

EFFECT OF FEEDING A RUMINANT GRADE MENHADEN FISH MEAL ON PRODUCTIVE AND REPRODUCTIVE PERFORMANCE OF LACTATING DAIRY COWS

Joan M. Burke, Charles R. Staples, Carlos A. Risco and William W. Thatcher
University of Florida, Gainesville 32611

Introduction

Delivery of fatty acids at various levels for metabolism can influence events important in dairy cow reproduction. A soybean oil emulsion (50% linoleic acid) was infused intravenously into Holstein heifers (Lucy et al., 1990). This resulted in increased plasma concentrations of prostaglandin F 2-alpha (PGF_{2α}) metabolite and altered follicular dynamics; that is, the numbers of ovarian follicles were increased and the size of the largest follicle was greater. In a second study, feeding rumen-protected fat (Megalac®, Church and Dwight Co. Inc., Princeton, NJ) to lactating dairy cows increased the numbers of 3 to 5 mm follicles and follicles greater than 15 mm in diameter and increased the size of the preovulatory follicle of a synchronized estrous cycle during the early postpartum period (Lucy et al. (1991). This effect of Megalac® on increased size of the preovulatory follicle was found in a later study to be the result of the fatty acids themselves rather than by improving energy balance of the cows (Lucy et al., 1993). In addition our lab has documented that feeding Megalac® at the rate of approximately .5 kg/day improved conception rates of lactating Holstein cows from 52 to 86% (Garcia-Bojalil, 1993).

In addition supplemental fat can influence uterine metabolism as well as that of the ovary. Peak plasma concentrations of PGF metabolite in response to a pulse dose (i.v.) of oxytocin on day 15 of an estrous cycle were depressed (48 vs. 90 ng/ml) in lactating dairy cows receiving an abomasal infusion of .45 kg/d of yellow grease (Oldick et al., 1995). Cows receiving yellow grease also demonstrated a larger and a faster growing dominant follicle compared to cows receiving tallow (2% linoleic acid).

Although the fatty acid makeup of the fat sources used in our experiments differed, one shared characteristic was significant concentrations of long chain, unsaturated fatty acids. Likewise fish meal contains approximately 8% fat of which two-thirds is long chain, unsaturated fatty acids. Typically unsaturated fatty acids are biohydrogenated by ruminal microorganisms, therefore preventing their delivery as-is to the lower gut for absorption. However, eicosapentaenoic (20:5n-3) and docosahexaenoic (22:6n-3) fatty acids found in fish oil appear to escape biohydrogenation (Ashes et al., 1992; Palmquist and Kinsy, 1994). Therefore feeding fish meal may result in uptake of these fatty acids for metabolism by reproductive tissues of the lactating cow. Indeed these fatty acids can inhibit cyclooxygenase activity and in turn decrease PGF_{2α} synthesis (Smith and Marnett, 1991).

Feeding fish meal to lactating dairy cows has proven to be beneficial (increased conception rates) although the mechanisms of action are not clear. Both Bruckental et al. (1990) and Armstrong et al. (1989) reported an improvement in conception rate of 20 percentage units by partial replacement of soybean meal with fish meal in the diet. Whether this response was due to a reduction in intake of ruminally degradable protein, an increase in intake of fat, or a combination is not known (Staples et al., 1991; 1993).

Of the available feedstuffs high in undegradable protein, fish meal appears to be more consistently effective in improving milk production than others. It has the greatest combined bypass value for two of the most limiting essential amino acids, methionine and lysine (Shaver et al., 1991). In a summary of eight studies in which corn silage was the main dietary forage source, milk production was increased an average of 1.6 kg/cow per day (Pike et al., 1993). Milk protein content has tended to be increased or unchanged; whereas, milk fat content has tended to be decreased or unchanged. Decreases in milk fat content were more common when the amount of fish meal fed was high (1 to 2.6 kg/d). Excess fish oil intake depressing fiber digestion via toxicity to ruminal microorganisms may account for the lowered fat content of milk. Feeding $\leq .75$ kg/d of fish meal is not likely to depress milk fat content. In addition, Sealac contains greater concentrations of the essential, often limiting amino acids, lysine and methionine, than other concentrated protein feedstuffs.

The objective of this study was to determine the efficacy of dietary fish meal for milk production and composition, body condition score (BCS), blood urea nitrogen (BUN), and reproductive performance of Holstein cows at two dairy farms in Florida.

Experimental Materials and Methods

Dairy A: The experiment was conducted on a Florida dairy herd from December, 1994 to May, 1995 using 341 multiparous lactating Holstein cows. Animals were housed in an open-sided, free-stall barn with a concrete floor and self-locking stanchions. Cows were assigned randomly by staff at the dairy to a control diet ($n = 166$) containing blood meal plus meat and bone meal as ruminally undegradable protein sources or to an experimental diet containing Sealac®, a ruminant grade Menhaden fish meal ($n = 175$; Zapata Protein, Inc., Hammond, LA). Composition of diets is listed in Table 1. Targeted intake of fish meal was 1.5 lb/cow/day. Cows were fed the diets starting at $25 \pm .5$ d postpartum (PP) and continued with the same diet for an average of 88 ± 2 d. Samples of totally mixed ration were collected weekly, dried at 55°C , composited monthly, and sent to Northeast DHIA Forage Testing Laboratory (Cornell University) for chemical analysis.

Cows were milked three times daily. Milk production was measured at one milking per month and daily milk production calculated by DHIA. The maximum number of milk production estimates per cow was five. Composition of milk was not measured.

The voluntary waiting period for breeding as set by the dairy was 60 d PP. At 30 ± 3 d PP, cows were injected with PGF_{2 α} (Lutalyse[®], 25 mg i.m.; The Upjohn Company, Kalamazoo, MI) to regress any existing corpus luteum and potentially increase the number of estrous cycles prior to first insemination. In addition, subsequent dilation of the cervix associated with estrus contributes to optimizing the uterine environment by reducing the occurrences of metritis and pyometra (Risco et al., 1995). Cows were synchronized to estrus with an injection of gonadotropin releasing hormone agonist (GnRHa; Buserelin, 8 μ g i.m.; Hoechst-Roussel Agri-Vet, Somerville, NJ) at 51 ± 3 d PP, followed 7 d later with an injection of PGF_{2 α} (Table 2). The system of estrus detection used in the herd was composed of visual observation of cows for estrus throughout the day, use of KaMar heat mount detectors (KaMar Marketing Group, Portland, ME), and visualization of tail heads that were chalked (All-weather Paintstick, LA-CO Industries, Chicago, IL). Cows were inseminated with frozen/thawed semen (37 bulls used in trial) within 12 h of detected estrus by one of five technicians. Semen source was used randomly across both treatments. Cows which did not show signs of estrus at the first synchrony were resynchronized one week later by injections with GnRHa at 65 ± 3 d PP followed by an injection of PGF_{2 α} 7-d later and bred to observed estrus. Any cows not showing estrus after two synchrony attempts were allowed to come into heat on their own and bred upon observation of standing heat. Cows which were bred at synchrony and did not conceive were rebred when returning to estrus. Pregnancy was diagnosed by palpation of the uterus and its contents per rectum at ≥ 42 d post-insemination.

