INTRODUCTION

Reproductive performance of lactating dairy cows worldwide has decreased markedly in association with selection for milk yield over the last 25 years. This condition is referred to as an “infertility syndrome” that hopefully is reversible with the application of current technology across the disciplines of dairy management, nutrition, herd health, genetics and physiology. Reproductive management systems have been developed that allow for a first service insemination rate following a synchronized ovulation of 35 to 40% under commercial conditions (Moore and Thatcher, 2006). However, it is evident that many of the cows presented for service are anovulatory and are not in a state to achieve optimal fertility. Occurrence of cyclicity is greater for multiparous than primiparous cows (e.g., 81.8 vs 69.5%) at 65 d postpartum. Cyclicity is influenced by milking frequency (twice = 82.7% vs thrice = 68.7%), BCS at both calving and AI, and change in BCS. As expected, more cyclic than anovular cows were pregnant at 30 (40.0 vs. 28.3%) and 58 (34.2 vs. 23.0%) days after AI (Rutigliano and Santos, 2005. Consequently, minimizing losses of BCS after calving and improving cyclicity early postpartum are expected to increase PR and enhance embryonic survival. The challenge is to manage body condition in healthy cows, with an inherent high dry matter intake, so that they have sufficient energy stores at parturition to meet the demands of ensuing negative energy status without a predisposition to fatty livers and metabolic disturbances and yet have sufficient body condition at the beginning of the breeding period such that they are cycling and able to sustain a pregnancy. Proper management to optimize restoration of uterine health in the postpartum period is essential but the impact of nutrition specifically on uterine health is limited. These associations target the importance of nutritional management from the time of “dry-off” to the time of insemination as being critical to herd reproductive performance.

The objective of this paper is to identify those physiological windows during the periparturient and postpartum periods that appear amendable to nutritional management that may improve subsequent fertility during the insemination period.
NUTRITIONAL ALTERATION OF PERIPARTURIENT REPRODUCTIVE EVENTS

Postpartum Immunosuppression and Response to Organic Selenium

During the immediate postpartum period, the cow’s immune system is challenged severely (Goff, 2006), and the innate and humoral defense systems are reduced. The incidence of diseases and disorders can be high during this time period and have a negative impact on reproductive performance. For example, the “risk” of pregnancy (odds ratio) was reduced if cows had retained fetal membranes (RFM) or lost one BCS (Loeffler et al., 1999). Reduction in adaptive and innate immunity at parturition increases the risk of health disorders such as RFM, metritis, and mastitis. Selenium has long been associated with immunity. Cattle supplemented with Se-yeast had an 18% increase of Se in plasma in comparison sodium selenite in some studies (Weiss, 2003). The state of Florida, USA is in a selenium deficient area, and lactating dairy cows are exposed to a seasonal period of heat stress that impacts reproductive performance and health.

We have conducted an experiment to evaluate a supplemental source of organic selenium on reproductive and immune responses by dairy cows during the summer heat stress period of Florida (Silvestre et al., 2006a; Silvestre et al., 2006b). Objectives were to evaluate effects of organic Se on PR at the first and second postpartum AI services, uterine health, and milk yield during the summer heat stress period. Cows were assigned (23 ± 8 days prepartum) to diets of organic Se (Se-yeast [SY; Sel-Plex®, Alltech; n = 289] or inorganic sodium Se [SS; n = 285]) fed at 0.3 ppm (DM basis) for >81 days postpartum. Rectal temperature was recorded each morning for 10 days postpartum (dpp). Vaginoscopies were performed at 5 and 10 dpp. Cows within diet were assigned randomly to 2 reproductive management programs (Presynch-Ovsynch vs. CIDR-Ovsynch [i.e., Ovsynch began 3 days after withdrawal of a 7 day-CIDR]). All cows were resynchronized for a second service with Ovsynch at 20 to 23 days after first service. An ultrasound pregnancy diagnosis was conducted at 27 to 30 days after first TAI. Cows in estrus following Presynchs were AI up to the second TAI service. Strategic blood sampling determined anovulatory status at Ovsynch and ovulatory response after TAI to first service. The PR at second service was determined by rectal palpation at ~42 dpp. In essence, we utilized this reproductive management program as a platform to examine pregnancy rates between the two treatments.

Blood was sampled for Se (n = 20 cows/diet) at -25, 0, 7, 14, 21, and 37 dpp. Plasma Se increased in SY-fed cows (0.087 vs. 0.069 ± .004 µg/ml; P < 0.01). Milk yield (35.6 kg/d for 81 days), milk somatic cells (291,618 cells/ml), and frequencies of retained fetal membrane (9.7%), mastitis (14.4%), anovulation (17.7%), and synchronized ovulation after TAI (82.5%) were not affected by diets or reproductive program. Diet failed to alter first service PR at ~30 days post AI (SY, 24.9% [62/249] vs. SS, 23.6% [62/262]) or pregnancy losses between ~30 and ~55 days post AI (SY, 39.3% vs. SS, 37.1 %). These low pregnancy rates and high embryonic losses are typical of cows managed during the summer heat stress.
period of Florida. Diet altered second service PR [SY, 17% (34/199) vs. SS, 11.3% (24/211); P < 0.05]. The benefit of SY on second service pregnancy rate is intriguing. We hypothesize that cows of the SY group were better able to reestablish an embryo-trophic environment at second service following either early or late embryonic losses. Diet altered frequency of multiparous cows detected with > 1 event of fever (rectal temperature > 39.5°C; SY, 13.3% [25/188] vs. SS, 25.5% [46/181]; P < 0.05) but the SY effect was not observed in primiparous cows which had a much higher frequency of fever (40.5%). Vaginoscopy discharge scores at 5 and 10 dpp were better for the SY group; namely, 47.1 (217/460) vs. 35.0% (153/437) clear, 43.4 [200/460] vs. 47.8% [209/437]) mucopurulent, and purulent (9.3% [43/460] vs. 17.1% [75/437]) discharge scores were affected by SY and SS, respectively (P<.05). Organic Se (Se-yeast, Sel-Plex®) improved uterine health and 2nd service PR during summer.

