Introduction

Uterine health is critical for establishment and maintenance of pregnancy in dairy cattle, particularly during first postpartum insemination when uterine involution and the uterine environment may be suboptimal (Melendez and Risco 2005). Prostaglandin F$_2$a (PGF$_{2a}$), a hormone from the family of prostanoids is synthesized primarily by the endometrial cells from arachidonic acid (C20 : 4), and it regulates the oestrous cycle in female cattle. Prostaglandin F$_{2a}$ is responsible for lysis of the corpus luteum (CL), and is also implicated in involution of the uterus after parturition (Kindahl et al. 1992). Cows that did not develop uterine infection had increased concentrations of PGF$_{2a}$, metabolite (PGFM: 13,14-dihydro-15 keto-PGF$_{2a}$) in plasma in the first 2 weeks postpartum, indicating that changes in PGF$_{2a}$ concentrations may influence development of uterine infection (Seals et al. 2002). The exact mechanism through which prostaglandins participate in involution of uterine tissue is not completely understood, but the increase in electrical activity of myometrial cells, and consequent increase in uterine contraction, has been implicated (Gajewski et al. 1999). In addition, exogenous administration of PGF$_{2a}$ to ewes increased lymphocyte proliferation independent of progesterone levels (Lewis and Wulster-Radcliffe 2006). Consistent with these findings, administration early postpartum of PGF$_{2a}$ analogue, dinoprost tromethamine, to cows diagnosed with metritis resulted in smaller diameter of uterine horn in primiparous cows, which was also associated with increased pregnancy rates, but a similar response was not observed in multiparous cows (Melendez et al. 2004).

Specific polyunsaturated fatty acids (PUFA) can modulate cellular metabolism beyond the supply of energy. In fact, PUFA were reported to influence cellular metabolism and gene expression in different tissues, such as inhibition of enzymes in mammary gland (Peterson et al. 2003), change in synthesis of metabolites and gene expression in endometrial cells (Mattos et al. 2004; Bilby et al. 2006b), and modification of gene expression in hepatocytes (Selberg et al. 2005). Increased intake of linoleic acid to 170% of the basal diet resulted in greater concentrations of PGFM in ewes during the last 3 days of gestation, in both maternal and foetal circulation, in response to dexamethasone administration (Elmes et al. 2005). Similar findings occurred in late lactation cows supplemented with linoleic acid at 225% of the basal diet, which resulted in larger plasma PGFM response to an oxytocin challenge (Petit et al. 2004). Supplementation with n-3 (Mattos et al. 2004; Bilby et al. 2006c; Moussavi et al. 2007) and n-6 fatty acids (FA; Burns et al. 2003) increased incorporation of these FA into endometrial tissue, which was, in some studies (Mattos et al. 2004), but not all (Moussavi et al. 2007), linked to changes in secretion of prostaglandins. Moreover, there is evidence that embryonic FA composition may impact embryo development. Human embryos that developed up to the blastocyst stage during in vitro incubation had more than twice the concentration of linoleic acid than embryos that arrested development at an earlier stage (Haggarty et al. 2006).

Because supplementation with linoleic acid has increased synthesis of PGF$_{2a}$, and the latter has been linked to enhanced uterine health and involution, it was hypothesized that supplementation with calcium salt of linoleic and trans-octadecenoic acids (LTFA) would increase uterine synthesis of prostaglandin in the first weeks of lactation and improve uterine health, which ultimately would result in increased pregnancy rates. The objective of this study was to evaluate effects of increasing intake of LTFA on uterine involution, synthesis of PGF$_{2a}$ and pregnancy rates in early lactation Holstein cows.
Materials and Methods

The University of California, Davis Institutional Animal Care and Use Committee approved all procedures involving cows in this study.

Animals, experimental design and feeding

Late gestation (233 ± 3 days) Holstein cows (n = 501) were assigned to one of the two treatments in a randomized incomplete block design. Cows were fed the same pre- and postpartum total mixed ration that differed only in the source of supplemental FA (Table 1). Treatments were a calcium salt of palm oil [PO; 2% of dry matter (DM); EnerG-II® Virtus Nutrition, LLC, Fairlawn, OH, USA] or a calcium salt rich in LTFA (2% of DM; EnerG-I Transition Formula®, Virtus Nutrition, LLC). Diets were formulated to meet, or exceed, National Research Council (NRC 2001) nutrient requirements for net energy for lactation, crude protein, fibre, mineral and vitamins, and provided an intake of 200 and 400 g/day of calcium salts for pre- and postpartum cows, respectively. A detailed description of housing, experiment design and diet composition is reported elsewhere (Juchem et al. 2004).