Dairy B: The experiment was conducted on a Florida dairy herd from January to June, 1995 using 300 multiparous lactating Holstein cows. Animals were housed in an opened-sided, concrete floor barn with old hay and recycled newspapers used as bedding. In addition, cows had 24-hour access to a sandlot and cooling pond. Cows with even-numbered ear tags were assigned to a control diet ($n = 146$) containing a mixture of four ruminally undegradable protein feedstuffs. Cows with odd-numbered ear tags were assigned to the Sealac[®] diet ($n = 154$) as described previously. Composition of diets is listed in Table 1. Feed samples were chemically analyzed as described previously. Cows were fed diets starting at 23 ± 5 d PP and continued with the same diet for 82 ± 2 d.

Cows were milked three times daily. Milk production was measured daily and averaged weekly using the S.A.E. AFIMILK system (Kibbutz Afikim 15148, Israel) for a maximum of 17 weeks per cow. Milk was measured biweekly for fat and protein content. Somatic cell count was measured monthly.

The synchronization and insemination program for the cows synchronized during the first weight weeks of the study (Period 1) was similar to that used at Dairy A, but a timed insemination program was utilized for the cows that were synchronized during the last six weeks ($n = 75$, Period 2). This change in breeding program was due to only a 50% estrus detection rate at first synchrony (Table 3). Previous research from our laboratory indicated that pregnancy and conception rates using a timed insemination program for primiparous and multiparous cows was comparable to

the program followed in Period 1 (Burke et al., 1995). The timed insemination program requires an additional injection of GnRHa 48 h after the PGF_{2α} injection at 58 d PP and cows are inseminated 16 h later without regard to estrus detection (Table 2). In Period 2 a second synchronization was not attempted because all cows were inseminated. The method of estrus detection in Period 1 was a combination of visual observation and pedometer readings using the AFIKIM system. Inseminations were performed by eight technicians using 80 bulls eight hours after the activity (steps per hour) of the cow reached two standard deviations above her average activity. Semen sources were used randomly across diets. Pregnancy diagnosis per rectal palpation occurred ≥ 45 d post-insemination. Pregnancy rate was defined as the proportion of experimental cows that were pregnant. Conception rate was the proportion of cows that were detected in estrus and inseminated that were pregnant.

Determination of body condition scores (BCS), progesterone, and blood urea nitrogen (BUN). Blood samples were collected from the tail vein immediately prior to injecting GnRHa (51 d PP) or PGF_{2α} (58 d PP). During Period 2 additional blood samples were collected from 56 cows at Dairy B just before the second injection of GnRHa to determine the degree of luteolysis. Collected blood samples were held constantly in ice, centrifuged (3,000 x g for 20 min) within 16 h of collection, and plasma was harvested. Plasma was stored at -20°C until analyzed for progesterone (Knickerbocker et al., 1986) and BUN. Cows were considered to have undergone luteolysis when plasma concentrations of progesterone declined to less than 1 ng/ml in the sample collected 48 h after PGF_{2α} injection (60 d PP).

Cows were scored for body condition (BCS; e.g., 1 to 5; Edmonson et al., 1989) between 0 and 10, at 30 ± 3 , and 58 ± 3 d PP for Dairies A and B. A final score was recorded when cows finished the study which was at 104 ± 1 d PP for Dairy A and 96 ± 2 d PP for Dairy B.

Statistical Analyses. Data were analyzed using the General Linear Models (GLM) procedure of SAS (1988). Cows were considered responders to first synchronization if signs of estrus were displayed within 7 d from injection of PGF_{2α}. The mathematical model used to analyze estrus detection and pregnancy rates included diet, month of synchronization (January to May for Dairy A, Periods 1 and 2 for Dairy B), parity, and synchrony (first or second). For analysis of conception rate, technician also was included in the model. For Dairy B, four technicians were pooled together since each inseminated less than 4 cows.

Other reproductive responses evaluated included number of days to first insemination, number of services per conception, overall pregnancy and conception rates by 120 d PP, number of days open for all cows (nonpregnant cows at 120 d PP were assigned a value of 120 d), and number of days open for only those conceiving by 120 d PP. These analyses examined effects of diet, and at Dairy B, period (Period 1 = breed at detected estrus, Period 2 = timed insemination). In addition, the interval between the injection of PGF_{2α} at 58 ± 3 d PP and insemination within 7 d was compared between dietary groups.

Body condition scores at 0 to 10, 30, and 58 d PP, and final BCS were analyzed using GLM with treatment, parity, and synchrony as independent variables for Dairy A. In addition to this mathematical model, a separate analysis included only diet and parity in order to include cows in Period 2 at Dairy B.

Regression analyses (SAS, 1988) was used to evaluate the relationships between BCS at 58 d PP and estrus detection, pregnancy rate, and conception rate as dependent variables, adjusted for the appropriate independent variables (e.g. diet, parity). Synchronization was included in the mathematical model; cows synchronized only once were compared with those cows not detected in estrus and resynchronized. Body condition scores were included in separate analyses as a continuous independent variable to examine their association with the least squares mean of milk production for the experimental period.

Data for milk production and composition was analyzed using GLM procedure (1988). The mathematical model included diet, cow within diet by parity, days in milk, and interactions. Orthogonal contrasts were calculated to determine effect of parity, diet, and diet by parity interactions. Analyses were performed that utilized the mature equivalent milk for all previous lactations as well as the cows milk weight measured just prior to start of the experimental diets as covariates. The response variable was the least squares mean for milk production for the experiment adjusted for days in milk.

RESULTS AND DISCUSSION

Reproductive responses. Pregnancy rate of a herd is a product of estrus detection rate and conception rate. How effective was the synchrony system in bringing cows into estrus? Fifty to 65% of the cows were detected in estrus following the program that was initiated at 51 ± 3 d PP (Table 3). Based on the number of cows at 51 d PP with concentrations of plasma progesterone of > 1 ng/ml and the number of cows expected to be in the nonluteal phase of the estrous cycle (23.8%), the proportion of cows estimated to be cycling at Dairy A was 97% but at Dairy B was only 81%. By 58 d PP (7 days after GnRHa injection), the number of cows having plasma progesterone concentrations > 1 ng/ml increased such that the proportion of cows estimated to be cycling was 100% for both dairies. Therefore the synchrony program used was effective to initiate ovarian activity for those anestrus cows at Dairy B. Cows which did not show signs of estrus at the first synchrony were resynchronized. Again a good percentage, 53 to 78%, showed signs of estrus. Therefore approximately 84 and 79% of the cows fed the control and Sealac® diets at Dairy A and 79 and 89% of the cows fed the control and Sealac® diets at Dairy B were inseminated between 55 and 78 days PP using the GnRHa, PGF_{2α}, and bred to standing heat system. In addition, all cows ($n = 75$) in Period 2 at Dairy B were bred at 61 d PP using the timed insemination system.

