Feeding n-6 and n-3 Fatty Acids to Dairy Cows: Effects on Immunity, Fertility and Lactation

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Introduction

Metritis and retained placenta are important risk factors for poor fertility. Dairy cows that develop metritis and retained placenta were 15% and 14% less likely to conceive (Grohn and Rajala-Schultz, 2000) and 3.3 times more likely to experience delayed conception (Kim and Kang, 2006) than normal cows. The incidence of metritis ranges from 2.2 to 37% (Melendez and Risco, 2005) and is dependent on transition cow management and case definition. Additionally, postpartum metritis has a major economic impact for dairy operations because of increased treatment expenses, milk loss, prolonged days open and culling rates (Esslemont and Peeler, 1993). Moreover, metritis and retained placenta are risk factors for subclinical endometritis in dairy cows (Rutigliano et al., 2008; Silvestre, 2008). Subclinical endometritis has marked negative effects on fertility (Kasimanickam et al., 2004; Gilbert et al., 2005; Rutigliano et al., 2008).

Another important event that determines the successful establishment of pregnancy is the ability of the conceptus to secrete interferon tau (IFN-τ), which inhibits secretion of $PGF_{2\alpha}$ by the uterine endometrium (Thatcher et al., 1994), and allows for maintenance of the corpus luteum. It is estimated that at least 40% of total embryonic losses occur between days 8 to 17 of pregnancy. This high proportion of losses precedes or occurs concurrently with the period when the embryo inhibits uterine secretion of $PGF_{2\alpha}$. Thus some loss of pregnancies may occur because non-viable or under-sized embryos secret sub-optimal quantities of IFN-τ needed to inhibit secretion of $PGF_{2\alpha}$ by the endometrium (Bilby et al., 2006a). Also, causes for pre-implantation embryo loss may involve both extra-uterine inflammatory mediators (i.e., tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), nitric oxide and $PGF_{2\alpha}$) that can affect embryonic development by acting either on the oocyte or on the developing embryo (Hansen et al., 2004).

An immunological suppression or tolerance is present during pregnancy; the fetus and fetal component of the placental unit is an allograft that needs to avoid rejection by the maternal unit for the duration of pregnancy (Siiteri and Stites, 1982).

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Maintenance of an immunologically favorable, immunosuppressive environment in the uterus is needed for embryo survival (Raghupathy, 2001; Hunt et al., 2005). An immune response to a foreign body starts with the induction of an inflammatory response that is amplified by cytokines produced by cells (i.e., epithelial cells, macrophages and later neutrophils) in the vicinity of the foreign body. Therefore strategies to partially inhibit secretion of $PGF_{2\alpha}$ and to cause less of a responsive immune response during breeding may increase pregnancy rates and embryonic survival.

Fatty Acids and their Synthesis of Effector Molecules

Fatty acids (**FAs**) are classified as lipids, which are biological compounds that are soluble in organic solvents. Lipids include cholesterol and fats such as triacylglycerols and phospholipids. Phospholipids are major components of cellular membranes and are a source of FAs for the synthesis of a variety of effector molecules such as the eicosanoids, a group of compounds that includes prostaglandins, thromboxanes and leukotrienes. Changes in chain length, degree of unsaturation and position of the double bonds in the acyl chain of FAs can have remarkable impacts on their function. The essential polyunsaturated FA, linoleic acid (**LN**; C18:2n-6) undergoes steps of chain elongation and desaturation forming differential n-6 products, such as dihomo-γ linolenic (C20:3n-6) and arachidonic (**AA**; C20:4n-6) acids. Likewise, α-linolenic acid (**ALN**; C18:3n-3) can form n-3 products such as eicosapentaenoic acid (**EPA**; C20:5n-3) and docosahexaenoic acid (**DHA**; C22:6n-3). These specific long chain polyunsaturated FAs produce eicosanoid products of the prostaglandin series PGF_1, PGF_2, and PGF_3, respectively, as well as various thromboxanes, leukotrienes, lipoxins, hydroperoxy-eicosatetraenoic acids and hydroxyeicosatetraenoic acids (HETE) that regulate inflammation and immunity.

Ruminant diets are supplemented with fat primarily to increase energy concentration and to enhance animal performance. However, when fat is fed in early lactation, cows often consume somewhat less of the diet and/or produce more milk. Therefore fat feeding during early postpartum seldom alters energy status even though a more energy dense ration is consumed. This suggests that specific biological responses are achieved by supplying differential FA's and altering substrate availability to the cow rather than simply attributing effects to additional energy. However, a major challenge in fat feeding is the inability to predict the delivery of specific FAs, particularly polyunsaturated FAs to the small intestine for absorption.

