME; Table 3.2). High concentrations of C18:3n3 are typical for a high-forage diet, such as a dry cow diet (Han et al., 2014). The postpartum ration had greater concentrations of total C18:1 *cis* (34.3 g / 100 g FAME) and total C18:2 *cis* (24.8 g / 100 g FAME) and a decreased C18:3n3 (4.7 g / 100 g FAME) concentration. The increase in total C18:2 *cis* and decrease in C18:3n3 from the prepartum to

postpartum ration were the primary contributors to the increase in total n6:n3 from 0.68 in the prepartum ration to 5.2 postpartum. This change is expected as the postpartum diet has decreased forage and increased corn, dry distillers grains, barley, calcium soaps of fatty acids, and especially canola meal that is high in C18:2 (Moser and Vaughan, 2010). Long chain FA C20:4 (0.13 vs. 0.03 g / 100 g FAME) and C22:6 (0.65 vs. 0.08 g / 100 g FAME) concentrations were greater in the prepartum ration as well. Although these FA only constitute a small fraction of detected FAME, it is important to note their changes across rations as their pro- and anti-inflammatory effects are dose dependent (Calder, 2008).

Individual FA in milk can arise from two main sources: *de novo* synthesis and uptake from circulation. These circulating free fatty acids can originate from dietary absorption or mobilization of adipose stores (Bauman and Davis, 1974). During early lactation when lipid mobilization is high, NEFA from adipose can account for approximately 40% of milk FA (Bell, 1995). In the current experiment, detection of milk FAME for *de novo* synthesized FA (less than 14 carbons in length) and many long chain FA, specifically C20:4 and C20:5, through gas chromatography was limited. The mean composition of the FAME detected in milk, therefore, do not fully reflect the FA profile of milk lipids. Of the FAME detected, concentration of C18:3n3 varied by treatments across time ($P < 0.01$) with control animals having greater concentrations than treatment animals (Figure 3.5). Concentrations in the control group peaked shortly after parturition before declining to similar concentrations of the treatment group. As mentioned, high-forage rations, similar to the control group ration prepartum, have high concentrations of C18:3n3 within the feed. Han et al. (2014) reported similar milk C18:3n3 concentrations from cows fed a high-forage diet.

Ratio of total n6 to n3 fatty acids varied across treatments by parity and time interaction ($P < 0.01$; Figure 3.4). Both total n6 and total n3 appear to be contributing factors for the overall changes in the ratio. Multiparous cows had higher ratios than primiparous cows after d +3. Within parity, treatment animals had a greater n6:n3 than control animals (Table 3.6). Benbrook and associates (2013) reported similar n6:n3 ratio in milk from cows fed conventional diets. High n6 intake is associated with increased inflammation through greater production of pro-inflammatory mediators (Calder, 2001). Supplementing diets with high concentrations of n3, such as C20:5 and C22:6 typically found in fish oils, are reported to have anti-inflammatory effects through decreased pro-inflammatory and increased anti-inflammatory mediator production (Calder, 2005). Only trace amounts of C22:6 and no C20:5 were detected in the milk FA profile in the current study. With the growing concern of rising n6:n3 in human diets and its associated potential for exacerbating cardiovascular disease, diabetes and inflammation (Elwood et al., 2010), it is interesting to note the effect of the high-energy, prepartum treatment ration in increasing the ratio in early lactating cows.

In the current study, serum lipids were fractionated into NEFA and PL and several FA differed because of the high-energy (prepartum ration). Nonesterified fatty acids typically constitute less than 1% of the total circulating lipid fractions (Quehenberger et al., 2010). In the NEFA fraction, sum of C18:1 *cis* ($P < 0.01$) and sum of C18:2 *cis* ($P < 0.01$) differed, and C18:0 ($P < 0.10$) tended to differ by treatment across parity and time (Table 3.7). Given the high degree of lipid mobilization in the form of NEFA to compensate for severe negative energy balance in the periparturient period (Goff, 2006) and the primary FA of subcutaneous adipose tissue being C16:0, C18:0 and C18:1 (Douglas et al., 2007), it is not surprising that these FA changed over time. Similar to milk C18:3n3 concentrations, the control group had

greater concentrations of C18:3n3 (1.3 vs. 1.0 ± 0.1 g / 100 g of FAME, $P = 0.04$) in serum NEFA than the treatment group (Figure 3.5). A proportion of the C18:3n3 from the serum NEFA fraction could have potentially been utilized by the mammary gland for milk fat synthesis; however, their true origin (diet or mobilized from lipid stores) remains unknown as FA were not labeled in the current study. Eicosanoid precursor, C20:4, tended to have greater concentrations in treatment animals (0.86 vs. 1.12 ± 0.10 g / 100 g of FAME, $P = 0.06$), potentially increasing available substrate for increased eicosanoid and pro-inflammatory synthesis. Several other long chain PUFA, such as C20:5 and C22:6 had no detectable differences between treatments. This is expected as PUFA have a relatively small concentration (approximately 11%) in the serum NEFA lipid profile in the current study.

Several FA associated with eicosanoid production in serum PL varied by treatment across parity and time as shown in Figure 3.6. Concentrations of C20:4 differed ($P < 0.01$) with multiparous cows having greater prepartum concentrations than primiparous cows. After parturition, concentrations were relatively similar between treatment and parity groups. Omega-3 FA C20:5 had concentrations greater in control animals prepartum and primiparous cows postpartum ($P = 0.04$). Contreras et al. (2010) reported values almost half the concentrations of C20:4, yet similar C20:5 concentrations were observed in this study in plasma PL. Greater C20:4 and decreased 20:5 concentrations in multiparous animals as compared with primiparous animals suggests the possibility of a pro-inflammatory state in multiparous animals because of increased available C20:4 substrate for synthesis of pro-inflammatory mediators and reduced inhibition of eicosanoid metabolism by C20:5. Ratio of n6 to n3 was very high for this fraction, primarily because of low total n3 and high C18:2 *cis* concentrations in the serum PL profile. The ratio was greater for treatment animals in the

prepartum period and multiparous cows in the postpartum period. Similar to the serum NEFA fraction and milk, concentrations of C18:3n3 varied by treatment × time ($P < 0.01$) with greater concentrations observed in the control group, prepartum and similar concentrations postpartum.

In the present study, few FA in the NEFA fraction of PBMC had significant changes in concentration because of treatment. As observed in the serum NEFA profile, concentrations of C20:4 in the NEFA fraction of PBMC were greater in treatment animals as compared with control animals (1.29 vs. 0.52 ± 0.27 g/100 g of FAME, $P = 0.02$). Concentrations of C20:4 also varied by treatment across parity and time (Figure 3.7; $P = 0.02$). Primiparous, treatment animals had a greater C20:4 prepartum concentration that dropped to concentrations similar to those observed in primiparous, control animals. Multiparous animals had greater C20:4 concentrations after parturition before returning to similar concentrations of the primiparous animals at d 21 postpartum. Although no significant difference was detected for sum of n3 and sum n6 (Table 3.12), the ratio of n6 to n3 varied by treatment across parity and time (Figure 3.7; $P = 0.05$). Concentrations peaked after parturition in primiparous animals as compared with multiparous animals around d 12 postpartum and continued increasing rapidly in treatment animals at 21 DIM. Predominant FA found in the NEFA fraction of PBMC were C16:0 (27.8 g/100 g lipid), C18:0 (26.6 g/100 g lipid) and C18:1 *cis* (8.0 g/100 g lipid), similar to the FA profile found in the serum NEFA fraction.

The FA profile of the PL fraction of PBMC have many roles in cellular function. Alteration to the FA composition can result in alterations to membrane properties, inter- and intracellular signaling and lipid mediator synthesis (Calder, 2008). The current experiment had few FA concentrations change in FA profile of the PL fraction of PBMC because of

treatment. Converse to PBMC NEFA C20:4 concentrations, the PL fraction had greater concentration of C20:4 in control group than in treatment group (10.1 vs. 8.2 ± 0.6 g / 100 g FAME, $P = 0.02$). As C20:4 functions as a substrate for eicosanoid synthesis in human immune cells (Calder, 2005), it is suggested that PBMC obtained from dairy cows fed a typical prepartum diet have the potential to produce more pro-inflammatory mediators than cows fed a high-energy prepartum diet. Increased pro-inflammatory mediators synthesis is essential for immune cells to respond to pathogen challenges; however, excess production can result in a chronic inflammatory state (Contreras, 2008). Concentrations of C18:3n3, a potential precursor for C20:5 and C22:6 synthesis, were greater in treatment animals than control animals (1.07 vs. 0.63 ± 0.20 g / 100 g FAME, $P = 0.04$). No significant difference was detected however in C20:5 and C22:6 concentrations. This observation was surprising as FA profile in the PL fraction of PBMC typically reflects that of the circulating FA profile (Contreras et al., 2010; Watts et al., 2013) and in the present study, greater serum concentrations of C18:3n3 in NEFA and PL were observed in control animals. Given the relatively small proportion of C18:3n3 in the PL fraction of PBMC, observed difference may be physiologically negligible. Fatty acids in greatest concentration in the PL fraction of PBMC were similar to those in the NEFA fraction with C16:0 (22.8 g/100 g lipid), C18:0 (19.5 g/100 g lipid) and C18:1 *cis* (16.3 g/100 g lipid).

Neutrophils, the primary cell-type in PMN, serve vital roles as phagocytes as well as production of cytokines and lipid mediators to signal and recruit nearby cells to aid in the immune response. Alterations to the FA profile of PMN result in similar changes to those observed in PBMC. In this study, several FA differed in the PL fraction of PMN. Although no difference was detected in C18:3n3 concentrations, C20:5 varied by treatment across time and

parity ($P < 0.01$). Primiparous cows had greater concentrations of C20:5 than multiparous cows and control group had greater quantities than treatment group. Greater available C20:5 can alter eicosanoid production and result in synthesis of less potent pro-inflammatory mediators, decreased cytokine production and more anti-inflammatory mediators. These mediators are beneficial in a chronic inflammatory state; however, decreased synthesis of cytokines and pro-inflammatory mediators can alter the immune response and lead to an immunosuppressed state. These reported concentrations of C20:5 are relatively small in the total PL fraction; therefore, the overall influence of C20:5 on PMN function in the immune response is likely to be minimal as FA influence is in a dose dependent manner (Calder, 2008). There is limited information on the FA profile of PMN. Kawakami et al. (2007) reported a limited FA profile from neutrophils isolated from humans with much higher quantities of C20:5. In the current study, ratio of n6 to n3 varied by treatment across time and parity as seen in several other fraction with similar response in that multiparous cows had greater concentrations than primiparous cows ($P < 0.01$; Figure 3.8).

Gene Expression

Treatment effects were detected for IL-1β mRNA expression in PBMC (Figure 3.9; $P = 0.04$). Expression of IL-1β was greater in the control group ($P = 0.04$) than that of the treatment group. In an immune response, activated macrophages secrete IL-1β to elicit many cellular activities such as T cell differentiation, B cell proliferation, leukocyte activation, increased ICAM expression, and synthesis and release of other pro-inflammatory cytokines (TNF-α and IL-6, for example; Netea et al., 2010). Decreased expression of IL-1β in treatment animals suggests a potentially reduced immune response to invading pathogens and increased risk for diseases, such as mastitis and metritis. A similar decrease in IL-1β

production was observed in human monocytes when diets were enriched in C18:3n3 (Caughey et al., 1996). Although differences in C18:3n3 between treatment groups in the PL fraction of PBMC were physiologically negligible, it may be postulated that PBMC expression of IL-1β was lower in treatment animals because of their higher concentration of C18:3n3 present in the PL fraction of PBMC. As reviewed by Calder (2005), monocytes and macrophages exposed to various n3 had decreased ICAM, IL-1β, TNF-α and IL-6 production because of altered eicosanoid metabolism (Chu et al., 1999; Wallace et al., 2003; Miles et al., 2000), resulting in altered immune cell response to pathogens. Although in the current study, no detectable treatment effects were observed for ICAM, IL-6 and TNF-α gene expression (Table 3.18), it is noted that the relatively low concentrations of n3 in the PL fraction of PBMC might not have been sufficient to elicit a decreased expression in all pro-inflammatory mediators.

In PMN, no treatment effects were detected for CASP, IL-8R and SELL mRNA expression. Lack of significant differences may relate to the relatively short life span of PMN of approximately 9 hours (Carlson and Kaneko, 1975) and the limited sampling in the current study. In comparison to the known effects of FA supplementation on inflammatory mediator production and mRNA expression of PBMC, the effects are almost unknown on PMN. A similar pattern of altered production and mRNA expression in PMN would also be expected, but not observed in the current study. Scalia et al. (2006) reported decreased viability and increased necrosis in PMN exposed to concentrations of NEFA typically observed in periparturient dairy cows. The physiological mechanism for these observations is not well defined but likely involves altered cytokine production from those analyzed in the current

study. Further research is needed to elucidate the effects of altered FA composition on PMN mRNA expression and protein abundance of pro-inflammatory mediators.

Conclusions

The transition period is a time of many changes for dairy cows. Parturition and initiation of lactation challenges all cows as nutrient supply cannot keep up with nutrient demand and therefore requiring cows to mobilize protein and lipid stores. High circulating NEFA concentrations aid to fulfill energy requirements in high-producing dairy cows when supply is limited from inadequate DMI. Cows with greater subcutaneous adipose stores as assessed by BCS usually mobilize more adipose stores and have greater concentrations of NEFA as compared to cows with lower BCS and adipose stores. These NEFA alter the physiology of the cow and are possibly a contributing factor to the higher incidence of metabolic disorders and infectious diseases often observed in cows with high BCS.

