Fatty Acids and Fertility – The Contributions of Dr. Charlie Staples

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Introduction

Dr. Charles (Charlie) Richard Staples accepted an Assistant Professor position at the University of Florida UF) in 1985. However, he began his industrious and interdisciplinary career during his postdoctoral program with Dr. Jimmy Clarke at the University of Illinois. We discussed the dynamics and importance of the postpartum period relative to restoration of reproductive competence in association with changes in energy balance and restoration of ovarian activity. It was a time at Florida when we were well on our way of being able to control the time of artificial insemination and had methodologies for hormonal and metabolic assays and the use of ultrasound to monitor ovarian activity and presence of the fetus. Furthermore, we realized that many cows presented for controlled breeding postpartum were anovulatory that contributed to poor herd fertility. Dr. Clarke commented that they were doing an extensive postpartum nutrition experiment at Illinois dealing with energy balance, and Charlie suggested measuring progesterone to establish postpartum patterns of ovarian activity. He collected samples throughout the experiment and progesterone measurements were analyzed at Florida. Thus, his inquisitive mind, willingness to share with others, and the value of interdisciplinary programs began early and was sustained throughout his academic career as a research scientist, teacher, mentor and sought-after speaker in the area of extension as well.

Postpartum Responses to Fat Supplementation

This first experiment introduced the importance of nutrition in utilization of dietary fats. Progesterone profiles in plasma were categorized such that anovular cows were compared with two cycling groups of 25 cows undergoing corpus luteum (**CL**) activity within 40 d after parturition and a second group of 14 cows expressing CL activity between 40 and 60 d postpartum. *Anovular cows ate less feed, produced less milk, and lost more body weight, resulting in a more negative energy status than cycling cows.* Differences in energy balance among cow groups were greatest the first 3 weeks. postpartum. Anovular cows obtained more energy from body reserves for milk production the first 2 weeks of lactation (i.e., greatest negative energy balance), than cows cycling prior to d 40. This focused our attention to the importance of potential nutritional strategies to be targeted in the peripartum and postpartum periods (Staples et al., 1990).

Charlie partnered with Matt Lucy a PhD student in my laboratory in those early years focusing on ovarian follicular dynamics in response to dietary supplementation

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with Ca salts of long-chain fatty acids (**Ca-LCFA**) for the first 60 days postpartum (Lucy et al., 1991). On d 25 postpartum, cows were injected with 25 mg of prostaglandin $F_{2\alpha}$ and treated for 15 d with an intravaginal controlled-internal drug release (**CIDR**) insert containing 1.9 g progesterone. During the first estrous cycle after CIDR removal, the average size of the largest (18.2 vs. 12.4 mm) and second largest (10.9 vs. 7.4 mm) follicles were greater in cows fed Ca-LCFA compared with control cows. Cows fed Ca-LCFA had more class 1 and 4 and fewer class 2 follicles during the estrous cycle after CIDR removal (**Figure 1**). The ability of larger follicles to influence the growth of smaller follicles is a phenomenon known as follicular dominance. Lactating cows fed Ca-LCFA had a reduction in the number of Class 2 medium-sized follicles indicative of the presence of large healthy dominant preovulatory follicles. Within this experimental model feeding of CA-LCFA stimulated follicular growth in restoration of estrous cycles in the postpartum period.

Charlie and his student Carlos Garcia-Bolalil demonstrated clearly that postpartum reproductive and metabolic responses were altered when multiparous Holstein cows were assigned randomly at calving to one of four treatment diets arranged in a 2 by 2 factorial (Garcia-Bolalil et al.,1998a;1998b). Factors were two dietary concentrations of ruminally degradable protein, 11.1 or 15.7% of dry matter (**DM**), and supplemental fat as Ca-LCFA from palm fatty acids distillated fed at 0 or 2.2% of dietary DM. Cows fed excess ruminally degradable protein had less ovarian follicular development, delayed first postpartum luteal activity (25.2 vs. 38.6 d), accumulated less luteal tissue, and had lower plasma progesterone concentrations. These changes were likely mediated by the greater body weight loss, increased plasma concentrations of nonesterified fatty acids and reduced concentrations of plasma insulin. In dairy cows fed a 15.7% degradable protein diet, supplementing Ca-LCFA doubled the number of CL, reduced the interval to first rise in progesterone by 6 d, doubled the number of normal luteal phases, and restored the pattern of accumulated plasma progesterone concentrations similar to that induced by lower ruminally degradable protein diets (**Figure 2**). Accumulated percent of cows pregnant by 120 d postpartum increased from 52.3 to 86.4% by supplemental fat. Therefore, in cows fed excess ruminally degradable protein, feeding Ca-LCFA diminished adverse changes in body weight, attenuation in ovarian activity, and pregnancy rate.

Meta-analysis was used to integrate a cross-section of smaller experiments and increase the statistical power over any single study to test the effects of dietary fats and diet composition on fertility using meta-regression method (Rodney et al., 2015). A total of 17 experiments (*i.e., 4 experiments contributed from Charlie Staples' program*) containing 26 comparisons were suitable for inclusion in statistical evaluations. Reproductive variables evaluated were risk of pregnancy (proportion pregnant), primarily to first service. A 27% overall increase in pregnancy to service was observed (relative risk = 1.27; 95% confidence interval = 1.09 to 1.45), and results were relatively consistent (small heterogeneity, I^2 = 19.9%). A strong indication of a reduction in calving to pregnancy interval was also identified, which was consistent across studies, supporting a conclusion that, overall, the inclusion of fats does improve fertility.

Fat and Fatty Acid Effects on Uterine Secretion of Prostaglandins

Prostaglandins (**PG**) are secreted profusely during two reproductive windows of the dairy cow: the peripartum period for 2 weeks after parturition and during subsequent estrous cycles associated with either regression or maintenance of the corpus luteum in cyclic or pregnant cows, respectively. One intensive area of investigation was whether fat feeding and specific fatty acids would modulate secretion of prostaglandins and other endocrine modulators in a manner that may subsequently benefit lactating cow lactational and reproductive performance.

