Nutrient Influences on Reproduction of Dairy Cows

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Introduction

Milk production per cow has increased consistently over the past decades with a concomitant decrease in conception rate and increases in health problems. Farm profit depends greatly upon these three factors. Although one may conclude that one factor progresses at the expense of the others, it is not necessarily a cause and effect relationship. In fact, milk production has been reported to be greater for cows that ovulate sooner postpartum (Staples et al., 1990) and have fewer days open (Emanuelson and Oltenacu, 1998; Lucy et al., 1992). Farms with greater milk production have been associated with higher incidence of diseases such as clinical mastitis, cystic ovaries, and silent estrus but the increased reporting of treating of these diseases may imply better management on those farms. That is, increased health disorders may suggest closer observation of and response to cow needs by farm personnel. It is not unreasonable to think that progress in all three areas is possible. As nutritionists, an increasing challenge will be to formulate diets based on the latest information that promote milk production as well as good reproductive health. Dietary protein, fat, and phosphorus are nutrients selected to discuss in terms of their potential impact on fertility.

Protein Effects

Increasing the protein concentration of the diet of lactating dairy cows can often increase milk production. Daily milk production increased linearly from 36.6 to 38.6 kg as dietary protein content increased from 13.8 to 23.9% (DM basis; Grings et al., 1991). However efficiency of use of dietary protein for milk production decreased as more protein was fed. Certainly dietary costs and environmental issues regarding nitrogen disposal are additional factors impending upon the desired target crude protein content of the diet. An additional consideration is the potential impact that overfeeding protein may have on reproductive health.

Dietary nitrogen is a source of nonprotein nitrogen, amino acids, and peptides for growth of ruminal microorganisms. The utilization of that dietary nitrogen depends heavily upon the supply of high energy carbohydrates in the diet. Ruminally degradable intake protein (**DIP**) that is consumed in greater amounts than can be utilized by the ruminal microorganisms is absorbed through the rumen wall, travels to the liver where it is converted to urea because of its potential toxicity. The urea leaves the liver via the bloodstream, equilibrates with body tissues, and is concentrated in the kidney for excretion in the urine. Urea also can be produced from ammonia derived from amino acids deaminated by the liver. These amino acids can originate from body tissues, as well as from the diet (ruminally undegradable intake protein, (**UIP**) and ruminal microbes that reach the small intestine. These amino acids in the circulatory system, that are not picked up by the mammary gland or deposited in tissues, are taken up by the liver and metabolized for energy. Therefore urea concentrations can increase in the animal's system if DIP or UIP is consumed in excess of metabolic need or if dietary energy is deficient, preventing full utilization of DIP by ruminal microbes.

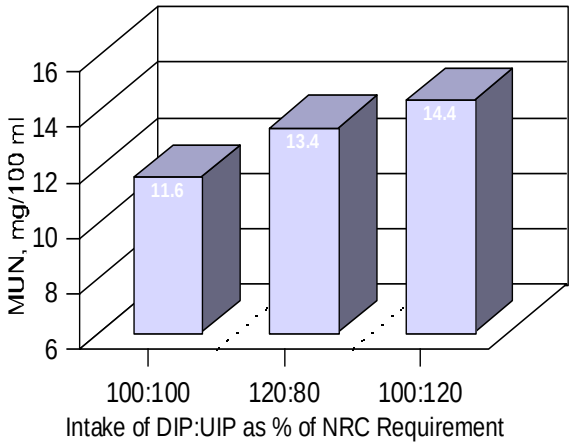
Assessing Urea Status

Accurate measurement of urea in body tissues and fluids has been used to reflect the status of protein intake and the protein-energy relationship within the ruminal environment. Urea has been measured in blood for years. Broderick and Clayton (1997) documented that concentration of urea in whole blood (**BUN**) and blood plasma (PUN) were virtually identical. Milk has been a popular target of late for urea analysis. In order to relate the earlier work to current work, efforts have been made to understand the relationship of blood urea to milk urea. The BUN concentration is often reported to be greater than the milk urea nitrogen (**MUN**) concentration. Using only 8 cows and sampling blood 3 hours after feeding, Oltner and Wiktorsson (1983) calculated the relationship of PUN to MUN from the a.m. milking as MUN = (0.908*PUN) - 0.09 ($r = 0.98$). Roseler et al. (1993) characterized the relationship of MUN to PUN using 15 cows sampled

for BUN 4 hours after feeding by the equation MUN = (0.88 * PUN) - 1.32 (r^2 = 0.79). Using data from 35 trials involving 482 cows fed 106 diets over a 15 year period, Broderick and Clayton (1997) reported the relationship to be: MUN = (0.62 * BUN) + 4.75 (r^2 = 0.84). The BUN values were determined on blood samples collected approximately 4 hours post feeding and MUN were done on milk samples composited from both daily milkings. All three equations have high r^2 values. Differences in equations are likely due to differences in time between sampling of blood and feeding and the sampling of milk and feeding, as significant feed intakes influence urea patterns in body fluids (Gustaffson and Palmquist, 1993). Urea will diffuse in and out of milk throughout the day. Cow A in their study demonstrates the situation that can exist that gives rise to widely differing BUN and MUN values. The average MUN value from two milkings was ~17 mg/100 ml with the one feeding occurring at about 2 h after the first milking and 7 h before the second milking. A blood sample taken 4 hours after feeding analyzed about 21 mg/100 ml, which is quite different from the MUN value. Although BUN and MUN values may be similar if samples are taken at the same time, they may be quite different if blood and milk samples are taken at different times in relation to time fed.

Figure 1. Intake of DIP:UIP Ratio on MUN

(Roseler et al. 1993. JDS 76:525.)