In the present study, a high-energy, prepartum diet was used to increase subcutaneous fat stores and lipid mobilization around parturition. This led to an altered physiological state compared to a typical prepartum diet, supplemented with monensin to minimize lipid mobilization. Several FA concentrations in milk and NEFA and PL fractions in serum, PBMC and PMN varied because of the high-energy prepartum treatment diet interacting with time and parity. Many important n3 and n6 FA, such as C20:5 (n3) and C20:4 (n6), known to alter eicosanoid and cytokine production in immune cells, varied due treatment and therefore, presumably altered PBMC and PMN response. Gene expression of several pro-inflammatory mediators that are indicative of cell function did not vary by treatment, except for decreased IL-1β expression in PBMC in treatment animals. Given the relatively brief lifespan of these immune cells, especially neutrophils in the PMN fraction, it is possible that effects were not

observed because of high cellular turnover in addition to the short sampling period. Further research is needed to fully understand the effects of changes to FA profile from excessive lipid mobilization as measured by circulating metabolites and dietary manipulation to influence subcutaneous fat stores through analysis of other inflammatory mediators and greater sampling of immune cells to observe physiological changes.

Figures

Figure 2.1. Serum nonesterified fatty acid concentrations (NEFA; LSM ± SEM in mEq L^{-1}) obtained from dairy cows (n = 22) at various time points through the transition period that were retrospectively dichotomized by body condition score (BCS) at d -5 to -7 before parturition. Closed squares present HIGH group (BCS ≥ 3.25) and open triangles present LOW group (BCS ≤ 3.0). * represents treatment means differed at those time-points specified, $P < 0.05$.

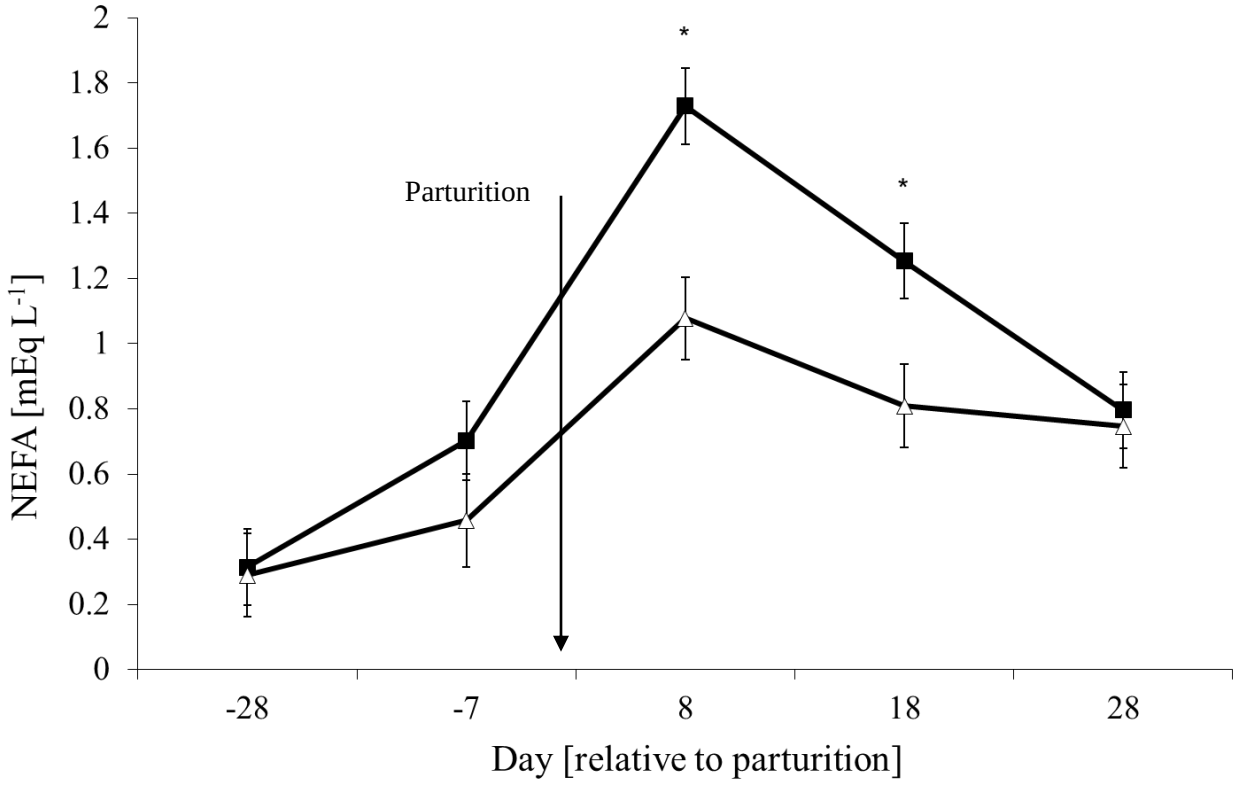


Figure 2.2. Pre- and postpartum mean weekly feed intakes (kg d^{-1}) obtained from dairy cows (n = 22) through the transition period that were retrospectively dichotomized by body condition score (BCS) at d -5 to -7 before parturition. Closed squares present HIGH group (BCS ≥ 3.25) and open triangles present LOW group (BCS ≤ 3.0). * represents treatment means differed at those time-points specified, $P < 0.05$.

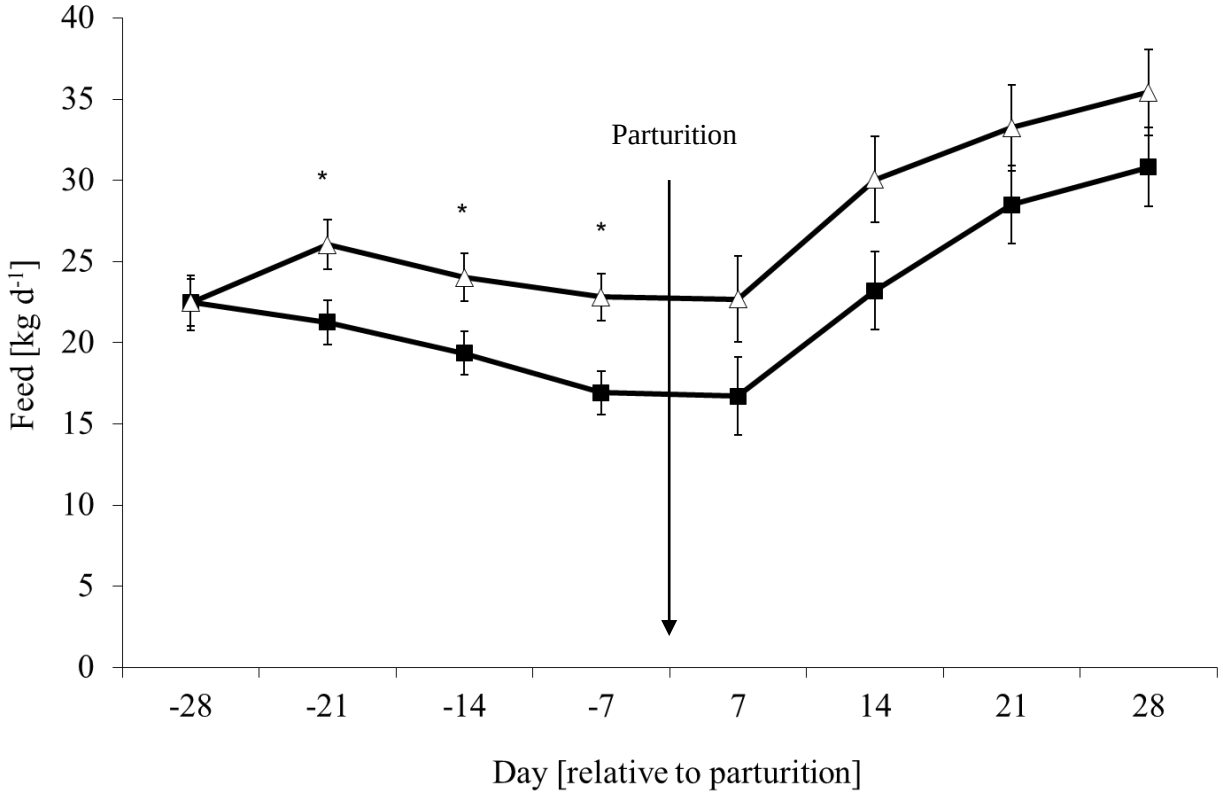


Figure 3.1. Least square means of dry matter intake in periparturient dairy cows [14 multiparous (solid line) and 20 primiparous (dashed line)] that received either the treatment (open circle; ○) of 10 kg of corn/hd per day prepartum and were fasted for 8 h on d +3 postpartum or control (closed square; ■) of 400 mg of monensin/hd per day prepartum.

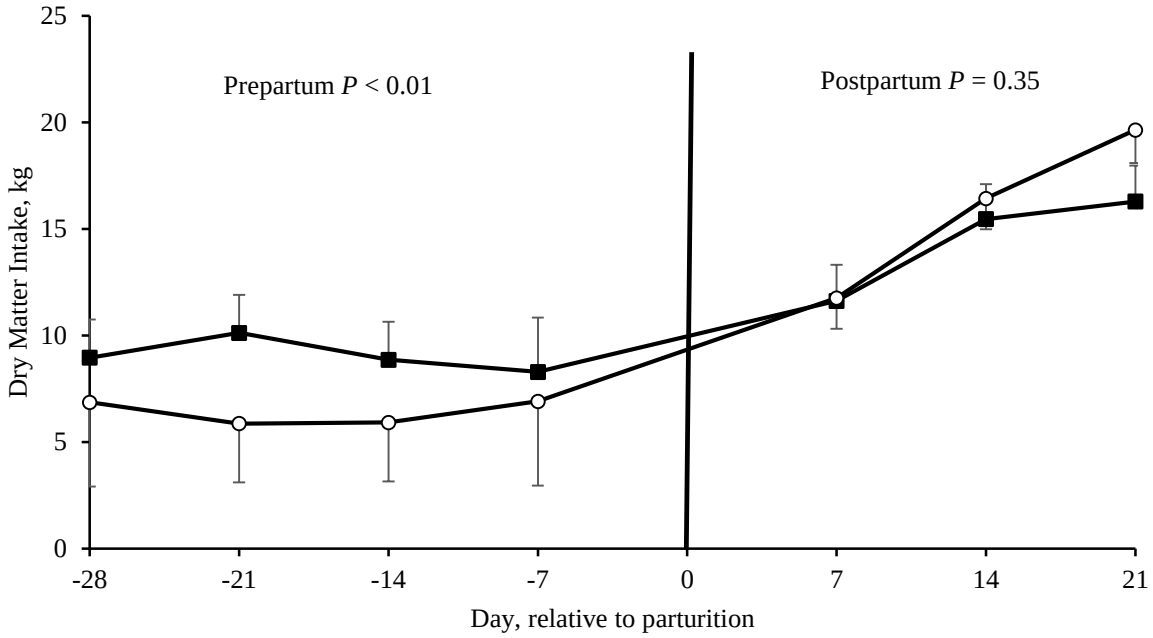


Figure 3.2. Least square means of body condition score and body weight of periparturient dairy cows [14 multiparous (solid line) and 20 primiparous (dashed line)] that received either the treatment (open circle; ○) of 10 kg of corn/hd per day prepartum and were fasted for 8 h on d +3 postpartum or control (closed square; ■) of 400 mg of monensin/hd per day prepartum.