A series of *in vitro* experiments were performed with uterine bovine endometrial (**BEND**) cells incubated for 24 h with fatty acids and then treated with phorbol ester to stimulate $PGF_{2\alpha}$ secretion (Mattos et al., 2003). The BEND cells were incubated with no fatty acid (control) and a variety of fatty acids that included C18:1n9, C18:2n-6, C18:3n-3, C20:4n-6, C20:5n-3, and C22:6n-3 at a concentration of 100 μ*M*. Only the omega-3 fatty acids (C18:3n-3, C20:5n-3, and C22:6n-3) suppressed synthesis of $PGF_{2\alpha}$, with C20:5n-3 and C22:6n-3 being the most suppressive (**Figure 3**). An additional experiment compared the inhibitory effects of the C20:5n-3 fatty acid with the bovine interferon-tau protein produced by the 17 to 25-day old embryo. Both suppressed $PGF_{2\alpha}$ secretion in this *in vitro* BEND cell system. These results raise the possibility that feeding diets enriched with omega 3-fatty acids that alter tissue composition may have an additive effect to improve pregnancy per insemination and reduce pregnancy losses. This concept is largely substantiated by *Charlie and his team of collaborators* in subsequent nutrition-reproduction experiments with lactating dairy cows.

One of the initial *"Charlie proofs"* of a fatty acid nutraceutical concept with lactating dairy cows was abomasum infusions of emulsions and solutions of either water, glucose (1 kg/d), tallow (0.45 kg/d) or yellow grease (0.45 kg/d) continuously (11.5 mL/minute) over a 16-h period each day. This was carried out as a 4 by 4 Latin square design with each period lasting 35 days: 14 d allowed for adjustment of treatments and 21 days allowed for data collection during a synchronized estrous cycle (Oldick et al., 1997). Plasma progesterone concentration peaked higher during the estrous cycle for cows infused with fat than for those infused with glucose. Mean growth rate and maximum size of the first wave dominant follicle were greater with tallow than with yellow grease. During the period of infusion of yellow grease and afterward, release of the metabolite of $PGF_{2\alpha}$, 13,14-dihydro-15-keto-PGF2α (**PGFM**) in response to an injection of oxytocin on d 15 of the estrous cycle was attenuated.

An additional study fed cycling multiparous cows (n = 32) diets containing menhaden fish meal: 0, 2.6, 5.2 or 7.8% fish meal for 56 days (Mattos et al., 2002). At day 15 of a synchronized estrous cycle (i.e., at 56 days of feeding the treatment diets) cows were injected with estradiol-17*β* (3 mg, i.v.) at 0900 h and oxytocin (100 IU, i.v.) at 1300 h. Plasma PGFM concentrations after oxytocin injection were reduced in cows fed diets containing fish meal compared with those fed no fish meal. Milk production (39.1 kg/d) and concentrations of fat, protein, or urea nitrogen in milk were not affected by diet. Feeding fish meal and fish oil containing C20:5n-3 and C22:6n-3 fatty acids

reduced the proportion of n-6 fatty acids and increased that of n-3 fatty acids in milk in a dose-responsive manner.

Whether feeding of omega-3 fatty acids would suppress synthesis of $PGF_{2\alpha}$ at a time when dairy cows naturally produce copious amounts of $PGF_{2\alpha}$, at the time of parturition was evaluated (Mattos et al., 2004). Holstein cows were fed diets containing either olive oil or fish oil (1.8 to 2% of dietary DM) from 21 days before expected calving date through 21 days postpartum. Blood samples were collected daily and analyzed for PGFM. Concentrations of C20:5n-3 and C22:6n-3 in caruncular tissue collected within 12 h of parturition were increased 5 to 6-fold in cows fed fish oil. Cows fed fish oil had reduced concentrations of PGFM in plasma during the period of maximum secretion of uterine $PGF_{2\alpha}$ in the early postpartum period compared with cows fed olive oil. Differences were significant ($P < 0.05$) at 0, 0.5, 2, and 2.5 days postpartum (**Figure 4**). The increased concentrations of C20:5n-3 and C22:6n-3 in caruncular tissue of cows fed fish oil suggest that these fatty acids may be the active compounds reducing secretion of uterine $PGF_{2\alpha}$.

Fats and their Nutraceutical Roles to Improve Reproductive Performance

Several characteristics and components of fats possibly contribute to inherent effects on reproduction. From an energy perspective, fat sources contain 2.5 times the caloric density of carbohydrate sources. Also, the polyunsaturated fatty acids such as linoleic acid (C18:2n-6), α-linolenic acid (C18:3n-3), eicosapentaenoic acid (**EPA**; C20:5n-3) and docosahexaenoic acid (**DHA**; C22:6n-3), and *trans* fatty acids partially escape rumen microbial biohydrogenation and then are absorbed in the small intestine into the blood stream. These absorbed long chain fatty acids are deposited into cells such as leucocytes or tissues such as endometrium, liver, and adipose depots (Bilby et al., 2006; Silvestre et al., 2011a).

In a Florida experiment, Silvestre et al. (2011a; 2011b) randomly allocated cows (n = 1,083) into two experimental transition diets beginning at approximately 30 days before the expected day of parturition and continued until 30 days postpartum. After 30 days, cows within each transition diet were allocated randomly into the experimental breeding diets that were fed until 160 days postpartum. Experimental transition and breeding diets differed only in the source of supplemental fatty acids. Transition diets consisted of Ca-LCFA containing palm oil (**PO**; EnerGII; 47% C16:0) or safflower oil (**SO**; Prequel 21; 64% C18:2n-6). The breeding diets consisted of PO or Ca-LCFA containing fish oil (**FO**, StrataG; 11% of C20:5n-3 + C22:6n-3). All Ca-LCFA were supplemented at 1.5% of the dietary DM. The combinational experimental diets fed during the transition and breeding periods were PO-PO, PO-FO, SO-PO, and SO-FO, respectively. Reproductive performance was evaluated extensively for first and second postpartum artificial inseminations after synchronizing the estrous cycles of cows. Neutrophil ratios of n-6:n-3 fatty acids were greater at 35 days postpartum in the SO diet and less at 85 days postpartum in FO compared with PO diets. Cows supplemented with Ca-LCFA containing SO had improved innate measures of immunity during the transition period (i.e., acute phase response, neutrophil function, and

cytokine production) to better cope with the bacterial challenges at calving. Conversely, cows fed Ca-LCFA containing FO had attenuated neutrophil cytokine production at 85 days postpartum, at the time of potential fertilization following a timed artificial insemination.

