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Nutritional Impact of Fatty Acids on Immune and Reproductive Responses of Lactating Dairy Cows

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INTRODUCTION

Ruminant diets are supplemented with fat primarily to increase energy concentration and to enhance animal performance. Dairy and beef cattle diets, without any supplemental fat, contain approximately 2% long-chain fatty acids (LCFA) of vegetable origin that are predominantly polyunsaturated. Because of the high energy density, fats are usually incorporated into cattle rations to improve production, growth and reproduction.

During early lactation, when lactating cows undergo a period of nutrient deficit, it was initially thought that incorporating supplemental fat to the diet would enhance energy intake and energy balance, which was expected to improve reproduction. Because early lactation cows mobilize large quantities of stored triacylglycerols in adipose tissue, concentrations of fatty acids (FA) in blood are usually high during the first weeks of lactation (Drackley, 1999). When fat is fed in early lactation, cows either consume less diet or production increases, thereby fat feeding early postpartum seldom alters energy status even though a more energy dense ration is consumed. Staples et al. (1998) indicated that feeding fat did not alter the energy status of dairy cows and suggested that reproductive responses were the result of supplying LCFA and altering substrate availability to the cow rather than simply an energy effect.

As with other nutrients, certain FA are essential for mammals. In 1929, George O. Burr and his wife were the first to describe the essentiality of FA in rats (Burr and Burr, 1929; Burr and Burr, 1930). They observed that growing rats fed diets low in fat ceased growing and experienced health problems and irregular ovulation, which were then reversed after feeding the polyunsaturated FA C18:2 n-6 (linoleic acid) and C18:3 n-3 (α-linolenic acid) (Burr and Burr, 1930). Therefore, the concept of essential FA was established and later understood that C18:2 n-6 and C18:3 n-3 could not be synthesized by mammalian cells because of lack of desaturase enzymes beyond the 9^{th} C in the acyl chain. Because of the essentiality of FA and the role of specific FA on reproductive processes, it is possible that reproduction in cattle may be more

influenced by the type of fat fed than fat feeding per se. This is particularly important and challenging as ruminants extensively hydrogenate polyunsaturated FA, thereby limiting the supply of dietary unsaturated FA for absorption in the small intestine.

Objectives of this presentation are: to review the basics of fats and in particular fatty acids, as to their potential impact on the immune system; to document the role of fatty acids, as a component of nutritional management, to improve health, reproductive efficiency and milk production.

Basics of Fatty Acid Structure and Function

Fats and Fatty Acids Defined

A fat is any organic compound of plant or animal origin that is not volatile, does not dissolve in water, and is oily or greasy. Chemically, fats are identical to animal and vegetable oils, consisting mainly of triglycerides (esters of glycerol with fatty acids). As we learned in organic chemistry, fats that are liquid at room temperature are called oils. Differences in melting temperature and physical state depend on the saturation of the fatty acids and the length of their carbon chains. The glycerides may have only a few different component fatty acids or many as found in butterfat. Almost all natural fats and oils incorporate only fatty acids that are constructed from two-carbon units and thus contain only even numbers of carbon atoms. Natural fats such as corn oil have small amounts of compounds besides triglycerides, including phospholipids, plant steroids, tocopherols (vitamin E), vitamin A, waxes, carotenoids, and many others, as well as their decomposition products. Sources of fats in foods include ripe seeds and some fruits (e.g., corn, peanuts, olives, avocados) and animal products (e.g., meat, eggs, milk). Fats contain more than twice as much energy (calories) per unit of weight as proteins and carbohydrates. Digestion of fats in foods, often partial, is carried out by enzymes called lipases. The breakdown products are absorbed from the intestine into the blood, which carries microscopic fat droplets reconstituted from digested fats (or synthesized in cells) to sites of storage or use. Fats are readily broken down — primarily into glycerol and fatty acids — by hydrolysis, a first step in their use.

The short-hand notation for identifying fatty acids is to give the number of carbons and double bonds in the molecule. Fats that have double bonds are classified as unsaturated fats. For example, a designation of 18:2 indicates a fatty acid of 18 carbons long having 2 double bonds. The term “omega” refers

to the location of the double bond in the carbon chain. An omega-6 fatty acid has its first double bond located between the 6th and 7th carbon counting from the methyl end of the chain. Likewise an omega-3 fatty acid has its first double bond located between the 3rd and 4th carbon counting from the methyl end of the carbon chain. Linoleic acid, abbreviated C18:2, is an omega-6 fatty acid. Linolenic acid, abbreviated C18:3, is an omega-3 fatty acid. Two additional omega-3 fatty acids are EPA (C20:5) and DHA (C22:6); but these are not considered essential for the cow because they can be synthesized from the omega-3 fatty acid, linolenic acid. Nevertheless EPA and DHA can play important roles in supporting good animal performance.

