

DIETARY FATTY ACID SUPPLEMENTATION AND REPRODUCTION IN DAIRY COWS

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During the last several decades genetic selection and improved management of dairy herds has markedly increased milk production, but fertility has decreased (Figure 1). Similar trends are documented around the world. Selection of dairy cattle for high milk yield has demonstrated that endocrine and metabolic controls of nutrient balance and reproductive processes are linked such that ovarian activity is compromised during negative energy balance (NEBAL) in early lactation (Butler, 2001; Butler, 2003). Successful management of lactating cows integrates nutrition and reproduction with a good postpartum herd health program to optimize both lactational and reproductive performance. Ideally, nutritional strategies are developed and incorporated that improve reproductive performance of dairy cows without detrimental effects on milk production. Some examples include maximizing dry matter intake (DMI) during the transition period, reducing the incidence of periparturient health problems, and adding supplemental fats to diets for manipulation of ovarian and uterine function.

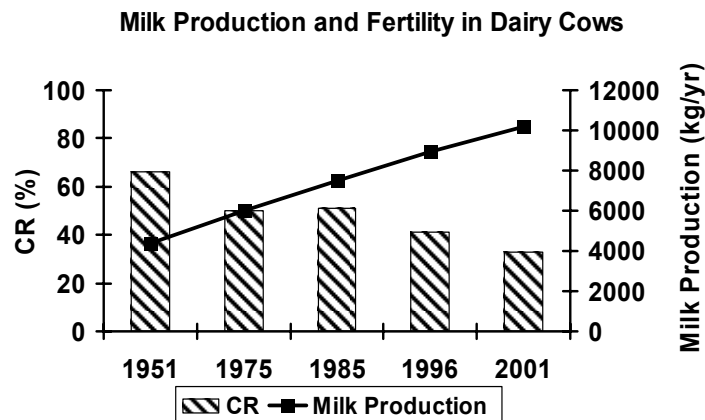


Figure 1. The inverse relationship between conception rate (CR%) and annual milk production of Holstein cows in New York

The effects of supplemental dietary fatty acids on reproductive performance and targeted reproductive tissues have been the subject of previous reviews (Mattos et al., 2000; Staples et al., 1998). Feeding fat is a common strategy to increase the energy density of the diet. Added fat provides calories, but also may impact metabolism and function of the ovary and uterus due to incorporation of specific fatty acids. Staples et al. (1998) reviewed 20 research studies on the effects of fat feeding and indicated that increasing dietary fat improved reproductive performance in more than half. The authors indicated that supplemental fat generally did not improve energy balance in cows, often

because of a nonsignificant depression in DMI among cows. Supplemental fat reduced insulin secretion in some studies due to decreased energy intake which could impair ovarian activity. Very few studies have recorded energy balance along with conception or pregnancy rates, but improved reproduction appeared not to be contingent on any improvement in energy status. Depending upon formulation, feeding supplemental fat early postpartum (PP) does not improve energy balance, often because of small to significant depression in DMI or increased production of milk or fat corrected milk (Beam and Butler, 1997; Beam and Butler, 1998; Block et al., 2005; Loften and Cornelius, 2004; Staples et al., 1998).

Supplementation with dietary fat during the early PP period in lactating cows generally stimulates ovarian function and follicular development (Staples et al., 1998; Thatcher et al., 2004). Calcium salts of fatty acids (CaSFA) seem to have the greatest effect on size of preovulatory follicles, but the number and size of dominant follicles was increased by feeding a blend of tallow and yellow grease at 2.2% of DMI (Beam and Butler, 1997). Addition of prilled fatty acids (2.6% of DM) did not alter follicle dynamics or the interval from parturition to first ovulation (Beam and Butler, 1998).

