EFFECTS OF THE TIMING OF INITIATION OF FAT SUPPLEMENTATION ON PRODUCTIVE AND REPRODUCTIVE RESPONSES OF PERIPARTURIENT DAIRY COWS DURING SUMMER

By

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This thesis is dedicated to my wonderful and patient husband, Gus, and to my mom who has always encouraged me to pursue my dreams.

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ABBREVIATIONS

AI	artificial insemination
ALK	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BW	body weight
BCS	body condition score
BHBA	β-hydroxy butyric acid
BUN	blood urea nitrogen
CSFA	calcium salts of fatty acids
CSLCFA	calcium salts of long chain fatty acids
CL	corpus leutem
CLA	conjugated linoleic acid
CV	coefficient of variation
DM	dry matter
DIM	days in milk
DMI	dry matter intake
EE	ether extract
FA	fatty acids
FCM	fat corrected milk
FSH	follicle stimulating hormone
GGT	gamma glutamyl transferase
GnRH	gonadatropin releasing hormone
LH	leutinizing hormone
LA	linoleic acid
LNA	linolenic acid
NEFA	nonesterified fatty acids
NFC	non fiber carbohydrates
$PGF_{2\alpha}$	prostaglandin $F_{2\alpha}$
PGFM	13, 14-dihydro-15-keto-$PGF_{2\alpha}$ metabolite
PHT	partially hydrogenated tallow
PUFA	polyunsaturated fatty acids
RIA	radioimmuno assay
RFM	retained fetal membranes
SCC	somatic cell count
TAG	triacylglycerol
TMR	total mixed ration
WCS	whole cottonseed

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Primiparous (n = 22) and multiparous (n = 25) Holstein cows were utilized in a completely randomized block design to determine the effects of timing of the initiation of feeding Ca salts of long chain fatty acids (**CSLCFA**) on cow performance the first 100 days in milk (**DIM**). Four treatments were as follows: control (no CSLCFA) and CSLCFA supplementation (2% of dietary DM) beginning at 28 d prior to expected calving date (CSLCFA prepartum), at 1 DIM, or at 28 DIM. Cows fed CSLCFA beginning in the prepartum period tended ($P > 0.05$ but ≤ 0.10) to produce more milk (42.2 vs. 37.1 kg/d) and had ($P \leq 0.05$) fewer small (2 to 5 mm) ovarian follicles during the first estrous synchronization using injection of gonadotropin-releasing hormone (44 ± 3 DIM) than cows fed CSLCFA initiated after calving. This milk increase was accompanied by a lower incidence of disease (mastitis, metritis, or retained fetal membranes) in the first 10 DIM (8 vs. 43%), elevated concentrations of triacylglycerol in

the liver dry matter (**DM**) at 14 ± 2 DIM (23.0 vs. 10.4%), lower expression of hepatic IGF binding protein-2 mRNA (0.08 vs. 0.12 arbitrary units/18S), and elevated concentrations of plasma bilirubin (0.36 vs. 0.23 mg/dl) compared to cows not fed CSLCFA prepartum. Expression of hepatic IGF-I and IGF-II mRNA did not differ among treatment groups. Multiparous cows appeared to benefit more from supplementation with CSLCFA than primiparous cows in that multiparous cows not fed CSLCFA at any time during the study had or tended to have lower concentrations of milk protein, a longer period and greater loss of body weight postpartum, greater concentrations of plasma beta-hydroxy butyric acid, and lower concentrations of plasma glucose, leptin, and IGF-I. In addition, multiparous cows fed fat prepartum tended to have fewer small (2 to 5 mm) and medium (6 to 9 mm) size but more larger size ovarian follicles (≥ 10 mm in size) at 21 DIM, more uterine tone at 21 DIM, a smaller cervical os at 28 DIM, and a slower decrease in plasma concentrations of 13, 14 dihydro-15 keto prostaglandin F-2α metabolite the first 10 d postpartum than multiparous cows not fed fat prepartum. Feeding CSLCFA in the prepartum period also benefited primiparous cows in that they tended to have lower concentrations of plasma fibrinogen. Initiation of fat-feeding at calving tended to result in fewer and smaller corpus luteum at 21 DIM compared to those fed CSLCFA prepartum. Estimated day of first ovulation postpartum based upon two consecutive days of progesterone exceeding 1.0 ng/ml did not differ among treatment groups (mean of 27 DIM). Multiparous cows fed CSLCFA had greater concentrations of plasma IGF-I at the time of artificial insemination and conception rate at first insemination tended to be better for all animals fed CSLCFA (58 vs. 27%) regardless of day of initiation of CSLCFA supplementation.

CHAPTER 1
INTRODUCTION

The transition from pregnancy to lactation in the dairy cow is a critical phase of the lactation cycle. The transition period, typically considered the time three weeks prepartum until three weeks postpartum, is marked by declining dry matter intake (**DMI**) as the cow approaches parturition and negative energy status in early lactation. Total energy intake in early lactation is usually less than what is required for maintenance and milk production (Staples et al., 1990); therefore the cow must mobilize adipose stores in the form of nonesterified fatty acids (**NEFA**) to help support lactation. Nonesterified fatty acids are removed from circulation by the liver. Once in the liver, NEFA are utilized for energy; however ruminants have reduced ability to export triglycerides from the liver and lipids can accumulate causing a metabolic disorder known as fatty liver (Drackley, 1999).

Supplemental fats can increase the energy density of the diet and reduce an energy deficit in early lactation. However milk production often increases when fats are added to the diet, resulting in no improvement of energy status. In addition, fat feeding can often result in a depression of DMI (Allen, 2000). The mechanisms by which supplemental fat depresses DMI are not apparent but could involve negative effects on ruminal fermentation and gut motility, release of gut hormones, oxidation of fat in the liver, and palatability of diets containing added fat (Allen, 2000). Feeding supplemental fat in the form of Ca salts of fatty acids (**CSFA**) makes the fat partially inert in the rumen and sometimes can prevent a depression in DMI. Feeding CSFA can allow specific fatty

acids (**FA**) to escape ruminal biohydrogenation which are then available in the small intestine for absorption and utilization. The modern dairy cow may be deficient in the essential FA, linoleic acid (**LA**) (Sanchez and Block, 2002). Providing LA in the form of CSFA may reduce a deficiency and act on target tissues in addition to increasing the energy density of the diet.

Supplemental fats have proven beneficial to reproductive efficiency of lactating dairy cows as well (Staples et al., 1998). Although reproductive performance is strongly associated with energy status (Staples et al., 1990), dietary fats can provide FA precursors for cholesterol and prostaglandin production, which have an affect on ovarian function, uterine function, and conception rates. Immune reactions have also been shown to be modulated by the diet including the FA composition by influencing cellular communication and activation through the synthesis of prostaglandins (Calder et al., 2002). Decreased incidence of disease in early lactation can result in increased milk production throughout the lactation and an increase in reproductive efficiency. The amount of a particular FA (e.g., LA) stored in the target tissue may control prostaglandin synthesis.

The modern dairy cow in the United States is most productive between the temperatures of 5 and 15°C and will begin to experience slight losses in milk production between 15 and 25°C (Hahn, 1985). Above 26°C, dramatic losses in production can occur although the humidity index will alter this upper critical temperature (Berman et al., 1985). In an attempt to dissipate additional body heat, the cow will increase her respiration rate, increase sweating rate, increase blood flow to skin, and decrease energy intake (Blackshaw and Blackshaw, 1994). Because fat has a low heat increment

associated with feeding, has a high energy density, and is utilized with high efficiency, it is an ideal feed additive during periods of heat stress. Reducing heat stress in lactating dairy cattle can increase milk production and reproductive efficiency. Heat stress can alter hormonal profiles and reduce the duration and intensity of estrus (Wolfenson et al., 1988). Using a large group of Isreali cows, Zeron and coworkers (2001) reported lowered conception rates in summer versus winter months which may have been due to differences in follicular dynamics and changes in the biological membranes. In the winter, more follicles per ovary and embryo development to the blastocyst stage, the two- to four-cell stage, and the morula stage were improved compared to summer months. In addition, the proportion of FA in the phospholipids of oocytes, granulosa cells, and follicular fluid was increased in the summer whereas the proportion of polyunsaturated FA in the phospholipids of oocytes and granulosa cells was increased in the winter. A study examining pregnancy rates in Florida and Georgia herds using DHIA records reported that high milk production exacerbated the already great drop in non-return rates during the summer months (Al-Katanani et al., 1999).

The objective of this thesis was to evaluate the timing of initiation of Ca salts of long chain FA on the production, plasma hormones and metabolites, and reproduction of periparturient Holstein cows in summer.

CHAPTER 2
LITERATURE REVIEW

Fatty Acids Defined

Linoleic acid (**LA**), the major fatty acid (**FA**) in most oilseeds, and linolenic acid (**LNA**), the major FA in fresh forages, are considered essential FA because they cannot be synthesized by mammals or ruminal microorganisms. They lack the Δ 12 and Δ 15 desaturase enzymes to convert oleic acid to LA and LNA. Both LA and LNA are polyunsaturated FA (**PUFA**) in that they have more than one point of unsaturation, or double bond. The shorthand notation system for identifying FA includes a description of the number of carbons present within the chain, the number of double bonds, and the location and configuration of the double bonds. In addition, the ω-numbering system begins numbering carbons starting at the methyl end of the FA. For example, LA is notated as C18:2, ω-6 because it has 18 carbons, two double bonds, and the first double bond is at the sixth carbon from the methyl end. The LNA is designated C18:3, ω-3. Lastly, FA can be classified in the ω-9 category such as oleic acid, C18:1. Omega families cannot be interconverted.

Fat supplementation is commonly used to increase the energy density of the diet of lactating dairy cows. During the first few weeks of lactation, dairy cows are limited by nutrient intake by which to meet the demands of lactation. Early postpartum dry matter intake (**DMI**) is limited by ruminal fill and does not peak until 10 to 14 wk postpartum, while milk production usually peaks at 4 to 8 wk postpartum (National Research Council [NRC], 2001).

Feeding diets with 3 to 5% added fat can increase energy intake without having a major negative effect on fiber digestion or milk fat (Palmquist and Jenkins, 1980). Supplying energy in the form of fat in place of carbohydrate can reduce microbial protein production since carbohydrates are the primary energy source for ruminal microbes. On the other hand, increasing the energy concentration of the diet by increasing the starch to an excessive concentration can have detrimental effects on digestion and animal health.

Feeding excessive unsaturated FA, which have a toxic effect on ruminal microbes, may lead to depressed fiber digestion and milk fat production (Brooks et al., 1954; Jenkins and Palmquist, 1984). Manufacturing Ca salts of fatty acids (**CSFA**) can make them partially inert in the rumen and can increase the energy density of the diet without hindering forage digestion (Jenkins and Palmquist, 1984). Preformed soaps of FA should not dissociate in the rumen, but dissociate in the abomasum at a low pH. The FA are then available in the small intestine for absorption and utilization by the body (Jenkins and Palmquist, 1984).

The studies reviewed in this thesis have been limited to those in which cows were fed the same experimental diets for the duration of the study. Important aspects of each study are summarized in Table 2.1 (ruminally inert fats), Table 2.2 (oilseeds) and Table 2.3 (rendered fats).

Effects of Supplemental Fat on Feed Intake and Production

Dry matter intake is often affected by the addition of fat to the diet (Allen, 2000; Jerred et al., 1990) The type of fat fed as well as the type and amount of forage will have an effect on the extent to which DMI is affected (Allen, 2000). Although the mechanisms are unclear, intake may be depressed when supplemental fats are fed due to decreased palatability, ruminal fill due to inhibition of fiber digestion, the metabolic

regulation of the gut hormone cholecystokinin on the brain satiety centers, and an increased rate of FA oxidation in the liver that can alter signals generated by hepatic vagal afferent nerves to brain centers signaling satiety (Allen, 2000). In addition, PUFA that reach the small intestine may decrease gut motility which could decrease DMI (Drackley et al., 1992). In a review of several studies, Allen (2000) reported that there was a linear decline in DMI such that for every 1% inclusion of tallow or CSFA in the diet there was a reduction of DMI of 1.2 and 2.5% respectively. Allen also reported that oilseeds negatively affected intake, although in a quadratic fashion with 2% of dietary FA coming from oilseeds resulting in maximum intake suppression.

In this review of studies where CSFA were fed to the same animals throughout the study (Table 2.1), the majority of experiments report no effect on DMI when CSFA were fed at 1.8 to 5% of dietary dry matter (**DM)** (Atwal et al., 1990; Chouinard et al., 1997; Erickson et al., 1992; Firkins and Eastridge, 1992; Holter et al., 1992; Moallem et al., 2000; Palmquist and Weiss, 1994; Schroeder et al., 2003; Skaar et al., 1989; Spicer et al., 1993). However, several studies reported a depression in DMI when cows were fed CSFA in diets in which alfalfa (silage or hay) was the sole forage source (Chouinard et al., 1997; Harrison et al., 1995; Jerred et al., 1990; Simas et al., 1995). Interestingly, a few studies reported DMI was depressed early in the experiment; however the effect disappeared after the cows consumed the diets for a longer period of time (Beam and Butler, 1998; Chouinard et al., 1997; Garcia-Bojalil et al., 1998), suggesting that there may be an adaptation period.

Depression of DMI was less commonly reported when cows were fed oilseeds as a fat source throughout the duration of the study. In two studies by Harrison and

coworkers (1995), intake was depressed when early lactation cows consumed diets of 12% whole cottonseed (**WCS**) and 2.7 or 5% of DM as CSFA (Table 2.2). In one of those studies, DMI was depressed when a diet of 12% WCS without CSFA was fed. When high amounts of whole soybeans (18% of dietary DM and 6.2% dietary ether extract (**EE**)) were fed in a diet containing 30% corn silage and 20% alfalfa silage, DMI was depressed 2.3 kg/d (9.7% of DMI) in comparison to controls (3.2% dietary EE). However the feeding of soybeans in the ground roasted form (18% of dietary DM) did not affect DMI (Pires et al., 1996). The roasting process may reduce exposure of the oil to the rumen microbes and therefore reduce the negative influence on rumen function. Escape of LA to the small intestine was increased in cows fed roasted versus raw soybeans (Dhiman et al., 1995; Tice et al., 1994). Similarly, the addition of rolled safflower seeds at 10% of dietary DM in combination with bST injections (5.5% dietary EE) depressed DMI by 4.6 kg/d (19.2% of DMI) in comparison to control cows receiving bST injections (2.3% dietary EE) in a diet of 25% corn silage and 25% alfalfa hay (DM basis). However, the replacement of safflower seeds with rolled sunflower seeds (6.2% dietary EE) in combination with bST injections did not affect DMI (Stegeman et al., 1992).

In this review of studies where rendered fats were fed to the same animals throughout the experiment, only two studies reported a suppression in DMI in comparison to the control or similar treatment without fat (Table 2.3). Bateman and coworkers (1996) reported a depression of 2.6 kg/d (10% of DMI) when tallow (2% of dietary DM) was fed in a 33% NDF diet in the winter (3.5 and 5.4% dietary EE for fat-supplemented and control diets, respectively) . However, tallow feeding had no effect on

DMI when included in the summer or in a diet with 40% NDF. Lastly, when lactating cows were fed diets of 0 or 3% tallow (DM basis) in a ration comprised of 33% alfalfa haylage and 17% corn silage, DMI was depressed 0.9 kg/d (3.6% of DMI) and 1.3 kg/d (5.4% of DMI) with the addition of 0 or 5% escape protein supplement, respectively (Son et al., 1996).

Changes in milk production can accompany changes in DMI. Often, milk production is increased due to increased energy intake when supplemental fats are fed, particularly in early lactation when the dairy cow must depend on her body reserves to help satisfy energy requirements for maintenance and lactation. Of 28 studies in which ruminal inert fat was fed to cows in continuously applied treatments, eleven studies did not report an increase in production of milk or fat-corrected milk (**FCM**) (Table 2.1). Reasons for the discrepancies between studies may be due in part to the day postpartum that fat feeding was initiated, the duration of the study, feed quality, amount of milk production, and combinations of these factors. In one study that did not report an increase in milk production, cows were supplemented CSFA while on pasture (Schroeder et al., 2003). In another study that did not report an increase in milk production, a 0.45 kg drench of CSFA was administered for only 4 d postpartum (Pickett et al., 2003). Cervantes and coworkers (1996) did not report an increase in milk production when feeding 0.4 kg/d of CSFA but the cows were in midlactation (average of 112 DIM) and were only fed diets for 38 d. Additionally, two studies that did not report an increase in milk production were already feeding high concentrate diets (> 61% of dietary DM) that included > 8.5% WCS and the additional energy provided by dietary fat may not have been beneficial (Simas et al., 1995; Spicer et al., 1993).

Similarly, in 11 of 17 studies in which oilseeds were fed to the same animals throughout the experiment, milk production or FCM increased (Table 2.2). Two studies that did not report an increase in milk production were already feeding high concentrate diets (> 60% of dietary DM) (Khorasani et al., 1991; Markus et al., 1996). Another study reported DMI depression of 9.7% when whole soybeans were fed, which may have contributed to the unaffected milk production (Pires et al., 1996).

When rendered fats were fed in continuous studies, six of 13 studies reported an increase in milk production or FCM yield (Table 2.3), and interestingly there was no suppression in DMI. Reasons for this variation in response across studies were not apparent.

Milk protein concentration was commonly reported to decline when ruminally inert fats or oilseed were fed (Tables 2.1 and 2.2) but less often when rendered fats were fed (Table 2.3). Although the mechanism is unclear, it could be due, in part, to less glucogenic precursors being consumed as starch is replaced with lipid. Less glucogenic precursors present in the diet is associated with decreased milk protein concentration (Rigout et al., 2003).

The response of milk fat concentration to supplemental fat seems to be dependent upon many factors. About 50% of the fat found in milk is synthesized in the mammary gland from acetate and butyrate, while the other 50% comes directly from fat absorbed from the blood (Ackers, 2002). Palmquist and coworkers (1993) published an equation predicting an increase in milk fat concentration of 0.18% from feeding an additional 0.5 kg of fat daily. Conversely, milk fat depression is often seen when fat is fed in diets in which corn silage was the sole forage source, resulting in a more acidic ruminal

environment than when fed in combination with alfalfa hay or haylage which has a greater buffering capacity (Onetti et al., 2004; Ruppert et al., 2003; Smith et al., 1993). This is thought to occur when PUFA are biohydrogenated in the rumen under acidic conditions to *trans* C18:1 FA rather than to C18:0. The enzymes responsible in the mammary gland to synthesize the short and medium chain fatty acids found in milk may be inhibited by these *trans* C18:1 FA, especially *trans*-10 C18:1, resulting in decreased milk fat concentration (Bauman and Griinari, 2003). In this review of studies in which supplemental fat was fed to the same cows throughout the experiment, an increase in milk fat concentration was more consistent when cows were fed CSFA in comparison to oilseeds or rendered fats.

Accompanying an increase in milk production due to fat supplementation may be an increase in 4% FCM yield (Erickson et al., 1992; Moallem et al., 2000; Salado et al., 2004). However, if milk fat concentration is unchanged or is depressed by fat supplementation, yield of 4% FCM may not significantly increase despite an increase in milk production (AbuGhazaleh et al., 2004; Pantoja et al., 1996; Selberg et al., 2004).

Timing of Initiation of Fat Feeding

Few studies in the literature examine when to start feeding fat during the periparturient period. If fat supplementation is begun in the dry period, benefits can be expected due to the adjustment of the animal's palate and ruminal microflora before the onset of the lactation. Skaar and workers (1989) fed 40 multiparous cows diets of 0 or 5% prilled fat containing 50% forage (equal amounts of corn silage and alfalfa silage, DM basis) beginning at 17 d before expected calving date and continued through 105 d in milk (**DIM**). Intake did not differ between fat-fed and control cows yet overall milk production tended to increase for cows fed fat. During the cool season, milk production

did not differ between treatments, however, during the warm season milk yield increased from 34.5 to 44.3 kg/d. Although cow performance was not negatively affected, liver biopsies revealed that cows fed fat beginning prepartum tended to have increased total hepatic lipid concentrations in comparison to controls (27.5 vs. 26.1%, DM basis at 1 DIM and 29 vs. 24%, DM basis at 5 weeks postpartum). Plasma nonesterified fatty acid (**NEFA**) concentrations did not differ between treatments indicating that the additional lipid in the liver may have been from dietary origin.

Waiting until calving to start feeding fat is another approach often tried because of the extra costs associated with fat supplementation. University of Florida workers (Garcia-Bojalil, et al., 1998) fed multiparous cows corn silage/alfalfa hay-based diets containing 0 or 2.2% calcium salts of palm oil beginning at calving and continuing through 120 DIM. Fat-fed cows began to produce more milk (~2 kg/d) after 3 weeks of supplementation and continued to produce more milk throughout the experiment.

Allowing cows to get through the period of negative energy balance before adding fat to the diet is one more strategy employed. Schingoethe and Casper (1991) summarized five studies in which whole sunflower or extruded soybeans were fed beginning at 4 weeks and continued through 16 weeks of lactation. Cows produced an average of 0.9 kg/d more milk while consuming the seeds, however, there was still a 3 to 4 week delay before a milk response was evident.

Salfer and coworkers in Minnesota (1995) evaluated when to initiate the feeding of partially hydrogenated tallow (**PHT**). Sixty-three animals were assigned to treatment at 14 d before expected calving date and remained on the same treatment until 151 DIM. Four dietary treatments were the following: 1) no PHT prepartum or postpartum, 2) 1%

PHT prepartum and 2% PHT postpartum, 3) 0% PHT prepartum and 2% PHT postpartum, and 4) 0% PHT prepartum and 2% PHT beginning at 35 DIM. Dry matter intake and milk production did not differ among treatments. When evaluating the first 35 d of lactation alone, cows fed fat beginning at calving had a 3.5 kg/d advantage of 3.5% FCM yield in comparison to cows fed fat beginning in the prepartum period, due in part to differences in the concentration of milk fat between the two treatments. Plasma NEFA concentrations, days to first estrus, pregnancy rate, days open, and incidence of disease did not differ among treatments.

Effects of Supplemental Fat on Adipose Tissue Lypolysis

When the energy needed for maintenance and lactation is greater than the energy provided in the diet, the dairy cow will begin to mobilize her body fat stores to lessen the energy deficit. Nonesterified fatty acids are released into the blood by adipose tissue and transported to hepatic and non-hepatic tissues. Once in the liver, NEFA have the three following fates: 1) oxidized to carbon dioxide to provide energy, 2) partially oxidized for energy but also producing ketone bodies such as β-hydroxy butyric acid (BHBA) that serve as fuel for other tissues, or 3) reconverted to triglycerides and stored (Drackley, 1999). When fat is added to the diet, plasma concentrations of NEFA routinely increase (Chilliard, 1993; Drackley, 1999; Grummer and Carroll, 1991). In a review of 50 treatment comparisons, Chilliard (1993) reported an average increase in concentration of plasma NEFA of 41 μM ($P < 0.005$) over controls when supplemental fat was fed. Likewise, Drackley (1999) reported an average increase in concentration of plasma NEFA of 81 μM over controls when supplemental fat was fed in reviewing seven studies. This increase due to dietary fat is much less than what is typically observed during the

transition period when NEFA concentrations may increase up to 1 mM or more (Grummer, 1993).

When NEFA concentrations in blood are high, as during the periparturient period, removal of NEFA by the liver may exceed the oxidative capacity of the liver and, when in combination with the low rate of export of triacylglycerol (TAG) out of the liver in ruminants, TAG accumulates in the liver (Grummer, 1993). Mechanisms to decrease hepatic lipid accumulation in cows fed fat might include decreased hepatic uptake of NEFA, increased oxidation of NEFA in the liver, decreased hepatic esterification of NEFA, or increased export of TAG from the liver (Grum et al., 1996).

Illinois workers (Grum et al., 1996) conducted a study in which cows were fed a control diet (80% oat hay, DM basis), a high concentrate diet (51% oat hay, DM basis), or a high fat diet (6.5% of DM as EE) for 50 d prepartum and a common diet postpartum. Cows fed fat during the prepartum period tended to have decreased plasma NEFA concentrations early postpartum and had decreased liver TAG concentrations at d 1 of lactation. They also reported a positive correlation between concentrations of plasma NEFA at 3 d prepartum and the concentration of TAG in the liver at 1 d postpartum. However, a similar study at Illinois (Douglas et al., 2004) in which cows were fed a moderate non-fiber carbohydrate (NFC) control diet, a low NFC diet with 4% choice white grease (Qual-Fat®) prepartum (DM basis), and a moderate NFC diet with 4% choice white grease (DM basis) beginning at 60 d prepartum revealed no treatment differences in DMI, milk production, plasma concentration of NEFA, or total hepatic lipid or TAG at 1 DIM.

During the periparturient period, the adipose tissue transfers from an anabolic state to a catabolic state. Tissue mobilization increases because the nutrient demands of the fetus and placenta are high and DMI declines during the last weeks of pregnancy (Grummer, 1993). Endocrine secretions have a major impact on lipogenesis and lipolysis. Insulin increases glucose uptake by the cell membrane and increases lipogenic enzymes to stimulate FA and TAG synthesis. Staples et al. (1998) reviewed 17 studies that reported insulin concentrations from fat-supplemented cows and found mixed results. In 8 studies, concentration of plasma insulin of cows fed fat were significantly depressed in comparison to controls, however differences were eliminated when adjusted for energy status of the animals.

Leptin is a hormone synthesized by adipose tissue that is positively regulated by adiposity and negatively by undernutrition. High concentrations of plasma leptin are associated with decreased feed intake and increased energy expenditure. Low concentrations of plasma leptin are associated with increased appetite and energy conservation. Concentrations of leptin are decreased around the time of parturition in concurrence with negative energy balance and a reduction in adipose stores and may be mediated by the reduction in plasma insulin (Block et al., 2003). Circulating concentrations of plasma leptin were not different between beef heifers fed a basal diet or diets with supplemental fat (4% corn oil or 2% Ca salts of conjugated LA (**CLA**), DM basis) for 32 or 60 d before slaughter (Gillis et al., 2004). However, leptin concentrations in adipose tissue were greater for heifers supplemented with 4% corn oil versus heifers fed the basal diet or 2% Ca salts of CLA (0.28, 0.18, and 0.17 μg/μg protein, respectively). Likewise, concentration of plasma leptin of late lactation dairy cattle

abomasally infused with cis-9, trans-11 or trans-10, cis-12 CLA isomers were not different (Baumgard et al., 2002).

Effects of Supplemental Fat on Immune Function

In response to an activated immune system, the liver will produce acute phase proteins such as ceruloplasmin, fibrinogen, and haptoglobin (Baumann and Gauldie, 1994). Haptoglobin is responsible for preventing the loss of body iron and concentrations are normally undetectable in bovine blood unless there is tissue damage. Ceruloplasmin is involved with copper transport and concentrations will increase due to an inflammatory response of the cow. Fibrinogen is involved with blood clotting and the formation of the fibrin matrix for tissue repair. Increased fibrinogen concentrations are detected during internal hemorrhage or tissue damage. Arthington and coworkers (2003) looked at the response of newly weaned beef calves to the stresses of transportation and co-mingling and reported increased concentrations of plasma fibrinogen, ceruloplasmin, and haptoglobin.

Immune reactions have been shown to be modulated by the diet, including the PUFA composition of the diet (Calder et al., 2002). Mechanisms involved in regulation are not yet understood, but evidence exists that PUFA composition of the diet influences cellular communication and activation through the synthesis of prostaglandins, tumor necrosis factor-α, and interferon-γ (Calder et al., 2002). Linoleic acid can be converted to arachidonic acid, the precursor for prostaglandin E_2 and leukotriene B_4 which are pro-inflammatory mediators. Similarly, LNA can be converted to eicosapentaenoic acid, the precursor for the synthesis of the inflammatory mediators prostaglandin E_3 and leukotriene B_5. Lessard et al. (2004) evaluated cellular immune functions of dairy cows fed supplemental fat during the transition period. Cows were fed diets of 2.7% Ca salts

of palm oil (Megalac), 5.9% flaxseed (n-3 FA) or 9.4% micronized soybeans (n-6 FA) from 6 wk prepartum to calving. From calving to 6 wk postpartum, cows were fed diets of 4.7% Megalac, 9.7% flaxseed, or 20.3% micronized soybeans. Serum antibody response to ovalbumin injections during the prepartum period did not differ among treatments. The lymphocyte response of blood mononuclear cells to mitogenic stimulation was lower in cows fed soybeans than in those receiving flaxseed or Megalac. The authors concluded that cellular immune functions were modulated around parturition; however feeding diets rich in n-3 or n-6 FA did not have a major impact on cellular immune function.

Immediately postpartum up to 90% of cows develop mild endometritis (Lewis, 1997). It is suggested by many researchers that a compromised immune system involving reduced effectiveness of neutrophilic movement and pathogen destruction may be the reason that some cows spontaneously recover while others develop severe uterine infections that reduce fertility (Lewis, 1997). Evaluation of cervical discharge using vaginoscopy can be used as a diagnosis tool of uterine bacterial infection (Dohmen et al., 1995). Clinical endometritis as described by a purulent or foul discharge after 20 d postpartum or a mucopurulent discharge after 26 d postpartum was associated with a reduction of pregnancy rates (LeBlanc et al., 2002). Abnormal vaginal discharge has been correlated with a delay in the first postpartum ovulation (Opsomer et al., 2000). Furthermore, if first ovulation occurs in the presence of a uterus with heavy contamination, it can lead to prolonged luteal phases which is also associated with lower fertility (Opsomer et al., 2000).

Effects of Supplemental Fat on Reproduction

A Historical Review

It is well established in nonruminants that animals with essential FA deficiencies have poor skin and hair, low growth rates, and reduced reproductive performance. In early studies, the essentiality of LA was documented primarily in nonruminants by causing then curing symptoms of deficiency. In the late 1920's Burr and Burr (1929) established that dietary fat was essential to the growing rat. After 70 d on purified diets, rats without lipid in the diet experienced dandruff, hair loss, cessation of growth, abnormal kidneys, blood in the urine, prolapsed penis, and irregular ovulation. All animals died after 120 to 230 d without dietary fat. The following year, Burr and Burr (1930) sought to identify specific FA responsible for normal reproductive function in rats. Growing rats were fed lipid-free diets and upon weight loss, individual lipid sources were supplemented at 1% of the diet. Of 22 females fed fat-free diets, 13 were not cycling or cycling irregularly. When three ovulatory rats that had been fed fat-free diets were bred, two produced litters; however no young lived more than a few hours. When four of the nonovulatory females were given five drops of either corn (41% LA), olive (7% LA), linseed (59% LA), or coconut (1% LA) oil daily, all resumed ovulation except for the rat supplemented with coconut oil. Six of the irregularly ovulating females fed fat-free diets were fed two drops of cod liver oil daily. After 4 wk all rats were cycling normally, were bred and produced normal litters. In response to their results, the authors stated, "…the resumption of ovulation is so rapid that growth has hardly begun. Synthesis of ovarian hormone ceases when fatty acids are eliminated from the diet." It was many years later that FA deficiency was studied in farm animals. In 1954 it was determined that the preweaned calf also requires FA (Lambert et al., 1954). Preruminant

calves were fed isocaloric, purified diets of synthetic milk devoid of lipid with or without hydrogenated soybean oil and lecithin. Calves fed diets without lipid developed symptoms of deficiency including scaly dandruff, long dry hair, hair loss, diarrhea, and low weight gain. Additionally, guinea pigs fed a basal fat-free diet for eight months had an average weight of 254 ± 28 g, incidence of dermatitis was 75% and mortality rate was 25% while guinea pigs raised on the same diet and supplemented with 0.4% methyl linoleate (1.31% of calories) had an average weight of 382 ± 15 g and had no dermatitis or mortality (Ried et al., 1964). Lastly, growing male pigs were fed 0, 0.25, 0.5, 1.0, 2.0, and 4.0% of dietary calories as LA using corn oil for ten wk (Sewell and McDowell, 1966). Concentration of LA in scrotal fat reflected dietary intake after five and ten wk on diet. Skin lesions appeared on pigs receiving 0, 0.25, and 0.5% of dietary calories as LA and they were then supplemented with methyl linoleate or methyl oleate at 1.0% of dietary calories. Skin lesion disappeared in pigs supplemented with methyl linoleate and remained on those supplemented with methyl oleate.

It has been documented in nonruminants, including the rat (Holtman, 1960), the guinea pig (Ried et al., 1964), and the pig (Sewell and McDowell, 1966) that a ratio of C20:3 to C20:4 in tissues/serum that exceeds 0.4 is indicative of a LA deficiency. The rational behind this ratio as an indicator of LA deficiency is that the synthesis of C20:3 n-9 from oleic acid increases when LA is deficient because of enzyme competition.

The Modern Dairy Cow

Quantification of LA available for use by the adult ruminant is difficult to predict because of ruminal biohydrogenation. The extent of ruminal biohydrogenation is variable and dependent upon many factors including the diet and ruminal conditions, however it is estimated by Chilliard et al. (2000) that 80% of dietary LA is

biohydrogenated. Feeding ruminally inert fats, such as CSFA, will partially protect FA from biohydrogenation, allowing greater escape to the small intestine.

Using the fat sub-model of the Cornell-Penn-Miner (CPM)-Dairy model, Sanchez and Block (2002) suggested that the amount of LA excreted in 45.5 kg of milk daily exceeds the post ruminal uptake from typical diets. Calculation of LA balance of the lactating dairy cow would be the following: LA absorbed from the diet – LA used for maintenance – LA used for milk production. Using the fat sub-model of the CPM-Dairy model, a cow consuming 25 kg of DM/d of a typical diet (no added fat source) would consume 225 g/d LA. Of the 225 g consumed, only 20% will escape biohydrogenation (45 g), and of that, 82% (37 g) will be absorbed in the small intestine. The LA requirement for maintenance of the mature lactating ruminant has not been defined. However, a calculation based on metabolic body weight using the nonlactating rat (Mattos and Palmquist, 1977) yields a maintenance requirement of 10.7 g/d for a cow weighing 607 kg. Milk output of LA of a cow producing 45 kg milk/d would be 54 g/d (3.4% milk fat containing 3.5% LA). In this situation, the LA balance would be -27.7 g/d (37 g/d absorbed from the diet – 10.7 g/d for maintenance – 54 g/d for milk production). To get this animal out of a deficient situation, a fat rich in LA must be supplemented. A possible explanation as to why numerous studies report an improvement in reproduction when additional fat is fed may be due to alleviating a LA deficiency of the modern high-producing dairy cow.