Many different types of supplemental fat have been fed to lactating cows. Some fat sources fed are listed in Table 1. Each fat source is composed of a different mix of individual fatty acids. Rendered fats include animal tallow and yellow grease (recycled restaurant grease) and are composed mainly of oleic acid (~43%). Granular fats are dry fats prepared commercially and are composed mainly of palmitic acid (36-50%). Examples include Energy Booster 100, EnerG-II, and Megalac-R. A variety of vegetable oils can be fed as free oil or in the seed form. The oil seeds contain from 18% oil (such as soybeans) to 40% oil (such as flaxseed). The selection of a vegetable oil will bring with it particular fatty acids. Canola oil is high in oleic acid. Cottonseed, safflower, sunflower, and soybean oils are high in linoleic acid. Flaxseed is high in linolenic acid. Linoleic acid and linolenic acid are essential fatty acids for the cow because neither her body nor her ruminal microorganisms can synthesize them. Fresh temperate grasses contain 1 to 3% fatty acids of which 55 to 65% is linolenic acid (Chilliard et al., 2001). Corn silage lipid contains much more linoleic acid (49%) than linolenic acid (4%) due to the presence of corn grain (Petit et al., 2004). Both linoleic and linolenic acid in forages can decrease during storage. As we have moved our dairy cows from pastures to barns and fed them stored forage, their intake of linolenic acid and possibly linoleic acid has likely decreased. The whole oil seed is frequently fed rather than the oil alone. Fish oil is unique that it contains eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), fatty acids found in fish tissue due to their consumption of marine plants.

Generally speaking, fat supplementation at 1.5% of the diet is usually safe in terms of cow performance with the exception of fish oil. Feeding fish oil at more than 1% of dietary dry matter will usually reduce feed intake and/or milk fat and protein concentration. If the fat concentration of the base diet without a fat supplement is 3 to 4%, then increasing it to 4.5 to 5.5% by fat supplementation should not be a problem if the dietary fiber is sufficient and

Table 1. Major fatty acid composition of select dietary fat sources.

	Fatty acid						
Fat source	C14:0 Myristic	C16:0 Palmitic	C16:1 Palmit-oleic	C18:0 Stearic	C18:1 Oleic	C18:2 Linoleic	C18:3 Linolenic
Tallow	3	25	3	18	43	3.8	<1
Yellow grease	2	21	4	11	44	14	<1
Energy Booster 100 [1]	3	40	1	41	10	2	<1
Megalac; EnerG-II [1]	1	50	<1	4	36	8	<1
Megalac-R[1]	1	36	<1	4	26	29	3
Canola oil	<1	4	<1	2	63	19	9
Cottonseed oil	1	23	1	3	18	54	1
Flaxseed oil	<1	5	<	3	20	16	55
Rapeseed oil	<1	5	<1	2	54	22	11
Safflower oil	<1	7	<1	2	12	78	<1
Soybean oil	<1	11	<1	4	23	54	8
Sunflower oil	<1	7	<1	5	19	68	1
Menhaden fish oil [2]	7	16	8	3	12	1	2
Prequel 21[1]	1	12.9	<1	4	17	64	<1
StrataG[1,3]	4	39	0	5	31	8	1

[1]Commercial preparations considered partially inert in the rumen.
[2]Also contains 14% C20:5 and 9% C22:6.
[3]Also contains 5.4% C20:5 and 5.3% C22:6.

effective. Certainly diets containing higher fat ingredients like distillers grains, hominy, or whole cottonseeds need to be watched closely so that the total fat content stays below 6%.

Dietary Fats Are Modified in the Rumen by Bacteria

The ruminal microbes will convert unsaturated fats to saturated fats by replacing the double bonds with single bonds between the carbons (called biohydrogenation). Some scientists have speculated that this act of biohydrogenation by bacteria is an attempt to protect themselves, as unsaturated fats can be toxic to bacteria, primarily the bacteria that digest fiber. The majority of the consumed unsaturated essential fatty acids, linoleic (C18:2) and linolenic (C18:3) acids, are converted by the bacteria to stearic acid (C18:0). During the process of biohydrogenation of unsaturated fats in the rumen, several intermediate forms of fatty acids, called trans fatty acids, also are formed. Some of the trans fatty acids, such as the trans-10, cis-12 conjugated linoleic acid

(CLA) and the trans-10 C18:1, can influence the cow's metabolism, including depressing milk fat synthesis. This intervention by ruminal bacteria to change essential fatty acids in the diet to other fatty acids has made the study of dietary fat effects on reproduction quite challenging. Calcium salts of fatty acids (CSFA) are insoluble at normal rumen pH and thus reach the abomasum where they are dissociated into Ca ions and free fatty acids due to the acidic pH. The free fatty acids are then absorbed in the duodenum and available to various tissues. Thus Calcium salts of fatty acids (Table 1) offer some protection from microbial biohydrogenation although the degree of protection from biohydrogenation is far less than complete (Jenkins and Bridges 2007).