The transition period for dairy cows, from 3 weeks precalving to 3 weeks post calving, is the most critical phase of the lactation cycle, affecting both milk production and reproductive performance. During the transition period, there is a decline in DMI around parturition, mobilization of body fat, and hormone changes for calving and onset of lactation. Increases in NEFA from fat stores can lead to liver and metabolic problems. Although most studies utilizing supplemental dietary fat started feeding cows at calving or somewhat later in lactation, feeding prepartum diets with higher energy density may offset the negative effects of lower DMI by reducing plasma NEFA concentrations and liver triglyceride accumulation. This idea was tested in a study by Doepel et al. (2002). Multiparous cows were fed one of two diets from 21 d before expected calving, energy levels 1.65 or 1.30 Mcal/kg NEL, and all cows were fed a common diet after calving. The high energy diet contained higher fat (4.8% including 2.2% tallow) and NFC and provided beneficial carry-over effects after calving of higher DMI, milk production and energy balance plus reduced NEFA and liver triglycerides.

Another recent trial (Frajblat and Butler, 2003) also considered the effects of adding fat to the prepartum diet. Multiparous cows (n=81) were fed two diets from 21 days before expected calving and a common diet PP. Prepartum diets were isoenergetic (2.9 or 4.6% fat) yielding NEL of 1.48 and 1.53 Mcal/kg. The inclusion of prilled saturated fatty acids had no significant effect on DMI or metabolic and hormonal parameters throughout the transition period and early lactation, nor on milk yield. However, the study provided some important information related to fertility. Monitoring NEBAL in dairy cows is typically done through body condition score (BCS). Greater NEBAL/BCS loss during the first 30 days of lactation delayed first ovulation (Figure 2). From other studies there is strong agreement that conception rate decreases with increased BCS loss. Cows remaining anovulatory until after 50 days of lactation had a higher risk of not becoming pregnant during lactation and, therefore, were more likely to be culled (Figure 2). The most striking carryover effects of feeding fat prepartum were reduction in days

open for pregnant cows (110 versus 141) and overall higher pregnancy rate @ 225 DIM (86% versus 58%). Another study is in progress to compare the effects of feeding saturated or unsaturated fat for 3 weeks prepartum on lactational performance, metabolic parameters, and ovarian and uterine function.

Early NEBAL&BCS loss delays first ovulation and relates to poor fertility/increased risk of culling

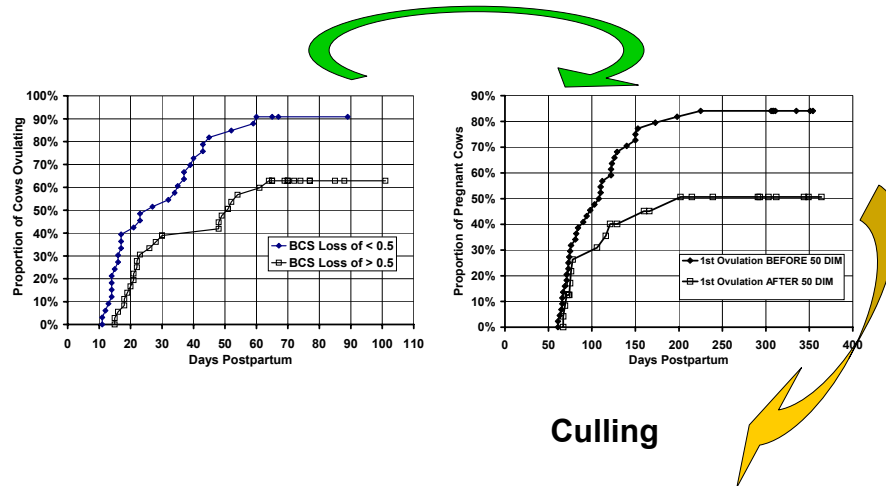


Figure 2. Negative energy balance results in body condition score (BSC) loss in early lactation dairy cows. Greater BCS loss (> 0.5 score) during the first 30 days of lactation delays the timing of first ovulation (survival analysis). Among the cows that had delayed ovulation until after 50 DIM, pregnancy rate during lactation was reduced and cows remaining open after ~225 days are likely to be culled.