Many studies report an improvement in reproductive performance of cows fed supplemental fat. In a review, Staples et al. (1998) reported an improvement in fertility rates in 11 of 20 articles and speculated that it was a result of dietary FA and not solely

due to an improvement in the energy status of the cows. Although reproductive performance is strongly associated with energy status (Staples et al., 1990), dietary fats can provide FA precursors for steroid (including cholesterol) and eicosanoid (including prostaglandins) production which have an affect on ovarian function, uterine function, and conception rates.

Dietary fats typically increase concentrations of circulating cholesterol, the precursor of progesterone (Grummer and Carroll, 1991). Ruminants fed supplemental fat often have a slight increase in blood progesterone concentration (Staples et al., 1998). Progesterone, secreted by the corpus luteum (**CL**), prepares the uterus for implantation of the embryo and helps maintain pregnancy by providing nourishment for the conceptus via induction of heterotrophic proteins from the endometrium. Work by Hawkins et al. (1995) suggests that the increase seen in circulating progesterone when cows are fed supplemental fat is from a reduced rate of clearance of progesterone rather than an increase in progesterone synthesis. Son et al. (1996) reported greater blood cholesterol and peak plasma progesterone concentration during the second ovulatory cycle in cows fed tallow at 2 vs. 0% of dietary DM. Workers at the University of Florida (Garcia-Bojalil et al., 1998) reported that accumulated plasma progesterone from 0 to 50 DIM was greater, pregnancy rates improved, and energy status did not change when cows were fed diets of 2.2% CSFA compared to non fat-supplemented cows.

Through a series of desaturases and elongases, LA (C18:2) can form dihomo-λ-linolenic acid, a direct precursor to the series 1 prostaglandins, or can be further desaturated to arachidonic acid (C20:4), a direct precursor to the 2 series prostaglandins. Prostaglandin $F_{2\alpha}$ (**$PGF_{2\alpha}$**), synthesized by endometrial tissue, is an important regulator

of parturition and the estrous cycle by causing regression of the CL. Upon conception, it is important to keep the CL from regressing in order to prevent early embryonic death. Immediately postpartum, 13, 14-dihydro-15-keto-$PGF_{2\alpha}$ metabolite (**PGFM**) is important in regressing the CL of pregnancy. If LA is supplemented in the diet prepartum, more arachidonic acid may be synthesized leading to higher concentrations of the series 2 prostaglandins and possibly a healthier uterine environment. Alternately, if excess LA is consumed, it can be converted to eicosadienoic acid (C20:2) instead of arachidonic acid (Kanduce et al., 1982), increasing the synthesis of the series 3 prostaglandins at the expense of the series 1 and 2 prostaglandins. It is thought that LA can compete with arachidonic acid for binding sites of a key enzyme, cyclooxygenase 2 (PGHS-2), that is necessary for the synthesis of $PGF_{2\alpha}$. The amount of a particular FA (e.g. LA) stored in the target tissue may control whether there is an inhibition or stimulation of prostaglandin synthesis. Reducing $PGF_{2\alpha}$ secretion through dietary fats could improve pregnancy rates by reducing early embryonic loss around the time of embryo recognition.

Plasma IGF-I concentrations are correlated positively with body condition and DMI. Low IGF-I concentrations are associated with an extended postpartum interval to estrus in beef cows and also with delayed puberty (Roberts et al., 1997; Rutter et al., 1989), indicating that IGF-I can be positively correlated with reproductive performance. Beam and Butler (1998) reported lower mean concentrations of plasma IGF-I from wk 1 to 3 postpartum in cows fed a diet containing 2.6% prilled fat compared to controls (37.6 vs. 47.7 ng/ml) despite no differences in energy balance. However, other studies reported no differences in concentration of plasma IGF-I when supplemental fat was fed (Salado et al., 2004; Spicer et al., 1993). Insulin-like growth factor I acts synergistically with

luteinizing hormone (**LH**) to promote follicular development (Lucy, 2001). However, if IGF-I is over stimulated there may be deleterious effects on embryo development, the uterine environment, and gene expression (Bilby et al., 2004). More specifically, overstimulation of IGF-I is detrimental to follicle and oocyte development (Armstrong et al., 2001).

Although the results are somewhat mixed, improvement in conception rates when fat is supplemented in the diet is often reported. In a study conducted in Wisconsin (Scott et al., 1995), five herds (n = 443) were fed CSFA at 0 or 450 g/d from 1 to180 or 200 DIM. They reported an increase in overall conception rate from 93 to 98%, and a tendency for more fat-supplemented cows to exhibit standing estrus (71.4 vs. 65.6%). In addition, they reported a tendency for less incidence of noncyclic ovaries in fat-fed cows. A study conducted in Pennsylvania and Israel by Ferguson et al. (1990) reported an improvement in first service conception rate when 253 cows over four herds were fed 0 or 2% ruminally inert fat from 0 to150 DIM (43 vs. 59%). Multiparous Holstein cows (n = 81) were fed isoenergetic diets containing 1.7% supplemental fat (prilled long chain FA) for 21 d prepartum and control or glucogenic-supplemented diets for 28 d postpartum (Frajblat and Butler, 2003). Fat supplementation prepartum did not affect follicle dynamics measured by ultrasonagraphy nor concentration of plasma progesterone, insulin, IGF-I, or NEFA. However, supplemental fat prepartum was associated with better pregnancy rates (86 vs. 58% for fat-supplemented and control cows respectively, P = 0.03). Recently, workers in Missouri (Oelrichs et al., 2004) reported no benefit for conception rates of Holstein cows (n = 64) fed raw, cracked soybeans beginning at 28 d prepartum or beginning at calving (fed at 1.9 and 2.9 kg of DM during

the prepartum and postpartum periods, respectively) despite an improvement in energy balance. Concentrations of plasma progesterone and PGFM, interval to first estrous cycle, and rates of cyclicity, ovulation, conception and pregnancy were not different from cows not fed soybeans. However, cows fed soybeans beginning either prepartum or at calving had fewer small (< 5 mm) follicles and tended to have more medium (6 to 9 mm) follicles than controls during the first synchronized estrous cycle. The high LNA and LA content in flaxseeds (57% LNA and 14% LA) may have been responsible for the improvement in conception rates (87.5 vs. 50.0%) of lactating dairy cows fed formaldehyde-treated whole flaxseed (17% of dietary DM) compared to those fed Ca salts of palm oil (5.6% of dietary DM) from 9 to 19 wk postpartum (Petit et al., 2001).

To investigate the theory that specific FA (e.g., LA) reaching target tissues could improve conception rates, Santos and coworkers (2004) supplemented dairy cows with a ruminally inert blend of LA and monoenoic *trans* FA or a Ca salt of palm oil from 25 d prepartum through ~55 d postpartum when cows were timed AI, then flushed 5 d after AI and recovered structures were evaluated. Cows fed the LA and monoenoic *trans* FA tended to have (P = 0.11) a greater fertilization rate (87 vs. 73%), had more accessory sperm per structure collected (34 vs. 21), and tended to have (P = 0.06) a greater proportion of embryos classified as high quality (73 vs. 51%). In an accompanying study, conception rate at first AI was greater for cows fed the blend of LA and monoenoic *trans* FA salt (38.9 vs. 25.9%).

In contrast, a few studies have reported a significant decrease in conception rates of cows fed supplemental fat. In reviewing three studies that reported decreased conception rates, Staples et al. (1998) noted that in all studies there was a dramatic improvement in

milk production. High milk production and negative energy balance have been linked to decreased fertility in dairy cattle.

Effects of Supplemental Fat on Follicle Development

In addition to an improvement in conception rates of lactating dairy cows, follicular development is improved often by fat feeding. Cows in negative energy balance or in poor body condition can experience reduced ovarian activity (Staples et al., 1990) which might be alleviated quicker by supplementing with fats. The mechanism by which ovarian activity is affected by energy status is likely at the hypothalamus-pituitary axis and perhaps at the ovary itself. Leutinizing hormone and follicle stimulating hormone (**FSH**) are secreted by the anterior pituitary gland upon stimulation by gonadotropin releasing hormone (**GnRH**) released from the hypothalamus to cause recruitment and growth of ovarian follicles. In early lactation and during the state of negative energy balance, ovarian activity is reduced by low pulsatile secretion of LH (Beam and Butler, 1999).

Simmetal cows (n = 12) were assigned to receive CSFA (0.5% of body weight) or an isocaloric control supplement in addition to prairie hay from parturition until the second postpartum ovulation. Calves were permanently removed at 25 d postpartum to assist with a quicker return to estrus. Concentrations of mean serum LH and total cholesterol for fat-supplemented cows was greater than for control animals. In addition, follicular development as determined by ultrasonography was affected in that growth of class 2 (6 to 9 mm) follicles into class 3 (10 to 15 mm) and 4 (> 15 mm) follicles was enhanced in cows receiving CSFA (Hightshoe et al., 1991).

At parturition, 18 cows were assigned to receive CSFA at 0 or 2.2% of dietary DM in a TMR containing 14.5% whole cottonseeds until 60 d postpartum (Lucy et al., 1991).

Prior to 25 d postpartum, CSFA-supplemented cows had a decreased number of 3 to 5 mm follicles and an increased number of 6 to 9 mm follicles. After d 25 postpartum, estrous was synchronized. The number of 3 to 5 mm follicles and follicles > 25 mm increased in CSFA-fed cows. In addition, the diameters of the largest and second largest follicles were greater in CSFA-supplemented cows.

Eighteen lactating Holstein cows were fed CSFA at 2.2% of dietary DM or an isoenergetic diet (Lucy et al., 1993). Although animals were in similar energy balance, cows fed CSFA had a larger second wave dominant follicle (18.7 mm) than did cows fed the 0% CSFA diet (16.1 mm).

Forty-five Holstein cows were fed a blend of tallow and yellow grease (88:12 wt/wt) at 0, 2.2, or 4.4% of the dietary DM from d 0 to 84 DIM (Beam and Butler, 1997). On d 14 postpartum, the number of follicles greater than 15 mm in diameter was dramatically increased in cows fed diets of 2.2 and 4.4% (~ 0.7) supplemental fat in comparison to the control (~ 0.3) and was not correlated with energy status. The diameter of the largest follicle from d 8 to 14 postpartum was greater in cows fed 2.2% supplemental fat (13.5 mm) versus controls (11.0 mm). If only the animals that ovulated their first wave dominant follicle were considered, all fat-supplemented cows increased the diameter of the largest follicle from d 8 to 14 postpartum.

At parturition, Holstein cows (n = 141) were allotted to one of three dietary treatments (Petit and Twagiramungu, 2002). The isonitrogenous, isoenergetic, and isolipidic diets contained whole flaxseed, Ca salts of palm oil, or micronized soybeans. The diameter of the CL of the cows fed flaxseed was larger than that of cows fed soybeans (19.7 vs.16.9 mm) but not larger than that of cows fed Ca salts of palm oil (17.5

mm). Embryo mortality from day 30 to 50 after AI tended to be lower ($P < 0.11$) when cows were fed flaxseed (0%) compared to Ca salts of palm oil (15.4%) or soybeans (13.6%).

The timing of initiation of fat supplementation during the periparturient period has received little attention. The beneficial results seen in various studies when feeding fat is initiated during the periparturient period is not consistent. There is only one published study (Salfer et al., 1995) conducted to evaluate the timing of initiation of fat supplementation in lactating dairy cows. The authors concluded that delaying the inclusion of partially hydrogenated tallow in the diet until 35 DIM had benefits on total milk production through improved persistency. The objective of the current experiment was to evaluate if initiating fat supplementation during the prepartum period, at parturition, or at 28 DIM would have a beneficial effect on milk production, liver function, and reproduction of Holstein cows during summer.

Table 2.1. Effects of feeding ruminally inert fat (Ca salts of fatty acids (CSFA) or prilled fat (PF)) to the same cows throughout the study on DMI, milk production, milk composition, and body weight (BW) change.

Reference	Treatments/ Fat source	Dietary EE, %	DIM	Diet, % of DM	DMI, kg/d	Milk, kg/d	4.0% FCM, kg/d	Milk protein, %	Milk fat, %	BW change, kg or kg/d
Erickson et al., 1992	1) Control 2) 12 g/d niacin (NA) 3) 3% CSFA 4) NA + CSFA	-------	15-98	35% alfalfa haylage, 10% corn silage, 55% concentrate	1) 18.5 2) 18.5 3) 17.5 4) 18.2	1) 36.2 2) 36.4 3) 38.2 4) 39.3 F**	1) 32.4 2) 32.7 3) 34.3 4) 35.4 F**	1) 2.71 2) 2.84 3) 2.55 4) 2.68 F**	1) 3.32 2) 3.32 3) 3.36 4) 3.35	1) 0.18 2) 0.11 3) -0.22 4) 0.07 (kg/d)
Moallem et al., 2000	1) Control 2) 0.55 kg/d CSFA 3) Control + bST	-------	0-150	9.5% wheat silage, 15% corn silage, 3.5% legume hay, 3.5% oat hay, 2.3% wheat straw, 66.2% concentrate	1) 23.5 2) 23.7 3) 24.5	1) 40.2 2) 42.4 3) 45.4 F**	1) 37.8 2) 40.8 3) 43.2 † F**	1) 2.92 2) 2.92 3) 2.94	1) 3.12 2) 3.25 3) 3.19 F**	N.S.
Firkins and Eastridge, 1992	1) Control 2) 7% soy hulls (SH) 3) 7% SH + 1% $NaHCO_3$ 4) 20% SH + 0.43% CSFA + 10.7% roasted soybeans	1) 2.39 2) 2.39 3) 2.34 4) 3.42 (FA)	28-133	Trt 1 and 4: 10% alfalfa silage, 31% corn silage, 59% concentrate Trt 2 and 3: 10% alfalfa silage, 20% corn silage, 70% concentrate	1) 24.4 2) 23.5 3) 23.2 4) 22.5	1) 37.0 2) 37.3 3) 35.1 4) 38.9	1) 33.8 2) 33.0 3) 31.2 4) 35.6 2 & 3 vs. 4*	1) 3.16 2) 3.18 3) 3.18 4) 2.88 F*	1) 3.50 2) 3.26 3) 3.39 4) 3.42 2 & 3 vs. 4*	1) 0.38 2) 0.43 3) 0.28 4) 0.18 (kg/d)
Garcia-Bojalil et al., 1998	1) 11.1% RDP 2) 11.1% RDP + 2.2% CSFA 3) 15.7% RDP 4) 15.7% RDP + 2.2% CSFA	1) 4.77 2) 6.65 3) 4.62 4) 6.20	0-120	34% corn silage, 13% alfalfa hay, 53 % concentrate [a]	1) 19.6 2) 19.0 3) 19.4 4) 19.8 F x RDP ** at 0-50 DIM	1) 27.1 2) 28.0 3) 25.5 4) 27.7 F** at 50-120 DIM	1) 25.5 2) 26.5 3) 24.1 4) 26.3	1) 3.06 2) 3.02 3) 3.06 4) 2.98 F** at 35-120 DIM	1) 3.63 2) 3.67 3) 3.66 4) 3.68	N.S.
Spicer et al., 1993	1) Control 2) 1.8% CSFA	-------	28-84	20% sorghum silage, 19% alfalfa hay, 61% concentrate [a]	1) 25.9 2) 24.4	1) 36.9 2) 36.0	1) 34.1 2) 32.7	-------	1) 3.54 2) 3.44	1) 1.4 2) 6.3 F**

† 3.5% FCM.
[a] Concentrate mix included 6-15% whole cottonseed.
F = effect of fat.
N.S. = not significant.
* P < .10.
** P < .05.

Table 2.1. Continued.

Reference	Treatments/ Fat source	Dietary EE, %	DIM	Diet, % of DM	DMI, kg/d	Milk, kg/d	4.0% FCM, kg/d	Milk protein, %	Milk fat, %	BW change, kg or kg/d
Chouinard et al., 1997	1) Control + $NaHCO_3$ 2) 2% CSFA + $NaHCO_3$ 3) 4% CSFA + $NaHCO_3$ 4) 4% CSFA	1) 3.9 2) 5.5 3) 6.6 4) 6.9	39-67	62% alfalfa silage, 38% concentrate	1) 23.6 2) 22.8 3) 21.4 4) 21.6 F** (linear)	1) 33.5 2) 35.2 3) 32.8 4) 32.0 F** (quadratic)	1) 31.2 2) 31.6 3) 28.9 4) 28.0 † F** (linear)	1) 2.96 2) 2.80 3) 2.66 4) 2.57 F**	1) 3.55 2) 3.38 3) 3.29 4) 3.43	-------
Chouinard et al., 1997	1) Control + $NaHCO_3$ 2) 2% CSFA + $NaHCO_3$ 3) 4% CSFA + $NaHCO_3$ 4) 4% CSFA	1) 4.0 2) 5.8 3) 7.5 4) 7.5	68-95	46% alfalfa silage, 54% concentrate	1) 23.4 2) 21.9 3) 21.5 4) 21.0	1) 33.6 2) 35.9 3) 34.0 4) 31.4 F x $NaHCO_3$*	1) 30.4 2) 31.3 3) 28.7 4) 26.7	1) 3.10 2) 2.96 3) 2.82 4) 2.74 F**	1) 3.41 2) 3.08 3) 2.93 4) 3.23 F** linear	-------
Harrison et al., 1995	1) Control 2) 12% WCS 3) 12% WCS and 2.7% CSFA	1) 2.5 2) 4.4 3) 6.0	21-119	23% alfalfa hay, 23% grass silage, 54% concentrate	1) 23.1 2) 23.9 3) 21.6 Trt**	-------	1) 38.1 2) 39.8 3) 39.5 Trt**	1) 3.08 2) 3.07 3) 2.91 Trt**	1) 3.24 2) 3.49 3) 3.74 Trt**	-------
Harrison et al., 1995	1) Control 2) 12% WCS 3) 12% WCS and 5% CSFA	1) 3.5 2) 5.1 3) 6.9	18-105	28% alfalfa hay, 18% grass silage, 54% concentrate	1) 23.6 2) 22.4 3) 22.2 Trt** Trt x P**	-------	1) 36.0 2) 37.2 3) 37.8 Trt** Trt x P**	1) 3.09 2) 3.11 3) 2.95 Trt** Trt x P**	1) 3.36 2) 3.65 3) 3.72 Trt** Trt x P**	-------
Holter et al., 1992	1) Control 2) 15% WCS 3) 15% WCS + 0.54 kg/d CSFA	-------	0-112	Ad libitum forage: 63% corn silage, 37% wilted grass silage. Concentrate was adjusted to milk production	1) 17.4 2) 16.6 3) 16.8	1) 35.2 2) 29.6 3) 32.5 Trt**	1) 31.6 2) 30.3 3) 31.9	1) 2.86 2) 2.88 3) 2.82 Trt**	1) 3.32 2) 4.14 3) 3.89 Trt**	-------

† 3.5% FCM.
WCS = whole cottonseed.
F = effect of fat.
Trt = effect of treatment.
P = effect of parity.
N.S. = not significant.
* P < .10.
** P < .05.

Table 2.1. Continued.

Reference	Treatments/ Fat source	Dietary EE, %	DIM	Diet, % of DM	DMI, kg/d	Milk, kg/d	4.0% FCM, kg/d	Milk protein, %	Milk fat, %	BW change, kg or kg/d
Selberg et al., 2004	1) Control 2) 225 g/d CaS conjugated linoleic acid 3) 225 g/d CaS trans-C18:1	Prepartum control: 4.3 Post-partum control: 5.2	-28-49	Prepartum: 13% bermuda grass hay, 39% corn silage, 52% concentrate Postpartum: 10% alfalfa hay, 29% corn silage, 61% concentrate	1) 21.6 2) 20.0 3) 20.2 1 vs. 3 at wk 4-6** 1 vs. 2 at wk 6**	1) 40.3 2) 41.5 3) 41.5 1 & 2 vs. 3 by wk**	1) 40.0 2) 37.4 3) 40.5 †	1) 2.89 2) 2.82 3) 2.81	1) 3.49 2) 2.99 3) 3.46 1 vs. 2**	-------
Hoffman et al., 1991	1)Degradable protein (DP) 2) Undegradable protein (UP) 3) DP + 2.8% sodium alginate treated tallow (SAT) 4) UP + 2.8% SAT	1) 3.1 2) 3.3 3) 5.7 4) 6.0	22-150	49% alfalfa silage, 51% concentrate	1) 22.7 2) 22.5 3) 22.7 4) 22.7 (group fed)	1) 31.8 2) 31.6 3) 33.1 4) 32.7 F x T**	1) 29.9 2) 29.9 3) 30.9 4) 31.2	1) 3.14 2) 3.03 3) 3.04 4) 3.00 F** F x T**	1) 3.67 2) 3.63 3) 3.60 4) 3.82	N.S.
Beam and Butler, 1998	1) Control 2) 2.59% PF	1) 4.8 2) 7.0	0-100	26% corn silage, 18% alfalfa haylage, 56% concentrate[a]	0-100 DIM N.S. 0-28 DIM 1) 15.5 2) 17.3 F**	F x T* (Control peaked ~41 vs. 43 kg/d for PF)	F x T* (Control peaked ~34 vs. 36 kg/d for PF)	-------	-------	1) -26.5 2) -42.6 F*
Jerred et al., 1990 [b]	1) Low silage (LS) 2) LS + 5% PF 3) Medium silage (MS) 4) MS + 5% PF 5) High silage (HS) 6) HS + 5% PF	1) 3.1 2) 6.5 3) 3.4 4) 7.2 5) 3.8 6) 7.2	5-105	Trt 1 & 2: 45% alfalfa silage, 55% concentrate Trt 3 & 4: 64% alfalfa silage, 36% concentrate Trt 5 & 6; 84% alfalfa silage, 16% concentrate	1) 23.6 2) 22.1 F** F x T**	1) 39.2 2) 38.8	1) 36.5 2) 37.8 F x T**	1) 2.89 2) 2.87 F x T**	1) 3.57 2) 3.88 F**	1) -0.36 2) -0.47 (kg/d)

† 3.5% FCM.
[a] Concentrate mix included 6-15% whole cottonseed.
[b] Note: there are no fat x forage interactions; results are presented as 1) control (treatments 1, 3 and 5) and 2) fat (treatments 2, 4, and 6).
F = effect of fat.
T = effect of time.
N.S. = not significant.
* P < .10.
** P < .05.

Table 2.1. Continued.

Reference	Treatments/ Fat source	Dietary EE, %	DIM	Diet, % of DM	DMI, kg/d	Milk, kg/d	4.0% FCM, kg/d	Milk protein, %	Milk fat, %	BW change, kg or kg/d
Skaar et al., 1989	1) Control 2) 12 g/d niacin (NA) 3) 5% PF 4) 12 g/d NA and 5% PF	1) 3.4 [b] 2) 3.4 [b] 3) 11.8 [b] 4) 11.8 [b]	-17-105	25% corn silage, 25% alfalfa silage, 50% concentrate	1) 19.3 2) 17.8 3) 19.2 4) 18.7	1) 38.4 2) 36.3 3) 42.0 4) 41.3 F x season** (fat - greater in summer)	1) 36.3 2) 34.5 3) 39.3 4) 38.2 † F x season** (fat was greater in summer)	1) 3.00 2) 2.87 3) 2.87 4) 2.87	1) 3.14 2) 3.19 3) 3.15 4) 3.12	F x T** (fat fed gained faster)
Pickett et al., 2003	1) Control 2) 500 ml/d propylene glycol (PG) drench 3) 0.45 kg/d CSFA drench 4) 500 ml/d PG + 0.45 kg/d CSFA drench	4.8	0-21	31% corn silage, 16% alfalfa hay, 9% alfalfa hay, 44% concentrate [a] Drenches were administered from 0-3 DIM	1) 17.2 2) 18.0 3) 16.9 4) 15.8	1) 36.5 2) 36.1 3) 32.5 4) 34.8	1) 42.9 2) 40.3 3) 38.1 4) 40.9 †	1) 3.68 2) 3.46 3) 3.66 4) 3.51	1) 4.66 2) 4.30 3) 4.64 4) 4.66	-------
Schroeder et al., 2003	1) TMR fed (control) 2) Pasture + 6.7 kg/d corn based concentrate 3) Pasture + 6.7 kg/d concentrate with 0.8 kg CSFA	1) 4.5 2) 6.1 3) 8.1	117-152	Control: 59% corn silage, 41% concentrate Treatments 2 and 3: 84% pasture, 16% concentrate	1) 23.7 2) 22.9 3) 21.5	1) 20.2 2) 19.2 3) 20.2	1) 19.5 2) 17.8 3) 16.1 1 vs. 3**	1) 3.70 2) 3.49 3) 3.41 1 vs. 2 & 3** Trt x T**	1) 3.91 2) 3.45 3) 2.56 Trt** Trt x T**	1) 23 2) -6 3) -10 1 vs. 2 & 3**
Moallem et al., 1999	1) Control 2) 0.55 kg/d CSFA 3) Control + bST	-------	0-150	8% wheat silage, 20% corn silage, 3% pea hay, 3% oat hay, 66% concentrate [a]	1) 24.0 2) 23.3 3) 24.7 (group fed)	1) 39.7 2) 42.5 3) 44.0 F**	1) 37.8 2) 40.9 3) 41.9 † F**	1) 2.98 2) 2.92 3) 2.92 F**	1) 3.18 2) 3.25 3) 3.19	Fat fed lost more BW (~7 kg) than control (P < 0.1)
Kim et al., 1993	1) Control 2) 17% extruded soybeans 3) 4.0% CSFA	1) 2.5 2) 5.1 3) 3.0	28-105	25% alfalfa hay, 25% corn silage, 50% concentrate	1) 17.8 2) 18.4 3) 16.6 2 vs. 3*	1) 29.2 2) 32.4 3) 31.8 F**	1) 25.4 2) 26.7 3) 28.7 F*	1) 2.99 2) 2.93 3) 2.81 F** 2 vs. 3**	1) 3.20 2) 2.69 3) 3.47 2 vs. 3**	-------

† 3.5% FCM.
[a] Concentrate mix included 6-15% whole cottonseed.
[b] concentrate mix only.
F = effect of fat.
T = effect of time.
Trt = effect of treatment.
N.S. = not significant.
* P < .10.
** P < .05.

Table 2.1. Continued.

Reference	Treatments/ Fat source	Dietary EE, %	DIM	Diet, % of DM	DMI, kg/d	Milk, kg/d	4.0% FCM, kg/d	Milk protein, %	Milk fat, %	BW change, kg or kg/d
Moallem et al., 1997	1) Control 2) 0.5 kg/d CSFA 3) Control + bST 4) 0.5 kg/d CSFA + bST	-------	0-150	10.4% wheat silage, 24% corn silage, 2.3% pea hay, 2.1% oat hay, 61.2% concentrate	1) 24.1 2) 24.3 3) 24.9 4) 24.7 (group fed)	1) 36.3 2) 39.8 3) 41.3 4) 42.9 F** F x bST**	1) 33.4 2) 36.9 3) 39.5 4) 40.8 F** F x bST**	1) 3.02 2) 2.99 3) 3.01 4) 2.99	1) 2.98 2) 3.03 3) 3.21 4) 3.20	1) 38.0 2) 43.2 3) 33.4 4) 35.4 (maximum BW loss)
Sklan et al., 1994	1) Control 2) 2.5% CSFA	1) 2.8 2) 4.9	0-120	14.3% wheat silage, 15.3% corn silage, 6.4% vetch hay, 64% concentrate [a]	1) 20.6 2) 20.3 (group fed)	1) 31.1 2) 35.0 F** F x T**	1) 29.4 2) 33.8 † F** F x T**	1) 2.97 2) 2.99 F x T**	1) 3.15 2) 3.26 F* F x T**	N.S.
Cervantes et al., 1996	1) Control 2) 0.4 kg/d CSFA 3) 12 g/d nicotinamide (NM) 4) 0.4 kg/d CSFA + 12 g/d NM	1) 3.1 2) 5.1 3) 3.0 4) 5.0	112-150	Varied dependent on stage of lactation. Forages (35-60% of diet) utilized were alfalfa hay, alfalfa haylage, and corn silage.	1) 20.3 2) 20.5 3) 24.0 4) 21.1 F x NM*	1) 30.7 2) 31.8 3) 33.5 4) 33.2	1) 28.3 2) 29.7 3) 29.7 4) 30.6	1) 3.21 2) 3.17 3) 3.31 4) 3.14 F**	1) 3.45 2) 3.57 3) 3.26 4) 3.46 F*	-------
Palmquist and Weiss, 1994	1) Control 2) 2.5% tallow + 2.5% CSFA (Also included 3 concentrations of RUP)	1) 3.08 2) 6.73 (FA)	0-60	25% corn silage, 25% alfalfa hay, 50% concentrate	1) 18.1 2) 18.8	1) 43.3 2) 42.4	1) 37.1 2) 37.1 †	1) 2.97 2) 2.93	1) 2.64 2) 2.78	1) -0.08 2) -0.23 (kg/d)
Atwal et al,, 1990	1) Control 2) 5% CSFA	1) 2.4	14-70	15% alfalfa silage, 10% alfalfa hay, 25% corn silage, 50% concentrate	1) 19.2 2) 19.7	1) 33.1 2) 33.3	1) 31.1 2) 32.5	1) 2.93 2) 2.94	1) 3.51 2) 3.80	1) 0.10 2) 1.35 (kg/d) F** (wk 1-4)

† 3.5% FCM.
[a] Concentrate mix included 6-15% whole cottonseed.
F = effect of fat.
T = effect of time.
* P < .10.
** P < .05.

Table 2.1. Continued.

Reference	Treatments/ Fat source	Dietary EE, %	DIM	Diet, % of DM	DMI, kg/d	Milk, kg/d	4.0% FCM, kg/d	Milk protein, %	Milk fat, %	BW change, kg or kg/d
Atwal et al,, 1990	1) Control 2) 5% CSFA	1) 2.3	1-56	25% alfalfa hay, 25% corn silage, 50% concentrate	1) 16.5 2) 15.1	1) 30.1 2) 29.7	1) 32.6 2) 30.8	1) 3.03 2) 3.01	1) 4.82 2) 4.37	1) -0.35 2) -1.85 (kg/d)
Simas et al., 1995	1) Dry rolled sorghum (DRS) 2) Steam flaked sorghum (SFS) 3) DRS + 2.5% CSFA 4) SFS + 2.5% CSFA	1) 5.7 2) 5.7 3) 7.5 4) 7.5	5-91	34% alfalfa hay, 66% concentrate[1]	1) 21.6 2) 23.1 3) 17.8 4) 19.5 F**	1) 34.3 2) 39.3 3) 33.4 4) 36.5	1) 31.6 2) 34.5 3) 31.2 4) 32.8 †	1) 2.93 2) 3.00 3) 2.79 4) 2.99 3 vs. others*	1) 3.16 2) 2.91 3) 3.23 4) 3.05	1) 0 2) 0.16 3) -0.2 4) -0.02 (kg/d) F*
Sklan et al., 1991	1) Control 2) 2.6% CSFA	-------	0-120	13.7% corn silage, 11.3% vetch hay, 75% concentrate [1]	1) 20.3 2) 20.2 (group fed)	1) ~37 2) ~39 (at peak) F** at 30 and 60 DIM	1) ~34 2) ~38 (at peak) F** until 90 DIM	N.S.	1) ~2.8 2) ~3.0 F** at 30 to 90 DIM	F** (CSFA cows lost more and faster)
Sklan et al., 1992	1) Control 2) 2% FA 3) 2.4% CSFA	1) 2.1 2) 4.2 3) 4.2 (FA)	93-213	1.4% oat hay, 20% wheat silage, 13.7% corn cobs, 64.9% concentrate	1) 21.2 2) 21.1 3) 20.9 (group fed)	1) 30.2^{b} 2) 31.3^{a} 3) 30.9^{ab} Trt x T** Trt x P**	1) 25.9^{c} 2) 27.7^{b} 3) 27.0^{a} † Trt x T** Trt x P**	1) 3.20 2) 3.13 3) 3.15 Trt x T** Trt x P**	1) 2.67^{a} 2) 2.81^{b} 3) 2.75^{ab} Trt x T** Trt x P**	-------
Sklan et al., 1992	1) 14.5% WCS 2) 1.8% FA 3) 2.1% CSFA	1) 5.0 2) 4.0 3) 4.0 (FA)	108-228	7.8% citrus silage, 32.7% wheat silage, 6.7% vetch hay, 52.8% concentrate	1) 20.1 2) 20.4 3) 20.3 (group fed)	1) 31.3^{b} 2) 32.5^{a} 3) 32.4^{ab} Trt x T**	1) 30.1 2) 29.7 3) 30.4 † Trt x T**	1) 3.02 2) 2.96 3) 2.98 Trt x T*	1) 3.25^{a} 2) 2.99^{b} 3) 3.17^{a}	-------

† 3.5% FCM.
[a,b,c] Means not followed by the same letter differ (P < .05).
[1] Concentrate mix included 6-15% whole cottonseed (WCS).
F = effect of fat.
Trt = effect of treatment.
T = effect of time.
P = effect of parity.
N.S. = not significant.
* P < .10.
** P < .05.

Table 2.2. Effects of feeding oilseeds alone or in combination with other fat sources to the same cows throughout the experiment on DMI, milk production, milk composition, and body weight (BW) change.

