

DIETARY INGREDIENTS AND NUTRITIONAL MANAGEMENT IMPACT FERTILITY IN DAIRY CATTLE

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ABSTRACT

Nutrition has an important impact on the reproductive performance of dairy and beef cattle. Energy is the major nutrient required by adult cattle. Inadequate energy intake has a detrimental effect on reproductive activity of female bovine. Cows under negative energy balance have lowered plasma glucose, insulin and IGF-I, reduced peak frequency of LH pulses, lowered plasma progesterone, and impaired ovarian activity. Incidence of postpartum anestrus and reduced fertility is magnified by losses of body condition during the early postpartum period. Inadequate, as well as excessive prepartum BCS extends postpartum anestrus and reduces conception and pregnancy rates during the breeding period. The effects of energy balance on fertility of cattle seem to be associated with metabolic and endocrine changes, as well as with reduced viability of the oocyte to be fertilized. Dietary manipulations that alter the supply of energy to high producing cows may ameliorate their reproductive performance. 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, alters PGF_{2α} secretion by the uterus, affects ovarian dynamics, enhances luteal function, and improves fertility. Although cottonseed feeding and gossypol intake seem 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 milk 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

Relationships between nutrition and reproduction in dairy and beef cattle have been extensively reviewed. The impact of nutrition on fertility of dairy herds may be caused by direct and indirect effects of nutrients on reproductive tissues. 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 when high levels of production are obtained. The peak milk yield occurs 4 to 6 weeks prior to the peak of dry matter intake (DMI) 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 negative energy balance (NEB), the blood concentration of nonesterified fatty acids 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 DMI might affect plasma concentrations of progesterone (Britt, 1994; Vasconcelos et al., 1998; Villa-Godoy et al., 1988; Wiltbank et al., 2000), which may interfere with 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 (Bonczek 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 in 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 proteins (RUP), and optimizing supply of ruminally fermentable carbohydrates during late gestation and early lactation will all maximize milk production and reproduction in dairy cattle.

Excess dietary crude protein (CP) and RDP has been associated with impaired reproductive performance in dairy cattle. Although it is not clear the mechanisms by which protein might impact fertility of dairy cows, when blood (BUN), plasma (PUN) or milk (MUN) levels of urea nitrogen are above 19 to 20 mg/dl, conception rates tend to decrease (Butler, 1998; Butler et al., 1996; Ferguson et al., 1993).

Energy reserves in dairy cattle as determined by body condition score (BCS) impact cyclicity and fertility during the breeding period. Reducing losses

of BCS during the first 60 d postpartum and minimizing the number of animals with a BCS below 2.75 (scale of 1 to 5) during the breeding period will maximize fertility. Although research has proposed improvements in reproductive performance of cows attributed to dietary manipulations, much of the published data originates from studies having nutritional rather than reproductive objectives (Staples et al., 1998a). Thus, conclusions must be taken with caution.

RESUMPTION OF POSTPARTUM CYCLICITY

The onset of lactation creates a huge drain of nutrients in high producing dairy cows, which in many cases antagonizes the resumption of reproductive cycles. Prolonged postpartum anestrus extends the period from calving to first artificial insemination and reduces fertility during the first postpartum service (Stevenson et al., 2001).

It has been well established that cows in early lactation cannot consume enough energy-yielding nutrients to meet the needs of production and maintenance (Butler, 2000; Butler and Smith, 1989). During the last weeks of gestation and early lactation, dairy cows experience a period of NEB (Ferguson, 1991; Santos, 1996). Homeorhetic controls in early lactation assure that body tissue, primarily adipose stores, will be mobilized in support of milk production. Extended periods of NEB challenges productivity. Delayed ovulation has been linked repeatedly with energy status (Beam and Butler, 1997, 1998; Butler and Smith, 1989; Canfield et al., 1990; Staples and Thatcher, 1997). Generally, the first postpartum ovulation in dairy cattle occurs 10 to 14 days after the nadir of NEB (Beam and Butler, 1997, 1998; Butler and Smith, 1989; Canfield et al., 1990; Santos, 1996), and severe weight and BCS losses, caused by inadequate feeding or illnesses, are associated with anestrus in dairy cattle.

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 15% of cows should be in anestrus after 60 days postpartum (Stevenson, 2001). Both estrus expression and conception improve with each estrous cycle through the third estrus postpartum. Thatcher and Wilcox (1973) observed that the greater the number of standing heats prior to breeding, the greater the chance for conception at first breeding (Table 1).

Resumption of ovarian activity in high producing dairy cows is determined by energy status of the animal. Therefore, feeding management that minimizes losses of body condition during the early postpartum and incidence of metabolic

Table 1. Effect of number of heats prior to first artificial insemination (first 60 d postpartum) on conception rates at first breeding.

Number of Heats	Number of Cows	Services per Pregnancy ^a	% Nonreturn to First Service ^a
0	294	2.60	34
1	459	2.58	39
2	362	2.32	44
3	90	2.21	47
4	4	1.75	25

Adapted from Thatcher and Wilcox (1973).

^a Linear effect of number of heats ($P < .05$).

disorders during early lactation should increase the number of cows experiencing a first ovulation during the first 4 to 6 weeks 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 to how much energy they can consume than it is 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 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.

In a recent review, Britt (1994) hypothesized that the effects of NEB on reproduction of dairy cows are associated not only with timing of first postpartum

ovulation, but also with the viability of the oocyte of the ovulatory follicle and the CL resultant of the ovulation of that follicle. According to Britt (1994), the period for a primordial follicle to develop into an ovulatory follicle can take anywhere from 80 to 100 days. 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. Kendrick et al. (1997) 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 BW. 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 higher intrafollicular IGF-I and plasma progesterone and tended to produce more oocytes graded as good.

There is some evidence that cows with higher DM intakes have lower plasma progesterone concentrations (Wiltbank et al., 2000) when under the same energy status. The greater DMI increases blood flow to the liver, which might increase progesterone clearance from the body. The lower plasma progesterone might affect follicular development and oocyte maturation, which may compromise fertility. Although cows with greater DMI have lower progesterone concentration, improving energy status by enhancing DMI increases peripheral concentrations of progesterone (Santos, 1996; Britt, 1994; Villa-Godoy et al., 1988), which benefits reproduction.