Estrus detection rates were not different between cows fed the two diets nor between the two synchronies at Dairy A. However estrus detection rate at the second

synchrony increased by a greater magnitude for cows fed Sealac® (50.6 vs. 78.3%) compared to control cows (50 vs. 58.0%; treatment by synchronization interaction, $P < .10$; Table 3). It appears that an additional two weeks of consumption of Sealac® improved expression of estrus at Dairy B.

Diet did not affect conception or pregnancy rates when cows were bred after synchrony of estrus nor when cumulative conception or pregnancy rates were determined by 120 d PP at Dairy A (Table 3). Overall conception and pregnancy rates were appreciably greater at Dairy A. At Dairy B, where fertility was lower, cows fed Sealac® tended to have greater ($P < .10$) overall pregnancy rates (39.5 vs. 30.6%).

Other reproductive measurements including number of days open, number of services per conception, number of days to first insemination, interval from injection of PGF_{2α} to insemination, number of observed heats prior to insemination, and proportion of cows detected in estrus prior to synchronization were unaffected by diet.

Some brief comments need to be made regarding the two reproductive management systems used in this study. Pregnancy rate to the first service was not different between cows at Dairy B that were timed inseminated and those that were inseminated at detected estrus; however conception rate was lowered by timed insemination ($33.1 \pm 5.3\%$ vs. $16.3 \pm 8.1\%$, $P < .07$). A lower conception rate for timed inseminated cow groups is expected because all cows are inseminated, including cows that may not have been cycling, or those that would not normally have responded to synchronization.

Month of the experiment did influence some reproductive measurements. Overall pregnancy ($P < .001$) and conception rates ($P < .001$) by 120 d PP declined and the number of days to first insemination increased ($P < .02$) as the study progressed at Dairy A, possibly due to a progressive increase in environmental temperature. Estrus detection rates during synchrony declined progressively ($P < .005$) from January ($72.8 \pm 5.6\%$) to April ($46.0 \pm 6.8\%$) at Dairy A perhaps due to changes in environmental temperature, management, or both. However pregnancy and conception rates were similar over time, indicative that fertility was not affected by environmental temperature.

Efficacy of Corpus Luteum Regression by PGF_{2α} Injection. The injection of PGF_{2α} acts to regress the corpus luteum (luteolysis). This regression assists with the final development of a newly recruited follicle induced by GnRHa injection. The very long chain fatty acids found in fish meal, eicosapentaenoic (20:5) and docosahexaenoic (22:6) acids, have been shown to inhibit synthesis of prostaglandin by ram seminal vesicles (Corey et al., 1983; Smith and Marnett, 1991). Therefore ingesting these fatty acids potentially could inhibit prostaglandin synthesis in the lactating dairy cow. If this occurred, dynamics of corpus luteum regression may be altered since uterine endogenous secretion of PGF_{2α} may be reduced and corpus luteum regression would be more dependent on the injected PGF_{2α}. Interestingly, blood samples collected 2 d after injection of PGF_{2α} (60-d PP) in Period 2 (Dairy B)

470 (P < .025)
revealed that plasma concentrations of progesterone tended to be greater ($P < .11$) in cows fed Sealac® (1.3 vs. $.6 \pm .3$ ng/ml). The proportion of cows with concentrations of plasma progesterone greater than 1 ng/ml was greater when Sealac® was fed compared to the control diet (30 vs. 5%, Figure 1), suggesting that Sealac® altered the dynamics of corpus luteum regression induced by the injection of PGF_{2α}. Regression of the corpus luteum was completed eventually based upon similarities in 1) estrus detection rates between diets and 2) number of days from PGF_{2α} injection and insemination.

Linoleic acid also has been shown to inhibit prostaglandin synthesis (Pace-Asciak and Wolfe, 1968). Abomasal infusion of yellow grease (17% linoleic acid) reduced the oxytocin-induced release of PGF_{2α} from the uterus compared with cows infused with tallow (2% linoleic acid), glucose, or water (Oldick et al., 1995).

Body condition scores. At Dairy A, cows scored 3.4 at calving, lost .4 BCS units by 30 d PP, and had essentially returned to their starting body condition by 104 d PP (Table 4). Parity influenced body condition as measured at 0 to 10 d PP ($P < .02$), 30 ± 3 d PP ($P < .02$), 58 ± 3 d PP ($P < .008$), and at 104 d PP ($P < .003$; Figure 2). Cows in their fourth lactation did not regain their body condition at calving by the end of the study as did the cows in the other parities. Cows in their fourth parity lost condition for a longer period of time (through 58 d PP) than did other parities and were thinnest at the end of the study. Cows in this parity produced the most milk of any parity group. They likely were relying more heavily on energy reserves to support the milk they produced. Second parity cows were among the thinnest throughout the study. These cows produced less milk (> 5 lb/d) during the trial as discussed later. Their requirement for growth plus a lack of energy reserves likely contributed to a lower milk production. Cows in their second or fourth parity were in lower body condition of all parities at the end of the study. Dietary treatment did not affect BCS at any time during the experiment.

Cows at Dairy B lost more condition than cows at Dairy A and did not return to their initial body condition by the end of the study as did the cows at Dairy A (Table 4). At 96 d post calving, cows were still .3 to .6 score units below their score at calving. Less condition during breeding may have contributed to the lower conception rates recorded at Dairy B (Table 3). In addition, cows fed Sealac® were in leaner body condition (2.7 vs. 2.9 BCS, $P < .03$) at 58 d PP (Table 4). However these cows appeared to be leaner at calving as well although different scores at calving were not significant. Body condition of cows was similar among parities at each point of measurement at Dairy B.

Body condition at 0 to 10 d PP positively influenced milk production of cows at Dairy B. For every increase in BCS at this time, mean milk production increased 6.1 pounds over the experimental period ($Y = 82.1 + 6.1X$, $R^2 = .04$, $P < .01$). Body condition score at 58 d PP was quadratically related to milk production. Milk production was maximized (103.5 lb/d) by cows scoring 2.5 at 58 d PP ($Y = 73.0 + 24.33X - 4.90X^2$, $P < .05$, $R^2 = .03$; Figure 3). Thinner cows (1.7 BCS) and more

conditioned cows (3.3 BCS) at the time of breeding produced less milk. These results would support the idea that overconditioned cows as well as underconditioned cows at calving result in lowered milk production.