Reference	Treatments/ Fat source	Dietary EE, %	DIM	Diet, % of DM	DMI, kg/d	Milk, kg/d	4.0% FCM, kg/d	Milk protein, %	Milk fat, %	BW change, kg or kg/d
Stegeman et al., 1992	1) Control 2) Control + bST 3) 10% rolled sunflower seeds + bST 4) 10% rolled safflower seeds + bST	1) 2.3 2) 2.3 3) 6.2 4) 5.5	104-216	25% corn silage, 25% alfalfa hay, 50% concentrate	1) 27.5 2) 23.9 3) 27.3 4) 19.3 3 vs. 4**	1) 29.5 2) 32.7 3) 40.0 4) 34.1 2 vs. 3 & 4** 3 vs. 4**	1) 25.0 2) 27.9 3) 32.3 4) 28.1 3 vs. 4**	1) 3.17 2) 3.27 3) 3.02 4) 3.08 F** 2 vs. 3 & 4**	1) 2.99 2) 3.06 3) 2.73 4) 2.86	1) 24.7 2) 19.4 3) 36.1 4) 39.9
AbuGhazaleh et al., 2004	1) Control 2) 10.64% extruded soybeans + 5.5% fish meal	1) 2.67 2) 4.93	103-173	25% alfalfa hay, 25% corn silage, 50% concentrate	1) 29.3 2) 27.7 F*	1) 34.5 2) 38.9 F** F x T**	1) 36.0 2) 36.4 †	1) 3.39 2) 3.18 F** F x T**	1) 3.74 2) 3.17 F** F x T**	-------
Kim et al., 1993	1) Control 2) 17% extruded soybeans 3) 4.0% CSFA	1) 2.5 2) 5.1 3) 3.0	28-105	25% alfalfa hay, 25% corn silage, 50% concentrate	1) 17.8 2) 18.4 3) 16.6 2 vs. 3*	1) 29.2 2) 32.4 3) 31.8 F**	1) 25.4 2) 26.7 3) 28.7 F*	1) 2.99 2) 2.93 3) 2.81 F** 2 vs. 3**	1) 3.20 2) 2.69 3) 3.47 2 vs. 3**	-------
Markus et al., 1996	1) Control 2) 7.1% whole sunflower seeds 3) 2.7% tallow	1) 1.8 2) 4.2 3) 4.1	16-112	12% corn silage, 14% alfalfa silage, 9.5% alfalfa hay, 64.5% concentrate	1) 22.2 2) 21.1 3) 21.6	1) 34.4 2) 34.6 3) 35.5	1) 30.0 2) 29.9 3) 31.6	1) 3.1 2) 3.0 3) 3.0	1) 3.2 2) 3.1 3) 3.3	-------
Weiss and Wyatt, 2003	1) Control 2) 12.3% whole roasted soybeans 3) 2.35% tallow (Also included 3 levels of vitamin E)	-------	160-188	38% corn silage, 8% alfalfa hay, 7% alfalfa silage, 47% concentrate	1) 22.3 2) 24.0 3) 22.0 2 vs. 3**	1) 35.1 2) 36.8 3) 37.5 F*	-------	1) 2.97 2) 2.92 3) 2.86	1) 3.76 2) 3.83 3) 3.08 F** 2 vs. 3**	1) 0.87 2) 1.29 3) 0.71 (kg/d) 2 vs. 3**

† 3.5% FCM.
F = effect of fat.
T = effect of time.
* P < .10.
** P < .05.

Table 2.2. Continued.

Reference	Treatments/ Fat source	Dietary EE, %	DIM	Diet, % of DM	DMI, kg/d	Milk, kg/d	4.0% FCM, kg/d	Milk protein, %	Milk fat, %	BW change, kg or kg/d
Drackley et al., 1998	1) Control 2) Control + 12 g/d niacin 3) 10% whole raw soybeans and 2.5% tallow 4) 10% whole raw soybeans, 2.5% tallow and 12 g/d niacin	1) 2.75 2) 2.75 3) 6.04 4) 6.04 (FA)	28-301	32.5% alfalfa haylage, 17.5% corn silage, 50% concentrate [1, 2]	1) 21.9 2) 21.7 3) 21.6 4) 22.2 F x T**	1) 30.5 2) 33.2 3) 31.8 4) 33.6 F x T**	1) 30.4 2) 32.9 3) 32.5 4) 34.2 † F x T**	1) 3.29 2) 3.16 3) 3.16 4) 3.13 F** F x T**	1) 3.56 2) 3.50 3) 3.68 4) 3.60 F x niacin x T**	
Sklan et al., 1992	1) Control 2) Control + 2.4% FA[3] 3) Control + 16.2% WCS	1) 1.8 2) 4.4 3) 4.4 (FA)	70-210	8.4% alfalfa hay, 17.2% corn silage, 74.4% concentrate	1) 21.4 2) 22.1 3) 21.4 (group fed)	1) 32.7 2) 33.6 3) 33.5 Trt x T**	1) 27.6[b] 2) 28.7[ab] 3) 30.4[a] † Trt x T **	1) 2.97 2) 2.95 3) 2.96 Trt x T*	1) 2.54[b] 2) 2.57[b] 3) 2.96[a] Trt x T*	-------
Sklan et al., 1992	1) 14.5% WCS 2) 1.8% FA[3] 3) 2.1% CSFA	1) 5.0 2) 4.0 3) 4.0 (FA)	108-228	7.8% citrus silage, 32.7% wheat silage, 6.7% vetch hay, 52.8% concentrate	1) 20.1 2) 20.4 3) 20.3 (group fed)	1) 31.3[b] 2) 32.5[a] 3) 32.4[ab] Trt x T**	1) 30.1 2) 29.7 3) 30.4 † Trt x T**	1) 3.02 2) 2.96 3) 2.98 Trt x T*	1) 3.25[a] 2) 2.99[b] 3) 3.17[a]	-------

[1] Concentrate mix included 6-15% whole cottonseed (WCS).

[2] After 175 DIM, diets were adjusted for decreased nutrient requirements. Forage content increased to 60% of DM. For treatments 3 and 4 whole raw soybeans were removed and tallow was decreased to 2.25% of the diet.

[3] Fatty acid (FA) source was mixed soapstock containing 86% free FA, of which 20% was linoleic acid.

[a,b,c] Means not followed by the same letter differ (P < .05).

† 3.5% FCM.

F = effect of fat.

Trt = effect of treatment.

T = effect of time.

* P < .10

** P < .05

Table 2.2. Continued.

Reference	Treatments/ Fat source	Dietary EE, %	DIM	Diet, % of DM	DMI, kg/d	Milk, kg/d	4.0% FCM, kg/d	Milk protein, %	Milk fat, %	BW change, kg or kg/d
Schingoethe and Casper, 1991	1) Control 2) Added fat from extruded soybeans or sunflower seeds (summary of 5 studies)	1) 2.6 2) 5.3 (average)	28-119	38% corn silage, 12% alfalfa hay, 50% concentrate	1) 20.8 2) 20.4	1) 3000 2) 3085 (kg)	-------	1) 2.99 2) 2.91 F**	1) 3.20 2) 2.95 F**	1) 0.18 2) 0.13 (kg/d)
Harrison et al., 1995	1) Control 2) 12% WCS 3) 12% WCS and 2.7% CSFA	1) 2.5 2) 4.4 3) 6.0	21-119	23% alfalfa hay, 23% grass silage, 54% concentrate	1) 23.1 2) 23.9 3) 21.6 Trt**	-------	1) 38.1 2) 39.8 3) 39.5 Trt**	1) 3.08 2) 3.07 3) 2.91 Trt**	1) 3.24 2) 3.49 3) 3.74 Trt**	-------
Harrison et al., 1995	1) Control 2) 12% WCS 3) 12% WCS and 5% CSFA	1) 3.5 2) 5.1 3) 6.9	18-105	28% alfalfa hay, 18% grass silage, 54% concentrate	1) 23.6 2) 22.4 3) 22.2 Trt** Trt x P**	-------	1) 36.0 2) 37.2 3) 37.8 Trt** Trt x P**	1) 3.09 2) 3.11 3) 2.95 Trt** Trt x P**	1) 3.36 2) 3.65 3) 3.72 Trt** Trt x P**	-------
Khorasani et al., 1991	1) Control 2) 4.5% jet-sploded whole canola seed (JSWCS) 3) 9% JSWCS 4) 13.2% JSWCS 5) 17.4% JSWCS	1) 2.2 2) 3.4 3) 4.4 4) 5.5 5) 6.7	36-92	30% alfalfa silage, 10% oat silage, 60% concentrate	1) 17.8 2) 18.8 3) 18.3 4) 16.2 5) 17.0	1) 32.5 2) 34.5 3) 34.0 4) 33.2 5) 29.8	1) 27.7 2) 28.2 3) 29.8 4) 26.9 5) 24.8	1) 2.95 2) 3.12 3) 2.89 4) 2.84 5) 2.71 F** (linear)	1) 3.08 2) 2.89 3) 3.06 4) 2.76 5) 2.84	-------
Faldet and Satter, 1991	1) 10% soybean meal 2) 13% raw soybeans (RS) 3) 13% heated soybeans (HS)	1) 3.3 2) 5.6 3) 5.6	15-119	50% alfalfa silage, 50% concentrate	1) 23.4 2) 22.3 3) 23.6	1) 34.5 2) 34.2 3) 38.9 HS**	1) 33.4 2) 34.7 3) 38.0 † HS**	1) 2.99 2) 2.89 3) 2.85 HS**	1) 3.41 2) 3.50 3) 3.41	1) 26 2) 2 3) 31 RS**

† 3.5% FCM.
WCS = whole cottonseeds.
F = effect of fat.
P = effect of parity.
Trt = effect of treatment.
* P < .10.
** P < .05.

Table 2.2. Continued.

Reference	Treatments/ Fat source	Dietary EE, %	DIM	Diet, % of DM	DMI, kg/d	Milk, kg/d	4.0% FCM, kg/d	Milk protein, %	Milk fat, %	BW change, kg or kg/d
Holter et al., 1992	1) Control 2) 15% WCS 3) 15% WCS + 0.54 kg/d CSFA	-------	0-112	Ad libitum forage: 63% corn silage, 37% wilted grass silage. Concentrate was adjusted to milk production	1) 17.4 2) 16.6 3) 16.8	1) 35.2 2) 29.6 3) 32.5 Trt**	1) 31.6 2) 30.3 3) 31.9	1) 2.86 2) 2.88 3) 2.82 Trt**	1) 3.32 2) 4.14 3) 3.89 Trt**	-------
Wu et al., 1994	1) Control 2) 12% WCS 3) 12% WCS + 2.2% safflower oil 4) 12% WCS +2.2% prilled tallow 5) 12% WCS +4.4% prilled tallow	1) 3.3 2) 5.2 3) 7.4 4) 7.4 5) 9.6	50-125	43% alfalfa hay, 57% concentrate	1) 28.2 2) 27.2 3) 28.8 4) 26.8 5) 24.1	1) 32.5 2) 32.6 3) 35.0 4) 34.3 5) 33.0 1 & 2 vs. 3 &4**	1) 32.4 2) 32.3 3) 33.4 4) 34.6 5) 33.0 † 1 & 2 vs. 3 &4**	1) 3.20 2) 3.03 3) 3.03 4) 3.08 5) 3.07 1 vs. 2 **	1) 3.49 2) 3.48 3) 3.26 4) 3.58 5) 3.51	1) 0.70 2) 0.82 3) 0.57 4) 0.64 5) 0.46 (kg/d)
Kim et al., 1991	1) Basal containing 14% soybean meal (SBM) 2) 17% extruded soybeans (ESB) 3) 17% ESB and 5% SBM	1) 2.6 2) 5.5 3) 5.1	28-112	25% corn silage, 25% alfalfa hay, 50% concentrate	1) 20.9 2) 20.7 3) 19.8	1) 33.0 2) 35.8 3) 34.2 F**	1) 28.5 2) 29.6 3) 30.2	1) 2.92 2) 2.88 3) 2.83	1) 3.20 2) 2.88 3) 3.17 2 vs. 3*	1) 0.31 2) 0.00 3) -0.02 (kg/d)
Pires et al., 1996	1) Control 2) 18% ground roasted soybean 3) 18% whole soybean 4) 2.7% blood meal 5) 2.7% blood meal +3% tallow	1) 3.2 2) 6.2 3) 6.2 4) 3.2 5) 6.2	21-126	30% corn silage, 20% alfalfa silage, 50% concentrate	1) 23.6a 2) 22.7ab 3) 21.3bc 4) 21.3bc 5) 20.4c	1) 39.6 2) 40.7 3) 36.4 4) 36.1 5) 39.3	1) 35.4 2) 35.0 3) 33.3 4) 33.9 5) 35.0	1) 3.03a 2) 2.83b 3) 2.88bc 4) 3.08a 5) 2.98ac	1) 3.33 2) 3.09 3) 3.50 4) 3.63 5) 3.29	-------

[a,b,c] Means not followed by the same letter differ (P < .05).
† 3.5% FCM.
WCS = whole cottonseeds.
Trt = effect of treatment.
* P < .10.
** P < .05.

Table 2.3. Effects of feeding rendered fats to the same cows throughout the study on DMI, milk production, milk composition and body weight (BW) change.

Reference	Treatments/ Fat source	Dietary EE, %	DIM	Diet, % of DM	DMI, kg/d	Milk, kg/d	4.0% FCM, kg/d	Milk protein, %	Milk fat, %	BW change, kg or kg/d
Tackett et al., 1996	1) 21% NDF 2) 21% NDF + 6% choice white grease (CWG) 3) 28% NDF 4) 28% NDF + 6% CWG	1) 2.8 2) 8.4 3) 2.7 4) 7.8	32-122	Treatment 1 & 2: 36% corn silage, 9% alfalfa hay, 55% concentrate Treatment 3 & 4: 36% corn silage, 16% alfalfa hay, 48% concentrate	1) 24.4 2) 21.7 3) 23.7 4) 21.5	1) 37.5 2) 38.9 3) 34.7 4) 38.0	1) 37.0 2) 35.4 3) 34.8 4) 36.3 †	1) 3.5 2) 3.3 3) 3.4 4) 3.4 F*	1) 3.4 2) 2.9 3) 3.5 4) 3.2 F** F x NDF x T**	1) 55 2) 53 3) 42 4) 29
Niagono et al., 1991	1) Control 2) Control + 1 kg yellow grease (Also included high and low degradability protein supplements)	1) 5.0 [a] 2) 16.3[a]	1-112	Ad libitum wheat silage and maximum of 16.4 kg/d of concentrate	1) 20.2 2) 19.0	1) 38.9 2) 39.8	1) 30.4 2) 29.4	1) 3.3 2) 3.4	1) 2.5 2) 2.4	1) -2.6 2) -24.4 F**
Bateman et al., 1996	1) 33% NDF, 0% tallow 2) 33% NDF, 2% tallow 3) 40% NDF, 0% tallow 4) 40% NDF, 2% tallow	1) 3.5 2) 5.4 3) 3.7 4) 5.8	120-240, winter	8% alfalfa hay, 22% corn silage, 14% alfalfa silage, 0 or 13% earlage, 56 or 43% concentrate	1) 25.2 2) 22.6 3) 23.3 4) 24.3 F x NDF*	1) 31.1 2) 31.2 3) 28.6 4) 30.3	1) 30.3 2) 30.2 3) 28.1 4) 28.9	1) 3.30 2) 3.17 3) 3.22 4) 3.09	1) 3.94 2) 3.90 3) 3.96 4) 3.73	-------
Bateman et al., 1996	1) 33% NDF, 0% tallow 2) 33% NDF, 2% tallow 3) 40% NDF, 0% tallow 4) 40% NDF, 2% tallow	1) 3.5 2) 5.4 3) 3.7 4) 5.8	120-240, summer	8% alfalfa hay, 22% corn silage, 14% alfalfa silage, 0 or 13% earlage, 56 or 43% concentrate	1) 21.7 2) 21.9 3) 22.7 4) 22.0	1) 29.4 2) 30.1 3) 30.7 4) 29.9	1) 26.9 2) 27.5 3) 29.8 4) 27.3	1) 3.14 2) 3.03 3) 3.25 4) 3.07	1) 3.52 2) 3.49 3) 3.86 4) 3.56	-------
Son et al., 1996	1) 0% tallow + 0% escape protein supplement (EP) 2) 0% tallow + 5% EP 3) 3% tallow + 0% EP 4) 3% tallow +5% EP	-------	14-84	33% alfalfa haylage, 17% corn silage, 50% concentrate	1) 25.1 2) 24.2 3) 24.1 4) 22.8 F** F x EP**	1) 32.5 2) 33.0 3) 33.0 4) 33.1	1) 31.4 2) 30.1 3) 31.1 4) 31.7 F x EP**	1) 2.97 2) 2.80 3) 2.87 4) 2.75	1) 3.78 2) 3.41 3) 3.61 4) 3.71	1) -30.2 2) -20.3 3) -17.3 4) -37.1 F x EP**

† 3.5% FCM
[a] concentrate mix only.
F = effect of fat.
T = effect of time.
*P < .10.
** P < .05.

Table 2.3. Continued.

Reference	Treatments/ Fat source	Dietary EE, %	DIM	Diet, % of DM	DMI, kg/d	Milk, kg/d	4.0% FCM, kg/d	Milk protein, %	Milk fat, %	BW change, kg or kg/d
Maiga et al., 1995	1) Control 2) 2% tallow 3) 8.3% tallow-molasses blend 4) 2% tallow + 5.4% whey	1) 2.9 2) 4.8 3) 4.7 4) 4.6	28-112	25% corn silage, 25% alfalfa hay, 50% concentrate	1) 23.1 2) 24.3 3) 24.5 4) 24.5	1) 31.9 2) 33.7 3) 33.7 4) 34.0 F**	1) 31.4 2) 33.9 3) 33.0 4) 33.4 †	1) 3.00 2) 2.98 3) 2.91 4) 2.86 2 vs. 3 & 4*	1) 3.48 2) 3.65 3) 3.52 4) 3.40 2 vs. 3 & 4*	1) 0.75 2) 0.42 3) 0.60 4) 0.61 (kg/d) F*
Pantoja et al., 1996	1) Control 2) 5% tallow 3) 5% tallow + partially hydrogenated tallow (PHT) (2:1 weight/weight) 4) 5% tallow + PHT (1:2 weight/weight) 5) 5% PHT	1) 2.90 2) 6.13 3) 6.80 4) 6.58 5) 6.54 (FA)	28-133	25% corn silage, 25% alfalfa silage, 50% concentrate	1) 22.3 2) 22.1 3) 21.2 4) 22.5 5) 23.9 2 vs. 5*	1) 35.6 2) 40.6 3) 36.9 4) 39.3 5) 38.0 F**	1) 33.6 2) 35.6 3) 33.6 4) 36.3 5) 36.3	1) 3.05 2) 2.86 3) 3.03 4) 2.98 5) 3.02 2 vs. 5**	1) 3.63 2) 3.17 3) 3.48 4) 3.56 5) 3.77 2 vs. 5**	1) -2.16 2) -0.53 3) 1.09 4) 0.13 5) 0.04 (kg/wk) F**
Salfer et al., 1995	1) Control 2) -14 – 0 DIM 1% partially hydrogenated tallow (PHT), 1-151 DIM 2% PHT 3) 1-151 DIM 2% PHT 4) 35-151 DIM 2% PHT	Prepartum 1) 3.01 2) 3.92 Post-partum: 1) 3.17 2) 4.99	1-151	Prepartum: 37% corn silage, 10% alfalfa silage, 14% grass hay, 39% concentrate Postpartum: 24% corn silage, 21% alfalfa silage, 55% concentrate	1) 19.9 2) 19.9 3) 20.6 4) 20.6	1) 30.7 2) 31.6 3) 31.9 4) 32.7	1) 32.0 2) 32.7 3) 33.2 4) 32.8 †	1) 3.04 2) 2.99 3) 2.97 4) 2.92	1) 3.81 2) 3.76 3) 3.83 4) 3.55	N.S.
Salfer et al., 1995	1) Control 2) -14 – 0 DIM 1% partially hydrogenated tallow (PHT), 1-151 DIM 2% PHT 3) 1-151 DIM 2% PHT	Prepartum 1) 3.01 2) 3.92 Post-partum: 1) 3.17 2) 4.99	1-35 (3 treatments initiated)	Prepartum: 37% corn silage, 10% alfalfa silage, 14% grass hay, 39% concentrate Postpartum: 24% corn silage, 21% alfalfa silage, 55% concentrate	1) 15.3 2) 15.5 3) 16.3	1) 30.2 2) 29.9 3) 30.6	1) 34.5 2) 33.4 3) 36.9 † 2 vs. 3*	1) 3.06 2) 3.08 3) 3.13	1) 4.45 2) 4.29 3) 4.81 2 vs. 3**	N.S.

† 3.5% FCM.
F = effect of fat.
N.S. = not significant.
* P < .10.
** P < .05.

Table 2.3. Continued.

Reference	Treatments/ Fat source	Dietary EE , %	DIM	Diet, % of DM	DMI, kg/d	Milk, kg/d	4.0% FCM, kg/d	Milk protein, %	Milk fat, %	BW change, kg or kg/d
Salado et al., 2004	1) Control 2) 0.7 kg/d partially hydrogenated vegetable oil	1) 6.7 [a] 2) 22.5 [a]	-15-75	Pasture and 5 or 4 kg of concentrate offered for treatments 1 and 2, respectively	1) 20.3 2) 19.7	1) 23.7 2) 25.0 F**	1) 22.5 2) 24.5 F**	1) 3.12 2) 3.14	1) 3.64 2) 3.86 F**	1) -0.40 2) -0.04 (kg/d)
Markus et al., 1996	1) Control 2) 7.1% whole sunflower seeds 3) 2.7% tallow	1) 1.8 2) 4.2 3) 4.1	16-112	12% corn silage, 14% alfalfa silage, 9.5% alfalfa hay, 64.5% concentrate	1) 22.2 2) 21.1 3) 21.6	1) 34.4 2) 34.6 3) 35.5	1) 30.0 2) 29.9 3) 31.6	1) 3.1 2) 3.0 3) 3.0	1) 3.2 2) 3.1 3) 3.3	-------
Weiss and Wyatt, 2003	1) Control 2) 12.3% whole roasted soybeans 3) 2.35% tallow (Also included 3 levels of vitamin E)	-------	160-188	38% corn silage, 8% alfalfa hay, 7% alfalfa silage, 47% concentrate	1) 22.3 2) 24.0 3) 22.0 2 vs. 3**	1) 35.1 2) 36.8 3) 37.5 F*	-------	1) 2.97 2) 2.92 3) 2.86	1) 3.76 2) 3.83 3) 3.08 F** 2 vs. 3**	1) 0.87 2) 1.29 3) 0.71 (kg/d) 2 vs. 3**
Wu et al., 1994	1) Control 2) 12% WCS 3) 12% WCS + 2.2% safflower oil 4) 12% WCS +2.2% prilled tallow 5) 12% WCS +4.4% prilled tallow	1) 3.3 2) 5.2 3) 7.4 4) 7.4 5) 9.6	50-125	43% alfalfa hay, 57% concentrate	1) 28.2 2) 27.2 3) 28.8 4) 26.8 5) 24.1	1) 32.5 2) 32.6 3) 35.0 4) 34.3 5) 33.0 1 & 2 vs. 3 & 4**	1) 32.4 2) 32.3 3) 33.4 4) 34.6 5) 33.0 † 1 & 2 vs. 3 & 4**	1) 3.20 2) 3.03 3) 3.03 4) 3.08 5) 3.07 1 vs. 2 **	1) 3.49 2) 3.48 3) 3.26 4) 3.58 5) 3.51	1) 0.70 2) 0.82 3) 0.57 4) 0.64 5) 0.46 (kg/d)
Pires et al., 1996	1) Control 2) 18% ground roasted soybean 3) 18% whole soybean 4) 2.7% blood meal 5) 2.7% blood meal + 3% tallow	1) 3.2 2) 6.2 3) 6.2 4) 3.2 5) 6.2	21-126	30% corn silage, 20% alfalfa silage, 50% concentrate	1) 23.6[a] 2) 22.7[ab] 3) 21.3[bc] 4) 21.3[bc] 5) 20.4[c]	1) 39.6 2) 40.7 3) 36.4 4) 36.1 5) 39.3	1) 35.4 2) 35.0 3) 33.3 4) 33.9 5) 35.0	1) 3.03[a] 2) 2.83[b] 3) 2.88[bc] 4) 3.08[a] 5) 2.98[ac]	1) 3.33 2) 3.09 3) 3.50 4) 3.63 5) 3.29	-------

[a] Concentrate mix only.
WCS = whole cottonseeds.
F = effect of fat.
* P < .10.
** P < .05.

Table 2.4. Effects of feeding ruminally inert fat (Ca salts of fatty acids (CSFA) or prilled fat (PF)) to the same cows throughout the study on concentration of plasma hormones and metabolites.

Reference	Treatments/ Fat source	Dietary EE, %	DIM	Diet, % of DM	NEFA, µeq/L	BHBA, mg/dl	Insulin, ng/ml	IGF-1, ng/ml	Other
Erickson et al., 1992	1) Control 2) 12 g/d niacin (NA) 3) 3% CSFA 4) NA + CSFA	-------	15-98	35% alfalfa haylage, 10% corn silage, 55% concentrate	1) 265 2) 238 3) 303 4) 352 F**	1) 6.50 2) 5.18 3) 7.82 4) 6.48 F**	-------	-------	-------
Moallem et al., 2000	1) Control 2) 0.55 kg/d CSFA 3) Control + bST	-------	0-150	9.5% wheat silage, 15% corn silage, 3.5% legume hay, 3.5% oat hay, 2.3% wheat straw, 66.2% concentrate	-------	-------	-------	-------	Progesterone based cyclicity, DIM 1) 27 2) 29 3) 26
Garcia-Bojalil et al., 1998	1) 11.1% RDP 2) 11.1% RDP + 2.2% CSFA 3) RDP 15.7% 4) 15.7% RDP + 2.2% CSFA	1) 4.77 2) 6.65 3) 4.62 4) 6.20	0-120	34% corn silage, 13% alfalfa hay, 53% concentrate [a]	RDP x F** [a]	-------	1) 0.69 2) 0.60 3) 0.55 4) 0.52 F*	-------	-------
Spicer et al., 1993	1) Control 2) 1.8% CSFA	-------	28-84	20% sorghum silage, 19% alfalfa hay, 61% concentrate [a]	-------	-------	-------	1) 36.1 2) 82.7	Progesterone 1) 6.03 2) 4.47 F**
Selberg et al., 2004	1) Control 2) 225 g/d CaS conjugated linoleic acid (CLA) 3) 225 g/d CaS trans-C18:1	Control prepartum: 4.3 Control post-partum: 5.2	-28-49	Prepartum: 13% bermuda grass hay, 39% corn silage, 52% concentrate Postpartum: 10% alfalfa hay, 29% corn silage, 61% concentrate	1 vs. 2 by week** (CaS CLA peaked higher at wk 1 postpartum)	1 vs. 2 by week** (CaS CLA peaked higher at wk 1 postpartum)	1 vs. 3 at week 6** (CaS trans-C18:1 cows were greater)	-------	Mean hepatic lipid and TAG concentrations did not differ among diets

[a] Concentrate mix included 6-15% whole cottonseed.

[b] NEFA concentrations from 0-49 DIM were elevated for cows fed 11.1% RDP + CSFA in comparison to 11.1% DIP, while15.7% RDP + CSFA decreased concentrations in comparison to the 15.7% RDP group. From 0-14 DIM cows fed 15.7% RDP diet had elevated NEFA concentrations in comparision to 15.7% RDP + CSFA.

F = effect of fat.

* $P < .10$.

** $P < .05$.

Table 2.4. Continued.

Reference	Treatments/ Fat source	Dietary EE, %	DIM	Diet, % of DM	NEFA, µeq/L	BHBA, mg/dl	Insulin, ng/ml	IGF-1, ng/ml	Other
Beam and Butler, 1998	1) Control 2) 2.59% PF	1) 4.8 2) 7.0	0-100	26% corn silage, 18% alfalfa haylage, 56% concentrate [a]	1) 554 2) 656	-------	1) 0.37 2) 0.33	1) 47.7 2) 37.6 F* (1-3 wk PP)	-------
Jerred et al., 1990 [c]	1) Low silage (LS) 2) LS + 5% PF 3) Medium silage (MS) 4) MS + 5% PF 5) High silage (HS) 6) HS + 5% PF	1) 3.1 2) 6.5 3) 3.4 4) 7.2 5) 3.8 6) 7.2	5-105	Trt 1 & 2: 45% alfalfa silage, 55% concentrate Trt 3 & 4: 64% alfalfa silage, 36% concentrate Trt 5 & 6; 84% alfalfa silage, 16% concentrate	-------	1) 13.3 2) 19.1 F**	-------	-------	-------
Skaar et al., 1989	1) Control 2) 12 g/d niacin (NA) 3) 5% PF 4) 12 g/d NA and 5% PF	1) 3.4 [b] 2) 3.4 [b] 3) 11.8 [b] 4) 11.8 [b]	-17-105	25% corn silage, 25% alfalfa silage, 50% concentrate	1) 298 2) 343 3) 320 4) 371	1) 10.8 2) 11.2 3) 10.2 4) 9.7	-------	-------	Increase in total hepatic lipids by fat feeding at 0 DIM and at 5 wk postpartum (P < 0.15); hepatic TAG = not significant
Pickett et al., 2003	1) Control 2) 500 ml/d propylene glycol (PG) drench 3) 0.45 kg/d CSFA drench 4) 500 ml/d PG + 0.45 kg/d CSFA drench	4.8	0-21	31% corn silage, 16% alfalfa hay, 9% alfalfa hay, 44% concentrate [a] Drenches were administered from 0-3 DIM	1) 643 2) 503 3) 602 4) 558	1) 10.8 2) 8.1 3) 9.4 4) 9.4	1) 0.66 2) 0.72 3) 0.63 4) 0.61	-------	Hepatic TAG % at 7 DIM 1) 10.8 2) 6.1 3) 10.0 4) 10.0

[a] Concentrate mix included 6-15% whole cottonseed .
[b] concentrate mix only.
[c] Note: there are no fat x forage interactions; results are presented as 1) control (treatments 1, 3 and 5) and 2) fat (treatments 2, 4, and 6).
F = effect of fat.
* P < .10.
** P < .05.

Table 2.4. Continued.

Reference	Treatments/ Fat source	Dietary EE, %	DIM	Diet, % of DM	NEFA, µeq/L	BHBA, mg/dl	Insulin, ng/ml	IGF-1, ng/ml	Other
Schroeder et al., 2003	1) TMR fed control 2) Pasture + 6.7 kg/d corn based concentrate 3) Pasture + 6.7 kg/d concentrate with 0.8 kg CSFA	1) 4.5 2) 6.1 3) 8.1	117-152	Control: 59% corn silage, 41% concentrate Trt 2 and 3: ~84% pasture, 16% concentrate	1) 349 2) 289 3) 311	-------	-------	-------	-------
Moallem et al., 1999	1) Control 2) 0.55 kg/d CSFA 3) Control + bST	-------	0-150	8% wheat silage, 20% corn silage, 3% pea hay, 3% oat hay, 66% concentrate [a]	1) 159 2) 152 3) 125 (measured only at 50 DIM)	-------	-------	-------	Progesterone, ng/ml 1) 33.0 2) 55.4 3) 30.0 F**
Moallem et al., 1997	1) Control 2) 0.5 kg/d CSFA 3) Control + bST 4) 0.5 kg/d CSFA + bST	-------	0-150	10.4% wheat silage, 24% corn silage, 2.3% pea hay, 2.1% oat hay, 61.2% concentrate	F** (fat feeding increased NEFA concentrations)	N. S.	-------	-------	DIM to first ovulation 1) 24.5 2) 26.9 3) 28.4 4) 27.5
Sklan et al., 1994	1) Control 2) 2.5% CSFA	1) 2.8 2) 4.9	0-120	14.3% wheat silage, 15.3% corn silage, 6.4% vetch hay, 64% concentrate [a]	1) 153 2) 172 F x P*	-------	-------	-------	1st conception rate was lower for primiparous cows fed CSFA F x P**
Cervantes et al., 1996	1) Control 2) 0.4 kg/d CSFA 3) 12 g/d nicotinamide (NM) 4) 0.4 kg/d CSFA + 12 g/d NM	1) 3.1 2) 5.1 3) 3.0 4) 5.0	112-150	Varied dependent on stage of lactation. Forages (35-60% of diet) utilized were alfalfa hay, alfalfa haylage, and corn silage.	1) 120 2) 157 3) 126 4) 151 F*	1) 3.9 2) 3.9 3) 3.8 4) 3.3	-------	-------	-------
Sklan et al., 1991	1) Control 2) 2.6% CSFA	-------	0-120	13.7% corn silage, 11.3% vetch hay, 75% concentrate [a]	Fat fed had greater concentrations until 40 DIM**	-------	-------	-------	-------

[a] Concentrate mix included 6-15% whole cotton seed.
F = effect of fat.
P = effect of parity.
N.S. = not significant.
* P < .10.
** P < .05.

Table 2.5. Effects of feeding oilseeds alone or in combination with other fat sources to the same cows throughout the study on concentration of plasma hormones and metabolites.

Reference	Treatments/ Fat source	Dietary EE, %	DIM	Diet, % of DM	NEFA, µeq/L	BHBA, mg/dl	Insulin, ng/ml	IGF-1, ng/ml	Other
Drackley et al., 1998	1) Control 2) Control + 12 g/d niacin 3) 10% whole raw soybeans and 2.5% tallow 4) Treatment 3 + 12 g/d niacin	1) 2.75 2) 2.75 3) 6.04 4) 6.04	28-301	32.5% alfalfa haylage, 17.5% corn silage, 50% concentrate [a]	1) 98 2) 117 3) 134 4) 122 F** F x niacin** F x niacin x T**	1) 4.9 2) 4.8 3) 4.6 4) 5.3 F x T* F x niacin x T**	-------	-------	-------
Pires, et al., 1996	1) Control 2) 18% ground soybean 3) 18% whole soybean 4) 2.7% blood meal 5) 2.7% blood meal + 3 % tallow	1) 3.2 2) 6.2 3) 6.2 4) 3.2 5) 6.2	21-126	30% corn silage, 20% alfalfa silage, 50% concentrate	1) 197 2) 248 3) 200 4) 194 5) 233	-------	-------	-------	-------

[a] After 175 DIM, diets were adjusted for decreased nutrient requirements. Forage content increased to 60% of DM. For treatments 3 and 4 whole raw soybeans were removed and tallow was decreased to 2.25% of the diet.