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, one might conclude that dairy cows should have at all times high quality palatable diet available to assure maximum DMI. However, DMI is limited during late gestation and early lactation (Santos, 1996), 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 DM (Staples and Thatcher, 1997). 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 in the portal

system, and decreased DMI. Diets with greater amounts of concentrate have higher content of 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 secretagogues. Diets with high ruminally degradable starch increase liver output of glucose (Theurer et al., 1999) and increase plasma concentrations of glucose and insulin (Santos et al., 2000). Improved energy status has been shown to increase IGF-I and insulin in plasma of cattle (Nebel and McGilliard, 1993; Spicer and Echternkamp, 1995). Insulin and IGF-I have direct effects on cultured ovarian cells. Some of the effects include stimulation of granulosa cell mitogenesis and granulosa and luteal cell progesterone production (Spicer and Echternkamp, 1995). Santos et al. (2000) observed that dairy cows fed diets with higher ruminally degradable starch had higher plasma progesterone during the first two postpartum estrous cycles. Feeding more ruminally degradable starch to early lactation dairy cows reduced loss of body condition, tended to improve energy balance and also tended to reduce the period cows experienced NEB (Santos et al., 2000).

Supplemental Fat

Ruminant diets are supplemented with fat primarily to increase their energy concentration and to enhance animal performance. Dairy and beef cattle diets without any supplemental fat contain approximately 2 to 3% long-chain fatty acids (LCFA) from vegetable origin that are predominantly polyunsaturated. A review of the effects of dietary fat on reproductive parameters of dairy cattle has been recently published (Staples et al. 1998a). Table 2 summarizes some of these results.

Fat Sources for Dairy Cows and Their Effects on $PGF_{2\alpha}$ Secretion

Many different types of supplemental fat have been fed to lactating dairy and beef cows. The fatty acid make up of these fat sources varies widely and, usually, vegetable oils are rich in polyunsaturated fatty acids, whereas animal fats are rich in saturated fatty acids. The triacylglycerols in fats and oils in the rumen are hydrolyzed, and unsaturated free fatty acids are biohydrogenated by rumen microorganisms. However, fats that are considered inert have a low pKa, which causes them to be poorly soluble in the rumen under normal pH. The polyunsaturated long chain fatty acids (PUFA) in fish oil are also poorly biohydrogenated in the rumen and, therefore, inert fats and fish oils deliver the greatest proportion of their unsaturated fatty acids to the small intestine.

Table 2. Effect of fat on performance of dairy cows

Treatment ¹	Cows/ TRT	kg/d		Days Open	CR ² 1 st AI	Overall		AI per Conception	
		DMI	Milk			CR	PR ³		
						%			
Control	120	20.6	30.3	116.0	NR ⁴	NR	NR	2.04	
WCS or SB	117	20.2	30.7	113.7	NR	NR	NR	2.02	
Control	629	20.5	31.1	109.0	43.1	74.0	65.8	1.91	
Inert fat	613	20.0	33.5	102.2	44.6	77.8	62.6	1.89	
Control	50	22.3	31.4	88.0	33.0	NR	69.0	1.36	
Tallow	50	22.0	32.3	95.0	44.0	NR	78.0	1.25	
Control	451	22.7	38.2	86.5	49.3	46.5	58.3	1.60	
FM	466	22.5	38.4	83.0	46.3	56.8	64.8	1.46	
Control	1250	21.4	32.7	102.1	43.5	63.3	65.3	1.81	
Fat	1246	21.0	34.0	98.4	45.3	69.0	66.3	1.61	

Adapted from Staples et al. (1998a) (Averages from 20 studies with 40 comparisons)

¹ WCS = whole cottonseed, SB = soybean, FM = fish meal

² CR 1st AI = conception rate at first artificial insemination

³ PR = pregnancy rate

⁴ Not reported

The essential fatty acid linoleic acid is a precursor for prostaglandin F_{2α} synthesis. Provision of inert fat in the diet of early postpartum dairy cows has been shown to enhance follicular growth and resumption of ovarian activity (Beam and Butler, 1997, 1998; Lucy et al., 1991). Lucy et al. (1991) hypothesized that the effects of fat on follicular dynamics were caused by the enhanced PGF_{2α} synthesis when linoleic acid was delivered to the small intestine. They synchronized estrus of postpartum dairy cows that were fed either a control diet or a diet with calcium salts. Feeding supplemental fat early postpartum increased PGF_{2α} secretion as measured by plasma levels of PGF metabolite (PGFM or 13,14-dihydro-15-keto-PGF_{2α}). Similar results were observed for postpartum beef cows fed fat-supplemented diets (Lammoglia et al., 1997; Lammoglia et al., 1996).

In recent years, however, addition of PUFA to diets of mid lactation dairy cows has been shown to inhibit PGF_{2α} secretion (Coelho et al., 1997; Mattos et al., 2000, 2001; Oldick et al., 1997; Staples et al., 1998a, 1998b, 2000; Thatcher et al., 1997). Therefore, the effects of dietary lipids on PGF_{2α} secretion by dairy and beef cows may be associated with the stage of lactation of these cows, as well as the fatty acid profile of the fat source.

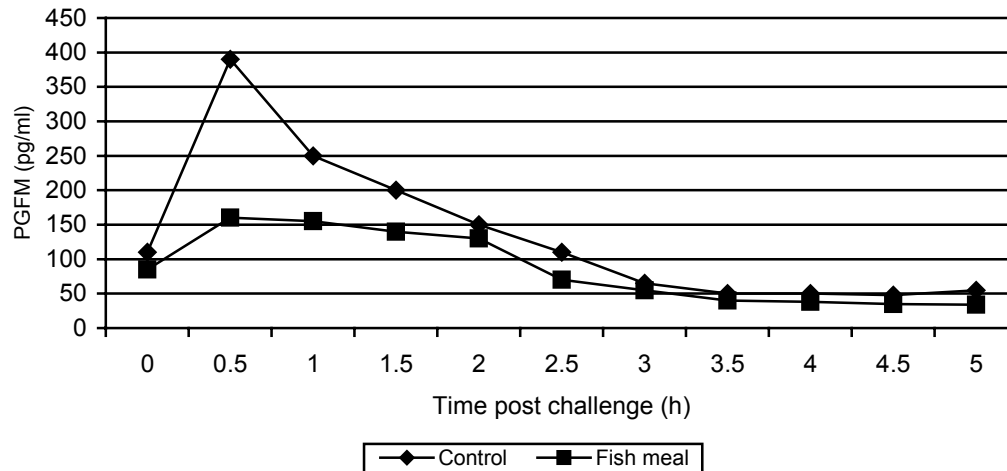
The essential fatty acids of the n-6 (linoleic acid; C18:2) and the n-3 (linolenic acid, C18:3; eicosapentaenoic - EPA, C20:5n-3; and docosahexaenoic - DHA, C22:6n-3) families can suppress the secretion of prostaglandins and other inflammatory mediators. Release of arachidonic acid, the precursor for prostaglandin synthesis, from the lipid pool in cell membranes is mediated by the enzyme phospholipase A₂. Once released from the phospholipid membranes arachidonic acid can now be processed by the prostaglandin synthase enzyme complex, which generates prostaglandins and other inflammatory mediators.