Juchem (2007) demonstrated that more than 70% of the LN and more than 85% of ALN fed to lactating cows were biohydrogenated in the rumen when fed as unprotected oils or as Ca salts (**CS**) of long chain FAs, respectively. Despite ruminal biohydrogenation, continual feeding of CS of fat enriched with fish oil (**FO**) increased concentrations of EPA and DHA in endometrium (associated with reduction in the proportion of AA), liver, mammary, muscle, subcutaneous and internal adipose tissues (Bilby et al., 2006b) of dairy cows. Therefore, daily feeding of CS of selective FAs is a practical approach in which tissue FA composition can be manipulated accordingly to the stage of the life cycle of the cow.

Neutrophils and Acute Phase Responses

Neutrophils are part of the innate immune system acting upon antigens in a non-specific manner as the first line of defense against pathogens. The migration of neutrophils from the vasculature is a stepwise process. Initially, capture and rolling adhesion mediated by the selectins (i.e. L-selectin) maintains a marginalization of neutrophils to the vascular endothelium. After this initial step, the neutrophil must be activated by chemoattractants (i.e., C5a, IL-8, TNF-α, leukotrienes) and up-regulation of integrins for firm adhesion or arrest of the neutrophils to the endothelium. The firm anchoring of the neutrophil at the site of inflammation allows these cells to evade the circulating blood and enter the tissues by diapedisis. Once at the site of infection neutrophils can internalize and kill many microbes by phagocytic activity that consists in the formation of a phagosome into which reactive oxygen species (i.e., O^{2-}, H_2O_2 and HOCl) and hydrolytic enzymes are secreted. The consumption of oxygen during the generation of reactive oxygen species has been termed “oxidative burst.”

Impaired neutrophil function during the peri-partum period increases the risk of retained fetal membranes (Kimura et al., 2002), metritis (Hammon et al., 2006), endometritis (Kim et al., 2005), and subclinical endometritis (Hammon et al., 2006) in dairy cows. Mechanisms of immunosuppression at parturition are related to the release of glucocorticoids (i.e. cortisol) during this time (Preisler et al., 2000) and reduced levels of L-selectin in neutrophils (Weber et al., 2001). Also, metabolic challenges imposed by the onset of lactation (Kimura et al., 1999), hypocalcemia (Kimura et al., 2006), and selenium deficiencies (Silvestre et al., 2006) can reduce neutrophil bactericidal activity during the peri-partum period.

The acute phase response of the liver is characterized by the secretion of acute phase proteins (i.e. haptoglobin and fibrinogen) when induced by pro-inflammatory cytokines (i.e., TNF-α, IL-1) produced by epithelial cells, macrophages and neutrophils present at the sites of inflammation (Petersen et al., 2004). The acute phase response is often used as a marker of stress; therefore often confused with an unwanted response. However, the acute phase response provides an early non-specific defense mechanism against insult before specific immunity is achieved (Peterson et al., 2004). Blood haptoglobin binds free hemoglobin restricting the availability of iron necessary for bacterial growth in the blood and prevents pro-oxidant activity of hemoglobin on tissues (Eaton et al., 1982). Fibrinogen is the precursor of fibrin, a protein that activates neutrophil toll like-receptors and $\alpha_M\beta_2$-integrin receptor to induce intracellular calcium mobilization, phosphorylation events, NF-kB activation (i.e., expression of cytokines, COX-2), and both cell adhesion and migration (Flick et al., 2004).

Effects of n-6 Fatty Acids in the Postpartum Period

During parturition, a very intense secretion of uterine $PGF_{2\alpha}$ occurs as evidenced by dramatic increases in concentration of plasma 13, 14 dihydro, 15-keto $PGF_{2\alpha}$ metabolite (**PGFM**) (Guilbault et al., 1984) in cows. Plasma concentrations of PGFM peaked at concentrations >1 ng/ml within 1 to 4 days after parturition and decreased

progressively until day 15 postpartum (Guibault et al., 1984) as the uterine size decreases and caruncular tissue (i.e., main source of uterine $PGF_{2\alpha}$) is sloughed. Seals et al. (2002) showed that concentrations of PGFM in jugular plasma were higher during the first 6 days postpartum (**dpp**) for cows that did not develop uterine infections (2160 pg/ml) in comparison to cows that developed endometritis (1450 pg/ml) between 15 to 21 dpp. Similarly, cows that were diagnosed with metritis had lower mean plasma concentrations of PGFM prior to initiation of systemic antibiotic treatments (on average at 5 dpp) compared to cows without metritis (Silvestre et al., in press).