Besides the use of fatty acids to increase caloric intake of dairy cows, there has been increased interest in various oil seeds and in designing rumen inert fat sources that will deliver polyunsaturated fatty acids of the omega-6 (linoleic acid, LA, C18:2) and omega-3 (α -linolenic acid, ALA, C18:3; eicosapentaenoic acid, EPA, C20:5; docosahexaenoic acid, DHA, C22:6) families to the lower gut for absorption. The strong interest in these polyunsaturated fatty acids is in their role as precursor substrates for biosynthesis of prostaglandins or as inhibitors of prostaglandins. LA and ALA are essential fatty acids found in forages and oil seeds, while EPA and DHA are found in marine products (fish meal, fish oil, etc.). EPA and DHA originally appeared in dairy cow diets when fish meal was used as a source of rumen undegradable protein, but now are valued for targeted manipulation and suppression of uterine synthesis of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; (Abayasekara and Wathes, 1999; Mattos et al., 2000; Thatcher et al., 2004). Manipulation of the fatty acid profile of the diet to amplify suppression of uterine $PGF_{2\alpha}$ during early pregnancy may be an important mechanism for reducing embryonic mortality and is perhaps the explanation for why feeding fish meal enhances reproductive performance (Staples et al., 1998). Literature results on

the regulation of uterine $\text{PGF}_{2\alpha}$ secretion in cows by oil seeds or forage-derived omega-6 and omega-3 fatty acids or their ratio are inconsistent. The uterine $\text{PGF}_{2\alpha}$ secretory response to oxytocin was either decreased (Cheng et al., 2001; Oldick et al., 1997) or increased (Fahey et al., 2002; Petit et al., 2004; Robinson et al., 2002) by omega-6 fatty acids. ALA, an omega-3 fatty acid, tended to reduce the $\text{PGF}_{2\alpha}$ response (Petit et al., 2002) or have no effect (Cheng et al., 2001; Robinson et al., 2002). Inconsistencies after dietary manipulation may be explained by variation in the availability of the specific fatty acids for incorporation into uterine tissue and the resulting ratio.

Detailed descriptions of uterine prostaglandin metabolism and mechanisms by which shifting the omega-6/omega-3 ratio may alter specific reproductive processes are presented in the several review articles listed earlier. A brief overview of fatty acids in relationship to prostaglandin metabolism is presented in Figure 3. Processing and metabolism of fatty acids in one family can only generate fatty acids of the same family. Dietary LA is converted to arachidonic acid ($\text{C}_{20:4}$) that is the immediate precursor of prostaglandins. The synthesis of $\text{PGF}_{2\alpha}$ from arachidonic acid is regulated by the key enzyme, prostaglandin endoperoxide synthase (PGHS). Increased availability of EPA may lead to suppression of uterine $\text{PGF}_{2\alpha}$ synthesis by competing for PGHS. Although DHA is not a substrate for PGHS, it is a strong inhibitor of the enzyme. Also the increased presence of EPA and DHA can inhibit the enzymes synthesizing arachidonic acid from LA. ALA can also compete with LA for the desaturase enzyme resulting in synthesis of more EPA and less arachidonic acid. Finally, the omega-3 fatty acids can displace arachidonic acid in the phospholipids of cell membranes reducing its availability for synthesis of $\text{PGF}_{2\alpha}$. Therefore, there are several possible mechanisms whereby omega-3 fatty acids can reduce $\text{PGF}_{2\alpha}$.