The exact mechanism by which certain fatty acids inhibit PGF_{2α} secretion is not completely clear. Some have suggested that the inhibition mechanism occurs by the competition of PUFA with arachidonic acid for binding to the enzyme cyclooxygenase. Inhibition of arachidonic acid production can also occur at the level of the Δ-6-desaturase enzyme (Staples et al., 1998a). In studies with laboratory animals, fatty acids of the n-3 family reduced PGF_{2α} secretion by inhibiting the Δ-5-desaturase enzyme. Both, the Δ-5-desaturase and the Δ-6-desaturase enzymes play key roles in the synthesis of arachidonic acid from linoleic and linolenic acids.

In studies with dairy cows, feeding PUFA in the diet inhibited synthesis of PGF_{2α} by the endometrium of dairy cows (Coelho et al., 1997; Mattos et al., 2001; Oldick et al., 1997; Staples et al., 2000; Thatcher et al., 1997). Oldick et al. (1997) infused dairy cows in the duodenum with water, glucose, yellow grease, or tallow. Infusion of a highly unsaturated fat source (yellow grease) decreased plasma PGFM in response to oxytocin challenge. Coelho et al. (1997) fed fish meal to 15 primiparous lactating dairy cows averaging 83 days in milk. These cows had their estrous cycle synchronized and were challenged with an injection of 3 mg of estradiol and 100 IU of oxytocin. Blood samples were taken sequentially at 15 min intervals for 1 h prior to and 5 h after oxytocin injection. Plasma PGFM was significantly reduced by feeding fish meal (Figure 1). Infusing yellow grease in the duodenum of dairy cows significantly increased the lifespan of the CL (Oldick et al., 1997).

The potency of different fatty acids to inhibit PGF_{2α} secretion by endometrial cells differs. Recent work from Florida (Mattos et al., 2001) utilizing endometrial cell cultures showed that EPA and DHA resulted in lower secretion of PGF_{2α} in comparison with linolenic acid. In a second experiment, they observed that EPA did not reduce the concentration of mRNA for cyclooxygenase-2, one of the enzymes of the prostaglandin synthase enzyme complex (Mattos et al., 2001). The same group (Staples et al., 2000) observed

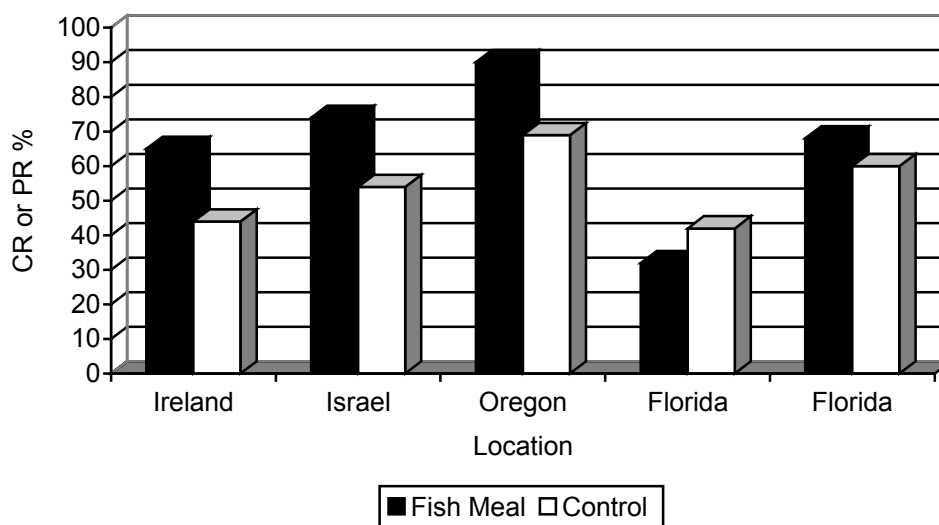
Figure 1. Effect of Fish Meal on PGFM Concentration After Estradiol 17B and Oxytocin Challenge (Coelho et al., 1997)



that feeding EPA and DHA reduced mRNA for prostaglandin endoperoxide synthase-2 relative to feeding linoleic acid. However, no differences were observed between EPA + DHA and the control diet. These data show that PUFA differ in their capacity to inhibit $\text{PGF}_{2\alpha}$ secretion by endometrial cells, and the mechanism probably does not involve gene transcription for synthesis of prostaglandin synthase. Therefore, it is possible that the mechanism by which PUFA inhibit $\text{PGF}_{2\alpha}$ secretion by bovine endometrial cells is mediated by inhibition of desaturase enzymes involved in the synthesis of arachidonic acid. Therefore, supplying fat sources that deliver polyunsaturated fatty acids to the small intestine decreases $\text{PGF}_{2\alpha}$ secretion and increases the lifespan of the CL.

During the first stages of pregnancy, the newly formed embryo signals the cow that it is present in the uterus at about day 16 to 17 of the estrous cycle (Thatcher et al., 1997). This signal prevents the endometrium from releasing $\text{PGF}_{2\alpha}$, which maintains the CL active. Supplying fatty acids that inhibit $\text{PGF}_{2\alpha}$ release by the uterus might improve the mechanism of embryo preservation, which may benefit rates of conception and embryo survival in cattle. Staples et al. (1998b) reviewed several studies in which included fish meal in the diet of dairy cows. Feeding fish meal increased conception or pregnancy rates in 4 of the 5 studies reviewed (Figure 2).