Body condition influenced reproductive responses at both Dairies A and B. At Dairy A, the BCS of cows requiring only one synchronization to initiate estrus behavior was greater (about .2 BCS units) at 30 ($P < .04$), 58 ($P < .001$), and 104 d PP ($P < .005$) compared with cows failing to show signs of estrus at the first synchrony and thus requiring a second synchrony (Figure 4). In a like fashion, BCS at 58 d PP was related positively ($P < .005$) to the estrus detection rate for cows synchronized a second time ($y_{\text{sync2}} = -14.1 + 23.84x$, $P < .01$, $R^2 = .05$) whereas BCS had little relationship to a successful first synchrony ($y_{\text{sync1}} = 98.9 + 1.39x$). For every .5 BCS unit increase at 58 d PP for cows synchronized twice, estrus expression was increased 11.9% compared to a .7% increase in cows synchronized once. A similar positive relationship existed between BCS at 58 d PP and pregnancy rate for cows synchronized twice versus only once at Dairy A, that is, $y_{\text{sync1}} = 46.7 + 1.43x$ vs. $y_{\text{sync2}} = -53.9 + 26.42x$ ($P < .01$, $R^2 = .04$). The same held true for conception rate with equations being $y_{\text{sync1}} = 39.0 - 1.53x$ vs. $y_{\text{sync2}} = -54.6 + 29.20x$, $P < .05$, $R^2 = .03$. For every .5 BCS unit increase at 58 d PP for cows synchronized twice, pregnancy rate increased 13.2% and conception rate increased 14.6%. This suggests that among cows that failed to respond to first synchrony, cows with lower body condition at 58 d PP were meeting metabolic demands for lactation prior to that for reproduction. Cows with lower BCS were less likely to express behavioral estrus when synchronized and perhaps fertility was impaired.

At Dairy B the relationship of BCS to estrus detection, pregnancy rate, or conception rate was not different between synchronies. For every .5 BCS unit increase at 58 d PP, pregnancy rate increased 7% ($y = -17.94 + 13.97x$, $P < .03$, $R^2 = .03$). Similarly, BCS at 58 d PP tended to be positively related to estrus detection rate ($P < .10$) as well as to conception rate ($P < .09$).

Blood Urea Nitrogen. At the time of first estrus synchronization, BUN values were similar between cows fed the two experimental diets (Table 4). This was true for both dairy farms. However cows at Dairy B appeared to have consistently greater concentrations of BUN compared to cows at Dairy A (21.3 vs. 17.2 mg/100 ml of plasma). Greater concentrations of BUN at Dairy B may have resulted from the use of lacto-whey in the diet which contains not less than 42% ammonium lactate. Blood samples taken at the end of the trial indicated that BUN values of cows fed Sealac® were lower ($P < .07$) at Dairy A, 17.0 vs. 16.4 mg/100 ml of plasma whereas no difference in BUN values were observed for cows at Dairy B at the end of the study. This .6 mg/100 ml difference likely was not of physiological significance. The relationship of BUN to milk production was not significant ($P > .10$) at either dairy farm. However at Dairy A, overall pregnancy rate tended to be decreased ($P < .12$) as BUN values taken at the end of the study increased. The relationship was $Y_{\text{preg}} = 93.6 - 1.8X$ ($R^2 = .01$) so that overall pregnancy rate declined 1.8 percentage units for every 1 unit increase in BUN concentration.

Feed intake and milk production and composition. Feed intake was very good at both dairy farms. Intake was 54.9 and 55.5 lb/d DM at Dairy A and 52.5 and 53.4 lb/d at Dairy B for cows fed control or Sealac®-supplemented diets respectively. Because cows were fed in groups and not individually, DM intake data could not be analyzed statistically.

Milk production was excellent at both dairy farms, averaging approximately 94 and 103 lb/cow/day at Dairies A and B respectively over the duration of the experiment (Table 5). As expected cows of different parities differed in milk produced ($P < .002$) with cows in their second parity being less productive. Diet did not influence milk production at Dairy A. However diet did influence milk production of cows at Dairy B but the effect was influenced by parity of the cows (diet by parity interaction, $P < .04$). Cows in their second lactation produced 5.1 lb/day more milk whereas cows in their fourth lactation produced 6.8 lb/day less milk compared to the control cows when Sealac® was included in the diet. If essential amino acids such as methionine and lysine coming from Sealac® are contributing to improvements in milk production, it might be predicted that cows in their first and/or second lactation might be the most responsive as they require amino acids for both growth and lactation. However Robert et al. (1994) reported multiparous cows to be more responsive (milk production) to rumen-protected methionine supplementation than primiparous cows. No primiparous cows were used in the current study.

When milk production was adjusted using each cow's previous mature equivalent weight as a covariate, dietary effects on milk production remained the same (Table 6). Milk production was unaffected by feeding Sealac® at Dairy A whereas milk production by second parity cows was stimulated by 5.0 lb/cow/day at Dairy B. Older cows did not respond to inclusion of Sealac® in the diet (diet by second vs. older cows interaction, $P = .0142$).

Using the milk weight immediately prior to assignment of cows to a particular diet as a covariate, the effect of Sealac® on milk production by second parity cows at Dairy B disappeared (Table 7). The positive response seen previously for second parity cows was reduced from 5.1 to 2.4 lb/d whereas the negative response for fourth parity cows was reduced from 6.8 to 1.1 lb/d. However the number of cows in each treatment was reduced substantially because many cows were started on experimental diets before a milk production was measured. Therefore cow numbers were reduced 44 and 33% for dairies A and B, respectively.

Only Dairy B analyzed milk for fat and protein content. Milk fat content averaged 3.19 and 3.27% for Dairies A and B across the experimental period (Table 8). Diet did not influence milk fat content although cows at each parity with the exception of the fourth parity produced milk of numerically greater fat content when fed Sealac®. This effect contributed to improved production of fat by all parities of cows except those in their fourth lactation (diet by parity interaction, $P = .0577$; Table 8).

Cows fed Sealac® produced 2.1 lb/d more 4% fat-corrected milk when averaged across all parities than controls (Table 9). Only cows in their fourth parity failed to produce more fat-corrected milk when fed Sealac® (diet by parity interaction, $P = .0442$).

Milk protein content usually has been a more sensitive variable than milk production in amino acid requirement studies. Fish meal has improved milk protein concentration in previous studies (Pike et al., 1993). Although milk protein concentration was not improved in the current study, production of milk protein tended to increase ($P = .1062$) when Sealac® replaced corn gluten meal, blood meal, and meat and bone meal in the diet (Table 10).

SUMMARY

Inclusion of fish oil in the diet appears to result in an alteration in regression dynamics of the corpus luteum as evidenced by a greater proportion of cows having elevated concentration of plasma progesterone post injection of $\text{PGF}_{2\alpha}$. Perhaps the increase in conception rate at Dairy B by cows fed Sealac® (39.5 vs. 30.6%; $P < .10$) could be attributable to increased survival of the embryo at the time of pregnancy recognition (e.g. when $\text{PGF}_{2\alpha}$ secretion is suppressed). Such an effect did not appear to be evident in Dairy A where herd fertility was greater. Lowered fertility at Dairy B may have resulted from greater duration of lower body condition. Body condition of cows at both dairies were related positively to estrus detection rate and conception rate. At Dairy A, BUN values were related negatively to overall pregnancy rate.

Replacement of typical undegradable protein sources (eg. blood meal, meat and bone meal, and corn gluten meal) with a ruminant grade Menhaden fish meal, fed at approximately 1.5 lb/day DM, resulted in positive benefits to animal performance. Cows fed Sealac® at Dairy B averaged greater production of fat, fat-corrected milk, and protein over the entire early postpartum period. Cows in their fourth lactation (16% of experimental cows) failed to produce more fat and fat-corrected milk when fed supplemental fish meal even though younger and older cows responded positively. Daily production of uncorrected milk was improved by second lactation cows only (38% of cows on trial). Sealac® provided no benefit to production at Dairy A; milk composition was not determined at this dairy farm. Differences for this response between dairies may be due to differences in milk production (10% more milk at Dairy B resulting in a greater amino acid requirement), inability of cows to regain lost body condition at the end of the study resulting in a greater reliance on dietary amino acids and less on tissue amino acids at Dairy B, or the replacement of a low lysine (corn gluten meal) with a high lysine (fish meal) protein feedstuff at Dairy B.