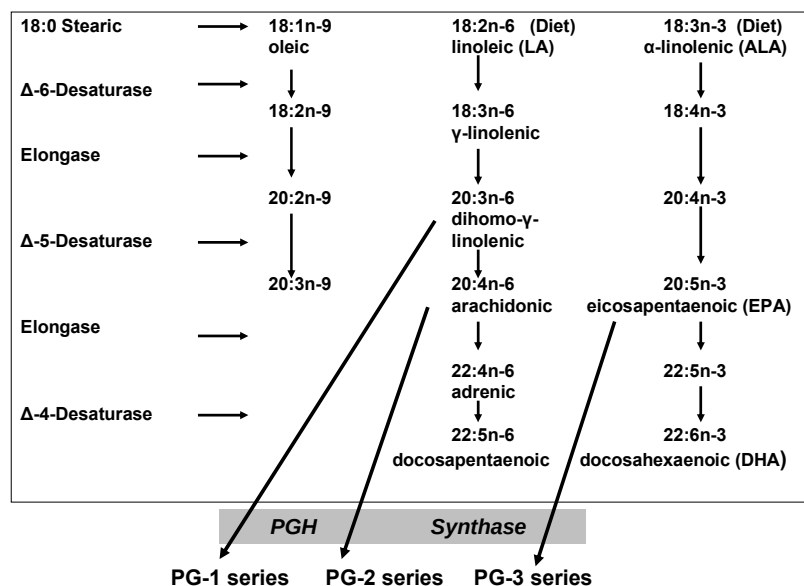


Figure 3. Metabolic transformations of the 3 major unsaturated fatty acid families by desaturation and elongation and linkage to the synthesis of prostaglandins

EFFECTS OF FEEDING FISH MEAL OR CALCIUM SALTS OF FISH OIL ON UTERINE PGF_{2α} AND REPRODUCTIVE PERFORMANCE IN LACTATING COWS

When lactating cows were fed fish meal at 2.7, 5.2 and 7.8% of DM for 35 days, uterine PGF_{2α} secretion induced by injection of oxytocin was attenuated compared to control cows not fed fish meal (Mattos et al., 2002). These results were very encouraging and we designed a study to compare the effects of feeding fish meal or a commercial fat supplement containing Ca salts of fish oil fatty acids (CaFOFA; EnerGII Repro, Bioproducts, Inc., Fairlawn, OH) on uterine PGF_{2α} secretion and lactational performance. From d 5 – 50 PP, cows were fed diets containing 0, 1.25, 2.5 and 5% menhaden fish meal (Sea-Lac, Omega Protein, Hammond, CA) or 2.3% CaFOFA on a DM basis. The diets were formulated to be isonitrogenous, isoenergetic and isolipid (adjusted to 5% lipids with Ca salts of palm oil fatty acids, EnerGII; Bioproducts, Inc., Fairlawn, OH). Rations were formulated to contain similar amounts of metabolizable protein (2645 g/d), net energy for lactation (1.78 Mcal/kg DM), and nonfiber carbohydrate (42%). Individual daily feed intakes were recorded and cows were milked 3x/d. Milk production, feed intake, uterine fatty acid composition (from uterine endometrial biopsies), and uterine PGF_{2α} production (PGFM) after oxytocin challenge are summarized in Table 1.

Milk production was significantly increased by feeding 5% fish meal or 2.3% CaFOFA, but milk composition remained similar across diets. Feed intake was significantly affected by diet. The increased availability of EPA and DHA from feeding fish meal or CaFOFA was reflected in increased concentrations of these fatty acids in uterine tissue confirming a previous report (Burns et al., 2003). However, PGF_{2α} secretion in response to oxytocin was not altered by increased dietary intake of EPA

Table 1. Milk production, feed intake, uterine fatty acid composition, and uterine PGF_{2α} response (PGFM) to oxytocin in lactating cows fed fish meal (FM) or CaFOFA

	Treatments				
	Control	1.25% FM	2.5% FM	5% FM	2.3% CaFOFA
Cows, n	6	6	6	6	7
Milk production, kg/d	48.2	49.8	48.6	53.5	52.3
DMI, kg/d	22.7	22.8	23.1	23.9	24.7
Uterine fatty acids ^a					
DHA	1.1	1.8	2.3	3.4	2.2
EPA	0.3	0.3	0.5	0.8	0.4
PGFM response ^b	4407±1246	3515±1376	5425±1243	3864±1110	5228±1030
^a g fatty acid/100g total fatty acids					
^b area under response curve units					

and DHA. This lack of attenuation of uterine $\text{PGF}_{2\alpha}$ production confirms another recent report (Wamsley et al., 2005), but is contradictory to the report by Mattos et al. (2002). The reason for this discrepancy is unclear.