Treatment diets were fed from −25 days prepartum to 80 days of lactation, after which all cows were fed the PO diet. Cows were scored for body condition utilizing the 1–5 scale with increments of 0.25 units (Ferguson et al. 1994) at −43 and −25 ± 3 days prepartum, at calving, and at 39, 66, 96 and 143 ± 3 days postpartum.

Reproductive management, pregnancy diagnosis and reproductive outcomes of interest

After calving, all cows were observed daily for signs of uterine diseases by the investigators. Rectal temperature was measured daily in all early lactation cows. Retained placenta was diagnosed in those cows that did not shed their foetal membranes within the first 24 h after calving. All cows were palpated per rectum in the first 2 weeks postpartum for diagnosis of metritis. Presence of watery, fetid vaginal discharge of uterine origin was utilized to diagnose cows with metritis. Cows that had metritis concomitantly with rectal temperature 39.5°C were diagnosed with puerperal metritis (Sheldon et al. 2004). Presence of previous pregnant horn to the diameter of the non-pregnant horn, and the relative change in diameter of the previous pregnant horn (i.e. diameter at week 3/diameter at week 2) were used to compare the rate of uterine involution between treatments. Because these measurements yielded similar results, the ratio of diameter of previously pregnant to non-pregnant horns are shown.

Cows assigned to be inseminated, 344, received an intramuscular (i.m.) injection of 25 mg of PGF2α (dino-prost tromethamine; 5 ml Lutalyme® Sterile Solution, Pfizer Animal Health, New York, NY, USA) at 50 ± 3 days in milk and were then enrolled in a timed artificial insemination (AI) protocol 10 days later, at 60 ± 3 days of lactation. The synchronization consisted of an injection of 100 μg of GnRH i.m. (Cysto- reline, 50 μg/ml gonadorelin diacetate tetrahydrate, Merial Ltd., Iselin, NJ, USA), followed 7 days later by an i.m. injection of 25 mg of PGF2α, and 24 h later by an i.m. injection of 1 mg of oestradiol cyanopine (2.0 mg/ml; Pfizer Animal Health), with timed AI performed 48 h after oestradiol injection, at 70 ± 3 days postpartum. Pregnancy was diagnosed at 27 days after AI by examination of the uterine horns and its contents by ultrasonography using a 7.5 MHz transrectal linear probe (Sonovet 2000, Universal Medical System, Bedford Hills, NY, USA). Presence of an amniotic vesicle with an embryo was used as an indicator of pregnancy. Cows diagnosed as pregnant on day 27 were re-evaluated 14 days later, at 41 days of gestation by palpation per rectum, whereas non-pregnant cows that had not been re-inseminated were re-enrolled in the timed AI protocol upon diagnosis of non-pregnancy. After the first postpartum AI, cows were observed once daily for signs of oestrus based on the removal of coloured chalk (All-weather Paintstick, LA-CO Industries, Chicago, IL, USA) from the tail head.

Blood sampling and analysis of prostaglandin metabolite

Blood was sampled four times weekly on Mondays, Wednesdays, Thursdays and Saturdays from 114 cows, 56 fed PO and 58 fed LTFA, during the first 7 days of lactation. In addition, 60 of the 114 cows, 30 fed PO and 30 LTFA, were also sampled four times weekly until 21 days postpartum, therefore concentrations of PGFM were analyzed from two groups of cows, the 114 cows collected in the first week postpartum and the 60 cows sampled in the first 3 weeks postpartum. Because