This illustrates the problem of characterizing a cow's *urea status*. Time of sampling (whether it's blood or milk) in relation to feeding, will influence the urea value obtained. Sampling both milkings for urea analysis is likely to provide a truer picture of average urea status than sampling of only one milking (Broderick and Clayton, 1997), but neither one may reflect the peak urea concentration the cow experiences. It may be the peak urea concentrations that indicate the dietary imbalance of protein and energy or the threat to reproductive function. How to obtain and use BUN and MUN values is still a work in progress. In order to more accurately measure the utilization of a diet's protein and energy by a herd, a minimum of four cows must be sampled in order to obtain a MUN value that is accurate for that diet within 2 mg/100 ml (95% confidence). To get to within 1 mg/100 ml of the actual value, 16 cows must be sampled (Broderick and Clayton, 1997).

What are typical BUN and MUN values? Wisconsin workers (Broderick and Clayton, 1997) reported mean MUN and BUN values of 14.8 and 16.2 mg/100 ml. Using their equations, the average dietary concentration of CP was 17.7% and of NE_L was 1.60 Mcal/kg. This ratio of CP to NE_L intake of 110 is greater than that recommended by the Dairy NRC. The NRC (1989) recommendations are approximately 100 to 105 g of CP per Mcal of NE_L intake. This ratio of 100 is thought to be a balance between dietary CP and energy; that is, energy intake is sufficient to allow ruminal microorganisms to efficiently utilize consumed protein. Therefore the average BUN and MUN values reported by Wisconsin workers may be overestimates of optimum values; that is, many cows on these trials were probably overfed protein or underfed energy; likely the former. A target MUN of 13.5 mg/100 ml is predicted to be a mean value for a cow producing 22,000 lb of milk over a 305-d lactation when fed according to the NRC guidelines (Jonker et al., 1998). Swedish workers reported a mean MUN of 13.9 mg/100 ml when cows were fed diets properly balanced for protein and energy (Oltner and Wiktorsson, 1983). The MUN went from 11.6 to 17.8 mg/100 ml when the ratio of CP to NE_L went from 99 to 117 g of CP/Mcal of NE_L (Roseler et al., 1993).

A drawback to relying on a dietary ratio (ie.100 g of CP per Mcal of NE_L) to ensure adequate utilization of dietary nitrogen and the prevention of elevated concentrations of circulating urea, is that the ratio is silent about the ruminal degradability of the dietary nitrogen and energy sources. Their form (UIP vs. DIP, fat vs. carbohydrate, etc.) can greatly influence the degree to which microbes can incorporate nitrogen and therefore affect urea production by the liver. Roseler et al. (1993)

demonstrated how the form of dietary protein can influence MUN concentrations as the ratio of dietary CP to NE_L was maintained. As the dietary concentration of CP (~15%) and NE_L (1.5 Mcal/kg) was kept the same but the DIP was overfed and the UIP underfed, the MUN increased from 11.6 to 13.4 mg/100 ml (Figure 1). The PUN increased from 14.8 to 16.5 mg%. Overfeeding UIP alone (120% of requirement) also elevated MUN to a similar value (14.4 vs. 13.4 mg%) as that of cows overfed DIP (Figure 1). A second drawback to using just the 100:1 ratio as a tool for evaluating diets for their balance of dietary CP and energy is that the ratio is not indicative of the amounts offered the cow in relation to her requirement. Diets that were excessive in both CP and NE_L relative to the cow's milk production resulted in MUN increasing from 13.8 to 15.8 mg/100 ml (Oltner and Wiktorsson, 1983). It is these greater MUN concentrations that may reflect an excessive nitrogen intake that may have a compromising effect on metabolism, specifically reproductive performance.

Urea Status and Reproductive Performance

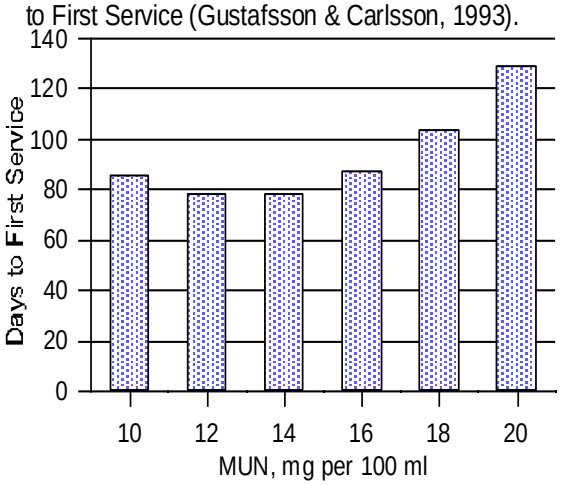
Elevated concentrations of urea in blood or milk have been associated with reduced reproductive performance of lactating dairy cows. Ten studies created differing BUN concentrations by feeding diets of different CP concentrations and looked at resulting fertility (Table 1). In 6 of the 10 studies, conception or pregnancy rates were depressed in the group of animals fed 19 to 21% CP diets. In a seventh study (Folman et al., 1981) 3 of 20 cows in the high CP group which had been inseminated a minimum of four times were culled prior to pregnancy diagnosis but were counted as pregnant in the final analysis; therefore the 44% conception rate was an optimum number and could have been as low as 30%. This study too may have reported a significant depression in fertility if the fate of those 3 cows were known. Although the main effect of diet was not significant, Barton et al. (1996) reported that conception of Jersey cows was negatively affected by consuming a high CP diet (84 vs. 17%) compared to Holstein cows (38 vs. 62%; diet by breed interaction). Although conception rates were similar, Carroll et al. (1988) reported a tendency ($P = 0.17$) for days open (72 vs. 82) and services per conception (1.5 vs. 1.8) to be greater for cows fed the high CP diet. Howard et al. (1987) reported no effect of CP intake on reproductive measurements in spite of elevated BUN concentrations.