A nutraceutical is defined as a product isolated or purified from feeds that is demonstrated to have a physiological benefit or provide protection against chronic disease. Specific classes of fatty acids exert direct regulatory effects on tissue function that impact milk production, immune status, and reproduction processes. The challenge is to understand these basic processes and to evaluate whether they have any benefit on reproductive efficiency. Dietary fat effects are not simply due to energy, but that specific nutraceutical effects likely are being manifested whereby certain fatty acids interact as substrates for specific enzymes (e.g., PGHS-2) and also interact with peroxisome proliferator-activated receptors (PPARs) to regulate gene expression. The essential polyunsaturated fatty acids, linoleic (18:2n-6) and α-linolenic (18:3n-3), undergo steps of chain elongation and desaturation forming differential n-6 products, such as dihomo-gamma linolenic (20:3n-6) and arachadonic (20:4n-6) acids, and n-3 products such as eicosapentaenoic acid (EPA; 20:5n-3). These specific long chain polyunsaturated fatty acids produce eicosonaoid products of the prostaglandin series PGF1, PGF2 and PGF3, respectively as well as various thromboxanes, leukotrienes, lipoxins, hydroperoxy-eicosatetraenoic acids and hydroxyeicosatetraenoic acids (HETE) that regulate inflammation and immunity (Mattos et al., 1999). The peroxisome proliferator-activated receptors (PPARs) are a family of nuclear receptors activated by specific fatty acids, eicosanoids and peroxisome proliferators. Previous studies have shown that they are involved in the regulation of genes affecting steroid and prostaglandin synthesis (MacLaren etal., 2006).

An example of a potential fatty acid nutraceutical is the ability of the *trans*-10, *cis*-12 conjugated linoleic acid (CLA) to induce milk fat depression in lactating dairy cows. Utilizing a bovine mammary cell line (MAC-T), Peterson and coworkers (2004) indicate that *trans*-10, *cis*-12 CLA reduces lipid synthesis in the bovine mammary gland through inhibition of the proteolytic activation of sterol response element-binding protein (SREBP-1) that led to a subsequent

reduction in transcriptional activation of lipogenic genes such as acetyl CoA carboxylase, fatty acid synthase, and stearoyl CoA desaturase. Consequently, supplemental diets enriched in polyunsaturated oils can cause a major reduction in milk fat content and this is due to the formation and absorption of *trans*-10, *cis*-12 CLA.

An important issue is whether feeding a by-pass fat, Ca salts of fatty acids enriched with fish oil, results in absorption of EPA and DHA that alters fatty acid concentrations among various tissues (Bilby et al., 2006c). Two diets were fed in which the oil of whole cottonseed (15% of dietary DM; control diet) was compared to oil prepared as a Ca salt containing fish oil (CaSFO) as one of the primary oils (1.9% of dietary DM; Virtus Nutrition). Cows fed CaSFO had an increased proportion of C20:5 and C22:6 in the endometrium, liver, and mammary tissue, and C22:6 was increased in the milk fat compared with cows not fed FO. However, proportions of C20:5 and C22:6 were not increased in s.c. and internal adipose tissues. An important observation was that the CaSFO diet reduced the concentrations of arachidonic acid (C20:4n-6) and preferentially increased the concentrations of EPA and DHA in the endometrium. Thus it is clear that EPA and DHA fatty acids of the CaSFO diet are being absorbed from the gastrointestinal tract and being preferentially taken up in the endometrial tissue.

Consequently, feeding by-pass fats appears to increase specific fatty acids to the tissues that may have profound regulatory effects on various biological functions possibly effecting production efficiency and health of the lactating dairy cow.

Fatty Acid Influence on the Immune System

Postpartum immune function

Dry matter intake is reduced greatly a few days before parturition and remains low for a few days after parturition at a time lactational demands contribute to a pronounced NEBAL. This period often represents a time when the immune system of the cow is suppressed severely, making them particularly vulnerable to diseases such as metritis and mastitis (Goff et al., 2006; Kimura et al., 2002; Hammon et al., 2006). Observations in mastectomized versus intact cows during the periparturient period indicate that the initiation of lactation and milk synthesis are factors contributing to immunosuppression in cows (Kimura et al., 1999). Neutrophil function declined in both intact and mastectomized cows as parturition approached, but rebounded very quickly in mastectomized cows after parturition. Neutrophil function remained depressed for several weeks after parturition in milk-producing cows. The implication of these

observations is that metabolic challenges experienced by the dairy cow at the onset of milk production impaired immune cell function. Indeed cows with puerperal metritis and clinical endometritis or subclinical endometritis had lower dry matter intake and higher concentrations of NEFA in plasma during the periparturient period, and higher BHBA during early lactation compared to cows with normal uterine health (Hammon et al., 2006).

