# IMPACT OF NUTRITION ON DAIRY CATTLE REPRODUCTION

**José Eduardo P. Santos, D.V.M., Ph.D.**
School of Veterinary Medicine, University of California - Davis

## Abstract

Nutrition has an important impact on the reproductive performance of dairy cattle. Energy is the major nutrient required by adult cattle and inadequate energy intake has a detrimental impact on reproductive activity of the female bovine. Cows under negative energy balance have lowered plasma glucose, insulin, and insulin - like growth factor-I (**IGF-I)**; reduced peak frequency of LH pulses; lowered plasma progesterone; and impaired ovarian activity. Incidence of postpartum anovulation and anestrus, as well as reduced fertility, is magnified by losses of body condition during the early postpartum period. Resumption of ovulatory cycles is associated with energy balance, but seems to be mediated by a rise in plasma IGF-I; which is linked to nutritional status and concentrations of insulin in blood. Feeding diets that promote higher plasma glucose and insulin may improve the metabolic and endocrine status of cows. Addition of supplemental fat to the diet improves energy intake, modulates $PGF_{2\alpha}$ secretion by the uterus, affects ovarian dynamics, enhances luteal function, and improves fertility. More specifically, some fatty acids might impact fertilization rate and embryo quality in dairy cows. Although gossypol intake seems to not affect lactation performance of dairy cows, it may affect fertility when the resulting plasma gossypol concentrations are excessive. Excessive intake of dietary crude protein or ruminally degradable protein increases urea nitrogen concentration in blood and tissues and alters uterine function, which may impair conception rates. Although evidence suggests that protein may interfere with reproduction, dietary changes should not be made in detriment to production and profitability.

## Introduction

Selection of dairy cattle for milk yield has linked the endocrine and metabolic controls of nutrient balance and reproductive events such that reproduction in dairy cattle is compromised during periods of nutrient shortage, such as in early lactation. The energy costs to synthesize and secrete hormones, to ovulate a follicle, and to sustain an early developing embryo are probably minimal compared to the energy needs for maintenance and lactation. However, the metabolic and endocrine cues associated with negative energy balance (**NEB**) impair resumption of ovulatory cycles, oocyte quality and embryo quality, and establishment and maintenance of pregnancy in dairy cattle.

As the demands for milk synthesis increase, reproductive functions may be depressed when no compensatory intake of nutrients is achieved. Numerous recent studies have reported that reproductive performance is compromised by the nutrient demands associated with high levels of production. Milk yield increases at a faster rate in the first 4 to 6 weeks after parturition than energy intake and high yielding cows will experience some degree of negative balance of energy and other nutrients during the early postpartum period. When cows experience a period of NEB, the blood concentrations of nonesterified fatty acids (**NEFA**) increases, at the same time that insulin-like growth factor-I **(IGF-I**), glucose, and insulin are low. These shifts in blood metabolites and hormones might compromise ovarian function and fertility. It has also been reported that energy balance and dry matter intake (**DMI**) might affect plasma concentrations of progesterone (Vasconcelos et al., 2003; Villa-Godoy et al., 1988), which may interfere with follicle development and maintenance of pregnancy.

During the last decades, genetic selection and improved management of herds have dramatically increased milk production of dairy cows, at the same time that fertility has decreased (Butler, 1998). Selection for higher milk production in dairy cattle has changed endocrine profiles of cows so that blood concentrations of bovine somatotropin and prolactin have increased, whereas insulin has decreased (Bonczeck et al., 1988). These hormonal changes and the increased nutrient demands for production might negatively impact reproduction of dairy cows. However, adequate nutrition and sound management has been shown to offset depression of fertility in herds with average milk production superior to 12,000 kg/cow/year (Nebel and McGilliard, 1993; Jordan and Fourdraine, 1993). Several nutritional strategies have been proposed to improve reproduction of dairy cattle with no detrimental effect on lactational performance. Maximizing DMI during the transition period, minimizing the incidence of periparturient problems, adding supplemental fat to diets, manipulating the fatty acid content of fat sources, balancing diets for adequate amounts of ruminally degradable (**RDP**) and undegradable (**RUP**) proteins, and optimizing supply of ruminally fermentable carbohydrates during late gestation and early lactation are expected to benefit reproduction in dairy cattle. However, factors such as high incidence of metabolic diseases early postpartum, poor body condition score (**BCS**) at first insemination, high milk urea N, and excessive gossypol concentrations in plasma are detrimental to fertility of dairy cattle.

## Resumption of Postpartum Cyclicity

The onset of lactation creates an enormous drain of nutrients in high producing dairy cows which, in many cases, antagonizes the resumption of ovulatory cycles. During early postpartum, reproduction is deferred in favor of individual survival. Therefore, in the case of the dairy cow, lactation becomes a priority to the detriment of reproductive functions.

During periods of energy restriction, oxidizable fuels consumed in the diet are prioritized toward essential processes such as cell maintenance, circulation, and neural activity (Figure 1; Wade and Jones, 2004).

Homeorhetic controls in early lactation assure that body tissue, primarily adipose stores, will be mobilized in support of milk production. Therefore, the early lactation dairy cow that is unable to consume enough energy-yielding nutrients to meet the needs of production and maintenance will sustain high yields of milk and milk components at the expense of body tissues. This poses a problem to reproduction as delayed ovulation has been linked repeatedly with energy status (Beam and Butler, 1998; Beam and Butler, 1997; Canfield et al., 1990; Butler and Smith, 1989). Energy deprivation reduces the frequency of pulses of luteinizing hormone (**LH**); thereby impairing follicle maturation and ovulation. Furthermore, undernutrition inhibits estrous behavior by reducing responsiveness of the central nervous system to estradiol by reducing the number of estrogen receptor α content in the brain (Figure 2; Hileman et al., 1999).

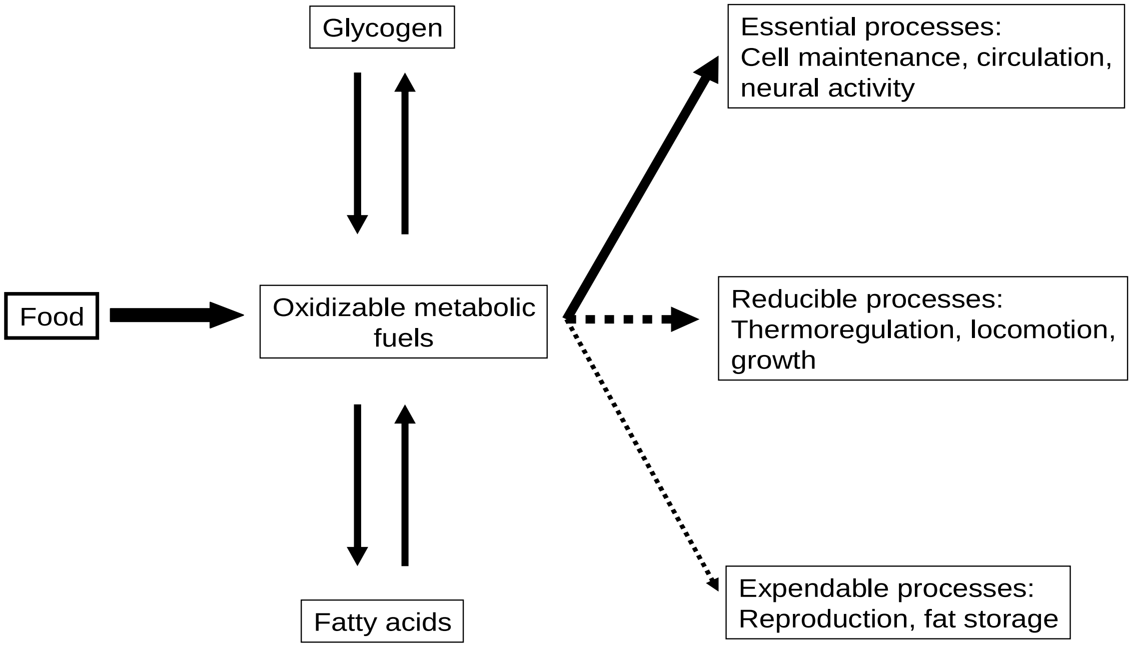

Figure 1. Partitioning of metabolic fuels according to priority (Wade and Jones, 2004).