Transition and breeding diets did not affect pregnancies per artificial insemination at 32 and 60 days after the first insemination (**Table 1**). Pregnancy loss from days 32 to 60 after first timed insemination was less ($P < 0.05$) for cows fed FO compared with that of cows fed PO. At second service (**Table 1**), breeding diet altered ($P < 0.05$) pregnancy per artificial insemination on day 32 and a tendency ($P = 0.10$) for a dietary interaction was detected between transition and breeding diets. The increase in pregnancy per insemination on day 32 caused by feeding FO was greater in cows fed the transition diet containing SO, whereas no increase in pregnancy per insemination in cows fed the PO breeding diet was found, regardless of transition diet. Likewise, a similar transition by breeding diet interaction ($P < 0.05$) was detected for the 60-day pregnancy per insemination. Accumulated proportion of cows pregnant (i.e., all artificial inseminations; **Table 1**) on day 32 after timed artificial insemination was not affected by transition or breeding diets, but tended to be significant ($P = 0.10$) for the interaction of diets. Accumulated proportion pregnant on day 60 after timed artificial insemination was not affected by transition diets, but it was greater ($P < 0.01$) for cows supplemented with FO compared with those supplemented with PO during the breeding period, and a tendency ($P = 0.07$) was observed for interaction between transition and breeding diets. Accumulated pregnancy losses were not affected by transition diets, but were less ($P < 0.01$) in cows supplemented with FO compared with those supplemented with PO). The greater pregnancy per artificial insemination at 32 and 60 d after the second service for FO-fed cows was detected partially during the warm season of the year and appeared to be preferentially beneficial when FO-supplemented cows were fed SO during the transition period before FO feeding (i.e., transition and breeding diet by season interaction). One possibility contributing to the interaction with season was that fewer cows received their second artificial insemination during the cool season (n = 193) than during the warm season (n = 411) of the year.

Sinedino et al., (2017) evaluated the effects of supplementing docosahexaenoic acid (C22:6n-3)-rich algae on reproduction of dairy cows. Holstein cows were assigned randomly to either a control (n = 373) or the same diet supplemented daily with 100 g/cow of an algae product containing 10% C22:6n-3 (algae, n = 366) from 27 to 147 days postpartum. Feeding algae increased resumption of estrous cyclicity (77.6 vs 65.9%) and pregnancy at first artificial insemination (47.6 vs 32.8%) in primiparous cows. Algae increased pregnancy per artificial insemination for all inseminations in both primiparous and multiparous cows (41.6 vs 30.7%), which reduced days to pregnancy by 22 days (102 vs 124 days) compared with control cows. Pregnant cows fed algae had greater expression of *RTP4* in blood leukocytes on day 19 after insemination compared with those in pregnant control cows. The change in expression of *RTP4,* a gene stimulated by conceptus interferon-tau, in cows fed C22:6n-3 rich supports an advancement of day 19 conceptuses in those cows. More advanced conceptuses would produce greater amounts of interferon-tau that would in turn stimulate interferon induced

genes such as *RTP4*. This would be emblematic of an overarching mechanism of cross-talk between the conceptus and maternal unit for embryo development and maintenance of pregnancy. The lack of an algae feeding effect on pregnancy per artificial insemination at the beginning of the breeding period in multiparous cows may be due to the amount of supplemental fatty acids fed was insufficient and longer feeding is necessary in cows with a larger body size and greater milk yield. Indeed, after 120 days of treatments, feeding algae increased pregnancy per artificial insemination and reduced days to pregnancy in both multiparous and primiparous cows. Feeding algae increased the incorporation of C20:5n-3, C22:6n-3, conjugated linoleic acid isomers *cis*-9 *trans*-11, *trans*-10 *cis*-12 and total n-3 fatty acids in phospholipids in plasma and milk fat. Yields of milk and true protein increased by 1.1 kg/day and 30 g/day respectively, whereas fat yield decreased 40 g/day in algae compared with control cows. Supplementing C22:6n-3 rich algae altered the fatty acid composition of lipid fractions and improved reproduction in dairy cows. The benefits on reproduction might be mediated by enhanced embryo development based on changes in interferon-stimulated gene expression.

Zenobi et al (2018) hypothesized that supplementation of ruminally protected choline (**RPC**) in the periparturient period would preferentially benefit those multiparous dairy cows that were more prone to develop a fatty liver postpartum. Experimental objectives were to evaluate the effect of prepartum energy intake on performance of dairy cows supplemented with or without ruminally protected choline (RPC; 0 or 12.9 g/d of choline ion in the form of choline chloride in a rumen-protected form; 0 or 60 g/d of ReaShure, Balchem Corp., New Hampton, NY). At 47 ± 6 days before the expected calving date, 93 parous Holstein cows were assigned randomly to 1 of 4 dietary treatments utilizing a 2 × 2 factorial arrangement of treatments. Cows were fed energy to excess (EXE; 1.63 Mcal of net energy for lactation/kg of diet DM) or to maintenance (MNE; 1.40 Mcal of net energy for lactation/kg of die DM) in *ad libitum* amounts throughout the dry period. The RPC was top-dressed from 17 ± 4.6 d prepartum through 21 d postpartum. After calving, cows were fed the same postpartum diet for the first 15 weeks of lactation that was supplemented with a blood-meal product enriched with lysine and methionine. Liver tissue was sampled at −14, 7, 14, and 21 d relative to parturition. Cows fed EXE or MNE diets, respectively, consumed 40 or 10% more Mcal/d than required at 15 d before parturition. Cows fed the MNE compared with the EXE diet prepartum consumed 1.2 kg/d more DM postpartum but did not produce more milk (41.6 vs.43.1 kg/d). Thus, postpartum cows fed the EXE diet prepartum were in greater mean negative energy balance, tended to have greater mean concentrations of circulating insulin, fatty acids, and β-hydroxybutyrate, and had greater triacylglycerol in liver tissue (8.3 vs. 10.7% of DM) compared with cows fed the MNE diet prepartum.