Table 1. Fatty acid profile from the supplemental fat sources

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>PO</th>
<th>LTFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>C16 : 0</td>
<td>45.0</td>
<td>11.1</td>
</tr>
<tr>
<td>C18 : 0</td>
<td>4.18</td>
<td>10.23</td>
</tr>
<tr>
<td>C18 : 1 total cis</td>
<td>37.47</td>
<td>17.73</td>
</tr>
<tr>
<td>C18 : 1 total trans</td>
<td>0.20</td>
<td>43.20</td>
</tr>
<tr>
<td>C18 : 2 e9, c12, n-6</td>
<td>10.93</td>
<td>15.50</td>
</tr>
<tr>
<td>C18 : 2 t10, c12</td>
<td>&lt;0.001</td>
<td>0.11</td>
</tr>
<tr>
<td>C18 : 3 e8, c12, c15, n-3</td>
<td>0.32</td>
<td>0.11</td>
</tr>
<tr>
<td>C20 : 5, n-3 (EPA)</td>
<td>&lt;0.001</td>
<td>0.02</td>
</tr>
<tr>
<td>C22 : 6, n-3 (DHA)</td>
<td>0.03</td>
<td>0.03</td>
</tr>
</tbody>
</table>

PO: calcium salt of palm oil; LTFA: calcium salt of linoleic and trans- octadecenoic acids; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid.

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cows were not sampled on the exact same day postpartum, day postpartum was analyzed to determine whether differences existed between treatments when blood was sampled. The mean day when a sample was collected was similar (p > 0.60) between treatments. The least squares means for days relative to parturition in which blood was sampled are presented in the x-axis of Fig. 1.

Approximately 10 ml of blood from the coccygeal vessels were collected into evacuated tubes containing 17.55 mg of K2 EDTA (Vacutainer, Becton Dickinson, Franklin Lakes, NJ, USA), and kept in ice until transported to the laboratory. Blood was centrifuged at 5°C and 2000 × g for 15 min for plasma separation. Plasma was stored at −25°C for future determination of PGFM. Concentration of PGFM in plasma utilized radioimmunoassay as described by Guilbault et al. (1984), and modified according to Mattos et al. (2004).

Fig. 1. Panel a, plasma concentrations of prostaglandin F2α, metabolite (PGFM; pmol/l) during the first 3 weeks of lactation for primiparous and multiparous cows supplemented with calcium salts of palm oil (PO; – □– ■, n = 30) or calcium salts of linoleic and trans-octadecenoic acids (LTFA; – ▲– ▼, n = 30). Panel b, plasma concentrations of PGFM in the first 2 days of lactation in primiparous (– □– ■, n = 15) and multiparous (– ▲– ▼, n = 15) cows fed PO, and primiparous (– ▲– ▼, n = 15) cows fed LTFA. Day postpartum indicate the least squares means at sampling. At day 1 postpartum, PO and LTFA cows were sampled (LSM ± SEM) at 0.6 ± 0.1 and 0.5 ± 0.1 day postpartum, respectively. Supplementation with the LTFA tended (p = 0.07, SEM = 1114) to increase concentration of PGFM in plasma on day 1 of lactation (Panel a). The interaction between treatment, day postpartum and parity (**p < 0.02) in the first 2 days of lactation is depicted on Panel b (Fig. 1; Panel a). Supplementation with LTFA increased (p = 0.02) PGFM in primiparous cows at day 1 (Fig. 1; Panel b) when compared with primiparous fed PO. Yet, in multiparous cows, concentrations of PGFM did not differ (p > 0.60) for cows fed PO or LTFA. Concentration of PGFM in plasma of the 60 cows sampled during the first 3 weeks of lactation for primiparous and multiparous cows supplemented with calcium salts of palm oil (PO; – □– ■, n = 30) or calcium salts of linoleic and trans-octadecenoic acids (LTFA; – ▲– ▼, n = 30). Panel b, plasma concentrations of PGFM in the first 2 days of lactation in primiparous (– □– ■, n = 15) and multiparous (– ▲– ▼, n = 15) cows fed PO, and primiparous (– ▲– ▼, n = 15) cows fed LTFA. Day postpartum indicate the least squares means at sampling. At day 1 postpartum, PO and LTFA cows were sampled (LSM ± SEM) at 0.6 ± 0.1 and 0.5 ± 0.1 day postpartum, respectively. Supplementation with the LTFA tended (p = 0.07, SEM = 1114) to increase concentration of PGFM in plasma on day 1 of lactation (Panel a). The interaction between treatment, day postpartum and parity (**p < 0.02) in the first 2 days of lactation is depicted on Panel b (Fig. 1; Panel a). Supplementation with LTFA increased (p = 0.02) PGFM in primiparous cows at day 1 (Fig. 1; Panel b) when compared with primiparous fed PO. Yet, in multiparous cows, concentrations of PGFM did not.