F = effect of fat.

T = effect of time.

* P < .10.

** P < .05.

Table 2.6 Effects of feeding rendered fats to the same cows throughout the study on concentration of plasma hormones and metabolites.

Reference	Treatments/ Fat source	Dietary EE, %	DIM	Diet, % of DM	NEFA, µeq/L	BHBA, mg/dl	Insulin, ng/ml	IGF-1, ng/ml	Other
Bateman et al., 1996	1) 33% NDF, 0% tallow 2) 33% NDF, 2% tallow 3) 40% NDF, 0% tallow 4) 40% NDF, 2% tallow	1) 3.5 2) 5.4 3) 3.7 4) 5.8	120-240, winter	8% alfalfa hay, 22% corn silage, 14% alfalfa silage, 0 or 13% earlage, 56 or 43% concentrate	1) 190 2) 220 3) 160 4) 210 F*	-------	-------	-------	-------
Bateman et al., 1996	1) 33% NDF, 0% tallow 2) 33% NDF, 2% tallow 3) 40% NDF, 0% tallow 4) 40% NDF, 2% tallow	1) 3.5 2) 5.4 3) 3.7 4) 5.8	120-240, summer	8% alfalfa hay, 22% corn silage, 14% alfalfa silage, 0 or 13% earlage, 56 or 43% concentrate	1) 450 2) 510 3) 460 4) 540 F*	-------	-------	-------	-------
Salado et al., 2004	1) Control 2) 0.7 kg/d partially hydrogenated vegetable oil	1) 6.7 [b] 2) 22.5 [b]	-15-75	Pasture and 5 or 4 kg of concentrate offered for treatments 1 and 2, respectively	1) 659 2) 691	-------	1) 0.52 2) 0.55	1) 140.1 2) 112.5	-------

F = effect of fat.
* P < .10.
** P < .05.

CHAPTER 3
EFFECTS OF THE TIMING OF INITIATION OF FEEDING CALCIUM SOAPS OF LONG CHAIN FATTY ACIDS ON PERIPARTURIENT HOLSTEIN COWS DURING SUMMER

Materials and Methods

Cows and Diets

Holstein cows, housed at the Dairy Research Unit near Hague, FL (29° 44' N latitude, 82° 26' W longitude) were blocked by predicted calving date, parity (primiparous or multiparous), body weight (**BW**), and milk production of the previous year for multiparous cows and assigned randomly to treatment at approximately 28 d prior to their due date. Parturition occurred between April 24 and August 9, 2003 for all cows. A total of 58 cows were assigned to the experiment and calved having consumed their dietary treatment for at least 10 d prior to calving. However, due to lameness (n = 3), displaced abomasum (n = 3), death due to respiratory infection (n = 1), severe mastitis (n = 1), severe vaginal tear (n = 1), ketosis, retained fetal membranes (**RFM**) and static rumen for 10 continuous d (n = 1), and an undiagnosed condition (n = 1), only 47 cows (n = 25 multiparous and n = 22 primiparous) were included in the final data set. Sample collections, housing conditions, and animal care met the requirements of the "Animals Used for Teaching and Research Protocol" approved by the Animal Care and Use Committee at the University of Florida, Gainesville.

Animals were housed on pasture with shade and cooling fans provided beginning at approximately 28 d prior to parturition. At approximately 7 d prior to parturition, cows were moved to a sand-bedded barn with cooling fans and sprinklers to allow for close

observation of signs of impending parturition. After parturition cows were housed in an open-sided free stall barn equipped with sand bedding, cooling fans, sprinklers and self-locking stanchions where they were fed their assigned dietary treatment.

Two diets were prepared and fed as a total mixed ration (**TMR**) (Table 3.1 and 3.2) in ad libitum amounts twice daily to allow for 5 to 10% refusal in the prepartum and postpartum periods, respectively. Calcium salts of long chain fatty acids (**CSLCFA**, Megalac-R®, Church & Dwight Co., Princeton, NJ) were fed at 0 or 2.0% of dietary dry matter (**DM**). The fatty acid (**FA**) profile of CSLCFA provided by the manufacturer was 17.4% C16:0, 2.1% C18:0, 32.1% C18:1 *cis*, 1.5% C18:1 *trans*, 30.5% C18:2, 2.4% C18:3, and 12.2% other FA. Four experimental treatments were the following: 0% CSLCFA (Control), CSLCFA fed starting at 28 d prepartum, CSLCFA fed starting at 1 d in milk (**DIM**), and CSLCFA fed starting at 28 DIM. Once initiated, all diets continued through 100 DIM. Those cows receiving CSLCFA beginning in the postpartum period were fed CSLCFA at 1% of dietary DM for 7 d in order to adapt cows to CSLCFA slowly.

Sample Collection

Representative samples were obtained of corn silage, bermudagrass hay, alfalfa hay, and concentrate mixes on a weekly basis. Corn silage was immediately dried at 55°C for 48 h in a forced air oven in order to calculate concentration of DM and maintain the same formulated forage to concentrate ratio. The weekly samples were composited on a monthly basis, ground through a 1-mm Wiley mill screen (A. H. Thomas, Philadelphia, PA) and analyzed for chemical composition using wet chemistry (Dairy One, Ithaca, NY). Cows were milked three times per day at 0200, 1000, and 1800 h. Milk production was recorded at each milking. Milk samples were collected weekly

from two consecutive milkings and analyzed by Southeast Milk lab (Belleview, FL) for fat, true protein, and somatic cell count (**SCC**) using a Bently 2000 NIR analyzer. Body weight was measured weekly after the 0900 h milking. Body condition scores (**BCS**) were assigned by the same two individuals at -4, 0, 3, 6, 9, 12, and 14 wk postpartum (Edmonson et al., 1989). Blood was collected three times weekly from parturition until artificial insemination (**AI)** (72 ± 3 DIM) and again at 7 d post AI from the coccygeal or the jugular vessels immediately before the 1000 h milking using 13 x 100 ml vacutainer tubes containing sodium heparin (Becton Dickinson Vacutainer systems, Franklin Lakes, NJ). Samples were put immediately on ice until centrifuged at 2619 g at 5°C for 30 min (RC-3B refrigerated centrifuge, H 600A rotor, Sorvall Instruments, Wilmington, DE). Plasma was decanted and frozen at -20°C. Liver samples were collected at 2, 14 ± 1, and 28 ± 1 DIM via liver biopsy, rinsed with sterile saline and immediately frozen in liquid nitrogen (-192°C) and stored at -80°C.

Reproductive Management

The previous pregnant uterine horn was determined as the longer horn with the greater diameter using a real time Ultrasound Aloka 500 scanner (Aloka Co., Ltd, Tokyo, Japan) equipped with a 5.0 MHz linear rectal transducer. The diameters of the uterine horn and cervix were measured at 21 ± 3 and 28 ± 3 DIM. The transducer was placed in a transverse position in relation to the horns, at approximately 4 cm past the bifurcation of the horns. When the transducer was positioned and the horns could be seen clearly, the image was fixed. Pressure with the transducer on the uterine horns was avoided in order to obtain a circular cross-section image of the horns. Machine calipers were activated such that a vertical line was extended from serosa to serosa of the uterine horn cross-section. Cervical diameter was measured by placing the transducer in a transversal

position in relation to the cervix at its middle section and the distance between two points was obtained as described above. A 7.5 MHz linear rectal transducer was used to monitor ovarian structures. Size, location and number of follicles were recorded at 21 and 28 DIM and categorized by size into Class 1 (2 to 5 mm), Class 2 (6 to 9 mm), and Class 3 (≥ 10 mm) follicles (Lucy et al., 1992). The location and size of corpus luteum (**CL**) (determined by measurement of length and width) were recorded.

At 21 ± 3 and 28 ± 3 DIM, uterine tonus was classified as none, moderate, and intense. Also, vaginal examinations were conducted to characterize uterine condition at 21 ± 3 and 28 ± 3 DIM using a glass speculum inserted into the vagina until the cervical os could be seen. The cervix was classified as open or closed, the cranial vagina was classified as pink or red, and vaginal discharge was classified according to amount (none, trace, slight, moderate, or copious) and quality (none, clear mucous, cloudy mucous, mucopurulent, or purulent).

Cows were enrolled in a Pre-synchronization/Ovsynch® protocol beginning at 44 ± 3 DIM by injecting gonadatropin releasing hormone (**GnRH**) (100 μg, Gonadorelin Diacetate Tetrahydrate, Cystorelin®, Merial Ltd., Athens, GA) and ovaries were scanned every other day for 7 d. At 51 ± 3 DIM, prostaglandin $F_{2\alpha}$ (**$PGF_{2\alpha}$**) (25 mg, Lutalyse®, Pharmacia Upjohn, Kalamazoo, MI) was injected. Ultrasound examination of the ovaries continued until the formation of a new CL. Eleven days later (62 ± 3 DIM), GnRH was injected again (100 μg), followed by $PGF_{2\alpha}$ (25 mg) 7 d later (69 ± 3 DIM), and GnRH (100 μg) 48 h following $PGF_{2\alpha}$ (71 ± 3 DIM). Ovaries were scanned by ultrasonagraphy at each injection day. All hormone injections were given intramuscularly (i.m.) after a blood collection from coccygeal vessels. Cows were inseminated at 72 ± 3 DIM (16 h

following GnRH) using the same batch of semen by the same trained inseminator. Immediately after AI, an injection of bST (POSILAC® Monsanto Co., St. Louis, MO) was given in the ischiorectal fossa and continued every 14 d thereafter. At 7 d post AI (79 ± 3 DIM), ovaries were examined by ultrasound for the presence of a CL. Cows with no CL were reenrolled in Ovsynch®. At 28 d post AI (100 ± 3 DIM), pregnancy was determined by identifying the presence of embryonic fluid, appearance of embryo and an embryonic heart beat using ultrasound. Pregnancy was confirmed by rectal palpation at 45 and 72 d post AI by the farm veterinarian.

Animal Health

Body temperature was measured the first 5 d postpartum using a rectal thermometer. Cows were diagnosed as having RFM if membranes were still attached 24 h after parturition. Treatment of RFM was penicillin (50 cc, i.m.) for 5 d. Cows having a displaced abomasum had corrective surgery performed by the farm veterinarian, followed by administration with dextrose (500 ml) and vitamin B complex (15 cc) intravenously plus calcium propionate (300 ml) and 30 g of Probios (Vets Plus Inc., Knapp, WI) orally. Ketosis was diagnosed using Ketostix® (Bayer, Pittsburgh, PA). Treatment for ketosis was dextrose (500 ml) and vitamin B complex (15 cc) intravenously and 30 g of Probios (Vets Plus Inc., Knapp, WI) orally. Cows having a rectal temperature > 39.4°C and with no other apparent health conditions were diagnosed as having metritis and treated with 0.23 to 0.45 mg/kg BW of Nexcel (ceftiofur sodium sterile powder, Pfizer Inc., New York, NY).

Sample Analysis

Plasma concentrations of nonesterified fatty acids (**NEFA**) (NEFA-C kit; Wako Fine Chemical Industries USA, Inc., Dallas, TX; as modified by (Johnson, 1993) and β-

hydroxy butyric acid (**BHBA**) (Pointe Scientific Inc., Lincoln Park, MI) were determined once weekly. A Technicon Autoanalyzer (Technicon Instruments Corp., Chauncey, NY) was used to determine weekly concentrations of blood urea nitrogen (**BUN**) (a modification of (Coulombe and Favreau, 1963) as described in Bran + Luebbe Industrial Method #339-01) and plasma glucose (a modification of (Gochman and Schmitz, 1972) as described in Bran + Luebbe Industrial Method #339-19).

A double antibody radioimmunoassay (**RIA**) was used to determine plasma concentrations of insulin (Badinga et al., 1991; Malven et al., 1987) and IGF-1 (Badinga et al., 1991) on every plasma sample collected. The sensitivity of the insulin assay was 0.3 ng/ml, and intra- and interassay coefficients of variation (**CV**) were 9.1 and 14.9%, respectively. The sensitivity of the IGF-1 assay was 50 pg/ml, and intra- and interassay CV were 11.4 and 12.1%, respectively. Weekly leptin concentrations were determined by RIA at the University of Missouri (Delavaud et al., 2000). Intra- and interassay CV were less than 10%. Concentrations of plasma progesterone were determined on every plasma sample collected using Coat-A-Count Kit (DPC® Diagnostic Products Inc., Los Angeles, CA) solid phase ^{125}I RIA. The sensitivity of the assay was 0.1 ng/ml and the intra- and interassay CV were 0.8 and 5.2%, respectively. A polyethylene glycol RIA procedure described by Meyer et al. (1995) was used to analyze the concentration of 15-keto-13,14-dihydro-prostaglandin F_2 (**PGFM**) on daily plasma samples collected during the first 10 DIM. The sensitivity of the assay was 31.25 pg/ml and the intra- and interassay CV were 8.2 and 20.6%, respectively.

The three plasma samples collected weekly were composited into one sample during each of the first 4 wk postpartum and analyzed for concentrations of alkaline

phosphatase (**ALK**) (Diagnostic Chemicals Ltd, Oxford, CN), alanine aminotransferase (**ALT**) (Abbott Diagnostics, Abbott Park, IL), aspartate aminotransferase (**AST**) (Abbott Diagnostics, Abbott Park, IL), gamma glutamyl transferase (**GGT**) (Thermo DMA, Arlington, TX), albumin (Diagnostic Chemicals Ltd, Oxford, CN), and total biliruben (Wako Chemicals USA, Inc., Richmond, VA) using a Hitachi 911 chemistry analyzer (Roche Diagnostics, Indianapolis, IN).

Acute phase protein concentrations were determined on two samples per week for 4 wk postpartum. Plasma fibrinogen was determined from a standard curve generated using a human reference (Sigma Diagnostics). Plasma haptoglobin concentrations were determined by measuring haptoglobin/hemoglobin complexing (Makimura and Suzuki, 1982). Ceruloplasmin oxidase activity was measured using colorimetric procedures described by Demetriou et al. (1974).

Total liver cellular RNA was isolated from samples collected at 2, 14, and 28 DIM from eight cows in the control, fat pre, and fat 1 DIM groups (n = 72) using TRIzol reagent (Life Technologies, Grand Island, NY) according to the manufacturer's directions. Ten μg of RNA was fractionated in a 1.0% agrose-formaldehyde gel and transferred overnight to BioTrans nylon membranes (ICN, Irvine, CA) by capillary action. The RNA was cross linked to the membrane by exposure to ultraviolet light for 90 sec and the membrane was heated at 80°C for 1 h. The membranes were prehybridized for 30 min with buffer (ULTRAhyb buffer, Ambion Inc., Austin, TX) to block non-specific binding sites. Membranes were then hybridized to ^{32}P-labeled IGF-I, IGF-II, and IGFBP-2 cDNA probes, respectively. After hybridization, filters were washed for 15 min in 50 ml of 2X SSC, 0.1% SDS at 50°C, followed by two 15-min

washes in 50 ml of 0.1X SSC, 0.1% SDS at 50°C. Filters were blotted dry and exposed to x-ray film (Super RX, Fugi Film, Japan) for 3 to 96 h at -80°C. Hybridization signals were quantified by densitometric analysis (Eastman Kodak, Rochester, NY).

Triacylglycerol (**TAG**) concentrations in liver samples (75 to 150 mg, wet weight) were determined colorimetrically (Foster and Dunn, 1973) by first extracting the total lipid as described by Drackley et al. (1992). Dry matter content of liver (~100 mg) was determined by drying in a forced air oven for 48 h at 55°C.

Statistical Analysis

Data were analyzed as a completely randomized design using the PROC MIXED procedure for repeated measurement of SAS (SAS software statistics, 2001) according to the following mathematical model:

$$Y_{ijkl} = \mu + D_i + P_j + DP_{ij} + C_{k\,(i\,j)} + W_l + DW_{il} + PW_{jl} + DPW_{iikl} + E_{ijkl}$$

where Y_{ijkl} is the observation, μ is the overall mean, D_i is the fixed effect of diet (i = 1, 2, 3, and 4), P_j is the fixed effect of parity (j = 1 and 2), DP_{ij} is the interaction of diet and parity, $C_{k\,(i\,j)}$ is random effect of cow within diet and parity (k = 1, 2, …n), W_l is the fixed effect of week (l = 0, 1, 2, …14), DW_{il} is the interaction of diet and week, PW_{jl} is the interaction of parity and week, DPW_{ijl} is the three way interaction of diet, parity and week, and E_{ijkl} is the residual error.

Results are reported as least square means. Significance was determined at $P \leq 0.05$ and tendencies included $P > 0.05$ and ≤ 0.10. Orthogonal contrasts used included 1) no CSLCFA versus CSLCFA (control vs. (CSLCFA pre, CSLCFA 1 DIM, plus CSLCFA 28 DIM)), 2) CSLCFA feeding initiated prepartum vs. CSLCFA feeding initiated postpartum (CSLCFA pre vs. (CSLCFA 1 DIM plus CSLCFA 28 DIM)), and 3) CSLCFA initiated at 1 DIM versus CSLCFA initiated at 28 DIM. These three contrasts

by parity interactions were tested also. In addition, for dependent variables measured only before 28 DIM, the cows assigned to the CSLCFA 28 DIM treatment were combined with control cows. For this data set (when only three treatments had been initiated), a separate set of orthogonal contrasts was used. Contrasts were 1) CSLCFA feeding initiated in the prepartum vs. no CSLCFA feeding in the prepartum period (CSLCFA pre vs. (Control plus CSLCFA 1 DIM plus CSLCFA 28 DIM)), and 2) no CSLCFA (Control plus CSLCFA at 28 DIM) vs. CSLCFA initiated at 1 DIM. These two contrasts by parity interactions were tested also.

Data for NEFA, BHBA, BUN, glucose, leptin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase, albumin, total biliruben, hepatic mRNA, hepatic TAG, milk production, BW, and BCS were evaluated using repeated measures of the Mixed procedure of the SAS software program. Data were tested to determine the structure of best fit, namely AR (1), ARH (1), CS, or CSH, as indicated by a lower Schwartz Baesian information criterion value (Littell et al., 1996). Orthogonal contrasts (cited above) were used to determine if the mean values were different. If main effects or contrasts were significant, the slice command was used to determine at which time points the treatments differed. If slice was significant, then the pdiff command was used to determine which treatments were different at that time point.

Data that did not have a common day in milk (insulin, IGF-I, P4, PGFM, fibrinogen, haptoglobin, and ceruloplasmin) were modeled using the CS structure as a polynomial function of time using regression analysis and coefficients were obtained to plot the curves after the level of order (linear, quadratic, cubic, quartic, or quintic) that

best fit the data was determined. Heterogeneity of regression was performed to determine if the curves differed for each of the orthogonal contrasts cited above.

Data for average milk protein concentration, average milk fat concentration, average milk protein yield, average milk fat yield, 3.5% fat corrected milk (**FCM**) yield, vaginal exams, and 21 DIM and 28 DIM ovarian maps were analyzed using the Proc GLM procedure of SAS.

Odds ratios and their confidence intervals were obtained using the Chi square and PROC LOGISTIC procedures for testing of first service conception rates, estrus cycling rates (the first day postpartum of ovulation was determined by plasma progesterone concentrations > 1 ng/ml for two consecutive sampling days) and incidence of disease.

Results and Discussion

Dry Matter Intake and Diets

Concentrations of nutrients in experimental diets were within or exceeded the acceptable targets for close-up nonlactating cows and lactating cows at 90 DIM (NRC, 2001) (Tables 3.1 and 3.2). The ether extract values of the diets containing CSLCFA increased 1.3 and 1.6 percentage units for the close-up and lactating diets respectively.

During the prepartum period, cows consumed an average of 10.0 and 10.2 kg of DM/d when offered the 0 or 2% CSLCFA diets, respectively. Cows were fed diets an average of 24.8 ± 7.4 d prior to parturition. During the postpartum period, cows consumed an average of 17.5 and 16.4 kg of DM/d when offered the 0 or 2% CSLCFA diets, respectively. In a review of several studies, Allen (2000) reported a linear decline in DMI of 2.5% for every 1% inclusion of CSLCFA in the diet DM. Although not statistically evaluated, the difference of 6.3% is close to the 5% depression of intake predicted by Allen's (2000) equations.

Production and Body Weight

As expected, multiparous cows were heavier (644 vs. 530 kg; P = 0.001) and produced more milk (43.4 vs. 41.0 kg/d; P = 0.041) than primiparous cows (Table 3.3). In addition, multiparous cows tended to have a greater SCC than did primiparous cows (586 vs. 354 x 1000/ml; P = 0.067; Table 3.3), however no treatment differences were detected.

It is well documented that milk production often increases when supplemental fats are fed. This may be due to increased energy intake. In the current study, cows fed CSLCFA in the prepartum period tended to produce more milk (42.2 vs. 37.1 kg/d; P = 0.059) and 3.5% FCM (41.3 vs. 36.5 kg/d; P = 0.087) the first 100 DIM than cows fed CSLCFA beginning in the postpartum period (Table 3.3). Feeding CSLCFA beginning prepartum probably increased milk production due to increased energy intake and because the cows did not have the additional stress of adjusting to a new dietary ingredient early in lactation. Frequently there is a three to four wk delay before milk production increases when supplemental fats are added during the postpartum period (Garcia-Bojalil et al., 1998; Schingoethe and Casper, 1991). In contrast to the results of this study, Salfer and coworkers (1995) did not detect any difference in milk yield or 3.5% FCM yield of cows fed diets of 0 or 2% partially hydrogenated tallow (**PHT**) beginning at 14 d prepartum, at 1 DIM, or at 35 DIM through 151 DIM. However, when only the first 35 DIM were considered, yield of 3.5% FCM by cows fed PHT beginning at 1 DIM tended to be greater than that of cows fed PHT beginning 14 d prepartum (33.4 vs. 36.9 kg/d).

Milk protein concentration was reported to not change (Atwal et al., 1990; Markus et al., 1996; Moallem et al., 2000) or to decline when supplemental fats were fed

(AbuGhazaleh et al., 2004; Cervantes et al., 1996; Drackley et al., 1998). Protein concentration in the milk of primiparous cows was 2.63% for both no CSLCFA and CSLCFA fed groups, however multiparous cows fed CSLCFA had increased milk protein concentration in comparison to cows not fed CSLCFA (2.70 vs. 2.47%) (no CSLCFA vs. CSLCFA by parity interaction, P = 0.091). This increase could be due to more ruminal microbes delivering protein for protein synthesis by the mammary gland. When fat is fed, bacteria numbers may increase in concurrence with a decrease in protozoal populations (Sutton et al., 1983). Likely due to increased milk production, milk protein yield tended to be greater by cows fed CSLCFA prepartum in comparison to cows fed CSLCFA beginning postpartum (1.15 vs. 1.02 kg/d; P = 0.08).

In this study, no treatment or parity differences were detected for average milk fat concentration (3.44%) or yield (1.32 kg/d) through 100 DIM. In contrast, some studies reported an increase in milk fat concentration when CSLCFA were included in the diet (Cervantes et al., 1996; Moallem et al., 2000; Sklan et al., 1994; Sklan et al., 1991). The response of milk fat concentration to dietary fat is dependent upon many factors including the fat concentration and composition in the basal diet and in the supplement as well as the forage source and amount. The ruminally inert fat fed in this study should not have interfered with ruminal digestion and the diet included sufficient fiber from both corn silage and alfalfa hay. About 50% of the fat found in milk is synthesized in the mammary gland from acetate and butyrate, while the other 50% comes directly from fat absorbed from the blood (Ackers, 2002). Increased uptake of long chain FA by the mammary gland for milk fat synthesis may inhibit the synthesis of short and medium chain FA (Bauman and Griinari, 2003). Because we did not analyze the FA present in

the milk, we can only speculate that there was a balance between increased dietary FA uptake and decreased synthesis of short and medium chain FA in the mammary gland such that total concentration of milk fat did not differ from control cows. Palmquist and coworkers (1993) published an equation based on 49 published experiments predicting an increase in milk fat concentration of 0.18% due to feeding an additional 0.5 kg of fat daily. In the present study, milk fat concentration was 0.11% units greater for cows fed CSLCFA versus controls however this was not significantly different.

In agreement with several other studies in which fat supplementation was initiated in the periparturient period (Garcia-Bojalil et al., 1998; Kim et al., 1993; Moallem et al., 2000), treatment did not affect mean postpartum BW (587 kg). Multiparous cows not fed CSLCFA continued to gradually lose BW for 12 wk whereas those fed CSLCFA reached nadir by two to three wk postpartum. The pattern of BW for primiparous cows appeared similar for all treatment groups (treatment by parity by week interaction, $P < 0.001$, Figure 3.2). A treatment by week interaction was detected for body condition score ($P = 0.007$, Figure 3.3). Cows fed CSLCFA beginning at 1 or 28 DIM reached their nadir sooner and began gaining body condition earlier postpartum than controls or cows fed CSLCFA beginning prepartum. Greater loss of body condition by these latter two groups of cows was most likely due to less energy intake and greater milk yield respectively.

Plasma Metabolites

Typically, plasma BHBA concentrations are not affected by fat supplementation, but can decrease slightly in fat-supplemented cows if cows on the basal diet have high BHBA concentrations (Grummer and Carroll, 1991). In this study, mean plasma concentration of BHBA was lower for cows fed CSLCFA in comparison to controls (8.7 vs. 12.5 mg/dl, respectively; $P = 0.007$; Table 3.4). This difference was particularly

apparent for control versus CSLCFA 1 DIM and 28 DIM at wk 3, 4, and 5 postpartum and at wk 5 for CSLCFA prepartum ($P < 0.05$; Figure 3.4). Control cows may have been mobilizing more of their adipose tissue early in lactation to support milk production due to the lower energy dense diet than cows fed CSLCFA. In addition, an interaction of treatment and parity was detected (Table 3.4). Multiparous cows fed CSLCFA had lower concentration of plasma BHBA than control cows whereas that of primiparous cows was greater but unaffected by diet (no CSLCFA vs. CSLCFA by parity interaction, $P = 0.044$).

When fat is supplemented, plasma concentrations of NEFA routinely increase (Drackley, 1999), although there are several reports of numeric increases in NEFA concentrations that are not significant (Beam and Butler, 1998; Salado et al., 2004). In this experiment, control cows did not differ from cows fed CSLCFA, probably due to control cows and CSLCFA prepartum following a similar pattern while cows fed CSLCFA beginning at 1 DIM and 28 DIM were similar but followed a different pattern. Mean plasma concentrations of NEFA were greater for cows fed CSLCFA in the prepartum period in comparison to cows fed CSLCFA beginning postpartum (456 vs. 294 meq/L; $P = 0.002$). This difference was particularly evident at wk 3 and 4 postpartum ($P < 0.05$, Figure 3.5). A large portion of the plasma NEFA in cows fed fat prepartum may have been from adipose mobilization to support greater milk production because plasma BHBA followed a similar pattern although not significant. Grum et al. (1996) reported lower concentrations of plasma NEFA postpartum when cows were fed supplemental fat (Qual-Fat®) beginning prepartum in comparison to cows not fed supplemental fat prepartum.

Concentrations of plasma glucose are not affected generally by fat supplementation (Grummer and Carroll, 1991). However in this study, concentration of plasma glucose was increased in cows fed CSLCFA in comparison to controls (65.6 vs. 60.4 mg/dl; P = 0.004) but this was observed mainly in multiparous cows. Similar to what was observed with BHBA, multiparous cows not fed CSLCFA tended to have lower concentrations of plasma glucose than those fed CSLCFA (55.2 vs. 65.5 mg/dl) but primiparous cows across diets did not differ in plasma glucose (no CSLCFA vs. CSLCFA by parity interaction, P = 0.054). Elevated blood ketones are associated often with lowered blood glucose. In addition, concentration of plasma glucose was lower for cows fed CSLCFA beginning prepartum versus postpartum (62.8 vs. 67.0 mg/dl; P = 0.025) and was greater for primiparous cows versus multiparous cows (66.8 vs. 61.7 mg/dl; P = 0.001). A tendency for an interaction of treatment and week was detected (P = 0.10) and treatment differences were evident at wk 3, 4, 5, and 6 postpartum (P < 0.05; Figure 3.6). Control cows had lower concentrations of plasma glucose than those fed CSLCFA at 1 DIM and 28 DIM at wk 3, 4, and 5 postpartum, than those fed CSLCFA prepartum at wk 5 postpartum, and than those fed CSLCFA at 1 DIM at wk 6 postpartum. Cows fed CSLCFA prepartum had lower plasma concentrations of glucose than those fed CSLCFA at 28 DIM at wk 3 and lower than those fed CSLCFA at 1 DIM at wk 6 postpartum. Differences in plasma glucose concentrations may be due to differences in DMI and milk production between treatments and parities. In a review of dietary fat and adipose tissue metabolism, Chilliard (1993) noted that in 52 comparisons the difference in plasma glucose between control and fat-supplemented groups was 0 g/L (± 0.03).

Mean plasma concentrations of BUN tended to be greater for cows fed CSLCFA starting prepartum in comparison to cows fed CSLCFA starting postpartum (12.9 vs. 10.6 mg/dl; P = 0.053). This was especially true for multiparous cows (15.3 vs. 10.9 mg/dl) compared to primiparous cows (10.4 vs. 10.2 mg/dl) (CSLCFA prepartum vs. CSLCFA postpartum by parity interaction, P = 0.081). A tendency for a treatment by week interaction was detected (P = 0.06; Figure 3.7), however multiple means contrasts did not detect any differences among means. Individual DMI were not measured however milk production was greater by cows fed CSLCFA beginning prepartum, so we can speculate that cows fed CSLCFA beginning prepartum were consuming more DM and therefore more dietary protein than cows fed CSLCFA beginning postpartum.

Plasma Hormones

Leptin is a hormone synthesized by adipose tissue that is stimulated by adiposity and inhibited by undernutrition. Concentrations of leptin were decreased around the time of parturition in concurrence with negative energy balance and a reduction in adipose stores and may have been mediated by the reduction in plasma insulin (Block et al., 2003). Primiparous cows fed CSLCFA tended to have a lower concentration of plasma leptin in comparison to controls (2.15 vs. 3.06 ng/ml) whereas multiparous cows fed CSLCFA tended to have greater concentrations of leptin in comparison to controls (2.39 vs. 1.65 ng/ml; Table 3.4; Figure 3.8) (no CSLCFA vs. CSLCFA by parity interaction, P = 0.078). Low concentrations of circulating plasma leptin were correlated highly with greater milk production (Liefers et al., 2003). This relationship appears to fit with animals not fed CSLCFA; that is the higher producing multiparous cows had lower plasma concentrations of leptin compared to the lower producing primiparous cows (1.65 vs. 3.06 ng/ml). However when CSLCFA were included in the diet, this inverse

relationship disappeared. Increasing the triglyceride concentration of the blood may possibly influence leptin synthesis and release.

Insulin-like growth factors play a diverse role physiologically and are very dependent upon the nutritional state of the animal. Plasma IGF-I concentrations are correlated positively with body condition and DMI. During the periparturient period when cows are often in a negative energy balance, circulating concentrations of IGF-I are low (Vega et al., 1991). As the cow continues through lactation and a positive energy status is restored, circulating concentrations of IGF-I will increase. Low IGF-I concentrations were associated with an extended postpartum interval to estrus in beef cows and also with delayed puberty (Roberts et al., 1997; Rutter et al., 1989), indicating that IGF-I can be correlated positively with reproductive performance. Insulin-like growth factor I acts synergistically with luteinizing hormone (LH) to promote follicular development (Lucy, 2001). Beam and Butler (1998) reported lower mean concentrations of plasma IGF-I (37.6 vs. 47.7 ng/ml) from wk 1 to 3 postpartum in lactating dairy cows fed a diet of 2.6% prilled fat compared to no supplemental fat despite no differences in energy balance. However, other studies reported no differences in concentration of plasma IGF-I when supplemental fat was fed (Salado et al., 2004; Spicer et al., 1993).

In the present study, primiparous cows had a greater mean concentration of IGF-I in comparison to multiparous cows when consuming the control diet (77.1 vs. 54.3 ng/ml); however when CSLCFA were fed, primiparous cows had a lower mean concentration (77.1 vs. 64.2 ng/ml) whereas concentration of IGF-I increased in multiparous cows (54.3 vs. 68.2 ng/ml) (interaction of treatment and parity; $P = 0.040$) (Table 3.4). Concentrations of IGF-I were lowest immediately postpartum for all

treatments and rose throughout the lactation period. The quadratic pattern of plasma IGF-I concentration over time was different for animals fed the control diet and animals fed CSLCFA ($P < 0.01$; Table A-1) and for animals fed CSLCFA beginning prepartum versus beginning postpartum ($P < 0.01$; Table A-1). Likewise, the pattern over time was different for cows fed the control diet and cows fed CSLCFA by parity ($P < 0.05$; Table A-4) and for cows fed CSLCFA beginning prepartum versus beginning postpartum by parity ($P < 0.01$; Table A-4). Primiparous cows fed no CSLCFA experienced a more rapid increase in concentrations of plasma IGF-I after parturition than CSLCFA-fed cows before reaching a plateau at 49 DIM; however plasma IGF-1 of cows fed CSLCFA continued to rise over time (Figure 3.9A). At 77 DIM there was no difference in concentrations of plasma IGF-I between the two treatment groups. Multiparous cows fed CSLCFA had greater plasma IGF-I concentrations at parturition than cows not fed CSLCFA and their concentrations rose steadily over time so that at 77 DIM, cows fed CSLCFA had a much greater concentration than control cows (Figure 3.9B). Concentrations of plasma IGF-I of primiparous cows fed CSLCFA beginning prepartum versus postpartum differed little over time (Figure 3.10A). However, multiparous cows fed CSLCFA beginning postpartum had greater concentrations of IGF-I at parturition and rose slightly but steadily over time whereas those fed CSLCFA beginning prepartum rose at a greater rate starting at 42 DIM to surpass cows fed CSLCFA beginning postpartum at 77 DIM (Figure 3.10B). The differences in circulating IGF-I concentrations may have been due to differences in energy balance.