The development of postpartum uterine disease depends on the immune response of the cow and type of bacteria that colonize the uterus (Sheldon et al., 2006). Postpartum endometritis and subclinical endometritis are a common reproductive disease which has been associated with a reduction in pregnancy per AI and extended interval to pregnancy in lactating dairy cows (Galvao et al 2009a; Gilbert et al. 2005; Kasimanickam et al., 2004; Rutigliano et al., 2008). A sequence of periparturient events leads to development of metritis in the first 2 weeks postaprtum, clinical endometritis after 3 weeks postpartum, and establishment of subclinical endometritis after 4 weeks postpartum, in which the subclinical inflammation of the endometrium is a chronic localized inflammatory process affecting 20 to 50% of the dairy cows in the first 60 to 80 days of lactation. It has been characterized by presence of increased proportion of neutrophils in uterine cytology (Gilbert et al., 2005; Kasimanickam et al., 2004). Suppression of immune function postpartum (Kehrli et al., 1989) is associated with increased risk for endometritis (Hammon et al., 2006). Early in lactation, bacteria contaminate the uterus of > 90% of dairy cows. Although most cows eliminate this bacterial contamination in the subsequent 5 weeks, a persistent inflammatory response continues to affect the uterus as either clinical or subclinical endometritis (Galvao et al., 2009b). Cows with subclinical endometritis have been treated with $PGF_{2\alpha}$ or intrauterine administration of antibiotics unsuccessfully (Galvao et al., 2009b,c). Alternative strategies of regulating immune function perhaps through the diet warrant investigation.

Innate Immune System

The immune system is comprised of two responsive component systems that work in a sequential-complementary manner to prevent infections. These are termed the innate and the acquired immune systems. The innate immune system is comprised of immediately available mechanisms which fight the first stages of infection associated with pathogens such as bacteria. Via implementation of this initial defensive response of the innate system, there is time gained to allow the acquired system to develop an antibody response against a specific pathogen to mediate specific cytotoxic effects for protection.

Innate immunity prevents infection by targeting general properties of pathogens as opposed to modifying cellular structure in response to the type of pathogen (Carroll and Forsberg, 2007). The phagocytic cells of the innate immune system identify pathogens by recognizing distinct pathogen-associated molecular patterns (PAMPs). Specifically, pathogens contain molecules not typically found in mammalian cells, and cells of the innate system are able to recognize these foreign cells. For example lipopolysaccharide from the gram-negative cell wall of bacteria is recognized by Toll-like receptors 2 and 4 on the surface of innate immune cells. Binding of PAMPs to Toll-like receptors initiates killing mechanisms by the neutrophils and macrophages.

The acute phase protein (APP) response is stimulated by the release of proinflammatory cytokines (IL-1, IL-6, and TNF-α) from macrophages and monocytes at the site of inflammation or infection. The initial release of proinflammatory cytokines is amplified by their paracrine actions, which cause further release of these cytokines and eventually results in a systemic release of cytokines. This increase in circulation of the proinflammatory cytokines stimulates release of acute phase proteins from the liver. The acute phase proteins have various biologic functions, such as proteinase inhibitors, enzymes, coagulation proteins, metal-binding proteins, and transport proteins. During an inflammatory response, proinflammatory cytokines, such as IL-1, IL-6, and TNF-α mediate the hepatocyte production and secretion of these proteins. The APPs become important mediators of immunologic functions and play an active role in pathogen trapping, tissue repair and remodeling. Consequently, they may be secreted in a dynamic manner during the peri-parturient period of lactating dairy cows that undergo marked changes in uterine involution, exposure to pathogens, and major alterations in rumen function with increases in feed intake.

Adaptive Immune System

The second arm of the immune system is termed the acquired or adaptive system and is characterized by the production of antibodies, which are directed against specific antigens and effector T-cells that target cells displaying specific antigens. Pathogens can be phagocytosed and digested by antigen-presenting cells (*e.g.*, macrophages, B- lymphocytes and dendritic cells). Digested pieces of pathogens are presented on the surface of the antigen-presenting cells to two large groups of T cells characterized by a co-receptor CD8 (i.e., these cells differentiate into CD8 cytotoxic T cells) or the \co-receptor CD4 T effector cells defined as TH1, TH2 or TH17. The TH1 cells can further activate macrophages to increase intracellular bacterial death; TH2 cells can activate antigen specific B lymphocytes that further differentiate into plasma cells that secrete specific

antibodies or stimulate clonal expansion of a B-cell lineage (i.e., memory cells that provide immune memory for future pathogenic insults). TH 17 cells induce localized epithelial and stromal cells to produce chemokines that recruit neutrophils to sites of infection early in the adaptive immune response. Consequently, there is an array of coordinated T-cell effector actions that are specifically generated. Depending upon the antigen presenting cell (e.g., macrophages, B lymphocytes and dendritic cells), a variety of cytokines may be secreted that cause differential development of effector T cells (i.e., IL-12 and interferon-gamma induce TH1 cells; IL-4 induces TH2 cells; TGF-β induces TH17 cells).

Sheldon and coworkers (2009) integrated the mechanisms of infection and immunity in the female reproductive tract of cattle that ultimately regulates reproductive efficiency. Cows postpartum that have uterine infections (metritis) are less likely to ovulate because growth of the dominant follicle is slower and there are lower concentrations of plasma estradiol. Furthermore, intrauterine infusion of *Escherichia coli* endotoxin suppressed the preovulatory surge of LH in heifers. If cows do ovulate then endometrial cytokines may alter steroidognesis of luteal cells that contribute to a lower secretion of progesterone or increase the PGE_2: PGF_2 ratio that may extend the luteal phase.