The effects of calcium salts of fish oil fatty acids has also been tested in a large commercial dairy herd (Santos, 2005). Holstein cows ($n = 738$) were assigned to one of two diets containing either tallow (1.65% of DM) or CaFOFA (1.8 % of DM) fed from d25 to 145 of lactation. Yield of fat corrected milk was higher for cows fed CaFOFA during the cooler season, but lower during heat stress. After analyzing the Ca salts samples, it was determined that the amount of free fat decreased with time in storage and that this time effect differed between the two seasons. This led the authors to suggest that some of the negative effects of CaFOFA during the heat stress season were consequent to higher availability of free polyunsaturated fatty acids in the rumen (i.e., unsaturated fatty acids have negative effects on ruminal fermentation). Because of the confounding effects of the free fat content during this trial, the same experiment was repeated during summer heat stress with additional 331 cows. Milk yield and reproductive performance were similar for both treatments.

Thus far, the results from feeding omega-3 fatty acids to lactating cows for the purpose of attenuating uterine $\text{PGF}_{2\alpha}$ production and consequently improving reproductive performance have not provided the expected positive outcomes. Additional controlled studies with large groups of cows are necessary to properly assess the possible beneficial effect of omega-3 fatty acids on reproduction as well as lactational performance.

EFFECT OF *TRANS*-FATTY ACIDS OR CONJUGATED LINOLEIC ACID (CLA) ON REPRODUCTION IN DAIRY COWS

Unsaturated fatty acids in the diet will be converted via biohydrogenation by ruminal microbes to saturated fats unless the fatty acids are modified to reduce their metabolism (eg., calcium salts of fatty acids and prilled fats). During biohydrogenation of unsaturated fatty acids in the rumen, the conversion to the saturated state is incomplete and intermediates include isomers of conjugated LA (CLA) and *trans*-fatty acids (Figure 4). *Trans*-10, *cis*-12 CLA can markedly decrease milk fat synthesis when absorbed from the rumen into the bloodstream and is then taken up by the mammary gland. When milk fat synthesis is reduced, the energy sparing effect could translate into increased milk production or improved reproductive performance. In addition CLA isomers reduce prostaglandin synthesis in several systems including reproductive tissues. Polyunsaturated fatty acids including CLA isomers are natural ligands for the family of nuclear transcription factors, peroxisome proliferator-activated receptors (PPARs). Three different PPAR isoforms are expressed in tissues as potential mediators of lipid effects. Recently, expression of PPAR_γ and its functional role has been demonstrated in ovarian theca and granulosa cells (Komar et al., 2001). Both PPAR_δ and PPAR_α are present in bovine endometrium (Thatcher et al., 2004). Therefore, PPARs may play a role in the effects of fatty acids on reproduction, especially in the case of CLA.