Figure 2. Effect of Fish Meal on Conception or Pregnancy Rates of Dairy Cows (Staples et al., 1998b)



Fat and follicular development

Positive effects of feeding fat on reproduction may occur through stimulation of ovarian follicular growth in association with increased energy balance (Lucy et al., 1991). A variety of fat sources have influenced size and number of ovarian follicles. The effects of feeding fat on follicular population have been observed for both dairy and beef cows at all stages of lactation (Tables 3 and 4). Inert fat (Ca-LCFA) was substituted for corn at 2.2% of diet DM, but energy density of the diet was not changed. Ultrasound scanning of the ovaries demonstrated an increase in the number of medium follicles prior to d 25 postpartum. On d 25, estrous was synchronized and the number of small and large follicles increased for fat-supplemented cows. Other authors (Beam and Butler, 1997, 1998) have observed similar effects of fat supplementation on the follicular population of dairy cows.

The effects of fat upon the number and size of ovarian follicles have been confirmed using postpartum beef cows (Table 4). In four of five studies, cows fed supplemental fat had greater numbers of medium size follicles, and in two of five studies cows had greater numbers of large size follicles. An increase in the number of smaller follicles may reflect a greater pool of follicles available for subsequent development. A greater number of large follicles may indicate an altered recruiting process (Staples and Thatcher, 1997). This effect of fat

Table 3. Effect of supplemental fat on number of follicle size classes from lactating dairy cows

Fat source	Measurement ²	Follicle size class (mm) ¹				Reference
		3 - 5	5 - 9	10-15	> 15	
Prilled fatty acids	4 x wk from d 8 PP to ovulation	NS	NS	NS	NR	Beam and Butler, 1998
Tallow + yellow grease	3 x wk from d 8 to 14 PP	NS	NS	NS	**	Beam and Butler, 1997
Tallow + yellow grease	Daily, during 1 EC	NS	**	NS	NR	Oldick et al., 1997
Ca salts LCFA	Before d 25 PP or at 1 st PP EC	**	**	NS	**	Lucy et al., 1991

** Increase from fat supplementation ($P < .05$); * Increase from fat supplementation ($P < .10$)

¹ NS: not significant; NR: not reported

² PP: postpartum, EC: estrous cycle

supplementation on follicular population is more significant when sources of unsaturated fatty acids are used (Williams and Stanko, 2000).

The impact of larger ovarian follicles due to the feeding of fat-supplemented diets on fertility has not been defined. When supplemental fat reduced DMI early in the postpartum period, size of the largest follicle did not influence ovulation rate (Beam and Butler, 1998). However, the same group observed in a previous study that follicles that ovulated in the first follicular wave postpartum were of greater diameter (Beam and Butler, 1997). Therefore, it is possible that increasing the number and the size of larger follicles by feeding fat can accelerate the interval from calving to first postpartum ovulation, which has been shown to be beneficial to fertility. Such results have been observed for postpartum beef cows (De Fries et al., 1998; Lammoglia et al., 1996, 1997).

Fat and luteal activity

Improved fertility in cattle has been associated with high circulating concentrations of progesterone during the luteal phases prior to and after breeding (Britt, 1994; Britt et al., 1996; Grummer and Carroll, 1988, 1991; Santos et al., 2001c). Addition of fat to dairy and beef diets has consistently been shown to

Table 4. Effect of supplemental fat on number of follicle size classes from beef cows

Fat source	Measurement ²	Follicle size class (mm) ¹				Reference
		3 - 5	5 - 8	> 8.1	Total	
Rice bran	D 15 to 29 PP	**	NS	NS	**	De Fries et al., 1998
	Wk 3 PP	NS	**	**	**	
Rice bran	1 st PP EC	NS	*	NS	NS	Lammoglia et al., 1997
	2 nd PP EC	NS	NS	NS	NS	
Animal tallow	After FSH		**			Thomas and Williams, 1996
Soybean oil	After FSH		**			
Rice bran	Wk 2 and 3 PP	**	**	**	**	Lammoglia et al., 1996
Soybean oil	First 10 d of EC	NS	**	NS	NS	Ryan et al., 1992
	After FSH	NS	NS	NS	NS	

**Increase from fat supplementation ($P < .05$); *Increase from fat supplementation ($P < .10$)

¹ NS: not significant; ² PP: postpartum; EC: estrous cycle

increase plasma cholesterol and cholesterol content in the follicular fluid and in the corpus luteum (Garcia-Bojalil et al., 1998a; Grummer and Carroll, 1988, 1991; Hawkins et al., 1995; Ryan et al., 1992; Staples et al., 1998a; Williams, 1989). The increase in plasma cholesterol in the bovine is due primarily to an increase in high-density lipoprotein (HDL) cholesterol (Grummer and Carroll, 1988, 1991; Hawkins et al., 1995), which is the only lipoprotein found in ovarian tissues (Williams and Stanko, 2000).

Grummer and Carroll (1991) reviewed several studies in which lipid was supplemented to dairy cows. They observed that in all of them plasma cholesterol levels were consistently increased in the treatment diet relative to the control diet. Using more recent data, we observed similar results as those described by Grummer and Carroll (1991) (Figure 3). Cholesterol serves as a precursor for the synthesis of progesterone by ovarian cells and hypercholesterolemia may increase

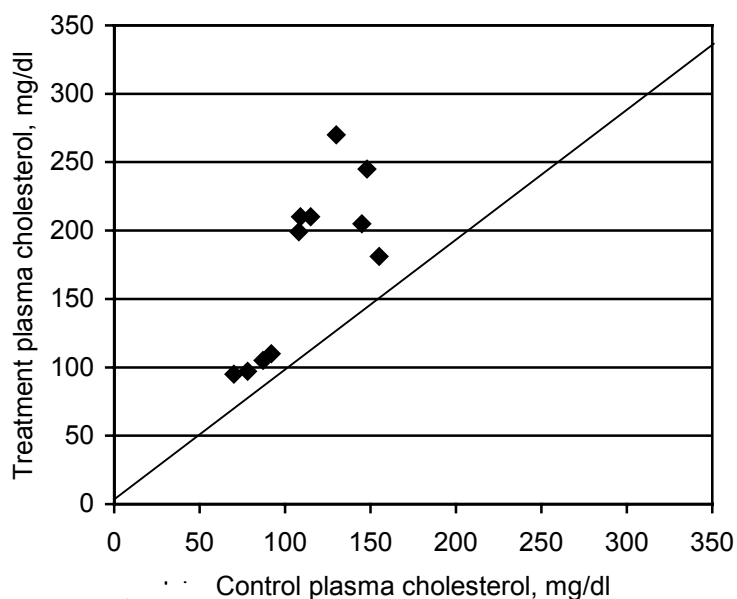


Figure 3. Effect of supplemental fat on plasma cholesterol concentrations. Adapted from Lammoglia et al. (1996); Hawkins et al. (1995); Ryan et al. (1992), and Williams (1989).

steroidogenesis by bovine CL. However, the mechanism seems to be associated with progesterone clearance, but not with its synthesis (Hawkins et al., 1995).