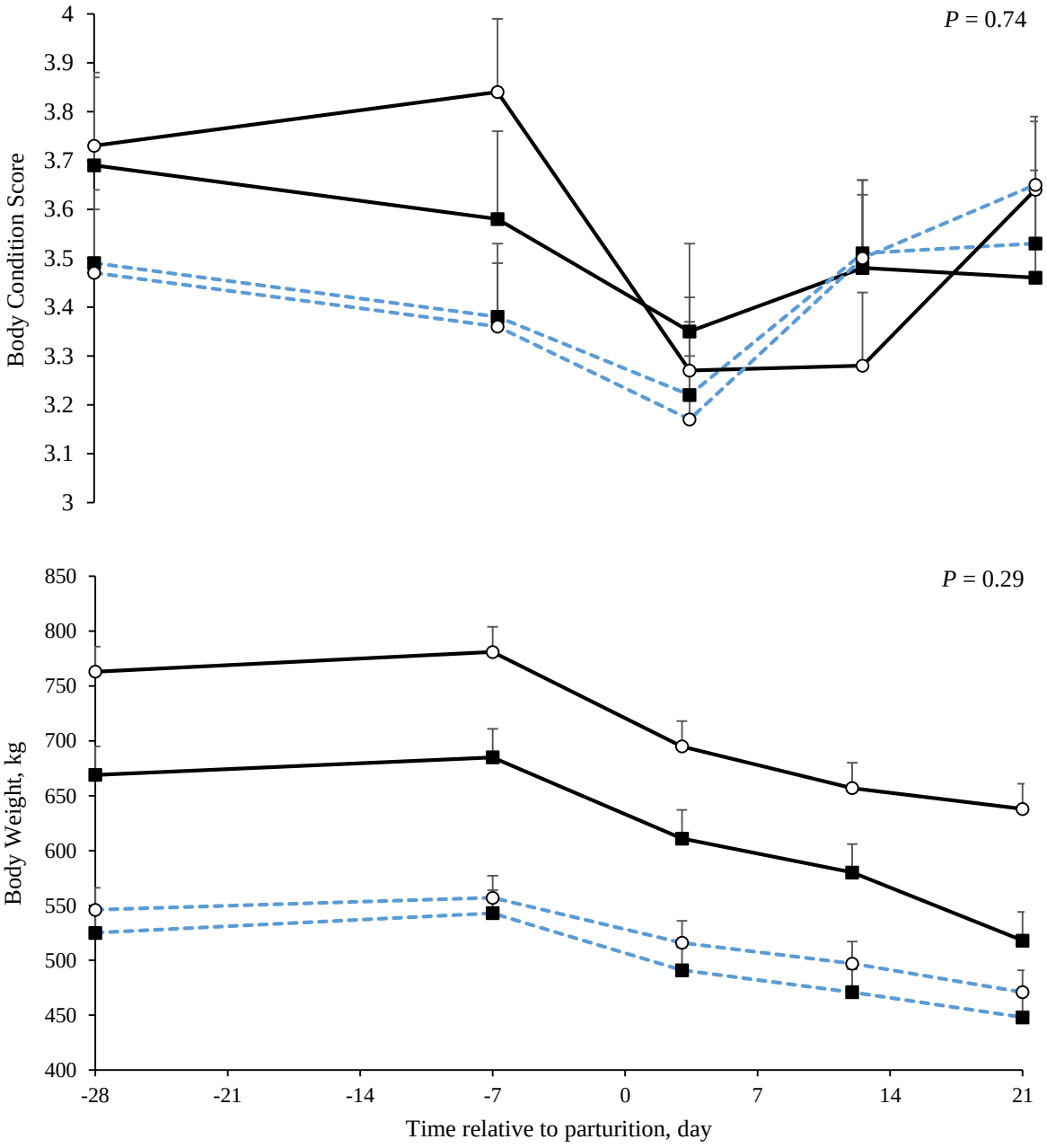


Figure 3.3. Least square means of energy corrected milk (kg / d), milk fat yield (kg / d) and somatic cell count of milk (×1000 / mL) samples obtained from periparturient dairy cows [14 multiparous (solid line) and 20 primiparous (dashed line)] that received either the treatment (open circle; ○) of 10 kg of corn/hd per day prepartum and were fasted for 8 h on d +3 postpartum or control (closed square; ■) of 400 mg of monensin/hd per day prepartum.

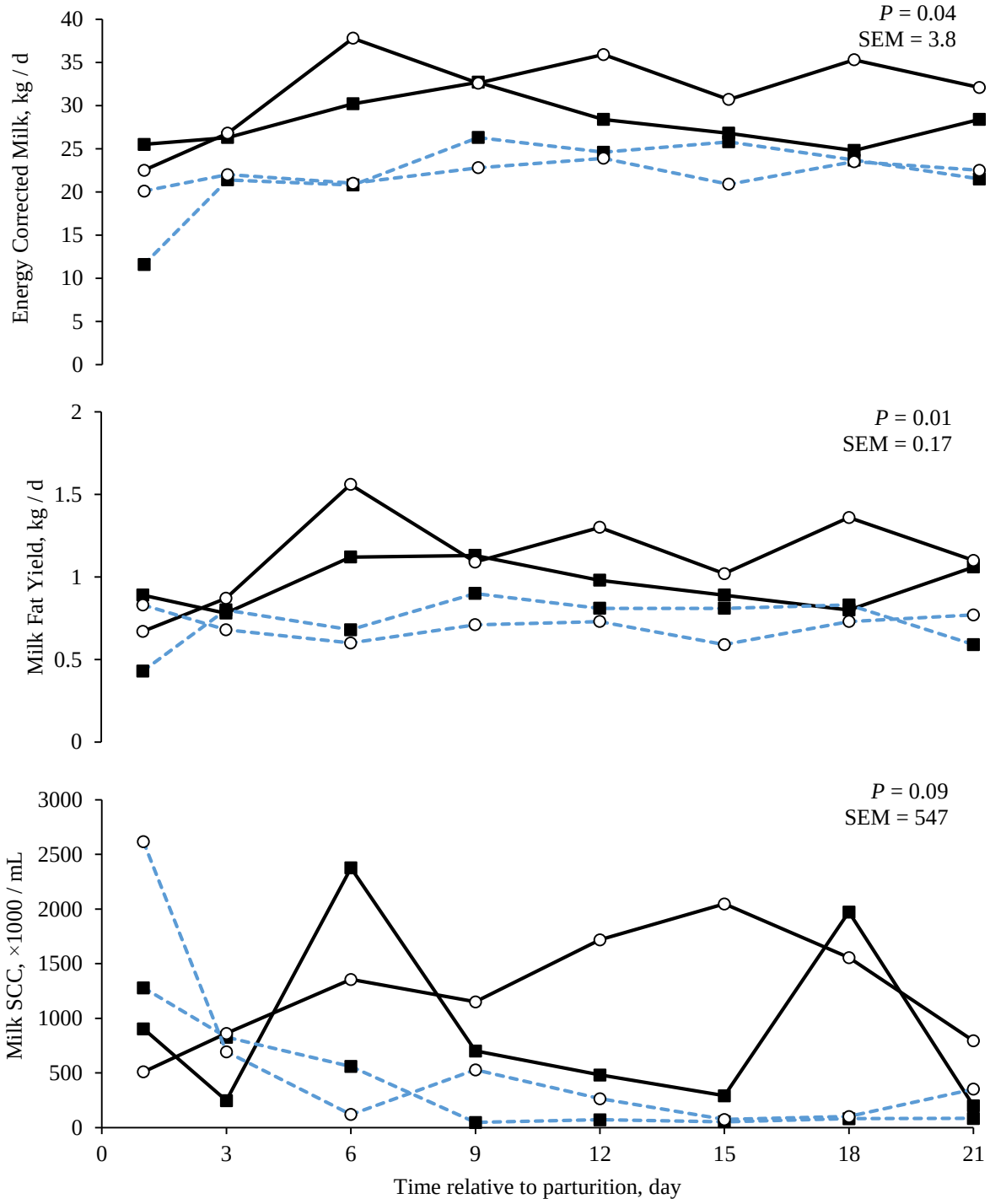


Figure 3.4. Least square means of n6:n3 in milk samples obtained from periparturient dairy cows [14 multiparous (solid line) and 20 primiparous (dashed line)] that received either the treatment (open circle; ○) of 10 kg of corn/hd per day prepartum and were fasted for 8 h on d +3 postpartum or control (closed square; ■) of 400 mg of monensin/hd per day prepartum.

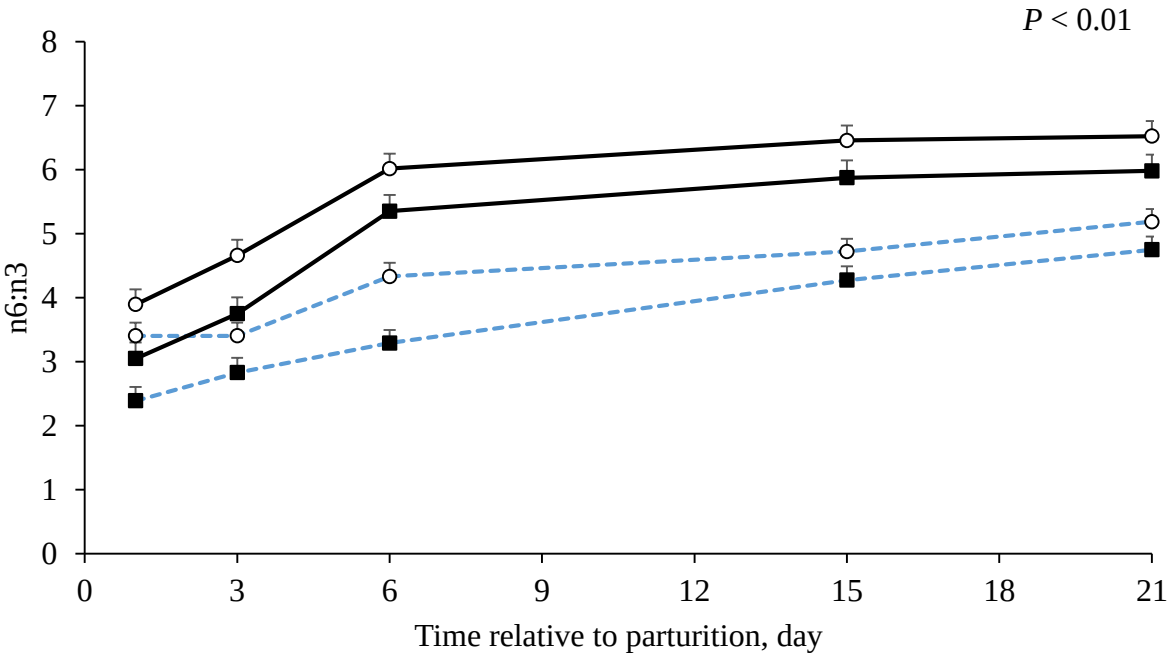


Figure 3.5. Least square means of C18:3n3 (g / 100 g FAME) of the nonesterified fatty acid and phospholipids fractions of serum and milk lipids from periparturient dairy cows [14 multiparous (solid line) and 20 primiparous (dashed line)] that received either the treatment (open circle; ○) of 10 kg of corn/hd per day prepartum and were fasted for 8 h on d +3 postpartum or control (closed square; ■) of 400 mg of monensin/hd per day prepartum.

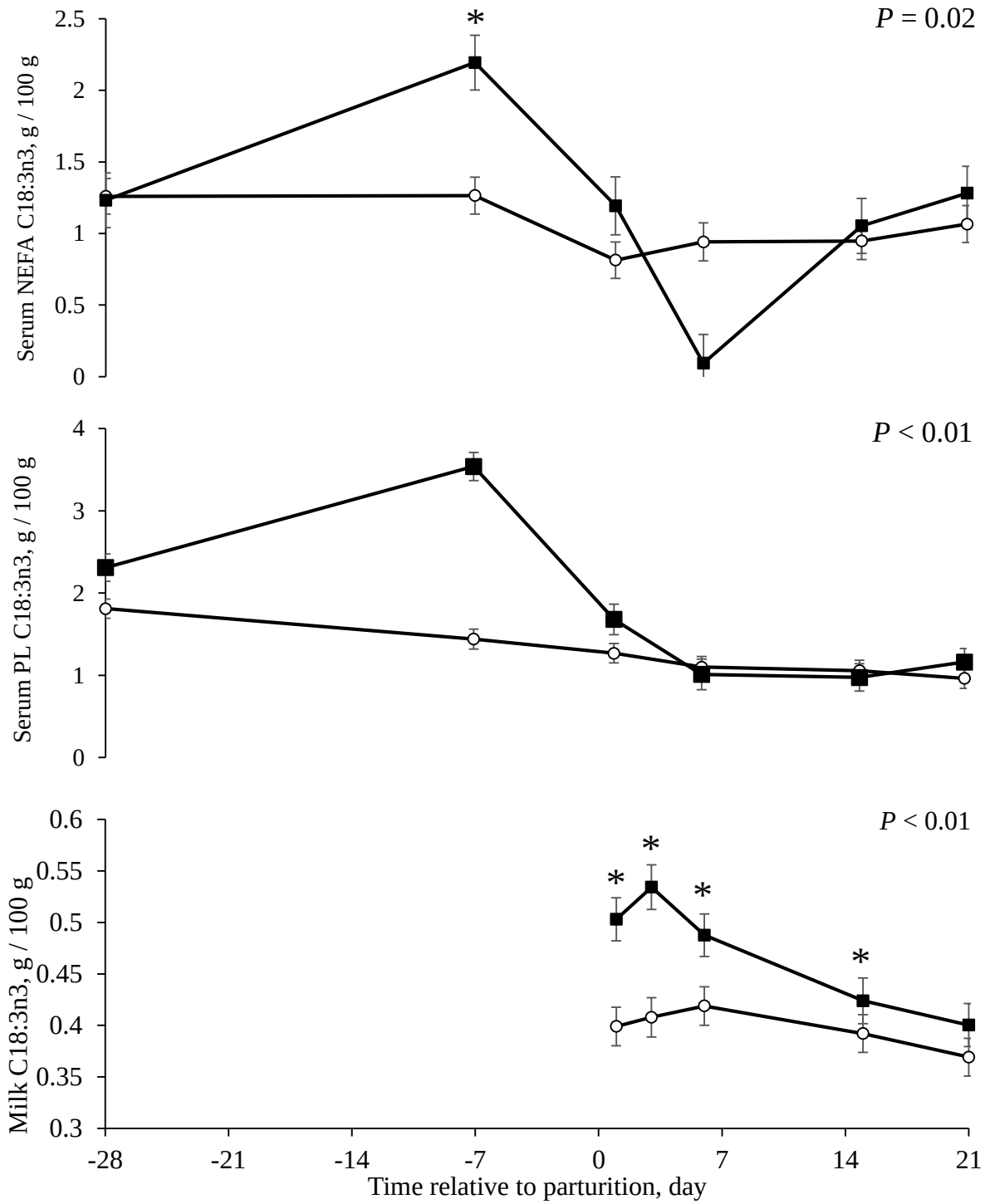


Figure 3.6. Least square means of fatty acid (g / 100 g FAME) profile of the phospholipids fraction of serum lipid samples obtained from periparturient dairy cows [14 multiparous (solid line) and 20 primiparous (dashed line)] that received either the treatment (open circle; ○) of 10 kg of corn/hd per day prepartum and were fasted for 8 h on d +3 postpartum or control (closed square; ■) of 400 mg of monensin/hd per day prepartum.