Results

Body condition score of cows was described in detailed in Juchem et al. (2004). Nevertheless, both BCS and change in BCS during the study were not influenced by treatment.

Of the initial 114 cows used to evaluate plasma PGFM concentrations, 2 were diagnosed with retained placenta, 10 with metritis and 8 with puerperal metritis. Mean days postpartum when blood samples were collected for plasma PGFM analysis did not differ (p > 0.60) for cows fed PO or LTFA. Concentration of PGFM in plasma of the 60 cows sampled during the first 3 weeks postpartum remained elevated during the first 2 weeks of lactation, with concentrations generally greater than 200 pm, and they returned to baseline values after the end of the second week postpartum (Fig. 1; Panel a). Supplementation with LTFA increased (p = 0.02) PGFM in primiparous cows at day 1 (Fig. 1; Panel b) when compared with primiparous fed PO. Yet, in multiparous cows, concentrations of PGFM did not.

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differ \((p = 0.11)\) between PO and LTFA cows. Similar responses in the first week postpartum were observed for PGFM concentrations when data from 114 cows sampled in the first 7 days postpartum were analyzed. The same interaction \((p < 0.01)\) between treatment and parity and day postpartum was observed, and supplementation with LTFA increased \((p = 0.03)\) concentrations of PGFM in plasma of primiparous cows in the first day postpartum, but no effect of diet was observed for multiparous cows.

Incidence of uterine disease was not affected \((p = 0.68)\) by diet in general, but severity of disease differed between treatments. Cows fed LTFA tended \((p = 0.08)\) to be less likely [adjusted odds ratio \((AOR) = 0.53\)] to develop puerperal metritis (Table 2). Consequently, the incidence of metritis was greater \((p = 0.02)\) for cows fed LTFA compared with those fed PO \((7.2\% \text{ vs } 15.5\%)\). Uterine involution, based on the absence of intrauterine fluid, tended \((p = 0.09)\) to be 3.2 days shorter for multiparous cows fed LTFA than PO, but there was no effect of diet for primiparous cows. The proportion of cyclic cows at 42 days postpartum was similar between PO and LTFA, and interval to first postpartum ovulation did not differ between treatments and averaged 31.3 days.

Diameter and rate of change of the non-pregnant horn were similar for cows fed PO and LTFA (Fig. 2). In the first 6 weeks postpartum, the diameter of the non-pregnant horn averaged 21.6 and 22.1 mm in cows fed PO and LTFA, respectively (Fig. 2). Likewise, the rate of involution of the previously pregnant horn was similar between PO and LTFA, and averaged 21.6 and 22.1 mm in cows fed PO and LTFA, respectively (Fig. 2). PO and LTFA, respectively (Fig. 2). In the first 6 weeks postpartum, the diameter of the non-pregnant horn were similar for cows fed PO and LTFA (Fig. 2). In the first 6 weeks postpartum, the diameter of the non-pregnant horn averaged 21.6 and 22.1 mm in cows fed PO and LTFA, respectively (Fig. 2). Likewise, the rate of involution of the previously pregnant horn was similar for cows receiving the different sources of FA. Multiparous cows had larger \((p = 0.02)\) previously pregnancy horn diameter than primiparous cows, but rate of involution did not differ between parity based on the lack of interaction \((p = 0.41)\) between parity and week postpartum and the parallelism of the lines in