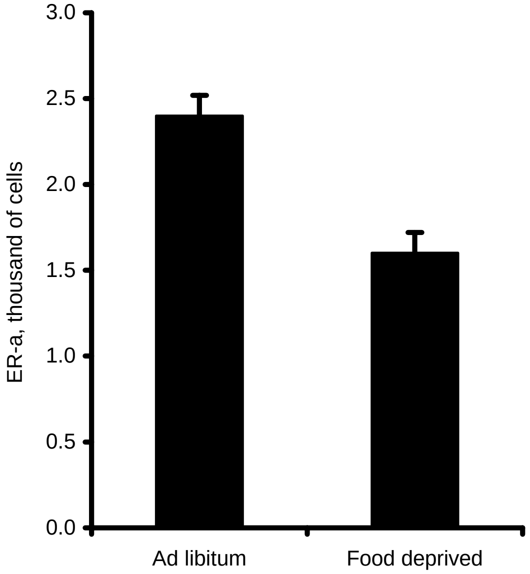

**Figure 2.** Changes in hypothalamic estrogen receptor-containing cells number in response to feed restriction in ewes (Hileman et al., 1999).

Generally, the first postpartum ovulation in dairy cattle occurs 10 to 14 d after the nadir of NEB (Beam and Butler, 1998; Beam and Butler, 1997; Canfield et al., 1990; Butler and Smith, 1989), and severe weight and BCS losses caused by inadequate feeding or illnesses are associated with anovulation and anestrus in dairy cattle. In fact, cows with low BCS at 60 d postpartum are more likely to be anovular (Figure 3; Santos et al., 2004a), which compromises reproductive performance at first postpartum insemination.

Prolonged postpartum anovulation or anestrus extends the period from calving to first artificial insemination and reduces fertility during the first postpartum service (Stevenson et al., 2001). In fact, anovular cows not only have reduced estrous detection and conception rates, but also have compromised embryo survival (Santos et al., 2004b). On the other hand, an early return to cyclicity is important in regard to early conception. The timing of the first postpartum ovulation determines and limits the number of estrous cycles occurring prior to the beginning of the insemination period. Typically, in most dairy herds, fewer than 20 % of cows should be anovulatory by 60 d postpartum (Stevenson, 2001). Estrus expression, conception rate, and embryo survival improved when cows were cycling prior to an estrous synchronization program for first postpartum insemination (Santos et al., 2004a; Santos et al., 2004b).

Resumption of ovarian activity in high producing dairy cows is determined by energy status of the animal. Therefore, feeding management that minimizes loss of body condition during the early postpartum period and incidence of metabolic disorders during early lactation should increase the number of cows experiencing a first ovulation during the first 4 to 6 wk postpartum.

## Energy and Reproduction

Energy intake appears to have the greatest impact on energy status of lactating dairy cows. Villa-Godoy et al. (1988) reported that variation in energy balance in postpartum Holstein cows was influenced most strongly by DMI (r = 0.73) and less by milk yield (r = -0.25). Therefore, differences among cows in the severity of energy balance are more related with how much energy they consume than with how much milk they produce.

During periods of NEB, blood concentrations of glucose, insulin, and IGF-I are low, as well as the pulse frequency of GnRH and LH. Plasma progesterone concentrations are also affected by the energy balance of dairy cows. These metabolites and hormones have been shown to affect folliculogenesis, ovulation, and steroid production *in vitro* and *in vivo*. The exact mechanism by which energy affects secretion of releasing hormones and gonadotropins is not well defined, but it is clear that lower levels of blood glucose, IGF-I, and insulin may mediate this process.

It has been suggested that NEB influences reproduction of dairy cows by impacting the quality and viability of the oocyte of the ovulatory follicle and the CL resultant of the ovulation of that follicle. Because there is substantial evidence that metabolic factors can influence early follicular development, it is conceivable that changes in metabolism during periods of NEB could influence preantral follicles destined to ovulate weeks later during the breeding period. To test this hypothesis, Kendrick et al. (1999) randomly assigned 20 dairy cows to one of two treatments formulated so that cows consumed either 3.6 % (high energy) or 3.2 % (low energy) of their body weight. Follicles were transvaginally aspirated twice weekly and oocytes were graded based upon cumulus density and ooplasm homogeneity. Cows in better energy balance (high energy) had greater intrafollicular IGF-I and plasma progesterone and tended to produce more oocytes graded as good. Therefore, NEB not only delays resumption of ovulatory cycles, but it might also influence the quality of occytes once cows are inseminated.

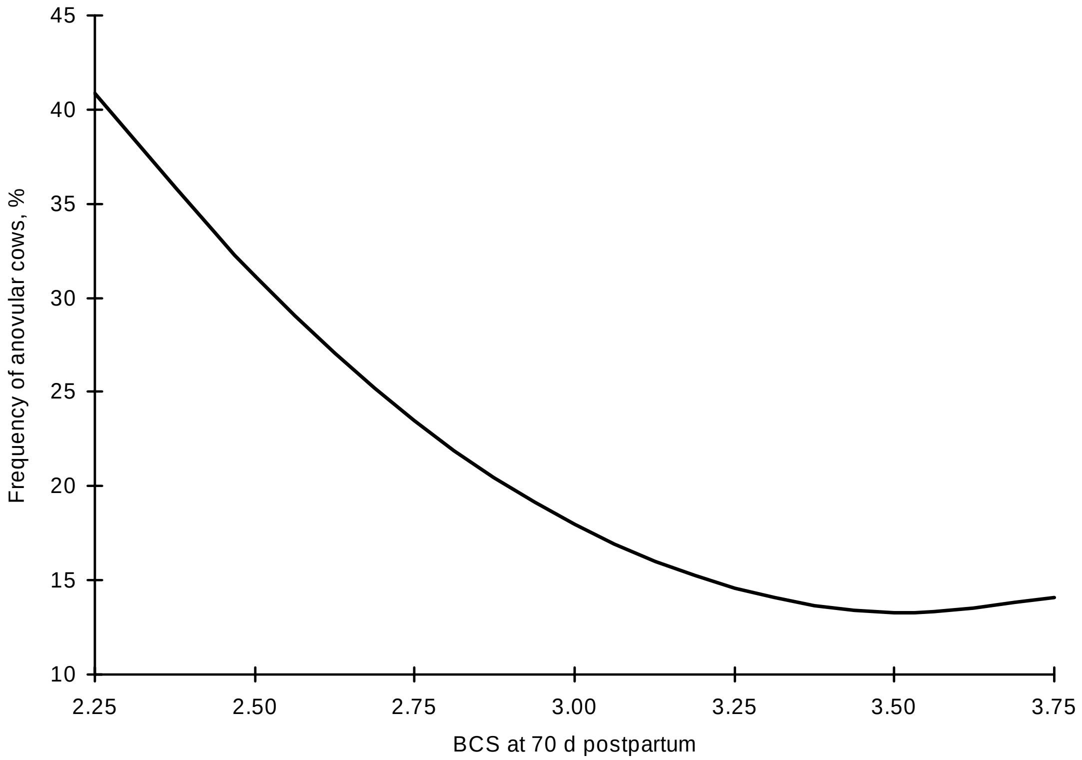

**Figure 3**. Relationship between the frequency of lactating dairy cows classified as anovulatory/anestrous based on plasma progesterone (< 1.0 ng/ml) at 51 and 63 days in milk and body condition score (BCS) at 70 DIM (Santos et al., 2004a).

## Nutritional Manipulation to Increase Energy Intake

Nutritional efforts to minimize the extent and duration of NEB may improve reproductive performance. The first and most important factor that affects energy intake in dairy cows is feed availability (Grant and Albright, 1995). Therefore, dairy cows should have at all times a high quality palatable diet available to assure maximum DMI. However, DMI is limited during late gestation and early lactation, which can compromise total energy intake and reproductive performance. Several nutritional management strategies have been proposed to increase energy intake during early lactation. Feeding high quality forages, increasing the concentrate:forage ratio, or adding supplemental fat to diets are some of the most common ways to improve energy intake in cows.

Intake of energy increases linearly with increasing grain in the diet up to 55 to 60 % of the dry matter (**DM**). Diets with more than 60 % concentrate and limited fiber content are associated with higher ruminal osmolarity, lower rumen pH, increased VFA in the rumen and portal system, and decreased DMI.