ACKNOWLEDGMENTS

Authors acknowledge the financial support for this work coming from the USDA,

International Fish Oil & Meal Manufacturers Association, Zapata Protein, Inc., The Upjohn Co., and Hoechst-Roussel Agri-Vet. Estelle Hirschert, Jesse Elliott, Jesse Johnson, Luzbel de la Sota, Eric Schmitt, and Monty Meyer at the University of Florida provided valuable technical assistance to the completion of the study. Many thanks to the owners, managers, and workers at the two Florida dairy farms for their cooperation and hard work in carrying out the study.

REFERENCES

Armstrong, J.D., E.A. Goodall, F.J. Gordon, D.A. Rice, and W.J. McCaughey. 1990. The effects of levels of concentrate offered and inclusion of maize gluten or fish meal in the concentrate on reproductive performance and blood parameters of dairy cows. *Anim. Prod.* 50:1.

Ashes, J. R., B.D. Siebert, S.K. Gulati, A.Z. Cuthbertson, and T.W. Scott. 1992. Incorporation of n-3 fatty acids of fish oil into tissue and serum lipids of ruminants. *Lipids*. Vol. 27. No. 8. Page 629.

Bruckental, I., D. Drori, M. Kalm, H. Lehrer, and Y. Folman. 1989. Effects of source and level of protein on milk yield and reproductive performance of high-producing primiparous and multiparous dairy cows. *Anim. Prod.* 48:319.

Burke, J.M., R.L. de la Sota, C.A. Risco, C.R. Staples, E.J.-P. Schmitt, W.W. Thatcher. 1995. Evaluation of timed insemination using a gonadotropin-releasing hormone agonist in lactating dairy cows. *J. Dairy Sci.* (Submitted).

Corey, E. J., C. Shih, and J. R. Cashman. 1983. Docosahexaenoic acid is a strong inhibitor of prostaglandin but not leukotriene biosynthesis. *Proc. Natl. Acad. Sci. USA* 80:3581.

Garcia-Bojall, C.M. 1993. Reproductive, productive, and immunological responses of Holstein dairy cows fed diets varying in concentration and ruminal degradability of protein and supplemented with ruminally inert fat. Ph.D. Dissertation. Univ. Florida. Gainesville.

Edmonson, A. J., I. J. Lean, L. D. Weaver, T. Farver, and G. Webster. 1989. A body condition scoring chart for Holstein dairy cows. *J. Dairy Sci.* 72:68.

Knickerbocker, J. J., W. W. Thatcher, F.W. Bazer, M. Drost, D. H. Barron, K. B. Fincher, and R. M. Roberts. 1986. Proteins secreted by day 16 to 18 conceptuses extend corpus luteum function in cows. *J. Reprod. Fertil.* 77:381.

Lucy, M.C., T.S. Gross, and W.W. Thatcher. 1990. Effect of intravenous infusion of soybean oil emulsion on plasma concentration of 15-keto-13,14-dihydro-prostaglandin F 2-alpha and ovarian function in cycling Holstein heifers. *Livestock Repro. Latin Amer., Internat. Atomic Energy Agency, Vienna.* p. 119.

Lucy, M.C., C.R. Staples, F.M. Michel, and W.W. Thatcher. 1991. Effect of feeding calcium soaps to early postpartum dairy cows on plasma prostaglandin F 2-alpha, luteinizing hormone, and follicular growth. *J. Dairy Sci.* 74:483.

Lucy, M.C., R.L. De La Sota, C.R. Staples, and W.W. Thatcher. 1993. Ovarian follicular populations in lactating dairy cows treated with recombinant bovine somatotropin (Sometribove) or saline and fed diets differing in fat content and energy. *J. Dairy Sci.* 76:1014.

Oldick, B.S., C.R. Staples, W.W. Thatcher, and P. Gyawu. 1994. Abomasal infusion of glucose and fats-effect on digestion, production, and ovarian and uterine function of cows. J. Dairy Sci. Submitted.

Pace-Asciak, C. and L. S. Wolfe. 1968. Inhibition of prostaglandin synthesis by oleic, linoleic and linolenic acids. Biochim. Biophys. Acta 152:787.

Palmquist, D.L. and D.J. Kinsey. 1994. Lipolysis and biohydrogenation of fish oil by ruminal microorganisms. J. Dairy Sci. (Suppl. 1) 77:350 (Abstr.).

Pike, I.H., E.L. Miller, and K. Short. 1993. The role of fish meal in dairy cow feeding. Draft Tech. Bulletin No. 27. Internat. Assoc. Fish Meal Manufacturers. Hertfordshire, England.

Risco, C. A., R. L. de la Sota, G. Morris, J. D. Savio, and W. W. Thatcher. 1995. Postpartum reproductive management of dairy cows in a large Florida dairy herd. Theriogenology 43:1249.

Robert, J.C., B.K. Sloan, and S. Bourdeau. 1994. The effects of supplementation of corn silage plus soybean meal diets with rumen protected methionine on the lactational performance of dairy cows in early lactation. J. Dairy Sci. 77(Suppl. 1):92 (Abstr.).

SAS®. 1988. In: SAS/STAT™ User's Guide (Release 6.03 Ed.) SAS Inst., Inc., Cary, NC.

Smith, W. L. and L. J. Marnett. 1991. Review. Prostaglandin endoperoxide synthase: structure and catalysis. Biochim. Biophys. Acta 1083:1.

Shaver, Randy, Bob Kaiser, and Dave Combs. 1991. How fish meal compares as bypass protein. Page 459 in Hoards Dairymen, May 25.

Smith, W.L. and L.J. Marnett. 1991. Prostaglandin endoperoxide synthase: structure and catalysis. Biochim. Biophys. Acta 1083:1.

Staples, C.R., Carlos Garcia-Bojaili, Bradley S. Oldick, William W. Thatcher, and Carlos A. Risco. 1993. Protein intake and reproductive performance of dairy cows: A review, a suggested mechanism, and blood and milk urea measurements. Page 37 in Proc. Fourth Annual Florida Ruminant Nutr. Symp., Univ. Florida, Gainesville, FL.

Staples, C.R., W.W. Thatcher, and M.C. Lucy. 1991. Effect of increasing dietary fat content on production and fertility of lactating dairy cows. Page 89 in Proc. Second Annual Florida Ruminant Nutr. Symp., Univ. Florida, Gainesville, FL.

Table 1. Ingredient and chemical composition of experimental diets used at Dairies A and B.