Caruncular and intercaruncular areas of the bovine uterus have chemo-attractant properties towards neutrophils, regardless of whether the cells were from the donor of the tissue or from other non-pregnant cows. This effect is seen consistently from 255 days of gestation to parturition, and it is more effective than other tissues, such as the skeletal muscle (Hoedemaker et al., 1992a). Moreover, cows in which the neutrophil chemotaxis response to caruncular supernatants is reduced were at increased risk to develop retained fetal membranes (Gunnink, 1984; Kimura et al., 2002).

Cullens (2005) supplemented cows beginning 24 days pre-partum with CS of FAs at 2% of DM. Linoleic acid was 30.5% of total fat supplemented. Plasma concentrations of PGFM were greater in the fat-supplemented cows compared with those not fed fat. Difference is possibly caused by the increased intake of the direct precursors of the 2 series prostaglandins. Similarly, Juchem et al. (in press) observed greater plasma concentrations of PGFM at day 1 postpartum when primiparous cows were supplemented pre-partum with CS of LN and trans-octadecenoic acids associated with less incidence of severe uterine infection (i.e., metritis associated with rectal temperature ≥ 39.5 ºC) compared with CS of palm oil.

Bovine neutrophils (i.e. *ex-vivo*) had greater direct chemotaxis responses to arachidonic metabolites (i.e. $PGF_{2\alpha}$, leukotriene-B_4, 5- and 15-hydroxyeicosatetraenoic) and improved phagocytic capacity towards labeled *S. aureus* in the presence of $PGF_{2\alpha}$ (Hoedemaker et al., 1992b). Also, $PGF_{2\alpha}$ increased bactericidal activity of neutrophils from ovariectomized mares (Watson, 1988). These aspects of neutrophil function are important for the first line of defense against pathogens. Therefore, feeding supplements enriched with the LN, may increase uterine production of $PGF_{2\alpha}$ and perhaps other immunostimulatory eicosanoids, and enhance innate immune functions to improve uterine health.

Strategic Feeding of Key Fatty Acids to Dairy Cows - A Florida Study

Design. In a recent Florida study, Silvestre et al. (2008a, 2008b) randomly allocated cows (n = 1,582) into two experimental transition diets beginning at approximately 30 days before the expected date of parturition and continued until 30 dpp. After 30 dpp cows within each transition diet were allocated randomly into the experimental breeding diets that were fed until 160 dpp. Experimental transition and breeding diets differed only in the source of supplemental FA.

Transition diets consisted of CS of palm oil (PO; EnerGII) or CS of safflower oil (SO; Prequel 21) and breeding diets consisted of CS of PO (EnerGII) or CS enriched in fish oil (FO, StrataG). All CS of FAs were manufactured by Virtus Nutrition (Corcoran, CA, USA) and supplemented at 1.5% of dietary DM. Diets were formulated to meet or exceed NRC (2001) nutrient requirements for net energy of lactation (NE_L), crude protein (CP), fiber, minerals and vitamins and fed to obtain intakes of 200 and 400 g/d of CS of FAs, for pre- and postpartum cows, respectively. Diets were fed as a total mixed ration twice daily targeting 5% orts.

Tissue FA Responses. Sub-samples of PO (n = 11) and SO (n = 12) cows were used for collection of cotyledonary-caruncular tissue that were separated manually and plunged into liquid nitrogen for further FA analysis. Only cows fed the pre-partum diet for more than 20 days were included. Collection of tissues was within 7 hours after parturition (average of 3 hours) and before placental expulsion. None of the cows developed a retained placenta. Total fatty acid concentration was lower ($P < 0.01$) in fetal cotyledonary tissue than maternal caruncular tissue (Table 1). Caruncular concentration of LN tended to be greater ($P < 0.10$) in cows fed SO (11.06%) compared to those fed PO (9.8%). Cotyledon concentration of LN was not different between cows fed PO (5.10%) or SO (5.53%). The predominant fatty acid in the cotyledon and caruncle was oleic acid (C18:1n-9; 24.5%) and stearic acid (C18:0; 20.8%), respectively. Saturated fatty acid concentration was less ($P < 0.01$) in cotyledon compared with the caruncle, and a greater ($P < 0.01$) concentration of unsaturated FAs was in the cotyledonary tissue (Table 1). The n-6:n-3 ratio was greater ($P < 0.05$) in caruncular tissue of SO compared to PO-supplemented cows (Table 1). Moreover, the cotyledonary concentration of LN was less ($P < 0.01$) compared to the caruncle. Consequently, the n-6: n-3 ratio was less in the cotyledon (Table 1).