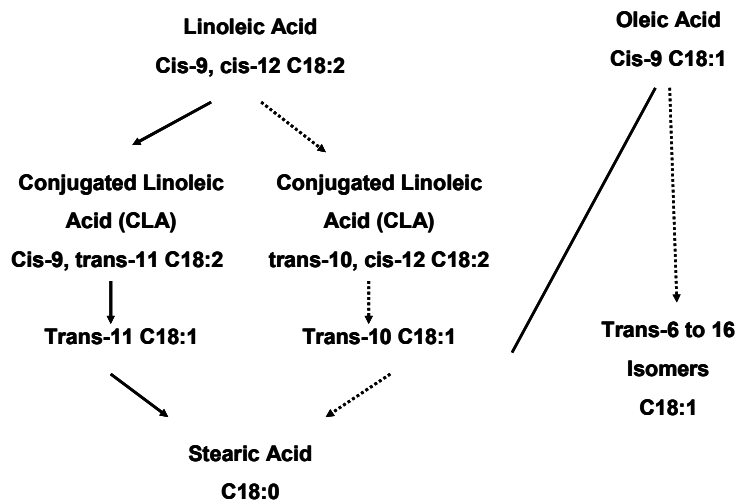


Figure 4. Biohydrogenation of fatty acids by rumen microbes (Staples and Cullens, 2005)

Recently Ca salts of CLA isomers have become available and provided the first opportunities to test the effects of dietary supplementation on reproductive function in dairy cows. Because reduction of milk fat secretion by use of CLA supplements may alleviate energy demands during early lactation, the objective of one study was to evaluate lactational performance, net energy balance, and reproductive response of dairy cows supplemented with two doses of CLA from 2 weeks before expected calving until 9 weeks PP (Castañeda-Gutiérrez et al., 2005). Supplements for all treatments provided 230 g/d of fat and varied in CLA content as described in Table 2 where the

Table 2. Effects of dietary supplement with calcium salts of CLA during transition and early lactation (9 weeks) on lactation and reproductive performance

	Treatment ^a			
	Control	CLA-1	CLA-2	P-value
Cows, n	16	16	16	
Prepartum DMI, kg/d	13.5	14.8	12.7	0.05
Postpartum DMI, kg/d	21.7	21.3	20.5	0.35
Milk yield, kg/d	45.4	43.8	43.8	0.98
Milk fat %	3.82	3.43	3.08	<.001
Milk energy output, Mcal/d	30.2	28.5	26.8	0.03
First ovulation PP, d				
Mean	32.6	27.3	41.4	0.05
Median	34.0	21.5	41.3	0.05
Days to pregnancy	160	135	151	0.81
Cows pregnant @ 185 DIM, %	44	81	53	0.07
^a Cows received a dietary supplement consisting of Ca-salts of palm fatty acids (control); or 32 g/d CLA isomers (t-8, c-10 [21.2%], c-9, t-11 [21.8%], t-10, c-12 [29.0%], c-11, t-13 [28.0%], (CLA-1); or 63 g/d CLA isomers (CLA-2).				

results are summarized. Supplementation with CLA resulted in an 11 and 21% decrease in milk fat yield for CLA-1 and CLA-2, respectively. Milk production and synthesis of other milk components did not differ among treatments. Milk energy output was significantly reduced by CLA-2, but net energy balance was unaffected. The effects of CLA on reproductive variables were inconsistent with CLA-1 generally being positive and CLA-2 being intermediate or similar to control. Any effects of CLA on reproduction were apparently independent of energy balance.

A second smaller study also tested whether CLA supplementation would affect energy metabolism in dairy cows, with treatment beginning during the transition period, but continuing until 20 weeks of lactation (Bernal-Santos et al., 2003). The results are summarized in Table 3. Feeding CLA reduced milk fat content by 12.5% during early lactation, however, milk production was increased by about 3 kg/d during the treatment period and energy balance remained unchanged. Although the number of cows was limited, there was a trend for CLA treatment to reduce the interval from parturition to first ovulation and to increase pregnancy rate. These effects once again seemed unrelated to energy balance.

Table 3. Effects of dietary supplement with calcium salts of CLA during transition and early lactation (20 weeks) on lactational and reproductive performance

	Treatment ^a		
	Control	CLA	P-value
Cows, n	15	15	
Prepartum DMI, kg/d	12.1	13.1	0.25
Postpartum DMI, kg/d	23.5	23.9	0.65
Milk yield, kg/d	44.3	47.1	0.13
Milk fat, %	3.60	3.15	<.001
First ovulation PP, d			
Mean	39.3	29.3	0.13
Median	32	20	0.07
Cows pregnant, n	8	12	0.21
CR % overall	27	42	
^a Cows received a dietary fat supplement consisting of Ca-salts of palm fatty acids or Ca-salts of CLA isomers (34 g/d; t-8, c-10 [9.2%], c-9, t-11 [25.1%], t-10, c-12 [28.9%], c-11, t-13 [16.1%].			