Cows fed RPC in transition tended to produce more milk (43.5 vs. 41.3 kg/d) and energy-corrected milk (44.2 vs. 42.0 kg/d) without increasing DM intake (23.8 vs. 23.2 kg/d) during the first 15 weeks postpartum. Supplementing RPC to cows resulted in a tendency for increased milk yield over the first 40 weeks postpartum (37.1 vs. 35.0 kg/d), although RPC supplementation stopped at 21 d postpartum. Also, response to RPC was observed regardless of amount of energy consumed during the dry period.

Energy balance of cows fed RPC was more negative at weeks 2, 3, and 6 postpartum, but mean circulating concentrations of fatty acids and β-hydroxybutyrate did not differ from those of cows not fed RPC. Despite differences in energy balance at 2 and 3 weeks postpartum, mean concentration of hepatic triacylglycerol did not differ between RPC treatments. Feeding RPC reduced the daily prevalence of subclinical hypocalcemia from 25.5 to 10.5%, as defined by concentrations of total Ca of <8.0 mg/dL in serum in the first 7 d postpartum. RPC treated cows tended to produce more milk over the first 40 weeks postpartum (37.1 vs. 35.0 kg/d) regardless of amount of energy consumed during the pregnant, nonlactating period.

Supplementation with RPC did not influence the proportion of cows cycling by 40 ±3 DIM (80.5 vs. 80.9% for −RPC and +RPC treatments, respectively). Feeding RPC tended to increase the proportion of cows pregnant at first artificial insemination (P = 0.09; 41.3 vs. 23.6%) but not at 40 weeks postpartum (69.8 vs. 62.5%). The hazard of pregnancy for +RPC and −RPC cows was 1.29 (95% CI = 0.44 to 1.35). The number of days open were 143 versus 123 d for −RPC and +RPC groups, respectively. Cox's proportional analysis of average days to pregnancy from the end of the voluntary waiting period until 210 DIM indicated that treatments did not affect (P = 0.14) rate at which cows became pregnant (**Figure 5**). Collectively, if these coordinated effects programed by RPC in the transition period were confirmed in subsequent experiments by Charlie's group at Florida (Arshad et al., 2020; Bollatti et al., 2020). His work reinforces the case for choline as an essential nutrient during the transition period for high-producing dairy cows.

Programming Effect of Dietary Fatty Acids on Performance of Holstein Heifers from Birth Through First Lactation: "Fatty Dancing: Mom and the Kids"

The concept that strategic feeding dairy cows' specific classes of fatty acids during late pregnancy would have potential programming effects on the newborn calf manifested during subsequent first lactation is a rather novel and insightful concept put forth by *Charlie* and his talented PhD student Dr. Miriam Garcia. This hypothesis was tested with supplementation of essential fatty acids, primarily linoleic acid (C18:2n-6), to prepartum cows during the last 2 months of pregnancy and to their newborn heifer calves. Productive and reproductive responses of heifers were followed for their first 3 years of life (Garcia et al., 2016).

During the last 8 weeks of pregnancy, Holstein cattle (n = 96) were fed either no fat supplement (**CTL**), a saturated fatty acid (**SFA**) supplement enriched in C16:0 and C18:0, or an unsaturated fatty acid supplement enriched in linoleic acid (**EFA**). Newly born heifers (n = 56) from these dams were blocked by dam diet and fed a milk replacer for 60 d with either a low linoleic acid (**LLA**) or high linoleic acid (**HLA**) concentration. The milk replacer was the sole feedstuff during the first 30 days of age. A grain mix with minimal linoleic acid was offered between 31 and 60 days of life. Profile of fatty acids in colostrum reflected that of dam diets. Profiles of fatty acids in plasma of heifers at 30 and 60 days of age reflected those of milk replacers consumed. Heifers fed HLA compared with LLA milk replacer had increased proportions (g/100 g of fatty acids) of

linoleic acid (45.8 vs. 40.7, $P < 0.01$), α-linolenic acid (0.83 vs. 0.66, $P < 0.01$) and lowered proportions ($P < 0.01$) of C12:0 and C14:0 in plasma. The maternal diet fed prepartum also did not change the plasma metabolic profile and productive performance of heifers, but heifers fed HLA compared with LLA milk replacer had or tended to have increased concentrations of anabolic metabolites and hormones coupled with a better body weight gain and greater conversion of dry matter intake into body weight. Heifers born from dams supplemented with fat prepartum tended to have a greater number of artificial inseminations at first pregnancy (2.53 vs. 1.85, P = 0.08). After correcting for predicting transmitting ability of the parents and body weight at calving, heifers born from dams supplemented with fat tended to produce more milk (9,100 vs. 8,415 kg, $P = 0.10$) and more protein (277 vs. 256 kg, $P = 0.09$) in a 305-day standardized lactation. Heifers fed HLA instead of LLA milk replacer produced more milk protein (283 vs. 258 kg, $P = 0.04$) and tended to produce more fat (350 vs. 319 kg, $P = 0.10$), coupled with a numerical increase in total milk yield (9161 vs. 8582 kg, $P = 0.14$). The increased number of artificial inseminations of primiparous diagnosed as pregnant by 300 DIM that were born from dams supplemented with fat (1.61 vs. 2.43) was not significant ($P = 0.14$). **Figure 6** shows the lactation curves for the effect of dam diet. Major effects ($P < 0.10$) due to dam diet were observed in early (weeks 7 to 11) and late lactation (weeks 33 to 43). These weekly milk yields were greater for heifers born from dams fed EFA compared to CTL. The SFA response was intermediate between CTL and EFA. The intermediate differences between SFA with control or EFA were only numerical. Strategic feeding of fatty acids during late uterine life and preweaning appears to enhance milk production of heifers in their first lactation. Future studies evaluating the impact of fat and fatty acid supplementation at different physiological stages of dairy animals on future productive and reproductive efficiency are warranted.