Immune Responses. Blood samples were collected from sub-samples of cows at enrollment prepartum (n = 18) and in the postpartum period (n = 47) at parturition (i.e., 2.8 ± 1.8 hours after delivery), 4 and 7 dpp for analyses of neutrophil activity and abundance of adhesion molecules using flow cytometry. Number of bacteria (*E. coli* and *S. aureus*) phagocytized per neutrophil was greater ($P < 0.01$) for cows fed SO at 4 dpp; this was associated with a greater ($P < 0.05$) intensity of H_2O_2 produced per neutrophil at 4 and 7 dpp in cows fed SO (Fig. 1). Neutrophil abundance of L-selectin (arbitrary units) was greater ($P < 0.05$) at 4 and 7 dpp for SO (1205.3 and 1134.2; S.E. = 96.2) compared with PO (862.5 and 892.8; S.E. = 95.8) supplemented cows, respectively. No effects of diet or day were observed in the abundance of β_2-integrin.

Neutrophil cytokine production and FAs profiles were measured in sub-samples of cows sampled at enrollment and at 35 dpp (n = 26) which was the last day of PO and SO feeding. Neutrophils were isolated from whole blood and incubated in media, with or without *lipopolysaccharide* (**LPS**), at 37°C in a 5% CO_2 incubator for 18 hours. Mean concentrations of TNF-α and IL-1β in supernatants of isolated neutrophils stimulated with LPS were greater ($P < 0.01$) for cows supplemented with SO (106.97 pg/ml and 1.45 ng/ml) compared with PO (63.25 pg/ml and 0.67 ng/ml), respectively. Concurrently, LN content of the neutrophil FAs, although numerically greater, was not significantly

greater (P = 0.19) in cows fed SO (23.2%) compared with those fed PO (20.6%). The predominant FAs in the neutrophils were linoleic, stearic, palmitic, oleic and erucic acids which comprised approximately 72% of all FAs. The ratio n-6 (C18:2 + C22:4): n-3 (C18:3 + C20:5 + C22:6) of FAs tended (P = 0.07) to be greater for cows fed SO (9.16 ± 0.73) compared with PO (7.16 ± 0.73).

Blood samples were collected daily from cows fed PO (n = 15) and SO (n = 17) from parturition to 10 dpp and continued thrice weekly until 35 dpp for analyses of plasma concentrations of PGFM and acute phase proteins (i.e., haptoglobin and fibrinogen), respectively. Plasma concentrations of PGFM were not affected by transition diets (Figure 2) except for days 4 and 7 postpartum, in which a greater (P < 0.05) concentration was detected for cows fed SO (2,809 ± 310 and 2,667 ± 314 pg/mL) compared with cows fed PO (2,081 ± 325 and 1,443 ± 325 pg/mL) diets, respectively. Additionally, mean plasma concentrations of haptoglobin and fibrinogen were greater (*P* < 0.05) for cows fed SO compared with PO diets (Figure 3).

Although feeding SO improved aspects of innate immunity (i.e., neutrophil function and acute phase response), cows fed SO (n = 562) or PO (n = 554) had similar frequency distributions of mucupurulent (10% and 14.4%) and purulent (30.4% and 28%) cervical discharges evaluated once between 8 to 10 dpp.

Collectively, feeding a LN-enriched diet, beginning in the close up ration pre-partum, changed FA profiles of tissues placing the cow in a “pro-inflammatory state.” Such a state involves a lower threshold for initiation of an inflammatory response and increased sensitivity of cells upon stimuli. Inflammation is the first step for initiation of an immune response.

Fertility Responses. In studies with variable designs and sample sizes, pregnancy rates were improved for postpartum lactating dairy cows supplemented with fish meal (Bruckental et al., 1989; Armstrong et al., 1990; Carrol et al., 1994 and Burke et al., 1997). Also, cows fed flaxseed, which is a source of ALN, had either increased first service pregnancy per artificial insemination (Petit et al., 2001), no effect (Fuentes et al., 2008) or reduced pregnancy loss from 30 to 50 days of pregnancy (Petit and Twagiramungu, 2006).