Fat supplementation has had mixed results on circulating concentration of plasma insulin (Staples et al., 1998). Florida workers (Garcia-Bojalil et al., 1998) reported

decreased insulin concentrations when periparturient cows were supplemented with CSLCFA at 2.2% of dietary DM, however others reported no difference between fat-fed cows and controls (Beam and Butler, 1998; Salado et al., 2004). Insulin has also stimulated ovarian follicle cell growth. When granulosa cells from small (1 to 5 mm) follicles were cultured, the addition of insulin increased cell numbers several fold and increased progesterone production in comparison to the control (Langhout et al., 1991). In the present experiment, plasma insulin concentrations increased gradually as week postpartum increased (Figure 3.11), however no treatment or parity effects for mean plasma concentration of insulin were detected (0.65 ng/ml, Table 3.4). Over time, eight individual quadratic curves for each treatment by parity combination fit the data significantly better than one pooled curve (Figure 3.11). However the orthogonal contrasts for treatment, parity, and treatment by parity were not significant for plasma concentrations of insulin over time (Table A-4). Although there were no treatment differences, it is noteworthy that plasma insulin and glucose concentrations followed a similar pattern.

Dietary fats typically increase concentrations of circulating cholesterol, the precursor of progesterone (Grummer and Carroll, 1991). Ruminants fed supplemental fat often have a slight increase in blood progesterone concentration (Staples et al., 1998). Progesterone, secreted by the CL, prepares the uterus for implantation of the embryo and helps maintain pregnancy by providing a nourishing environment for the conceptus. At breeding, greater concentrations of plasma progesterone has been associated with higher conception rates (Butler et al., 1996). Work by Hawkins et al. (1995) suggested that the increase seen in circulating progesterone when cows were fed supplemental fat was from

a reduced rate of clearance of progesterone rather than from an increased synthesis of progesterone. Son et al. (1996) reported greater blood cholesterol and peak plasma progesterone concentrations during the second ovulatory cycle in cows fed tallow at 2 vs. 0% of dietary DM accompanied by a tendency of improved conception. Workers at the University of Florida (Garcia-Bojalil et al., 1998) reported that accumulated plasma progesterone from 0 to 50 DIM was greater, pregnancy rates improved, and energy status did not change when cows were fed diets of 2.2% calcium salts of palm oil compared to non fat-supplemented cows.

The cubic pattern over time of plasma accumulated progesterone was different between parities when cows were fed the control versus the CSLCFA-supplemented diet ($P < 0.05$; Table A-4). Primiparous cows fed CSLCFA had a slightly greater rise in plasma concentration of accumulated progesterone beginning at 25 DIM compared to cows fed no CSLCFA whereas multiparous cows fed no CSLCFA had a slightly greater rise in plasma concentration of accumulated progesterone beginning at 23 DIM than cows fed CSLCFA (Figure 3.12). Cows were inseminated at 72 ± 3 DIM when there was very little difference in accumulated progesterone concentrations among treatment and parity groups. Patterns over time were detected to be different between parities when fed CSLCFA beginning at 1 DIM or at 28 DIM ($P < 0.01$; Table A-4). Upon closer examination of the data, two primiparous cows fed CSLCFA beginning at 1 DIM did not ovulate until 40 and 60 DIM (determined by two consecutive plasma progesterone concentrations > 1.0 ng/ml) which could have skewed the accumulated progesterone concentrations of the group. To determine if differences existed before cows were enrolled in a synchronization program, accumulated plasma progesterone concentrations

before the first hormone injection (46 DIM ± 3) were analyzed. The proportion of cows that ovulated before the first GnRH injection (44 ± 3 DIM) were 91% (10/11), 75% (9/12), 83% (10/12) and 100% (12/12) for control, CSLCFA prepartum, CSLCFA at 1 DIM, and CSLCFA at 28 DIM respectively and did not differ. The DIM at first ovulation (Table 3.4) did not differ among treatments either (mean of 27 DIM). The quadratic pattern over time differed for accumulated plasma progesterone concentrations of cows fed the control diet and cows fed CSLCFA by parity when only 1 to 46 DIM were included ($P < 0.01$; Table A-4; Figure 3.13); however patterns over time were not different between cows fed CSLCFA beginning at 1 DIM and at 28 DIM (Table A-4). We concluded that the difference in accumulated progesterone over time between cows fed CSLCFA beginning at 1 DIM and at 28 DIM was not different but was due to two animals that experienced delayed ovulation.

Through a series of desaturases and elongases, linoleic acid (**LA**) (C18:2) can form dihomo-λ-linolenic acid, a direct precursor to the series 1 prostaglandins, or can be further desaturated to arachidonic acid (C20:4), a direct precursor to the 2 series prostaglandins (Biochemistry of Lipids, Lipoproteins, and Membranes, 1996). Prostaglandin $F_{2\alpha}$, synthesized by endometrial tissue, is an important regulator of parturition and the estrous cycle by causing regression of the CL. Immediately prepartum, $PGF_{2\alpha}$ is important in regressing the CL of pregnancy and circulating $PGF_{2\alpha}$ concentrations decline as the postpartum uterus declines in size. Concentrations of PGFM may be associated with immune functions such as cellular immunity and neutrophil function. If LA is supplemented in the diet prepartum, more arachidonic acid

may be synthesized leading to higher concentrations of the series 2 prostaglandins and possibly a greater immune competence.

Using heterogeneity of regression, the cubic patterns of plasma concentrations of PGFM over time were different for cows fed CSLCFA prepartum in comparison to cows not fed CSLCFA prepartum ($P < 0.05$, Table A-2). However parity status also influenced this contrast (parity by no CSLCFA prepartum vs. CSLCFA prepartum interaction, $P < 0.05$, Table A-5). Primiparous cows fed CSLCFA prepartum had similar initial plasma PGFM concentrations as primiparous cows not fed CSLCFA prepartum, however peak concentrations (at ~4 DIM) were greater for cows fed CSLCFA prepartum and declined at a slower rate (Figure 3.14A). Multiparous cows not fed CSLCFA prepartum had high initial plasma PGFM concentrations, continued to decline and stabilized at 10 DIM. Multiparous cows fed CSLCFA prepartum had peak concentrations of plasma PGFM at 4 DIM, slowly declined and stabilized at 12 DIM (Figure 3.14B). The reason why there was an interaction of treatment and parity is unclear. When cows that experienced RFM and/or metritis were excluded from this analysis, the patterns and statistical significance were unchanged. Synthesis of PGFM in cows fed diets high in LA prepartum was greater postpartum possibly due to the increased intake of the direct precursors to the 2 series prostaglandins.

Reproductive Measurements

Size of the previous pregnant horn at 21 and 28 DIM did not differ between treatments or parities, nor did the change in uterine horn size from 21 to 28 DIM (Table 3.5). At 21 DIM, mulitparous cows fed CSLCFA prepartum had more uterine tonus than multiparous cows not fed CSLCFA prepartum, however this was not evident in primiparous animals (CSLCFA prepartum vs. no CSLCFA prepartum by parity

interaction; P = 0.050). This pattern did not carry over to measurements taken at 28 DIM. There were no differences between treatments or parities in the size of the cervical os at 21 DIM (3.38 cm). However at 28 DIM, multiparous cows fed CSLCFA prepartum tended to have a smaller cervical os than those not fed CSLCFA prepartum (2.97 vs. 3.30 cm) whereas this did not occur in primiparous cows fed CSLCFA prepartum versus those not fed CSLCFA (3.12 vs. 2.89 cm) (parity by CSLCFA prepartum vs. no CSLCFA prepartum interaction, P = 0.089). There were no differences between treatments or parities in whether the cervix was open or closed at 21 and 28 DIM (Table 3.5). At 21 DIM, cervical color was red only for some primiparous cows fed CSLCFA prepartum. Therefore the CSLCFA prepartum vs. no CSLCFA prepartum by parity interaction was significant (P < 0.001). Cervical color at 28 DIM was not affected by treatment or parity (Table 3.5).

Clinical endometritis as described by a purulent or foul discharge after 20 d postpartum or a mucopurulent discharge after 26 d postpartum was associated with a reduction of pregnancy rates (LeBlanc et al., 2002). In addition, abnormal vaginal discharge has been correlated with a delay in the first postpartum ovulation (Opsomer et al., 2000). Furthermore, if the first ovulation occurred in the presence of a uterus with heavy contamination, it led to prolonged luteal phases which was also associated with lower fertility (Opsomer et al., 2000). Primiparous cows had a greater (more purulent discharge) average score for vaginal discharge amount at 21 DIM in comparison to multiparous cows (2.32 vs. 1.77; P = 0.034), however at 28 DIM there were no differences between treatments or parities (Table 3.6). Primiparous cows had a greater average score for vaginal discharge quality (greater evidence of infection) at 21 DIM in

comparison to multiparous cows (3.37 vs. 1.42; P < 0.001), however at 28 DIM differences among treatments or between parities were not detected (Table 3.6).

Primiparous cows had less class 1 (P = 0.048) and class 2 follicles (P = 0.034) at 21 DIM in comparison to multiparous cows (Table 3.7), but the number of class 3 follicles tended to be greater at 28 DIM (P = 0.099) in primiparous compared to multiparous cows (Table 3.7). The number of class 1 (8.0 vs. 15.5; P = 0.09) and class 2 (0.7 vs. 2.8; P = 0.10) follicles at 21 DIM was less in multiparous cows fed CSLCFA prepartum compared to cows not fed CSLCFA prepartum (Table 3.7). However, there were no such changes in primiparous cows thus accounting for the interaction. These changes in follicle dynamics at 21 DIM reflects stimulation in the number of class 3 follicles for multiparous cows due to fat feeding prepartum. Number of class 3 follicles at 21 DIM were increased in multiparous cows fed CSLCFA prepartum (2.3 vs. 1.4; P = 0.06) with no differences among diets for the number of class 3 follicles of primiparous cows. This early stimulus in pre-ovulatory follicles (class 3) due to fat feeding prepartum was eliminated among the other groups by 28 DIM with primiparous cows tending to have greater overall number of class 3 follicles (2.2 vs. 1.6; P = 0.10). At 21 DIM, the number of CL tended to be fewer (0.4 vs. 0.9) and smaller (16.9 vs. 25.2 mm) for cows fed CSLCFA starting at 1 DIM compared to those not fed CSLCFA (Table 3.7). The number and size of CL present on the ovaries at 28 DIM did not differ between treatments or parities.

Stimulation of ovarian follicle development in cows fed supplemental fat often has been reported (Staples et al., 1998). Fat supplementation for 21 d prepartum did not affect follicle dynamics in cows fed isoenergetic diets containing 1.7% supplemental fat (prilled long chain FA) (Frajblat and Butler, 2003). However supplemental fat prepartum

resulted in greater pregnancy rates (86 vs. 58%). In contrast, workers in Missouri (Oelrichs et al., 2004) recently reported that cows fed soybeans beginning either prepartum or at calving had fewer small (< 5 mm) follicles and tended to have more medium size (6 to 9 mm) follicles than controls during the first synchronized estrous cycle. During the first estrous synchronization in the present study, there was no difference in number of class 2 follicles (6 to 9 mm), class 3 follicles (> 10 mm), number of CL, or size of CL present as determined by ultrasound on the day that GnRH was injected (d 0), nor on d 2, 4, 6, or 7 following GnRH (Table 3.8). Such changes were evident in the present study through 21 and 28 d postpartum. However, changes thereafter during the synchronization period were not detected. For example, during the same time frame, the number of class 1 follicles (2 to 5 mm) was fewer for cows fed CSLCFA prepartum compared to cows fed CSLCFA beginning in the postpartum period (10.4 vs. 14.8 averaged across the five measurement periods; P = 0.008). This may reflect a greater turnover of follicles in this group.

Improvement in conception rates when fat is supplemented in the diet is reported often (Staples et al., 1998). No change in conception rates due to fat feeding also is regularly reported (Holter et al., 1992; Oelrichs et al., 2004). In contrast, a few studies have reported a decrease in conception rates of cows fed supplemental fat (Erickson et al., 1992; Sklan et al., 1994), although it may have been due to greater milk production and a more negative energy balance which have been strongly linked to decreased fertility in dairy cattle. In this study, first service conception rates of cows that responded to synchronized ovulation were 27% (3/11), 40% (4/10), 70% (7/10), and 63% (7/11) for cows fed no CSLCFA, CSLCFA prepartum, CSLCFA beginning at 1 DIM, and CSLCFA

beginning at 28 DIM, respectively. Cows fed CSLCFA tended to have greater first service conception rates compared to cows not fed CSLCFA (58 vs. 27%, respectively, $P < 0.10$).

The number of cows per treatment in this study were too low to have a lot of confidence in the conception rate results however a 31% increase is noteworthy. Although improvement in first service conception rate was not due to differences in follicular dynamics during the first synchronized estrous, IGF-I acts synergistically with LH to promote follicular development (Lucy, 2001). Greater concentrations of plasma IGF-I around the time of breeding in multiparous cows fed CSLCFA (Figure 3.9) may have contributed to increased first service conception rates.

Hepatic Measurements

In the present study, mean hepatic TAG concentrations tended to be greater for cows fed CSLCFA beginning prepartum in comparison to cows not fed CSLCFA prepartum (16.7 vs. 10.4% of dry liver weight; $P = 0.080$; Table 3.9). Hepatic TAG concentrations in cows fed CSLCFA prepartum and control diets peaked at 14 DIM, however, cows fed CSLCFA beginning at 1 DIM did not decrease at 28 DIM but remained elevated (treatment by day interaction; $P = 0.04$; Figure 3.15). At 14 DIM the TAG concentration of cows fed CSLCFA prepartum tended to be greater than that of cows not fed CSLCFA prepartum ($P = 0.084$; Figure 3.15). Plasma NEFA concentrations were greater in cows fed CSLCFA prepartum than cows not fed CSLCFA prepartum in early lactation, which may account for the differences in TAG accumulation. In contrast, Illinois workers (Grum et al., 1996) reported decreased liver TAG concentrations at 1 DIM and a tendency for decreased plasma NEFA concentrations early postpartum in cows fed 6.5% of DM as fat for 50 d prepartum. They also reported a

positive correlation between concentrations of plasma NEFA at 3 d prepartum and the concentration of TG in the liver at 1 d postpartum. However, a later study (Douglas et al., 2004) in which cows were fed a moderate non-fiber carbohydrate (**NFC**) control diet, a low NFC diet supplemented with 4% fat prepartum and 2% fat postpartum, or a moderate NFC fat-supplemented diet beginning at 60 d prepartum revealed no treatment differences in DMI, milk production, plasma concentration of NEFA, or total hepatic lipid or TAG at 1 DIM. Additionally, recent research conducted at the University of Florida found no difference in hepatic TAG accumulation in cows fed control, 225 g/d of Ca salts of conjugated linoleic acid (**CLA**), or 225 g/d of Ca salts of *trans* C18:1 beginning at 28 d prepartum despite differences in plasma NEFA concentrations at 1 wk postpartum (Selberg et al., 2004). While others have reported a negative effect of elevated hepatic TAG on day to first estrus (Jorritsma et al., 2000) or to first ovulation (Marr et al., 2002), days to first ovulation in this study was not greater for the treatment group with elevated hepatic TAG concentration (CSLCFA prepartum).

Neither treatment nor parity affected hepatic IGF-I or IGF-II mRNA expression (Table 3.9; Figures 3.17 and 3.18). The liver is the primary source of circulating IGF-I and decreased hepatic IGF-I mRNA expression results in decreased circulating concentrations of IGF-I in cattle (Wang et al., 2003). In this study, the additional hepatic lipid present postpartum in cows fed CSLCFA prepartum was not detrimental to IGF-I or IGF-II mRNA expression. Cows fed CSLCFA beginning prepartum tended to have less IGFBP-2 mRNA expression in comparison to cows not fed CSLCFA prepartum (0.078 vs. 0.121 arbitrary units/18S; $P = 0.097$; Figure 3.19). Steady state expression of IGF-II mRNA expression, which is an indirect measurement of synthesis, was not affected by

treatment and steady state IGFBP-2 expression was lower in cows fed CSLCFA prepartum, indirectly implying that circulating IGF-II in cows fed CSLCFA prepartum was increased. Recently, University of Florida workers reported no treatment differences in hepatic IGF-I mRNA expression in cows fed a control diet, 225 g/d of calcium salts of CLA, or 225 g/d of *trans* C18:1 during early lactation (Selberg et al., 2003). However, IGF-II mRNA and IGFBP-2 abundance were greater for cows fed *trans* C18:1 than for cows fed the control diet or CLA. Expression of mRNA of IGF-1 (Figure 3.17), IGF-II (Figure 3.18), and IGFBP-2 (Figure 3.19) did not differ among biopsy days nor were treatment by day interactions detected to be significant for these three dependent variables.

Measurements of Immune Status

Immune reactions have been shown to be modulated by the diet, including the PUFA composition of the diet (Calder et al., 2002). Mechanisms involved in regulation are not yet understood, but evidence exists that the poly unsaturated FA composition of the diet influences cellular communication and activation through the synthesis of prostaglandins, tumor necrosis factor-α, and interferon-γ (Calder et al., 2002). Linoleic acid can be converted to arachidonic acid, the precursor for prostaglandin E_2 and leukotriene B_4 which are pro-inflammatory mediators. Lessard et al. (2004) evaluated cellular immune functions of dairy cows fed supplemental fat during the transition period. Cows were fed a diet containing 2.7% Ca salt of palm oil, 5.9% flaxseed (n-3 FA), or 9.4% micronized soybeans (n-6 FA) from 6 wk prepartum to calving followed by diets containing and 4.7% Ca salt of palm oil, 9.7% flaxseed, or 20.3% micronized soybeans from calving to 6 wk postpartum. The authors concluded that cellular immune functions were modulated around parturition; however feeding diets rich in n-3 or n-6 FA

did not have a major impact on cellular immune function. In the present study, incidence of disease (mastitis, metritis, or RFM) during the first 10 DIM was lower ($P < 0.05$) for cows fed CSLCFA prepartum (8%, 1/12) versus those not fed CSLCFA prepartum (35% [8/23] for the no CSLCFA treatment and 58% [7/12] for the CSLCFA at 1 DIM treatment) which could have had beneficial effects on milk production and reproduction.

In response to immunological stress, the liver will produce the acute phase proteins ceruloplasmin, fibrinogen, and haptoglobin (Baumann and Gauldie, 1994). It is unclear whether increased concentrations of acute phase proteins in lactating dairy cattle is due to greater stress (indicating an adverse state) or due to a greater immune response (indicating a healthier state). Fibrinogen is involved with blood clotting and the formation of the fibrin matrix for tissue repair. Increased fibrinogen concentrations are detected during internal hemorrhage or tissue damage. Normal values in cattle range from 100 to 600 mg/dl (The Merck Veterinary Manual, 1997). In this study, the range was 26 to 402 mg/dl. The concentrations rose, plateaued, then decreased during the first 4 wk postpartum. Mean plasma concentrations of fibrinogen during the first 27 d postpartum tended to be greater for control cows than for cows fed CSLCFA beginning at 1 DIM (123.8 vs. 100.2 mg/dl; $P = 0.07$; Table 3.10). Primiparous cows had greater fibrinogen concentrations when CSLCFA was withheld prepartum than when fed prepartum (114.4 vs. 70.5 mg/dl); however fibrinogen concentrations were not different for multiparous cows regardless of when CSLCFA feeding was initiated (no CSLCFA prepartum vs. CSLCFA prepartum by parity interaction; $P = 0.029$; Table 3.10).

Haptoglobin is responsible for binding iron, a limiting nutrient in bacterial growth. Because haptoglobin concentrations are normally undetectable in bovine blood unless

there is tissue damage, it can be a good indicator of the immunological stress response to parturition. In this study, the range was 0 to 329 mg of HbB/ 100 ml (the amount of hemoglobin bound by haptoglobin/ 100 ml of plasma). Mean plasma concentrations of haptoglobin during the first 27 d postpartum were greater for primiparous than multiparous cows (31.9 vs. 17.0 mg of HbB/ 100 ml of plasma; P = 0.043; Table 3.10), indicating that parturition caused a greater immune response in primiparous cows. Using heterogeneity of regression, the cubic pattern of plasma haptoglobin over time was affected by feeding CSLCFA and parity (Table A-5). Primiparous cows not fed CSLCFA prepartum had greater initial plasma concentrations of haptoglobin than cows fed CSLCFA prepartum and declined at a faster rate so that both treatment groups reached their nadir at 23 DIM (Figure 3.20A). Multiparous cows fed CSLCFA prepartum had very low initial concentrations of haptoglobin, rose to peak concentrations at 7 DIM and reached their nadir at 23 DIM. Multiparous cows not fed CSLCFA prepartum had higher initial concentrations and steadily declined through 27 DIM (Figure 3.20B). The trends over time indicate cows fed CSLCFA prepartum had less of an acute phase protein response to parturition than cows not fed CSLCFA prepartum, although it is unclear why haptoglobin concentrations in multiparous cows fed CSLCFA prepartum did not peak until 7 DIM.

Ceruloplasmin is involved with copper transport to tissues utilizing its antioxidant properties and concentrations will increase due to an inflammatory response of the cow. Normal values in cattle range from 16.8 to 34.2 mg/dl (The Merck Veterinary Manual, 1997). In this study, the range was from 9.0 to 51.0 mg/dl, suggestive of an acute inflammatory response to parturition. Mean plasma concentrations of ceruloplasmin

were greater for primiparous than for multiparous cows during the first 27 d postpartum (24.4 vs. 20.7 mg/dl; P = 0.007) indicating that parturition caused a greater immune response in primiparous cows. The pattern over time was not different among treatments or between parities (Figure 3.21).

Elevations in plasma concentrations of total biliruben are due to severe hepatic damage or extrahepatic obstruction, and following pregnancy. Normal values in cattle range from 0 to 0.5 mg/dl (The Merck Veterinary Manual, 1997). In this study, the range was 0.0 to 1.3 mg/dl. Mean plasma concentrations of total biliruben were greater for cows fed CSLCFA prepartum than for cows not fed CSLCFA prepartum (0.36 vs. 0.23 mg/dl; P = 0.04), although values were still within the normal range. An effect of week postpartum was detected (P < 0.001; Figure 3.22).

Elevations in plasma concentrations of ALT are due to hepatic disease although it is not a good indicator in cattle because of its low activity and it is not liver specific (The Merck Veterinary Manual, 1997). Mean plasma concentrations of ALT did not differ among treatments or between parities (21.6 IU/L; Table 3.10), however an effect of week postpartum was detected (P = 0.003; Figure 3.23).

Elevations in plasma concentrations of ALK are due to liver lesions or bile duct obstruction, however in cattle there is a very wide range of normal activity between animals (The Merck Veterinary Manual, 1997). Mean plasma concentrations of ALK were lower for multiparous cows in comparison to primiparous cows (31.7 vs. 44.1 U/L; Table 3.10). An effect of week postpartum was detected (P < 0.001; Figure 3.24).

Concentrations of AST are present in most tissues and it is not liver specific. Damage to the liver and the postparturient period in cattle cause leakage of large amounts

of AST into blood (The Merck Veterinary Manual, 1997). Mean plasma concentrations of AST did not differ among treatments or between parities (90.5 IU/L; Table 3.10), however an effect of week postpartum was detected (P = 0.01; Figure 3.25).

Plasma concentrations of GGT are of hepatic origin and can be a good indicator of liver and bile duct damage in cattle (The Merck Veterinary Manual, 1997). Mean plasma concentrations of GGT did not differ among treatments or between parities (30.0 U/L; Table 3.10), however an effect of week postpartum was detected (P = 0.01; Figure 3.25). At wk 4 postpartum cows fed CSLCFA prepartum had greater plasma concentrations of GGT than cows not fed CSLCFA prepartum (P = 0.03; Figure 3.26).

Plasma concentrations of albumin decrease due to the failure of hepatic parenchymal synthesis. In cattle, only 5% of serum albumin levels are synthesized per day, therefore it takes time to see hepatic damage (The Merck Veterinary Manual, 1997). Concentration of plasma albumin increased slightly with wk postpartum (P = 0.003; Figure 3.27). Initiation of CSLCFA supplementation at calving stimulated albumin concentrations in plasma of primiparous cows but reduced it in multiparous cows (no CSLCFA vs. CSLCFA 1 DIM by parity interaction; P = 0.018; Table 3.10).

Parity Effects

As expected, primiparous cows weighed less, produced less milk, and tended to have less SCC in the milk than multiparous cows. In addition, primiparous cows had greater circulating glucose and tended to have lower circulating BUN then multiparous cows. Parturition, removal of the calf, and the milking process is probably more stressful on first calf heifers than on multiparous cows. Indicative of a higher stress level, heifers had a greater immune response soon after parturition than cows as indicated by greater circulating concentration of the acute phase proteins, haptoglobin and ceruloplasmin, and

the liver enzyme ALK. At 21 DIM, primiparous cows had more vaginal discharge, a more purulent vaginal discharge, and fewer class 1 and class 2 follicles and tended to have more class 3 follicles at 28 DIM than multiparous cows.

Conclusion

Holstein cows began consuming a diet at 0 or 2% of dietary DM as CSLCFA at ~28 d prepartum. Cows fed the 0% CSLCFA diet either remained on a CSLCFA-free diet at parturition or were shifted to a 2% CSLCFA diet at either 1 or 28 DIM and remained on said diet until 100 DIM. Animals fed CSLCFA in the prepartum period continued to receive CSLCFA throughout the lactation period. Cows fed CSLCFA beginning in the perpartum period tended to produce more milk. This milk increase was accompanied by a tendency for elevated concentrations of TG in the liver at 14 DIM, a tendency for lower expression of hepatic IGFBP-2 mRNA, and elevated concentrations of plasma biliruben compared to cows not receiving CSLCFA prepartum. Multiparous cows appeared to benefit more from supplementation with CSLCFA than primiparous cows in that multiparous cows not fed CSLCFA at any time during the study had or tended to have lower concentrations of milk protein, longer and greater loss of BW, greater concentrations of plasma BHBA, and lower concentrations of plasma glucose, leptin, and IGF-I. In addition, multiparous cows fed CSLCFA prepartum tended to have fewer small and medium size but more larger size follicles (≥ 10 mm in size), more uterine tone at 21 DIM , a smaller cervical os at 28 DIM, and a slower decrease in plasma concentrations of PGFM the first 10 d postpartum than multiparous cows not fed CSLCFA prepartum. Multiparous cows fed CSLCFA had greater concentrations of IGF-I at the time of AI and all cows fed CSLCFA tended to have greater conception rate at first service regardless of day of initiation of CSLCFA supplementation.

Table 3.1: Ingredient and chemical composition of diets fed to nonlactating cows.

	Control	CSLCFA[1]
Ingredient, % of DM		
Corn silage	50	50
Bermudagrass hay	10	10
Ground corn	16.9	14.4
Citrus pulp	5.0	5.1
Soybean meal	11.8	12.4
Megalac- R®[1]	0.0	2.0
Mineral mix [2]	6.2	6.2
Trace mineral salt [3]	0.009	0.009
Chemical		
DM%	39.8	39.8
NE_L, Mcal/kg of DM	1.58	1.61
CP, % of DM	15.1	14.3
Ether extract, % of DM	3.1	4.4
NDF, % of DM	36.2	36.7
ADF, % of DM	21.3	21.6
Ca, % of DM	2.2	2.1
P, % of DM	0.33	0.32
K, % of DM	1.1	1.1
Mg, % of DM	0.35	0.33
Na, % of DM	0.24	0.25
Cl, % of DM	1.04	0.92
S, % of DM	0.26	0.26
Mn, mg/kg of DM	34	33
Cu, mg/kg of DM	12	14
Zn, mg/kg of DM	42	47
Fe, mg/kg of DM	257	249
Mo, mg/kg of DM	1.4	1.6

[1] Megalac- R®, Church & Dwight Co. Inc., Princeton, NJ.

[2] Mineral Mix contained 22.8% CP, 22.9% Ca, 0.20% P, 0.2% K, 2.8% Mg, 0.7% Na, 2.4% S, 8% Cl, 147 mg/kg of Mn, 27 mg/kg of Fe, 112 mg/kg of Cu, 95 mg/kg of Zn, 7 mg/kg of Se, 8 mg/kg of I, 11 mg/kg of Co, 268,130 IU of vitamin A/kg, 40,000 IU of vitamin D/kg, and 1129 IU of vitamin E/kg (DM basis).

[3] Minimum concentrations of 40% Na, 55% Cl, 0.25% Mn, 0.2% Fe, 0.033% Cu, 0.007% I, 0.005% Zn, and 0.0025% Co (DM basis).

Table 3.2: Ingredient and chemical composition of diets fed to lactating cows.

	Control	CSLCFA[1]
Ingredient, % of DM		
Corn silage	37.5	37.5
Alfalfa hay	10	10
Ground corn	24.2	21.9
Citrus pulp	5.0	5.0
Cottonseed hulls	2.5	2.5
Soybean meal	9.1	9.4
Soy Plus [2]	6.8	6.8
Bio Phos [3]	0.4	0.4
Megalac- R®[1]	0.0	2.0
Mineral mix [4]	4.5	4.5
Chemical		
DM%	46.2	46.3
NE_L, Mcal/kg of DM	1.67	1.71
CP, % of DM	16.9	16.7
Ether extract, % of DM	3.1	4.7
NDF, % of DM	30.9	30.2
ADF, % of DM	18.9	18.8
Ca, % of DM	1.15	1.51
P, % of DM	0.49	0.46
K, % of DM	1.27	1.27
Mg, % of DM	0.29	0.31
Na, % of DM	0.44	0.53
Cl, % of DM	0.34	0.39
S, % of DM	0.23	0.24
Mn, mg/kg of DM	71	86
Cu, mg/kg of DM	23	23
Zn, mg/kg of DM	88	106
Fe, mg/kg of DM	236	227
Mo, mg/kg of DM	2.1	1.6

[1] Megalac- R®, Church & Dwight Co. Inc., Princeton, NJ.

[2] West Central Soy, Ralston, IA.

[3] IMC-Agrico, Bannockburn, IL.

[4] Mineral Mix contained 26.4% CP, 10.2% Ca, 0.90% P, 3.1% Mg, 1.5 % S, 5.1% K, , 8.6 % Na, 11698 mg/kg of Zn, 512 mg/kg of Cu, 339 mg/kg of Fe,2231 mg/kg of Mn, 31 mg/kg of Co, 26 mg/kg of I, 7.9 mg/kg of Se, 147,756 IU of vitamin A/kg, and 787 IU of vitamin E/kg (DM basis).

Table 3.3. Milk yield, milk composition, postpartum body weight, and postpartum body condition score of Holstein cows fed diets without calcium salts of long chain fatty acids (CSLCFA) (no CSLCFA) or diets with CSLCFA starting prepartum, at 1 day in milk (DIM) or at 28 DIM.

	Treatments									Orthogonal contrasts[1], P =						
Measure	No CSLCFA		CSLCFA prepartum		CSLCFA 1 DIM		CSLCFA 28 DIM		SE	A	B	C	Parity	D	E	F
	P[2]	M[3]	P	M	P	M	P	M								
Milk yield, kg/d	36.0	41.0	39.5	44.8	35.4	38.3	34.7	39.9	3.1	0.924	0.059	0.896	0.041	0.933	0.816	0.710
3.5% FCM, kg/d	36.1	39.7	40.2	42.5	34.7	37.3	35.4	38.5	3.3	0.947	0.087	0.779	0.215	0.861	0.920	0.941
Milk fat, %	3.45	3.28	3.55	3.33	3.53	3.41	3.57	3.42	0.13	0.487	0.789	0.871	0.195	0.977	0.782	0.949
Milk protein, %	2.63	2.47	2.58	2.69	2.56	2.70	2.75	2.71	0.56	0.102	0.525	0.241	0.846	0.091	0.679	0.261
Milk fat yield, kg/d	1.27	1.36	1.43	1.44	1.21	1.28	1.26	1.34	0.09	0.912	0.114	0.659	0.451	0.865	0.783	0.967
Milk protein yield, kg/d	1.03	1.06	1.06	1.25	0.92	1.06	1.01	1.10	0.06	0.756	0.080	0.439	0.067	0.487	0.649	0.797
Milk SCC, x 1000/ml	407	750	463	466	264	700	282	428	121	0.324	0.760	0.465	0.067	0.614	0.400	0.407
BW, kg	530	644	539	621	518	646	533	664	28	0.991	0.676	0.555	< 0.001	0.992	0.325	0.957
BCS	3.06	2.77	2.88	3.10	3.02	3.31	3.13	2.95	0.19	0.363	0.501	0.534	0.937	0.220	0.626	0.231

[1] Orthogonal contrast of means were the following: A = No CSLCFA vs. CSLCFA, B = CSLCFA prepartum vs. (CSLCFA 1 DIM plus CSLCFA 28 DIM), C = CSLCFA 1 DIM vs. CSLCFA 28 DIM, D = contrast A by parity, E = contrast B by parity, and F = contrast C by parity.

[2] Primiparous cows.

[3] Multiparous cows.

Table 3.4. Concentration of plasma hormones and metabolites and day of first ovulation of Holstein cows fed diets without calcium salts of long chain fatty acids (CSLCFA) (no CSLCFA) or diets with CSLCFA starting prepartum, at 1 day in milk (DIM) or at 28 DIM.