Diets with greater amounts of concentrate have higher content of non-fiber carbohydrate (**NFC**). Starch is the most important NFC in diets for high producing cattle. Degradation of starch in the rumen increases the proportion of propionate relative to other VFA and enhances glucose synthesis by the liver. Both glucose and propionate are insulin secretagogue, and diets with high ruminally degradable starch increase liver output of glucose (Theurer et al., 1999) and plasma concentrations of glucose and insulin (Santos et al., 2000).

A number of studies have demonstrated the importance of insulin as a signal mediating the effects of acute changes in nutrient intake on reproductive parameters in dairy cattle. In early postpartum dairy cattle under NEB, reduced expression of hepatic growth hormone receptor 1A (**GHR-1A)** is thought to be responsible for the lower

**Table 1.** Effect of diets designed to alter plasma insulin concentrations on reproductive parameters of high- and low-genetic merit postpartum dairy cows (Gong et al., 2002).

| Genetic merit | Diet: Low insulin | | Diet: High insulin | |
|---|---|---|---|---|
| | Low | High | Low | High |
| Plasma insulin,[1] ng/mL | 0.34 | 0.21 | 0.48 | 0.32 |
| Ovulation in first 50 d postpartum,[1] % | 60 | 50 | 100 | 80 |
| Days to first ovulation | 43 | 54 | 28 | 41 |
| Conception rate to first AI, % | 62.5 | 37.5 | 66.7 | 44.4 |

[1] Effect of high-insulin diet ($P < 0.05$).

concentrations of IGF-I in plasma of cows (Radcliff et al., 2003). Because IGF-I is an important hormonal signal that influences reproductive events such as stimulation of cell mitogenesis, hormonal production, and embryo development, among other functions; increasing concentrations of IGF-I early postpartum are important for early resumption of cyclicity and establishment of pregnancy. It is interesting to note that insulin mediates the expression of GHR-1A in dairy cows (Butler et al., 2003; Rhoads et al., 2004), which results in increased concentrations of IGF-I in plasma. Because IGF-I and insulin are important for reproduction in cattle, feeding diets that promote greater insulin concentrations should benefit fertility.

Gong et al. (2002) fed cows of low- and high-genetic merit isocaloric diets, that differed in the ability to induce high or low insulin concentrations in plasma. The authors observed that diets that induced high insulin reduced the interval to first postpartum ovulation, increased the proportion of cows ovulating in the first 50 d postpartum, and improved conception rate at first AI (Table 1).

Recently, monensin (Rumensin 80®, monensin sodium, Elanco, Greenfield, IN) was approved for use in lactating dairy cattle to enhance milk production efficiency. However, although not labeled for treatment of subclinical ketosis, monensin supplementation of transition dairy cows increases plasma glucose concentrations and reduces subclinical ketosis. Because monensin increases propionate production in the rumen and glucose concentrations in plasma, it is expected that monensin supplementation will improve plasma insulin concentrations and impact reproductive performance. When cows were treated with a controlled release capsule that delivers approximately 335 mg of monensin/d, risk for ketosis, displaced abomasum and multiple illnesses were reduced, but reproductive parameters were not altered (Table 2; Duffield et al., 1999).

**Table 2.** Effect of monensin in a controlled release capsule on conception rates of dairy cows (Duffield et al., 1999).

| | Treatment: Monensin | Treatment: Control |
|---|---|---|
| Cows, n | 406 | 403 |
| Conception rate, % | | |
| First AI | 35.2 | 34.5 |
| Second AI | 37.6 | 41.5 |
| Third AI | 40.7 | 38.6 |
| > 3 AI | 60.0 | 64.1 |

## Feeding Fat and Fatty Acids

Feeding fat is a common method to increase the energy density of the diet. However, supplemental fat sources not only provide calories, but also impact tissue metabolism by altering gene expression (polyunsaturated fatty acids; Sumida et al., 1993), by supplying precursors (acetyl Co-A) for synthesis of other fatty acids or steroids (Staples et al., 1998), or by competing with other cell components (Thatcher et al., 2004).

The use of fat in diets of dairy cattle usually increases the energy density of the ration and improves lactation and reproduction (Table 3; Ferguson et al., 1990).

**Table 3.** Effect of feeding 500 g of saturated free fatty acids on reproduction of dairy cows (Ferguson et al., 1990).

| | Treatment | |
|---|---|---|
| | Control, no supplemental fat | 500 g/d saturated free fatty acids |
| Cows, n | 138 | 115 |
| Conception rate 1st AI, % | $42.6^a$ | $59.1^b$ |
| Overall conception rate, % | $40.7^a$ | $59.3^b$ |
| Pregnant, % | $86.2^c$ | $93.0^d$ |

$^{a,b}$ Superscripts in the same row differ ($P < 0.05$).

$^{c,d}$ Superscripts in the same row differ ($P = 0.08$).

Some have suggested that fat feeding improves reproduction in spite of provision of calories (Staples et al., 1998). These effects might be mediated by the fatty acid make up of the fat source, as different fatty acids can have distinct effects at the tissue and cellular level.

There are different commercial sources of rumen inert fats including hydrogenated fatty acids and calcium salts of fatty acids. These fat sources were originally designed to increase the caloric intake of dairy cows with minimal impact on rumen microbial activity. More recently, there has been an increased interest in designing fat sources rich in the polyunsaturated fatty acids of ω-6 (linoleic acid, *cis*-9 *cis*-12 $C18:2_{n6}$) and ω-3 (α-linolenic acid, $C18:3_{n3}$; eicosapentaenoic (**EPA**), $C20:5_{n3}$; docosahexaenoic (**DHA**), $C22:6_{n3}$) for delivery to the lower gut for absorption. Because microbial activity in the rumen resulting in lipolysis and biohydrogenation alters the make-up of fat sources rich in polyunsaturated fatty acids, methods to protect fatty acids from microbial activity in the rumen have been developed. Staples et al. (1998) reviewed the literature on the effects of fat feeding on reproduction in dairy cows and indicated that increasing dietary fat in the ration usually resulted in improvements in reproductive performance of cows. The authors indicated that the beneficial effects of supplemental fat were observed in spite of provision of calories (Staples et al., 1998).

Unfortunately, the commercial methods available today to *protect* lipids from microbial biohydrogenation are not very effective at preventing saturation of polyunsaturated fatty acids in the rumen (Lundy III et al., 2004). In spite of that, we attempted to increase the supply of polyunsaturated fatty acids in a Ca salt form in 4 experiments to improve reproductive performance of lactating dairy cows (Bruno et al., 2004; Cerri et al., 2004; Juchem et al., 2004a, Juchem et al., 2004b, Juchem et al., 2007).

### *Effect of Calcium Salts of Fish and Palm Oil on Lactational and Reproductive Performance of Dairy Cows*

Seven-hundred and thirty eight multiparous Holstein cows from a commercial dairy farm were assigned to one of the two different diets in a randomized complete block design (Juchem et al., 2007a; Juchem et al., 2007b). Cows were blocked according to parity and previous lactation milk yield and, within each block, randomly assigned to one of the two treatment diets containing either tallow (1.65 % diet DM) or Ca salts of fish oil and palm oil (CaSFO, 1.8 % diet DM) to provide equal amounts of fatty acids. All cows received a pre-treatment diet during the first 25 d in lactation that consisted of a blend of each fat supplement (0.8 % of tallow + 0.9 % of CaSFO) and remained in the treatment diets until 145 d in lactation. The supplemental fat sources were designed to supply no ω-3 fatty acids as EPA, ($C20:5_{n3}$) and DHA, ($C22:6_{n3}$) when tallow was fed or approximately 10 g of EPA and 10 g of DHA when calcium salts were fed. Based on previous studies (Thatcher et al., 2004), we hypothesized that feeding a calcium salt of ω-3 fatty acids would modulate uterine prostaglandin synthesis and benefit embryo survival in lactating dairy cows.