Table 1: Conception or pregnancy rates (CR) and BUN of lactating dairy cows or virgin heifers[1] fed diets of moderate or elevated crude protein (CP) concentration.

	Animal No.	13 to 17% CP diets		19 to 21% CP diets	
Reference		*CR, %*	*BUN, mg%*	*CR, %*	*BUN, mg%*
Jordan & Swanson, 1979	30	53[a]	---	40[b]	18
Folman et al., 1981	39	56	9	44	15
Kaim et al., 1983	250	79[a]	9	65[b]	17
Howard et al., 1987	109	87	~15	85	~25
Carroll et al., 1988[2]	57	64	10	56	24
Bruckental et al., 1989	139	65[a]	25	52[b]	32
Canfield et al., 1990[2]	65	48[a]	12	31[b]	19
Elrod and Butler, 1993[1,2]	80	82[a]	~14	61[b]	~24
Barton et al., 1996[2]	64	41	9	44	21
McCormick et al., 1999	119	75[a]	20	53[b]	25
Average		65	14	53	22

[a,b] Means in the same row with different superscripts differ, $P < 0.05$. [2] First service.

Figure 2. Relationship of Bulk Tank MUN and Days to First Service (Gustafsson & Carlsson, 1993).



Several studies fed diets that were isonitrogenous but differed in DIP content (Table 2). Conception rates were depressed or the number of days to first ovulation were greater when cows consumed more DIP, although BUN concentrations did not always differ between treatments.

Others have examined the relationship of BUN to reproductive performance among farms or among cows within a farm. Nine Pennsylvania dairy farms contributed 332 cows to a study examining the relationship between serum urea nitrogen (**SUN**) and conception rate (Ferguson et al., 1993). Most of the herds were fed about a 16.5% CP diet. The SUN concentrations, measured every 2 weeks for each cow, were averaged between 50 and 150 days postpartum. Sixty, 25, and 15% of the cows were classified as having an average SUN value of <14.9, 15 to 19.9, and > 20 mg/100 ml, respectively. The likelihood of conception rate decreased with increasing SUN concentration above 20 mg/100 ml using one type of statistical analysis but another type of analysis indicated a lowered probability of conception when SUN was >14.9 mg/100 ml. Farms with overall lower conception rates were more sensitive to conception failure due to high SUN values compared to farms with overall higher conception rates.

Diets containing from 17.5 to 19% CP were fed to 160 multiparous cows at the Cornell University farm (Butler et al., 1996). Average PUN on the day of first AI (post 60 days of lactation) was 18.9 ± 0.3 mg/100 ml. The pregnancy rate of cows with an above average PUN value was lower compared to cows with a below average PUN value (53 vs. 35%). They repeated the study using 155 cows, only MUN values were determined on the day of AI instead of PUN. The mean MUN value was 22.3 ± 0. 4 mg/100 ml. The mean pregnancy rate of cows having a MUN value of < 19 mg/100 ml was 68% which was greater than the 47% for cows having a MUN > 19 mg/100 ml.

Table 2: Reproductive performance of lactating dairy cows fed diets of high CP content with moderate or elevated concentrations of ruminally degradable intake protein (DIP).

Reference	Diet CP %	DIP, % CP	BUN, mg%	Reproductive measurement
Garcia-Bojalil et al.,	20.5	54	17	25 d to 1st ovulation[a]
1998b	20.7	76	22	39 d to 1st ovulation[b]
	20	60	20	34 d to 1st ovulation[a]
Figueroa et al., 1992	20	65	21	50 d to 1st ovulation[b]
Bruckental et al., 1989	21.6	FM replaces	~28	72% pregnancy rate[a]
Carroll et al., 1994	21.6	some SBM	~33	52% pregnancy rate[b]
	20.8	61	23	71% conception 1st AI[c]
Westwood et al., 1998	20.7	67	23	68% conception 1st AI[c]
	19.3	63	---	lower conception at 1st AI for
	19.3	85	---	high DIP diet

[a,b] Means with different superscripts within an experiment are different, P < 0.05.

[c] A diet by location interaction; cows fed the low DIP diet had greater conception (P = 0.04) when fed using a feed bunk line but lower conception when fed using Calan gate feeders.

Virgin heifers too have demonstrated a lower first service conception rate when PUN values were

elevated by increasing the CP of the diet from 15.5 to 21.8% (Elrod and Butler, 1993). First service conception rates were 82 and 61% for the two groups respectively. A dividing of the heifers into three groups, based upon whether their PUN values were below (<9.9), within (9.9 to 16), or above (> 16 mg/100 ml) one standard deviation from the mean, showed conception rate to decrease most dramatically when PUN exceeded 16 mg/100 ml, 87.5%, 72.5%, and 42.8%, respectively.

Swedish workers measured the MUN concentrations from bulk tank samples collected weekly over a 4 to 5 month period involving 29 herds producing an average of 17,400 lb of FCM (Gustafsson and Carlsson, 1993). The average MUN was 11.2 $\pm$ 0.2 mg/100 ml. The number of days to first service was found to be related to the average MUN concentration as described by the equation $Y = 247 - 73.9x + 8.02x^2$, where Y is days to first service and x is MUN concentration (mM; 10 mg/100 ml = 3.57 mM). A MUN concentration between 10 and 16 mg% was associated with the fewest days to first service (~80 days), with days to first service increasing to 128 days when MUN averaged 20 mg% (Figure 2).