	Treatments									Orthogonal contrasts [1], P =						
Measure	No CSLCFA		CSLCFA prepartum		CSLCFA 1 DIM		CSLCFA 28 DIM		SE	A	B	C	Parity	D	E	F
	P[2]	M[3]	P	M	P	M	P	M								
BHBA, mg/dl	10.7	14.2	10.4	9.7	10.2	6.7	8.4	6.9	1.6	0.007	0.146	0.629	0.635	0.044	0.501	0.520
NEFA, meq/L	384	450	432	480	400	241	289	245	56	0.149	0.002	0.348	0.588	0.217	0.130	0.316
Glucose, mg/dl	65.5	55.2	65.2	60.4	68.7	64.8	67.8	66.5	2.1	0.004	0.025	0.846	0.001	0.054	0.553	0.533
Blood urea nitrogen, mg/dl	10.2	11.5	10.4	15.3	9.8	10.9	10.6	11.0	1.3	0.682	0.053	0.744	0.055	0.706	0.081	0.808
Leptin, ng/ml	3.06	1.65	2.83	2.62	1.91	1.99	1.70	2.57	0.39	0.846	0.156	0.729	0.669	0.078	0.465	0.470
IGF-I, ng/ml	77.1	54.3	62.2	60.6	64.3	69.9	66.2	74.1	7.5	0.953	0.261	0.683	0.641	0.040	0.487	0.885
Insulin, ng/ml	0.62	0.54	0.65	0.52	0.71	0.75	0.74	0.63	0.09	0.236	0.102	0.608	0.320	0.975	0.536	0.449
Accumulated progesterone, 1 to 77 DIM, ng/ml	22.0	27.4	24.0	24.0	21.9	26.2	26.5	23.3	4.0	0.948	0.884	0.779	0.555	0.516	0.906	0.602
Accumulated progesterone, 1 to 46 DIM, ng/ml	7.3	11.1	8.7	10.9	7.9	9.7	9.1	8.0	2.4	0.942	0.806	0.930	0.467	0.606	0.931	0.847
DIM at first ovulation	27.8	26.3	29.7	27.8	32.2	27.5	22.8	24.6	4.0	0.910	0.560	0.125	0.587	0.987	0.955	0.417

[1] Orthogonal contrast of means were the following: A = No CSLCFA vs. CSLCFA, B = CSLCFA prepartum vs. (CSLCFA 1 DIM plus CSLCFA 28 DIM), C = CSLCFA 1 DIM vs. CSLCFA 28 DIM, D = contrast A by parity, E = contrast B by parity, and F = contrast C by parity.

[2] Primiparous cows.

[3] Multiparous cows.

Table 3.5. Concentration of plasma PGF2α metabolite (PGFM) the first 14 DIM and the size and characteristics of the uterus and cervix of Holstein cows fed diets without calcium salts of long chain fatty acids (CSLCFA) (no CSLCFA) or diets with CSLCFA starting prepartum, or at 1 day in milk (DIM).

	Treatments							Orthogonal contrasts [1], P =				
Measure	No CSLCFA		CSLCFA prepartum		CSLCFA 1 DIM		SE	A	B	Parity	C	D
	P[2]	M[3]	P	M	P	M						
PGFM, pg/ml	1004	1186	1443	1412	1536	955	244	0.261	0.722	0.418	0.509	0.111
Previous pregnant horn size at 21 DIM, cm	2.43	2.52	2.38	2.62	2.55	3.95	0.18	0.500	0.133	0.114	0.984	0.376
Previous pregnant horn size at 28 DIM, cm	2.24	2.60	2.50	2.35	2.42	2.59	0.20	0.841	0.655	0.452	0.256	0.613
Change in uterine horn size from 21 to 28 DIM, cm	-0.20	0.08	0.12	-0.27	-0.13	-0.36	0.20	0.683	0.357	0.456	0.269	0.199
Uterine tonus[4] at 21 DIM	1.80	1.77	1.50	2.17	2.17	1.50	0.28	0.923	0.855	0.964	0.050	0.237
Uterine tonus[4] at 28 DIM	1.70	1.69	1.33	2.00	1.67	1.83	0.26	0.815	0.832	0.209	0.227	0.731
Size of cervical os at 21 DIM, cm	3.20	3.59	3.35	3.54	3.25	3.38	0.19	0.610	0.663	0.140	0.852	0.480
Size of cervical os at 28 DIM, cm	3.12	3.23	3.12	2.97	2.67	3.38	0.18	0.727	0.387	0.136	0.089	0.086
Cervix closed (1) or open (2) at 21 DIM	1.20	1.00	1.00	1.00	1.00	1.00	0.07	0.460	0.164	0.277	0.460	0.164
Cervix closed (1) or open (2) at 28 DIM	1.10	1.00	1.00	1.00	1.00	1.00	0.05	0.622	0.350	0.467	0.622	0.350
Cervical color, pink (1) or red (2) at 21 DIM	1.00	1.00	1.50	1.00	1.00	1.00	0.07	< 0.001	1.00	0.007	< 0.001	1.00
Cervical color, pink (1) or red (2) at 28 DIM	1.00	1.08	1.17	1.00	1.00	1.00	0.08	0.367	0.606	0.639	0.152	0.606

[1] Orthogonal contrast of means were the following: A = (No CSLCFA plus CSLCFA 1 DIM) vs. CSLCFA prepartum, B = No CSLCFA vs. CSLCFA 1 DIM, C = contrast A by parity, and D = contrast B by parity.

[2] Primiparous cows.

[3] Multiparous cows.

[4] Score of 1 = no tonus, 2 = moderate tonus, and 3 = tonus.

Table 3.6. Vaginal observations at 21 and 28 DIM of Holstein cows fed diets without calcium salts of long chain fatty acids (CSLCFA) (no CSLCFA) or diets with CSLCFA starting prepartum, or at 1 day in milk (DIM).

	Treatments							Orthogonal contrasts [1], P =				
Measure	No CSLCFA		CSLCFA prepartum		CSLCFA 1 DIM		SE	A	B	Parity	C	D
	P[2]	M[3]	P	M	P	M						
Vaginal discharge amount[4] at 21 DIM	2.30	2.15	2.33	1.50	2.33	1.67	0.30	0.481	0.441	0.034	0.445	0.377
Vaginal discharge amount[4] at 28 DIM	2.40	1.46	1.83	2.00	2.17	1.83	0.33	0.872	0.828	0.182	0.189	0.344
Vaginal discharge quality[5] at 21 DIM	3.60	1.92	2.67	1.00	3.83	1.33	0.60	0.133	0.759	< 0.001	0.702	0.479
Vaginal discharge quality[5] at 28 DIM	2.50	1.08	1.50	1.67	3.33	2.00	0.69	0.313	0.193	0.137	0.228	0.946

[1] Orthogonal contrast of means were the following: A = (No CSLCFA plus CSLCFA 1 DIM) vs. CSLCFA prepartum, B = No CSLCFA vs. CSLCFA 1 DIM, C = contrast A by parity, and D = contrast B by parity.

[2] Primiparous cows.

[3] Multiparous cows.

[4] Score of 1 = none, 2 = trace, 3 = slight, 4 = moderate, or 5 = copious.

[5] Score of 0 = none, 1 = clear, 2 = cloudy, 3 = mucous with pus, 4 = mucopurulent, or 5 = purulent.

Table 3.7. The number and size[1] of ovarian structures at 21 and 28 DIM of Holstein cows fed diets without calcium salts of long chain fatty acids (CSLCFA) (no CSLCFA) or diets with CSLCFA starting prepartum, or at 1 day in milk (DIM).

	Treatments							Orthogonal contrasts[2], P =				
	No CSLCFA		CSLCFA prepartum		CSLCFA 1 DIM							
Measure	P[3]	M[4]	P	M	P	M	SE	A	B	Parity	C	D
Number of class 1 follicles at 21 DIM	8.4	12.8	9.0	8.0	10.7	18.3	2.2	0.050	0.074	0.048	0.087	0.451
Number of class 2 follicles at 21 DIM	1.1	2.8	0.8	0.7	0.5	2.8	0.7	0.111	0.695	0.034	0.100	0.627
Number of class 3 follicles at 21 DIM	1.4	1.5	1.0	2.3	1.8	1.3	0.4	0.718	0.780	0.358	0.057	0.437
Number of CL[5] at 21 DIM	0.7	1.2	0.8	1.0	0.3	0.5	0.3	0.442	0.080	0.278	0.756	0.555
Size of CL at 21 DIM, mm	22.9	27.5	23.0	29.0	12.3	21.5	4.6	0.256	0.084	0.103	0.915	0.616
Number of class 1 follicles at 28 DIM	14.0	14.3	10.5	9.7	12.0	16.3	2.3	0.058	0.995	0.504	0.454	0.365
Number of class 2 follicles at 28 DIM	2.0	2.5	1.3	1.5	0.7	1.3	0.7	0.723	0.055	0.411	0.723	0.921
Number of class 3 follicles at 28 DIM	2.1	1.7	2.7	1.8	1.8	1.5	0.4	0.181	0.530	0.099	0.505	0.919
Number of CL at 28 DIM	0.9	0.9	0.7	1.2	0.8	1.2	0.3	0.880	0.746	0.227	0.536	0.571
Size of CL at 28 DIM, mm	27.1	24.3	27.0	34.4	33.2	26.9	5.3	0.581	0.396	0.903	0.295	0.729

[1] Class 1 follicles were 2 to 5 mm, class 2 follicles were 6 to 9 mm, and class 3 follicles were ≥ 10 mm.

[2] Orthogonal contrast of means were the following: A = (No CSLCFA plus CSLCFA 1 DIM) vs. CSLCFA prepartum, B = No CSLCFA vs. CSLCFA 1 DIM, C = contrast A by parity, and D = contrast B by parity.

[3] Primiparous cows.

[4] Multiparous cows.

[5] Corpus luteum.

Table 3.8. Ovarian structures present on the ovaries of Holstein cows fed diets without calcium salts of long chain fatty acids (CSLCFA) (no CSLCFA) or diets with CSLCFA starting prepartum, at 1 day in milk (DIM) or at 28 DIM where day equals the days after GnRH injection for estrous synchronization (44 ± 3 DIM).

		Treatments				
Measure	Day	No CSLCFA	CSLCFA prepartum	CSLCFA 1 DIM	CSLCFA 28 DIM	SE
Number of class 1 follicles [a]	0	14.0	9.1	15.2	13.4	1.5
(2 to 5 mm)	2	11.2	11.5	15.3	19.1	1.4
	4	12.6	9.9	16.3	13.8	1.4
	6	12.3	11.0	15.7	12.1	1.4
	7	12.8	10.5	16.4	10.3	1.4
Number of class 2 follicles	0	1.5	1.0	3.25	2.1	0.5
(6 to 9 mm)	2	2.2	3	3.4	2.1	0.5
	4	2.3	1.4	1.4	3.2	0.5
	6	2.4	2.3	0.9	2.4	0.5
	7	1.8	1.9	2	1.6	0.5
Number of class 3 follicles	0	2.3	1.8	2.5	2.0	0.3
(≥ 10 mm)	2	2.1	1	1.3	1.1	0.3
	4	2.2	2.1	2.3	1.9	0.3
	6	2.4	2.3	1.6	1.9	0.3
	7	2.8	2.5	2.0	2.0	0.3
Number of corpus luteum [b]	0	1.0	0.7	0.7	1.0	0.2
	2	1.0	0.9	0.9	1.2	0.2
	4	1.2	1.25	1.2	1.3	0.2
	6	1.3	1.3	1.0	1.7	0.2
	7	1.4	1.5	1.2	1.7	0.2
Size of corpus luteum [c]	0	21.3	11.6	13.4	21.8	3.6
(mm)	2	21.3	16.7	15.4	25.0	3.5
	4	25.8	21.0	21.7	26.6	3.5
	6	27.3	25.3	20.2	34.6	3.5
	7	28.7	29.0	25.5	29.6	3.5

[a] CSLCFA prepartum vs. CSLCFA postpartum, P = 0.008.
[b] CSLCFA 1 DIM vs. CSLCFA 28 DIM, P = 0.099.
[c] CSLCFA 1 DIM vs. CSLCFA 28 DIM, P = 0.092.

Table 3.9. Concentrations of hepatic triacylglycerol (TAG), and hepatic IGF-I, IGF-II, and IGF binding protein (BP) -2 mRNA levels of Holstein cows fed diets without calcium salts of long chain fatty acids (CSLCFA) (no CSLCFA) or diets with CSLCFA starting prepartum, or at 1 day in milk (DIM).

	Treatments							Orthogonal contrasts [1], P =				
Measure	No CSLCFA		CSLCFA prepartum		CSLCFA 1 DIM		SE	A	B	Parity	C	D
	P[2]	M[3]	P	M	P	M						
TAG, % of wet weight	2.8	3.0	5.3	6.0	2.7	5.6	1.3	0.102	0.317	0.257	0.725	0.266
TAG , % of dry weight	8.6	9.8	16.2	17.2	8.3	14.7	3.8	0.080	0.531	0.364	0.693	0.478
IGF-I mRNA, arbitrary units	0.024	0.020	0.013	0.003	0.011	0.048	0.073	0.110	0.550	0.494	0.231	0.110
IGF-II mRNA, arbitrary units	0.292	0.288	0.269	0.224	0.296	0.331	0.043	0.159	0.591	0.892	0.429	0.670
IGFBP-2 mRNA, arbitrary units	0.087	0.136	0.091	0.065	0.149	0.111	0.028	0.097	0.534	0.837	0.519	0.142

[1] Orthogonal contrast of means were the following: A = (No CSLCFA plus CSLCFA 1 DIM) vs. CSLCFA prepartum, B = No CSLCFA vs. CSLCFA 1 DIM, C = contrast A by parity, and D = contrast B by parity.

[2] Primiparous cows.

[3] Multiparous cows.

Table 3.10. Concentration of plasma acute phase proteins and liver enzymes of Holstein cows fed diets without calcium salts of long chain fatty acids (CSLCFA) (no CSLCFA) or diets with CSLCFA starting prepartum, or at 1 day in milk (DIM).

	Treatments							Orthogonal contrasts [1], P =				
Measure	No CSLCFA		CSLCFA prepartum		CSLCFA 1 DIM		SE	A	B	Parity	C	D
	P[2]	M[3]	P	M	P	M						
Fibrinogen, mg/dl	131.7	115.8	70.5	121.6	97.1	103.2	13.4	0.175	0.070	0.211	0.029	0.353
Haptoglobin, mg HbB/100 ml[4]	31.6	15.4	27.5	18.2	36.5	17.5	8.4	0.750	0.980	0.043	0.646	0.872
Ceruloplasmin, mg/dl	24.4	21.1	25.1	19.0	23.7	21.9	1.6	0.634	0.974	0.007	0.195	0.675
Total biliruben, mg/dl	0.22	0.26	0.32	0.39	0.23	0.22	0.06	0.040	0.837	0.529	0.658	0.640
Alanine aminotransferase, IU/L	22.5	20.2	24.7	20.7	22.7	18.5	2.7	0.482	0.7749	0.122	0.883	0.719
Alkaline phosphatase, U/L	39.8	32.4	45.8	33.8	46.7	29.0	3.9	0.431	0.646	< 0.001	0.947	0.172
Aspartate aminotransferase, IU/L	111.9	89.6	106.9	114.8	119.6	82.5	19.3	0.576	0.986	0.289	0.294	0.692
Gamma glutamyl transferase, U/L	30.8	25.4	32.8	33.4	26.5	31.0	4.5	0.264	0.880	0.976	0.900	0.265
Albumin, g/dl	2.55	2.73	3.01	2.78	2.95	2.45	0.15	0.104	0.672	0.130	0.772	0.018

[1] Orthogonal contrast of means were the following: A = (No CSLCFA plus CSLCFA 1 DIM) vs. CSLCFA prepartum, B = No CSLCFA vs. CSLCFA 1 DIM, C = contrast A by parity, and D = contrast B by parity.

[2] Primiparous cows.

[3] Multiparous cows.

4 Amount of hemoglobin bound by haptoglobin/100 ml of plasma.

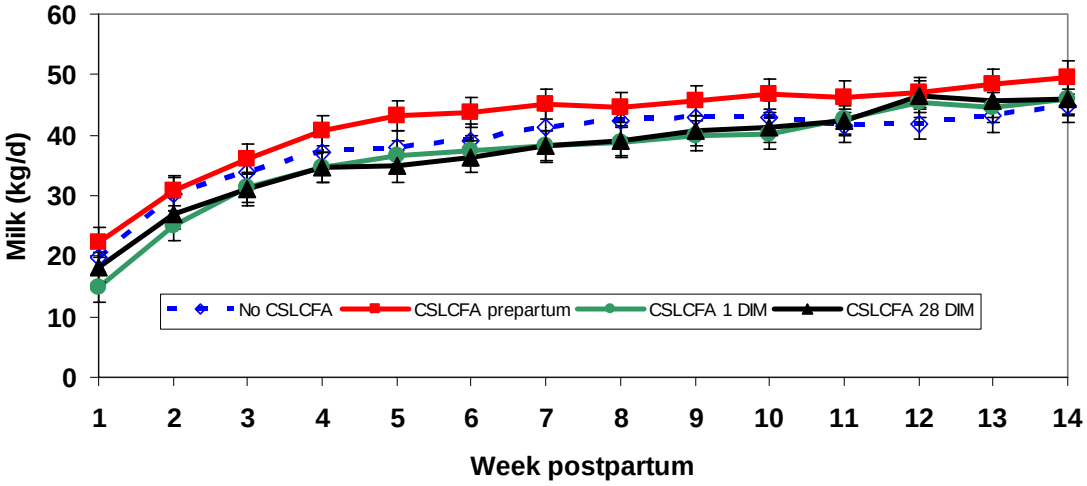

Figure 3.1. Least squares means for milk production of cows fed diets without calcium salts of long chain fatty acids (CSLCFA) (no CSLCFA) or diets with CSLCFA starting prepartum, at 1 day in milk (DIM), or 28 DIM.

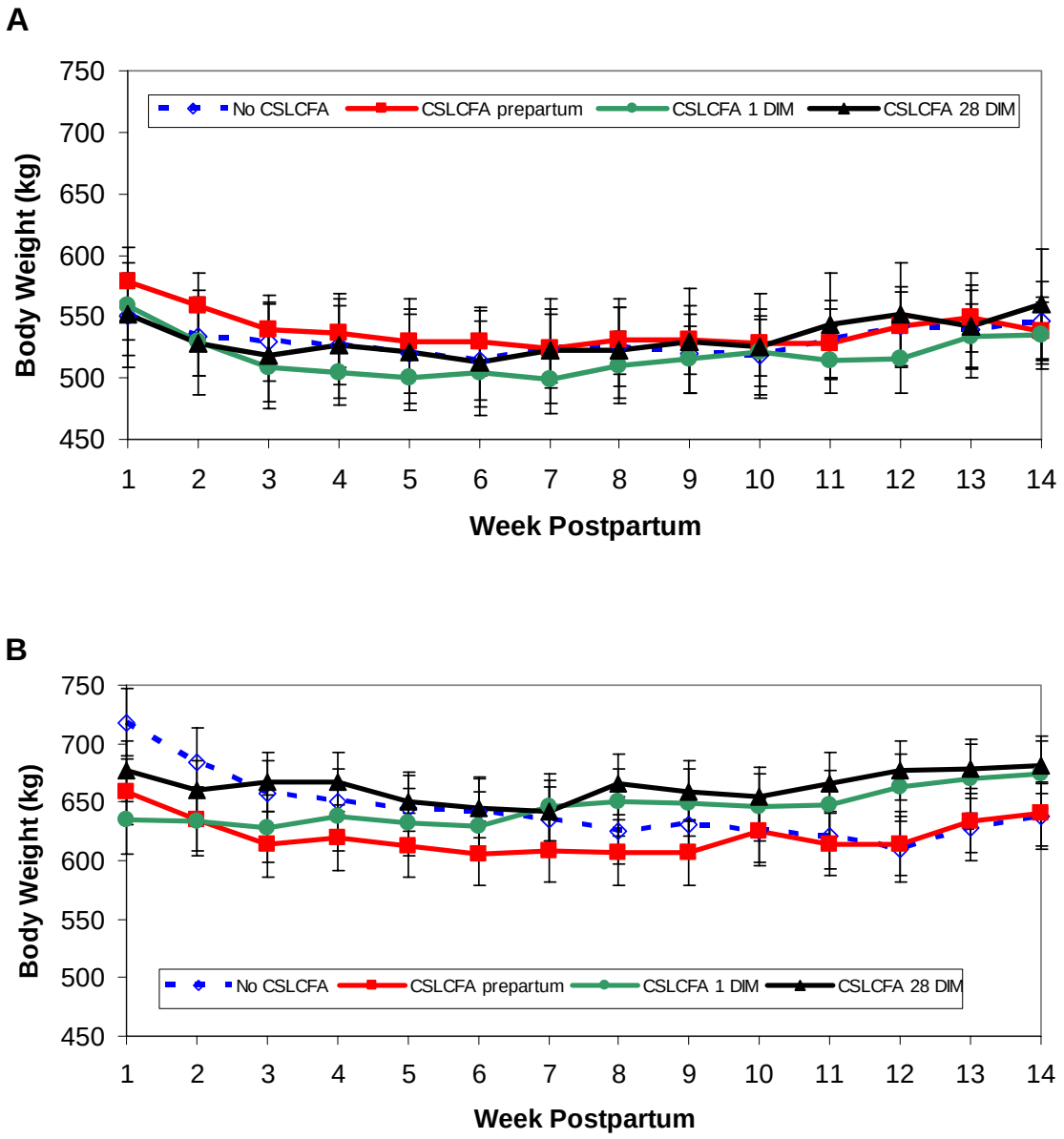

Figure 3.2. Least squares means for body weight of primiparous (A) and multiparous (B) cows fed diets without calcium salts of long chain fatty acids (CSLCFA) (no CSLCFA) or diets with CSLCFA starting prepartum, at 1 day in milk (DIM), or 28 DIM. There was a treatment x parity x week interaction ($P < 0.001$).

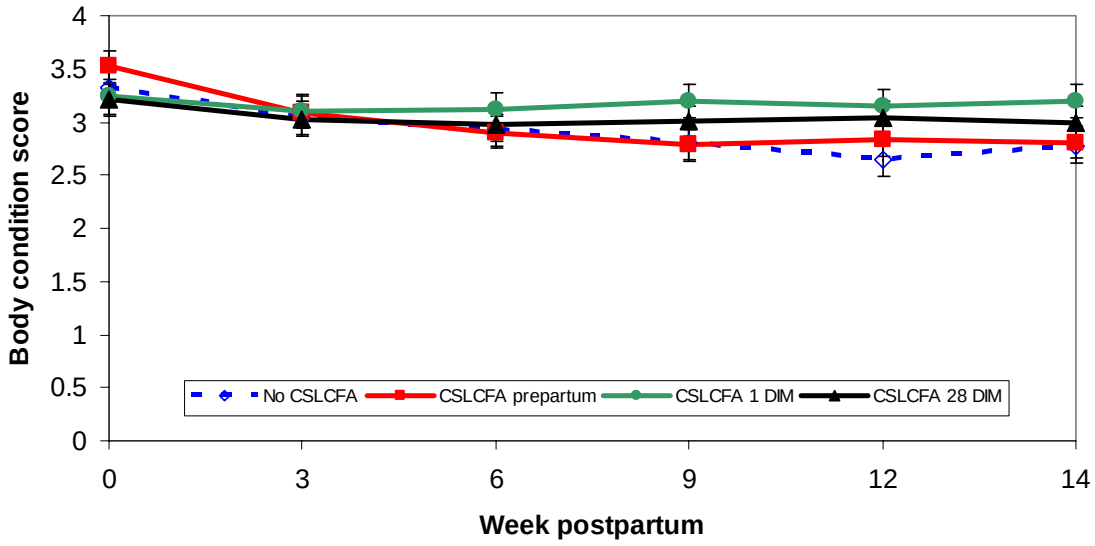

Figure 3.3. Least squares means for body condition score of cows fed diets without calcium salts of long chain fatty acids (CSLCFA) (no CSLCFA) or diets with CSLCFA starting prepartum, at 1 day in milk (DIM), or 28 DIM. There was a treatment x week interaction (P = 0.007).

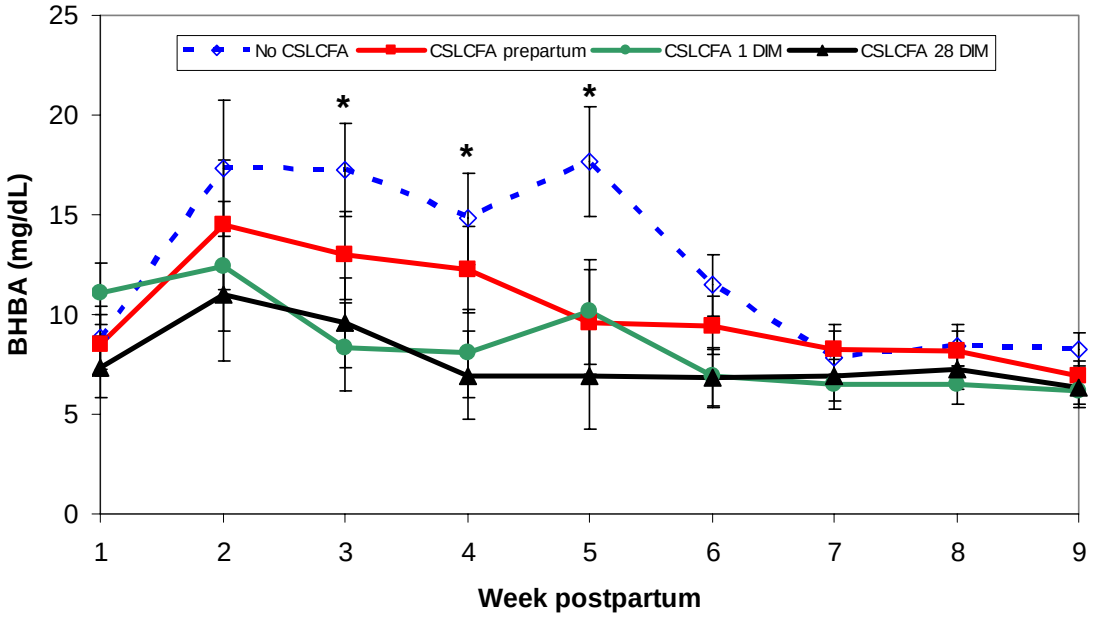

Figure 3.4. Least squares means for plasma BHBA concentration of cows fed diets without calcium salts of long chain fatty acids (CSLCFA) (no CSLCFA) or diets with CSLCFA starting prepartum, at 1 day in milk (DIM), or 28 DIM. The asterisks indicate the treatment no CSLCFA is different than CSLCFA 1 DIM and CSLCFA 28 DIM at wk 3, 4, and 5 of lactation and different than the treatment CSLCFA prepartum at wk 5 postpartum ($P < 0.05$).

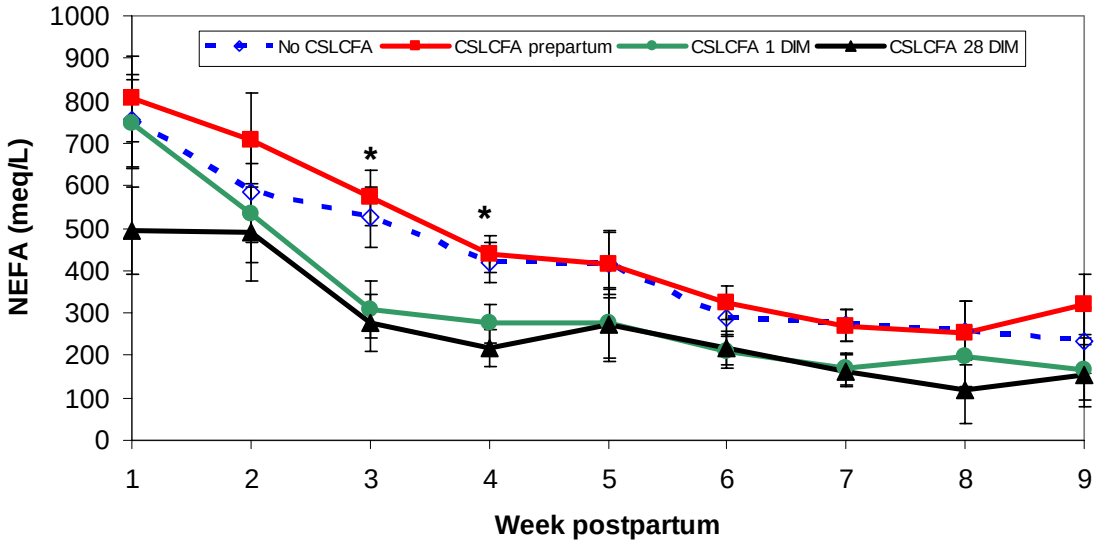

Figure 3.5. Least squares means for plasma NEFA concentration of cows fed diets without calcium salts of long chain fatty acids (CSLCFA) (no CSLCFA) or diets with CSLCFA starting prepartum, at 1 day in milk (DIM), or 28 DIM. The asterisks indicate that the treatments no CSLCFA and CSLCFA prepartum are different than CSLCFA 1 DIM and CSLCFA 28 DIM at wk 3 and 4 postpartum ($P < 0.05$).

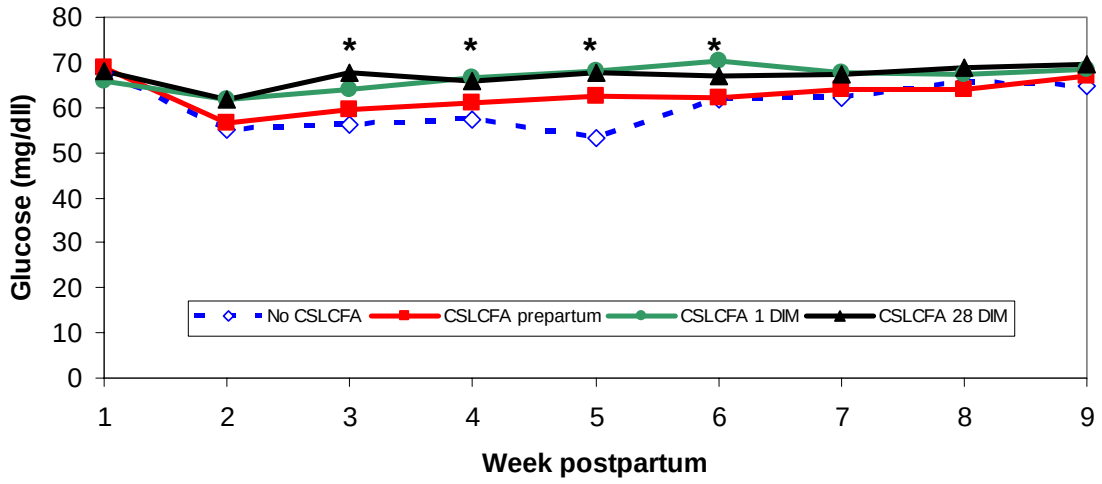

Figure 3.6. Least squares means for plasma glucose concentration of cows fed diets without calcium salts of long chain fatty acids (CSLCFA) (no CSLCFA) or diets with CSLCFA starting prepartum, at 1 day in milk (DIM), or 28 DIM. There tended to be a treatment x week interaction ($P = 0.10$). The asterisks indicate treatment differences at week 3, 4, 5, and 6 postpartum ($P < 0.05$). The treatment no CSLCFA is different than CSLCFA 1 DIM and CSLCFA 28 DIM at wk 3, 4, and 5 postpartum, different than CSLCFA prepartum at wk 5 postpartum, and different than CSLCFA 1 DIM at wk 6 postpartum. The treatment CSLCFA prepartum is different than CSLCFA 28 at wk 3 and different than CSLCFA 1 DIM at wk 6 postpartum.

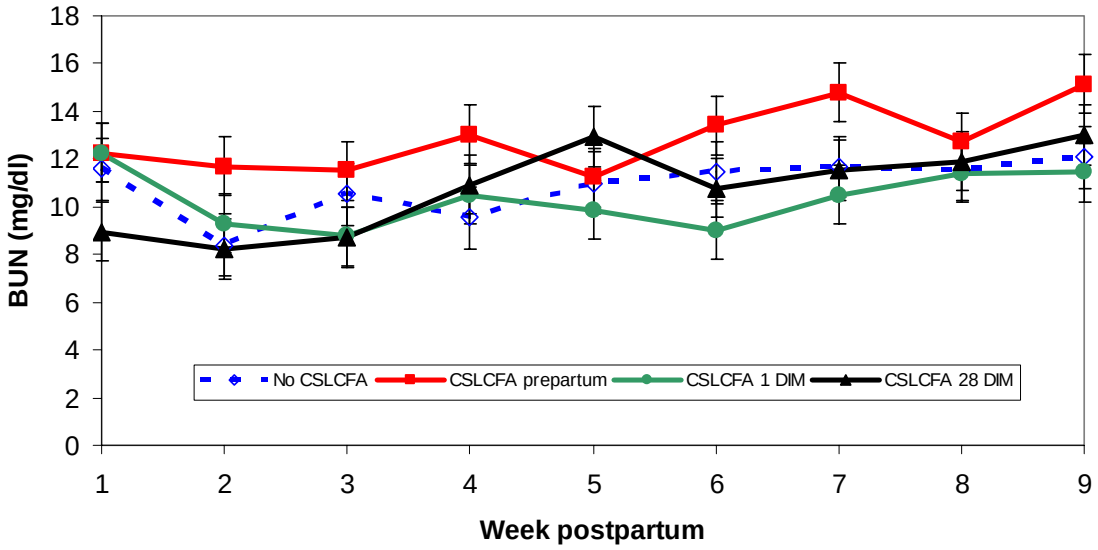

Figure 3.7. Least squares means for blood urea nitrogen (BUN) concentration of cows fed diets without calcium salts of long chain fatty acids (CSLCFA) (no CSLCFA) or diets with CSLCFA starting prepartum, at 1 day in milk (DIM), or 28 DIM. There was a tendency for a treatment x week interaction (P = 0.06)

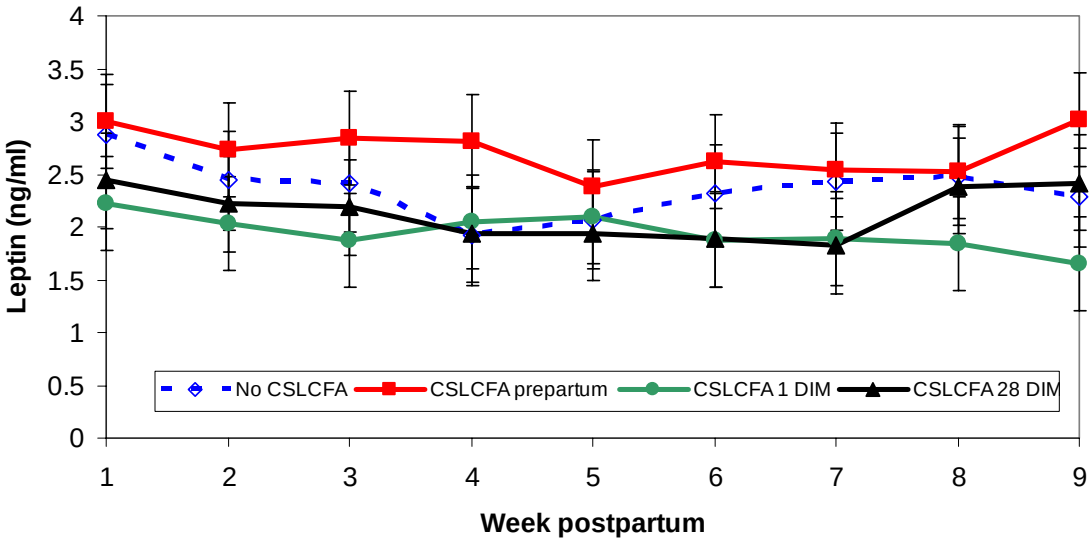

Figure 3.8. Least squares means for plasma leptin concentration of cows fed diets without calcium salts of long chain fatty acids (CSLCFA) (no CSLCFA) or diets with CSLCFA starting prepartum, at 1 day in milk (DIM), or 28 DIM.