Yields of 3.5 % fat-corrected milk (**FCM**) and milk fat were not affected by source of fatty acids, but yields of true protein were greater for cows fed tallow. Feeding CaSFO altered the fatty acid composition of milk fat and increased linoleic, EPA, DHA, and *trans*-9 *cis*-11 CLA. Body condition score was smaller for cows fed CaSFO than tallow. Individual cow DM intake at 52 and 100 d postpartum was similar ($P = 0.50$) for CaSFO and tallow and it averaged 26 kg/d. Digestibility of DM in the total tract was higher for cows fed CaSFO, but efficiency of feed utilization (3.5 % FCM/ DMI) was similar for both treatments. No treatment effect was observed on plasma concentrations of glucose and NEFA.

Reproductive performance of dairy cows was generally not affected by treatment (Table 4).

**Table 4.** Reproductive responses at first and second AI for multiparous cows fed calcium salts of fish and palm oils (CaSFO) or tallow (Experiment 1).

| Item | Treatment[2] |  | OR (95% CI)[1] | P value |
|---|---|---|---|---|
|  | CaSFO | Tallow |  |  |
| Cycling,[2] % | 82.2 (343) | 83.2 (356) | 0.98 (0.7 – 1.5) | 0.68 |
| First AI |  |  |  |  |
| Pregnancy rate, % |  |  |  |  |
| D 28 | 35.9 (343) | 40.7 (356) | 0.81 (0.9 – 1.7) | 0.19 |
| D 39 | 31.2 (340) | 35.7 (356) | 0.81 (0.9 – 1.7) | 0.21 |
| D 67 | 26.3 (335) | 32.0 (353) | 0.76 (0.9 – 1.8) | 0.10 |
| Pregnancy loss, % |  |  |  |  |
| D 28 to 39 | 11.7 (120) | 12.4 (145) | 0.96 (0.5 – 2.3) | 0.85 |
| D 39 to 67 | 12.9 (101) | 8.9 (124) | 1.44 (0.3 – 1.5) | 0.34 |
| D 28 to 67 | 23.5 (115) | 20.4 (142) | 1.18 (0.5 – 1.5) | 0.55 |
| Second AI |  |  |  |  |
| Pregnancy rate, % |  |  |  |  |
| D 39 | 19.9 (176) | 23.0 (178) | 0.96 (0.7 – 2.0) | 0.47 |
| D 67 | 18.7 (176) | 17.4 (178) | 1.20 (0.5 – 1.6) | 0.74 |
| Pregnancy loss, % | 5.7 (35) | 24.4 (41) | 0.23 (1.1 – 26.2) | 0.04 |
| Pregnant 140 DIM, % | 49.8 (343) | 54.2 (356) | 0.86 (0.9 – 1.6) | 0.25 |

[1] OR = odds ratio; CI = confidence interval; Tallow was utilized as the reference group.

[2] Number in parenthesis represent the total number of cows.

After analyzing Ca salts samples, we determined that the amount of free fat decreased as the storage time increased. In the first few days after manufacturing, the free fat content was approximately 12 %, and it decreased to less than 6 % after 60 d. Most of the negative effects of CaSFO on embryonic survival were observed for cows inseminated under heat stress, and when the free fatty acid content of the calcium salts was high. Therefore, we suspect that some of the negative effects observed were consequent to the higher availability of free polyunsaturated fatty acids in the rumen.

### *Effect of Calcium Salts of Fish and Palm Oil on Lactational and Reproductive Performance of Dairy Cows under Heat Stress*

Because of the negative effects observed in cows receiving CaSFO under heat stress in the previous experiment and the higher free fat content of the calcium salts fed during the heat stress period, we replicated the same experiment in the summer of 2003 (Bruno et al., 2004).

Dietary treatment and experimental design was the same as described previously. Three-hundred and thirty-one multiparous Holstein cows were assigned to treatments from May 1st to August 31st, and the study was conducted from May to December of 2003.

Group DMI averaged 25.7 kg/d. Yields of milk, 3.5 % FCM, and milk fat were similar for both treatments; but cows fed CaSFO produced milk with lower concentrations of true protein, resulting in lower yields of milk protein. Similarly, concentrations of lactose and solids nonfat were also lower for cows fed CaSFO. Reproductive performance as determined by conception rates, pregnancy loss, days open, and proportion of cows pregnant at the end of the study were similar for both treatments.

### *Effect of Feeding Ca Salts Rich in $\omega$-6 and trans Fatty Acids during Transition on Lactation, Health, and Reproduction of Dairy Cows*

Four-hundred and twenty-three prepartum Holstein dairy cows were assigned to one of the two treatment diets that differed in the supplemental fat source (Juchem et al., 2004a; 2004b). The study started 25 d before the expected calving date, and cows were fed the supplemental fat sources until 70 to 75 d postpartum. The supplemental fat sources were added at equal amounts for both treatments during the pre- (1.9 % of the diet DM; ~250 g/cow/d) and postpartum periods (1.5 % of the diet DM; ~250 g/cow/d) and they consisted of Ca salts of palm oil (**PO**) or calcium salts of linoleic and a blend of monoenoic trans fatty acids (**LTFA**). The calcium salts were the only supplemental fat source fed to cows throughout the entire study.

Yields of milk were similar for both treatments throughout the 70 d experiment. However, because milk fat content decreased beginning at 3 wk postpartum, 3.5 % FCM production was lower for cows fed LTFA. Yields of milk protein were similar between treatments, but milk from cows fed LTFA had higher milk true protein content (2.78 vs 2.74 %; $P < 0.01$). Feeding LTFA altered the fatty acid profile of milk fat as early as 2 wk postpartum. Cows fed LTFA had milk fat with greater concentration of linoleic acid, cis-9 cis-11 CLA, and trans fatty acids. Conception rate at first postpartum insemination was higher for cows fed LTFA than those fed PO (36.1 vs 28.1 %; $P = 0.09$).

### *Effect of Fat Sources Differing in Fatty Acid Profile on Fertilization Rate and Embryo Quality in Lactating Dairy Cows*

Because of the positive effects of LTFA on conception rates of lactating dairy cows, we evaluated the effects of these fatty acids on fertilization rate and embryo quality. One hundred and fifty-four lactating Holstein dairy cows were assigned to one of the two treatment diets as described in the previous experiment. After synchronizing the estrous cycle and ovulation, cows were inseminated and uteri flushed 5 d after AI (Cerri et al., 2004). A total of 161 ovulations were detected in 154 cows, and 14 (18.7 %) and 12 (15.2 %) cows fed PO and LTFA, respectively, experienced double ovulation when inseminated. The number of structures recovered was 45 and 41 for PO and LTFA, respectively; and the recovery rate (number of structures/number of corpora lutea) was similar for both treatment, averaging 53.4 %. Fertilization rate tended to be higher ($P = 0.11$) for cows fed LTA than those fed PO (87.2 vs 73.3 %). Similarly, the number of accessory sperm per structure collected was greater ($P < 0.001$) for cows fed LTA than PO (34.3 vs 21.5), which might partially explain the greater fertilization rate. Cows fed Ca salts of LTFA throughout the transition period and early lactation had a greater ($P = 0.06$) proportion of embryos classified as high quality compared to cows fed Ca salts of PO (73.5 vs 51.5 %). Furthermore, the number of total cells (19.4 vs 14.0; $P = 0.13$) and the proportion of live cells (94.2 vs 85.3 %; $P = 0.09$) tended to be greater for cows fed LTA than those fed PO. Results from this experiment indicate that improvements in fertility of dairy cows at first postpartum AI observed in the previous study might be attributed to improvements in fertilization rate and embryo quality when cows are fed fat sources containing linoleic and monoenoic trans fatty acids during the transition period.

## Source of Se and Reproduction

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 unit (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 to sodium selenite in some studies (Weiss, 2003). Some regions of the US are deficient in Se, particularly the Southeast; whereas other states such as California are mostly adequate in Se.