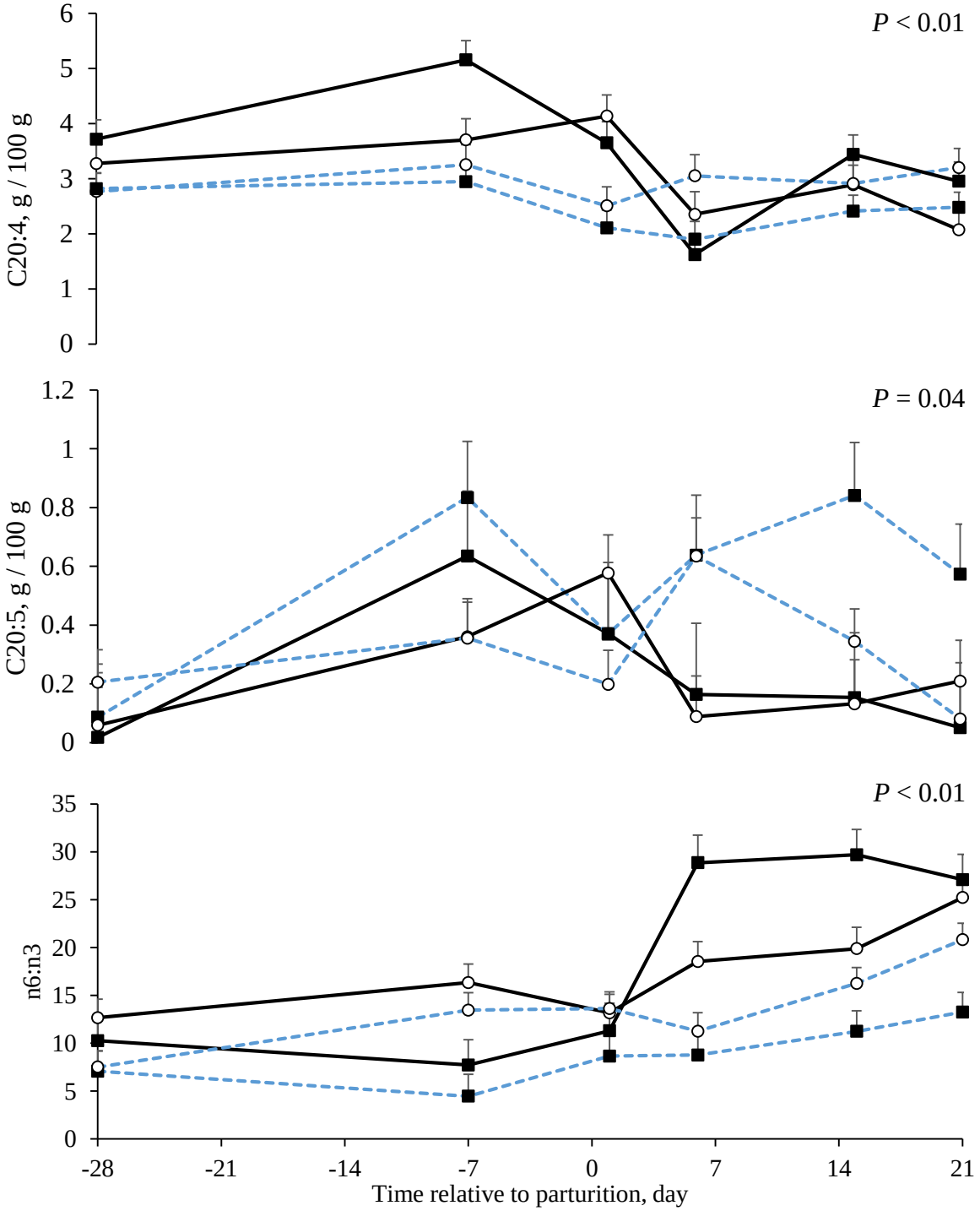


Figure 3.7. Least square means of C20:4 (g / 100 g FAME) profile and n6:n3 of the nonesterified fatty acid fraction of peripheral blood mononuclear cell samples obtained from periparturient dairy cows [14 multiparous (solid line) and 20 primiparous (dashed line)] that received either the treatment (open circle; ○) of 10 kg of corn/hd per day prepartum and were fasted for 8 h on d +3 postpartum or control (closed square; ■) of 400 mg of monensin/hd per day prepartum.

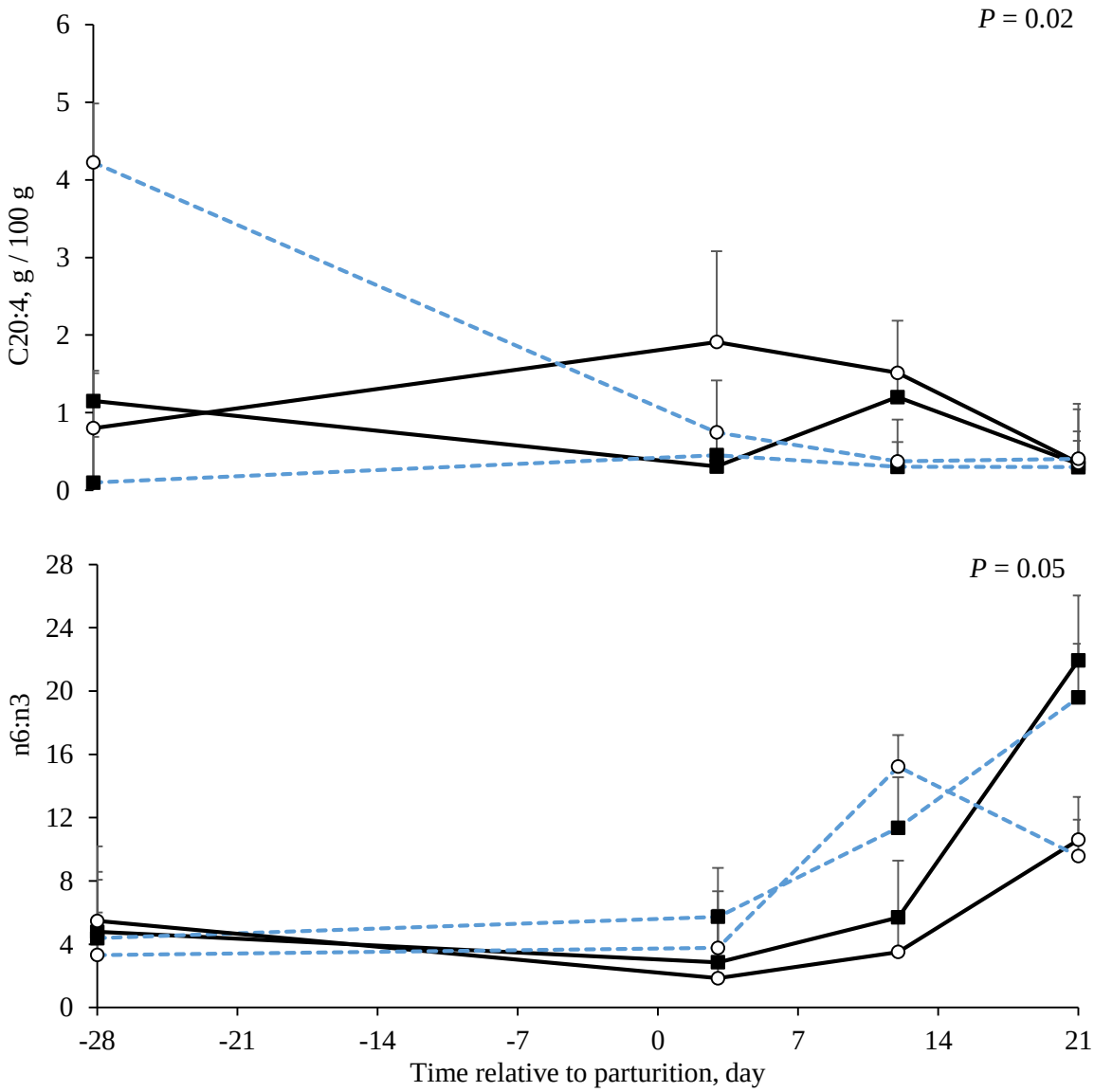


Figure 3.8. Least square means of fatty acid (g / 100 g FAME) profile of the phospholipids fraction of polymorphonucleocyte samples obtained from periparturient dairy cows [14 multiparous (solid line) and 20 primiparous (dashed line)] that received either the treatment (open circle; ○) of 10 kg of corn/hd per day prepartum and were fasted for 8 h on d +3 postpartum or control (closed square; ■) of 400 mg of monensin/hd per day prepartum.

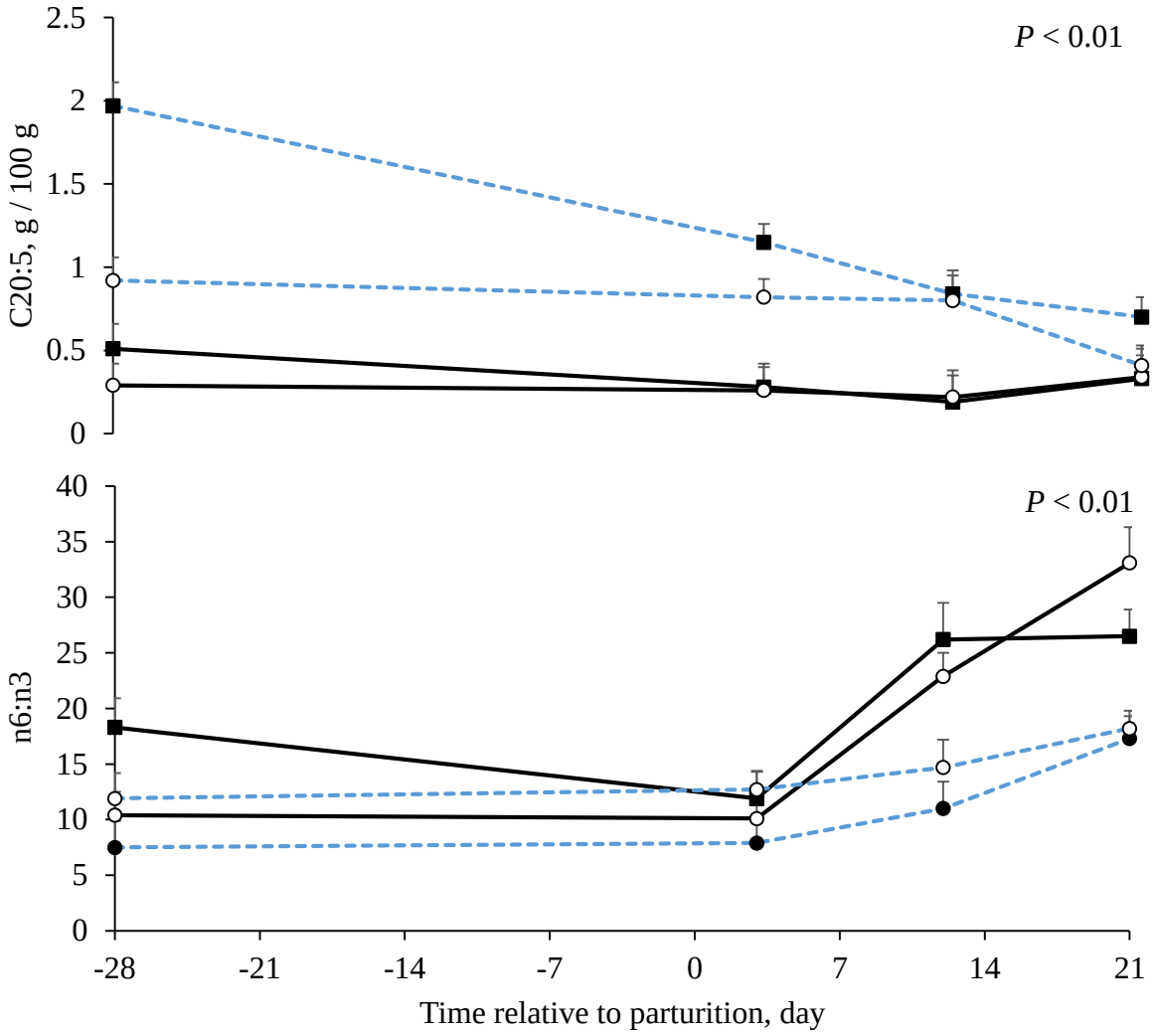
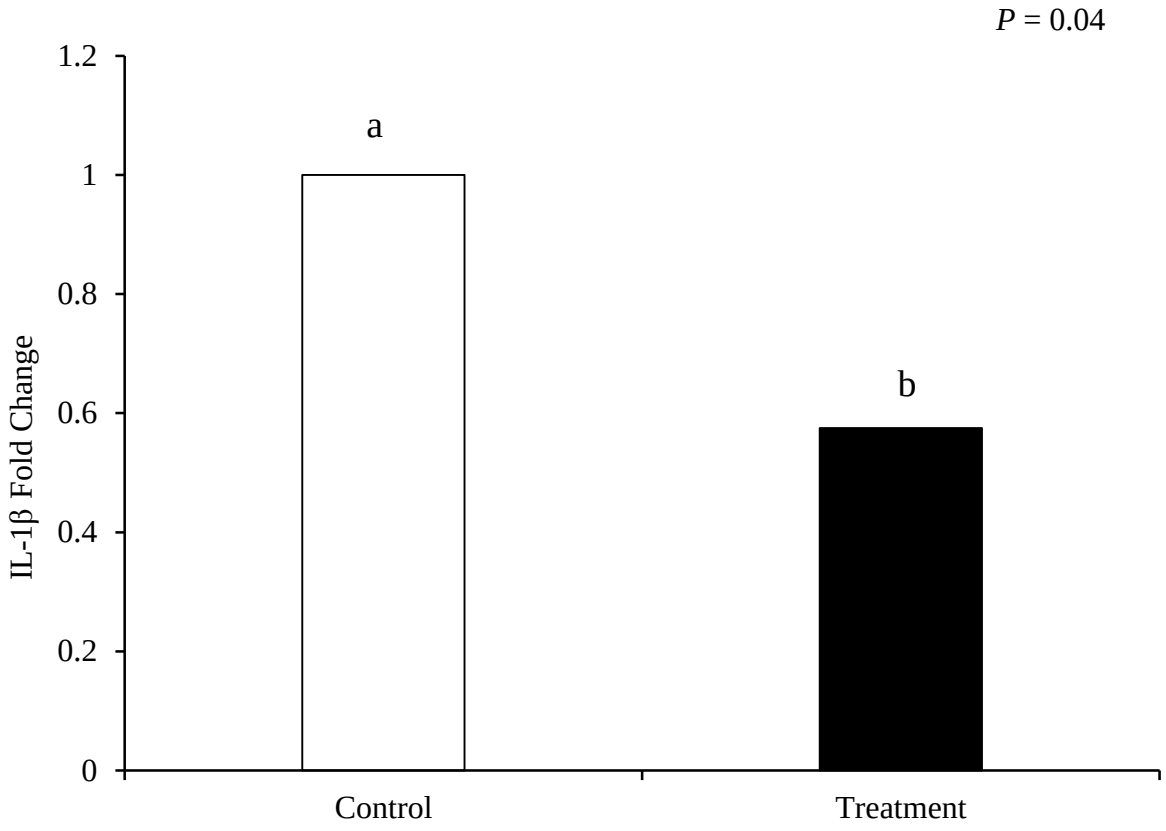