Florida Experiment with Targeted Feeding of N-3 and n-6 fatty acids

Design and Rationale

In a recent Florida study, Silvestre et al. (2008a, 2008b and 2008c) 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 of fish oil (FO, StrataG). All CS of FAs were manufactured by Virtus Nutrition (Corcoran, CA, USA) and supplemented at 1.5% of dry matter. Diets were formulated to meet or exceed NRC (2001) nutrient requirements for net energy of lactation (NE_L), crude protein (CP), fiber, mineral 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/Cellular, Immunological and Hormonal 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 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 2). Caruncular concentration of LN tended to be greater ($P < 0.10$) in SO (11.06%) compared to PO diets (9.8%). Cotyledon concentration of LN was not different between PO (5.10%) and SO (5.53%) diets. The predominant fatty acid in the

Table 2. 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	Int
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).
†$P \leq 0.10$; *$P \leq 0.05$; **$P \leq 0.01$; NS = non-significant.

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 2). 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 linoleic acid was less ($P < 0.01$) compared to the caruncle. Consequently, the n-6: n-3 ratio was less in the cotyledon (Table 2).

Blood samples were collected from sub-samples of cows at enrollment (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 in the SO at 4 dpp associated with a greater ($P < 0.05$) intensity of H_2O_2 produced per neutrophil at 4 and 7 dpp in cows fed SO fat supplement (Figure 1). Neutrophil abundance of L-selectin (arbitrary units) was increased ($P < 0.01$) after parturition (752.75) and 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 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. Neutrophil LN content of the FAs, although numerically greater, was not significantly greater ($P = 0.19$) in cows fed SO (23.23%) compared with PO (20.61%). 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) supplements.

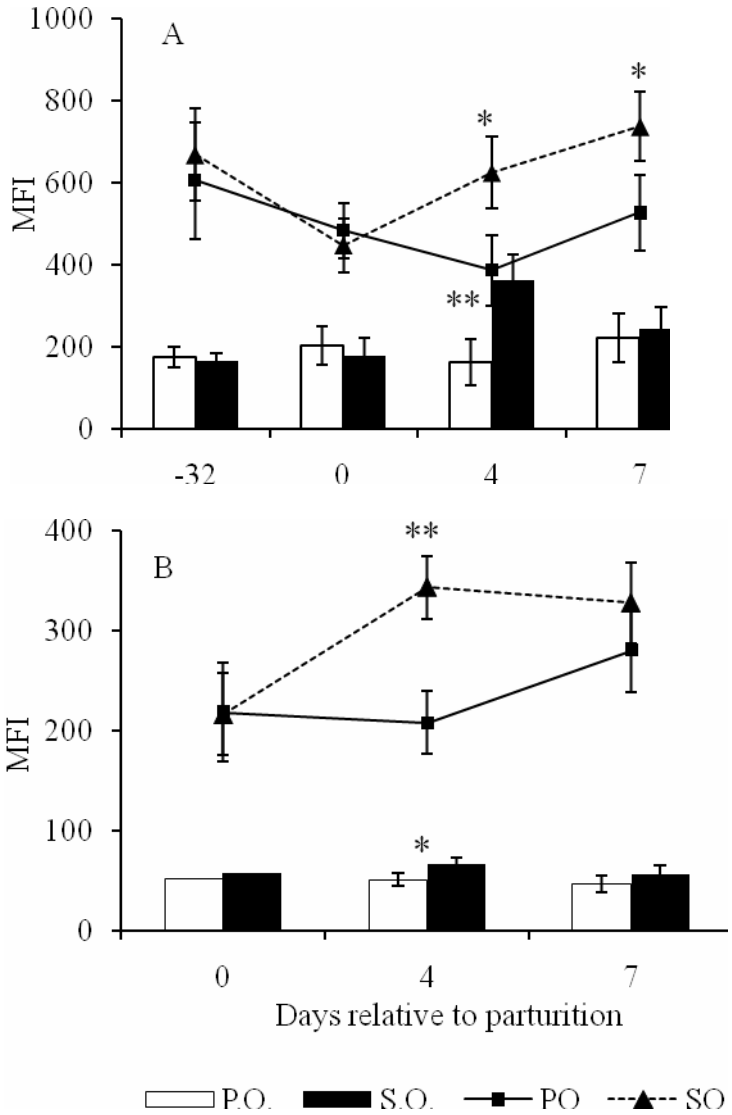

Figure 1. Least squares means (± S.E.) of neutrophil mean fluorescence intensity (MFI) for number of bacteria phagocytised per neutrophil (bars), and for intensity of H_2O_2 produced per neutrophil (lines) in whole blood stimulated with *E. coli* (A) or *S. aureus* (B). Cows were supplemented with palm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

Blood samples were collected from PO (n = 15) and SO (n = 17) cows daily 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 the SO (2,809 ± 310 and 2,667 ± 314 pg/mL) compared with 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 transition diets (Figure 3).