A field trial in a commercial herd (n = 423 cows) was performed to compare the effects of Ca-salts of palm oil fatty acids versus Ca-salts of LA plus a blend of monoenoic *trans*-fatty acids (Santos, 2005). The diets were fed from 3 weeks before expected calving until ~75 DIM and contained ~250 g/cow/d of fat supplement. Milk yields were similar for both treatment groups, but milk fat % was decreased by feeding *trans*-fatty acids (3.3% vs. 3.6% in control). Conception rate at first AI was higher for cows fed *trans*-fatty acids (33.5 vs. 25.6% in controls, P < 0.09). The results of a follow-

up study suggested that the improved CR % was likely due to increased fertilization rate of oocytes and higher embryo quality.

Thus far, the increases in conception rate and pregnancy rates for cows fed supplemental CLA or *trans*-fatty acids seems quite promising for enhancing reproductive performance in lactating dairy cows. By decreasing milk fat synthesis, these fatty acids may spare energy utilization as one means to enhance reproduction, but the actions of these unsaturated fatty acids through PPAR mediated pathways in the uterus and ovary have not been investigated.

REFERENCES

- Abayasekara, D. R. E. and D. C. Wathes. 1999. Effects of altering dietary fatty acid composition on prostaglandin synthesis and fertility. *Prost. Leuk. Essent. Fatty Acids* 61:275-287.
- Beam, S. W. and W. R. Butler. 1997. Energy balance and ovarian follicle development prior to the first ovulation postpartum in dairy cows receiving three levels of dietary fat. *Biol. Reprod.* 56:133-142.
- Beam, S. W. and W. R. Butler. 1998. Energy balance, metabolic hormones, and early postpartum follicular development in dairy cows fed prilled lipid. *J. Dairy Sci.* 81:121-131.
- Bernal-Santos, G., J. W. Perfield, D. M. Barbano, D. E. Bauman, and T. R. Overton. 2003. Production responses of dairy cows to dietary supplementation with conjugated linoleic acid (CLA) during the transition period and early lactation. *J. Dairy Sci.* 86:3218-3228.
- Block, E., W. Chalupa, E. Evans, T. Jenkins, P. Moate, D. Palmquist, and C. Sniffen. 2005. Calcium salts are highly digestible. *Feedstuffs* July 25:20-25.
- Burns, P. D., T. E. Engle, M. A. Harris, R. M. Enns, and J. C. Whittier. 2003. Effect of fish meal supplementation on plasma and endometrial fatty acid composition in nonlactating beef cows. *J. Anim. Sci.* 81:2840-2846.
- Butler, W. R. 2003. Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows. *Livest. Prod. Sci.* 83:211-218.
- Butler, W. R. 2001. Nutritional effects on resumption of ovarian cyclicity and conception rate in postpartum dairy cows. *Animal Science Occasional Publication No. 26* (Vol.1):133-145.
- Castañeda-Gutiérrez, E., T. R. Overton, W. R. Butler, and D. E. Bauman. 2005. Dietary supplements of two doses of calcium salts of conjugated linoleic acid during the transition period and early lactation. *J. Dairy Sci.* 88:1078-1089.
- Cheng, Z., R. S. Robinson, P. G. A. Pushpakumara, R. J. Mansbridge, and D. C. Wathes. 2001. Effect of dietary polyunsaturated fatty acids on uterine prostaglandin synthesis in the cow. *J. Endocrin.* 171:463-473.
- Doepel, L., H. Lapierre, and J. J. Kennelly. 2002. Peripartum performance and metabolism of dairy cows in response to prepartum energy and protein intake. *J. Dairy Sci.* 85:2315-2334.
- Fahey, J., J. F. Mee, J. J. Murphy, and D. O'Callaghan. 2002. Effects of calcium salts of fatty acids and calcium salt of methionine hydroxy analogue on plasma

