

Effects of Dietary Fat Supplementation on Reproduction in Lactating Dairy Cows

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■ Take Home Messages

- Fat content of the diet should be increased above the typical 3% of diet DM for cows in the early postpartum period in order to better meet the energy density recommendations of the NRC. Additional benefits such as increased milk, better management of body condition, and/or improved pregnancy rates may occur.
- Feeding Calcium Salts of Long Chain Fatty Acids (CaLCFA) statistically improved pregnancy or conception rates in a number of studies. Feeding fish meal, possibly due to its unique fatty acids, also appears to hold promise for enhanced fertility.
- Feeding 0.454 kg/day of CaLCFA or fish meal at approximately 3% of diet DM has improved performance.
- Improvement in fertility apart from improved energy status suggests that fat supplementation mediates its positive effect through other physiological mechanisms such as : progesterone concentrations in plasma are enhanced by fat supplementation and may enhance embryo survival; certain Poly Unsaturated Fatty Acids (PUFA), such as linoleic acid and eicosapentanoic acids may reduce uterine secretion of $\text{PGF}_{2\alpha}$.
- Nutritional management programs can be integrated with reproductive management programs.

■ Introduction

Milk production is an energy expensive biological process. Fat is an energy dense nutrient. Therefore feeding supplemental fat to lactating dairy cows has been of interest for many years. Reviews of experiments using supplemental fat for lactating cows began very early in modern animal husbandry, the first appearing in 1907 (Kellner as cited by Sundstol, 1974). Other reviews naturally

have followed (Warner, 1960; Palmquist and Jenkins, 1980; Grummer and Carroll, 1991). These reviewers have concentrated on the influence of fat supplementation on milk production and composition. The effect of dietary lipid on the reproduction of the cow has received much less attention by the scientific community. A significant success story in the dairy industry over the last 35 years is the consistent improvements in milk production, with production per cow tripling. On the other hand, conception rates to artificial insemination have dropped from 66 to 40 - 50% over this same time period. The metabolic demands for increasing milk production represents a classic scientific example of goal-oriented nutrient partitioning. Dietary nutrients and nutrients from body tissues are directed to milk production. During this same time, the uterus, ovary, and hypothalamus/pituitary glands of the cow undergo a process of recovery and rebuilding for the establishment of subsequent pregnancy. From this historic trend, it appears that production may proceed at the temporary expense of reproduction. Unique dietary formulations targeted for the benefit of reproductive performance represents a challenging new area of postpartum research. Fat supplementation is slowly developing a track record of data for reproductive effects. However our understanding of this relationship is not clear as much of the published data comes from studies that had nutritional rather than reproductive objectives. Despite these shortcomings, it is important to examine the data and to try to draw some conclusions.

■ Fat Sources and Their Fate in the Rumen

Many different types of supplemental fat have been fed to lactating cows under experimental conditions. The fatty acid makeup of these fat sources varies widely. Coppock and Wilks (1991) specified the fatty acid profile of many of the commonly used fats (Table 1). Many of the whole oil seeds and unprocessed vegetable oils contain a large proportion of long chain, polyunsaturated fatty acids (PUFA) such as linoleic acid (C18:2). The rendered fats such as tallow and yellow grease contain a large proportion of the monounsaturated fatty acid, oleic acid (C18:1). Tallow can vary greatly in the ratio of saturated to unsaturated fatty acids and in proportion of linoleic acid (range of 2 to 8.5%). Unfortunately, most published studies in which tallow was fed did not report the fatty acid profile of that tallow source. Granular fats such as calcium soaps of fatty acids and prilled fats contain mainly the saturated fats, palmitic and stearic acids.

TABLE 1. Fatty acid composition of supplemental fat sources (Coppock and Wilks, 1991).

Fatty acid	Supplemental fat source							
	Cotton seeds	Soy-bean	Sun-flower	Cod liver oil	Tallow	Yellow grease	Ca salt of palm oil	Prilled fat
	----- % of fatty acids -----							
C12:0	--	--	--	--	0.3	0.7	--	--
C14:0	0.8	0.1	--	2.9	3.4	2.4	2.1	2.2
C16:0	22.7	10.3	5.9	10.6	25.6	18.0	60.5	48.6
C16:1	0.8	0.2	--	9.1	4.9	4.2	0.1	0.5
C18:0	2.3	3.8	4.5	2.3	15.1	12.0	4.7	35.1
C18:1	17.0	22.8	19.5	24.6	40.9	46.8	29.4	12.8
C18:2	51.5	51.0	65.7	3.7	8.5	12.8	3.3	0.7
C18:3	0.2	6.8	--	--	1.0	2.9	--	--
C20:1	--	--	--	10.0	--	--	--	--
C20:5	--	--	--	12.8	--	--	--	--
C22:6	--	--	--	12.3	--	--	--	--

■ Reproductive Effects of Dietary Fat

Ovarian Follicle Development

A variety of fat sources have influenced the size and number of ovarian follicles. Normally follicle development progresses through stages of recruitment, selection, and dominance during each estrous cycle (Thatcher et al., 1996). In the initial days of the estrous cycle, a group of follicles grow up from which a single follicle (called the dominant follicle) continues to grow while the others undergo atresia. At approximately day 10 to 11 of the cycle, this dominant follicle regresses, and this process of recruitment and selection reoccurs. A second dominant follicle arises and ovulates. This is the normal

sequence for cows having a two follicular wave estrous cycle. Three consecutive dominant follicles would arise if cows experienced a three wave estrous cycle. As follicles are recruited and grow in diameter, they increase from a detectable size of 3 mm up to about 15 to 18 mm before regressing or ovulating.