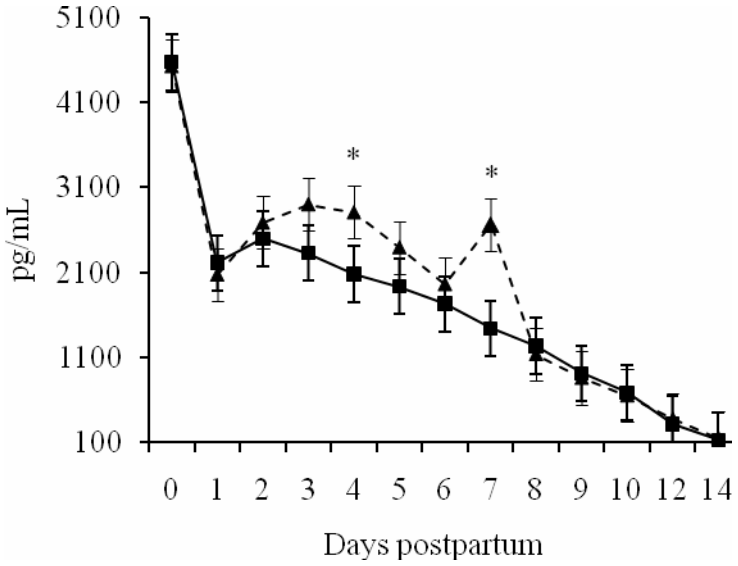

Figure 2. Least squares means (± S.E.) for plasma concentrations of 15-keto-13,14-dihydro-prostaglandin-$F_{2\alpha}$ (PGFM) for the first 14 days postpartum in a sub-sample of cows fed calcium salts of palm oil (—; n = 15) or safflower oil (---; n = 17) during the pre-partum period (at least 20 days)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

Although feeding SO improved aspects of innate immunity (i.e., neutrophil function and acute phase response), SO (n = 562) and PO (n = 554) cows 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.

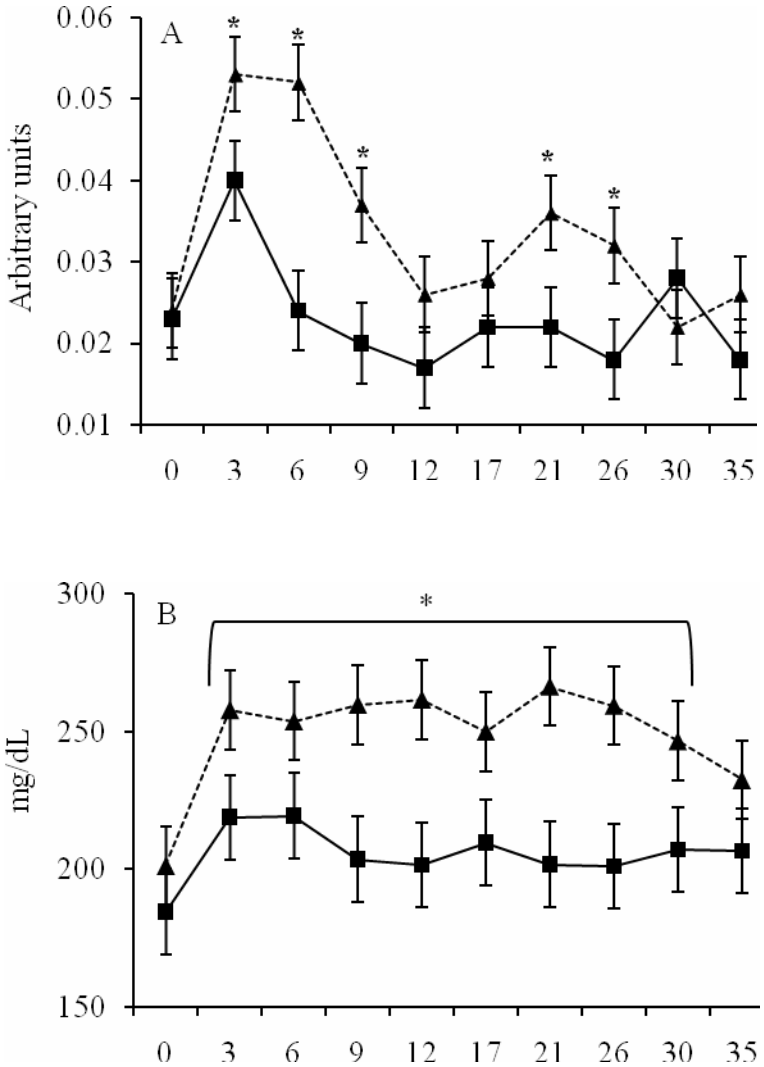

Figure 3. Least squares means (± S.E.) for plasma concentration of haptoglobin (A) and fibrinogen (B) for cows fed calcium salts of palm oil (■; n =15) or safflower oil (▲; n = 17) during the pre-partum period (at least 20 days) to 35 days postpartum. Cervical discharge score was examined at 8 days postpartum.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

Mean concentration of TNF-α in supernatants of isolated neutrophils at 35 DIM was greater ($P < 0.05$) for cows supplemented with SO compared with PO when cells were stimulated or not with LPS (Figure 4A). Because the concentration of TNF-α was constitutively (no LPS) greater in supernatants of neutrophils from SO supplemented cows, the TNF-α increase ($P < 0.01$) after LPS stimulation was greater for SO supplemented cows although the mass increase did not differ between diets. In contrast at 85 DIM, 55 days after initiation of breeding diets (i.e., PO and FO), the mean concentration of TNF-α before LPS stimulation did not differ between diets (Figure 4B). However, at this time TNF-α production in response to LPS was attenuated ($P < 0.05$) in cows fed FO compared with those fed PO (Figure 4B). Consequently, the mass increase in response to LPS was less ($P < 0.01$) in the FO compared with PO supplemented cows (Figure 6B).