- prostaglandin F_{2α} metabolite and milk fatty acid profiles in late lactation holstein-friesian cows. *Theriogenology* 58:1471-1482.
- Frajblat, M. and W. R. Butler. 2003. Effect of dietary fat prepartum on first ovulation and reproductive performance in lactating dairy cows. *J. Anim. Sci.* 81(Suppl.1):119. (Abstr.)
- Komar, C. M., O. Braissant, W. Wahli, and T. E. Curry, Jr. 2001. Expression and localization of PPARs in the rat ovary during follicular development and the periovulatory period. *Endocrinology* 142:4831-4838.
- Loften, J. R. and S. G. Cornelius. 2004. Responses of supplementary dry, rumen-inert fat sources in lactating dairy cow diets. *The Professional Animal Scientist* 20:461-469.
- Mattos, R., C. R. Staples, and M. J. Thatcher. 2000. Effects of dietary fatty acids on reproduction in ruminants. *Rev. Reprod.* 5:38-45.
- Mattos, R., C. R. Staples, J. Williams, A. Amorcho, M. A. McGuire, and W. W. Thatcher. 2002. Uterine, ovarian, and production responses of lactating dairy cows to increasing dietary concentrations of menhaden fish meal. *J. Dairy Sci.* 85:755-764.
- Oldick, B. S., C. R. Staples, W. W. Thatcher, and P. Gyawu. 1997. Abomasal infusion of glucose and fat - effect on digestion, production, and ovarian and uterine functions of cows. *J. Dairy Sci.* 80:1315-1328.
- Petit, H. V., R. J. Dewhurst, N. D. Scollan, J. G. Proulx, M. Khalid, W. Haresign, H. Twagiramungu, and G. E. Mann. 2002. Milk production and composition, ovarian function and prostaglandin secretion of dairy cows fed omega-3 fats. *J. Dairy Sci.* 85:889-899.
- Petit, H. V., C. Germiquet, and D. Lebel. 2004. Effect of feeding whole, unprocessed sunflower seeds and flaxseed on milk production, milk composition, and prostaglandin secretion in dairy cows. *J. Dairy Sci.* 87:3889-3898.
- Robinson, R. S., P. G. A. Pushpakumara, Z. Cheng, A. R. Peters, D. R. E. Abayasekara, and D. C. Wathes. 2002. Effects of dietary polyunsaturated fatty acids on ovarian and uterine function in lactating dairy cows. *Reproduction* 124:119-131.
- Santos, J. E. P. 2005. Nutritional management strategies to improve reproductive efficiency in dairy cattle. *Proceedings 2005 Intermountain Nutrition Conference Pub 192, UT Agric Exp Sta, Logan, UT*:101-121.
- Staples, C. R., J. M. Burke, and W. W. Thatcher. 1998. Influence of supplemental fats on reproductive tissues and performance of lactating cows. *J. Dairy Sci.* 81:856-871.
- Staples, C. R. and F. M. Cullens. 2005. Implications of fat-feeding practices for lactating dairy cows -- effects on milk fat. *Adv. Dairy Tech.* 17:277-295.
- Thatcher, W. W., T. Bilby, C. R. Staples, L. MacLaren, and J. Santos. 2004. Effects of polyunsaturated fatty acids on reproductive processes in dairy cattle. *Southwest Nutrition and Management Conference Feb. 26-27*:1-13.
- Wamsley, N. E., P. D. Burns, T. E. Engle, and R. M. Enns. 2005. Fish meal supplementation alters uterine prostaglandin F_{2α} synthesis in beef heifers with low luteal-phase progesterone. *J. Anim. Sci.* 83:1832-1838.