Innate immunity (i.e., neutrophil function) was determined by phagocytic and oxidative burst capacity of neutrophils in whole blood using a dual color flow cytometric method. Samples were collected in a sub-sample of 38 cows at -26, 0, 7, 14, 21 and 37 dpp for neutrophil function. Adaptive immunity (ability to induce an antibody response) was monitored with anti-IgG to Ovalbumin (Ovalb) following vaccination with Ovalb antigen (1 mg [i.m.]) dissolved in an E. coli J5 endotoxemia preventive vaccine at -60 and -22 ± 6 dpp (day of initiating of SY [n=38] and SS [n=47] diets) and again at parturition (day 0) with Ovalb dissolved in PBS with Quil-A adjuvant. Serum samples were collected on days of immunization and at 21 and 42 dpp.

Percentage of gated neutrophils that phagocytized E. coli and underwent oxidative burst did not differ between diet groups at -26 dpp (44.6 ± 4.6%). For subsequent samples, a diet*parity*day interaction was detected (P<0.05; Figure 1): SY [Organic Se (Se-yeast, Sel-Plex®)] improved neutrophil function at parturition in multiparous cows (42 ± 6.14% > 24.3 ± 7.2%) and at 7, 14 and 37 dpp in primiparous cows (53.9 > 30.7, 58.6 > 41.9, 53.4 > 34.8%, respectively; pooled SE=6.8%). It is clear that neutrophil function is suppressed in primiparous cows at the time of parturition and it is not restored until between 7 to 14 days postpartum (Figure 1). In contrast, the multiparous cows did not have a restoration in neutrophil function until between 14 to 21 days postpartum (Figure 2). Organic Se improved phagocytosis and killing activity of neutrophils in both primiparous and multiparous cows (Figures 1 and 2). However, the primiparous cows seemed to be more responsive in that SY stimulated neutrophil function throughout the period of day 0 to 21 whereas, SY stimulation in multiparous cows was evident on only the day of parturition. In most all of our postpartum experiments, we detect distinct differences between primiparous and multiparous cows for a multiplicity of responses.

Anti-IgG to Ovalb did not differ between diets at -60 and -22 dpp (0.18 ± 0.01 and 0.97 ± 0.04 OD). Although Anti-IgG to Ovalb concentration did not differ between diet groups for primiparous cows (1.40 ± 0.08 OD), concentrations were higher in SY cows at 21 and 42 dpp (1.91 ± 0.1 > 1.24 ± 0.07, 1.44 ± 0.7 > 0.99 ± 0.07 OD, respectively; P<0.01). Thus our measurement of adaptive immunity was improved in multiparous dairy cows in response to SY but not in primiparous cows.
Figure 1. Phagocytosis and killing activity of neutrophils in whole blood from periparturient multiparous dairy cows fed an inorganic selenium (control) or organic selenium (Sel-Plex®) supplements.

Figure 2. Phagocytosis and killing activity of neutrophils in whole blood from periparturient primiparous dairy cows fed an inorganic selenium (control) or organic selenium (Sel-Plex®) supplements.
Mean milk yield and milk composition determined from monthly samples for cows receiving supplemental inorganic selenium (sodium selenite, SS) and organic selenium (selenium yeast, SY) indicated that both monthly milk yield (37.1 > 36.4 kg/d) and fat-corrected milk (36.2 > 35.3 kg/d) were greater for cows receiving SY (Silvestre et al., 2007). Milk production was monitored on a daily basis for the first 81 days of lactation and mean daily milk yield did not differ between treatments (35.6 kg/d). However, monthly milk yields were elevated in the SY or Sel-Plex group in later stages of lactation (i.e., between 6-8 months of lactation). The increase in milk production in later stages of lactation occurred primarily in primiparous cows but not in multiparous cows.

Our findings indicated that feeding organic SE as Organic Se (Se-yeast, Sel-Plex®) beginning at 26 days prepartum, elevated plasma Se concentrations, increased neutrophil function at the time of parturition, improved immuno-responsiveness in multiparous cows, improved uterine health and increased 2nd service PR during summer in a Florida environment that is selenium deficient.