When cows were supplemented during the transition period with SO, mean concentration of IL1-β in supernatants of isolated neutrophils at 35 DIM was greater ($P < 0.01$) when cells were stimulated with LPS (Figure 4A), and a greater mass increase of IL1-β was detected ($P < 0.01$, Figure 4A). At 85 DIM, concentrations of IL1-β did not differ between FO and PO diets when neutrophils were either stimulated or not with LPS (Figure 4B).

Collectively, feeding a linoleic fatty acid enriched diet, beginning in the close up ration pre-partum and continued to 30 days postpartum, changes fatty acid 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. Conversely, feeding a fish oil fatty acid enriched diet after 30 days postpartum induces an “anti-inflammatory response” that may supplement the anti-inflammatory effects of the conceptus during early pregnancy.

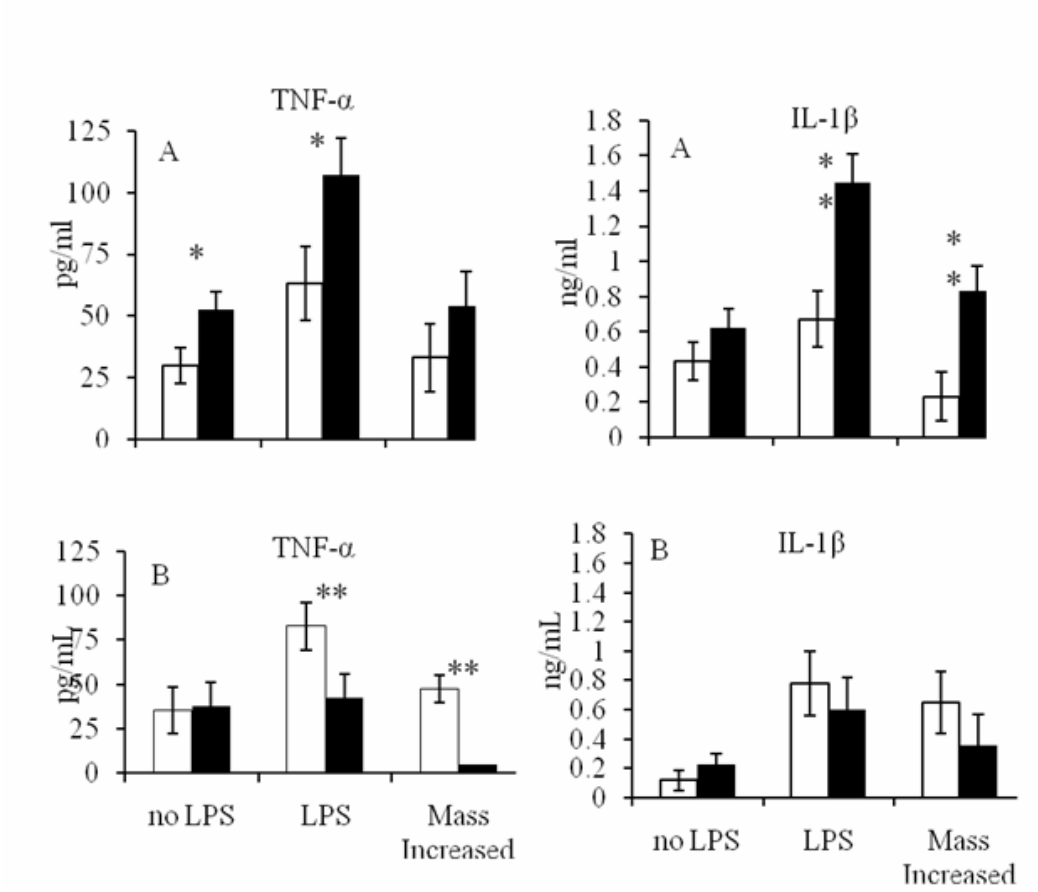

Figure 4. Least squares means (± SEM) for TNF-α and IL-1β concentration in supernatants of isolated neutrophils cultured for 18 h in RPMI-1640 stimulated or not with LPS incubated at 37° C and 5% CO_2 at 35 DIM for cows supplemented with calcium salts of palm oil (n = 13; □) or safflower oil (n = 13; ■) during the transition period (A) or at 85 DIM for cows supplemented with calcium salts of palm oil (n = 14; □) or fish oil (n = 14; ■) from 35 to 85 DIM (B); * $P < 0.05$ and ** $P < 0.01$.

Fatty Acid Influence on Reproductive Performance

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 (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.