We have conducted an experiment to evaluate a supplemental source of organic selenium on reproductive and immune responses by dairy cows in FL and CA (Silvestre et al., 2006a; Silvestre et al., 2006b; Rutigliano et al., 2006a; Rutigliano et al., 2006b). Objectives were to evaluate effects of organic Se on health and reproductive performance of dairy cows. Cows were assigned prepartum at approximately 25 d prior to expected day of calving to one of two sources of Se, organic Se (Se-yeast [SY; Sel-Plex®, Alltech) or inorganic sodium Se (sodium selenite, SS) fed at 0.3 ppm (DM basis) until 80 d posptartum. In both sites, cows followed the same study protocol and health was monitored daily throughout the study. Rectal temperature was recorded each morning for 10 d postpartum. In FL, vaginoscopic evaluation of the reproductive tract was performed at 5 and 10 DIM. Cows were evaluated for incidence of RFM, metritis, puerperal metritis, subclinical endometritis by uterine cytology, ketosis, displacement of abomasum, and mastitis. Cows were subjected to one of the two ovulation synchronization programs, a presynchronized Ovsynch protocol, in which presynchronization was performed with 2 $PGF_{2\alpha}$ given 14 d apart, with the last $PGF_{2\alpha}$ injected

12 d prior to initiation of the Ovsynch, or a CIDR-presynchronized Ovsynch protocol, in which the cows received a CIDR for 7 d, $PGF_{2\alpha}$ at CIDR removal, and Ovsynch was initiated 3 d later.

Plasma Se concentrations increased with days postpartum, but source of Se did not influence Se concentrations in cows in CA. However, in FL, feeding SY improved plasma Se concentrations (0.087 vs 0.069 ± .004 µg/ml; $P < 0.01$). Incidence of postpartum diseases did not differ between treatments in both sites, but cows fed SY had smaller incidence of purulent vaginal discharge than those fed SS in FL. Diet altered frequency of multiparous cows detected with > 1 event of fever (rectal temperature > 39.5 ºC; 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 d postpartum 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 9.3 (43/460) vs 17.1 % (75/437) purulent for SY and SS groups, respectively ($P < 0.05$). Feeding organic Se (Se-yeast, Sel-Plex®) improved uterine health and second service PR during summer.

Diet failed to alter first service pregnancy rates in CA and FL, and second service pregnancy rate in CA. However, second service pregnancy rate in FL was greater for cows fed SY than SS [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.

Innate immunity (i.e., neutrophil function) was determined by phagocytic and oxidative burst capacity of neutrophils in whole blood in both sites. In CA, source of supplemental Se had no effect on neutrophil phagocytic or killing activities, but in FL cows fed SY had improved neutrophil function compared with cows fed SS. Percentage of gated neutrophils that phagocytized Escherichia coli and underwent oxidative burst did not differ between dietary groups at 26 d prepartum (44.6 ± 4.6 %). For subsequent samples, a diet by parity by day interaction was detected ($P < 0.05$); namely, SY improved neutrophil function at parturition in multiparous cows (42 ± 6.14 % vs 24.3 ± 7.2 %) and at 7, 14 and 37 d postpartum in primiparous cows (53.9 vs 30.7 %, 58.6 vs 41.9 %, and 53.4 vs 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 d postpartum. In contrast, the multiparous cows did not have a restoration in neutrophil function until between 14 to 21 d postpartum (Figure 1). Organic Se improved phagocytosis and killing activity of neutrophils in both multiparous and primiparous cows. However, the primiparous cows seemed to be more responsive in that SY stimulated neutrophil function throughout 0 to 21 d postpartum; whereas, SY stimulation in multiparous cows was evident on only the day of parturition. In most of our postpartum experiments, we detect distinct differences between primiparous and multiparous cows for a multiplicity of physiological and biochemical responses.

Anti-IgG to Ovalbulmin did not differ between dietary groups at -60 and -22 d postpartum (0.18 ± 0.01 and 0.97 ± 0.04 optical density). Although Anti-IgG to Ovalbulmin concentration did not differ between dietary groups for primiparous cows (1.40 ± 0.08 optical density), concentrations were higher in SY cows at 21 and 42 d postpartum (1.91 ± 0.1 vs 1.24 ± 0.07; 1.44 ± 0.7 vs 0.99 ± 0.07 optical density, 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.

Our findings indicated that feeding SY improved measures of humoral and cellular immunity, uterine health, and second service pregnancy rate in cows in FL; which is known as a Se deficient state. However, in CA source of Se had no impact on health, measures of immune response, and reproductive performance.

## Gossypol and Reproduction

Gossypol was first discovered by Chinese scientists after noticing that no birth of a child happened for more than a decade in a village where people cooked food with cottonseed oil. Since then, innumerous reports in the literature have confirmed the anti-fertility effect of gossypol in mammals. Gossypol disrupts cell membrane metabolism, affects glycolysis, influences mitochondrial and energy metabolism in the cell, and increases fragility of cell membranes; such as in the red blood cells. In fact, erythrocyte fragility has been one of the indicators of potential gossypol toxicosis.

Risco et al. (1992) were one of the first to show that gossypol can be toxic and even kill growing cattle. They fed rations to bull calves with 200, 400 or 800 mg/kg of free gossypol (**FG**) for 120 days. The diets with 400 and 800 mg/kg of FG were considered to be toxic and could potentially cause the death of growing ruminants. Baby calves have little ability to detoxify gossypol and toxicity can be easily induced by feeding cotton products.

The negative effects of gossypol on fertility of ruminants are clear in males. Studies at the University of Florida and at Kansas State University have shown that as little as 8 g/d of FG fed to young bulls reduced sperm quality and sexual activity (Chenoweth et al., 2000; Velasquez-Pereira et al., 1998).

Little information is available on the effects of cottonseed or gossypol on fertility of dairy cows. The female ruminant seems to be relatively insensitive to the anti-fertility effect of gossypol because of rumen detoxification; but *in vitro* data indicate some inhibition of embryonic development and ovarian steroidogenesis (Randel et al., 1992). However, a recent study from the University of Florida (Brocas et al., 1997) observed a distinct effect of gossypol on gametes and embryos of cattle. Feeding cottonseed meal to dairy cows did not affect the number of oocytes collected per cow, cleavage rate after *in vitro* maturation and fertilization, or the proportion of oocytes or embryos that developed to blastocysts. When oocytes were exposed *in vitro* to physiological levels of gossypol (similar to those observed in plasma of cows fed cotton products), cleavage rate or subsequent development was not affected. In contrast, addition of a higher gossypol concentration to the media reduced embryo cleavage rate. Based on these data, the authors concluded that developing embryos are sensitive to high gossypol concentrations and diets that increase plasma gossypol level may impact fertility of cows.

Three experiments were conducted to determine the effects of three concentrations of FG in the diet of Holstein dairy heifers on reproductive parameters by assessing follicle development, luteal function, embryo quality, and embryo development (Coscioni et al., 2003a; Coscioni et al., 2003b; Villaseñor et al., 2003). In all 3 experiments, postpubertal heifers were randomly assigned to one of three isocaloric and isonitrogenous diets differing only in their FG content (from cracked Pima cottonseed): control (C; 0 mg of FG/kg of BW); medium (M; 20 mg of FG/kg of BW); and high (H; 40 mg of FG/kg of BW).

In experiment 1 (Coscioni et al., 2003a), emergence of first and second follicular waves (**FW**) were similar (P>0.15) for C (1.1 and 9.1 d), M (1.0 and 8.9 d), and H (1.9 and 8.8 d). Deviation of the dominant follicle (**DF**) after emergence for the first (C=3.5 vs M=3.5 vs H=3.4; P=0.99) and second FW (C=4.0 vs M=4.6 vs H=4.5; P=0.61) was not affected by treatments. Treatment had no effect on CL growth throughout the estrous cycle (P=0.68). Estrous cycle length (P=0.60), maximum follicle diameter for the DF of the first and second FW (P=0.96, P=0.64), period of follicle dominance for the DF of the first and second FW (P=0.99, P=1.0), and diameter of the ovulatory follicle (P=0.36), were not influenced by dietary gossypol intake.