Conclusion

Dr. Charles Richard Staples developed and provided leadership to a basic and applied program in ruminant nutrition that has bridged the disciplines of applied ruminant nutrition, nutritional physiology, statistics, endocrinology and molecular biology. His integrated research efforts have focused primarily on strategic management of dietary nutrients to positively impact reproductive, mammary, digestive, and metabolic tissues to coordinate health, well-being and production performance of lactating dairy cows. He has accomplished this through his leadership as a nutritionist, strong functional participation in interdisciplinary programs, mentorship of graduate students in ruminant nutrition and associated programs at the University of Florida. Furthermore, he has team taught in 3 undergraduate and 2 graduate courses. His breadth of vision, understanding and application to dairy cattle production and management is evident in this presentation today dealing solely with *fatty acids and fertility*. Improved fertility has been achieved through feeding dietary fatty acids leading to recrudescence of ovarian follicle development, ovulation, and programing of the corpus luteum/uterine tissues to support fetal development and programing of both the calf and lactating maternal unit. A presentation on *"Fats and Fertility"* is only one

cornerstone of many in describing Dr. Charles Staples contributions in ruminant nutrition. His humble and giving personality combined with scientific excellence has contributed to his priorities in life: Love of Family, Faith in God and Mankind, and Science.

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Table 1. First, second, and accumulated pregnancy (% and number of cows) per AI at 32 and 60 d after AI and pregnancy loss of cows fed fat supplements in 4 different sequences (Silvestre et al., 2011a)

	Diet[1]				Diet contrast[2] (*P*-value)		
AI	PO-PO	SO-PO	PO-FO	SO-FO	C1	C2	C3
First AI							
d 32	38.7 (107/276)	35.8 (96/268)	39.1 (103/263)	35.8 (89/248)	NS	NS	NS
d 60	33.7 (92/273)	29.7 (79/266)	37.0 (97/262)	32.8 (81/247)	NS	NS	NS
Loss	11.5 (12/104)	15.9 (15/94)	4.9 (5/102)	7.9 (7/88)	NS	<0.05	NS
Second AI							
d 32	27.7 (43/155)	26.7 (41/154)	30.3 (44/154)	43.3 (65/150)	NS	<0.05	0.10
d 60	21.0 (38/152)	22.5 (34/151)	27.3 (39/143)	41.3 (62/150)	NS	<0.01	<0.05
Loss	5.0 (2/40)	10.0 (4/38)	7.1 (3/42)	4.6 (3/65)	NS	NS	NS
All AI							
d 32	54.4 (152/279)	50.5 (138/273)	53.8 (147/273)	59.5 (154/259)	NS	NS	0.10
d 60	48.3 (132/273)	42.5 (114/268)	50.3 (136/270)	55.4 (143/258)	NS	<0.01	0.07
Loss	9.6 (14/146)	14.3 (19/133)	5.5 (8/144)	6.5 (10/153)	NS	<0.01	NS

[1]PO (palm oil; EnerGII); SO (safflower oil; Prequel 21); FO (fish oil; StrataG). All fat supplements were manufactured as calcium salts by Virtus Nutrition, LLC, Corcoran, CA.

[2]Contrasts are C1 (transition diets; PO-PO + PO-FO vs. SO-PO + SO-FO); C2 (breeding diets; PO-PO + SO-PO vs. PO-FO + SO-FO); and C3 (interaction of diets; PO-PO + SO-FO vs. PO-FO + SO-PO). NS = nonsignificant.