Because the benefits of feeding fat may originate from specific FA (Staples et al., 1998; Staples and Thatcher, 2005), and absorption of unsaturated FA is limited in ruminants because of microbial biohydrogenation in the rumen (Juchem, 2007), studies have evaluated whether feeding FA differing in the degree of saturation might influence fertility of dairy cows (Table 3). When cows were fed 0.75 kg of fat from flaxseed, a source rich in C18:3 n-3, or sunflower seed, a source rich in C18:2 n-6, pregnancy tended ($P = 0.07$) to be greater for cows fed n-3 FA (Ambrose et al., 2006). However, a similar response to flaxseed was not observed by others (Fuentes et al., 2008; Petit and Twagiramungu, 2006). Similarly, feeding n-3 FA from fish oil as Ca-LCFA did not improve pregnancy at first postpartum AI when compared with beef tallow (Juchem, 2007). Juchem (2007) evaluated the effect of feeding pre- and postpartum cows Ca-LCFA of palm oil or a blend of C18:2 n-6 and trans-octadecenoic FA. Cows fed unsaturated FA were 1.5 times more likely to be pregnant at 27 or 41 d after AI compared with cows fed palm oil. Improvements in pregnancy when cows were fed C18:2 n-6 and trans-octadecenoic FA were supported by increased fertilization and embryo quality in non superovulated lactating dairy cows (Cerri et al., 2009).

Table 3. Effect of fatty acid (FA) supplementation on pregnancy per AI in lactating dairy cows

Reference	Cows	Amount/day	FA source[1]		
			Saturated	n-6 FA	n-3 FA
			Pregnancy per AI, %		
Ambrose et al. (2006)	121	0.75 kg of fat	---	32.2	48.4[¶]
Fuentes et al. (2008)	356	0.40 kg of fat	---	39.2	38.8
Juchem (2007)	699	0.40 kg of FA	40.7	---	35.9
Juchem (2007)	323	0.40 kg of FA	22.8	---	24.8
Juchem (2010)	344	2% of ration	28.6	37.9[¶]	---
Petit and Twagiramungu (2006)	110	0.6 - 0.8 kg of FA	55.9	40.0	44.4

[¶] Within a row, effect of source of FA ($P < 0.07$).
[*] Within a row, effect of source of FA ($P < 0.05$).
[1] Saturated = mostly saturated and monounsaturated FA; n-6 FA = source rich in C18:2 n-6; n-3 FA = source rich in C18:3 n-3 or C20:5 n-3 + C22:6 n-3.

Table 4. Effect of fatty acid (FA) supplementation on pregnancy losses after postpartum AI in lactating dairy cows

Reference	Pregnancies	Amount/day	FA source[1]		
			Saturated	n-6 FA	n-3
			Pregnancy loss, %		
Ambrose et al. (2006)	77	0.75 kg of fat	---	27.3	9
Juchem (2007)	257	0.40 kg of FA	20.4	---	2
Juchem (2007)	77	0.40 kg of FA	5.4	---	1
Juchem (2010)	114	2% of the ration	9.8	6.3	-
Petit and Twagiramungu (2006)	51	0.6 - 0.8 kg of FA	21.1	12.5	

[*] Within a row, effect of source of FA ($P < 0.05$).
[1] Saturated = mostly saturated and monounsaturated FA; n-6 FA = source rich in C18:2 n-6; n-3 FA = source rich in C18:3 n-3 or C20:5 n-3 + C22:6 n-3.

Because n-3 FA suppress uterine secretion of $PGF_{2\alpha}$ (Mattos et al. 2002; 2003; 2004), it may improve embryonic survival in cattle (Mattos et al., 2000). In 2 of 4 experiments, feeding the n-3 FA C18:3 n-3 (Ambrose et al., 2003; Petit and Twagiramungu, 2006) reduced pregnancy losses in lactating dairy cows (Table

4). On the other hand, when n-6 FA were fed as Ca-LCFA, pregnancy losses were similar to those observed for cows fed Ca-LCFA of palm oil (Juchem, 2007).

In the recent Florida study described above for immune responses (Silvestre et al., 2008a, 2008c), 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, 2008a, 2008c).

Transition, breeding and interaction of diets did not affect pregnancy per AI at 32 and 60 days after TAI for first service (Table 5). 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 5). 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 5). 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 5). 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 5).

Table 5. 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.

In this Florida study (Silvestre, 2008a 2008c), 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.

Collectively, these data indicate that feeding fat to dairy cows generally improves fertility and responses are observed with the energy increment in the diet; also, these data suggest that fertility responses to fat feeding are altered according to the type of dietary FA, although responses are not always consistent. Feeding n-3 FA from oilseeds or as Ca-LCFA improved pregnancy per AI in some, but not all studies. Similarly, feeding Ca-LCFA rich in n-6 FA improved pregnancy per AI in one of two experiments with lactating dairy cows. Although feeding n-3 FA has not consistently increased the risk of pregnancy, it has reduced pregnancy losses in dairy cows.

Conclusion

The threshold for triggering an immune response (i.e., creating a pro-inflammatory state that can respond greatly upon challenge) due to feeding FAs precursors of pro-inflammatory eicosanoids can benefit the 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 FAs 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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