**DIETARY FATS ARE MODIFIED IN THE RUMEN BY BACTERIA WITH DIFFERENTIAL POST-RUMINAL ABSORPTION AND BIOLOGICAL EFFECTS IN TISSUES:**

The ruminal microbes will convert unsaturated fats to saturated fats by replacing the double bonds with single bonds between the carbons (called biohydrogenation). Speculation is that bacterial biohydrogenation is an attempt to protect the bacteria, as unsaturated fats can be toxic especially to fiber digesters. The majority of the consumed unsaturated essential fatty acids, C18:2 and C18:3 are converted by the bacteria to C18:0. For example when approximately 20 g of C18:0, 280 g of C18:2, and 40 g of C18:3 are consumed daily, approximately 370 g of C18:0, 40 g of C18:2, and 4 g of C18:3 leave the rumen daily because of biohydrogenation. Several intermediate forms of fatty acids, called trans fatty acids, also are formed during biohydrogenation. 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 a depression in milk fat synthesis. This intervention by ruminal bacteria to change essential fatty acids in the diet to other fatty acids raises the issue whether potentially active unsaturated fatty acids can escape the rumen and be available to the tissues such as the uterus and mammary gland.

**Ca Calt Containing Fish Oil (CaSFO)**

We examined the effects of Ca salts of fatty acids enriched with fish oil and bST injection (500 mg; Posilac, Monsanto) on endocrine responses, ovarian-uterine function, expression of various genes in the uterus, and conceptus development on day 17 after estrus in cyclic and pregnant lactating dairy cows (Bilby et al., 2006a; Bilby et al., 2006b). 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). Formulated concentrations of ether extract (5.7%), nonstructural carbohydrate (36%), crude protein (18%), and net energy of lactation (1.7 Mcal/kg) were similar between diets (100% DM basis). Ingredients (corn silage, alfalfa hay, cottonseed hulls, ground corn, citrus pulp, soybean meal, expeller soybean meal, whole cottonseeds, calcium salt of fat, and mineral/vitamin premix) were fed as a totally mixed ration twice daily. All cows (n = 35) were fed the control diet for the first 10 dpp. Starting on day 11 of lactation, 8 of these cows were temporarily assigned to a transition diet containing 0.95% CaSFO to initially adjust cows to eating CaSFO. On day 18 postpartum, the CaSFO was increased to 1.9% of dietary DM on which they remained for the duration of the experiment. Ad libitum feed intakes were measured daily on a group basis. Daily feed intake by the cows fed CaSFO averaged 20.9 kg of DM/cow over the entire period of feeding. The calculated intake of EPA and DHA was 7.4 g/d for each fatty acid (i.e., 14.8 g/d total). Cows were milked thrice daily and individual milk weights recorded daily. All cows were presynchronized and started an Ovsynch protocol at approximately 7 days after a detected estrus. Cows assigned to bST treatment received an injection of bST at the Timed Artificial Insemination (TAI; i.e., cows were either TAI [pregnant] or not TAI [cyclic]) and 11 days later. The second injection at day 11 was done to insure that GH concentrations would be sustained until the time of slaughter on day 17. All cows that were designated for slaughter had to have ovulated and formed a CL when evaluated at day 7 following the second GnRH injection of the Ovsynch protocol.

Dietary supplementation with CaSFO increased milk production compared with control cows fed lipid in the form of whole cottonseeds during the postpartum period before TAI or bST treatment. Although milk yield increased, body condition score and body weight were not influenced by diet. The cows fed CaSFO produced as much as 3 kg more milk than cyclic control-fed cows. Moussavi et al. (2007) also reported that feeding CaSFO also increased milk production.

An important point is whether feeding a by-pass fat enriched in fish oil results in absorption of EPA and DHA that alters fatty acid concentrations among various tissues (Figure 3). The endometrium and liver had highest concentrations of C18:2n-6, C20:4n-6, C18:3n-3, EPA, and DHA compared to milk fat, mammary tissue, muscle, and both subcutaneous and internal fat tissues (Bilby et al., 2006c). 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 (see Table 1 in Bilby et al., 2006c). 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 to decrease the n6:n3 ratio of fatty acids. These dynamic tissue responses to CaSFO are comparable to those reported by Moussavi et al., 2007.

Supplementation with n-3 PUFA-rich fish oil was associated with reduced steady state concet rations of PPARδ mRNA. In contrast, the CaSFO diet did not alter abundance of PPARα mRNA. Immunohistochemistry to localize PPARδ indicated that the protein is
expressed in the luminal epithelium, glandular epithelium, subepithelial stroma and, to a lesser extent, in the adluminal stroma. The decrease in abundance of PPARδ mRNA associated with cows fed the CaSFO diet also was reflected with immunolocalization in that there was a reduction in moderate staining intensity of PPAR δ protein in the luminal epithelium of cows fed FO with or without bST treatment.