Mechanisms of Action

Several hypotheses have been proposed to explain why overfeeding protein might negatively influence reproductive performance. One that has received some attention is that the uterine environment may be adversely modified by overfeeding protein so that the normal processes leading to fertilization, embryo development, and implantation of the conceptus are hampered. Uterine secretions (17.2 vs. 6.4 mg/100 ml) and plasma (16.8 vs. 4.8 mg/100 ml) contained elevated concentrations of urea when cows (n = 18) were fed diets of 23 vs. 12% CP starting at 40 days postpartum (Jordan et al., 1983). Although authors did not report conception rates, their data support the fact that elevated PUN concentrations are associated with elevated urea concentrations in the fluids of the reproductive tract ($r^2 = 0.80$). Starting dietary treatments at 5 days postpartum, lactating cows (n = 57) fed a diet of 20% CP had greater concentrations of urea in plasma (24.5 vs. 10.0 mg/100 ml) and vaginal mucosa (20.9 vs. 8.2 mg/100 ml) compared to cows fed a 13% CP diet (Carroll et al., 1988). However, first service conception rates of cows differing in vaginal urea concentrations were similar. Associated with the elevated concentrations of uterine urea is a decreasing uterine pH. Uterine pH decreased approximately 0.1 pH units for each 5 mg/100 ml increase in PUN concentration (Elrod et al., 1993; Butler, 1998). In addition to pH changes, ion concentrations (K, Mg, and P) were reported to decrease when CP of diets increased from 12 to 23% (Jordan et al., 1983). Because the uterine environment influences embryo development, these changes may compromise normal fertility processes. In other words, cows may be conceiving equally well when fed high CP diets, but the embryos are not surviving. Early embryonic mortality was suspected in Holstein heifers (n = 80) that were fed 21.8% compared to 15.5% CP diets (Elrod and Bulter, 1993). Of the 16 heifers fed the high CP diet that did not conceive to first service, 7 heifers demonstrated extended interestrus intervals of 26 to 36 days; whereas the 7 control heifers that did not conceive had normal interestrus intervals of 18 to 22 days. Further work in this area is needed to test this hypothesis.

Another hypothesis to explain the negative impact of high concentrations of systemic nitrogen on reproductive performance states that the energy costs of detoxifying large amounts of ammonia to urea may aggravate an existing energy shortage postpartum such that metabolic attention is diverted away from ovarian activity. Energy expended for ammonia detoxification has been reported to be 7.2 kcal of ME/g of excess N (NRC, 1989) and energy losses of 11.95 kcal of NE_L/g of excess N (Twigge and Van Gils, 1988).

Those energy costs of ammonia detoxification are rarely observed in reduced milk production by cows fed the higher CP diets, possibly because of homeorhesis. However, increased loss of body weight (**BW**) is not an unusual response of cows fed greater amounts of unutilized nitrogen. Approximately 50 kg of BW were lost during the first 28 d postpartum by cows fed diets of 15.7% DIP compared with only 21 kg during the first 21 d postpartum by cows fed diets of 11.1% DIP (Garcia-Bojalil et al., 1998a). Likewise, cows fed a 20% CP diet lost more BW in early postpartum compared to cows fed 14 to 15% CP diets (Holtz et al., 1986; Sonderman and Larson, 1989). Average daily gain of BW between nadir of BW and 24 weeks postpartum was 60 g/d less for cows fed 21.6% compared to 17% CP diets containing soybean meal (Bruckental et al., 1989). None of these responses of greater BW loss can be attributed to greater milk production by cows fed more protein. The greater loss of BW by cows fed greater amounts of CP may reflect a greater reliance on body tissue reserves to maintain milk

production because of greater energy expenditure for ammonia detoxification.

That high dietary protein may mediate its negative effects on reproduction through energetic mechanisms is supported by the work of Garcia-Bojalil et al. (1998b). Cows fed a diet of 15.7% DIP without inert fat (Megalac®) had 17 more days to first service, fewer corpora lutea, and less accumulated plasma progesterone the first 50 days postpartum than cows fed diets of 11.1% DIP. The inclusion of inert fat at 2.2% of diet DM into the high DIP diet, shortened the time to first service by 6 days, increased the number of CL, and alleviated plasma progesterone depression caused by feeding high DIP alone (DIP by fat interaction).

Another aspect of energy metabolism at the level of the liver may be partly responsible. Liver function may be compromised by excess ammonia such that important energy transactions are inhibited. A reduction in the capacity of the liver to synthesize glucose from propionate in the presence of excess ammonia has been observed (Overton et al., 1998). In addition, glucose utilization can be impaired by overfeeding protein. A reduction in the rate of glucose utilization and elevated blood glucose was observed in Holstein steers following a pulse dose of urea (0.4 g/kg BW) into the rumen (Spires and Clark, 1979). Plasma glucose was increased and plasma insulin was depressed in cows fed high amounts of degradable protein (Garcia-Bojalil et al., 1998a). Decreased production or utilization of glucose can result in greater use of body reserves for milk production. Impairment of glucose metabolism can negatively affect ovarian function (Staples et al., 1998).

The development of fatty liver in cows can reduce the ability of the liver to detoxify ammonia. Ureagenesis of liver cells in vitro was linearly decreased as the triglyceride content of the liver increased (Strang et al., 1998). Cows fed high CP diets that develop fatty liver may experience greater systemic ammonia concentrations and therefore experience a more adverse effect of ammonia on tissue function.

Lastly, excess dietary CP may inhibit fertility by suppression of the immune system through some nitrogenous compound that reduces the cow's included reduced conception, more days open, or delayed ovulation accompanied, in some cases, by lower plasma progesterone concentrations, greater response to an antigenic stressor (Barton et al., 1996). Primiparous cows recovering from uterine infections experienced more days to first ovulation (39 vs. 18 d) when fed a 20 vs. 13% CP diet (Carroll et al., 1988). Likewise, cows fed a high CP diet (20 vs. 13%) tended to increase days open when they had health problems compared to healthy cows, as determined using survival analysis (Barton et al., 1996).