### Table 2. Incidence of uterine diseases and resumption of cyclicity in cows supplemented with calcium salts of palm oil (PO) or calcium salts of linoleic and trans-octadecenoic acids (LTFA)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>PO</th>
<th>LTFA</th>
<th>AOR 95% CI</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cows (n)</td>
<td>246</td>
<td>255</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retained placenta (%)</td>
<td>6.5</td>
<td>6.7</td>
<td>1.02</td>
<td>0.50–2.07</td>
</tr>
<tr>
<td>Uterine disease (%)</td>
<td>22.3</td>
<td>24.4</td>
<td>1.14</td>
<td>0.66–1.88</td>
</tr>
<tr>
<td>Puerperal metritis (%)</td>
<td>15.1</td>
<td>8.8</td>
<td>0.53</td>
<td>0.26–1.06</td>
</tr>
<tr>
<td>Metritis (%)</td>
<td>7.2</td>
<td>15.6</td>
<td>2.38</td>
<td>1.17–5.00</td>
</tr>
<tr>
<td>Uterine involution (%)</td>
<td>90.8</td>
<td>91.2</td>
<td>1.05</td>
<td>0.47–2.35</td>
</tr>
<tr>
<td>Uterine involution, day ± SEM</td>
<td>30.3 ± 0.7</td>
<td>28.3 ± 0.7</td>
<td>—</td>
<td>0.16</td>
</tr>
<tr>
<td>First ovulation, day ± SEM</td>
<td>27.7 ± 0.8</td>
<td>29.6 ± 0.8</td>
<td>—</td>
<td>0.13</td>
</tr>
<tr>
<td>Cycling cows (%)</td>
<td>89.7</td>
<td>83.8</td>
<td>0.58</td>
<td>0.27–1.20</td>
</tr>
</tbody>
</table>

Metrits, cows with fetid vaginal discharge of uterine origin during the first 3 weeks of lactation; puerperal metritis, metritis associated with rectal temperature ≥39.5°C; uterine disease, the sum of cows diagnosed with metritis and puerperal metritis; cycling, cows that had a corpus luteum visualized by ultrasound examination of the ovaries during the first 6 weeks postpartum. Cows were considered to have had the uteri completely involuted when no uterine fluid was visualized by ultrasound examination in the first 6 weeks postpartum.

AOR, adjusted odds ratio; CI, confidence interval. PO is the reference category.

**Discussion**

Incorporation of PUFA in endometrial cells can impact synthesis of prostaglandins (Mattos et al. 2004) and alter uterine gene expression (Bilby et al. 2006b). Furthermore, some have suggested that trans-octadecenoic fatty acids might influence uterine health and reproductive efficiency of early lactation dairy cows by altering prostaglandin release by the uterus (Rodriguez-Sallaberry et al. 2007).

Cows fed LTFA were supplemented with a calcium salt that contained 42% more linoleic acid than PO,
which consistently increased the concentration of PGFM in plasma of primiparous cows (Fig. 1); however, the increase in PGFM was of short duration, limited to the first 2 days after calving. Release of PGF$_2$\alpha from the uterus occurs in a pulsatile pattern, and PGFM in plasma from cows with uncomplicated puerperium returned to baseline values considerably quick, at 12 (Mattos et al. 2004) to 20 days after parturition (Seals et al. 2002). Feeding a source of unsaturated FA to transition cows increased PGFM concentrations in plasma and the increase was attributed to the high content of trans-octadecenoic fatty acids (Rodriguez-Sallaberry et al. 2007). Yet, the fat sources fed in the current study also differed in their content of linoleic acid, which is known to stimulate uterine prostaglandin release (Elmes et al. 2005). Even when large doses of FA were supplemented, approximately 5 times greater than the basal diet, changes in prostaglandin secretion were limited to the first 2 days after calving. Release of PGF$_2$\alpha from the uterus occurs in a pulsatile pattern, and PGFM in plasma from cows with uncomplicated puerperium returned to baseline values considerably quick, at 12 (Mattos et al. 2004) to 20 days after parturition (Seals et al. 2002). Feeding a source of unsaturated FA to transition cows increased PGFM concentrations in plasma and the increase was attributed to the high content of trans-octadecenoic fatty acids (Rodriguez-Sallaberry et al. 2007). Yet, the fat sources fed in the current study also differed in their content of linoleic acid, which is known to stimulate uterine prostaglandin release (Elmes et al. 2005). Even when large doses of FA were supplemented, approximately 5 times greater than the basal diet, changes in prostaglandin secretion were
of short duration, limited to the first week after parturition (Scholljegerdes et al. 2007). Whether such limited increase in uterine synthesis of prostaglandins has a biological role is not clear, but cows that developed metritis had smaller PGFM concentrations during the first week postpartum compared with cows with healthy uteri (Seals et al. 2002), suggesting that short increases in synthesis of prostaglandin during the immediate postpartum might be sufficient to benefit uterine defence mechanisms. In the present study, concentration of PGFM during the first 6 days post-partum had no effect on change in diameter of the uterine horns (Fig. 3) and on the interval to clearance of uterine fluid. It is possible that the lack of effect was because increased concentrations of PGFM in plasma were not as persistent as reported in previous studies. Severe metritis, associated or not with systemic signs, compromised rate of uterine involution, and increased the score of intrauterine fluid during the first 4 weeks after parturition compared with cows that did not develop uterine disease (Mateus et al. 2002). In contrast, mild endometritis had no detrimental effects on change in uterine diameter, and slightly increased uterine fluid score in the same study (Mateus et al. 2002). Cows fed LTFA tended to have less complicated cases of metritis (Table 2), which is possibly associated with improved defence against bacterial infection and reduction of pyrexia in those cows, as suggested by others (Rodriguez-Sallaberry et al. 2007).