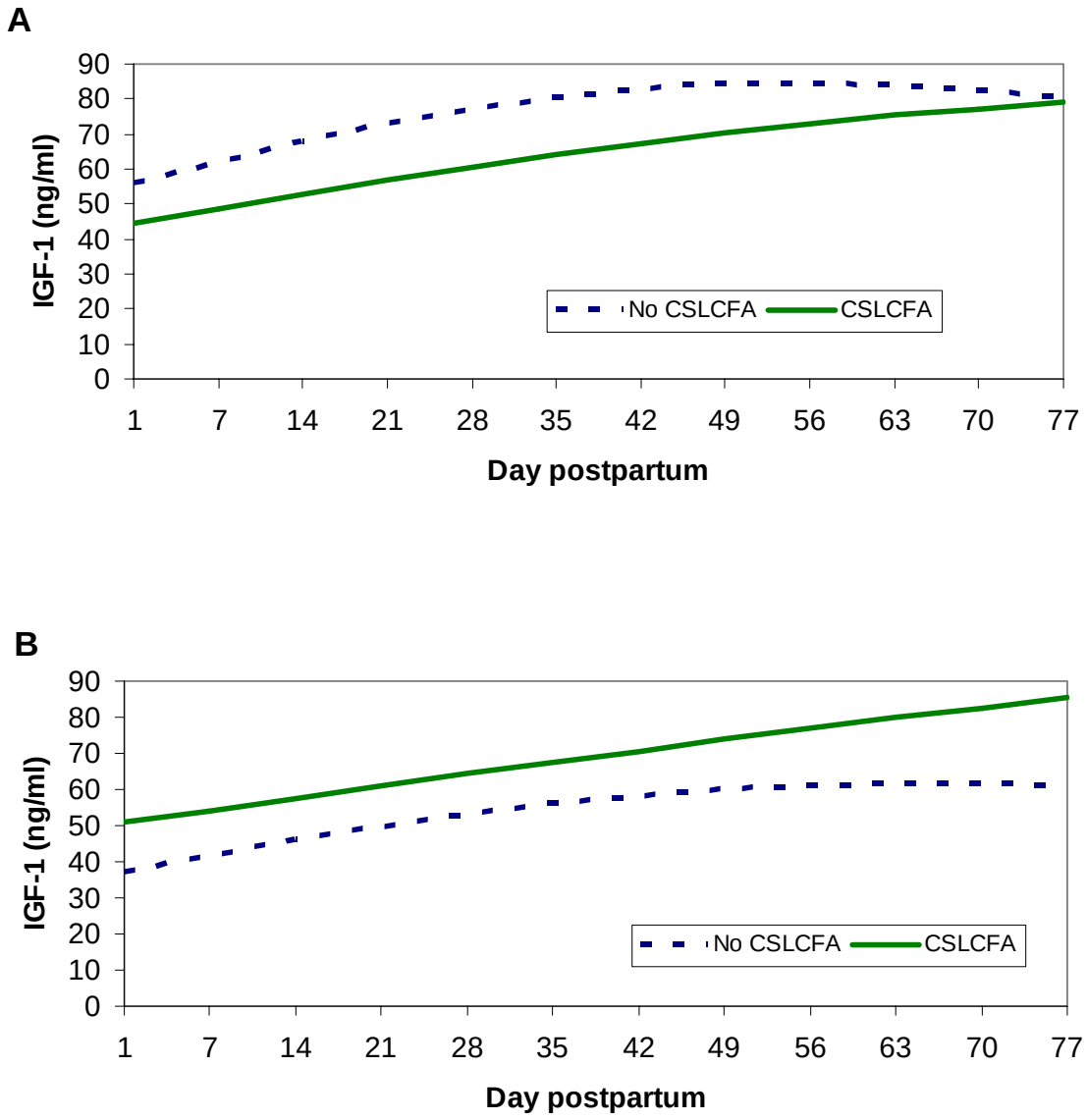

Figure 3.9. Regression plot of plasma IGF-1 concentration of primiparous (A) and multiparous (B) cows fed diets without calcium salts of long chain fatty acids (CSLCFA) (no CSLCFA) or cows fed CSLCFA starting prepartum, at 1 day in milk (DIM), or 28 DIM. There was a treatment x parity x day interaction for this contrast (second order polynomial, $P < 0.01$). The pooled SE of primiparous cows fed no CSLCFA or CSLCFA was 7.9 and 4.3 ng/ml, respectively. The pooled SE of multiparous cows fed no CSLCFA or CSLCFA was 7.2 and 4.1 ng/ml, respectively.

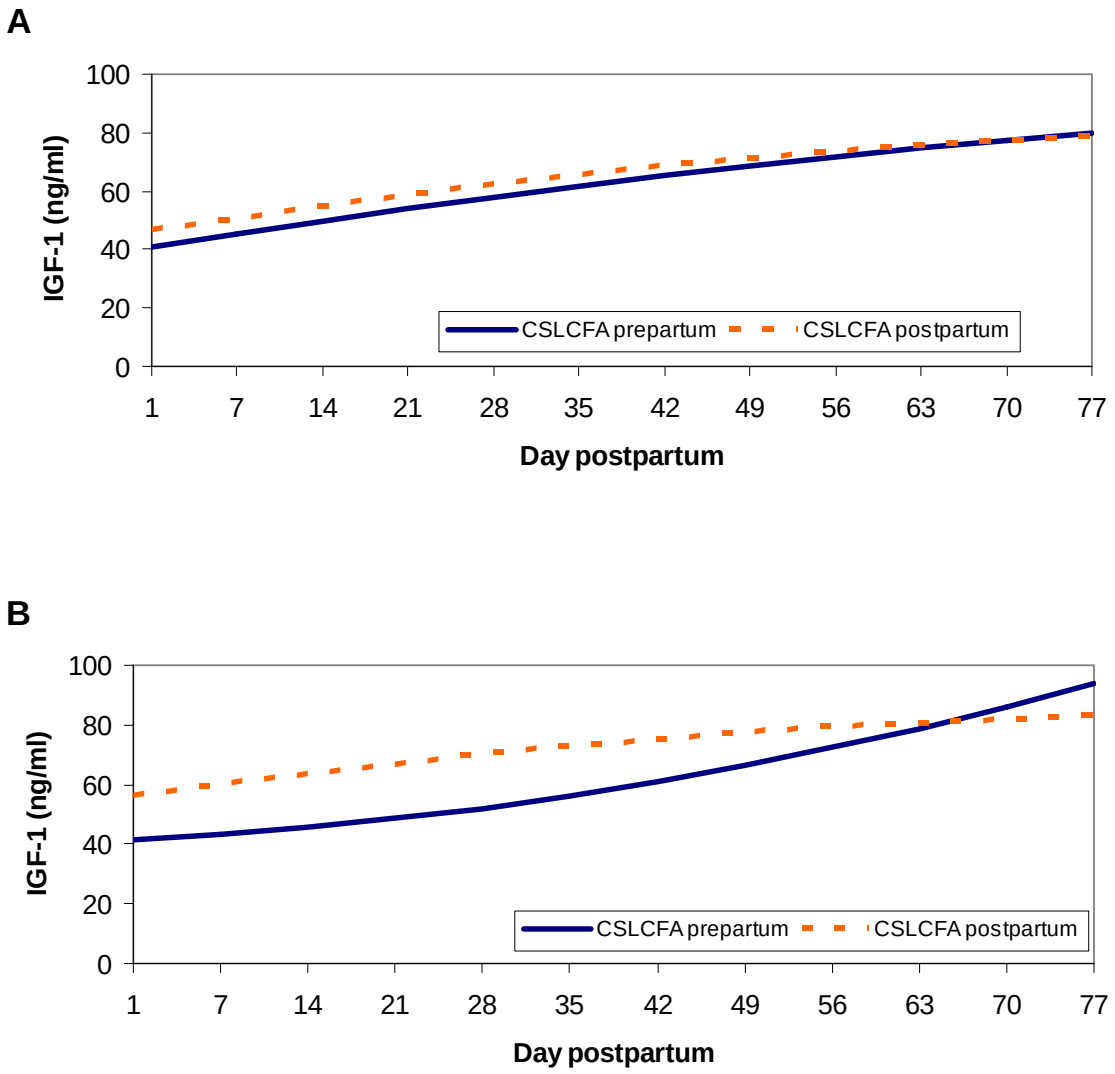

Figure 3.10. Regression plot of plasma IGF-1 concentration of primiparous (A) and multiparous (B) cows fed diets with calcium salts of long chain fatty acids (CSLCFA) starting prepartum (CSLCFA prepartum) or cows fed CSLCFA beginning at 1 d in milk (DIM) or 28 DIM (CSLCFA postpartum). There was a treatment x parity x day interaction for this contrast (second order polynomial, $P < 0.01$). The pooled SE of primiparous cows fed CSLCFA prepartum or CSLCFA postpartum was 7.5 and 4.4 ng/ml, respectively. The pooled SE of multiparous cows fed CSLCFA prepartum or CSLCFA postpartum was 6.7 and 2.6 ng/ml, respectively.

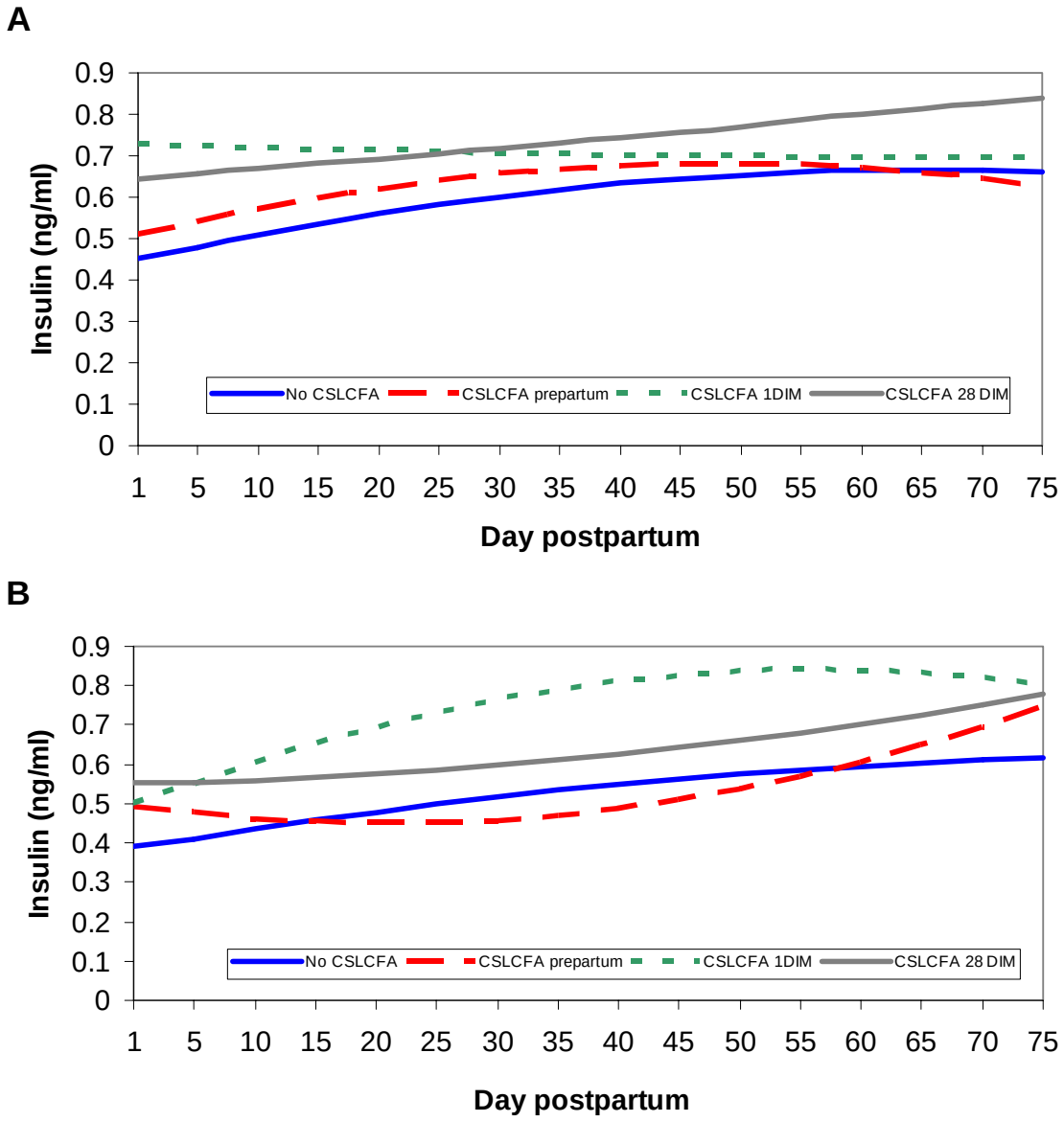

Figure 3.11. Regression plot of plasma insulin concentration of primiparous (A) and multiparous (B) cows fed diets without calcium salts of long chain fatty acids (CSLCFA) (no CSLCFA), or diets with CSLCFA starting prepartum, at 1 day in milk (DIM), or 28 DIM. There was a treatment x parity x day interaction (second order polynomial, $P < 0.01$). The pooled SE of primiparous and multiparous cows was 0.05 and 0.05 ng/ml, respectively.

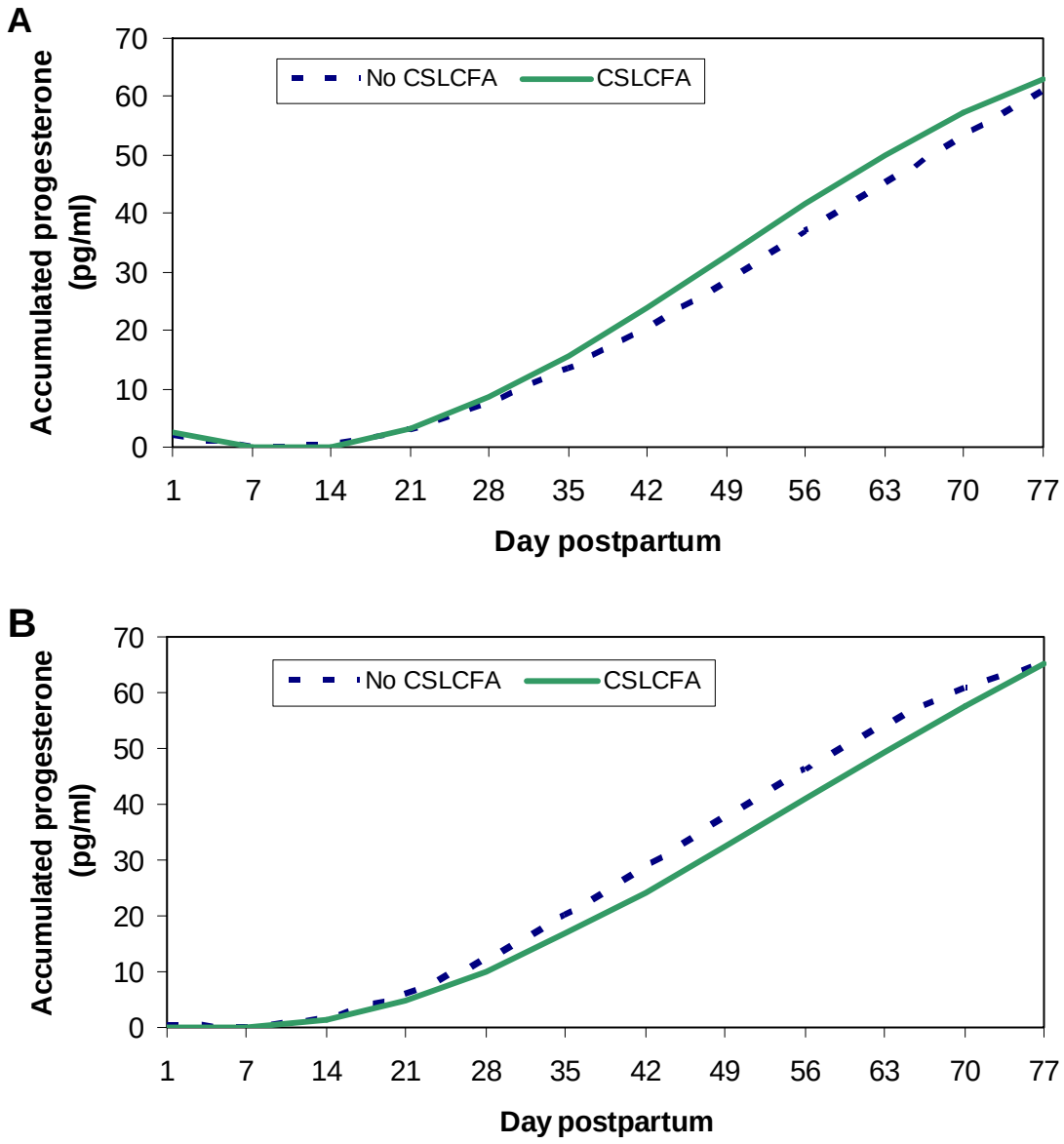

Figure 3.12. Regression plot of accumulated plasma progesterone concentration from d 1 to 77 postpartum of primiparous (A) and multiparous (B) cows fed diets without calcium salts of long chain fatty acids (CSLCFA) (no CSLCFA) or cows fed CSLCFA (starting prepartum, at 1 day in milk (DIM), and 28 DIM). There was a treatment x parity x day interaction for this contrast (third order polynomial, $P < 0.05$). The pooled SE of primiparous fed no CSLCFA or CSLCFA was 4.7 and 4.2 pg/ml, respectively. The pooled SE of multiparous cows fed no CSLCFA or CSLCFA was 2.5 and 2.4 pg/ml, respectively.

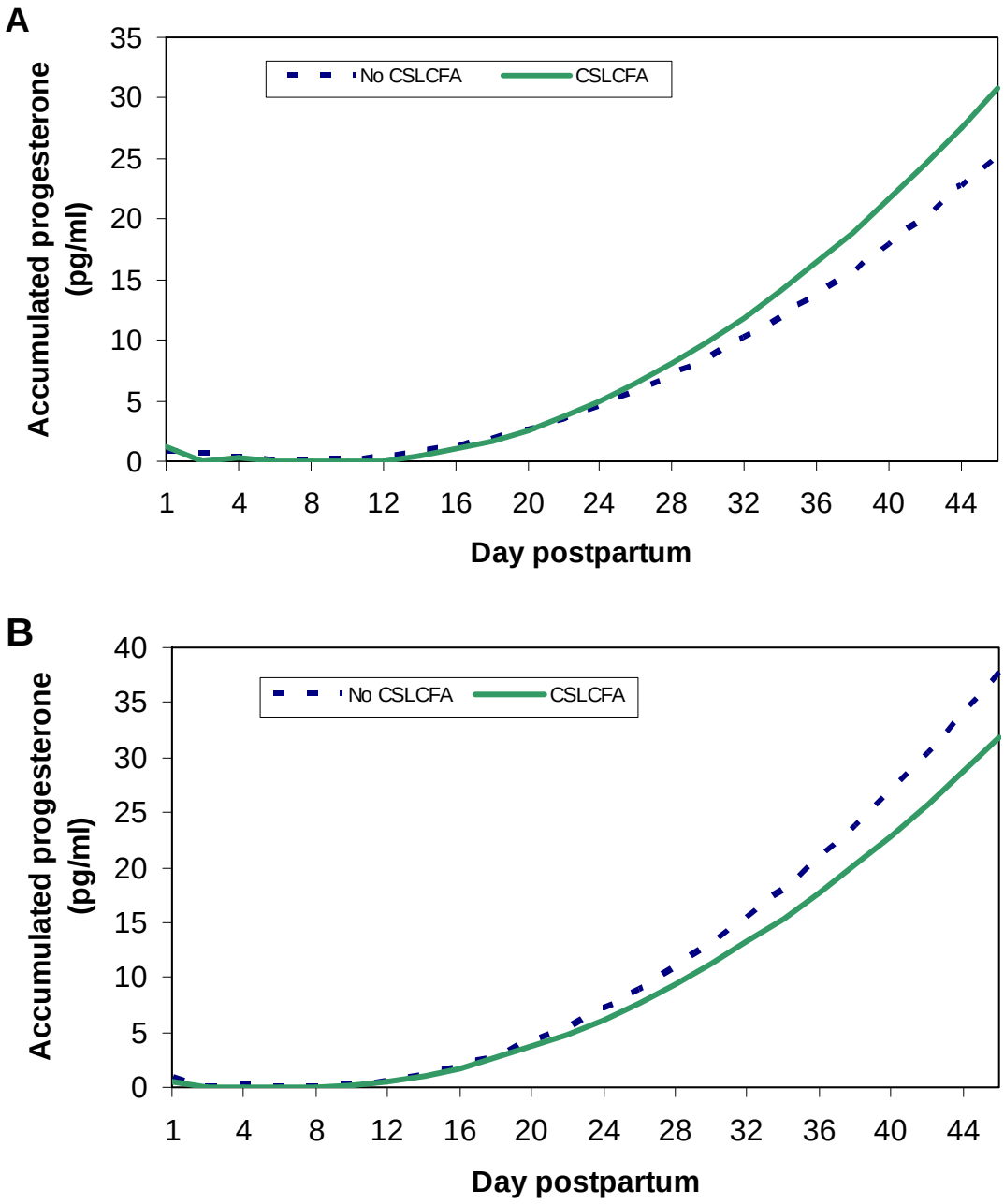

Figure 3.13. Regression plot of accumulated plasma progesterone concentration from d 1 to 46 postpartum of primiparous (A) and multiparous (B) cows fed diets without calcium salts of long chain fatty acids (CSLCFA) (no CSLCFA) or cows fed CSLCFA (starting prepartum, at 1 day in milk (DIM), and 28 DIM). There was a treatment x parity x day interaction for this contrast (second order polynomial, $P < 0.01$). The pooled SE of primiparous cows fed no CSLCFA or CSLCFA was 2.5 and 1.4 pg/ml, respectively. The pooled SE of multiparous cows fed no CSLCFA or CSLCFA was 2.3 and 1.3 pg/ml, respectively.

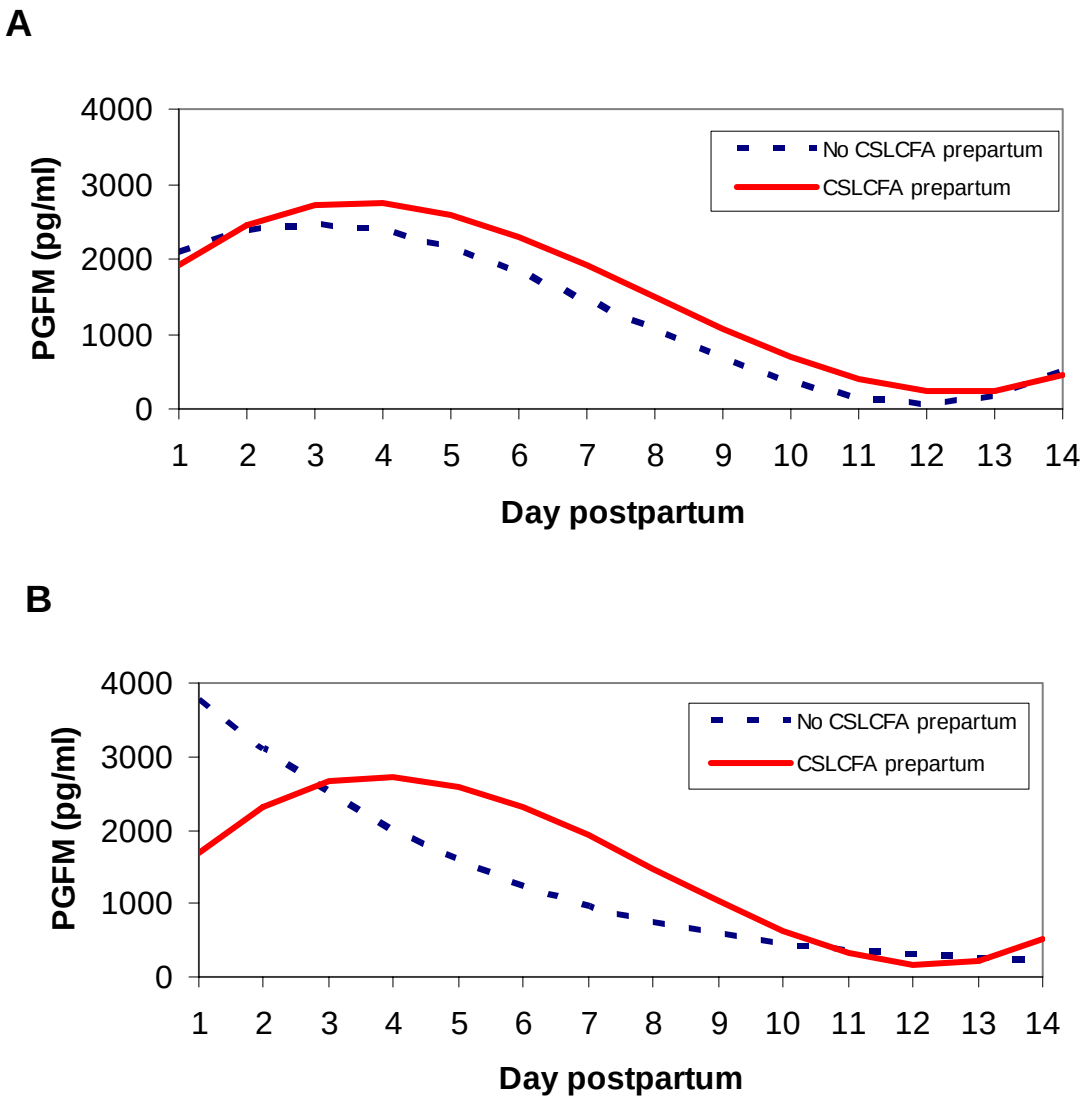

Figure 3.14. Regression plot of concentration of plasma PGF2α metabolite (PGFM) of primiparous (A) or multiparous (B) cows fed diets without calcium salts of long chain fatty acids (CSLCFA) prepartum (no CSLCFA plus CSLCFA starting at 1 d in milk (DIM) and 28 DIM), or CSLCFA prepartum. There was a treatment x parity x day interaction for this contrast (third order polynomial, $P < 0.05$). The pooled SE of primiparous cows fed no CSLCFA prepartum or CSLCFA prepartum was 166 and 273 pg/ml, respectively. The pooled SE of multiparous cows fed no CSLCFA prepartum or CSLCFA prepartum was 154 and 271 pg/ml, respectively.

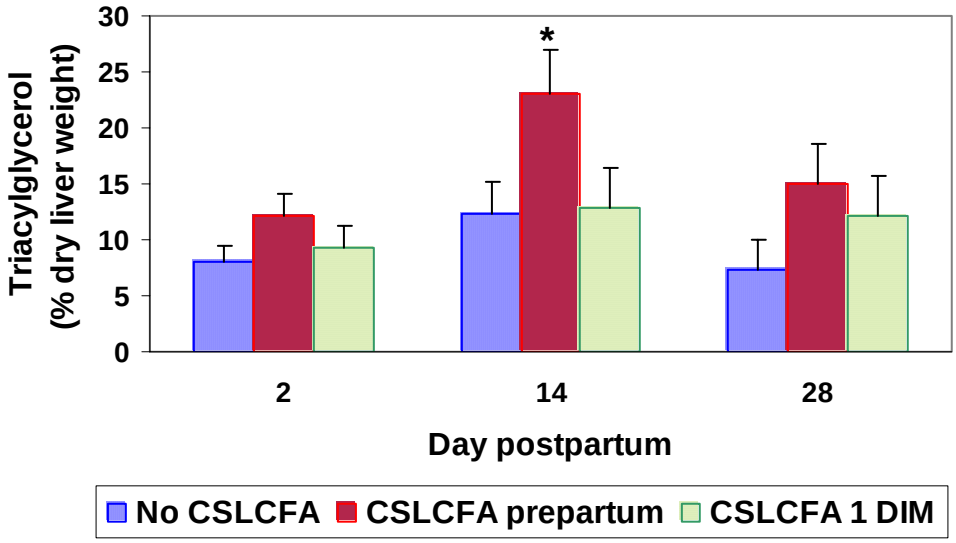

Figure 3.15. Least squares means for hepatic triacylglycerol concentration (% of dry liver weight) of cows fed diets without calcium salts of long chain fatty acids (CSLCFA) (no CSLCFA) or diets with CSLCFA starting prepartum or at 1 day in milk (DIM). There was a treatment x day interaction (P = 0.04). The asterisk indicates that there was a tendency for CSLCFA prepartum to differ from no CSLCFA and CSLCFA 1 DIM at 14 DIM (P = 0.084).

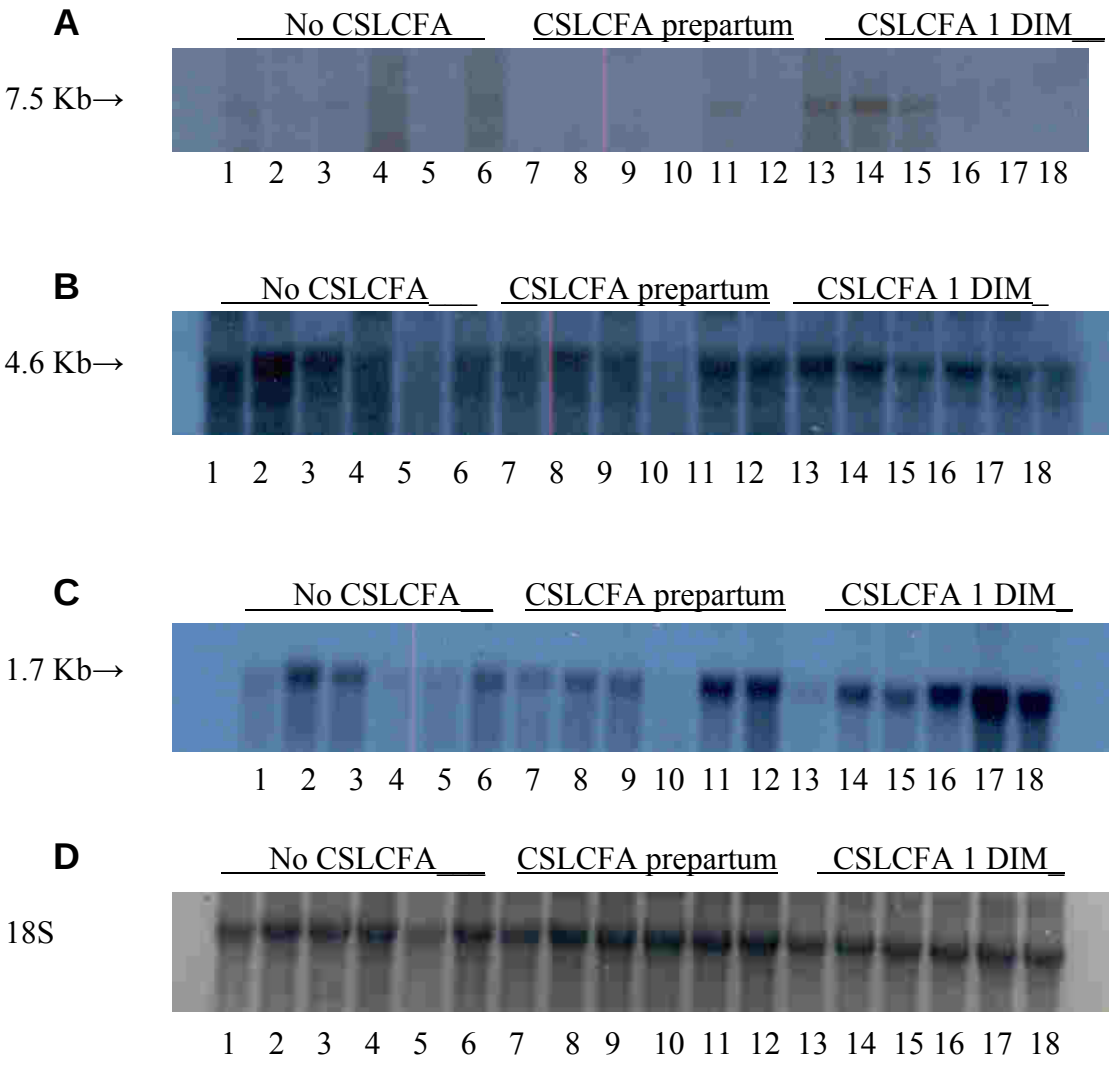

Figure 3.16 Ten micrograms of total cellular RNA isolated from livers of cows fed diets without CSLCFA (lanes 1 to 6), or diets with CSLCFA starting prepartum (lanes 7 to 12) or at 1 DIM (lanes 13 to 18) were subjected to Northern blot analysis. Representitive Northern blots for IGF-I (A), IGF-II (B), or IGFBP-2 (C) mRNA expression are shown. Within each dietary treatment, two cows are represented at 2 DIM (lanes 1 and 4; 7 and 10; 13 and 16), 14 DIM (lanes 2 and 5; 8 and 11; 14 and 17), and 28 DIM (lanes 3 and 6; 9 and 12; 15 and 18).