Several studies involving either dairy or beef cows have reported that fat supplementation increased the number of follicles of different class sizes. An increase in the number of smaller follicles may reflect a greater pool of follicles available for subsequent development. A greater number of larger follicles may indicate an altered selection process. In addition to the increased numbers of follicles due to fat supplementation, the size of the dominant follicle has commonly been increased. Larger follicles usually occur under conditions of low concentrations of progesterone and high estradiol 17- β . As will be discussed later, this hormonal profile is just the opposite of what is seen typically when fat is supplemented. The impact of larger ovarian follicles due to the feeding of supplemental fat on conception rate has not been defined. If a follicle becomes too large (> 25 mm), it can become cystic and fail to ovulate. Only one study reported greater occurrence of cystic follicles when fat was fed (Salfer et al., 1995). The size of a healthy follicle may have no relationship to the amount of estradiol it secretes or to the secretion of progesterone by the subsequent CL formed. The mechanism by which dietary fats stimulate ovarian activity has yet to be determined.

■ Conception Rates

What does the scientific literature report concerning supplemental fat effects on reproduction of lactating dairy cows? Of the studies reporting conception or pregnancy rate data (See review of Staples et al., 1998), 11 studies reported an improvement ($P < 0.10$ or ≥ 15 percentage unit difference between means) either in first service conception rate or in the overall conception/pregnancy rate. Mean improvement was 17 percentage units. Feedstuffs stimulatory to reproduction included calcium soaps of long chain fatty acids (**CaLCFA**) ($n = 6$), fish meal ($n = 4$), and tallow ($n = 1$). Besides improved conception rate, one large study ($n = 443$ from five herds in Wisconsin) reported other positive benefits (Scott et al., 1995). A greater proportion of cows fed CaLCFA showed stronger signs of estrus (71.4 vs. 65.6% exhibited standing heat), had cycling ovaries (75.4 vs. 69.5% as determined by rectal palpation done every 2 to 4 wk), and required less exogenous PGF_{2 α} to induce estrus (43.7 vs. 55.7%).

Supplemental fat was sometimes detrimental to conception rate at first service as reported by Carroll et al. (1994), Erickson et al. (1992), and Sklan et al. (1994). In each case, a lowered conception rate was accompanied by a large increase in milk production (range of 2.2 to 4.5 kg/d). High milk production has been linked to lowered fertility in lactating dairy cows but milk production is

inferior to energy status (**ES**) as an influence on reproductive performance. The inclusion of some fat source(s) into the ration within the first 30 d postpartum (**PP**) has resulted in dramatic improvements in the conception/pregnancy rates of over half of the studies cited in Staples et al., 1998.

■ Mechanisms by Which Fats May Improve Fertility

Several hypotheses have been proposed regarding the mechanism(s) by which fat supplementation improves reproductive performance. These include 1) an amelioration of a negative ES thus leading to an earlier return to estrus postpartum and therefore improved fertility, 2) an increase in progesterone production/secretion favorable to improved fertility, and 3) a stimulation or inhibition of PGF_{2α} production/release which influences the persistence of the CL.

Fat Supplementation and Energy Status

Those lactating dairy cows which experience a prolonged and intense negative ES have a delayed resumption of estrous cycles which can increase the number of days open. If fat supplementation can help increase energy intake, then possibly the negative ES can be lessened and estrous cycles start sooner. The more cycles a cow goes through prior to reaching the insemination period, the more likely she will conceive at that first insemination.

Does the scientific literature report improved energy status in the early postpartum period of fat-supplemented dairy cows? Most of the time the answer is no because of a nonsignificant depression in feed intake and/or an increase in milk production resulting in no change in energy status (Beam, 1995; Cummins and Sartin, 1987; Harrison et al., 1995; Jerred et al., 1990; Lucy et al., 1993; Spicer et al., 1993b).