Figure 3.9. Gene expression (fold change) of interleukin-1β (IL-1β) of periperipheral blood mononuclear cells obtained from periparturient dairy cows [14 multiparous and 20 primiparous] that received either the treatment (closed bar) of 10 kg of corn/hd per day prepartum and were fasted for 8 h on d +3 postpartum or control (open bar) of 400 mg of monensin/hd per day prepartum. Least square means are illustrated in fold change ($2^{-\Delta\Delta Ct}$ method) relative to control treatment. Original Δ cycle threshold values were used for statistical analysis.



Tables

Table 2.1. Mean fatty acid composition of nonesterified fatty acids fraction of serum lipids obtained from dairy cows (n = 22) at various time points throughout the transition period that were retrospectively dichotomized by body condition score at day -5 to -7 before parturition.

Fatty acid,[1] g 100 g^{-1}	day, relative to parturition					SEM[2]	*P* - value
	-28	-7	8	18	28		
C14:0	2.6[a]	2.2[bc]	2.3[ab]	1.9[c]	2.1[bc]	0.2	0.01
C16:0	17.2[a]	19.2[ac]	21.8[b]	21.5[bc]	20.3[bc]	1.0	0.01
C16:1	0.8[a]	1.2[ab]	2.1[c]	2.2[c]	1.5[b]	0.2	0.001
C18:0	21.1	24.9	22.3	23.1	23.4	1.4	0.37
C18:1 *cis*	13.1	18.1[a]	22.9[ab]	27.0[b]	21.8[a]	2.1	0.001
C18:2 *trans*	15.4[a]	11.0[ab]	9.3[b]	2.7[c]	7.6[bc]	2.2	0.01
C18:2 *cis*	6.7[a]	6.4[a]	4.6[b]	5.7[ab]	6.7[a]	0.5	0.02
C20:1	3.6[a]	2.7[ac]	2.4[ac]	0.9[b]	1.8[bc]	0.5	0.001
C20:3n3	0.65[ab]	0.76[a]	0.39[c]	0.48[bc]	0.58[ac]	0.09	0.03

[a,b,c,d] Means in a row without a common letter differ, $P < 0.05$.

[1]C14:0 = myristic acid, C16:0 = palmitic acid, C16:1 = palmitoleic acid, C18:0 = stearic acid, C18:1 = oleic acid, C18:2 = linoleic acid, C20:1 = eicosenoic acid, C20:3n3 = eicosatrienoic acid.

[2]Largest SEM reported

Table 2.2. Mean fatty acid composition of phospholipids fraction of serum lipids obtained from dairy cows (n = 22) at various time points throughout the transition period that were retrospectively dichotomized by body condition score at day -5 to -7 before parturition.

Fatty acid,[1] g 100 g^{-1}	day, relative to parturition					SEM[2]	*P* - value
	-28	-7	8	18	28		
C14:0	1.8	1.6	1.5	1.7	1.6	0.3	0.94
C16:0	18.6^{a}	18.7^{ab}	23.5^{b}	22.3^{ab}	22.4^{b}	1.6	0.05
C16:1	1.1^{a}	0.8^{a}	0.7^{a}	1.1^{a}	1.7	0.2	0.01
C18:0	20.2^{a}	15.8^{bc}	16.0^{c}	19.8^{ab}	19.2^{ac}	1.5	0.05
C18:1 *cis*	7.6^{a}	7.8^{a}	10.9^{b}	12.2^{b}	12.0^{b}	0.8	0.001
C18:2 *trans*	0.3	2.6	0.9	1.0	0.1	1.0	0.36
C18:2 *cis*	28.6	26.5	28.5	25.0	28.0	1.7	0.43
C20:1	0.15	0.07	0.01	0.54	0.16	0.26	0.62
C20:3n3	2.7	1.8^{a}	1.1^{b}	1.6^{ab}	2.0^{a}	0.3	0.01

^{a,b,c}Means in a row without a common letter differ, $P < 0.05$.

[1]C14:0 = myristic acid, C16:0 = palmitic acid, C16:1 = palmitoleic acid, C18:0 = stearic acid, C18:1 = oleic acid, C18:2 = linoleic acid, C20:1 = eicosenoic acid, C20:3n3 = eicosatrienoic acid.

[2]Largest SEM reported

Table 2.3. Mean dry matter intake (DMI), body condition score (BCS), milk composition and yield of dairy cows (n = 22) throughout the transition period that were retrospectively dichotomized by BCS at day -5 to -7 before parturition

	BCS[1]				
	HIGH	LOW	SEM[2]	Treatment *P* - value	Treatment × Time *P* - value
DMI (kg d^{-1})					
Prepartum	20.0	23.8	1.1	0.02	0.09
Postpartum	24.8	30.4	2.2	0.09	0.85
Milk					
Protein %	3.1	3.2	0.1	0.29	0.59
Protein Yield	1.0	1.1	0.1	0.50	0.91
Fat %	2.8	3.1	0.4	0.62	0.89
Fat Yield	0.8	1.0	0.1	0.19	0.81
Lactose %	4.7	4.8	0.1	0.36	0.34
SNF	8.7	8.8	0.2	0.86	0.22
SCC[3]	134.8	213.1	56.0	0.33	0.50
Milk Yield (kg d^{-1})	33.5	34.1	2.8	0.89	0.53
ECM (kg d^{-1})[4]	28.4	32.1	2.5	0.88	0.52

[1]BCS dichotomization: HIGH BCS ≥ 3.25, LOW BCS ≤ 3.0
[2]Larger SEM reported
[3]SCC: somatic cell count (×1000/mL)
[4]ECM: 0.327 × milk yield kg + 12.95 × fat yield kg + 7.21 × protein yield kg

Table 3.1. Ingredient composition (% of DM) of prepartum and postpartum rations

	Ration	
Item	Prepartum	Postpartum
Alfalfa hay	12.89	6.42
Grass hay	9.99	6.47
Triticale silage[1]	44.25	30.03
Oat and pea silage[2]	14.77	-
Cracked corn, dry	3.97	7.20
Rolled barley	8.97	16.39
Dry distiller corn grain with solubles	-	8.97
Canola meal	-	17.06
Sodium bicarbonate	-	0.52
Liquid mineral/vitamin pre-mix[3]	4.99	4.50
Calcium soaps of fatty acids[4]	-	2.25
Salt	0.17	0.19
Chemical Analysis		
DM	46.4	62.3
NDF	51.6	38.4
ADF	34.4	24.5
CP	12.1	17.6
Soluble (as % of CP)		
Ether extract	2.8	5.6
NFC	24.8	32.9
Ash		
Ca	0.7	0.9
P	0.3	0.5
Mg	0.2	0.3
K	2.5	1.8
NE_L, Mcal/kg of DM	1.39	1.64

[1]Triticale silage 37% DM (as fed).
[2]50:50 Oat and pea silage 32% DM (as fed).
[3]Performix, Caldwell, ID.
[4]EnerGII; Virtus Nutrition, Corcoran, CA.

Table 3.2. Fatty acid (g / 100 g FAME) composition of basal rations fed to dairy cows (n = 34) that received either the treatment of 10 kg of corn/hd per day prepartum and were fasted for 8 h on d +3 postpartum or control of 400 mg of monensin/hd per day prepartum. All cows received the same postpartum ration after parturition.

	Ration	
Fatty acid (g / 100 g)	Prepartum	Postpartum
C 14:0	2.2	0.97
C 14:1	0.12	0.02
C 15:0	0.36	0.11
C 15:1	0.04	ND[1]
C 16:0	24.8	28.9
C 16:1	0.53	0.36
C 17:0	0.26	0.15
C 17:1	0.28	0.07
C 18:0	2.8	3.16
Σ C 18:1 *trans*	0.16	0.48
Σ C 18:1 *cis*	11.4	34.3
Σ C 18:2 *trans*	0.05	ND
Σ C 18:2 *cis*	20.8	24.8
C 18:3n6	0.05	ND
C 20:0	0.95	0.47
C 18:3n3	31.6	4.73
C 20:1	0.30	0.38
C 21:0	0.14	0.05
C 20:2	0.14	0.07
C 22:0	0.04	0.03
C 20:3n6	0.96	0.29
C 20:3n3	0.09	0.02
C 22:1	ND	0.02
C 20:4	0.13	0.03
C 23:0	0.33	0.21
C 22:2	ND	ND
C 20:5	ND	ND
C 24:0	0.60	0.25
C 24:1	0.26	0.05
C 22:6	0.65	0.08
Σ SFA[2]	32.4	34.3
Σ UFA[3]	67.6	65.8
Σ MUFA[4]	13.1	35.7
Σ PUFA[5]	54.5	30.0
Σ n-6[6]	22.1	25.2
Σ n-3[7]	32.4	4.8
n-6:n-3	0.68	5.2
SFA:UFA	0.48	0.52

[1]ND = not detected
[2]SFA = saturated fatty acids
[3]UFA = unsaturated fatty acids
[4]MUFA = monounsaturated fatty acids
[5]PUFA = polyunsaturated fatty acids
[6]n-6 = omega-6 fatty acids that included C18:2 *trans*, C18:2 *cis*, C18:3n6, C20:2, C20:3n6, C20:4, and C22:2
[7]n-3 = omega-3 fatty acids that included C18:3n3, C20:3n3, C20:5 and C22:6

Table 3.3. Least square means of the production performance of dairy cows [14 multiparous (M) and 20 primiparous (P)] that received either the treatment of 10 kg of corn/hd per day prepartum and were fasted for 8 h on d +3 postpartum (TRT) or control of 400 mg of monensin/hd per day prepartum (CON) throughout the transition period.

	TRT		CON			*P* - value			
	M	P	M	P	SEM[1]	TRT	TRT × Time	TRT × Parity	TRT × Parity × Time
Milk Yield (kg/d)	27.2	21.6	24.9	21.4	2.6	0.57	0.61	0.65	0.13
ECM (kg/d)[2]	21.1	21.7	27.5	21.7	2.4	0.38	0.29	0.39	0.04
ECM / DMI (kg/d)	2.94	1.85	4.09	1.97	0.74	0.26	0.73	0.35	0.33
Composition									
Protein									
%	4.26	3.69	3.95	3.43	0.12	0.01	<0.01	0.81	0.94
Yield, (kg/d)	1.08	0.76	0.96	0.71	0.09	0.30	0.81	0.01	0.19
Fat									
%	4.37	3.56	3.95	3.57	0.29	0.44	0.43	0.43	<0.01
Yield, (kg/d)	1.12	0.70	0.95	0.73	0.08	0.34	0.35	0.19	0.01
Lactose									
%	4.23	4.47	4.32	4.51	0.10	0.48	0.76	0.80	0.54
Yield, (kg/d)	1.18	0.99	1.08	0.99	0.12	0.67	0.52	0.66	0.45
SNF, %	9.34	9.04	9.13	8.82	0.17	0.20	<0.01	0.99	0.97
SCC[3]	1244	594	896	375	363	0.78	0.11	0.84	0.09
SCS[4]	2.43	2.00	2.33	1.92	0.20	0.62	0.64	0.95	0.28
MUN[5]	16.8	21.1	18.7	19.1	1.5	0.99	0.01	0.14	0.87

[1]Largest SEM reported

[2]ECM: energy corrected milk = 0.327 × milk yield kg + 12.95 × fat yield kg + 7.65 × protein yield kg; Dairy Record Management Systems (2013).