Summary of Protein Effects

How should milk or blood be collected in order to determine the *urea status* of a group of cows fed a particular diet? Blood and milk that are collected at the same time of day will likely have very similar urea concentrations. However the timing of sampling of body fluids in relation to timing of feeding can greatly influence the urea values obtained. The BUN and MUN values may be quite different if blood and milk samples were taken at different times in relation to time fed. For example, blood sampled 2 hours after feeding will have greater concentration of urea than milk sampled at the parlor 7 hours after feeding. To determine the peak urea concentration, blood or milk should be sampled approximately 2 to 3 hours after a major feeding event. The MUN value determined from a composited milk sample will likely be more representative of the cow's *urea status* during the day than a BUN or a MUN value measured once daily. Approximately 16 cows should be sampled in order to accurately assess their utilization of the diet being fed.

What role can BUN or MUN play in evaluating cow management? Both MUN and BUN can potentially serve as indicators of a diet formulated properly for the correct ratio and amount of protein and energy as well as the correct proportions of DIP and UIP. Based upon the current literature, cows fed a well formulated diet would be expected to have a MUN value between approximately 11.5 and 14 mg/100 ml. Values greater than these suggest that dietary nitrogen is being used inefficiently and an adjustment in dietary protein and/or energy is likely warranted.

Cows fed CP or UIP in significant excess of need have often, but not always, demonstrated reduced reproductive performance. This has

loss or slower gain of body weight, and decreased pH and concentrations of K, Mg, and P in the uterus. Concentrations of BUN of >19 to 20 mg/100 ml for

cows and >16 mg/100 ml for virgin heifers may indicate that the animal is at risk of reduced reproductive performance. Cows that experience health disorders may be at greater risk of reproductive harm when diets containing excessively high CP or DIP are fed. Great attention should be given to practice excellent reproductive management skills on farm and to formulate and feed appropriate amounts of nitrogen in order to minimize potential negative effects of feeding high CP diets on fertility. The mechanism(s) by which excess dietary nitrogen negatively affects fertility are reviewed in the paper and continues to be studied.

Supplemental Fat Feeding

In an effort to support an energy-demanding function (milk production), an energy dense nutrient (fat) is often included in the diet in small amounts (3 to 5% of diet DM). Production of milk is often stimulated by this strategy (Table 3). An additional positive response to fat supplementation has been improved fertility (Staples et al., 1998). Seven studies reported a significant increase or a ≥15% unit increase in conception/pregnancy rate when a ruminally inert fat (n = 6) or tallow (n = 1) was fed at ≤ 3% of diet DM starting very early in lactation. The inclusion of fish meal in the diet also has stimulated fertility (n = 4; Staples et al., 1998). The oils in the fish are hypothesized to be responsible for this positive response, hence their inclusion in the current discussion. What accounts for this improved fertility of cows supplemented with fat?

How Might the Feeding of Additional Fat Improve Fertility? Three main hypotheses have been proposed regarding the mechanism(s) by which fat supplementation may improve reproductive performance. They are listed below as a group and then discussed individually in the following paragraphs.

1. The feeding of additional energy in the form of fat reduces the cow's negative energy status so that she returns to estrus earlier after calving and therefore conceives sooner.
2. Cows fed fat produce or secrete more progesterone, a hormone necessary for the implantation and nutrition of the newly formed embryo.
3. Specific individual long chain fatty acids found in some fats inhibit the production or release of prostaglandin $F_{2\alpha}$ (**$PGF_{2\alpha}$**) by the uterus. This prevents the regression of the corpus luteum **(CL)** on the ovary so that the newly formed embryo survives.

Does fat supplementation improve energy status? Those lactating dairy cows, which experience a prolonged and intense negative energy status, have a delayed resumption of estrous cycles after parturition, which can increase the number of days open. If fat supplementation can help increase energy intake, then possibly the negative energy state can be lessened and estrous cycles resume sooner.

Table 3: Effect of fat on performance factors related to energy status in studies reporting improved fertility due to feeding of tallow (Son et al., 1996) or calcium salts of long chain fatty acids **(CaLCFA)** (all other references).

Reference	DM intake, lb/day	Fat corrected milk, lb/day	Body weight or energy status (ES)
Son et al., 1996	↓ 2.6	↑ 1.3	More negative ES
Sklan et al., 1991	↓ 0.2	↑ 3.7	↑ loss of weight
Scott et al., 1995	NR	↑ 2.9	No change
Garcia-Bojalil et al., 1998	↓ 0.2	↑ 3.5	No change
Ferguson et al, 1990	NR	↑ 2.0	NR
Sklan et al., 1989	NR	↑ 3.1	↑ loss, ↑ gain weight
Schneider et al., 1988	↓ 2.0	↑ 6.4	↑ gain of weight

NR = not reported

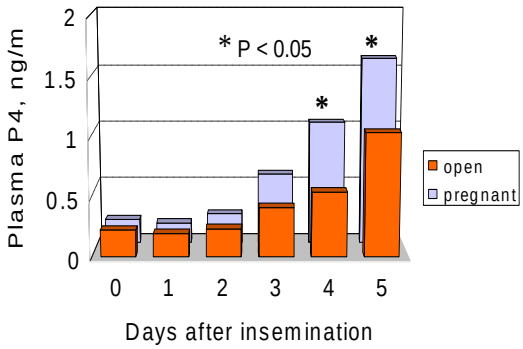
Does the scientific literature report improved energy status in the early postpartum period of fat-supplemented dairy cows? Most of the time the answer is no because of a nonsignificant depression in feed intake and/or an increase in milk production resulting in no change in energy status (Beam and

Butler, 1997; Cummins and Sartin, 1987; Harrison et al., 1995; Jerred et al., 1990; Lucy et al., 1993; Spicer et al., 1993).