Was an improved ES responsible for the improvement in conception or pregnancy rates reported by studies summarized in Staples et al., 1998? Unfortunately, ES of experimental cows was calculated in only two studies (Carroll et al., 1994; Son et al., 1996). Dairy cows fed tallow at 3% of dietary DM had a greater pregnancy rate despite having a more negative calculated mean net ES from wk 2 to 12 PP compared to controls (Son et al., 1996). Conception rate at first AI decreased from 67 to 33% when fish meal replaced soybean meal in diets fed to cows via Calan gates with no change in mean ES (Carroll et al., 1994). If changing BW or BCS is used as indicators of ES, cows experiencing improved conception rates did so either without an improvement in BW or BCS (Armstrong et al., 1990; Garcia-Bojalil, 1998a,b; Carroll et al., 1994) or in spite of a worsening BW or BCS (Sklan et al., 1991; Burke et al.,

1997). Improved BW did match improved conception rates of fat-fed cows in two studies (Schneider et al., 1988 for Israeli cows; Bruckental et al., 1989). Although there is evidence that the feeding of fat can improve the ES of cattle, an improvement in reproductive performance occurred in several instances apart from an improving ES of the experimental animals. Therefore fat supplementation likely is mediating its positive influence on reproductive performance by other means.

Fat Supplementation and Progesterone

Does the additional supply of fatty acids for metabolism influence the production/secretion of hormones responsible for the growth and recruitment of ovarian follicles and the development of the corpus luteum (CL)? Between 25 and 55% of mammalian embryos die in early gestation. Much of the early embryonic loss occurring in mammals is due to inadequate function of luteal cells (Niswender and Nett, 1994). The main function of the luteal cells in the CL is to synthesize progesterone. Progesterone helps prepare the uterus for implantation of the embryo and also helps maintain pregnancy by providing nourishment to the conceptus. Increased concentrations of plasma progesterone have been associated with improved conception rates of lactating ruminants. Likewise, progesterone concentration prior to insemination has been associated with greater fertility. Therefore if fat supplementation can improve progesterone synthesis, then fertility may be improved. The main precursor for the synthesis of progesterone is cholesterol. The circulating concentration of cholesterol is increased consistently by fat supplementation (Grummer and Carroll, 1991). Cholesterol is an important compound in the formation of chylomicrons during the process of absorption of fat from the small intestine. As fat intake increases, more chylomicrons form and thus more cholesterol likely is needed. As more cholesterol is synthesized, more progesterone is potentially synthesized, especially if cholesterol supply had been limiting.

Not only are concentrations of cholesterol increased in the blood, but follicular fluid concentrations have been increased during fat supplementation. Both soybean oil fed at 5.4% of diet DM (Ryan et al., 1992) and whole cottonseed (Wehrman et al., 1991) increased the concentration of high density lipoprotein cholesterol in the follicular fluid of beef cows. In addition, the fat content of luteal cells was increased by fat supplementation. Electron microscopic examination of slices of CL tissue revealed that lipid occupied a greater percentage of cell area in luteal cells from beef heifers fed CaLCFA from 100 d prepartum to their third estrous cycle postpartum than cows not fed CaLCFA (Hawkins et al., 1995).

Has increased cholesterol concentrations due to fat supplementation resulted in increased progesterone concentrations as well? Dairy cows fed supplemental fat (tallow, CaLCFA, or prilled fatty acids) often have small increases in blood

progesterone concentration (Table 2). From these studies the implication is that additional circulating cholesterol stimulated by the feeding of fat increases the synthesis of progesterone by follicular and luteal cells.

TABLE 2. Concentration of plasma progesterone is increased by feeding supplemental fat to lactating dairy cows.

Reference	Time of measurement	Diet		SEM
		Control	Fat	
		----- ng/ml -----		
Lucy et al., 1993	1 - 12 d of estrous cycle	4.2	5.2	0.8 ^a
Carroll et al., 1990	9 - 15 d of estrous cycle	6.6	7.7	0.3 ^b
Sklan et al., 1991	8 - 20 d of estrous cycle	Greater accumulation ^b		
Spicer et al., 1993	5 - 12 wk PP	4.5	6.0	0.5 ^b
Garcia-Bojalil,1998b	1 - 7 wk PP	Greater accumulation ^b		
Son et al., 1996	2 - 12 wk PP	4.2	4.8	0.3 ^a

^{a,b}Means in the same row with different superscripts are different.

Recent work by Hawkins et al. (1995) suggests that increases in plasma progesterone in cows fed fat-supplemented diets may not be due to increased synthesis but rather reduced clearance of progesterone from circulation. On d 12 to 13 of the third cycle, heifers were ovariectomized. Repeated blood samples taken immediately before and after ovariectomy indicated that the half-life of serum progesterone was increased in heifers fed fat. Authors suggested that prolonged elevated concentrations of progesterone was due to a reduced rate of clearance from the blood rather than an increased rate of secretion.

Effect of Fat Supplementation on Secretion of Prostaglandin F_{2α} (PGF_{2α}) and Fertility

Prostaglandins play an important role in reestablishing estrous cycles both immediately after parturition and thereafter until conception occurs. The uterus releases PGF_{2α} during each estrous cycle to regress each newly formed CL if the cow is not pregnant. This initiates a new estrous cycle. During the period of CL regression, concentrations of PGF_{2α} and progesterone are related inversely. If the cow does conceive, release of PGF_{2α} from the uterus is prevented in order to preserve the CL on the ovary and maintain pregnancy (eg. prevent early embryonic death).