[3]SCC: somatic cell count (×1000/mL)

[4]SCS: somatic cell score = log (SCC)

[5]MUN: milk urea nitrogen

Table 3.4. Least square means of dry matter intake (DMI), body condition score (BCS) and body weight (BW) obtained from dairy cows [14 multiparous (M) and 20 primiparous (P)] that received either the treatment of 10 kg of corn/hd per day prepartum and were fasted for 8 h on d +3 postpartum (TRT) or control of 400 mg of monensin/hd per day prepartum (CON) throughout the transition period.

	TRT		CON			*P* - value			
	M	P	M	P	SEM[1]	TRT	TRT × Time	TRT × Parity	TRT × Parity × Time
DMI (kg/d)									
Prepartum	6.2	6.6	7.8	10.0	0.9	<0.01	0.92	0.01	-
Postpartum	17.7	14.8	13.8	15.6	1.9	0.35	0.07	0.16	0.20
DMI (as % of BW)									
Prepartum	0.81	1.61	1.39	1.82	0.06	<0.01	<0.01	<0.01	<0.01
Postpartum	3.0	3.2	2.9	3.3	0.4	0.95	0.82	0.75	0.38
BW (kg)	707	517	612	496	25	0.01	0.49	0.10	0.29
ΔBW (kg)									
Prepartum[2]	39	26	35	40	22	0.81	-	0.63	-
Postpartum[3]	-126	-101	-206	-95	25	0.10	-	0.05	-
BCS[4]	3.55	3.43	3.50	3.43	0.13	0.82	0.42	0.85	0.74
ΔBCS									
Prepartum	0.11	-0.10	-0.10	-0.11	0.18	0.48	-	0.51	-
Postpartum	0.38	0.50	0.10	0.31	0.17	0.12	-	0.80	-

[1]Largest SEM reported

[2]Change between d -28 to -7, relative to parturition

[3]Change between d +3 to -21, relative to parturition

[4]Based on a 1-5 scale

Table 3.5. Least square means of fatty acid (g / 100 g FAME) of milk obtained from dairy cows [14 multiparous (M) and 20 primiparous (P)] that received either the treatment of 10 kg of corn/hd per day prepartum and were fasted for 8 h on d +3 postpartum (TRT) or control of 400 mg of monensin/hd per day prepartum (CON) throughout the transition period.

	TRT		CON			*P* - value			
Fatty Acid (g / 100 g)	M	P	M	P	SEM[1]	TRT	TRT × Time	TRT × Parity	TRT × Parity × Time
C 14:0	6.5	6.5	6.7	7.2	0.4	0.28	0.95	0.57	0.62
C 14:1	0.71	0.63	0.61	0.67	0.05	0.47	0.82	0.11	0.76
C 15:0	-	-	-	-	-	-	-	-	-
C 15:1	-	-	-	-	-	-	-	-	-
C 16:0	31.9	31.1	31.6	31.9	0.6	0.65	0.87	0.37	0.02
C 16:1	2.4	1.7	2.1	1.6	0.1	0.06	0.27	0.39	0.56
C 17:0	0.69	0.94	0.79	0.97	0.05	0.10	0.45	0.36	0.02
C 17:1	0.42	0.51	0.46	0.50	0.04	0.58	0.81	0.56	0.15
C 18:0	14.1	16.7	14.6	16.9	0.8	0.57	0.78	0.81	0.81
Σ C 18:1 *trans*	1.4	2.0	1.4	2.1	0.2	0.83	0.79	0.77	0.30
Σ C 18:1 *cis*	34.5	32.1	34.2	30.1	1.3	0.30	0.90	0.45	0.23
Σ C 18:2 *trans*	-	-	-	-	-	-	-	-	-
Σ C 18:2 *cis*	3.0	2.7	2.9	2.4	0.1	0.03	<0.01	0.14	0.06
C 18:3n6	-	-	-	-	-	-	-	-	-
C 20:0	0.13	0.15	0.11	0.17	0.02	0.94	0.84	0.26	0.79
C 18:3n3	0.34	0.45	0.41	0.53	0.02	<0.01	<0.01	0.79	0.06
C 20:1	0.43	0.52	0.40	0.53	0.03	0.67	0.82	0.41	0.04
C 21:0	-	-	-	-	-	-	-	-	-
C 20:2	-	-	-	-	-	-	-	-	-
C 22:0	-	-	-	-	-	-	-	-	-
C 20:3n6	-	-	-	-	-	-	-	-	-
C 20:3n3	0.24	0.23	0.25	0.20	0.02	0.57	0.56	0.14	<0.01
C 22:1	-	-	-	-	-	-	-	-	-
C 20:4	-	-	-	-	-	-	-	-	-
C 23:0	-	-	-	-	-	-	-	-	-
C 22:2	0.04	0.06	0.05	0.09	0.01	<0.01	0.21	0.16	0.65
C 20:5	-	-	-	-	-	-	-	-	-
C 24:0	0.003	0.010	0.003	0.014	0.003	0.54	0.17	0.41	-
C 24:1	0.03	0.03	0.02	0.02	0.01	0.47	0.55	0.65	0.88
C 22:6	0.007	0.017	0.003	0.021	0.004	0.99	0.75	0.31	-

[1]Largest SEM reported

Table 3.6. Summary of various fatty acid profiles of milk obtained from dairy cows [14 multiparous (M) and 20 primiparous (P)] that received either the treatment of 10 kg of corn/hd per day prepartum and were fasted for 8 h on d +3 postpartum (TRT) or control of 400 mg of monensin/hd per day prepartum (CON) throughout the transition period.

	TRT		CON			*P* - value			
Fatty Acid (g / 100 g)	M	P	M	P	SEM[1]	TRT	TRT × Time	TRT × Parity	TRT × Parity × Time
Σ SFA[2]	56.2	58.8	56.9	61.0	1.2	0.17	0.99	0.50	0.22
Σ UFA[3]	43.7	41.1	43.0	38.9	1.2	0.18	0.89	0.49	0.22
Σ MUFA[4]	39.9	37.4	39.1	35.4	1.3	0.22	0.89	0.58	0.21
Σ PUFA[5]	3.8	3.7	3.8	3.5	0.1	0.32	0.03	0.29	-
Σ n-6[6]	3.1	2.8	3.0	2.5	0.1	0.04	<0.01	0.18	0.08
Σ n-3[7]	0.58	0.70	0.66	0.76	0.03	0.01	0.44	0.66	-
n-6:n-3	5.5	4.2	4.8	3.5	0.2	<0.01	0.34	0.98	<0.01
SFA:UFA	1.4	1.5	1.4	1.7	0.1	0.22	0.84	0.20	-

[1]Largest SEM reported

[2]SFA = saturated fatty acids

[3]UFA = unsaturated fatty acids

[4]MUFA = monounsaturated fatty acids

[5]PUFA = polyunsaturated fatty acids

[6]n-6 = omega-6 fatty acids that included C18:2 *trans*, C18:2 *cis*, C18:3n6, C20:2, C20:3n6, C20:4, and C22:2

[7]n-3 = omega-3 fatty acids that included C18:3n3, C20:3n3, C20:5 and C22:6

Table 3.7. Least square means of fatty acid (g / 100 g FAME) of the nonesterified fatty acid fraction of serum obtained from dairy cows [14 multiparous (M) and 20 primiparous (P)] that received either the treatment of 10 kg of corn/hd per day prepartum and were fasted for 8 h on d +3 postpartum (TRT) or control of 400 mg of monensin/hd per day prepartum (CON) throughout the transition period.

	TRT		CON			*P* - value			
Fatty Acid (g / 100 g)	M	P	M	P	SEM[1]	TRT	TRT × Time	TRT × Parity	TRT × Parity × Time
C 14:0	3.6	2.8	3.1	3.4	0.4	0.76	0.46	0.17	0.18
C 14:1	2.0	1.9	1.9	2.0	0.2	0.99	0.18	0.49	0.75
C 15:0	0.74	0.74	0.76	0.98	0.11	0.19	0.24	0.26	0.24
C 15:1	0.32	0.46	0.54	0.59	0.18	0.22	0.27	0.72	0.57
C 16:0	28.0	27.4	27.9	27.6	0.7	0.89	0.70	0.76	0.04
C 16:1	2.9	2.9	3.4	3.3	0.4	0.18	0.01	0.87	0.19
C 17:0	1.3	1.4	1.4	1.6	0.1	0.24	0.12	0.74	0.06
C 17:1	0.26	0.56	0.34	0.37	0.14	0.59	0.70	0.18	0.95
C 18:0	23.0	24.8	24.4	25.0	1.0	0.41	0.22	0.48	0.10
Σ C 18:1 *trans*	1.42	2.1	1.6	1.9	0.3	0.97	0.54	0.59	0.24
Σ C 18:1 *cis*	22.7	21.8	21.9	19.0	1.3	0.13	0.41	0.42	<0.01
Σ C 18:2 *trans*	0.07	0.08	0.08	0.08	0.02	0.81	0.26	0.89	0.07
Σ C 18:2 *cis*	7.9	7.4	7.6	6.9	0.6	0.42	0.30	0.91	0.01
C 18:3n6	0.06	0.08	0.07	0.08	0.02	0.86	0.36	0.81	0.29
C 20:0	-	-	-	-	-	-	-	-	-
C 18:3n3	1.1	1.0	1.1	1.5	0.2	0.04	0.02	0.05	0.29
C 20:1	0.10	0.14	0.06	0.19	0.07	0.94	0.14	0.47	0.71
C 21:0	0.12	0.09	0.16	0.17	0.05	0.10	0.93	0.62	0.19
C 20:2	0.08	0.10	0.08	0.18	0.07	0.44	0.43	0.41	0.87
C 22:0	0.36	0.20	0.26	0.33	0.08	0.74	0.13	0.10	0.14
C 20:3n6	0.70	0.86	0.64	0.82	0.10	0.63	0.95	0.92	0.59
C 20:3n3	0.18	0.19	0.26	0.30	0.09	0.19	0.52	0.78	<0.01
C 22:1	0.31	0.35	0.27	0.22	0.08	0.19	0.32	0.50	0.17
C 20:4	1.26	0.99	0.83	0.89	0.16	0.06	0.25	0.22	0.01
C 23:0	0.30	0.27	0.26	0.34	0.09	0.84	0.39	0.48	0.74
C 22:2	0.06	0.07	0.06	0.17	0.07	0.26	0.72	0.25	0.23
C 20:5	0.33	0.32	0.26	0.38	0.09	0.92	0.62	0.38	0.48
C 24:0	0.20	0.19	0.20	0.28	0.07	0.47	0.10	0.47	0.24
C 24:1	0.08	0.05	0.04	0.14	0.07	0.66	0.60	0.20	0.31
C 22:6	0.04	0.18	0.01	0.27	0.19	0.88	0.47	0.74	0.73

[1]Largest SEM reported

Table 3.8. Summary of various fatty acid profiles of nonesterified fatty acid fraction of serum obtained from dairy cows [14 multiparous (M) and 20 primiparous (P)] that received either the treatment of 10 kg of corn/hd per day prepartum and were fasted for 8 h on d +3 postpartum (TRT) or control of 400 mg of monensin/hd per day prepartum (CON) throughout the transition period.

	TRT		CON			*P* - value			
Fatty Acid (g / 100 g)	M	P	M	P	SEM[1]	TRT	TRT × Time	TRT × Parity	TRT × Parity × Time
Σ SFA[2]	58.1	58.6	59.2	60.7	1.4	0.19	0.93	0.66	0.19
Σ UFA[3]	41.9	41.4	40.8	39.3	1.4	0.19	0.93	0.66	0.19
Σ MUFA[4]	30.1	30.2	29.9	27.7	1.7	0.37	0.64	0.45	<0.01
Σ PUFA[5]	11.8	11.2	10.9	11.6	0.9	0.75	0.87	0.44	<0.01
Σ n-6[6]	8.8	8.5	8.4	8.1	0.6	0.50	0.29	0.95	0.06
Σ n-3[7]	1.6	1.7	1.6	2.5	0.4	0.25	0.53	0.21	0.18
n-6:n-3	6.8	6.1	5.9	4.5	0.5	0.01	0.11	0.42	0.82
SFA:UFA	1.5	1.5	1.5	1.6	0.1	0.15	0.95	0.54	-

[1]Largest SEM reported

[2]SFA = saturated fatty acids

[3]UFA = unsaturated fatty acids

[4]MUFA = monounsaturated fatty acids

[5]PUFA = polyunsaturated fatty acids

[6]n-6 = omega-6 fatty acids that included C18:2 *trans*, C18:2 *cis*, C18:3n6, C20:2, C20:3n6, C20:4, and C22:2

[7]n-3 = omega-3 fatty acids that included C18:3n3, C20:3n3, C20:5 and C22:6

Table 3.9. Least square means of fatty acid (g / 100 g FAME) of the phospholipids fraction of serum obtained from dairy cows [14 multiparous (M) and 20 primiparous (P)] that received either the treatment of 10 kg of corn/hd per day prepartum and were fasted for 8 h on d +3 postpartum (TRT) or control of 400 mg of monensin/hd per day prepartum (CON) throughout the transition period.