Fatty acids of the n-3 family are thought to reduce uterine pulsatile secretion of $PGF_{2\alpha}$ that can possibly delay luteolysis (Mattos et al., 2004). Both EPA and DHA FAs inhibited secretion of $PGF_{2\alpha}$ when endometrial cells were stimulated with phorbol ester in vitro (Mattos et al., 2003). Suppression of luteolytic $PGF_{2\alpha}$ secretion and maintenance of the CL are obligatory steps for establishment of pregnancy in cows (Thatcher et al., 1994). Also, FAs such as EPA and DHA inhibited production of IL-1β and of TNF-α by human monocytes (Sinha et al., 1991; Purasiri et al., 1997). Caughey *et al.* (1996) demonstrated that a diet enriched with flaxseed followed by FO inhibited IL-1 and TNF-α production by monocytes that was correlated negatively with the EPA content of these cells.

In this recent Florida study (Silvestre, 2008), cows at 43 dpp began a Presynch protocol with two injections of $PGF_{2\alpha}$ (25 mg, dinoprost tromethamine, i.m., Lutalyse®; Sterile Solution; Pfizer Animal Health, New York, NY) injected 14 days apart. The Ovsynch protocol was initiated 14 days after the second injection of $PGF_{2\alpha.}$ of the Presynch with a GnRH injection (100 µg; gonadorelin diacetate tetrahydrate, i.m., Cystorelin®, Merial Ltd., Athens, GA) followed 7 days later by an injection of $PGF_{2\alpha}$ and a final injection of GnRH 56 hours later. Timed artificial insemination (**TAI**) for first service was performed 16 hours after the second GnRH injection of the Ovsynch protocol.

All cows received a controlled internal drug-releasing device (**CIDR**, EAZI-BREED; Pfizer Animal Health, New York, NY) containing 1.38 g of progesterone at 18 days after the first TAI followed 7 days later by removal of the CIDR device and an 100 µg injection of GnRH. At 32 days after first TAI, cows were examined for pregnancy by per-rectum ultrasonography to identify presence of an embryo and an embryonic heart beat. Non-pregnant cows were injected with 25 mg of $PGF_{2\alpha}$ and then injected with 100 µg of GnRH 56 hours later. A TAI was performed 16 hours after the last GnRH for the second service. Cows were examined for pregnancy by per-rectum ultrasonography at 32 days after second service. All cows diagnosed pregnant after first and second services were re-examined by per-rectum ultrasonography at 60 days after insemination to determine pregnancy losses.

Pregnancy per AI, pregnancy losses, and cumulative proportion of pregnant cows after two services were analyzed using pre-determined statistical contrasts to test the effects of the transition diets (PO-PO + PO-FO vs. SO-PO + SO-FO), breeding diets (PO-PO + SO-PO vs. PO-FO + SO-FO) and the interaction of transition and breeding diets (PO-PO + SO-FO vs. PO-FO + SO-PO) accordingly with the experimental feeding design described above (Silvestre, 2008).

Transition diets, breeding diets, and interaction of diets did not affect pregnancy per AI at 32 and 60 days after TAI for first service (Table 2). However, pregnancy loss from day 32 to day 60 after the first TAI was less ($P < 0.05$) in FO compared to PO supplemented cows during the breeding period (Table 2). For second service, breeding diet altered ($P < 0.05$) the 32 day estimates of pregnancy per AI and a tendency ($P < 0.10$) for an interaction was detected between transition and breeding diets (Table 2). The increase in day 32 pregnancy per AI caused by FO was greater in cows fed the SO transition diet, whereas there was no increase in pregnancy per AI in cows fed the PO breeding diet regardless of transition diet (Table 2). Both breeding diet and a transition by breeding diet interaction ($P < 0.05$) were detected for the 60 day pregnancy per AI response in which FO stimulated pregnancy rate per AI but the response to FO was greater in cows fed the SO transition diet (Table 2).

Immune Responses During Breeding Period. Cytokine production and profiles of FAs in neutrophils were measured in a sub-sample of cows (n = 28) at 85 dpp at a time when cows were fed the breeding diets (i.e., PO or FO) for approximately 55 days. Culture procedures for neutrophils were as described previously. Mean concentration of

TNF-α, but not IL-1β, in supernatants of isolated neutrophils was less (P < 0.01) for cows supplemented with FO (42.55 pg/ml and 0.6 ng/ml) compared with PO (82.68 pg/ml and 0.78 ng/ml) in response to LPS, respectively. Concurrently, the neutrophil content of EPA (1.5% and 0.30%), DPA (C22:5n-3; 3.48% and 2.33%) and DHA (1.65% and 0.11%) FAs were greater (P < 0.01) in cows fed FO compared with PO diets, respectively. Consequently, the ratio of n-6 (C18:2 + C22:4): n-3 (C18:3 + C20:5 + C22:6) FAs were less (P < 0.01) in cows fed FO (3.75) compared with those fed PO (8.48).