Some PUFA can serve as a substrate for the synthesis of $\text{PGF}_{2\alpha}$. These include cis-linoleic acid (C18:2) that is commonly found in natural fat sources. It can be desaturated and elongated to form arachidonic acid (C20:4) which serves as an immediate precursor for the series two prostaglandins of which $\text{PGF}_{2\alpha}$ is a key member. Key regulatory enzymes for these conversions include delta six desaturase and cyclooxygenase. These same fatty acids also can inhibit prostaglandin synthesis by competitive inhibition with these key enzymes. Linoleic acid has been shown to be an inhibitor of prostaglandin synthesis that is produced by the endometrium in response to the presence of a conceptus in order to preserve the integrity of the conceptus (Danet-Desnoyers, 1993; Thatcher et al., 1994). Other fatty acids besides linoleic acid can play inhibitory roles. Arachidonic, eicosapentaenoic (C20:5), and docosahexanoic (C22:6) acids have been shown to inhibit cyclooxygenase activity (Smith and Marnett, 1991). The amount of particular fatty acids reaching the target tissues likely influence whether prostaglandins synthesis is stimulated or inhibited.

If release of $\text{PGF}_{2\alpha}$ is inhibited, the life span of the CL and the length of the estrous cycle should be prolonged. The life span of the CL was increased in dairy cows abomasally infused with yellow grease (17% linoleic acid) compared to tallow (2% linoleic acid; Oldick, 1997) and in beef cows fed whole cottonseeds (Williams, 1989). In addition, lactating dairy cows fed CaLCFA had more CL and the CL were of greater diameter than cows not fed CaLCFA (Garcia-Bojalil, 1998b).

Fatty acids unique to fish products like menhaden fish meal also can reduce the conversion of arachidonic acid to $\text{PGF}_{2\alpha}$. These include eicosapentaenoic (C20:5) and docosahexanoic (C22:6) acids. They make up 11.6% and 6.6% of the fatty acids in menhaden fish meal (8.4% ether extract; Sealac®, Zapata Haynie, Hammond, LA). Unlike shorter chain fatty acids, these fatty acids appear to escape biohydrogenation in the rumen (Ashes et al., 1992; Palmquist and Kinsey, 1994). Inclusion of fish meal in diets for lactating dairy cows often has improved conception rates (Staples et al., 1998). The repression of $\text{PGF}_{2\alpha}$ by these fatty acids may account for the improved conception rates.

We recently completed an experiment (Mattos R. and W.W. Thatcher, Unpublished observations, 1999) with cycling multiparous cows ($n=31$), averaging 116 days in milk, that were assigned to one of four Menhaden fish meal diets in a study conducted from May to June, 1999. Diets contained 0, 2.6, 5.2 or 7.8% Menhaden fish meal (dry matter basis), and were formulated to contain similar amounts of crude protein and net energy of lactation. Fish oil (0.22% of diet DM) was added to the diet containing 7.8% fish meal to further increase the intake of 20:5n-3 and 22:6n-3. An ovulatory synchronization program was initiated involving the injection of GnRH (Cystorelin, 100 μg) followed 7 d later by administration of $\text{PGF}_{2\alpha}$ (25 mg; im). A second injection of $\text{PGF}_{2\alpha}$ (15 mg; im) was administered 24 h later. At 24 h following the second

injection of $\text{PGF}_{2\alpha}$, hCG (1000 IU, iv; 2000 IU, im) was given to induce ovulation. At 15 d after the injection of hCG (40 to 44 days of feeding Menhaden fish meal), all cows received an injection of oestradiol- 17β (3 mg, iv) at 0900 h and oxytocin (100 units, iv) at 1300h. Blood samples were taken from an indwelling jugular vein catheter at 15 min intervals for 1 h prior to and 4 h after the oxytocin injection to monitor uterine secretion of $\text{PGF}_{2\alpha}$, and differences among the PGFM response curves were analysed by homogeneity of regression analyses. Feeding of Menhaden fish meal caused an inhibition of plasma PGFM concentrations in response to injection of oxytocin (Control versus Menhaden fish meal diets, $P < .025$; Figure 1). Furthermore, there were differences among the fish meal diets (2.6% vs 5.2%, 7.8% not-significant; $5.2\% < 7.8\%$ $P < .05$). The greatest attenuation in PGFM response was observed in the diet with the lowest Menhaden fish meal (2.6%). Clearly if the PGFM response of the 7.8% Menhaden fish meal diet is less than the 5.2% diet then the PGFM response of the 2.6% diet is less than the response to the 5.2% Menhaden fish meal diet (Figure 1). The responses to Menhaden fish meal