Ruminants fed fat-supplemented diets have been shown to consistently have higher plasma progesterone and these effects are observed for beef and dairy cows. Concentration of progesterone in follicular fluid and luteal tissue of cows is increased in some (Ryan et al., 1992, 1995), but not all studies when fat is supplemented in the diet (Lammoglia et al., 1997).

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 to reduced clearance of progesterone from circulation. These results might explain why dairy and beef cows have increased plasma progesterone, with no effect on CL number or area when fed fat-supplemented diets. Several studies with beef and dairy cows have shown higher plasma progesterone for cows fed additional fat, but size and number of CL have not been changed (Garcia-Bojalil et al., 1998b; Lammoglia et al., 1997; Ryan et al., 1995; Thomas and Williams, 1996). The precise mechanism by which supplemental lipids affect progesterone clearance deserves further investigation.

Progesterone concentration before and after AI has been associated with improved fertility in cows. The recovery of embryos 7 d after estrus in lactating dairy cows increased as plasma progesterone concentration increased just prior to

AI (Britt et al., 1996). Also, increasing plasma progesterone during mid luteal phase increased conception rates in dairy cows (Santos et al., 2001c).

COTTONSEED FEEDING, GOSSYPOL AND REPRODUCTION

Gossypol is a yellow, polyphenolic compound found primarily in the pigment glands of the cotton plant. Gossypol exists in the free and bound forms. In the intact whole seed, gossypol is mostly found as free gossypol (FG). However, when cottonseed is processed, gossypol binds to proteins, possibly to the epsilon-amino group of lysine. In addition to the free and bound forms of gossypol, this pigment can be present in two distinct stereoisomer forms, the plus isomer (+) and the minus isomer (-).

Cottonseed is often fed at 10 to 15% of the total diet DM for dairy cows as a source of energy, primarily from fat, protein and fiber. A primary concern with feeding large amounts of cottonseed is the possibility of gossypol toxicity in cows. Although ruminants have well developed rumen microbial populations able to detoxify gossypol by converting free to bound gossypol within the rumen and impeding its absorption into the blood, it is possible that feeding excessive amounts of the toxin in the free form may overcome this protective mechanism and impair animal performance.

It is well established in males that gossypol intake reduces semen quality and impairs fertility. However, little data is available on the effects of cottonseed and gossypol intake on fertility in dairy cows. A large field study was designed to determine the effects of two types of cottonseed and differing gossypol intakes on plasma gossypol and reproductive performance in dairy cows (Santos et al., 2001a, 2001b). A total of 832 Holstein cows, 3 to 25 DIM in 3 commercial dairy farms, were randomly assigned to one of the two treatment diets for 170 d. Cottonseed represented 10% of the diet dry matter, and treatments consisted of replacing whole Upland cottonseed (WUP) with a 1:2 blend of WUP and cracked Pima (BUPCP) cottonseed. Blood was collected from all cows at 63 and 93 DIM for measurements of plasma gossypol. Cows were housed in 10 freestall barns and reproductive management was as follows: All cows received 2 injections of $\text{PGF}_{2\alpha}$ at 33 ± 3 and 47 ± 3 d postpartum. Estrus was detected once daily, both visually and by tail chalking after 47 DIM. Within each dairy, cows found in estrus were artificially inseminated in the morning by the same technician. Pregnancy was diagnosed by rectal palpation between 35 and 45 d after AI. Pregnancies were reconfirmed when cows were 170 d postpartum, immediately prior to completing the experimental period. Those cows not found in estrus after the second injection of $\text{PGF}_{2\alpha}$ were then enrolled in a timed AI protocol at 70 d

postpartum. A cow was considered as a success in responding to PGF_{2α} when found in estrus in the 7 d following the second PGF_{2α} injection. Incidence of abortions was determined by the number of pregnant cows that were later diagnosed as open during the course of the study. Cystic ovaries were diagnosed by rectal palpation or ultrasonography by the presence of a large fluid filled structure (> 25 mm in diameter) in the absence of a corpus luteum.

Intakes of FG increased 32% in cows receiving the BUPCP diet. Concentrations of total gossypol, as well as the proportion of total gossypol as minus (-) isomer in plasma were higher for cows fed BUPCP than WUP. Lactation performance did not differ between the two treatments, and yields of milk and 3.5% FCM were greater in cows with higher plasma gossypol. Replacement of whole linted Upland cottonseed with a 1:2 blend of whole linted Upland and cracked Pima cottonseed fed at 10% of the diet DM had no effect on estrus detection and first postpartum AI conception rate, but it decreased conception for all services. In addition, cows fed the high gossypol diet had a lower overall pregnancy at d 170 postpartum. Furthermore, incidence of abortion was increased by feeding the high gossypol diet. Cows that aborted or were not pregnant at the end of the study had a higher plasma gossypol concentration.

It is not clear the exact mechanism by which gossypol might affect fertility in dairy cattle. In vitro studies (Brocas et al., 1998) have shown that gossypol concentrations of 10 µg/ml can disrupt embryo development. This is similar to the concentration of gossypol in plasma observed by Santos et al. (2001a) for cows fed a high gossypol diet. In addition to the effects on the embryo, some have suggested that gossypol interferes with the ability of luteal cells to produce progesterone, which may impair maintenance of pregnancy. Therefore, it is possible that high concentrations of plasma gossypol in dairy cows decrease conception and increase pregnancy loss caused by changes in embryo development and luteal function.

PROTEIN AND REPRODUCTION

It has been repeatedly shown that increasing dietary CP intake will lead to increased milk production (NRC, 2001). Because intake is depressed during early lactation, maximum microbial protein synthesis is not reached until 8 to 12 weeks postpartum. NRC (2001) recommends an 18 to 19% CP diet during the first weeks of lactation to compensate for the limited DMI. A slightly higher percentage of UIP (38 to 40% of CP) is fed to compensate for any potential depression in microbial protein yield in providing essential amino acids for milk and milk protein synthesis.