Was an improved energy state responsible for the improvement in conception or pregnancy rates reported by studies in Table 3? Unfortunately, very few studies calculated the energy status of the experimental cows. Dairy cows fed tallow at 3% of dietary DM had a greater pregnancy rate despite having a more negative calculated mean net energy status from weeks 2 to 12 postpartum compared to controls (Son et al., 1996). If changing body weight or body condition score is used as an indicator of energy status, cows experiencing improved conception rates did so either without an improvement in body weight or body condition (Scott et al., 1995; Garcia-Bojalil et al., 1998) or in spite of a worsening body weight or body condition (Sklan et al., 1991; Table 3). Improved body weight did match improved conception rates of fat-fed cows in one study (Schneider et al., 1988 for Israeli cows; Table 3). Lactating cows in the Sklan et al. (1989) study lost more weight initially but gained more thereafter when fed fat, so the effect of fat on energy status is a mixed one. Although there is evidence that the feeding of fat can improve the energy status of cattle, an improvement in reproductive performance occurred in several instances apart from an improving energy status of the experimental animals. Therefore fat supplementation likely is improving reproductive performance by other means.

Does fat supplementation increase progesterone secretion/production? Progesterone is called the hormone of pregnancy; that is, progesterone is continually synthesized during pregnancy. The CL formed by the ovulated follicle remains on the ovary throughout pregnancy and is responsible for synthesizing progesterone. Progesterone helps prepare the uterus for implantation of the embryo and also helps maintain pregnancy by providing nourishment to the embryo. Increased concentrations of plasma progesterone have been associated with improved conception rates of lactating ruminants (Figure 3). Likewise,

Figure 3. Plasma Progesterone (P_4) of Lactating Cows After First AI (Butler et al., 1996)



progesterone concentration prior to insemination has been associated with greater fertility. Between 25 and 55% of mammalian embryos die in early gestation. An inadequate functioning of CL cells has been blamed for these losses (Niswender and Nett, 1994). Therefore if fat supplementation can improve progesterone synthesis, then fertility may be improved.

A number of studies have reported that dairy cows fed supplemental fat (tallow, CaLCFA, prilled fatty acids, or whole cottonseeds) had elevated concentrations of blood progesterone (Table 4). What is the connection between fat supplementation and progesterone? Some have suggested that the key is cholesterol. Progesterone is synthesized from cholesterol. The circulating concentration of cholesterol is increased consistently by fat supplementation (Grummer and Carroll, 1991) probably because cholesterol is needed for absorption of dietary fat. In addition, HDL-cholesterol increased in follicular fluid of beef cows fed whole cottonseed (Wehrman et al., 1991). Moallem et al. (1999) found progesterone to be in higher concentration in follicular fluid of dairy cows fed calcium salts of palm oil. 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. However, cholesterol may not be a limiting substrate for the synthesis of progesterone. Elevated blood progesterone concentrations may reflect a slower clearance rate for progesterone rather than a greater rate of synthesis.

Table 4: Concentration of plasma progesterone was 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[a]	5.2[b]	0.8
Carroll et al., 1990	9 - 15 d of estrous cycle	6.6[a]	7.7[b]	0.3
Sklan et al., 1991	8 - 20 d of estrous cycle	Greater accumulation[a,b]		
Spicer et al., 1993	5 – 12 wk postpartum	4.5[a]	6.0[b]	0.5
Garcia-Bojalil et al., 1998	1 – 7 wk postpartum	Greater accumulation[a,b]		
Son et al., 1996	2 – 12 wk postpartum	4.2[a]	4.8[b]	0.3
Adams, 1998	2 – 9 wk postpartum	Greater accumulation[a,b]		

[a,b] Means in the same row with different superscripts are different.

Table 5: Effect of fat supplementation on the diameter of the dominant ovarian follicle of lactating dairy cows.

Reference	Fat source	Experimental diets	
		Control	Fat
		-------- mm -------	
Lucy et al., 1991	CaLCFA	12.4	18.2
Lucy et al., 1990	Soybean oil	7.0	10.2
Lucy et al., 1993	CaLCFA	16.0	18.6
Oldick et al., 1997	Yellow grease	16.9	20.9
Beam and Butler, 1997	Tallow -Yellow grease	11.0	13.5
Staples et al., 2000	Soybean oil, fish oil	14.3	17.1
Average		12.9	16.4

Rather than increased cholesterol being the mediator of increased progesterone, the formation of larger follicles and thus a larger CL by fat feeding may be the cause for greater progesterone. Using ultrasonography, the size of the dominant follicle has been found to be larger in lactating dairy cows receiving supplemental fat (Table 5). A variety of dietary fat sources have had this effect. The size of the dominant follicle was 3.5 mm larger (27% increase) on average in fat-supplemented cows compared to control cows. Does the ovulation of a larger follicle increase the chances of a successful

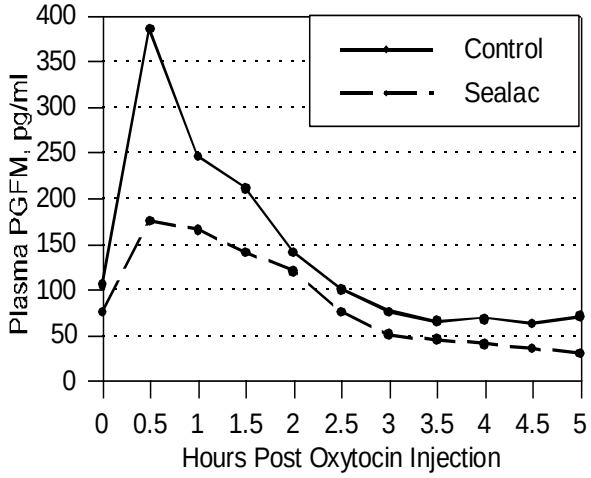
conception? Recent work has indicated that a larger ovulating dominant follicle resulted in a larger CL which was associated with a tendency for greater circulating concentrations of progesterone and greater pregnancy rates (Vasconcelos et al., 1998). In addition, lactating dairy cows fed Megalac® had CL of 5 mm greater diameter than control cows (17.2 vs. 12.2 mm; Garcia-Bojalil et al., 1998).