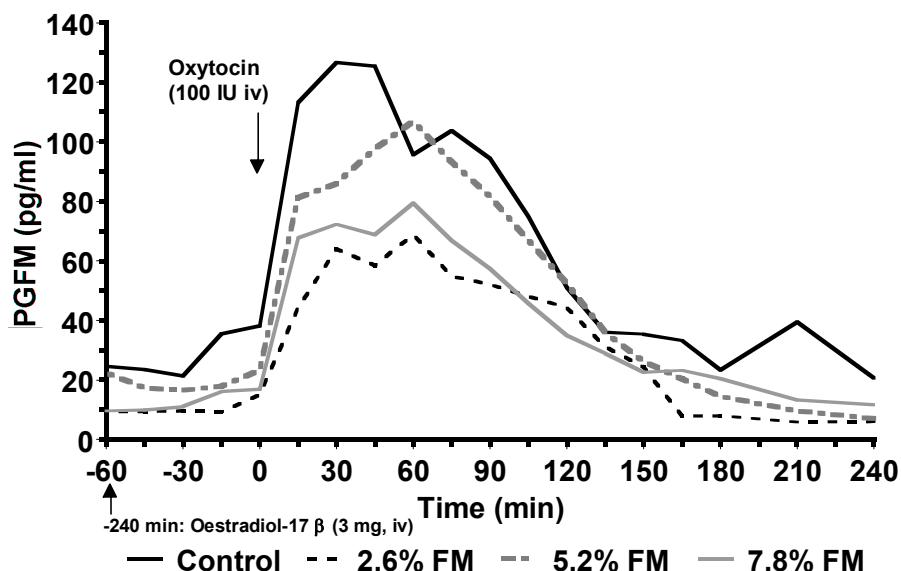


Figure 1. Response of PGFM to sequential injections of estradiol oxytocin in cows fed 0% (control), 2.6%, 5.2% and 7.8% Menhaden meal (dry matter basis); Pooled ± 12.5 pg/ml

were not a classical dose response. This may be attributable partially to variation among cows within a treatment although cow variability is considered

in the statistical analyses. However, it is clear that the early phase response, through 60 minutes following oxytocin, is attenuated for cows fed Menhaden fish meal. This reduction in concentrations of plasma PGFM supports the concept that antiluteolytic mechanisms could be enhanced through the diet. This likely occurred due to an alteration in lipid status of the endometrium. Collectively, these findings suggest that increasing dietary PUFAs can possibly alter the synthesis of prostaglandins of the 2 series by: partial replacement of arachidonic acid in phospholipid pools, which limits the amount of arachidonic acid precursor available for prostaglandin synthesis; decreasing arachidonic acid biosynthesis by inhibition of $\Delta 6$ and $\Delta 5$ desaturase enzymes that are necessary for conversion of linoleic acid to arachidonic acid, by acting as a direct competitive inhibitor with arachidonic acid for PGHS (Prostaglandin H Synthase), and directly decreasing gene expression of PGHS. The potential to selectively alter nutrient availability to enhance the antiluteolytic effect in early pregnancy is a strategy to be combined with various reproductive technologies to enhance embryo survival.

Estrogen helps in the regression of the CL by stimulating the uterus to secrete $\text{PGF}_{2\alpha}$. Estrogen also makes the CL more sensitive to $\text{PGF}_{2\alpha}$ thus obtaining a more complete regression of the CL. Several studies have reported that fat supplementation has lowered concentrations of estradiol- 17β in plasma of dairy cows infused with yellow grease or tallow (Oldick, 1997), in serum of beef cows fed CaLCFA (Hightshoe et al., 1991), and in follicular fluid of beef cows fed soybean oil (Ryan et al., 1992).

A few papers have reported a benefit of feeding fish meal on fertility of lactating dairy cows. Was this improvement in conception due to the reduction of overfeeding degradable protein or to the oils that are unique in fish meal? Diets for lactating cows ($n = 240$) were elevated from 17 to 21% CP by adding either additional soybean meal or fish meal (7.3% of diet DM; 1.5 kg/d) (Bruckental et al., 1989). The BUN values at 3 hours post feeding were elevated for cows fed additional soybean meal, 25.7, 32.6, and 26.9 mg/100 ml for multiparous cows and 24.0, 32.4, and 29.9 mg/100 ml for primiparous cows fed the three diets. By 16 weeks postpartum, cows fed the additional soybean meal had lower conception rates (52%) than cows fed the 17% CP diet (65%). Conception rates were returned to normal (72%) when fish meal replaced the soybean meal. Researchers did not feed a diet of 17% CP that included fish meal so the reproductive impact of fish meal in diets of more typical CP content was not tested. When a situation was established to allow excess dietary protein to negatively impact conception, the inclusion of fish meal rectified the negative situation. Is this ability of fish meal to improve fertility only evident when diets are quite high in CP? Two field studies conducted at Florida testing fish meal (Burke et al., 1997) help support this idea. Fish meal replaced other UIP (Undegradable Intake Protein) feedstuffs so that diets were similar in concentrations of CP and UIP. The concentration of dietary CP (18.1%) and BUN (~ 17 mg/100 ml) of cows at Dairy A were not likely high enough to

potentially suppress conception rates and so the inclusion of fish meal in the diet did not overcome any negative effects of urea. At Dairy B, concentrations of dietary CP (~19.5%) and BUN (~ 21 mg/100 ml) were high enough to depress pregnancy rate (Ferguson et al., 1993; Butler et al., 1998). The cows fed fish meal at this dairy farm did show an improvement in pregnancy rate (41 vs. 32%) without BUN changing.