In experiments 2 and 3 (Coscioni et al., 2003b, Villaseñor et al., 2003), number of structures collected per heifer were, respectively, 9.4, 8.4, and 8.8 for C, M, and H (P=0.88). Number of embryos grades 1 and 2 were similar for all treatments (P=0.87), and averaged 3.5, 3.6, and 3.3 for C, M, and H, respectively. However, heifers receiving the H diet (5.8) had a higher number (P<0.01) of grade 3 and degenerated embryos than those receiving the C (3.6) and M (3.2) diets. Number of unfertilized oocytes were higher (P<0.01) for heifers fed diets containing gossypol than for controls. High dietary gossypol retarded embryo development, and heifers fed C and M had a lower proportion of embryos classified as morula than those fed H (33.3 vs 20.2 vs 47.7 %; P<0.02). Total number of cells in fresh stained embryos were similar for C, M, and H (16.6 vs 14.8 vs 16.4; P=0.40). However number of live cells was higher for C compared to M, but did not differ from H (13.5 vs 10.4 vs 12.2; P=0.05). Similarly, percent of total live cells was higher for C compared to M, but did not differ from H (80.2 vs 72.2 vs 78.1; P=0.02). Hours of development for *in vitro* cultured embryos was reduced by gossypol acetic acid (77.1 vs 64.9 h; P=0.05), but no interaction between heifer diet and culture medium was observed (P=0.50). Number of cells after culture was higher for C than M and H (23.1 vs 20.3 vs 14.6; P<0.01), but culture medium did not influence cell numbers (P=0.50). Results indicate that consumption of up to 40 mg of FG/kg of BW does not influence follicle and CL development in dairy heifers, but feeding a diet with 40 mg of FG/kg of BW increased the number of degenerated embryos and hindered embryo development by day 7 after AI. Furthermore, concentrations of gossypol *in vitro* of 10 µg/ml resulted in delayed embryo development.

Results from these studies indicate that FG intake up to 40 mg/kg of BW/d does not affect

**Table 5**. Effect of type of cottonseed and dietary gossypol on reproductive parameters in dairy cows (Santos et al., 2003).

| Parameter | Treatment |  |  |
|---|---|---|---|
|  | Whole Upland | Blend of whole Upland and cracked Pima | P < |
| Estrous detection rate, % | 51.5 | 57.0 | 0.10 |
| Conception rate at first AI, % | 28.2 | 29.3 | 0.73 |
| Proportion pregnant, % | 79.1 | 70.6 | 0.01 |
| Abortion, % | 3.3 | 7.9 | 0.01 |

follicle and CL development in Holstein heifers, but it can affect fertility by decreasing fertilization and delaying embryo development. Reduced conception and increased pregnancy loss in dairy cattle fed high dietary gossypol, previously demonstrated by our laboratory, might be related to reduced embryo quality and viability. When lactating dairy cows received embryos from heifers fed either no gossypol or > 20 mg of free gossypol/kg of body weight, pregnancy rates were reduced (P = 0.02) in those cows receiving embryos from gossypol-fed heifers (Galvão et al., 2004). In fact, when cows were fed 720 or 950 mg/kg of FG in the diet for the first 180 d in lactation, gossypol consumption increased

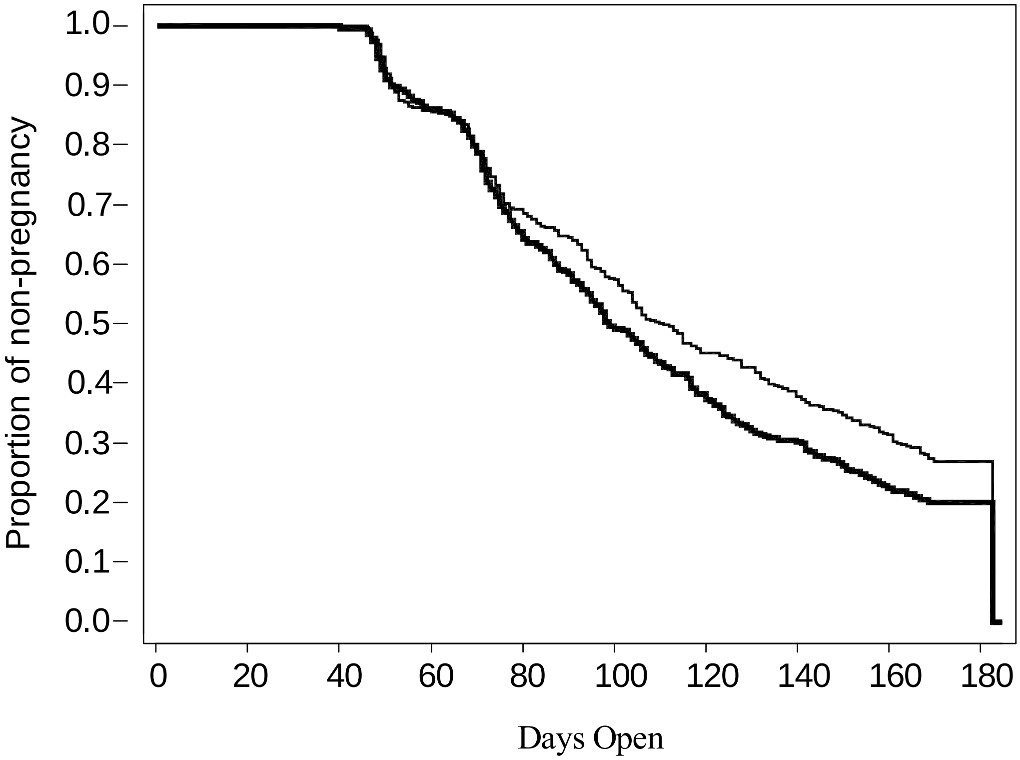

**Figure 4.** Survival curves for the proportion of cows remaining nonpregnant when fed the low gossypol diet with whole Upland cottonseed (thick line; LSM = 111.2; SEM = 2.35) and of cows fed the high gossypol diet with a blend of whole Upland and cracked Pima cottonseed (thin line; LSM = 119.1; SEM = 2.59). The high gossypol diet reduced (P < 0.01) pregnancy rates (Santos et al., 2003).

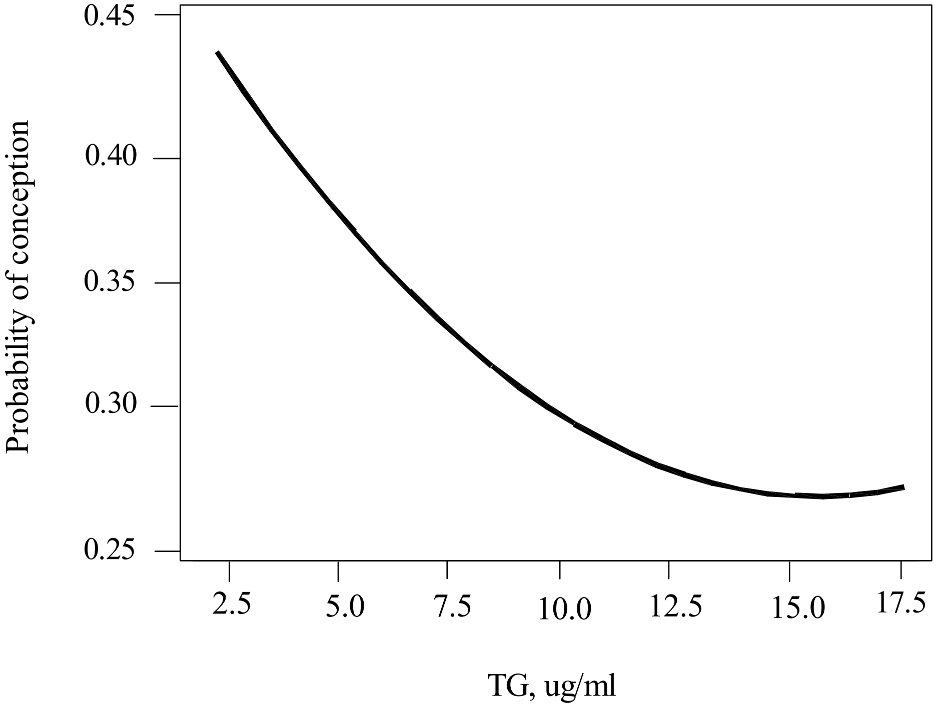

**Figure 5**. Probability of conception after the first postpartum artificial insemination (AI) according to plasma total gossypol (TG) concentrations (µg/ml). Quadratic effect of plasma TG on the probability of conceiving after the first postpartum AI was significant ($P < 0.006$).

from approximately 17 to 24 g/d throughout the course of the study, which resulted in marked differences in plasma gossypol concentrations in the first 150 d postpartum (Santos et al., 2002). These changes in plasma gossypol concentrations reduced conception rates and increased pregnancy losses (Table 5), which resulted in reduced overall pregnancy rates during the entire study (Figure 4; Santos et al., 2003).