Ingredient	Dairy A		Dairy B	
	Control	Sealac®	Control	Sealac®
Corn silage	21.5	21.5	21.8	21.8
Alfalfa hay	7.2	7.2	5.4	5.4
Cottonseed hulls	-	-	5.6	5.6
Bermudagrass hay	4.7	4.7	-	-
Hominy	30.9	30.9	34.0	34.0
Whole cottonseed	12.6	12.6	8.7	8.7
Wet brewers grains	5.4	5.4	11.2	11.2
Lacto-whey	-	-	3.7	3.7
Sealac®	-	2.7	-	2.8
Soybean meal	7.7	7.7	2.0	2.0
Meat and bone meal	.9	-	.3	-
Blood meal	.9	-	.5	-
Corn gluten meal	-	-	.7	-
Minerals & Vitamins	5.0	4.1	6.1	4.8
Chemical composition				
Dry matter	58.1	58.1	52.9	52.9
NEL, Mcal/kg	1.71	1.71	1.71	1.71
Crude protein, % DM	18.1	18.1	19.8	19.5
UIP, % DM	7.0	7.1	6.9	6.9
Fat, % DM	5.6	5.7	6.7	6.7
Neutral detergent fiber, % DM	28.9	28.8	35.8	35.1
Acid detergent fiber, % DM	18.3	18.3	18.9	18.4
Calcium	.98	1.01	1.05	1.00
Phosphorus	.52	.57	.52	.52
Potassium	1.4	1.4	1.6	1.5
Magnesium	18.3	18.3	18.9	18.4

Table 2. Synchronization and insemination program for Dairy A (Period 1 only) and Dairy B.

Range of d PP for injection	Period 1		Period 2	
	Injection	Approximate time	Injection	Approximate time
30 ± 3	PGF _{2α} ¹	1200 h	PGF _{2α}	1200 h
51 ± 3	GnRHa ²	1200 h	GnRHa	1200 h
58 ± 3	PGF _{2α}	1200 h	PGF _{2α}	1200 h
60 ± 3			GnRHa	1200 h
61 ± 3	AI at detected estrus		Timed AI	0600 h
65 ± 3 ³	GnRHa	1200 h		
72 ± 3	PGF _{2α}	1200 h		
	AI at detected estrus			

¹Prostaglandin F 2-alpha (Lutalyse).

²Gonadotropin releasing hormone (Buserelin).

³Cows not detected in estrus were resynchronized at 65 d PP, Period 1.

Table 3. Estrus detection, conception and pregnancy rates and other reproductive responses of cows consuming control or Sealac diets. Standard errors follow means. Least square means are adjusted for appropriate effects in model.

Response	Dairy A		Dairy B	
	Control	Sealac	Control	Sealac
Cows in study	166	175	146	154
Estrus detection rate				
First Synchrony	65.3 ± 4.7 (n = 166)	57.1 ± 4.8 (n = 175)	50.0 ± 5.2† (n = 109)	50.6 ± 5.7† (n = 116)
Second Synchrony	58.7 ± 7.1 (n = 57)	53.3 ± 6.4 (n = 76)	58.0 ± 8.2 (n = 40)	78.3 ± 7.6 (n = 58)
Pregnancy rate				
First Synchrony	28.7 ± 4.2 (n = 166)	20.7 ± 4.2 (n = 175)	14.4 ± 3.8 (n = 109)	18.4 ± 4.2 (n = 116)
Second Synchrony	23.5 ± 6.3 (n = 56)	24.1 ± 5.7 (n = 76)	10.2 ± 6.0 (n = 40)	18.4 ± 4.2 (n = 58)
Conception rate				
First Synchrony	40.1 ± 6.4 (n = 109)	32.9 ± 6.8 (n = 99)	31.0 ± 7.6 ‡ (n = 56)	37.8 ± 7.8 ‡ 29.8 (n = 50)
Second Synchrony	36.4 ± 10.1 (n = 31)	38.5 ± 9.5 (n = 39)	11.0 ± 11.6 30.5 (n = 23)	26.2 ± 9.2 13.8 (n = 40)
Overall pregnancy rate to 120 d PP	64.8 ± 5.8 (n = 161)	65.4 ± 6.1 (n = 174)	30.6 ± 5.3 (n = 144)	39.5 ± 5.8* (n = 150)
Overall conception rate to 120 d PP	66.5 ± 5.7 (n = 155)	70.1 ± 6.1 (n = 159)	33.4 ± 6.5 (n = 133)	41.3 ± 6.4 (n = 141)
First insemination only	42.3 ± 6.2 (n = 159)	40.9 ± 6.6 (n = 160)	19.7 ± 4.8 (n = 133)	22.1 ± 5.2 (n = 150)
Days open to 120 d PP, All cows in study	92.1 ± 3.0 (n = 161)	92.2 ± 3.1 (n = 174)	105.7 ± 2.6 (n = 144)	102.8 ± 2.8 (n = 150)
<u>Only cows that conceived</u>	78.0 ± 2.8 (n = 106)	79.5 ± 2.5 (n = 105)	74.0 ± 2.8 (n = 46)	71.5 ± 2.5 (n = 62)
Services per conception	1.5 ± .10 (n = 106)	1.4 ± .09 (n = 105)	1.4 ± .12 (n = 46)	1.4 ± .10 (n = 62)
Days to first insemination ^a	69.4 ± 2.2 (n = 166)	74.0 ± 2.3 (n = 175)	64.8 ± 1.3 (n = 133)	64.6 ± 1.4 (n = 141)
Interval from PGF _{2α} to insemination	3.22 ± .12 (n = 103)	3.22 ± .13 (n = 97)	3.06 ± .14 (n = 88)	3.12 ± .14 (n = 87)
Number of heats prior to insemination	.53 ± .05 (n = 166)	.43 ± .05 (n = 174)	NE ^b	NE
Cows detected in estrus prior to 48 d PP	40.9 ± 3.7 (n = 166)	42.7 ± 3.7 (176)	NE	NE
Progesterone (ng/ml) 2 d post PGF _{2α}	NE	NE	.57 ± .3 (n = 25)	1.26 ± .3 (n = 31)

^aDays to first service includes all cows that were inseminated by 120 d PP.

^bNot estimated.

†Column means within Dairy differ for synchrony, $P < .04$, and diet by synchronization interaction detected, $P < .10$.

‡Column means within Dairy differ for synchrony, $P < .07$.

*Diet effect, $P < .10$.

Table 4. Body condition scores and blood urea nitrogen concentrations of cows consuming control or Sealac® diets at two Florida dairy farms. Standard errors follow means. Least square means are adjusted for appropriate effects in model.

Day of measurement	Dairy A		Dairy B	
	Control	Sealac®	Control	Sealac®
Body condition score				
0 - 10 d PP	3.4 ± .06 (n = 69)	3.4 ± .07 (n = 81)	3.4 ± .07 (n = 79)	3.2 ± .07 (n = 90)
30 d PP	3.0 ± .04 (n = 164)	3.0 ± .05 (n = 169)	2.8 ± .05 (n = 79)	2.8 ± .06 (n = 90)
58 d PP	3.1 ± .05 (n = 142)	3.1 ± .05 (n = 158)	2.9 ± .05 (n = 124)	2.7 ± .06* (n = 134)
Final ^a	3.3 ± .05 (n = 142)	3.4 ± .06 (n = 140)	2.8 ± .05 (n = 138)	2.9 ± .06 (n = 145)
Blood urea nitrogen, mg/100 ml plasma				
58 d PP	17.2 ± .2 (n = 162)	17.3 ± .2 (n = 167)	21.4 ± .4 (n = 124)	21.3 ± .4 (n = 134)
Final ^a	17.0 ± .2 (n = 140)	16.4 ± 2** (n = 142)	21.5 ± .4 (n = 138)	21.3 ± .5 (n = 145)

^aFinal BCS and blood sample taken at 104 ± d at Dairy A and 96 ± d at Dairy B.