The size of the previously pregnant uterine horn at 5 weeks of lactation was associated with risk of pregnancy (Fig. 4) and, therefore, utilized as a parameter to evaluate uterine involution. The average PGFM concentration during the first week postpartum was similar for cows categorized as having the previously pregnant uterine horn diameter $<$ 22.21 mm or $>$ 22.20 mm at 5 weeks postpartum. Because concentration of PGFM during the first week of lactation did not affect the rate of uterine involution, the initial study hypothesis that stimulating increased prostaglandin concentrations in the immediate postpartum would benefit uterine involution is not supported. Yet, this possibility cannot be completely ruled out because the increase in synthesis of PGFM was only observed on the day after parturition. Plasma PGFM increased for approximately 7 days postpartum in cows that did not develop uterine infection (Seals et al. 2002), which is considerably longer than in the present study. Therefore, the improvement in pregnancy rates observed by feeding LTFA is more likely to be a direct effect of these FA on factors other than uterine involution and health. Potential candidates are oocyte and embryo quality (Santos et al. 2008).

Metritis and retained placenta are important risk factors for poor fertility. Cows that developed metritis were 15% less likely to conceive (Gröhn and Rajala-Schultz 2000), and 3.3 times more likely to experience delayed conception (Kim and Kang 2006). A compilation of different studies that evaluated incidence of metritis observed that the median incidence was 10.1%, although the range was wide, from 2.2% to 37.3% (Melendez and Risco 2005). It is possible that marked differences in incidence of metritis are the result of differences in postpartum cow management, case definition and knowledge of the person making the diagnosis. The incidence of uterine disease in the current study was similar for cows fed PO and LTFA, and within the range reported for cows fed PO. Melendez and Risco (2005), although cows fed LTFA tended to have less complicated cases of metritis than cows fed PO. Because incidence of other diseases that have been shown to increase the risk of uterine disease did not differ between treatments (Juchem et al. 2004), the trend to reduced incidence of puerperal metritis can be interpreted as potential benefit of LTFA supplementation. In addition, it is likely that this benefit was independent of uterine prostaglandin synthesis, although prostaglandins can upregulate uterine immune defences when the uterus is exposed to the immunosuppressive effects of progesterone (Lewis and Wulster-Radcliffe 2006). The leucocytes that infiltrate the uterine lumen are an important mechanism of defence against bacterial infection.

Degree of metritis is related to the extent of bacterial contamination and integrity of host defence mechanisms. Leucocytes from cows that did not develop metritis had increased phagocytic capacity compared with those from cows that had uterine disease (Kim et al. 2005). Supplementation with linoleic acid as phophatidylcholine for 3 days increased in vitro phagocytosis and killing of Candida albicans in healthy adult humans (Jannace et al. 1992). The latter study demonstrated the ability of PUFA to modulate cellular immune response, and it is reasonable to suggest that altering the dietary FA fed to cows might have influenced innate immune function, thereby resulting in reduced risk of puerperal metritis in cows fed LTFA.