The mechanism for improved fertility when fish meal is fed may occur at the level of the reproductive tract. Recent work (Butler, 1998) demonstrated that urea can compromise the dynamic relationship of progesterone and $\text{PGF}_{2\alpha}$ at the level of the endometrial cells of the uterus. Endometrial cells produce $\text{PGF}_{2\alpha}$, yet incubation of these cells with progesterone and estradiol suppressed this production in vitro. The presence of urea, however, restimulated the synthesis of $\text{PGF}_{2\alpha}$. In application, when a cow conceives, the endometrial cells do not produce $\text{PGF}_{2\alpha}$, partly because the corpus luteum produces progesterone to suppress $\text{PGF}_{2\alpha}$ and the embryo survives. However if a cow is fed a high CP diet so that uterine urea concentrations are up, the urea may counteract this effect of progesterone so that $\text{PGF}_{2\alpha}$ is released and embryo survival is reduced. Now if the high CP diet contains fish meal, the oils in fish meal may help prevent the synthesis of $\text{PGF}_{2\alpha}$ so that the negative influences of high uterine urea on endometrial cells is overcome. That is, the polyunsaturated fatty acids (PUFA) in fish meal may help suppress $\text{PGF}_{2\alpha}$ release from endometrial cells and counteract the effect of urea to stimulate $\text{PGF}_{2\alpha}$ release, thus early embryonic mortality is reduced. Replacing soybean meal with fish meal may have improved fertility by both lowering circulating urea and providing PUFA to overcome the negative effects of urea on endometrial cells.

However fish meal has improved conception rates even when fish meal increased BUN and nitrogen intake. Irish workers fed ryegrass silage ad libitum along with concentrates at 0.8, 4.0, or 7.2 kg/d to lactating cows (n = 80) starting at parturition (Armstrong et al., 1990). Fish meal was fed at 0.8 kg/d and replaced an equal amount of a barley-soybean meal supplement. The group of cows fed fish meal had a greater conception rate than those not fed fish meal (64 vs. 44%) despite the fact that intake of nitrogen was approximately 57 g/d greater and BUN were greater. The control diet was approximately 18% CP (70% DIP) and that containing the fish meal was 20.4% CP (63% DIP) (calculated from data provided in paper).

■ Interaction of Fat Supplementation and Elevated Crude Protein Intake on Reproduction

Postpartum changes in uterine regression, restoration of ovarian activity and increased milk production are accompanied by dramatically changing energy

and protein states of the animal. In fact, changes in these nutritional conditions have been shown to influence physiological changes associated with reproduction. If more crude protein (CP) is fed than can be utilized by the cow, urea concentrations in body tissues can be elevated. Feeding of diets containing 19 to 21% CP result in elevated BUN concentrations and frequently in lowered conception rates compared with cows fed 15 to 16% CP diets. Older cows are more likely to be affected negatively by elevated dietary CP than younger cows. Not only has the total CP content of a diet proven important for reproductive performance but also the dietary concentration of degradable intake protein (DIP). Replacing soybean meal with a less ruminally degradable protein feedstuff such as fish meal, corn gluten meal, etc. often alleviated some reproductive inefficiency, including delayed first ovulation, lowered conception rates, and elevated embryonic deaths. In general, feeding of excess protein leading to elevated BUN concentrations resulted in some reduction in reproductive performance of lactating dairy cows.

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. The need to detoxify ammonia by animal tissues can be energetically costly. Feeding 100 g of unutilized CP results in a loss of 0.2 Mcal of energy (Twigge and Van Gils 1998). If 500 to 1000 g of excess protein is consumed, energy costs could be a quite substantial 2 Mcal/d (up to 7% of NEL requirement for maintenance and production of 30 kg of milk). With energy status averaging about -11 Mcal/d during the first three weeks postpartum (Staples et al., 1990), an additional 1 to 2 Mcal/d cost is not small. 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.

To test the effects of intake of energy and DIP on reproductive performance of lactating dairy cows, 45 cows were assigned at calving to 20% CP diets containing either 15.7% or 11.1% DIP and 0 or 2.2% CaLCFA (Megalac^R; Garcia-Bojalil et al., 1998a, Garcia-Bojalil 1998b). Crude protein intake was 1100 g greater than required for milk produced (Nat. Res. Council 1989). Treatments continued through 120 days in milk. Cows fed the highly degradable protein diets had greater BUN values (22.0 vs. 17.3 mg%; $P=.01$). Based upon progesterone concentrations of blood samples taken three times per week, cows fed the 15.7% DIP diets experienced more days to first luteal phase postpartum than cows fed other diets (39 vs. 25 days; $P=.002$). All cows on experiment were synchronized to estrus between days 50 and 57. Cows not cycling prior to synchronization were assigned 50 days to first luteal activity. If cows had not been synchronized, the number of days to first luteal activity likely would have been even greater for cows fed the 15.7% DIP diets. 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 recrudescence of ovarian activity and the anestrus