The uterus (i.e., endometrium) undergoes an antiluteolytic pathway in the presence of a conceptus (i.e., decrease in ER-α and oxytocin receptors) that leads to maintenance of the corpus luteum in pregnancy. In the present study, feeding CaSFO appeared to induce subtle antiluteolytic effects when examining immunohistochemistry spatial responses for the progesterone and estradiol-α receptors and PGHS-2 proteins (Bilby et al., 2006b). Staining for progesterone receptors was evident in the superficial glandular epithelial cells of the cyclic cows at day 17 whereby CaSFO increased the moderate and heavy staining. Conversely, moderate to heavy staining intensity for the ER-α receptor was reduced in
Table I. Effect of Feeding Various Oilseeds on the Essential Fatty Acid Concentration of Milk Fat From Dairy Cows.1

<table>
<thead>
<tr>
<th>Reference</th>
<th>Seed Type</th>
<th>C18:2</th>
<th>C18:3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dhiman et al., 1995</td>
<td>0% vs. 16% soybeans</td>
<td>3.2%</td>
<td>6.2%*</td>
</tr>
<tr>
<td>Holter et al., 1992</td>
<td>0% vs. 15% whole cottonseeds</td>
<td>4.0%</td>
<td>4.2%</td>
</tr>
<tr>
<td>Markus et al., 1996</td>
<td>0% vs. 7.1% whole sunflower seeds</td>
<td>2.3%</td>
<td>2.8%*</td>
</tr>
<tr>
<td>Petit et al., 2004b</td>
<td>0% or 9.6% whole sunflower seeds</td>
<td>3.2%</td>
<td>3.8%</td>
</tr>
<tr>
<td>Stegeman et al., 1992</td>
<td>0% or 10% rolled sunflower seeds</td>
<td>2.2%</td>
<td>3.3%*</td>
</tr>
<tr>
<td>Tice et al., 1994</td>
<td>19.7% raw vs. roasted whole soybeans</td>
<td>5.5%</td>
<td>6.7%*</td>
</tr>
<tr>
<td>Stegeman et al., 1992</td>
<td>0% or 10% rolled safflower seeds</td>
<td>2.2%</td>
<td>3.1%*</td>
</tr>
</tbody>
</table>

* Values under the oilseed column having an asterisk were significantly different from the control values.

the luminal epithelial cells of CaSFO-fed cows. This antiluteolytic pattern was associated with an attenuated effector response reflected by a decrease in heavy staining for PGHS-2 protein in the luminal epithelium of cyclic cows (Bilby et al., 2006b). Perhaps activation of the PPARδ receptor normally attenuates expression of the progesterone receptor and enhances expression of the ER-α receptor and PGHS-2 protein. Present results indicate that feeding by-pass fat enriched in fish oil (i.e., EPA and DHA) decreased PPARδ receptors which would reverse these responses related to the progesterone receptor, the ER-α receptor and PGHS-2 protein. It is clear that EPA antagonizes the stimulatory effects of arachidonic acid on PGF2α secretion (Mattos et al., 2003). As a consequence, a dietary manipulation of a nutraceutical complex of fatty acids has possibly altered the cellular and intracellular processes to sustain embryo development or pregnancy. The take home message is that feeding a by-pass fat enriched with fish oil appears to mediate specific effects in the uterus of cyclic cows that may benefit the processes of early pregnancy. Such results indicate that a by-pass fat diet can alter expression of a complement of genes in the uterus that impinge on uterine responses that support maintenance of pregnancy and development of the conceptus. Although GH in plasma was markedly elevated in cyclic cows fed CaSFO that received bST, their concentrations of IGF-1 remained relatively low throughout the sampling period. This reflects the potential dietary interactions of fat feeding on endocrine and metabolic responses.
in lactating dairy cows. Perhaps the higher milk production and lower plasma concentrations of insulin in cows fed the CaSFO (Bilby et al., 2006a) altered liver responsiveness to exogenous bST to reduce peripheral secretion of IGF-1 as compared to the control fed cyclic cows treated with bST.

An additional 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.

**Oil Seeds Supply Unsaturated Fatty Acids**

Oil seeds are also candidates for potential sources of unsaturated fatty acids and those seeds that can deliver the key fatty acids past the rumen may be good candidates for dietary supplementation to influence fertility. Although the oil in many oil seeds contains more than 50% linoleic acid (Table 1), the delivery of linoleic acid past the rumen to the small intestine is not the same for all oil seeds. If we use an increase in the linoleic acid concentration of milk as an indicator that an oil seed can delivery linoleic acid to the tissues, then soybeans appear to be most effective and cottonseeds seem to be ineffective (Table 1). Sunflower seeds and safflower seeds also can increase the linoleic acid of milk fat, but not quite as effectively as that of soybeans. The processing of whole seeds also can influence their ability to deliver unsaturated fat past the rumen. Roasting of soybeans and rolling of sunflowers seemed to increase delivery of linoleic acid. Regarding linolenic acid, whole flaxseeds fed at about 10% of the diet can deliver some of its omega-3 fatty acid to the tissues. Grinding the flaxseed or heat-treated linseed (i.e., flaxseed) may deliver even more linolenic acid to the tissues (Table 1). In the United Kingdom, a process has been developed in which cracked linseeds or soybeans are processed with steam in order to create Maillard products, which help to protect the seed’s unsaturated fatty acids from ruminal microbes (Robinson et al., 2002).