Diets that supply excess of CP or DIP, lack ruminally fermentable carbohydrates, or present asynchrony between protein degradation and energy availability in the rumen promote greater concentration of urea nitrogen in the blood and greater excretion of urea in milk and urine (Ferguson and Chalupa, 1989; Garcia-Bojalil et al., 1998a; Roseler et al., 1993). Surplus of protein intake increases urea concentration in the blood, and because urea can easily pass from the blood to the milk, there is a close relationship ($r = 0.88$ to 0.96) between BUN and MUN (Hof et al., 1997; Roseler et al., 1993). MUN represents 80 to 90% of BUN in lactating dairy cows (Roseler et al., 1993).

The effect of dietary concentration of CP or DIP (Barton et al., 1996; Blanchard et al., 1990; Bruckental et al., 1989; Canfield et al., 1990; Carroll et al., 1988; Elrod and Butler, 1993; Elrod et al., 1993; Ferguson and Chalupa, 1989; Ferguson et al., 1988; Folman et al., 1981; Garcia-Bojalil et al., 1994, 1998b; Howard et al., 1987; Jordan et al., 1983; Jordan and Swanson, 1979; Kaim et al., 1983), PUN or MUN (Butler, 1998, 2000; Butler et al., 1996; Ferguson et al., 1993), and ammonia (Visek, 1984) on fertility of dairy cows has been questioned. In some, but not all studies, increasing dietary CP has resulted in decreased conception rates of lactating dairy cows (Table 5). Although Table 5 shows a decrease in conception for cows fed high CP diets, these data should be interpreted carefully. The lack of information on milk production and the limited number of animals in each study limits the value of the pregnancy data.

The suggested mechanisms by which dietary CP might affect fertility of dairy cows are: 1) Toxic compounds of nitrogen metabolism (ammonia or urea) may affect uterine environment and impair sperm, ova, or early embryo survival; 2) Magnification of the effects of early postpartum negative energy balance; 3) Reduced plasma progesterone concentration; and 4) Exacerbation of $\text{PGF}_{2\alpha}$ secretion.

Although several studies have tried to elucidate the mechanisms by which the excess of CP or DIP may negatively impact fertility, the outcomes have not been conclusive. Ferguson et al. (1993) reported that the conception rates in dairy herds are decreased when PUN level is above 20 mg/dl. This hypothesis was furthered by Canfield et al. (1990) and Butler et al. (1997), who reported that PUN and MUN levels were higher in animals that did not conceive compared to those that conceived. Although these studies reported a relationship between urea nitrogen and fertility, others have not observed the same (Barton et al., 1996; Carroll et al., 1988; Howard et al., 1987).

Table 5. Conception rates and PUN concentrations of lactating cows fed moderate or elevated CP

Reference	Dietary CP, %			
	13 - 16		19 - 21	
	CR, %	PUN, mg/dl	CR, %	PUN, mg/dl
Jordan and Swanson, 1979 ¹	53	NR ³	40	NR
Folman et al., 1981	56	8.8	44	15.4
Kaim et al., 1983 ¹	57	9	43	17
Howard et al., 1987	87	15	85	26
Carroll et al., 1988 ²	64	11	56	24
Bruckental et al., 1989	65	25	52	32
Canfield et al., 1990 ^{1,2}	48	12	31	19
Elrod and Butler, 1993 ^{1,2}	83	< 16	62	> 16
Barton et al., 1996 ²	40.6	8.5	43.7	22.1
Average	61.5	12.8	50.7	22.2

¹ $P < .05$

² First service conception rate

³ Not reported

Research has not been able to determine what levels of CP or DIP in the diet will elevate blood or milk concentrations of urea nitrogen above 20 mg/dl without affecting performance. However, it has been well established that diets with excessive CP or DIP contents will significantly elevate PUN (Garcia-Bojalil et al., 1998a). Barton et al. (1996) divided 64 dairy cows in two treatments (32 cows/treatment). Cows were fed either a 13% or a 20% CP. PUN was elevated by the higher CP (21 vs 8.6 mg/dl) diet, but days to first ovulation, days to first AI, days open, services per conception, and conception rates were not different between treatments. Garcia-Bojalil et al. (1998a, 1998b) fed 45 early postpartum

dairy cows a 20.6% CP diet with either 11.1% or 15.7% DIP (% of CP). Protein degradability had no effect on performance of cows, but the already excessive CP content of the diet associated with excessive DIP resulted in higher PUN and glucose, but lower plasma insulin. Cows fed high DIP diet lost twice as much body weight (BW) as cows fed adequate DIP diet.

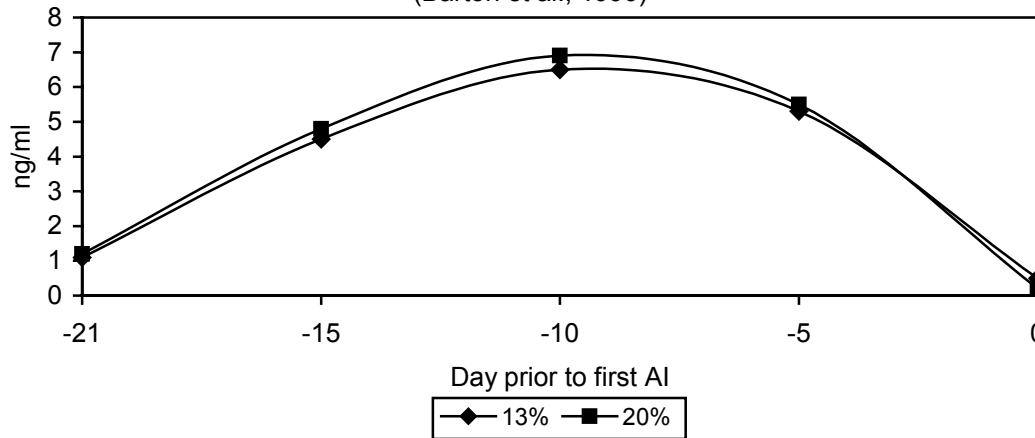
Postulated mechanisms

Despite the fact that several studies have been conducted to evaluate the effects of protein on reproductive tissues, the exact mechanism by which excess of CP or DIP may negatively impact fertility has not been elucidated. Jordan et al. (1983) fed 18 cows isocaloric diets containing either 13% or 23% CP starting on day 40 postpartum. Uterine secretions and blood samples were collected during different stages of the estrous cycle after day 50 postpartum. Plasma and uterine concentrations of urea as well as blood concentration of ammonia were elevated by the high CP diet. Uterine secretion of minerals was also affected by dietary protein content. The same group (Jordan and Swanson, 1979) observed that cows fed higher CP diets had higher serum LH during the first postpartum estrous cycle and after GnRH challenge.