Therefore, feeding Holstein heifers diets that supply > 20 mg of FG/kg of BW can suppress embryo development. When these embryos are transferred into lactating cows, maintenance of pregnancy is compromised. Furthermore, when lactating cows are fed diets that result in plasma gossypol concentrations greater than 5 µg/ml, conception rates and maintenance of pregnancy are reduced. In fact, when the impact of plasma gossypol concentrations on conception rates after the first postpartum insemination was evaluated, conception declined in a quadratic manner as plasma gossypol concentrations increased (Figure 5, Santos et al., 2003). Therefore, it is prudent to feed lactating dairy cows amounts of cottonseed that result in low plasma gossypol concentrations.

## Implications

Inadequate intake of nutrients and inadequate body reserves during early lactation are the major factors affecting reproductive performance of dairy cows. Improving energy balance by increasing energy intake through additional NFC or supplemental fat in the diet reduces days to first ovulation and improves conception postpartum. Growing evidence indicates that the delivery of supplemental unsaturated fatty acids to the lower gut for absorption may target reproductive tissues to alter reproductive function and fertility. We presented results from 4 experiments indicating that reproductive performance of dairy cows is influenced by dietary fatty acids when the energy concentration in the ration is maintained. However, better methods to protect these fatty acids are required if precise calculations of the supply of unsaturated lipids are to be utilized in dairy cattle ration formulation to improve fertility. Source of Se might influence health and reproduction of dairy cows, but response seems to be dependent upon the background Se concentrations in dietary ingredients. Lastly, although lactating dairy cows can consume substantial amounts of gossypol with no detrimental effects on health and lactation, when plasma gossypol concentrations increase above 5 µg/ml, embryo development and establishment and maintenance of pregnancy are compromised.

## References

Beam, S.W., and W.R. Butler. 1998. Energy balance, metabolic hormones, and early postpartum follicular development in dairy cows fed prilled lipid. J. Dairy Sci. 81:121-131.

Beam, S.W., and W.R. Butler. 1997. Energy balance and ovarian follicle development prior to first ovulation postpartum in dairy cows receiving three levels of dietary fat. Biol. Reprod. 56:133-142.

Bonczeck, R.R., C.W. Young, J.E. Wheaton, and K.P. Miller. 1988. Responses of somatotropin, insulin, prolactin, and thyroxine to selection for milk yield in Holsteins. J. Dairy Sci. 71:2470-2478.

Brocas, C, R.M. Rivera, F.F. Paula-Lopes, L.R. McDowell, M.C. Calhoun, C.R. Staples, N.S. Wilkinson, A.J. Boning, P.J. Chenoweth, and P.J. Hansen. 1997. Deleterious actions of gossypol on bovine spermatozoa, oocytes, and embryos. Biol. Reprod. 57:901-907.

Bruno, R.G.W., K.N. Galvão, S.O. Juchem, W.W. Thatcher, E.J. DePeters, D. Luchini, and J.E.P. Santos. 2004. Effect of Ca salts of palm and fish oils on lactation and reproduction of dairy cows under heat stress. J. Dairy Sci. 87(Suppl. 1):336 (Abstr.).

Butler, S.T., A.L. Marr, S.H. Pelton, R.P. Radcliff, M.C. Butler, and W.R. Butler. 2003. Insulin restores GH responsiveness during lactation-induced negative energy balance in dairy cattle: effects on expression of IGF-I and GH receptor 1A. J. Endocrinol. 176:205-217.

Butler, W. R. 1998. Review: effect of protein nutrition on ovarian and uterine physiology in dairy cattle. J. Dairy Sci. 81: 2533-2539.

Butler, W.R., and R.D. Smith. 1989. Interrelationships between energy balance and postpartum reproductive function. J. Dairy Sci. 72:767-783.

Canfield, R.W., C.J. Sniffen, and W.R. Butler. 1990. Effects of excess degradable protein on postpartum reproduction and energy balance in dairy cattle. J. Dairy Sci. 73:2342-2349.

Cerri, R.L.A., R.G.S. Bruno, R.C. Chebel, K.N. Galvão, H. Rutgliano, S.O. Juchem, W.W. Thatcher, D. Luchini, and J.E.P. Santos. 2004. Effect of fat sources differing in fatty acid profile on fertilization rate and embryo quality in lactating dairy cows. J. Dairy Sci. 87(Suppl. 1):297 (Abstr.).

Chenoweth, P.J., C.C. Chase, Jr., C.A. Risco, and R.E. Larsen. 2000. Characterization of gossypol-induced sperm abnormalities in bulls. Theriogenology. 53:1193-203.

Coscioni, A.C., M. Villaseňor, K.N. Galvão, J.E.P. Santos, B. Puschner, and L.M.C. Pegoraro. 2003a. Effect of gossypol intake and plasma gossypol concentrations on follicle development and luteal function in dairy heifers. J. Dairy Sci. 86(Suppl. 1):240 (Abstr.).

Coscioni, A.C., M. Villaseňor, K.N. Galvão, R. Chebel, J.E.P. Santos, J.H. Kirk, B. Puschner, and L.M.C. Pegoraro. 2003b. Effect of gossypol intake on plasma and uterine gossypol concentrations and on embryo quality and development in superovulated Holstein dairy heifers. J. Dairy Sci. 86(Suppl. 1):240 (Abstr.).

Duffield, T.F., K.E. Leslie, D. Sandals, K. Lissemore, B.W. McBride, J.H. Lumsden, P. Dick, and R. Bagg. 1999. Effect of a monensin-controlled release capsule on cow health and reproductive performance. J. Dairy Sci. 82:2377-2384.

Ferguson, J.D., D. Sklan, W.V. Chalupa, and D.S. Kronfeld. 1990. Effects of hard fats on in vitro and in vivo rumen fermentation, milk production, and reproduction in dairy cows. J. Dairy Sci. 73:2864-2879.

Galvão, K.N., A.C. Coscioni, S.O. Juchem, M. Villaseñor, and J.E.P. Santos. 2004. Effect of hCG on d 5 of the estrous cycle on luteal function and pregnancy rates (PR) in lactating dairy cows receiving embryos from gossypol fed donor heifers. J. Dairy Sci. 87(Suppl. 1):102 (Abstr.).

Goff, J. P. 2006. Major advances in our understanding of nutritional influences on bovine health. J. Dairy Sci. 89:1292-1301.

Gong, J. G., D. G. Armstrong, G. Baxter, C. O. Hogg, P. C. Garnsworthy, and R. Webb. 2002. The effect of increased dietary intake on superovulatory response to FSH in heifers. Theriogenology. 57:1591-1602.

Grant, R.J., and J.L. Albright. 1995. Feeding behavior and management factors during the transition period in dairy cattle. J. Anim. Sci. 73:2791.

Hileman S.M., L.S. Lubbers, H.T. Jansen, and M.N. Lehman. 1999. Changes in hypothalamic estrogen receptor-containing cell numbers in response to feed restriction in the female lamb. Neuroendocrinology. 69:430-437.

Jordan, E.R., and R.H. Fourdraine. 1993. Characterization of the management practices of the top milk producing herds in the country. J. Dairy Sci. 76:3247-3256.

Juchem, S.O., R.L.A. Cerri, M. Villaseñor, K.N. Galvão, R. Bruno, H.M. Rutigliano, A.C. Coscioni, E.J. DePeters, W.W. Thatcher, D. Luchini, and J.E.P. Santos. 2004a. Effect of feeding Ca salts of palm oil (PO) or of a blend of linoleic and monoenoic trans fatty acids (LTFA) on lactation and health of Holstein cows. J. Dairy Sci. 87(Suppl. 1):95-96 (Abstr.).

Juchem, S.O., R.L.A. Cerri, R. Bruno, K.N. Galvão, E.W. Lemos, M. Villaseñor, A.C. Coscioni, H.M. Rutigliano, W.W. Thatcher, D. Luchini, and J.E.P. Santos. 2004b. Effect of feeding Ca salts of palm oil (PO) or of a blend of linoleic and monoenoic trans fatty acids (LTFA) on uterine involution and reproductive performance in Holstein cows. J. Dairy Sci. 87(Suppl. 1):310 (Abstr.).