Milk Responses. In the Florida study milk weights were recorded once a month for all cows. The single measurement of milk production for each month was considered as the average for the month. Data from the first 5 months of lactation were used. Average milk yield for combinations of transition and breeding diets were PO-PO (41.1 ± 0.6 Kg/day; n = 295), PO-FO (41.3 ± 0.7 Kg/day; n = 280), SO-PO (41.7 ± 0.6 Kg/day; n = 302) and SO-FO (42.1 ± 0.7 Kg/day; n = 289). Average milk yield was affected (P = 0.02) by transition diets such that cows supplemented with SO (41.9 ± 0.4 Kg/day; n = 591) during the transition period had a greater average milk yield for the 5 months postpartum compared with cow fed PO (41.2 ± 0.4 Kg/day; n = 575). Average milk yield was not affected by breeding diet or the interaction between transition and breeding diets.

Summary

The threshold for triggering an immune response (i.e. creating a pro-inflammatory state that can respond greatly upon challenge) due to feeding FA precursors of pro-inflammatory eicosanoids can benefit postpartum immunity (innate immunity and secretion of acute phase proteins) of dairy cows. Conversely, following a healthy transition period, supplementation of FO can increase the threshold for triggering an anti-immune response during the breeding period by exerting an anti-inflammatory state that may attenuate immune responses in early pregnancy to benefit pregnancy rate and survival of embryos.

The integration of the disciplines of ruminant nutrition, reproductive physiology, immunology and clinical medicine has the potential to provide useful alternatives to improve postpartum health and fertility in dairy cows in a scenario of increasing milk production. Therefore, we propose that sequential feeding of diets rich in LN followed by diets rich in EPA and DHA during the peri-parturient and breeding periods, respectively, impacted FA composition of tissues, altered immune-responses that can benefit overall cow performance and fertility. Such feeding strategies warrant economic analyses to evaluate cost-benefit.

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Table 1. Least squares means and pooled SE for total fatty acids (g/100 g of freeze-dried tissue) and different fatty acid percentages (% of the total fatty acid; g/100 g of fatty acids) in the cotyledon and caruncle tissues collected at the time of parturition for cows supplemented with palm oil or safflower oil.

	Palm oil		Safflower oil					
Fatty acid	Cotyledon	Caruncle	Cotyledon	Caruncle	SE	Diet	Tissue	Intxn
Total	4.3	8.6	4.7	8.7	0.26	NS	**	NS
SFA	39.9	46.8	38.8	44.5	0.40	**	**	NS
UNSFA	46.5	42.6	45.7	43.0	0.60	NS	**	NS
MUSFA	34.7	28.9	34.5	28.1	0.57	NS	**	NS
PUFA	11.8	13.8	11.2	14.9	0.50	NS	**	NS
n-6/n-3	1.3	5.4	1.6	6.3	0.04	*	**	NS

Diet fed from 33 days pre-partum to 30 days postpartum.
Palm oil (EnerGII) and Safflower oil (Prequel 21). All fat supplements were manufactured as calcium salts by Virtus Nutrition, LLC (Corcoran, CA, USA) and supplemented at 1.5% of the dry matter.
SFA = Saturated fatty acids, UNSFA = unsaturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids.
n6/n3 = (C18:2 + C22:4)/(C18:3 + C20:5 + C22:6).
†P ≤ 0.10; *P ≤ 0.05; **P ≤ 0.01; NS = nonsignificant.

Table 2. First and second services pregnancies per AI at 32 and 60 days after insemination and pregnancy loss for experimental diets

	Diets				Diet contrasts[1] (P – value)		
	PO-PO	SO-PO	PO-FO	SO-FO	C1	C2	C3
First service % (n=)							
D32	38.7 (107/276)	35.8 (96/268)	39.1 (103/263)	35.8 (89/248)	NS	NS	NS
D60	33.7 (92/273)	29.7 (79/266)	37.0 (97/262)	32.8 (81/247)	NS	NS	NS
Loss	11.5 (12/104)	15.9 (15/94)	4.9 (5/102)	7.9 (7/88)	NS	< 0.05	NS
Second service % (n=)							
D32	27.7 (43/155)	26.7 (41/154)	30.3 (44/154)	43.3 (65/150)	NS	< 0.05	= 0.10
D 60	21.0 (38/152)	22.5 (34/151)	27.3 (39/143)	41.3 (62/150)	NS	< 0.01	< 0.05
Loss	5.0 (2/40)	10.0 (4/38)	7.1 (3/42)	4.6 (3/65)	NS	NS	NS