*Row means within Dairy are different, $P < .03$.

**Row means within Dairy are different, $P < .07$.

Table 5. Effect of feeding Sealac® on uncorrected milk production by lactating dairy cows at two Florida dairy farms.

Parity	Dairy A			Dairy B		
	Control	Sealac®	SEM	Control	Sealac®	SEM
	----- lb/d (n) -----					
All ^a	94.1 (162)	94.4 (174)	1.2	103.0 (147)	102.6 (156)	1.1 ^b
Second	93.1 (55)	90.2 (85)	1.6	94.6 (61)	99.7 (54)	1.6
Third	98.0 (46)	98.1 (28)	2.3	104.4 (34)	102.8 (50)	2.0
Fourth	95.2 (26)	98.0 (30)	2.7	110.9 (24)	104.1 (25)	2.5
≥ Fifth	90.2 (35)	91.1 (31)	2.4	102.2 (28)	103.8 (27)	2.5

^aParity; P = .0017 for Dairy A; P = .0001 for Dairy B.

^bDiet by parity interaction, P = .0325.

Table 6. Effect of feeding Sealac® on milk production (adjusted with mature equivalent milk weight as a covariate) by lactating dairy cows at two Florida dairy farms.

Parity	Dairy A			Dairy B		
	Control	Sealac®	SEM	Control	Sealac®	SEM
	----- lb/d (n) -----					
All	93.7 (160)	94.7 (174)	1.0	102.5 (143)	102.7 (153)	.9
Second	92.9 (55)	91.3 (85)	1.5 ^a	95.6 (57)	100.6 (51)	1.5 ^{b,c}
Third	94.5 (46)	97.8 (28)	2.1	103.8 (34)	102.3 (50)	1.7
Fourth	94.7 (26)	95.3 (30)	2.3	108.2 (24)	104.5 (25)	2.2
≥ Fifth	92.6 (33)	94.2 (31)	2.2	102.6 (28)	103.3 (27)	2.0

^aParity, Second vs. older cows, P = .0488.

^bParity, Second vs. older cows, P = .0001.

^cDiet by second vs. older cows interaction, P = .0142.

Table 7. Effect of feeding Sealac® on milk production (adjusted with milk weight prior to treatment assignment as a covariate) of lactating dairy cows at two Florida dairy farms.

Parity	Dairy A			Dairy B		
	Control	Sealac®	SEM	Control	Sealac®	SEM
	lb/d (n)					
All ^a	93.1 (83)	91.2 (105)	1.3	102.5 (94)	102.4 (108)	1.1
Second	93.4 (25)	91.9 (52)	1.9	97.7 (44)	100.1 (36)	1.5 ^a
Third	95.1 (26)	94.6 (19)	2.4 ^b	103.8 (19)	101.9 (37)	1.9
Fourth	90.5 (14)	91.7 (19)	2.8	105.5 (13)	104.4 (12)	2.7
≥ Fifth	93.4 (18)	86.6 (15)	2.8	103.0 (18)	103.1 (23)	2.1

^aParity, Second vs. older cows, $P = .0011$.

^bThird vs. older cows, $P = .0539$.

Table 8. Effect of feeding Sealac® on milk fat percent and production by lactating dairy cows at Dairy B.

Measurement	Control	Sealac®	SEM
	% (n)		
Milk fat percent			
All lactations	3.19 (133)	3.27 (149)	.05
Second	3.20 (58)	3.28 (51)	.08
Third	3.20 (28)	3.40 (48)	.09
Fourth	3.25 (23)	3.12 (24)	.12
≥ Fifth	3.10 (24)	3.28 (26)	.12
Milk fat production			
	lb/d (n)		
All lactations ^{a,b}	3.38 (133)	3.49 (148)	.06
Second	3.13 (58)	3.38 (50)	.09
Third	3.38 (28)	3.59 (48)	.11
Fourth	3.73 (23)	3.38 (24)	.15
> Fifth	3.27 (24)	3.62 (26)	.15

^aParity, $P = .0449$.

^bDiet by parity interaction, $P = .0577$.

Table 9. Effect of feeding Sealac® on 4% fat-corrected milk production by lactating dairy cows at Dairy B.

Parity	Control	Sealac®	SEM
	----- lb/d (n) -----		
All parities ^{a,b}	93.2 (133)	95.3 (148)	1.3
Second	86.1 (58)	92.3 (50)	2.0
Third	93.3 (28)	96.2 (48)	2.3
Fourth	101.9 (23)	94.4 (24)	3.0
≥ Fifth	91.7 (24)	98.6 (26)	3.1

^aParity; P = .0018.

^bTreatment by parity interaction; P = .0442.

Table 10. Effect of feeding Sealac® on milk protein percent and production by lactating dairy cows at Dairy B.

Measurement	Control	Sealac®	SEM
	----- % (n) -----		
Milk protein percent			
All parities ^a	2.80 (133)	2.85 (149)	.02
Second	2.83 (58)	2.88 (51)	.03
Third	2.87 (28)	2.87 (48)	.04
Fourth	2.73 (23)	2.88 (24)	.05
≥ Fifth	2.78 (24)	2.76 (26)	.05
Milk protein production	----- lb/d (n) -----		
All parities ^{b,c}	2.96 (133)	3.04 (148)	.04
Second	2.76 (58)	2.98 (50)	.05
Third	3.04 (28)	3.03 (48)	.06
Fourth	3.11 (23)	3.14 (24)	.08
≥ Fifth	2.95 (24)	3.03 (26)	.08

^aParity, P = .0547.

^bControl vs. Sealac, P = .1062.

^cParity, P = .0010.