Juchem, S.O., J.E.P. Santos, R.L.A. Cerri, R.C. Chebel, K.N. Galvão, E.J. DePeters, W.W. Thatcher, and F. Silvestre. 2007a. Reproduction and uterine prostaglandin secretion of dairy cows fed calcium salts of fish oil. J. Dairy Sci. (submitted March 2007).

Juchem, S.O., J.E.P. Santos, R.L.A. Cerri, R.C. Chebel, K.N. Galvão, R. Bruno, E.J. DePeters, T. Scott, W.W. Thatcher, and D. Luchini. 2007b. Effect of calcium salts of fish and palm oils on lactational performance of Holstein cows. Anim. Feed Sci. Technol. *In press.*

Kendrick, K.W., T.L. Bailey, A.S. Garst, A.W. Pryor, A. Ahmadzadeh, R.M. Akers, W.E. Eyestone, R.E. Pearson, and F.C. Gwazdauskas. 1999. Effects of energy balance on hormones, ovarian activity, and recovered oocytes in lactating Holstein cows using transvaginal follicular aspiration. J. Dairy Sci. 82:1731-1740.

Lundy, III, F.P., E. Block, W.C. Bridges, Jr., J.A. Bertrand, and T.C. Jenkins. 2004. Ruminal biohydrogenation in Holstein cows fed soybean fatty acids as amides or calcium salts. J. Dairy Sci. 87:1038-1046.

Loeffler, S. H., M. J. de Vries, and Y. H. Schukken. 1999. The effects of time of disease occurrence, milk yield, and body condition on fertility of dairy cows. J. Dairy Sci. 82:2589-2604.

Nebel, R.L., and M.L. McGilliard. 1993. Interactions of high milk yield and reproductive performance in dairy cows. J. Dairy Sci. 76:3257-3268.

Radcliff, R.P., B.L. McCormack, B.A. Crooker, and M.C. Lucy. 2003. Plasma hormones and expression of growth hormone receptor and insulin-like growth factor-I mRNA in hepatic tissue of periparturient dairy cows. J. Dairy Sci. 86:920-3926.

Randel R.D., C.C. Chase Jr., and S.J. Wyse. 1992. Effects of gossypol and cottonseed products on reproduction of mammals. J. Anim. Sci. 70:1628-1638.

Rhoads, R.P., J.W. Kim, B.J. Leury, L.H. Baumgard, N. Segoale, S.J. Frank, D.E. Bauman, and Y.R. Boisclair. 2004. Insulin increases the abundance of the growth hormone receptor in liver and adipose tissue of periparturient dairy cows. J. Nutr. 134:1020-1027.

Risco, C.A., C.A. Holmberg, and A. Kutches. 1992. Effect of graded concentrations of gossypol on calf performance: toxicological and pathological considerations. J. Dairy Sci. 75:2787-2798.

Rutigliano, H.E., R.L.A. Cerri, F.S. Lima, L.F. Vettorato, D.B. Araujo, J. Hillegass, W.W. Thatcher, and J.E.P. Santos. 2006a. Effects of source of supplemental Se on health and immune status of periparturient dairy cows. J. Dairy Sci. 89(Suppl. 1):165 (Abstr.).

Rutigliano, H.M., F.S. Lima, R.L.A. Cerri, L.F. Greco, J.M. Vilela, V. Magalhaes, J. Hillegass, W.W. Thatcher, and J.E.P. Santos. 2006b. Effects of source of supplemental Se and method of presynchronization on reproduction and lactation of dairy cows. J. Dairy Sci. 89(Suppl. 1):151 Abstr.).

Santos, J.E.P., S.O. Juchem, R.L.A. Cerri, K.N. Galvão, R.C. Chebel, W.W. Thatcher, C. Dei, and C. Bilby. 2004a. Effect of bST and reproductive management on reproductive and lactational performance of Holstein dairy cows. J. Dairy Sci. 87:68-881.

Santos, J.E.P., W.W. Thatcher, R.C. Chebel, R.L.A. Cerri, and K.N. Galvão. 2004b. The effect of embryonic death rates in cattle on the efficacy of estrous synchronization programs. Anim. Reprod. Sci. 82-83C:513-535.

Santos, J.E.P., M. Villaseňor, E.J. DePeters, P.H. Robinson, and C.H. Holmberg. 2003. Type of cottonseed and gossypol in diets of lactating dairy cows: Plasma gossypol, reproduction, and health. J. Dairy Sci. 86:892-905.

Santos, J.E.P., M. Villaseñor, E.J. DePeters, P.H. Robinson, and B.C. Baldwin. 2002. Effect of type of cottonseed and gossypol intake on plasma gossypol and performance of lactating Holstein dairy cows. J. Dairy Sci. 85:1491-1501.

Santos, J.E.P., J.T. Huber, C.B. Theurer, C.M. Nussio, L.G. Nussio, M. Tarazon, and D. Fish. 2000. Effects of grain processing and bovine somatotropin on metabolism and ovarian activity of dairy cows during early lactation. J. Dairy Sci. 83:1004-1015.

Staples, C.R., J.M. Burke, and W.W. Thatcher. 1998. Influence of supplemental fats on reproductive tissues and performance of lactating cows. J. Dairy Sci. 81:856-871.

Stevenson, J.S. 2001. Reproductive management of dairy cows in high milk-producing herds. J. Dairy Sci. 84(E. Suppl.):E128-E143.

Silvestre, F.T., D.T. Silvestre, C. Crawford, J.E.P. Santos, C.R. Staples, and W.W. Thatcher. 2006a. Effect of selenium (Se) source on innate and adaptive immunity of periparturient dairy cows. Biol. Repro. 39th Annual Meeting. Special Issue. p 132.

Silvestre, F.T., D.T. Silvestre, J.E.P. Santos, C. Risco, C.R. Staples, and W.W. Thatcher. 2006b. Effects of selenium (Se) sources on dairy cows. J. Anim. Sci. 89 (Suppl. 1):52.

Sumida, C., R. Graber, and E. Nunez. 1993. Role of fatty acids in signal transduction: modulators and messengers. Prost. Leukot. Essent. Fatty Acids 48:117-122.

Thatcher, W.W., T. Bilby, C.R. Staples, L. MacLaren, and J. Santos. 2004. Effects of polyunsaturated fatty acids on reproductive processes in dairy cattle. *In:* Proc. Southwest Nutrition & Management Conference, Bioproducts, Inc. Pre-Conference Symposium. Phoenix, AZ, February 26.

Theurer C.B., J.T. Huber, A. Delgado-Elorduy, and R. Wanderley. 1999. Invited review: summary of steam-flaking corn or sorghum grain for lactating dairy cows. J. Dairy Sci. 82:1950-1959.

Vasconcelos, J.L.M., S. Sangsritavong, S.J. Tsai, and M.C. Wiltbank. 2003. Acute reduction in serum progesterone concentrations after feed intake in dairy cows. Theriogenology. 60:795-807.

Velasquez-Pereira, J., P.J. Chenoweth, L.R. McDowell, C.A. Risco, C.A. Staples, D. Prichard, F.G. Martin, M.C. Calhoun, S.N. Williams, and N.S. Wilkinson. 1998. Reproductive effects of feeding gossypol and vitamin E to bulls. J. Anim Sci. 76:2894-2904.

Villa-Godoy, A., T.L. Hughes, R.S. Emery, T.L. Chaplin, and R.L. Fogwell. 1988. Association between energy balance and luteal function in lactating dairy cows. J. Dairy Sci. 71:1063-1069.

Villaseňor, M., A.C. Coscioni, K.N. Galvão, S.O. Juchem, J.E.P. Santos, and B. Puschner. 2003. Effect of gossypol intake on plasma and uterine gossypol concentrations and on embryo development and viability *in vivo* and *in vitro.* J. Dairy Sci. 86(Suppl. 1):240 (Abstr.).

Wade, G.N., and J.J. Jones. 2004. Neuroendocrinology of nutritional infertility. Am. J. Regul. Integr. Comp. Physiol. 287:1277-1296.

Weiss, W. P. 2003. Selenium nutrition of dairy cows: comparing responses to organic and inorganic selenium forms. *In*: Nutritional Biotechnology in the Feed and Food Industries, Alltech Inc., Lexington, KY. pp. 333-343.