Carroll et al. (1988) observed that cows fed a 20% CP diet had higher concentrations of PUN, uterine urea nitrogen, and ruminal ammonia than cows fed a 13.5% CP diet. However, reproductive performance was not affected by CP content in the diet, despite the increased fat corrected milk (FCM) production during the experimental period. Plasma progesterone concentration prior to first service was lower for the high CP diet. In this study, energy intake was not affected by dietary treatments, but cows fed 20% CP diet produced more milk than cows fed 13% CP diet. This response in milk production with no compensatory energy intake can affect energy balance. It has been shown that NEB can decrease plasma progesterone concentrations in dairy cows (Britt, 1994; Santos, 1996; Villa-Godoy et al., 1988). In a subsequent study (Barton et al., 1996), dietary CP (13% vs 20% CP) did not affect FCM yield, BW change and plasma progesterone of lactating dairy cows (Figure 4).

Garcia-Bojalil et al. (1998b) observed that feeding a diet high in ruminally degradable protein increased the interval from calving to first postpartum luteal phase, reduced the length of the first luteal phase and decreased peak and accumulated plasma progesterone concentrations. Nevertheless, cows fed high DIP diet lost twice as much BW as the cows fed adequate DIP diet. Therefore, the effects of CP on progesterone concentrations seem to be associated with an effect on energy balance, but not a direct mechanism of CP or toxic metabolites on corpus luteum activity. Garcia-Bojalil et al. (1994) superovulated nonlactating

Figure 4. Effect of CP on plasma progesterone of lactating dairy cows
(Barton et al., 1996)



Holstein cows that were fed either a control (12.3% CP) diet or a diet with excessive CP content (27.4% CP). Dietary CP had no effect on plasma progesterone concentration before or after superovulatory treatment. No effects were observed for plasma estradiol either.

Elrod and Butler (1992) examined the ion flux across caruncular endometrium with urea or ammonia infusions. Treatment with ammonia increased K and Na flux across the endometrium. Urea decreased pH in the mucosal media and Na transport across the endometrium, while increasing K and P transport. The same group (Elrod and Butler, 1993) used 80 Holstein heifers to test the effects of increased CP from RDP source on fertility during two experimental periods. In experiment one, heifers were fed the experimental diets (15.5% vs 21.8% CP) for 4 weeks before the first AI. PUN was increased by 4.6 mg/dl for the high CP/RDP diet. First service conception rate was 25% lower for the high CP/RDP compared with the control diet (82% vs 61%). In experiment two, heifers were fitted with catheters in the jugular vein, in the bladder, and in the uterus. Heifers fed high CP/RDP had higher PUN and urinary urea nitrogen. Plasma ammonia and urinary ammonia did not differ between treatments. Uterine pH tended to be lower on the day of estrus, and it was significantly lower on day 7 of the estrous cycle for heifers fed the high protein diet. Ammonia is a weak base and high concentrations of ammonia in fluids would increase pH. Based on the findings of these studies, the authors hypothesized that high CP/RDP diet will increase nitrogen compounds in the body, which may interfere with uterine pH by changes in ion

concentrations. These changes in uterine environment may affect fertilization or embryo survival.

Elrod et al. (1993) conducted another study to further examine the effects of dietary CP content and degradability on uterine environment. They assigned 36 early lactation Holstein cows to 3 dietary treatments: a balanced CP, a high UIP, and a high DIP. High CP diets, both from high UIP and DIP, increased PUN by 3.5 and 7.1 mg/dl, respectively, compared with balanced CP diet. Dietary treatments had no effects on body fluids during the day of estrus. However, on day 7 of the estrous cycle, high CP diets significantly decreased uterine pH. This data further supported previous findings of the same group (Elrod and Butler, 1993).

Excess of protein intake did not affect follicular activity of dry cows (Garcia-Bojalil et al., 1994). The number of follicles of different class sizes (class 1 = 4 mm; class 2 = 5 to 9 mm; class 3 > 9 mm) did not differ between treatments during and after superovulatory treatment. However, during early postpartum, lactating dairy cows fed high DIP diet had lower number of total follicles in the ovaries (Garcia-Bojalil et al., 1998b). Surprisingly, follicle number in the lactating cow study was limited to a maximum average of only 2.8 follicles per cow.

The effects of dietary CP on fertilization and embryo survival of superovulated cows were examined by Garcia-Bojalil et al. (1994) and Blanchard et al. (1990) (Table 6). In the Florida study, high CP diet did not affect the number of transferable and nontransferable embryos, the number of unfertilized ova or the percentage of transferable embryos. However, using vital stain DAPI (4,6-diamidino-2-phenylindole), the percentage of embryos with disrupted membrane and dead blastomeres tended to be higher for the low CP/UIP diet (Garcia-Bojalil et al., 1994). In the other study (Blanchard et al., 1990), protein degradability did not affect the number of transferable and nontransferable embryos, the number of unfertilized ova, but the percentage of transferable embryos was reduced by the high DIP diet.

Excess ammonia production in the rumen requires additional energy for its metabolism to urea in the liver. The energy required to metabolize ammonia to urea in the liver and to excrete excess of urea in the urine could exacerbate energy deficits during early lactation. This might explain why cows fed higher DIP had similar milk production and DMI, but lost more BW than those fed lower DIP diets (Garcia-Bojalil et al., 1998a).

Table 6. Effect of CP or DIP on embryo quality of superovulated cows.

Diet CP (DIP), % CP	Cows	Transf. Embryos.	Nontransf. Embryos	Unfert. Ova	Transf. Embryos %	DAPI ¹ Negative, %	Reference
12.3 (59.7)	22	4.0	1.6	1.8	49.7	53.1 ^b	Garcia- Bojalil et al., 1994
27.4 (70.7)	22	4.9	2.0	1.8	54.0	66.7 ^a	
16.0 (73.0)	19	4.5	4.0	3.1	44.2 ^b	NR ²	Blanchard et al., 1990
16.1 (64.0)	19	5.5	3.3	2.3	66.9 ^a	NR	

^{a, b} Means in a column within study with different superscript differ ($P < 0.10$)

¹ 4,6-diamidino-2-phenylindole vital stain

² Not reported

Although there is no clear relationship between protein intake and reproduction, cows fed diets that result in high urea nitrogen concentrations in blood might have reduced conception or pregnancy rates. Therefore, data indicate that nutritional management should be considered when PUN or MUN are consistently high. Changes in dietary management should not be made to the detriment of milk production and profitability. Feeding excessive amounts of CP/DIP might increase energy costs for ammonia metabolism and it might also impact on BW losses during early lactation. Balancing diets for CP, ruminally degradable and undegradable proteins, and ruminally degradable carbohydrates will improve performance of cows and minimize any potential negative effect of protein on reproductive performance of cows.