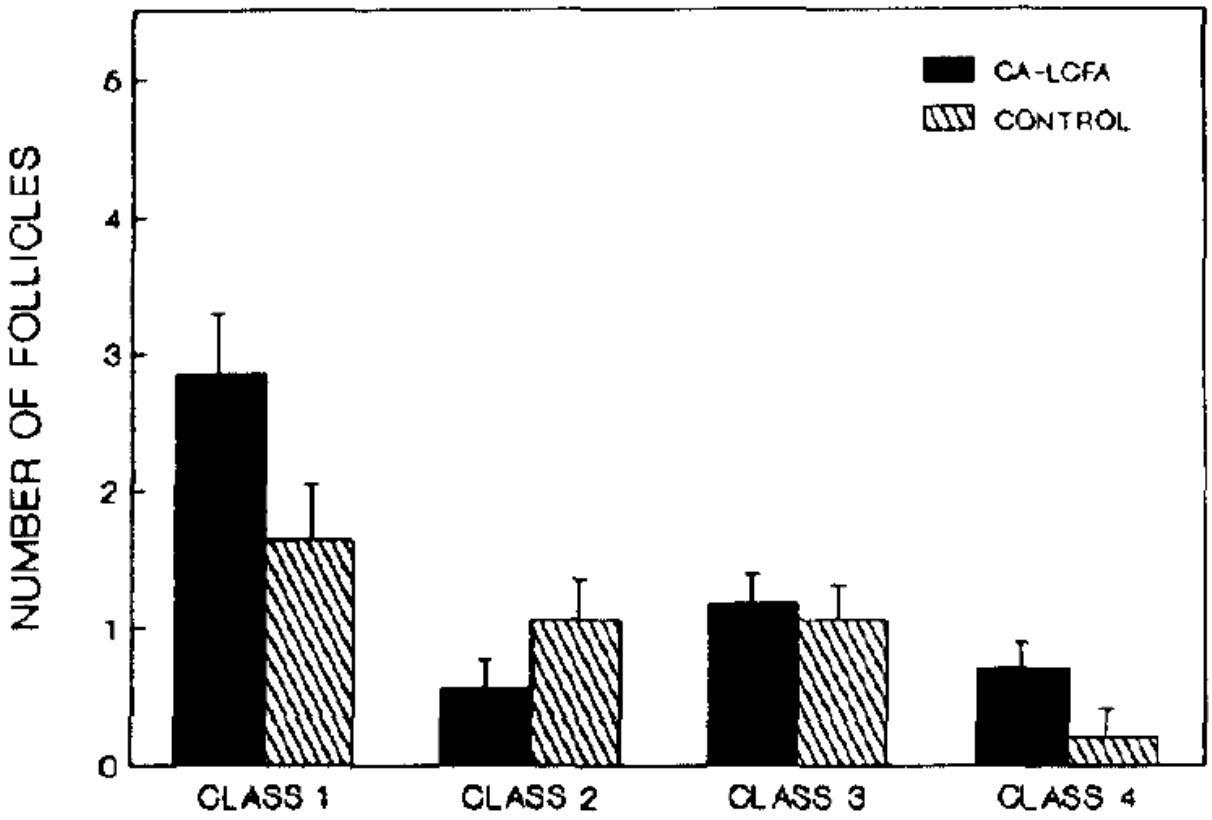

Figure 1. Average number of follicles within different follicle *size* classes (class 1,3 to 5 mm; class 2,6 to 9 mm; class 3, 10 to 15 *mm;* class 4, >15 mm) during an estrous cycle (d 6, 12, and 18) after removal of the CIDR insert for lactating dairy cows fed diets with (CA-LCFA) and without (CONTROL) calcium salts of long-chain fatty acids. Adapted from Lucy et al. (1991).

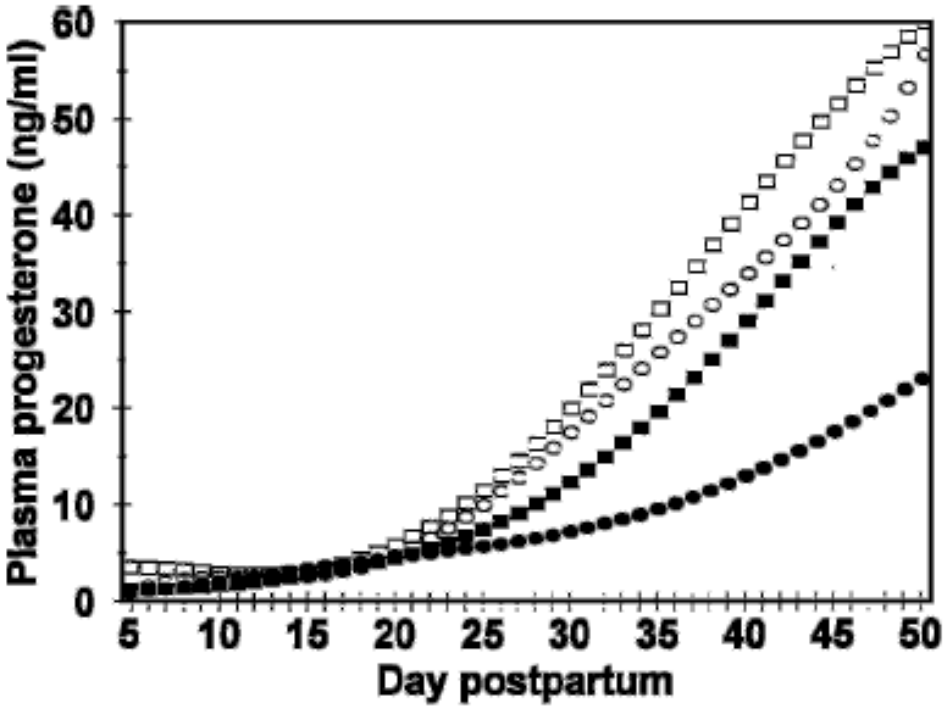

Figure 2. Fifth-order polynomial regression curves of accumulated plasma progesterone concentrations (ng/mL) from lactating Holstein cows fed diets containing 11% degradable intake protein (DIP) and 0% Ca salts of long-chain fatty acids (□), 15.7% DIP and 0% Ca salts of long-chain fatty acids (●), 11.1% DIP and 2.2% Ca salts of long chain fatty acids (○), and 15.7% DIP and 2.2% Ca salts of long chain fatty acids (■). An interaction (*P* = 0.001) between DIP and Ca salts of long chain fatty acids was detected. The standard error of the mean was 0.9. Adapted from Garcia-Bojalil et al. (1998b).