**FAT FEEDING ON CONCEPTION RATES**

According to the scientific literature, a variety of fat supplements have benefited conception rates of lactating dairy cows (Table 2). The conception rates are sometimes reported for first insemination or for accumulated inseminations. Feedstuffs stimulatory to conception included calcium salts of palm oil distillate, tallow, Energy Booster (prilled tallow), flaxseed (formaldehyde-treated or rolled), MegaPro Gold (which is a calcium salt
of palm oil plus rapeseed meal and whey permeate) fed to grazing cows, calcium salt of a mixture of soy oil and monounsaturated trans fatty acids, Megalac-R, CLA, and fish meal. The average improvement in conception rate was 21 percentage units. This is not to imply that the feeding of one of these feedstuffs to cows on a commercial dairy farm will increase herd conception rate by 21 percentage units. Any benefit experienced on a commercial dairy farm will likely be less than 10 percentage units because management is usually not as tight as that exercised on an experiment at a research station. In contrast, some of the research station experiments are comprised of a small number of cows or experimental units. Other

Table 2. Studies Reporting Improved Conception Rates (first service or cumulative services) of Lactating Dairy Cows Fed Supplemental Fatty Acids (P < 0.10). Unless otherwise indicated with a footnote, the control diet did not contain a fat supplement.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Fat source and Concentration or Amount in Diet</th>
<th>Number of Cows in Trial</th>
<th>Control Treatment</th>
<th>Fat Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferguson et al., 1990</td>
<td>2% Ca-palm oil</td>
<td>253</td>
<td>43</td>
<td>59&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sklan et al., 1991</td>
<td>2.6% Ca-palm Oil</td>
<td>99</td>
<td>62</td>
<td>82</td>
</tr>
<tr>
<td>Scott et al., 1995</td>
<td>1 lb/d Ca-palm oil</td>
<td>443</td>
<td>93</td>
<td>98</td>
</tr>
<tr>
<td>Garcia-Bojalil et al., 1998</td>
<td>2.2% Ca-palm oil</td>
<td>43</td>
<td>52</td>
<td>86</td>
</tr>
<tr>
<td>Son et al., 1996</td>
<td>3% tallow</td>
<td>68</td>
<td>44</td>
<td>62</td>
</tr>
<tr>
<td>Frajblat and Butler, 2003</td>
<td>1.7% Energy Booster</td>
<td>81</td>
<td>58&lt;sup&gt;2&lt;/sup&gt;</td>
<td>86</td>
</tr>
<tr>
<td>Petit et al., 2001</td>
<td>17% formaldehyde-treated flaxseed</td>
<td>30</td>
<td>50&lt;sup&gt;3&lt;/sup&gt;</td>
<td>87&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Petit et al., 2004a</td>
<td>Whole unprocessed flaxseed</td>
<td>30</td>
<td>29</td>
<td>59&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ambrose et al., 2006b</td>
<td>9% rolled flaxseed</td>
<td>121</td>
<td>32&lt;sup&gt;2&lt;/sup&gt;</td>
<td>48&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>McNamara et al., 2003</td>
<td>3.3 lb/d MegaPro Gold</td>
<td>129</td>
<td>35</td>
<td>54</td>
</tr>
<tr>
<td>Juchem et al., 2004</td>
<td>1.5% (Soy + Trans C18:1)</td>
<td>397</td>
<td>26&lt;sup&gt;3&lt;/sup&gt;</td>
<td>34&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cullens, 2004</td>
<td>2% Megalac-R</td>
<td>42</td>
<td>27</td>
<td>58&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Castaneda-Gutierrez et al.,</td>
<td>0.3 lb/d Ca-CLA</td>
<td>32</td>
<td>44&lt;sup&gt;3&lt;/sup&gt;</td>
<td>81</td>
</tr>
<tr>
<td>Bruckental et al., 1989</td>
<td>7.3% fish meal</td>
<td>132</td>
<td>52</td>
<td>72</td>
</tr>
<tr>
<td>Armstrong et al., 1990</td>
<td>1.8 lb/d fish meal</td>
<td>80</td>
<td>44</td>
<td>64</td>
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<tr>
<td>Carrol et al., 1994</td>
<td>3.5% fish meal</td>
<td>44</td>
<td>68</td>
<td>89&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Burke et al., 1997</td>
<td>2.8% fish meal</td>
<td>300</td>
<td>32</td>
<td>41</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>49.0</td>
<td>70.6</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> First insemination.

<sup>2</sup> Control diet contained equal energy to fat-supplemented diet. Fat was fed prepartum only.

<sup>3</sup> Control diet contained Ca salt of palm oil distillate.

<sup>4</sup> Control diet contained rolled sunflower seeds.
studies have reported no positive pregnancy benefit to fat-supplementation (Table 3). The average response was 44.6 vs. 41.8% for control and test-fat treatment groups.

From the studies listed in Table 2, it is very difficult to determine which fat supplements or which fatty acid(s) may be most efficacious. When cows fed fats containing mainly palmitic and oleic acids (tallow, Energy Booster, and Ca salts of palm oil distillate) were compared against a no supplemental fat control, the fat-supplemented cows had better conception rates. In four head-to-head comparisons of fat supplements, cows fed calcium salts of palm oil distillate did not conceive as well as those fed formaldehyde-treated flaxseed (Petit et al., 2001), unprocessed whole flaxseed (Petit et al., 2004a), a calcium salt mixture of soybean oil and monounsaturated trans fatty acids (Juchem et al., 2004), or CLA (Castaneda-Gutierrez et al., 2005; Table 2). Therefore fats containing mainly palmitic and oleic acids may not be as effective.