BODY CONDITION SCORE AND REPRODUCTION

Body condition scoring has become a standard management tool used by dairy professionals to quantify the energy reserves of cows during strategic periods of the lactation cycle. Scoring cows for body condition is based on visual appraisal and palpation of specific areas to subjectively evaluate subcutaneous deposits of adipose tissue and muscle mass. Nutritional management is a major factor controlling reproduction in dairy and beef cows. Reduced nutrient intake is associated with loss of BW, which in turn is manifested by changes in BCS, delayed ovulation, decreased luteal activity, and anestrus (Butler, 2000; Butler and Smith, 1989; Butler et al., 1982).

In dairy cattle, the 1 to 5 scale to score body condition developed by Wildman et al. (1982) is the most commonly used. In Holstein cows, a 1-unit change in BCS in the 1 to 5 scale represents a 50 to 60 kg change in BW (Ferguson, 1991; Santos, 1996).

In dairy cows, excessive body condition (> 4.0) during the dry period and at calving can influence DMI and negative energy balance during late gestation and early lactation (Santos, 1996). Although very few controlled studies have been able to reproduce the negative effects of high degree of fatness prepartum on postpartum performance, it is recommended that dairy cows gain very little body condition during the dry period and that their BCS should be between 3.25 to 3.75 at the time of calving. Changes in BCS during the first weeks postpartum might be more important than the BCS itself at calving. Changes in BCS reflect changes in energy status. Cows that lose BCS during the breeding period, or have a low BCS during insemination have reduced conception rates and are less likely to respond to estrus synchronization (Santos et al., 2001b) and ovulation synchronization protocols (Moreira et al., 2000).

The reestablishment of normal LH pulse patterns is the key factor in resumption of ovulation postpartum. The mechanism by which NEB delays ovarian activity, according to Butler and Smith (1989), probably manifests itself by impinging pulsatile secretions of LH. It has been shown that a lowered availability of glucose and insulin might decrease LH pulse frequency and may limit the responsiveness of the ovary to gonadotropins (Butler, 2000).

Body condition score affects cyclicity. Estrus response to two injections of PGF_{2α} given 14 days apart during the early postpartum period (33 and 47 DIM) was dramatically reduced by low BCS (Santos et al., 2001b). Only 38.0% of the cows with a BCS equal to or lower than 2.75 were observed in estrus in the 7 days following the second PGF_{2α} injection, compared with 56.8% of the cows with a BCS greater than 2.75. Santos et al. (2001c) observed that cows with a BCS at the time of insemination equal to or lower than 2.75 had a lower conception rate than those with BCS greater than 2.75 (Figure 5). Similarly, Moreira et al. (2000) observed that low BCS reduced pregnancy rates when cows were subjected to a timed AI protocol. Therefore, dietary manipulation that minimizes losses of BCS and the number of cows with a low BCS during the breeding period should reduce incidence of anestrus and increase pregnancy rates in dairy cows.

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. Metabolic and endocrine changes associated with feeding higher energy diets might explain their impacts on reproduction. Balancing diets for CP and RDP reduces nitrogen wastage, improves energy utilization, and enhances performance and reproduction of cows. Use of protein sources containing long chain unsaturated fatty acids such as fish meal has been consistently shown to improve milk production and reproductive performance of dairy cows. The fat content of the diet should be increased above the typical 3% of diet DM for cows in the early postpartum period. Feeding supplemental fat sources that deliver greater amounts of PUFA, especially eicosapentaenoic and docosahexaenoic might improve conception and reduce embryonic loss. Such a strategy will be made possible by the design of fat products containing large amounts of PUFA that are resistant to rumen biohydrogenation.

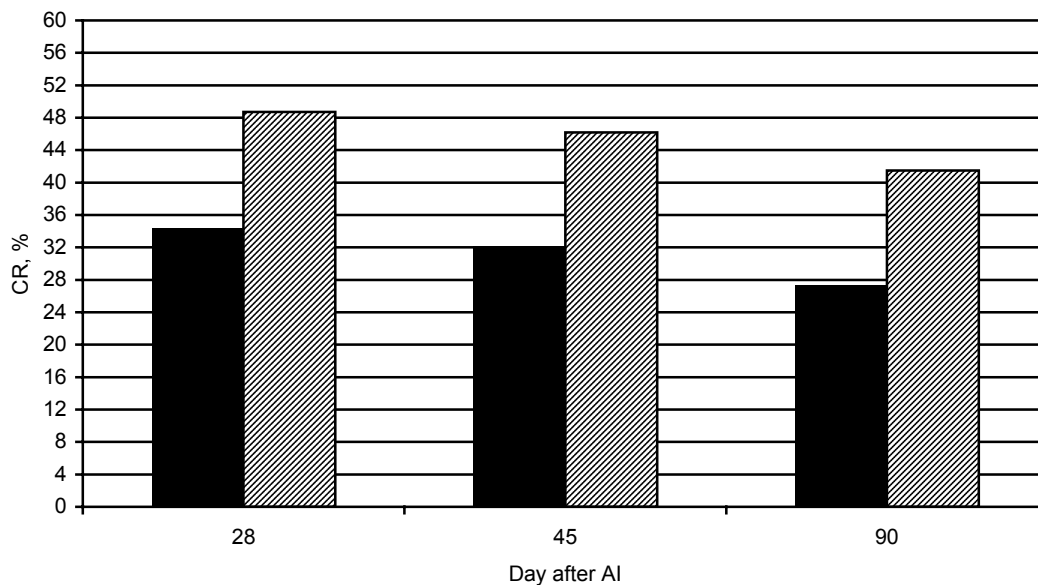


Figure 5. Effect of body condition score (BCS) at the time of AI on conception rates (CR) of dairy cows on d 28, 45 and 90 after AI. Cows were divided into two groups, those with BCS equal to or lower than 2.75 (black solid bars) and those with a BCS greater than 2.75 (white bars with diagonal shading). Body condition score at AI affected CR ($P < 0.0001$). Adapted from Santos et al. (2001c).

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