condition were matched with greater loss of body weight and body condition by these cows. Cows fed 15.7% DIP diets lost more body weight and for a longer period of time compared with cows fed 11.1% DIP diets. The absence of CaLCFA resulted in a 10 kg greater loss in BW of cows fed 15.7% DIP diets. In addition, body condition loss was greater and more prolonged by cows fed the CaLCFA-free, 15.7% DIP diet. 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. Days to first estrous was reduced by 6 days when CaLCFA was fed with 15.7% DIP diets. Accumulated progesterone concentrations throughout the postpartum period are depicted in Figure 2. 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 (interaction among dietary treatments, $P < .0001$). Results indicate that 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. The fact that elevated intakes of protein may exert its effect through increased energy costs to the animal is supported by the work of Elrod and Butler (1993). They demonstrated that feeding excess CP (21 vs. 15% of diet) lowered conception rates of heifers from 82 to 61% when heifers were fed an energy deficient diet (70% of ME requirements).

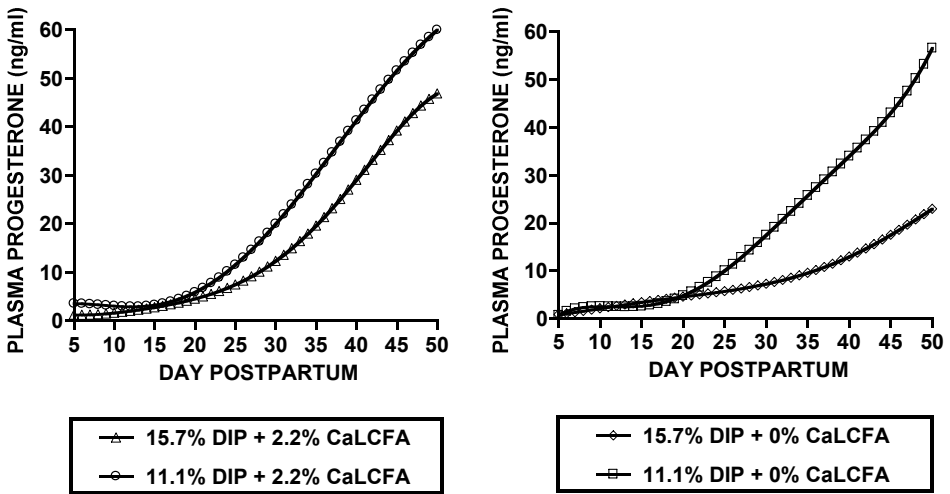


Figure 2. Regression curves of accumulated plasma progesterone concentrations from lactating Holstein cows fed diets containing 11.1% and 15.7% degradable intake protein and/or 0% and 2.2% CaLCFA. The standard error of the mean was 0.9. The interaction of DIP and CaLCFA differed among dietary treatments ($P < .0001$).

■ Nutritional-Reproductive Management

Exciting strategies have been developed to integrate nutritional and reproductive management. The example above demonstrates that the detrimental effects of feeding a high DIP diet on reproduction can be alleviated by supplemental fat feeding (CaLCFA). Fats (concentrated energy sources) can be incorporated into the diet of cows in early postpartum in order to try to minimize the differences between energy intake and energy output. Absorption of total fatty acids by the ruminant is linear up to 1200 g/day (Staples et al., 1993) which is about 6% of DMI. Typical nonfat-supplemented diets contain about 2 to 3% fat. Therefore it appears that there is significant room to increase the use of fat in diets without loss of efficiency. Because fat is an energy dense nutrient, it is natural to suppose that supplemental fat would improve energy status of the cow. However, this has not been the result in many cases. Oftentimes energy status is not affected by feeding fat because either DMI is depressed or milk production is increased (Holter et al., 1992, Skaar et al., 1989, Spicer et al., 1993b). Nevertheless, feeding supplemental fat has proven effective in improving reproductive performance of lactating dairy cows (Staples et al., 1998).

Integration of nutritional and reproductive management programs is essential for successful management of the dairy operation. An example of this is a study involving 186 cows that evaluated effects of whole cotton seed (WCS) feeding and low doses of bST on reproduction during the postpartum period of lactating dairy cows (Adams et al., 1998). The diets were total mixed rations (TMRs) formulated according to the requirements for lactating Holstein cows. Within 24 h after calving, cows received one of two experimental diets ad libitum. All cows that were on bST treatment received 208 mg (0.5 ml) of bST (Posilac®, Protiva Co., St. Louis, MO) subcutaneously every 2 wk starting within 7 d of calving. This dose of bST is 50% of the standard commercial dose rate. Beginning October 1996, apparently healthy cows were assigned randomly to one of four treatments (T) in a 2 x 2 factorial design. Treatments were WCS diet group (15 % of DM) with (+WCS +bST; T3) or without (+WCS - bST; T1) bST and no WCS diet groups with (-WCS +bST; T2) or without bST (-WCS -bST; T0). All cows received PGF_{2α} (25 mg im, Lutalyse®, Pharmacia-Upjohn Co., MI) at 30 ± 3 d postpartum. Blood samples were collected three times a week from calving until initiation of the Ovsynch/TAI protocol. The Ovsynch/TAI protocol was initiated on 65 ± 3 days postpartum, and cows were timed inseminated at day 75. On day 111 postpartum (36 days after insemination) cows were diagnosed for pregnancy by ultrasound examination. If cows were not pregnant the Ovsynch/TAI protocol was repeated and second insemination was made at day 121 postpartum. Thus all cows received their first insemination on day 75 postpartum and both inseminations required no heat detection. Following second service, cows were watched for heats for subsequent services.