[1]Contrast are C1 (transition diets [PO-PO + PO-FO vs. SO-PO + SO-FO]), C2 (breeding diets [PO-PO + SO-PO vs. PO-FO + SO-FO]) and C3 (interaction of diets [PO-PO + SO-FO vs. PO-FO + SO-PO]).
PO (Palm oil; EnerGII); SO (Safflower oil; Prequel 21); FO (Fish oil; StrataG). All fat supplements were manufactured as calcium salts by Virtus Nutrition, LLC (Corcoran, CA, USA) and supplemented at 1.5% of the dry matter. NS = non-significant.

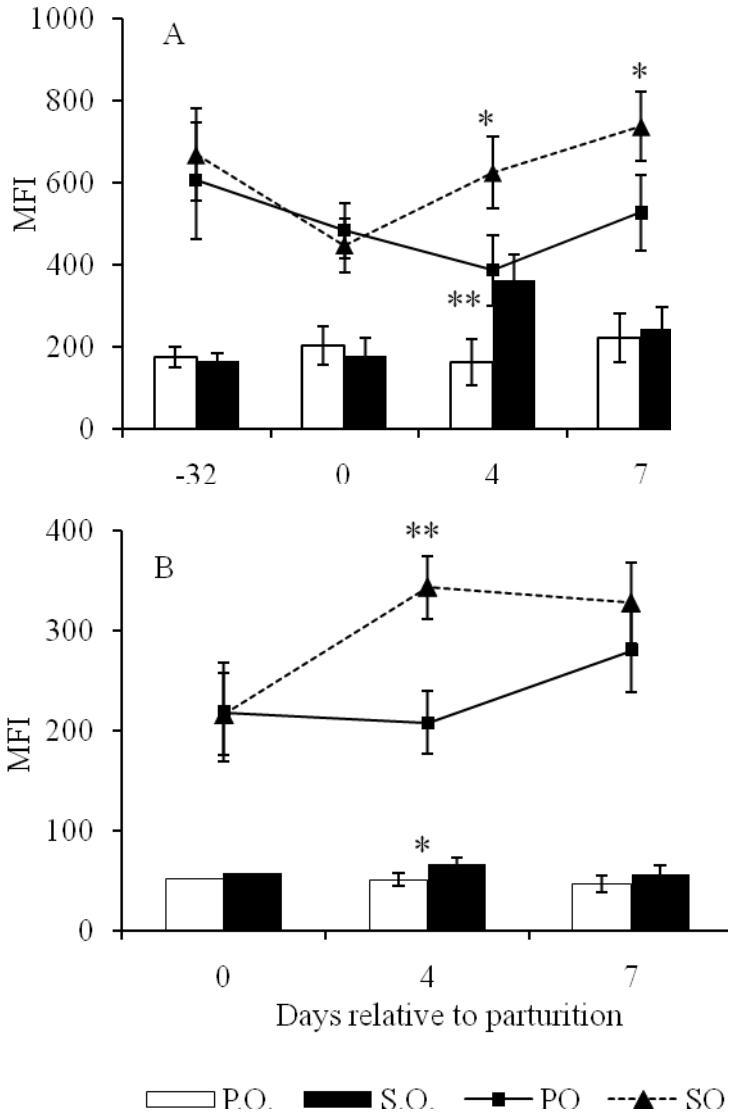

Figure 1. Least squares means (± S.E.) of neutrophil mean fluorescence intensity (MFI) for number of bacteria phagocytised per neutrophil (bars), and for intensity of H_2O_2 produced per neutrophil (lines) in whole blood stimulated with *E. coli* (A) or *S. aureus* (B). Cows were supplemented with palm oil (PO; n = 23) or safflower oil (SO; n = 24) during the transition period. *$P < 0.05$ and ** $P < 0.01$