Flaxseeds are a source of the n-3 fatty acid, α-Linolenic Acid (C18:3) and have been evaluated as a stimulator of reproductive performance of lactating dairy cows with mixed results. Although the fatty acids in fresh grass can contain a high proportion of linolenic acid, flaxseeds are the only concentrated source of linolenic acid (~20% of DM) available. First service conception rate was increased from 50 to 87% when lactating cows in the United Kingdom were fed formaldehyde-treated flaxseed at 17% of a ryegrass silage-based diet between 9 and 19 weeks postpartum (Petit et al., 2001). Control cows were fed a calcium salt of palm oil (5.6% of diet) and flaxseed meal. Cows had been on their diets for 6 weeks prior to insemination. Production of uncorrected milk (41.0 vs. 43.7 lb/day) and 4% fat-corrected milk (44.5 vs. 50.5 lb/day) was less for cows fed flaxseed but DM intake was not changed. In a Canadian study involving 121 Holstein cows (Ambrose et al., 2006b), cows fed coarsely rolled flaxseed at 9% of the diet had a better first service conception rate (P < 0.07) compared to the control cows fed rolled sunflower seeds at 8.7% of dietary DM (48.4 vs. 32.2%). Although the overall pregnancy rates (i.e., cumulative pregnancy rate to 1st and 2nd TAI) were not different between the two groups (67.7 vs. 59.3%), the overall pregnancy loss from day 32 of pregnancy to calving was less for cows fed flaxseed (9.8 % < 27.3 %; P < 0.05). Diets were fed for 28 days prior to insemination using a timed AI protocol and continued for 32 days after AI. Dry matter intake (49.6 vs. 47.0 lb/day) but not milk yield (80.9 vs. 79.4 lb/day) tended to be greater by cows fed flaxseeds. In a second Canadian study conducted on two commercial dairy farms, conception rate was not different between cows fed whole flaxseed at 10.6% of the diet and those fed micronized soybeans starting at calving (Petit and Twagiramungu, 2006). However those fed flaxseed had less (P < 0.07) embryonic loss. From the same lab, embryos collected from cows fed whole unprocessed flaxseed had a better gestation rate when transferred to heifers than embryos from cows fed Ca salt of palm oil distillate (58.8 vs. 29.3%; Petit et al., 2004a).

Three recent studies involving a greater number of dairy cows did not report any pregnancy advantage to cows fed flaxseed. Holstein cows (n = 356) on a commercial dairy in Spain were fed diets of either 5.5% extruded whole flaxseed or 4.9% extruded soybeans plus 1% calcium salts of palm oil between 4 to 20 weeks postpartum (Fuentes et al., 2007).
Cows were detected in estrus using visual observation and the Afimilk system. First service (39 vs. 39%) and overall conception rates (40 vs. 34%) did not differ between soybean and flaxseed groups, respectively. Yield of 4% fat-corrected milk was less for cows fed flaxseeds (83.1 vs. 78.0 lb/day) due to a lower milk fat concentration (2.65 vs. 2.86%). A commercial dairy in Oregon (n = 309 cows) was used to evaluate rolled flaxseed, fed from about 32

### Table 3. Studies Reporting a Negative Effect or No Improvement in Conception Rates (first service or cumulative services) of Lactating Dairy Cows Fed Supplemental Fatty Acids. Unless otherwise indicated with a footnote, the control diet did not contain a fat supplement.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Fat Source and Concentration or Amount in Diet</th>
<th>Number of Cows in Trial</th>
<th>Control Treatment</th>
<th>Fat Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schneider et al., 1988</td>
<td>1.1 lb/d Ca-palm oil</td>
<td>108</td>
<td>43</td>
<td>60^1</td>
</tr>
<tr>
<td>Sklan et al., 1989</td>
<td>1.1 lb/d Ca-palm oil</td>
<td>108</td>
<td>28</td>
<td>44^1</td>
</tr>
<tr>
<td>Carroll et al., 1990</td>
<td>5% prilled fat</td>
<td>46</td>
<td>33</td>
<td>75^1</td>
</tr>
<tr>
<td>Holter et al., 1992</td>
<td>1.2 lb/d Ca-palm oil</td>
<td>38</td>
<td>50^2</td>
<td>44^1</td>
</tr>
<tr>
<td>Lucy et al., 1992</td>
<td>3% Ca-palm oil</td>
<td>40</td>
<td>44</td>
<td>12^1</td>
</tr>
<tr>
<td>Sklan et al., 1994</td>
<td>2.5% Ca-palm oil primiparous cows</td>
<td>40</td>
<td>74</td>
<td>33^1,a</td>
</tr>
<tr>
<td>Sklan et al., 1994</td>
<td>2.5% Ca-palm oil multiparous cows</td>
<td>62</td>
<td>42</td>
<td>33^1</td>
</tr>
<tr>
<td>Salfer et al., 1995</td>
<td>2% partially hydrogenated tallow</td>
<td>32</td>
<td>32</td>
<td>33^1</td>
</tr>
<tr>
<td>Bernal-Santos et al., 2003</td>
<td>0.3 lb/d Ca-CLA</td>
<td>30</td>
<td>27^4</td>
<td>42</td>
</tr>
<tr>
<td>Bruno et al., 2004</td>
<td>1.5% (Ca-palm + fish oils)</td>
<td>331</td>
<td>26^1</td>
<td>27^1</td>
</tr>
<tr>
<td>Petit and Twagiramungu, 2006</td>
<td>10.6% whole flaxseed</td>
<td>70</td>
<td>58^3</td>
<td>64</td>
</tr>
<tr>
<td>Ambrose et al., 2006a</td>
<td>9% rolled flaxseed</td>
<td>309</td>
<td>37^6</td>
<td>26^1</td>
</tr>
<tr>
<td>Ambrose et al., 2006 personal comm.</td>
<td>8% rolled flaxseed</td>
<td>266</td>
<td>42^7</td>
<td>43</td>
</tr>
<tr>
<td>Fuentes et al., 2007</td>
<td>5.5% extruded flaxseed</td>
<td>356</td>
<td>39^8</td>
<td>39^1</td>
</tr>
<tr>
<td>Carroll et al., 1994</td>
<td>3.5% fish meal</td>
<td>18</td>
<td>67</td>
<td>33^1,a</td>
</tr>
<tr>
<td>Burke et al., 1997</td>
<td>2.7% fish meal</td>
<td>341</td>
<td>65</td>
<td>60</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>44.2</td>
<td>41.8</td>
</tr>
</tbody>
</table>