Whole cotton seed is a common supplemental fat source that is fed in the Southeastern USA. Since increases in IGF-1 (Insulin Growth Factor-1) appear to be stimulatory to follicle and ovarian development (Thatcher et al., 1996), we were interested in administering bST at a low dose to evaluate ovarian activity and subsequent fertility. Although early ovarian activity may be associated with subsequent increases in fertility, we feel that it is important not to sustain a long period of progesterone exposure during the period of uterine involution. Consequently, we routinely inject PGF_{2α} at day 30 postpartum to regress any CL and reduce progesterone concentrations. This stimulates turnover of CL and ovarian follicles, permits clearance of uterine contents, and reduces exposure to progesterone that may inhibit uterine defense mechanisms and predisposes the uterus to infection.

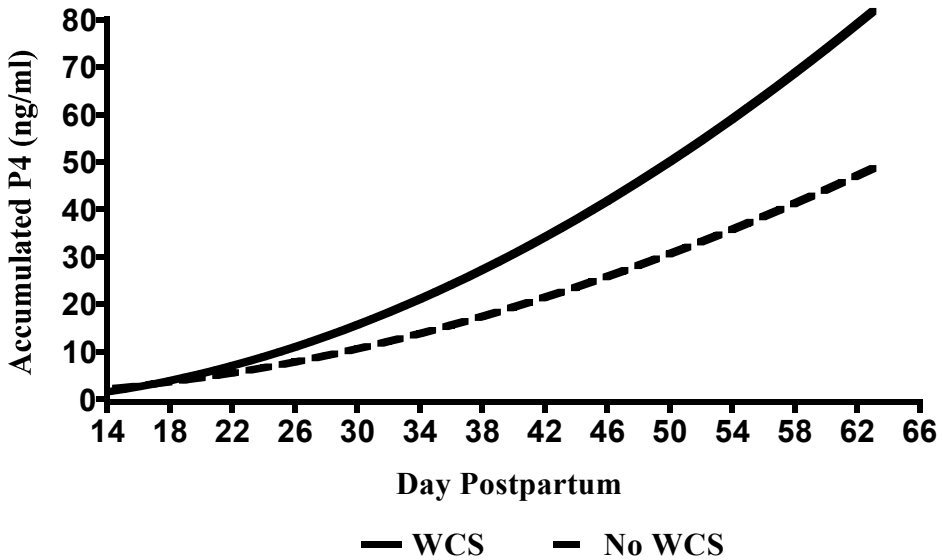


Figure 3. Effect of whole cotton seed on accumulated plasma progesterone ($P < .02$).

Feeding WCS diets clearly stimulated ovarian activity based upon a greater accumulation of progesterone during the postpartum period up to 62 days postpartum when the Ovsynch/TAI program was initiated (Figure 3.). The increase in accumulated progesterone associated with WCS diets was associated with an earlier occurrence of a progesterone rise following $\text{PGF}_{2\alpha}$ injection on day 30 (39.2 < 43.5 days, $P < .05$), and a higher peak progesterone elevation during the rise after $\text{PGF}_{2\alpha}$ injection (11.4 > 9.25 ng/ml, $P < .05$). The increase in ovarian activity as measured by accumulated progesterone concentrations may have been associated with higher plasma concentrations of HDL-cholesterol in the WCS treatment group (107.4 > 83.5 mg/100ml). Although ovarian activity differed significantly between diets with and without WCS, pregnancy rates did not differ following timed inseminations to either the first, second, or accumulative pregnancy rate to first and second service (Table 3). Pregnancy responses demonstrate the advantage of integrating a reproductive management program with nutritional management. Although the diet without WCS was associated with a lower level of ovarian activity, implementation of the Ovsynch/TAI protocol stimulated and controlled ovarian activity such that there was no dietary treatment effect on fertility. Indeed the Ovsynch/TAI protocol permitted a very precise first service for all cows, and the re-synchronized Ovsynch/TAI procedure for cows that did not conceive to first service guaranteed a second service within a 46 day period for all open cows.

Table 3. Least square means for pregnancy rates at day 45 after insemination for cows fed diets of 0 or 15% WCS and injected with 0 or 208 mg of bST at 14 days interval.

Treatment	Total cows	1st TAI (%)	2nd TAI (%)	1st and 2nd TAI (%)
0% WCS; no bST	50	37.1	23.6	51.3
15% WCS; no bST	45	33.6	26.4	51.8
0% WCS; +bST	43	27.1	35.8	51.1
15% WCS; +bST	48	27.3	26.2	46.8

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