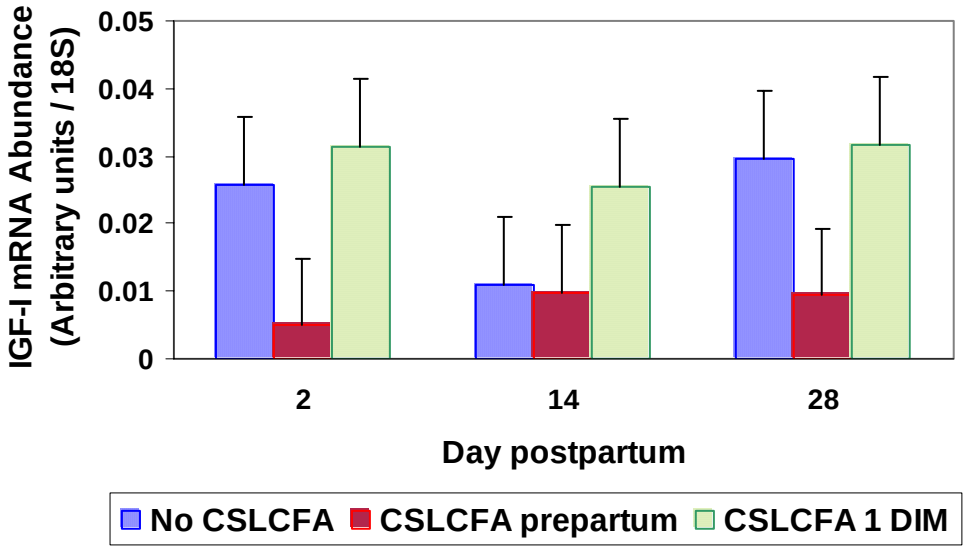

Figure 3.17. Least squares means for hepatic IGF-I mRNA abundance of cows fed diets without calcium salts of long chain fatty acids (CSLCFA) (no CSLCFA) or diets with CSLCFA starting prepartum or at 1 day in milk (DIM). Each bar represents 8 cows per collection period. Values are expressed as ratios over densitometric values for 18S.

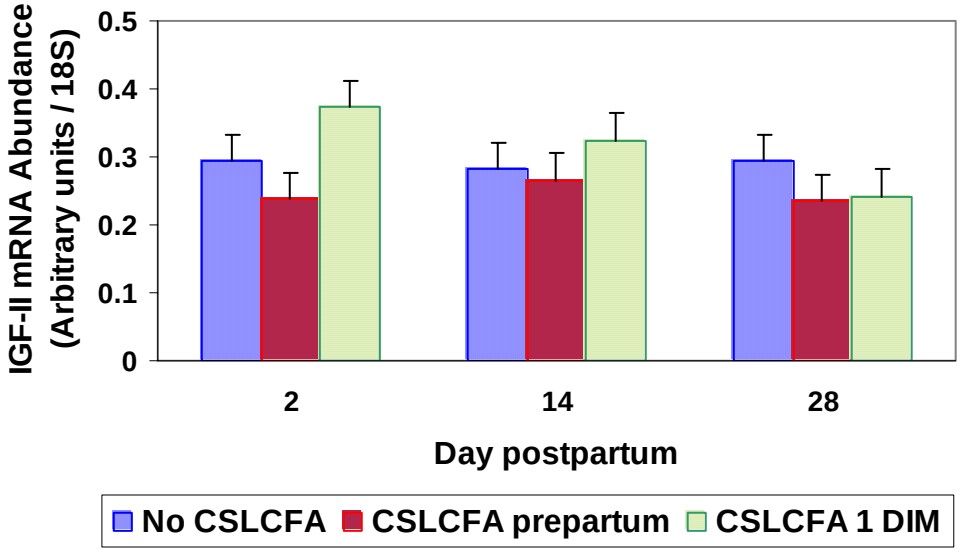

Figure 3.18. Least squares means for hepatic IGF-II mRNA abundance of cows fed diets without calcium salts of long chain fatty acids (CSLCFA) (no CSLCFA) or diets with CSLCFA starting prepartum or at 1 day in milk (DIM).). Each bar represents 8 cows per collection period. Values are expressed as ratios over densitometric values for 18S.

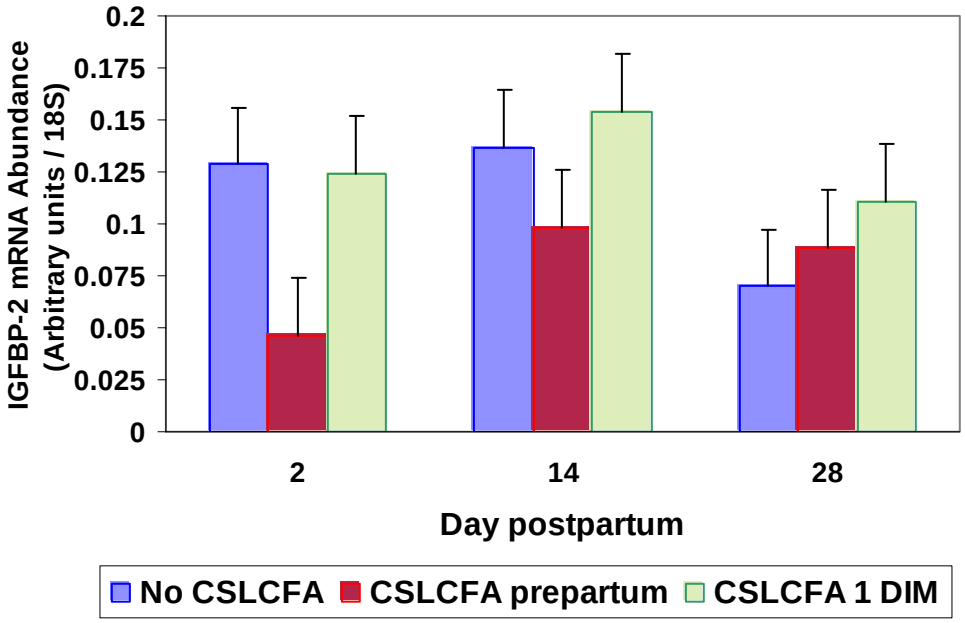

Figure 3.19. Least squares means for hepatic IGFBP-2 mRNA abundance of cows fed diets without calcium salts of long chain fatty acids (CSLCFA) (no CSLCFA) or diets with CSLCFA starting prepartum or at 1 day in milk (DIM).). Each bar represents 8 cows per collection period. Values are expressed as ratios over densitometric values for 18S.

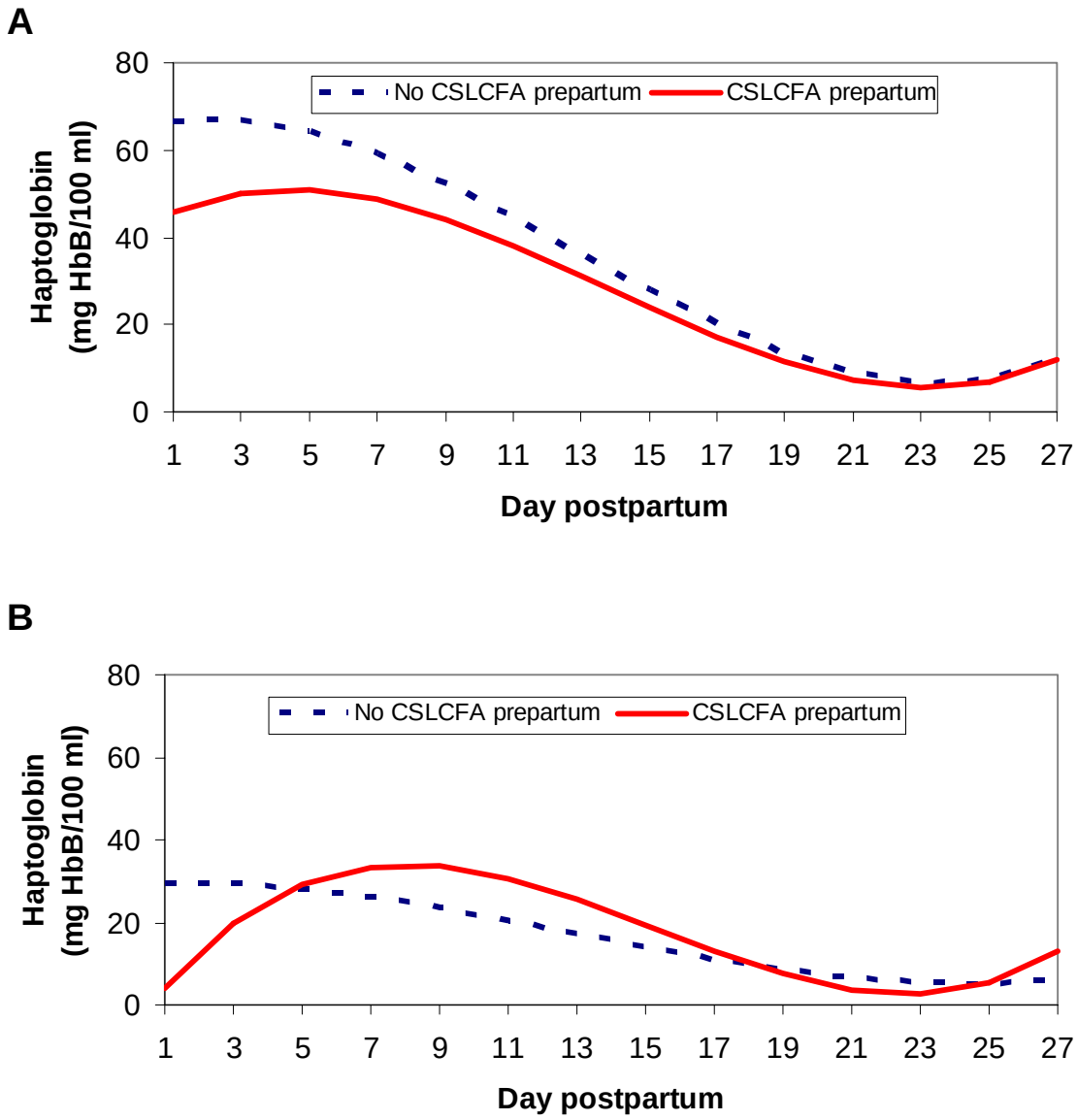

Figure 3.20. Regression plot of plasma concentration of haptoglobin of primiparous (A) and multiparous (B) cows fed diets without calcium salts of long chain fatty acids (CSLCFA) prepartum (no CSLCFA plus CSLCFA starting at 1 d in milk (DIM) and 28 DIM) or CSLCFA prepartum. There was a treatment x parity x day interaction for this contrast (third order polynomial, $P < 0.05$). The pooled standard error (SE) of primiparous cows fed no CSLCFA prepartum or CSLCFA prepartum was 5.5 and 9.0 mg HbB/100 ml (amount of hemoglobin bound by haptoglobin/100 ml of plasma), respectively. The pooled SE of multiparous cows fed no CSLCFA prepartum or CSLCFA prepartum was 5.0 and 9.0 mg HbB/100 ml, respectively.

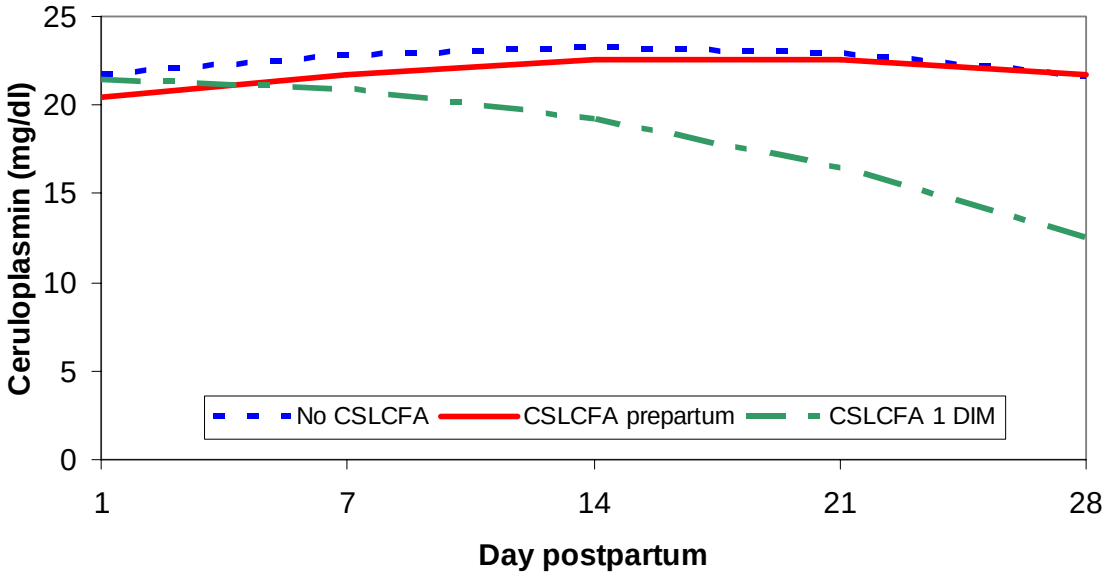

Figure 3.21. Regression plot of plasma concentration of ceruloplasmin of cows fed diets without calcium salts of long chain fatty acids (CSLCFA) (no CSLCFA) or diets with CSLCFA starting prepartum or at 1 day in milk (DIM). This was a third order polynomial (day x day x day, P = 0.003) and the pooled standard error of no CSLCFA, CSLCFA prepartum, and CSLCFA 1 DIM was 7.3, 10.2, and 10.1mg/dl, respectively.

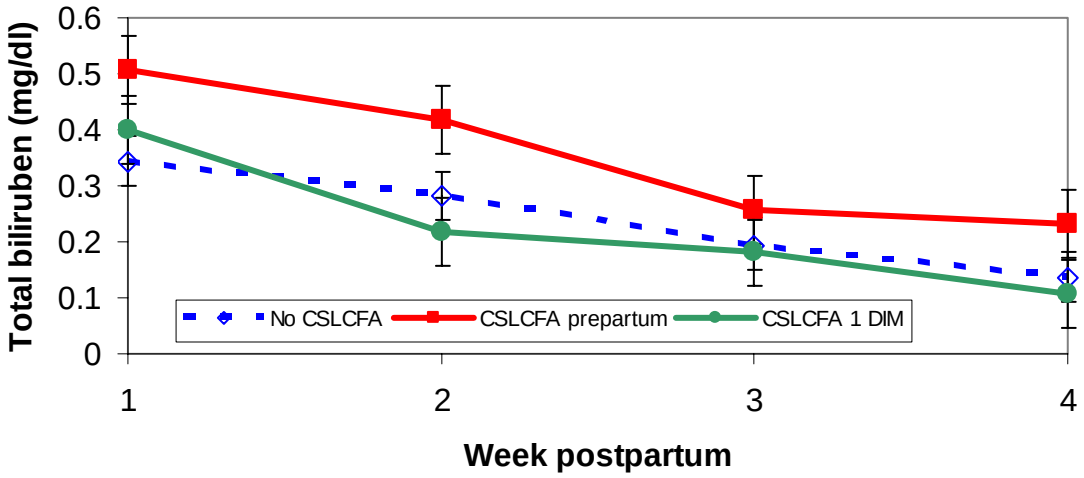

Figure 3.22. Least squares means for plasma concentration of total biliruben of cows fed diets without calcium salts of long chain fatty acids (CSLCFA) (no CSLCFA) or diets with CSLCFA starting prepartum or at 1 day in milk (DIM). Week postpartum was significant ($P < 0.001$).

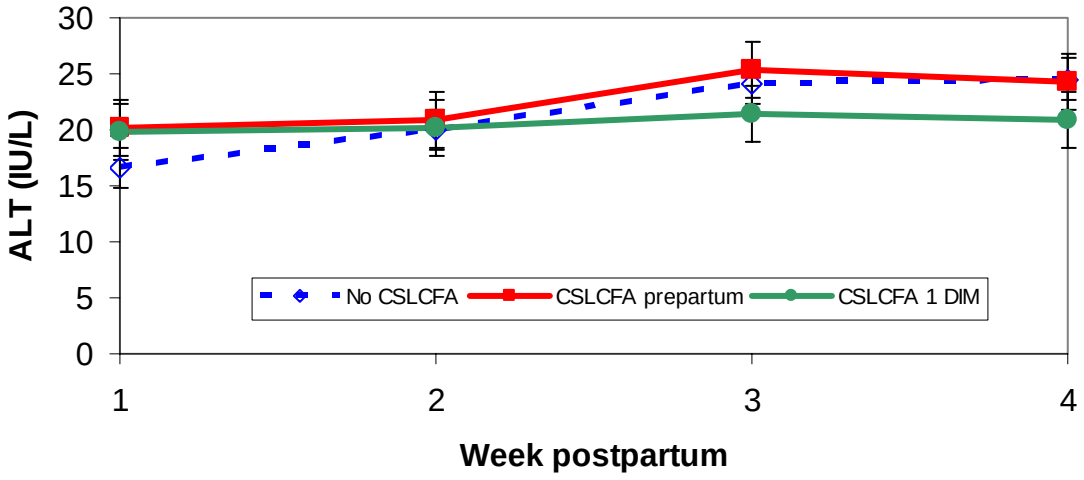

Figure 3.23. Least squares means for plasma concentration of alanine aminotransferase (ALT) of cows fed diets without calcium salts of long chain fatty acids (CSLCFA) (no CSLCFA) or diets with CSLCFA starting prepartum or at 1 day in milk (DIM). Week postpartum was significant (P = 0.003).

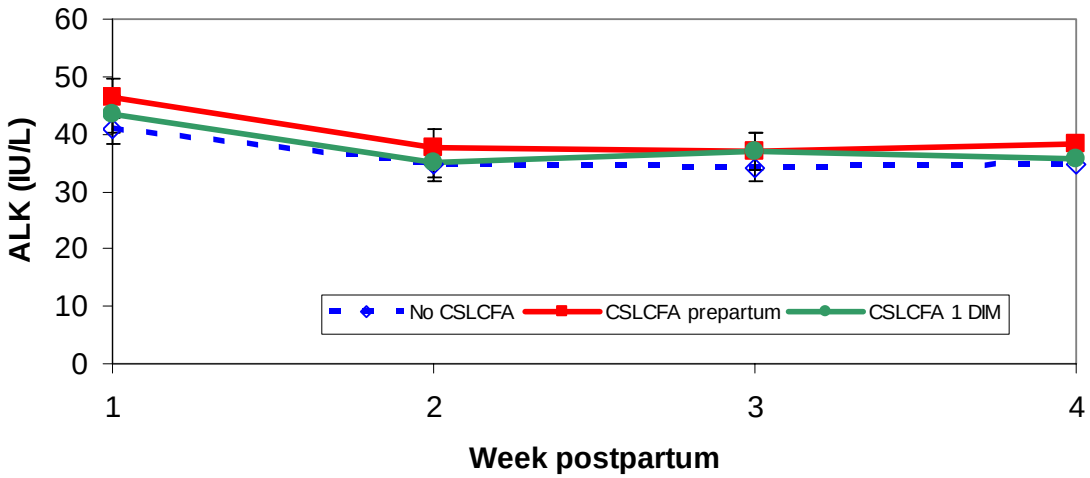

Figure 3.24. Least squares means for plasma alkaline phosphatase (ALK) concentration of cows fed diets without calcium salts of long chain fatty acids (CSLCFA) (no CSLCFA) or diets with CSLCFA starting prepartum or at 1 day in milk (DIM). Week postpartum was significant ($P < 0.001$).

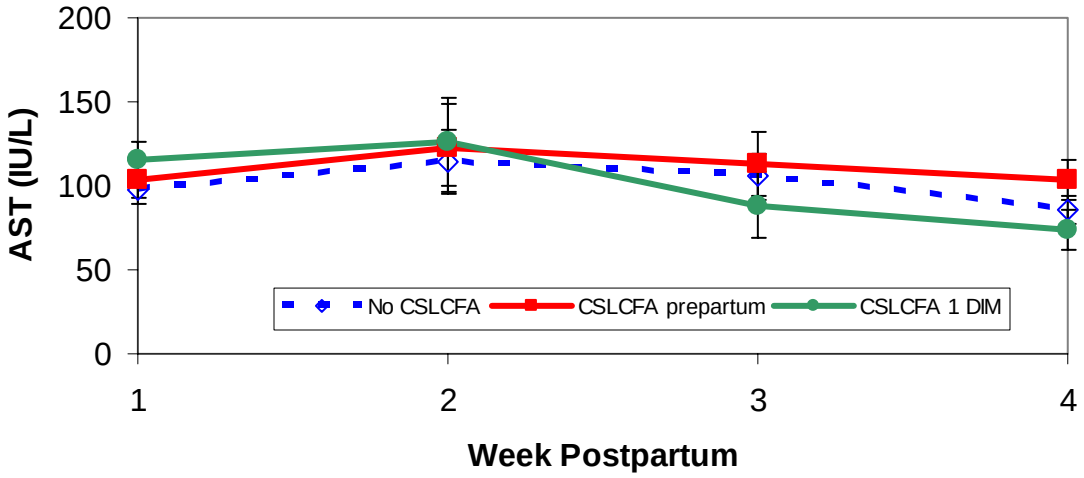

Figure 3.25. Least squares means for plasma concentration of aspartate aminotransferase (AST) of cows fed diets without calcium salts of long chain fatty acids (CSLCFA) (no CSLCFA) or diets with CSLCFA starting prepartum or at 1 day in milk (DIM). Week postpartum was significant (P = 0.01).

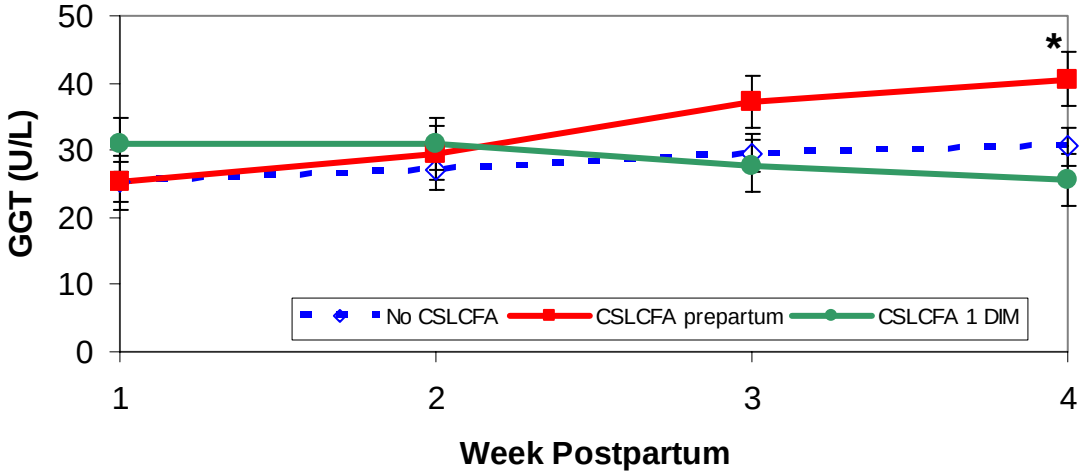

Figure 3.26. Least squares means for plasma concentration of gamma glutamyl transferase (GGT) of cows fed diets without calcium salts of long chain fatty acids (CSLCFA) (no CSLCFA) or diets with CSLCFA starting prepartum or at 1 day in milk (DIM). There was a treatment x week interaction (P = 0.01). The asterisk indicates that the treatment CSLCFA prepartum is different than no CSLCFA and CSLCFA 1 DIM at wk 4 (P = 0.03).

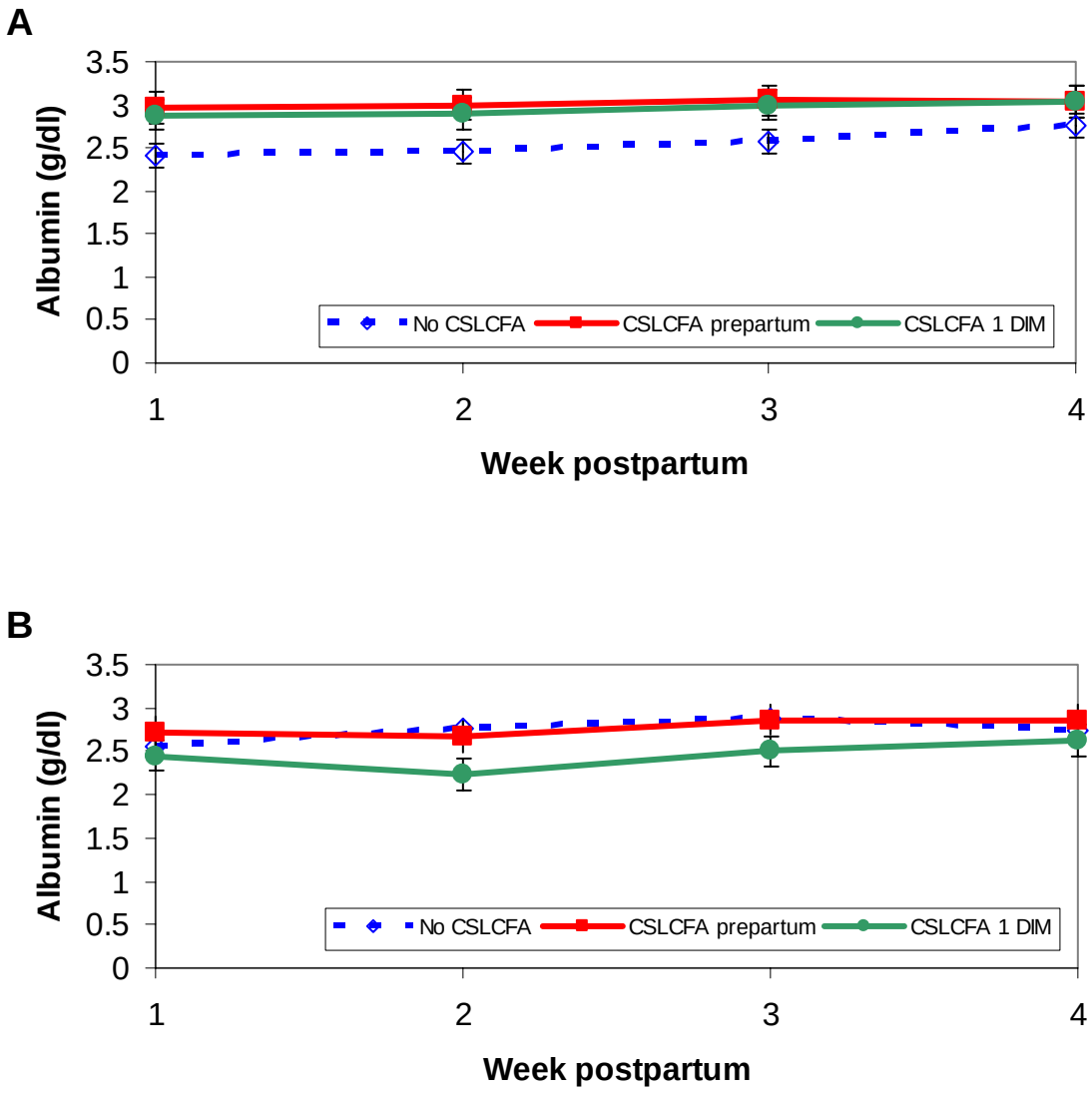

Figure 3.27. Least squares means for concentration of plasma albumin of primiparous (A) or multiparous (B) cows fed diets without calcium salts of long chain fatty acids (CSLCFA) (no CSLCFA) or diets with CSLCFA starting prepartum or at 1 day in milk (DIM). Week postpartum was significant (P = 0.003).

APPENDIX
TESTS OF HETEROGENITY OF REGRESSION

Table A-1. Tests of homogeneity of regression and orthogonal contrasts for treatment effects on plasma Insulin, IGF-I and accumulated progesterone concentrations of Holstein cows fed diets without calcium salts of long chain fatty acids (CSLCFA) or diets with CSLCFA starting prepartum, at 1 day in milk (DIM) or at 28 DIM.

		Error term[2]		Treatment[3]		Orthogonal contrasts[1]		
Response Variable	Order of regression	df	MS	df	MS	A	B	C
Insulin	2	1401	0.058	6	0.058	NS[4]	NS	NS
IGF-I	2	1404	226	6	1521**	**	**	NS
Accumulated progesterone, 1 to 77 DIM	3	1448	73.9	9	91.3	NS	NS	NS
Accumulated progesterone, 1 to 46 DIM	2	845	40.5	6	20.6	NS	NS	NS

[1] Orthogonal contrast of curves were the following: A = No CSLCFA vs. CSLCFA, B = CSLCFA prepartum vs. (CSLCFA 1 DIM plus CSLCFA 28 DIM), and C = CSLCFA 1 DIM vs. CSLCFA 28 DIM.

[2] Error degrees of freedom (df) and error mean squares (MS) when individual curves were generated for each treatment.

[3] Degrees of freedom (df) and mean square (MS) for the difference in residuals between fitting one pooled curve vs. fitting an individual curve for each treatment.

[4] NS = not significant.

* $P < 0.05$.

** $P < 0.01$.

Table A-2. Tests of homogeneity of regression and orthogonal contrasts for treatment effects on plasma PGF2α metabolite (PGFM), haptoglobin, and ceruloplasmin concentrations of Holstein cows fed diets without calcium salts of long chain fatty acids (CSLCFA) or diets with CSLCFA starting prepartum, at 1 day in milk (DIM) or at 28 DIM.

		Error term[2]		Treatment[3]		Orthogonal contrasts[1]	
Response Variable	Order of regression	df	MS	df	MS	A	B
PGFM	3	210	834800	6	224903*	*	NS[4]
Haptoglobin	3	313	850	6	1488	NS	NS
Ceruloplasmin	2	314	10.4	4	8.45	NS	NS

[1] Orthogonal contrast of curves were the following: A = (No CSLCFA plus CSLCFA 1 DIM) vs. CSLCFA prepartum, B =No CSLCFA vs. CSLCFA 1 DIM.
[2] Error degrees of freedom (df) and error mean squares (MS) when individual curves were generated for each treatment.
[3] Degrees of freedom (df) and mean square (MS) for the difference in residuals between fitting one pooled curve vs. fitting an individual curve for each treatment.
[4] NS = not significant.
* $P < 0.05$.

Table A-3. Tests of homogeneity of regression and orthogonal contrasts for parity effects on plasma insulin, IGF-1, accumulated progesterone, PGF2α metabolite (PGFM), haptoglobin, and ceruloplasmin concentrations of Holstein cows fed diets without calcium salts of long chain fatty acids (CSLCFA) or diets with CSLCFA starting prepartum, at 1 day in milk (DIM) or at 28 DIM.

		Error term[1]		Parity[2]	
Response Variable	Order of regression	df	MS	df	MS
Insulin	2	1405	0.058	2	0.215*
IGF-I	2	1408	232	2	200
Accumulated progesterone, 1 to 77 DIM	3	1454	74.0	3	136
Accumulated progesterone, 1 to 46 DIM	2	849	40.1	2	165*
PGFM	3	213	864500	3	1506833
Haptoglobin	3	316	826	3	4632**
Ceruloplasmin	2	316	10.2	2	29.2

[1] Error degrees of freedom (df) and error mean squares (MS) when individual curves were generated for each parityt.
[2] Degrees of freedom (df) and mean square (MS) for the difference in residuals between fitting one pooled curve vs. fitting an individual curve for each parity.
* $P < 0.05$.
** $P < 0.01$.

Table A-4. Tests of homogeneity of regression and orthogonal contrasts for treatment by parity interaction effects on plasma insulin, IGF-1 and accumulated progesterone concentrations of Holstein cows fed diets without calcium salts of long chain fatty acids (CSLCFA) or diets with CSLCFA starting prepartum, at 1 day in milk (DIM) or at 28 DIM.

		Error term[2]		Treatment by parity[3]		Orthogonal contrasts[1]		
Response Variable	Order of regression	df	MS	df	MS	A	B	C
Insulin	2	1393	0.058	14	0.132**	NS[4]	NS	NS
IGF-I	2	1396	224	14	934**	*	**	NS
Accumulated progesterone, 1 to 77 DIM	3	1436	72.0	21	214**	*	NS	**
Accumulated progesterone, 1 to 46 DIM	2	837	39.6	14	85.4**	**	NS	NS

[1] Orthogonal contrast of curves were the following: A = No CSLCFA vs. CSLCFA by parity, B = CSLCFA prepartum vs. (CSLCFA 1 DIM plus CSLCFA 28 DIM) by parity, and C = CSLCFA 1 DIM vs. CSLCFA 28 DIM by parity.

[2] Error degrees of freedom (df) and error mean squares (MS) when individual curves were generated for each treatment and parity.

[3] Degrees of freedom (df) and mean square (MS) for the difference in residuals between fitting one pooled curve vs. fitting an individual curve for each treatment and parity.

[4] NS = not significant.

* $P < 0.05$.

** $P < 0.01$.

Table A-5. Tests of homogeneity of regression and orthogonal contrasts for treatment by parity interaction effects on plasma PGF2α metabolite (PGFM), haptoglobin, and ceruloplasmin concentrations of Holstein cows fed diets without calcium salts of long chain fatty acids (CSLCFA) or diets with CSLCFA starting prepartum, at 1 day in milk (DIM) or at 28 DIM.

		Error term[2]		Treatment by parity[3]		Orthogonal contrasts[1]	
Response Variable	Order of regression	df	MS	df	MS	A	B
PGFM	3	201	834079	15	1407712*	*	NS[4]
Haptoglobin	3	304	830	15	1512*	*	NS
Ceruloplasmin	2	308	10.4	10	10.1	NS	NS

[1] Orthogonal contrast of curves were the following: = (No CSLCFA plus CSLCFA 1 DIM) vs. CSLCFA prepartum by parity, B =No CSLCFA vs. CSLCFA 1 DIM by parity.

[2] Error degrees of freedom (df) and error mean squares (MS) when individual curves were generated for each treatment.

[3] Degrees of freedom (df) and mean square (MS) for the difference in residuals between fitting one pooled curve vs. fitting an individual curve for each treatment.

[4] NS = not significant.

* $P < 0.05$.

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BIOGRAPHICAL SKETCH

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