^1 First insemination.  
^2 Control diet contained whole cottonseed at 15% of dietary dry matter.  
^3 Control diet contained tallow.  
^4 Control diet contained Ca salt of palm oil distillate.  
^5 Control diet contained micronized soybeans.  
^6 Control diet contained Ca salt of palm oil distillate and High Fat Product from ADM.  
^7 Control diet contained Ca salt of palm oil distillate and tallow.  
^8 Control diet contained extruded soybeans and Ca salt of palm oil distillate.  
^a Significant dietary effect, P < 0.05.
days postpartum through 31 days after timed AI (Ambrose et al., 2006a). Cows were on diets at least 28 days prior to AI. Conception rates at 94 days after AI were not different, being 36.7% for controls and 25.6% for cows fed flaxseeds when all cows were considered. When only cows that responded to synchronization were included in the data set (n = 169), conception rate was lower for cows fed flaxseed at 31 days post AI (51.2 vs. 35.3%). Loss of embryos between 31 and 94 days post AI was not affected by diet but 9 control cows lost their embryos whereas 4 flaxseed-fed cows lost their embryos. Lastly, lactating dairy cows fed rolled flaxseed (8% of diet DM) had a similar conception rate (43.3%; n=141) to those fed a mixture of tallow and Ca salt of palm oil distillate (41.6%; n=125) at 35 days post AI (Ambrose et al., 2006, personal communication). Although not different, embryo loss was 8% vs. 16% for cows fed flaxseed vs. control fat. Although the evidence is not strong, it appears that feeding flaxseed may not improve initial pregnancy rates but may reduce embryonic loss.

Although the main nutrient in fish meal is protein and not fat, it is included here because the oils unique to fish may play a role in establishing pregnancy. The inclusion of fish meal in the diet (2.7 to 7.3% of dietary DM) has improved either first service or overall pregnancy rate in four studies. In some of these studies, fish meal partially replaced soybean meal resulting in a reduction of an excessive intake of ruminally degradable protein. Therefore, the improved conception rates may have been due to the elimination of the negative effect of excessive intake of ruminally degradable protein on conception. However, in a field study in which the concentration of ruminally undegradable protein was kept constant between dietary treatments, cows fed fish meal had a better conception rate (Burke et al., 1997) suggesting that the positive response was due to something other than a reduction in intake of ruminally degradable protein. Indeed, our present ongoing studies with CaSFO demonstrate clear effects of fatty acids in fish oil on endocrine and uterine endometrial tissue responses. Presently, we are examining CaSFO effects on reproductive and milk production responses of lactating dairy cows on a commercial dairy (Silvestre, F.T. and Thatcher, W.W. experiment in progress).