	TRT		CON			*P* - value			
Fatty Acid (g / 100 g)	M	P	M	P	SEM[1]	TRT	TRT × Time	TRT × Parity	TRT × Parity × Time
C 14:0	2.2	1.8	1.7	1.9	0.4	0.55	0.52	0.15	0.24
C 14:1	0.58	0.59	0.56	0.60	0.07	0.86	0.27	0.71	0.47
C 15:0	0.51	0.57	0.46	0.65	0.05	0.70	<0.01	0.15	0.14
C 15:1	0.27	0.37	0.26	0.33	0.05	0.62	0.99	0.66	0.23
C 16:0	22.4	21.9	22.6	22.0	0.6	0.76	0.66	0.89	0.81
C 16:1	1.2	1.2	1.4	1.4	0.1	0.13	0.26	0.88	0.47
C 17:0	0.83	1.11	0.94	1.15	0.10	0.31	0.55	0.66	0.43
C 17:1	0.88	0.65	0.49	0.70	0.16	0.13	0.24	0.06	0.65
C 18:0	23.5	24.7	23.8	25.4	1.4	0.68	0.21	0.83	0.31
Σ C 18:1 *trans*	1.2	1.3	1.2	1.7	0.3	0.45	0.56	0.28	0.92
Σ C 18:1 *cis*	13.4	13.6	12.6	14.0	0.7	0.77	0.39	0.34	0.41
Σ C 18:2 *trans*	0.17	0.13	0.17	0.18	0.02	0.22	0.01	0.20	0.76
Σ C 18:2 *cis*	20.9	19.7	19.6	17.3	1.2	0.10	0.01	0.61	0.55
C 18:3n6	0.34	0.26	0.28	0.24	0.03	0.18	0.44	0.60	<0.01
C 20:0	0.14	0.16	0.17	0.21	0.02	0.06	0.03	0.69	0.07
C 18:3n3	1.3	1.3	1.6	1.9	0.1	<0.01	<0.01	0.16	0.07
C 20:1	0.15	0.16	0.09	0.14	0.02	0.04	0.61	0.20	0.11
C 21:0	0.08	0.03	0.05	0.05	0.02	0.62	0.27	0.17	0.37
C 20:2	0.10	0.12	0.12	0.07	0.04	0.73	0.97	0.31	0.24
C 22:0	1.4	1.4	1.3	1.5	0.2	0.94	0.73	0.70	0.29
C 20:3n6	2.7	2.2	2.5	2.0	0.2	0.15	0.09	0.84	<0.01
C 20:3n3	0.10	0.08	0.17	0.09	0.06	0.36	0.30	0.46	0.15
C 22:1	-	-	-	-	-	-	-	-	-
C 20:4	3.1	3.0	3.4	2.4	0.2	0.64	0.05	0.01	<0.01
C 23:0	0.39	0.31	0.37	0.49	0.05	0.05	<0.01	0.02	0.57
C 22:2	0.26	0.59	0.34	0.48	0.09	0.90	0.89	0.23	0.88
C 20:5	0.24	0.30	0.23	0.56	0.09	0.09	0.40	0.08	0.04
C 24:0	0.09	0.07	0.06	0.06	0.04	0.42	0.65	0.65	0.24
C 24:1	0.33	0.23	0.38	0.21	0.05	0.78	0.90	0.42	0.06
C 22:6	0.20	0.46	0.14	0.48	0.05	0.60	0.60	0.34	0.86

[1]Largest SEM reported

Table 3.10. Summary of various fatty acid profiles of phospholipids fraction of serum obtained from dairy cows [14 multiparous (M) and 20 primiparous (P)] that received either the treatment of 10 kg of corn/hd per day prepartum and were fasted for 8 h on d +3 postpartum (TRT) or control of 400 mg of monensin/hd per day prepartum (CON) throughout the transition period.

	TRT		CON			*P* - value			
Fatty Acid (g / 100 g)	M	P	M	P	SEM[1]	TRT	TRT × Time	TRT × Parity	TRT × Parity × Time
Σ SFA[2]	51.5	52.1	51.5	53.4	1.7	0.67	0.48	0.63	0.57
Σ UFA[3]	47.4	46.2	45.5	45.0	1.7	0.31	0.10	0.82	0.24
Σ MUFA[4]	18.0	18.2	17.0	19.1	1.0	0.94	0.25	0.19	0.69
Σ PUFA[5]	29.3	28.1	28.4	25.8	1.3	0.22	0.05	0.54	0.07
Σ n-6[6]	27.5	25.9	26.4	22.7	1.3	0.07	0.03	0.03	0.11
Σ n-3[7]	1.8	2.1	2.1	3.1	0.2	<0.01	<0.01	0.06	0.04
n-6:n-3	17.6	13.8	19.2	8.9	1.3	0.11	<0.01	<0.01	<0.01
SFA:UFA	1.2	1.3	1.5	1.4	0.2	0.34	0.11	0.38	0.14

[1]Largest SEM reported

[2]SFA = saturated fatty acids

[3]UFA = unsaturated fatty acids

[4]MUFA = monounsaturated fatty acids

[5]PUFA = polyunsaturated fatty acids

[6]n-6 = omega-6 fatty acids that included C18:2 *trans*, C18:2 *cis*, C18:3n6, C20:2, C20:3n6, C20:4, and C22:2

[7]n-3 = omega-3 fatty acids that included C18:3n3, C20:3n3, C20:5 and C22:6

Table 3.11. Least square means of fatty acid (g / 100 g FAME) of the nonesterified fatty acid fraction of peripheral blood mononuclear cells obtained from dairy cows [14 multiparous (M) and 20 primiparous (P)] that received either the treatment of 10 kg of corn/hd per day prepartum and were fasted for 8 h on d +3 postpartum (TRT) or control of 400 mg of monensin/hd per day prepartum (CON) throughout the transition period.

	TRT		CON			*P* - value			
Fatty Acid (g / 100 g)	M	P	M	P	SEM[1]	TRT	TRT × Time	TRT × Parity	TRT × Parity × Time
C 14:0	5.7	5.6	4.3	5.4	0.6	0.16	0.24	0.28	0.07
C 14:1	0.85	0.58	0.47	0.87	0.14	0.70	0.13	0.01	0.06
C 15:0	1.4	1.3	1.0	1.3	0.1	0.05	0.23	0.10	0.07
C 15:1	0.94	1.01	0.91	0.74	0.17	0.33	0.82	0.44	0.55
C 16:0	27.9	27.9	27.4	28.1	1.4	0.91	0.78	0.80	0.41
C 16:1	2.1	2.1	1.7	2.1	0.2	0.32	0.65	0.31	0.20
C 17:0	1.3	1.7	1.4	1.4	0.5	0.85	0.99	0.52	0.96
C 17:1	1.27	0.99	1.06	0.94	0.23	0.54	0.10	0.68	0.10
C 18:0	25.1	24.7	29.4	26.8	2.1	0.08	0.45	0.55	0.84
Σ C 18:1 *trans*	2.3	2.2	1.6	2.0	0.5	0.33	0.07	0.62	0.61
Σ C 18:1 *cis*	7.8	7.3	7.6	8.6	1.5	0.72	0.81	0.58	0.35
Σ C 18:2 *trans*	-	-	-	-	-	-	-	-	-
Σ C 18:2 *cis*	2.3	2.7	2.2	2.7	0.9	0.95	0.60	0.96	0.82
C 18:3n6	0.18	0.09	0.02	0.04	0.07	0.04	0.58	0.29	0.99
C 20:0	0.67	0.57	0.62	0.74	0.11	0.49	0.12	0.23	0.90
C 18:3n3	2.5	2.4	2.1	1.8	0.8	0.41	0.40	0.82	0.18
C 20:1	0.55	0.31	0.81	0.38	0.18	0.30	0.01	0.55	0.19
C 21:0	0.53	0.47	0.65	0.31	0.22	0.93	0.23	0.45	0.70
C 20:2	0.72	1.06	0.71	0.54	0.37	0.39	0.49	0.39	0.25
C 22:0	3.4	3.4	3.1	3.0	1.1	0.72	0.82	0.92	0.14
C 20:3n6	5.9	7.4	7.6	7.7	1.7	0.55	0.66	0.67	0.18
C 20:3n3	1.00	0.75	0.81	0.56	0.31	0.51	0.04	0.99	0.66
C 22:1	2.6	2.1	2.1	2.0	0.7	0.58	0.43	0.79	0.05
C 20:4	1.14	1.44	0.75	0.29	0.43	0.02	0.12	0.23	0.02
C 23:0	0.26	0.20	0.22	0.05	0.06	0.11	0.99	0.36	0.44
C 22:2	0.28	0.52	0.44	0.58	0.20	0.52	0.81	0.78	0.30
C 20:5	0.05	0.36	0.02	0.24	0.27	0.69	0.34	0.79	<0.01
C 24:0	0.20	0.21	0.20	0.23	0.07	0.90	0.79	0.88	0.10
C 24:1	0.90	0.47	0.68	0.80	0.25	0.81	0.52	0.24	0.72
C 22:6	-	-	-	-	-	-	-	-	-

[1]Largest SEM reported

Table 3.12. Summary of various fatty acid profiles of the nonesterified fatty acid fraction of peripheral blood mononuclear cells obtained from dairy cows [14 multiparous (M) and 20 primiparous (P)] that received either the treatment of 10 kg of corn/hd per day prepartum and were fasted for 8 h on d +3 postpartum (TRT) or control of 400 mg of monensin/hd per day prepartum (CON) throughout the transition period.

	TRT		CON			*P* - value			
Fatty Acid (g / 100 g)	M	P	M	P	SEM[1]	TRT	TRT × Time	TRT × Parity	TRT × Parity × Time
Σ SFA[2]	66.4	66.0	68.5	67.2	2.5	0.49	0.59	0.87	0.79
Σ UFA[3]	33.6	34.0	31.5	32.8	2.5	0.49	0.59	0.87	0.79
Σ MUFA[4]	19.3	17.2	16.9	18.4	2.0	0.72	0.65	0.31	0.98
Σ PUFA[5]	14.2	16.9	14.6	14.4	2.4	0.62	0.67	0.48	0.46
Σ n-6[6]	10.7	13.3	11.7	11.8	1.9	0.90	0.45	0.48	0.56
Σ n-3[7]	3.5	3.6	2.9	2.6	1.0	0.29	0.33	0.81	0.18
n-6:n-3	5.4	8.0	8.8	10.3	2.1	0.12	0.04	0.75	0.05
SFA:UFA	2.4	2.3	2.6	2.8	0.4	0.35	0.69	0.67	0.75

[1]Largest SEM reported

[2]SFA = saturated fatty acids

[3]UFA = unsaturated fatty acids

[4]MUFA = monounsaturated fatty acids

[5]PUFA = polyunsaturated fatty acids

[6]n-6 = omega-6 fatty acids that included C18:2 *trans*, C18:2 *cis*, C18:3n6, C20:2, C20:3n6, C20:4, and C22:2

[7]n-3 = omega-3 fatty acids that included C18:3n3, C20:3n3, C20:5 and C22:6

Table 3.13. Least square means of fatty acid (g / 100 g FAME) of the phospholipids fatty acid fraction of peripheral blood mononuclear cells obtained from dairy cows [14 multiparous (M) and 20 primiparous (P)] that received either the treatment of 10 kg of corn/hd per day prepartum and were fasted for 8 h on d +3 postpartum (TRT) or control of 400 mg of monensin/hd per day prepartum (CON) throughout the transition period.