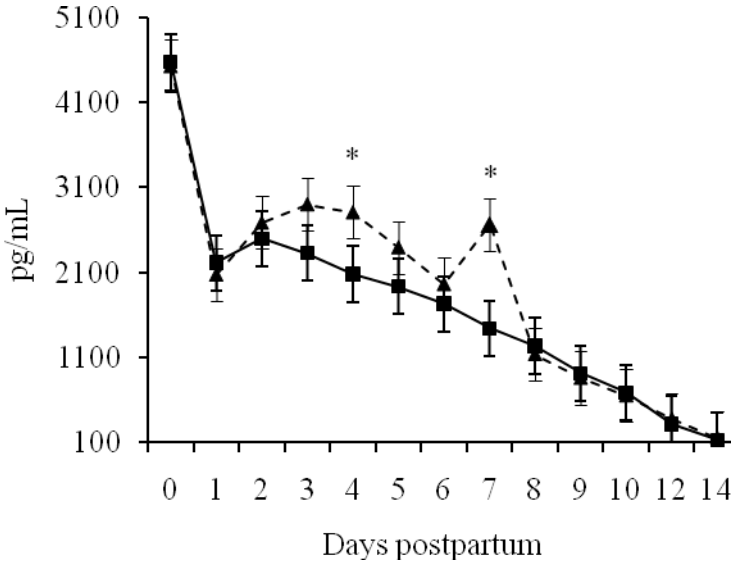

Figure 2. Least squares means (± S.E.) for plasma concentrations of 15-keto-13,14-dihydro-prostaglandin-$F_{2\alpha}$ (PGFM) for the first 14 days postpartum in a sub-sample of cows fed calcium salts of palm oil (—; n = 15) or safflower oil (---; n = 17) during the pre-partum period (at least 20 days) to 35 days postpartum. *P < 0.05

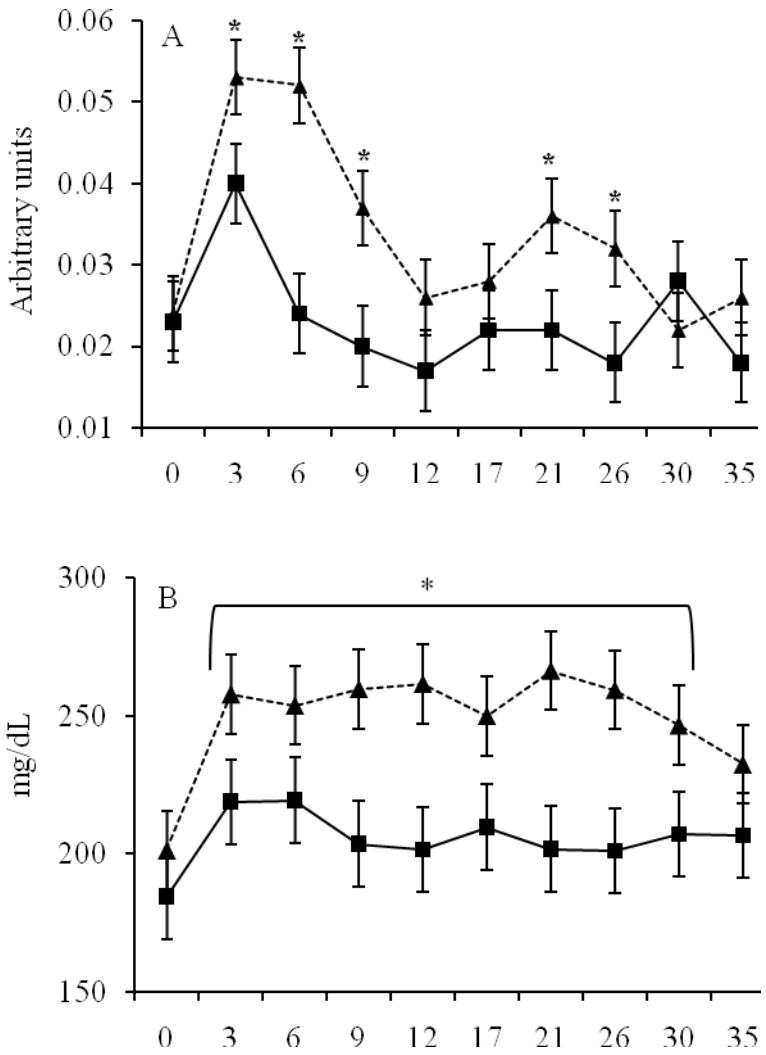

Figure 3. Least squares means (± S.E.) for plasma concentration of haptoglobin (A) and fibrinogen (B) for cows fed calcium salts of palm oil (■; n =15) or safflower oil (▲; n = 17) during the pre-partum period (at least 20 days) to 35 days postpartum. Cervical discharge score was examined at 8 days postpartum. *P < 0.01

SESSION NOTES