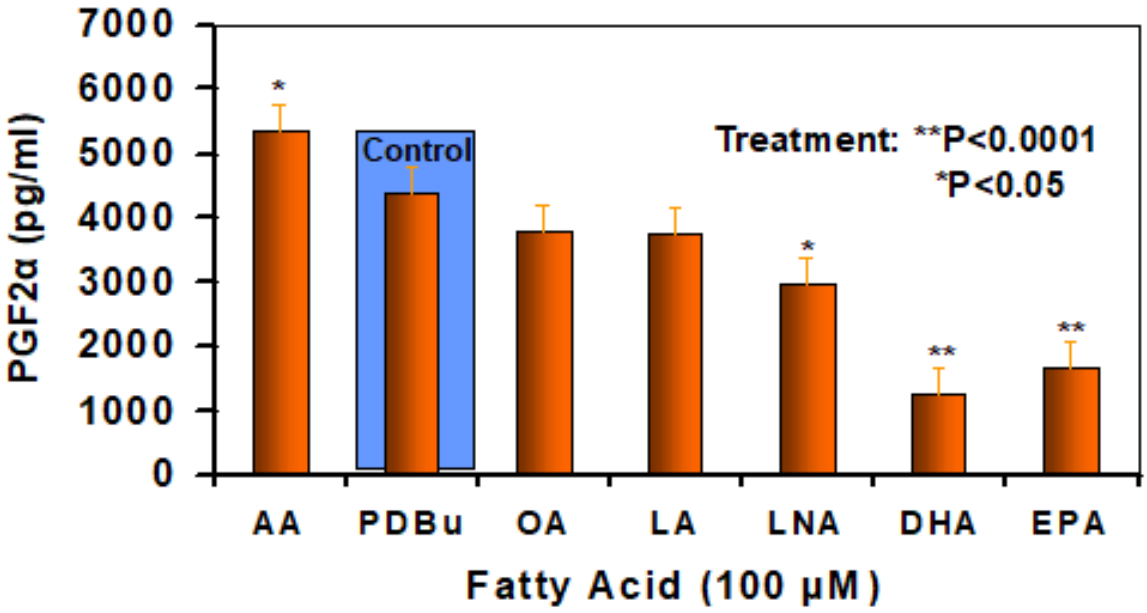

Figure 3. Synthesis of prostaglandin (PG) $F_{2\alpha}$ by bovine endometrial cells incubated with a variety of fatty acids. AA = arachidonic acid; OA = oleic acid; LA = linoleic acid; LNA = linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid. Difference between each fatty acid and control: *P < 0.05; **P <0.01. Adapted from Mattos et al. (2003).

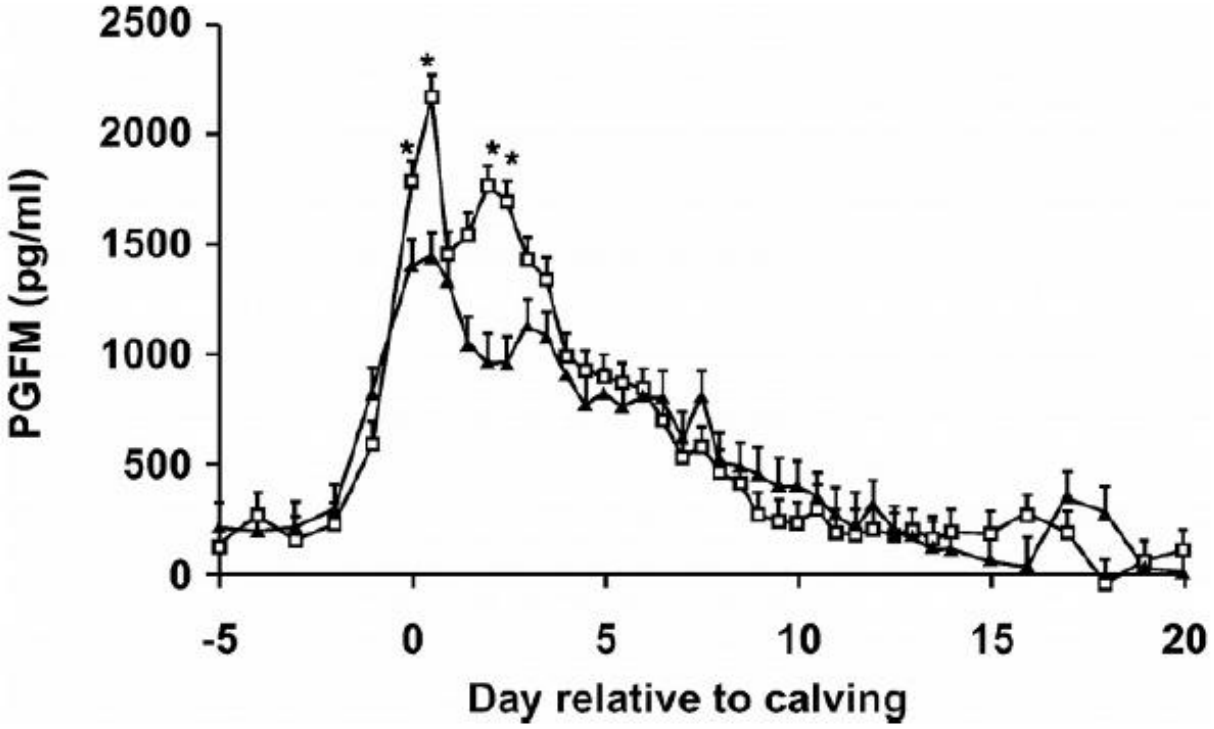

Figure 4. Pre- and postpartum plasma concentrations of prostaglandin$F_{2\alpha}$ metabolite (PGFM) of cows fed fish oil (▲, n = 6) or olive FM concentrations were lower in fed fish oil at 0, 0.5, 2, and 2.5 d after parturition (*, $P < 0.05$). Adapted from Mattos et al. (2004).