**Influence of Dietary Protein on Reproductive Performance**

The rumen generates ammonia due to protein digestion by ruminal microbes. The ammonia is converted into urea after hepatic uptake. One possibility of how high protein feeding may adversely affect reproductive performance is the increased energy costs to the animal for detoxification of ammonia resulting in a “weakening” of the cow’s energy state. This energy cost is likely to push early postpartum cows even further into negative or less positive energy states, thus delaying return to normal ovarian activity. Garcia-Bojalil and co-workers (1998) demonstrated how fat feeding interacted with high degradable intake protein (DIP) to influence reproductive responses in postpartum dairy cows. Dairy cows (n=45) were assigned at parturition to 20% CP diets containing either 15.7% or 11.1% DIP and 0 or 2.2% CaLCFA (Calcium salts of Long Chain Fatty Acids; MegalacR). Crude protein intake was
1100 g greater than required for milk produced. Treatments continued through 120 days in milk with BUN being higher for cows fed the highly degradable protein diets (22.0 vs. 17.3 mg %). Cows fed the 15.7% DIP diets experienced more days to first luteal phase postpartum than cows fed other diets (39 vs. 25 days). All cows on experiment were synchronized to estrus between days 50 and 57. Four out of 10 cows fed 15.7% DIP diet without CaLCFA were anestrus at synchronization compared with only three out of 35 cows fed the other dietary treatments. These prolonged days to restoration of ovarian activity and the anestrus condition were matched with greater loss of body weight and body condition by these cows. The absence of CaLCFA resulted in a 10 kg greater loss in BW and loss of body condition in cows fed 15.7% DIP diets. The additional energy costs of detoxifying ammonia from highly degradable dietary protein possibly led to a greater reliance on body energy stores for milk production. This resulted in a more severe energy state that delayed ovarian activity. By including CaLCFA in the diet, the energy shortage was somewhat alleviated, allowing cows to rely more on feed energy and less on body reserves for milk production. The detrimental effect of 15.7% DIP diets was alleviated markedly by supplementation of CaLCFA, but supplementation of CaLCFA to the 11.1% diet was not stimulatory. Consequently, dynamics of postpartum ovarian activity can be suppressed indirectly by feeding of high DIP (15.7%), but this adverse effect can be alleviated partially by feeding of CaLCFA. Also of interest was the observation that pregnancy rate by 120 days postpartum was increased from 52.3% to 86.4% when CaLCFA was supplemented and evaluated as a main effect across diets.

More direct and detrimental effects of high protein feeding on embryo-maternal reproductive processes was reviewed recently by Santos et al., 2008. Increasing circulating concentrations of urea N by manipulating the dietary energy and protein reduced conception rates of heifers (Butler, 1998), and embryos collected from lactating dairy cows fed a diet containing excess protein had reduced pregnancy rates in nonlactating recipients (Rhoads et al., 2006). Increased concentrations of ammonia and urea in the reproductive tract may influence embryo viability by altering follicular and oviductal environments. Kenny et al. (2002) observed that elevated concentrations of ammonia and urea did not influence concentrations of glucose, lactate, K, Na, and Mg in the oviducts, but concentrations of Ca were reduced. One of the consistent effects of excessive dietary protein is a reduction in uterine lumen pH during the early luteal phase (Butler, 1998), which was associated with reduced conception rate. Infusion of urea increased plasma urea and reduced uterine luminal pH in dairy cows (Rhoads et al., 2004). Maturation of oocytes in the presence of increasing concentrations of urea in vitro did not affect subsequent cleavage of zygotes, but seemed to have a negative effect on the proportion of blastocysts. However, incubation of zygotes with the same concentrations of urea had no effect on embryo development (Ocon and Hansen, 2003). Results from in vivo embryo production studies in dairy cows were not consistent necessarily with the in vitro studies. When dry cows were superovulated and received diets with concentrations of protein and rumen degradable protein much higher than recommended for high producing lactating dairy cows, no negative effects on embryo quality and viability were observed (Garcia-Bojalil et al., 1994). Similarly, excess protein (fed as urea) did not
affect embryo quality in superovulated lactating cows (Rhoads et al., 2006). When lactating diets differing in protein degradability were fed, the ration with higher rumen degradable protein tended to reduce the proportion of transferable embryos, even though the number of transferable embryos was not affected (Blanchard et al., 1990). In vivo-produced embryos from dairy cattle fed excess protein often appear unaffected. Nevertheless, embryos of similar grade quality resulted in reduced pregnancy rate after transfer when they originated from cows consuming excess dietary protein (Rhoads et al., 2006). Due to possible detrimental effects of excess feeding of protein on re-establishment of ovarian cycles postpartum as well as adverse alterations in the oviductal and uterine environment of the developing embryo, it is recommended that lactating dairy cows should not be fed in excess of their needs for maintenance, growth and lactation.

SUMMARY

Major advancements have been made for programmed reproductive management. However, reproductive performance is not optimal in the modern day lactating dairy cow. Major attention needs to focus on management of the cow during the peripartum and postpartum period to optimize the transition from the nonlactating to the lactating state in a manner that improves general health, immune function, and restoration of subsequent reproductive competence. Development of programs to optimize health, lactational, and reproductive performance will entail integration of herd health, nutrition and reproductive management programs.

Under summer heat stress conditions in the selenium deficient region of Florida, selenium yeast (SY) fed in the ration (Sel-Plex®; 0.33 mg/Kg), beginning at 26 days prepartum, elevated plasma Se concentrations, increased neutrophil function at the time of parturition, improved immuno-responsiveness in multiparous cows, improved uterine health, and increased second service PR.

Growing evidence indicates that the design and delivery of supplemental unsaturated fatty acids to the lower gut for absorption (specifically linoleic acid, linolenic acid, EPA and DHA) may target reproductive tissues to alter reproductive function and fertility. As summarized in Table 2, lactating dairy cows can benefit reproductively when fed a moderate amount of supplemental fat. Fat sources enriched in omega-6 or omega-3 fatty acids that deliver these fats to tissues beyond the rumen may be the most effective ones to feed. Studies showing a beneficial effect of fat feeding on conception rates fed fats at a minimum of 1.5% of the diet. Fat feeding can start when the cows enter the close-up group since there appears to be beneficial effects on cow health.

REFERENCES