In summary, fat supplementation can increase the concentration of fat, cholesterol, and progesterone in blood and ovarian structures of ruminants as well as increase the size of ovulating follicles. Improved fertility may result from more progesterone being available to improve embryo survival and health of fat-fed cows.

Does fat supplementation influence the secretion of prostaglandin F-2alpha (PGF-2α)?
Prostaglandin F-2alpha plays an important role in reestablishing estrous cycles both immediately after parturition and thereafter until conception occurs. The uterus releases $PGF_{2\alpha}$ towards the end of 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 $PGF_{2\alpha}$ and progesterone are related inversely. If the cow does conceive, release of $PGF_{2\alpha}$ from the uterus is prevented in order to preserve the CL on the ovary and maintain pregnancy (e.g. prevent early embryonic death). If fats can help suppress the release of $PGF_{2\alpha}$ from the uterus around the time of conception, then the embryo has an increased chance of survival. Some fatty acids found in fish oils as well as linoleic acid, a fatty acid found in high concentrations in cotton and soybean seeds, have suppressed the synthesis of $PGF_{2\alpha}$ in the laboratory by acting on key enzymes (Mattos et al., 2000).

If release of $PGF_{2\alpha}$ is partially inhibited by fat feeding, the life span of the CL and the length of the estrous cycle should be prolonged. The life span of the CL was increased by 1.2 days in dairy cows abomasally infused with yellow grease (17% linoleic acid) compared to tallow (2% linoleic acid; Oldick et al., 1997).

Figure 4. Effect of Fish Meal on Concentration of Plasma Prostaglandin Metabolite (PGFM)



Fatty acids unique to fish products like menhaden fish meal (Sealac®, Zapata Haynie, Hammond, LA) appear to escape biohydrogenation **(BH**) 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 $PGF_{2\alpha}$ by these fatty acids may account for the improved conception rates, as suggested by a study done on a Florida dairy farm. Starting at approximately 25 d postpartum, menhaden fish meal (Sealac®) replaced a mixture of corn gluten meal, fish meal, meat and bone meal, and blood meal in a totally mixed ration for lactating dairy cows, such that the undegradable intake protein content of the diets were similar (Burke et al., 1997). An injection of GnRH was given at 51 ± 3 d postpartum to start the process of recruiting a new follicle. Seven days later, $PGF_{2\alpha}$ was injected to regress the existing CL and help ovulate the new follicle. Blood samples were taken 2 d after injection of $PGF_{2\alpha}$ and measured for progesterone. Cows fed fish meal tended to have greater concentrations of plasma progesterone (1.3 vs. 0.6 ng/ml) and a greater proportion of cows had concentrations > 1 ng/ml compared to cows fed the other protein feedstuffs (29 vs. 4%). Overall pregnancy rate at 120 d PP was increased from 32 to 41%. In a second study at Florida, primiparous lactating dairy cows were fed diets containing menhaden fish meal (Sealac®) at 0 or 5.4% of diet DM. After 25 days of feeding and on day 15 of a synchronized estrous cycle, cows were injected with estradiol (3 mg) and oxytocin (100 IU). Frequent blood sampling indicated that the release of $PGF_{2\alpha}$ by the uterus was reduced in cows fed fish

meal (Figure 4; Thatcher et al., 1997). Thus feeding menhaden fish meal potentially can prolong the life of the CL and enhance embryo survival.

Conclusions of how dietary fats may improve fertility. Based on the experiments done at this time, it appears that dietary fats may increase the size and the life span of the CL. The larger size of the dominant follicle in fat-supplemented cows may result in a larger CL. More CL cells produce more progesterone. Greater progesterone should improve implantation and nutrition of the embryo. In addition, certain fatty acids such as linoleic acid and those found in fish may partially suppress secretion of $PGF_{2\alpha}$ by the uterus at the time of conception so that the CL is not regressed and embryo survival is potentially enhanced.

Delivery of Polyunsaturated Fatty Acids to Small Intestine for Absorption

When linoleic acid is consumed, the rumen microbes convert it to a different fatty acid such as stearic acid. On average, about 60 to 85% of the linoleic acid is converted. The greater the degree of unsaturation of a fatty acid, the greater the extent of BH in the rumen. Yet some fat sources appear to be more resistant to microbial changes than others. Both soybeans and cottonseeds contain about 10% linoleic acid. However the transfer of linoleic acid to the milk was greater when soybeans were fed than when cottonseeds were fed. Table 6 indicates that cows fed soybeans produced a milk that contained twice as much linoleic acid as cows not fed soybeans. On the other hand, cows fed cottonseeds or canola seeds did not produce a milk that had greater linoleic acid content. Feeding fat in the calcium soap form does provide partial protection from BH. The feeding of a calcium salt of palm oil, Megalac®, (~8.5% linoleic acid) resulted in more linoleic acid being incorporated into milk fat than controls. Uptake of more linoleic acid by the mammary gland suggests that other tissues such as the uterus may take up more linoleic acid when soybeans or Megalac® is fed.