	TRT		CON			*P* - value			
Fatty Acid (g / 100 g)	M	P	M	P	SEM[1]	TRT	TRT × Time	TRT × Parity	TRT × Parity × Time
C 14:0	2.5	2.2	1.5	1.9	0.4	0.08	0.19	0.23	0.19
C 14:1	0.57	0.43	0.50	0.53	0.13	0.87	0.02	0.37	0.23
C 15:0	0.97	1.06	0.78	0.97	0.17	0.33	0.18	0.71	0.36
C 15:1	0.43	0.55	0.62	0.48	0.10	0.53	0.98	0.18	0.71
C 16:0	22.6	23.4	21.4	23.1	1.0	0.37	0.46	0.62	0.34
C 16:1	1.39	1.22	1.00	0.95	0.18	0.02	0.32	0.66	0.53
C 17:0	1.1	1.3	1.0	1.1	0.2	0.39	0.70	0.69	0.79
C 17:1	0.95	0.64	0.47	0.74	0.14	0.11	0.57	0.02	0.19
C 18:0	19.6	18.5	21.0	19.6	1.4	0.29	0.66	0.87	0.49
Σ C 18:1 *trans*	2.6	2.6	2.0	1.9	0.2	<0.01	0.03	0.98	0.26
Σ C 18:1 *cis*	16.5	16.2	15.1	16.2	0.8	0.36	0.84	0.34	0.08
Σ C 18:2 *trans*	0.05	0.03	0.07	0.03	0.03	0.73	0.18	0.54	0.07
Σ C 18:2 *cis*	9.3	8.8	9.1	9.2	0.9	0.93	0.97	0.70	0.20
C 18:3n6	0.10	0.12	0.08	0.09	0.03	0.50	0.74	0.84	0.03
C 20:0	0.77	0.61	0.86	0.57	0.14	0.83	0.15	0.62	0.47
C 18:3n3	0.87	1.27	0.63	0.64	0.30	0.04	0.41	0.35	0.25
C 20:1	1.3	1.0	1.3	1.1	0.1	0.80	0.36	0.97	0.82
C 21:0	-	-	-	-	-	-	-	-	-
C 20:2	0.42	0.64	0.44	0.42	0.09	0.13	<0.01	0.07	0.34
C 22:0	2.0	2.1	2.4	2.0	0.3	0.52	0.04	0.41	0.39
C 20:3n6	2.6	3.0	2.9	2.5	0.5	0.79	0.60	0.41	0.08
C 20:3n3	0.04	0.20	0.14	0.01	0.11	0.65	0.14	0.13	0.26
C 22:1	1.00	0.85	0.51	0.88	0.22	0.23	0.80	0.17	0.09
C 20:4	8.3	8.0	10.5	9.6	0.9	0.02	0.08	0.69	0.21
C 23:0	0.10	0.17	0.18	0.10	0.04	0.79	0.94	0.03	0.15
C 22:2	0.36	0.45	0.53	0.43	0.12	0.40	0.18	0.32	0.68
C 20:5	1.05	1.07	0.99	1.35	0.15	0.43	0.63	0.22	0.49
C 24:0	0.16	0.16	0.12	0.17	0.05	0.37	0.38	0.57	0.07
C 24:1	1.8	2.7	3.3	2.8	0.8	0.19	0.92	0.23	0.03
C 22:6	0.44	0.65	0.50	0.61	0.18	0.91	0.72	0.70	0.97

[1]Largest SEM reported

Table 3.14. Summary of various fatty acid profiles of the phospholipids fatty acid fraction of peripheral blood mononuclear cells obtained from dairy cows [14 multiparous (M) and 20 primiparous (P)] that received either the treatment of 10 kg of corn/hd per day prepartum and were fasted for 8 h on d +3 postpartum (TRT) or control of 400 mg of monensin/hd per day prepartum (CON) throughout the transition period.

	TRT		CON			*P* - value			
Fatty Acid (g / 100 g)	M	P	M	P	SEM[1]	TRT	TRT × Time	TRT × Parity	TRT × Parity × Time
Σ SFA[2]	50.0	49.5	49.4	49.5	1.4	0.84	0.94	0.83	0.24
Σ UFA[3]	50.0	50.5	50.6	50.5	1.4	0.84	0.94	0.83	0.24
Σ MUFA[4]	26.4	26.2	24.7	25.6	1.0	0.20	0.59	0.56	0.03
Σ PUFA[5]	23.6	24.3	25.8	24.9	1.7	0.33	0.91	0.58	0.02
Σ n-6[6]	21.2	21.1	23.6	22.3	1.6	0.20	0.79	0.67	<0.01
Σ n-3[7]	2.4	3.2	2.3	2.6	0.4	0.26	0.67	0.49	0.88
n-6:n-3	10.7	10.2	12.7	10.8	1.5	0.30	0.41	0.58	0.47
SFA:UFA	1.0	1.0	1.0	1.0	0.1	0.77	0.98	0.90	0.31

[1]Largest SEM reported

[2]SFA = saturated fatty acids

[3]UFA = unsaturated fatty acids

[4]MUFA = monounsaturated fatty acids

[5]PUFA = polyunsaturated fatty acids

[6]n-6 = omega-6 fatty acids that included C18:2 *trans*, C18:2 *cis*, C18:3n6, C20:2, C20:3n6, C20:4, and C22:2

[7]n-3 = omega-3 fatty acids that included C18:3n3, C20:3n3, C20:5 and C22:6

Table 3.15. Least square means of fatty acid (g / 100 g FAME) of the phospholipids fatty acid fraction of polymorphonucleocytes obtained from dairy cows [14 multiparous (M) and 20 primiparous (P)] that received either the treatment of 10 kg of corn/hd per day prepartum and were fasted for 8 h on d +3 postpartum (TRT) or control of 400 mg of monensin/hd per day prepartum (CON) throughout the transition period.

	TRT		CON			*P* - value			
Fatty Acid (g / 100 g)	M	P	M	P	SEM[1]	TRT	TRT × Time	TRT × Parity	TRT × Parity × Time
C 14:0	2.2	1.4	1.5	1.3	0.4	0.27	0.80	0.49	0.16
C 14:1	0.37	0.31	0.24	0.27	0.06	0.09	0.38	0.37	0.40
C 15:0	0.46	0.54	0.39	0.47	0.07	0.21	0.55	0.99	0.08
C 15:1	-	-	-	-	-	-	-	-	-
C 16:0	20.4	19.8	18.2	17.8	1.1	0.04	0.79	0.96	0.19
C 16:1	0.86	0.53	0.84	0.71	0.20	0.59	0.11	0.49	0.29
C 17:0	0.64	0.72	0.63	0.73	0.04	0.90	0.62	0.74	0.18
C 17:1	0.21	0.28	0.25	0.27	0.04	0.62	0.95	0.44	0.15
C 18:0	19.3	19.8	21.2	19.5	1.0	0.32	0.24	0.15	0.28
Σ C 18:1 *trans*	2.0	1.8	1.5	1.6	0.2	0.09	0.20	0.45	0.10
Σ C 18:1 *cis*	19.7	17.4	15.8	15.7	1.3	0.02	0.18	0.34	0.12
Σ C 18:2 *trans*	-	-	-	-	-	-	-	-	-
Σ C 18:2 *cis*	23.1	25.5	27.4	27.5	2.0	0.09	0.48	0.53	0.11
C 18:3n6	0.12	0.08	0.09	0.14	0.03	0.56	0.15	0.15	-
C 20:0	0.32	0.31	0.33	0.34	0.04	0.52	0.34	0.98	0.54
C 18:3n3	1.6	1.1	1.1	1.7	0.5	0.89	0.60	0.10	0.46
C 20:1	0.84	0.68	0.80	0.64	0.06	0.40	0.80	0.97	0.15
C 21:0	0.30	0.21	0.19	0.20	0.13	0.52	0.54	0.61	0.08
C 20:2	0.18	0.28	0.23	0.26	0.04	0.68	0.59	0.30	0.55
C 22:0	1.4	1.4	1.9	1.6	0.2	0.02	0.01	0.38	0.07
C 20:3n6	0.53	0.52	0.71	0.58	0.14	0.29	0.33	0.63	0.23
C 20:3n3	0.47	0.22	0.31	0.44	0.14	0.78	0.31	0.11	0.53
C 22:1	-	-	-	-	-	-	-	-	-
C 20:4	4.4	5.4	5.4	5.9	0.7	0.20	0.01	0.69	0.12
C 23:0	0.01	0.02	0.01	0.07	0.01	0.10	0.03	0.03	<0.01
C 22:2	0.12	0.24	0.19	0.22	0.07	0.68	0.64	0.45	0.20
C 20:5	0.27	0.74	0.33	1.16	0.10	0.01	<0.01	0.05	<0.01
C 24:0	0.06	0.11	0.06	0.05	0.02	0.15	0.16	0.10	0.51
C 24:1	0.34	0.26	0.42	0.34	0.08	0.24	0.04	0.97	0.56
C 22:6	0.14	0.38	0.14	0.49	0.06	0.32	0.31	0.31	-

[1]Largest SEM reported

Table 3.16. Summary of various fatty acid profiles of the phospholipids fatty acid fraction of polymorphonucleocytes obtained from dairy cows [14 multiparous (M) and 20 primiparous (P)] that received either the treatment of 10 kg of corn/hd per day prepartum and were fasted for 8 h on d +3 postpartum (TRT) or control of 400 mg of monensin/hd per day prepartum (CON) throughout the transition period.

	TRT		CON			*P* - value			
Fatty Acid (g / 100 g)	M	P	M	P	SEM[1]	TRT	TRT × Time	TRT × Parity	TRT × Parity × Time
Σ SFA[2]	45.0	44.4	44.3	42.0	1.3	0.17	0.33	0.47	0.15
Σ UFA[3]	55.0	55.6	55.7	58.0	1.3	0.17	0.33	0.47	0.15
Σ MUFA[4]	24.3	21.2	20.0	19.6	1.6	0.03	0.24	0.32	0.12
Σ PUFA[5]	30.7	34.4	35.8	38.5	2.6	0.05	0.24	0.82	0.21
Σ n-6[6]	28.2	31.9	33.9	34.6	2.4	0.06	0.24	0.49	0.12
Σ n-3[7]	2.5	2.5	1.9	3.8	0.6	0.46	0.82	0.03	0.22
n-6:n-3	19.1	14.4	20.7	10.9	1.3	0.44	0.42	0.04	<0.01
SFA:UFA	0.87	0.82	0.82	0.74	0.04	0.10	0.29	0.73	-

[1]Largest SEM reported

[2]SFA = saturated fatty acids

[3]UFA = unsaturated fatty acids

[4]MUFA = monounsaturated fatty acids

[5]PUFA = polyunsaturated fatty acids

[6]n-6 = omega-6 fatty acids that included C18:2 *trans*, C18:2 *cis*, C18:3n6, C20:2, C20:3n6, C20:4, and C22:2

[7]n-3 = omega-3 fatty acids that included C18:3n3, C20:3n3, C20:5 and C22:6

Table 3.17. Taqman® gene expression assay bovine primer/probe sets used for real-time polymerase chain reactions.

Gene[1]	Assay Identification	Accession Number
Peripheral Blood Mononuclear Cell		
ICAM	Bt03213910_g1	NM_174348.2
IL-1β	Bt03212745_m1	NM_174903.1
IL-6	Bt03211905_m1	NM_173923.2
TNF-α	Bt03272017_m1	NM_001101152.2
Polymorphonucleocyte		
SELL	Bt03223211_m1	NM_174182.1
Endogenous Control		
GAPDH	Bt03210913_g1	NM_001034034.2
RPS9	Bt03272017_m1	NM_001101152.2

[1]ICAM: intercellular adhesion molecule, IL-1β: interleukin-1β, IL-6: interleukin-6, TNF-α: tumor necrosis factor-α, SELL: L-selectin, GAPDH: glyceraldehyde 3-phophate dehydrogenase, RPS9: ribosomal protein S9

Table 3.18. Least square means of delta Ct values of intracellular adhesion molecule (ICAM), interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) of peripheral blood mononuclear cells obtained from dairy cows [14 multiparous (M) and 20 primiparous (P)] that received either the treatment of 10 kg of corn/hd per day prepartum and were fasted for 8 h on d +3 postpartum (TRT) or control of 400 mg of monensin/hd per day prepartum (CON) throughout the transition period.

	TRT		CON			*P* - value			
Gene	M	P	M	P	SEM[1]	TRT	TRT × Time	TRT × Parity	TRT × Parity × Time
ICAM	8.4	7.3	8.7	7.2	0.4	0.91	0.23	0.61	0.32
IL-1β	5.7	3.7	4.7	3.1	0.4	0.04	0.26	0.40	0.24
IL-6	13.6	11.8	13.3	11.5	0.6	0.55	0.16	0.96	0.41
TNF-α	4.8	3.6	4.3	3.5	0.4	0.39	0.16	0.56	0.33

[1]Largest SEM reported

Table 3.19. Least square means of delta Ct values of caspase-1 (CASP), interleukin-8 receptor (IL-8R), and L-selectin (SELL) of polymorphonulceocytes obtained from dairy cows [14 multiparous (M) and 20 primiparous (P)] that received either the treatment of 10 kg of corn/hd per day prepartum and were fasted for 8 h on d +3 postpartum (TRT) or control of 400 mg of monensin/hd per day prepartum (CON) throughout the transition period.

	TRT		CON			*P* - value			
Gene	M	P	M	P	SEM[1]	TRT	TRT × Time	TRT × Parity	TRT × Parity × Time
CASP	6.4	6.3	6.4	6.4	0.2	0.72	0.89	0.89	0.48
IL-8R	-0.23	-0.64	0.43	-0.48	0.42	0.28	0.20	0.51	0.68
SELL	0.76	0.54	0.91	0.56	0.18	0.58	0.52	0.67	0.23

[1]Largest SEM reported

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Appendix

University of Idaho
Animal Care and Use Committee

Date: Thursday, November 29, 2012
To: Pedram Rezamand
From: University of Idaho
Re: Protocol 2011-24
Effect of elevated lipid mobilization on fatty acid composition of blood and immune cells

Your requested amendment to the animal care and use protocol shown above was reviewed and approved by the University of Idaho on Thursday, November 29, 2012.

This protocol was originally submitted for review on: Thursday, December 16, 2010
The original approval date for this protocol is: Wednesday, January 19, 2011
This approval will remain in affect until: Friday, November 29, 2013
The protocol may be continued by annual updates until: Sunday, January 19, 2014

Comments

Your request to increase animal numbers to 40 head per pen due to space constraints at the dairy has been approved.

Federal laws and guidelines require that institutional animal care and use committees review ongoing projects annually. For the first two years after initial approval of the protocol you will be asked to submit an annual update form describing any changes in procedures or personnel. The committee may, at its discretion, extend approval for the project in yearly increments until the third anniversary of the original approval of the project. At that time, the protocol must be replaced by an entirely new submission.

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208-885-8958