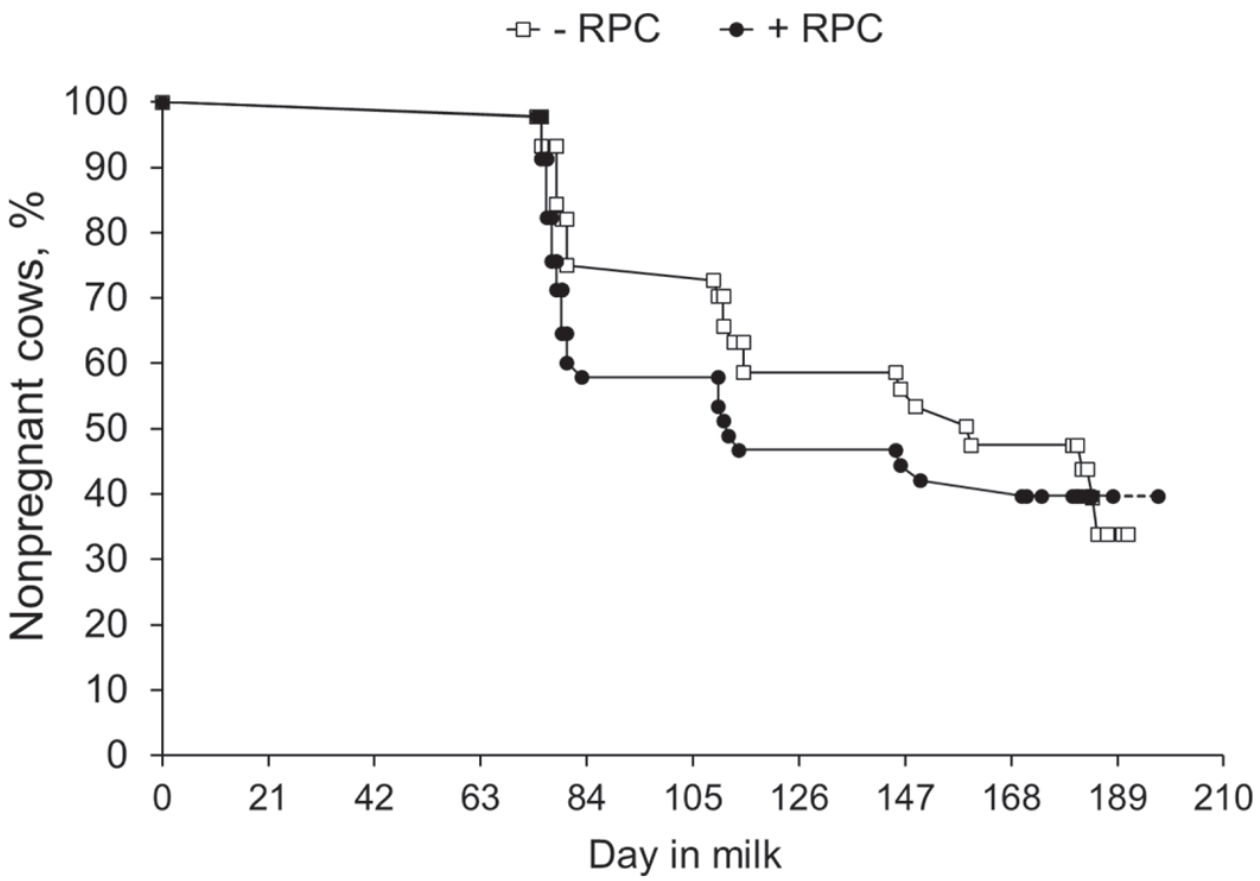

Figure 5. Survival curves for days to pregnancy up to 210 DIM for multiparous Holstein cows supplemented with or without ruminally protected choline (+RPC or –RPC, respectively). Only cow's synchronized and artificial insemination were included in the analysis (n = 91). Effect of RPC (P= 0.14) was not detected. Average (143 vs. 123) and median (160 vs. 112) days to pregnancy for −RPC and +RPC treatments, respectively. Hazard ratio was 1.29 (95% CI = 0.74–2.26). Pregnancy by 210 DIM was 55.4 vs. 59.7% for –RPC vs +RPC, respectively. Adapted from Zenobi et al. (2018).

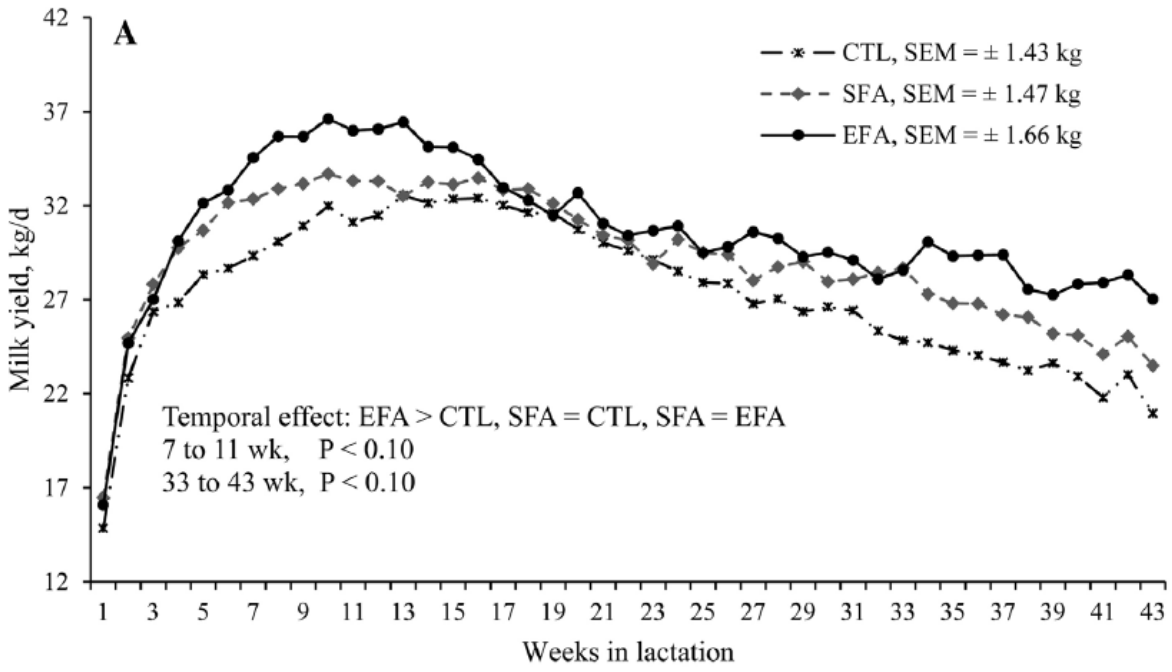

Figure 6. Effect of prepartum dam diets (A) and milk replacer (B) on lactation curves of primiparous Holstein cows fed milk replacer containing low linoleic acid (LLA) or high linoleic acid (HLA) when baby calves from 1 to 60 days of age. Primiparous cows were born from dams fed diets supplemented with no fat (CTL), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 weeks before expected calving date. Adapted from Garcia et al. (2014).