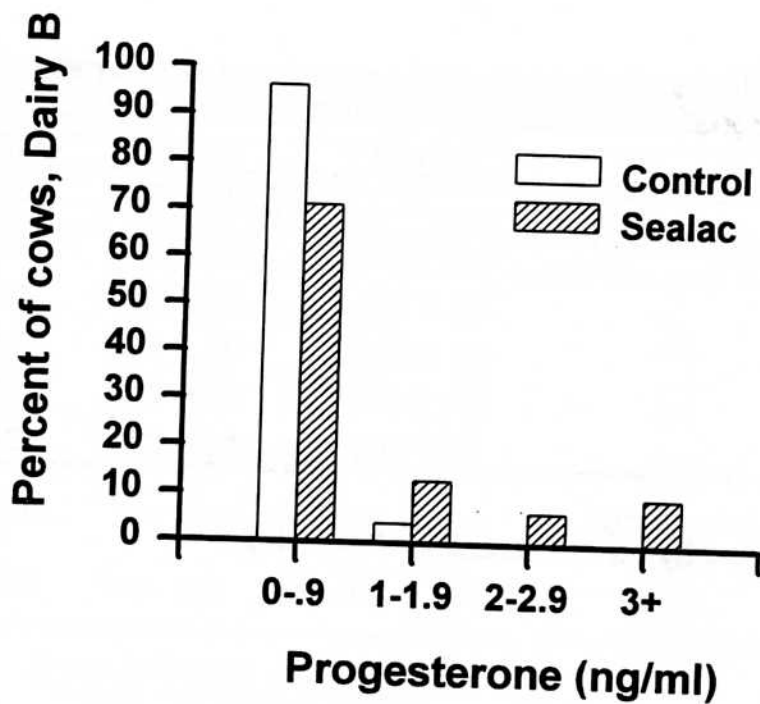


Figure 1. Plasma concentrations of progesterone 2 days after injection of PGF_{2α} in Dairy B consuming control or Sealac diet.

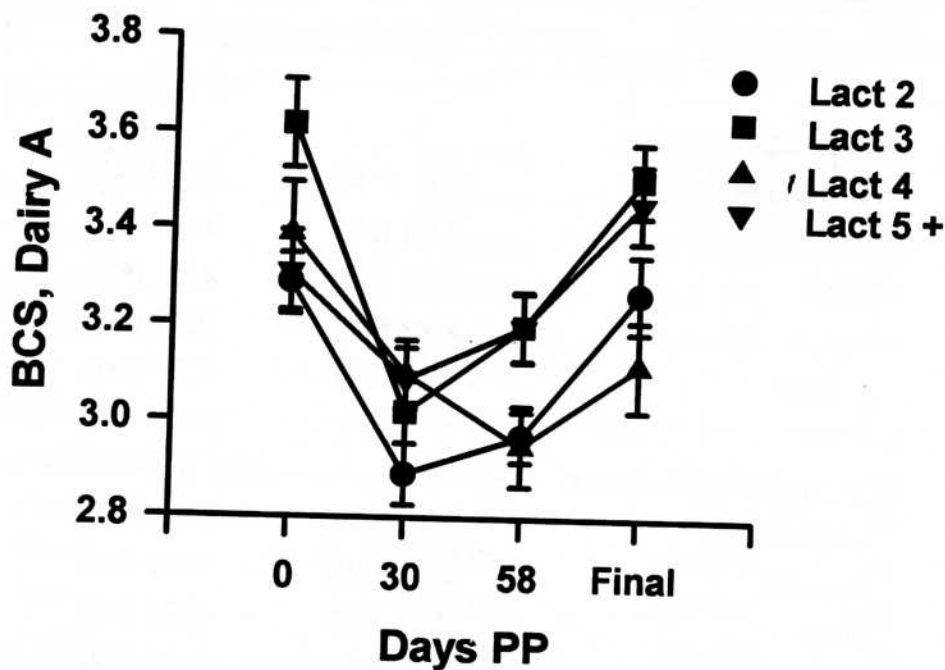


Figure 2. Body condition score (\pm standard error) of cows in Dairy A at 0 to 10 d PP, 30 and 58 d PP, and final BCS.

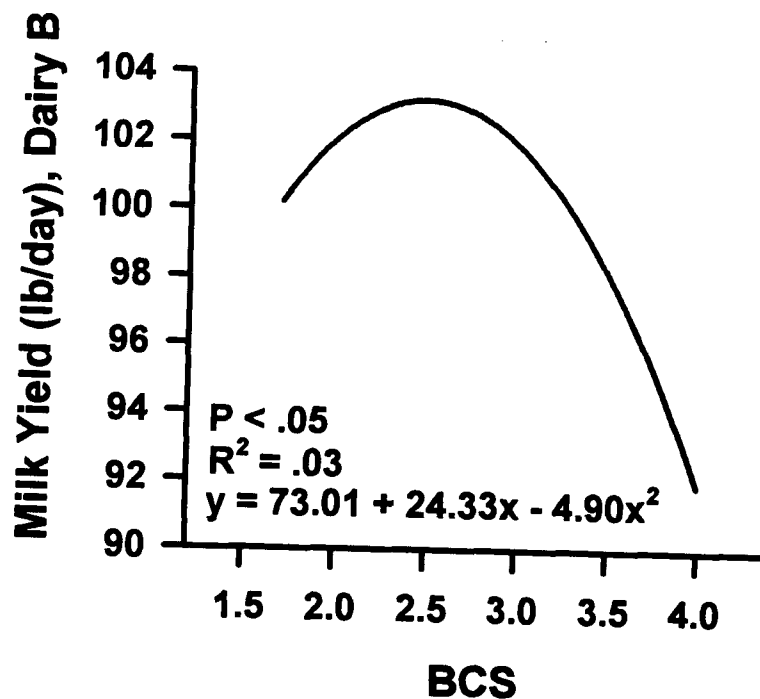


Figure 3. Regression of least squares mean of milk yield vs. BCS at 58 d PP for cows in Dairy B.

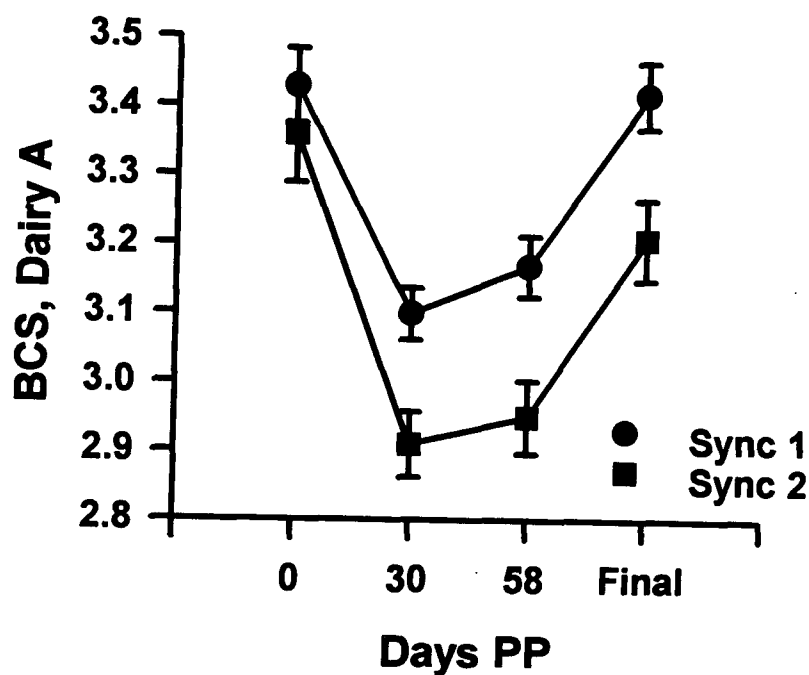


Figure 4. Body condition scores (\pm standard error) at 0 to 10 d PP, 30 and 58 d PP, and final BCS for cows synchronized once or twice in Dairy A.