Dietary fat is known to modify the FA composition of the endometrium (Burns et al. 2003; Mattos et al. 2004; Moussavi et al. 2007), follicular fluid (Zeron et al. 2002), but not oocytes (Zeron et al. 2002). Incorporation of PUFA into the phospholipid bilayer alters membrane fluidity, and is one of the mechanisms by which FA can impact cell metabolism. In addition, some FA control transcriptional factors that regulate biochemical pathways involved in lipid metabolism and insulin sensitiveness (Bocher et al. 2002) and pregnancy recognition (MacLaren et al. 2006). Heat stress dramatically changed the FA composition of oocytes, granulosa cells and follicular fluid, and was associated with a sizeable decline in risk of pregnancy in dairy herds (Zeron et al. 2001). Cows that had ovarian follicles aspirated during the winter had increased concentrations of PUFAs, particularly oleic and linoleic acids, whereas concentration of palmitic acid was reduced compared with cows with follicles aspirated in the summer months. Concentration of linoleic acid was 3.6 and 4.1 times greater in the oocytes and granulosa cells, respectively, during the winter than summer (Zeron et al. 2001). Increased concentrations of PUFAs during the winter months were associated with increased pregnancy rate, almost threefold that of observed for cows in the summer months (14.2% vs 41.6%). Whether PUFA were simply associated with increased pregnancy, or were the causative event of altering fertility needs to be determined. Nevertheless, a similar pattern of FA compositional change occurred in human embryos.
cultured *in vitro*, and those that developed beyond the 4-cell stage had increased concentrations of unsaturated FA (Haggarty et al. 2006).

Feeding calcium salts of fish oil to ewes increased incorporation of PUFA in the follicular fluid and in the cumulus cells, but FA composition of oocytes were not altered (Zeron et al. 2002). Increased PUFA content was linked to better oocyte quality and improved their resistance to chilling. However, because diets were not isoenergetic, it is not possible to separate effects of FA, total fat content or net energy density of the diet on oocyte quality. Other studies *in vitro* supplemented PUFA in isoenergetic diets and did not report any benefit on oocyte and embryo quality (Bilby et al. 2006a). Yet, it is unlikely that embryo responses to FA *in vitro* mimic those *in vivo*; generally, feeding unsaturated FA improved embryo quality *in vivo* (Santos et al. 2008). In support of these observations, Cerri et al. (2008) fed non-supersaturated dairy cows the same combination of FA fed in the current study, 2% of the diet DM as PO or LTFA and observed a tendency to increased fertilization and number of accessory spermatozoa. Furthermore, feeding LTFA resulted in improved embryo quality (Cerri et al. 2008). Consequently, improvements in pregnancy rates observed in the current study might be mediated, at least in part, by improved fertilization and embryo quality.

It is interesting to note that effects of LTFA on pregnancy rates did not persist after removal of the supplemental fat, as demonstrated by similar risk of pregnancy at second postpartum AI and lack of overall effect on time to pregnancy. Therefore, benefits to altering the FA profile in the diet of dairy cows may be limited to the period in which they consume the respective FA.

**Conclusions**

Feeding calcium salts of LTFA compared with a calcium salt of PO increased pregnancy rates following the first postpartum insemination. Synthesis of PGF<sub>2α</sub> based on concentration of PGFM in plasma was slightly increased on the first day postpartum, but improvements in uterine involution were modest and not associated with changes in plasma PGFM. Whether increases in PGF<sub>2α</sub> directly improve uterine environment such that embryo survival is favoured deserves further investigation. Feeding LTFA improved pregnancy, which apparently was independent of uterine involution, and possibly through improvement in embryo quality, or uterine health or both. In conclusion, feeding LTFA during late gestation and early lactation improves fertility of dairy cows, but benefits seem to be restricted to the period in which FA are fed.

**Acknowledgements**

This research was supported by grants from the Center for Food Animal Health of the School of Veterinary Medicine, University of California Davis, Virtus Nutrition, LLC, and the National Research Initiative Competitive Grant no 2004-35203-14137 from the USDA Cooperative State Research, Education, and Extension Service. Our thanks are extended to the staff of Corcoran State Prison Dairy for use of their facilities and animals and to Daniel Luchini for suggestions during the conduct of the experiment.

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Submitted: 04 Jun 2008

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