The unique long chain fatty acids found in fish are thought to be largely resistant to microbial changes (Ashes et al., 1992; Palmquist and Kinsey, 1994). Therefore they should pass through the rumen with modest changes and be absorbed from the small intestine and stored in uterine tissues to potentially play a positive role at the time of conception.

Extent of BH of unsaturated fatty acids is pH sensitive. It is thought that this increased resistance to the saturation process is due more to a reduction in lipolysis than to a reduction in BH. The relationship of ruminal pH and degree of BH is the reverse for calcium soaps of unsaturated fatty acids; that is, the more acidic the pH, the greater the dissociation of the calcium from the fat leading to increased BH.

Reasons for the inconsistent influence of dietary fats on reproductive performance are open to speculation. Factors influencing ruminal pH such as forage source, forage particle size, feeding management, dietary buffers, etc. could influence BH of PUFA. Source of fat and its fatty acid profile are likely important. Storage and turnover of fatty acid pools in tissues of experimental animals potentially influence fat effects on fertility. The length of time of feeding a fat source prior to their assignment to a newly initiated fat-feeding experiment may influence and complicate interpretation of results. Effects of particular fatty acid intakes by cattle on the phospholipid pools of fatty acids may lead to carry over effects since phospholipids can function as a storage site for essential fatty acids. The duration that fatty acid profiles of phospholipids remain unaltered once supplemental fat intake is eliminated needs to be examined. Carry over effects of phospholipid pools potentially influence synthesis of the prostaglandins. These factors as well as others yet unidentified may make the impact of dietary fat on reproductive tissues and performance less than predictable.

Summary of Fat Feeding

Should fat be included in the diet in hopes of improving reproduction? The fat content of the diet should be increased above the typical 3% of diet DM for cows in the early postpartum period. This enables the diet to better meet the energy density recommendations of the National Research Council (1989). Benefits from this additional fat can be additional milk, better management of body condition, and/or improved pregnancy rates.

What kind of fat is most likely to provide a boost in reproductive performance? Only CaLCFA and fish meal have been evaluated to any extent for reproductive effects. Both feedstuffs have

Table 6: Source of dietary fat influences linoleic acid content of milk fat

	Diet

Reference	Fat source	Control	+ fat source
		- - - - % of total fatty acids in milk - - - -	
Dhiman et al., 1995	16% soybeans	3.2 [a]	6.2 [b]
Tice et al., 1994	20% soybeans	2.9 [a]	5.5 [b]
Holter et al., 1992	15% cottonseeds	4.0	4.2
Harrison et al., 1995	12% cottonseeds	3.4	3.5
Aldrich et al., 1997	11% canola seeds	3.4	3.3
Dhiman et al., 1995	3.4% Megalac®	3.2 [a]	3.5 [b]
Holter et al., 1992	3.2% Megalac®	4.2 [a]	5.3 [b]

improved pregnancy or conception rates in a number of studies. The unique fatty acids in fish meal may be responsible for enhanced fertility. Too few studies have been conducted to determine whether whole oil seeds (cottonseed, soybean or sunflower) have potential to improve reproductive function. If linoleic acid is a limiting fatty acid postruminally, then fat sources containing high concentrations of this fatty acid would be a good choice. Soybeans appear to deliver more linoleic acid to the small intestine than cottonseeds. Roasting of soybeans may be an effective way to reduce BH in the rumen.

How much fat should be fed in hopes of eliciting a positive response? Feeding 1 to 1.5 lb/day of CaLCFA or fish meal at approximately 2 to 3% of dietary DM has proven beneficial.

What is the mechanism by which dietary fat stimulates fertility? In some studies, fat feeding has improved pregnancy rate without improving energy status of the lactating cow. This suggests that fat supplementation mediates its positive effect through other physiological mechanisms. Based on the experiments done at this time, it appears that dietary fats may increase the size and the life span of the CL. The larger size of the dominant follicle in fat-supplemented cows may result in a larger CL. More luteal cells produce more progesterone. Greater progesterone should improve implantation and nutrition of the embryo. In addition, certain fatty acids such as linoleic acid and those found in fish may partially suppress secretion of $PGF_{2\alpha}$ by the uterus at the time of conception so that the CL is not regressed and embryo survival is enhanced.

Phosphorus

A survey of dairy nutritionists in the U.S. found that diets are being formulated at an average P concentration of 0.48% of DM. This is 17 to 30% above the NRC (1989) recommendation for cows past the first 3 weeks postpartum. Reasons for this level of feeding include concerns over the availability of P in feedstuffs and about reduced reproductive performance of cows fed lower levels of dietary P. Wisconsin researchers have conducted several long-term studies using graded dietary concentrations of P and measured both production and reproduction. For 308 d, Holstein cows (n = 26) were fed a diet of either 0.31, 0.40, or 0.49% P by increasing the amount of monosodium phosphate in the diet (Wu et al., 2000). The number of days to first estrus and first AI were greatest for cows fed the 0.40% P diet. The number of services per conception by 206 days in milk increased linearly as dietary P increased. Overall milk production was not different (24,310 lb average) although cows fed the 0.31% P diet produced less milk during the last third of lactation. In a second study, Holstein cows were fed diets of either 0.31 to 0.38% P (n = 14) or 0.44 to 0.48% P (n = 16) over two consecutive lactation cycles (Wu and Satter, 2000). In year one, dietary P concentration did not influence any productive or reproductive measurement. All cows were pregnant by 230 days in milk. In year 2, cows fed the greater P diet, tended to have more days to first estrus and a lower conception rate at first service and at 230 days in milk. Again milk production and composition were unaffected by diet. When scientists combined their study with that of others feeding *low* and *high* P diets to include a total of 785 cows, all measurements of reproductive performance were similar. Authors suggested that dietary P would need to be < 0.25% of DM for reproductive performance to possibly be impaired.

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