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

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EFFECT OF FISH MEAL FEEDING ON DAIRY COW FERTILITY - TRIALS ON TWO LARGE DAIRY FARMS IN FLORIDA

SUMMARY AND CONCLUSIONS

Trials on two large dairy farms (A and B) in Florida involving over 600 cows to investigate the effect of fish meal on fertility have been undertaken. No significant effects of fish meal on milk production, body condition or fertility were seen at Farm A. Milk yields were greater at Farm B (mean 46.4 kg/d v 42.3 kg/d) and fertility poorer (pregnancy rate to 120 days 36.7% v 62.7%) compared with Farm A.

At Farm B, fish meal increased milk yield in second calving cows (38% of cows on trial) where the response was an extra 2.3 kg/d but not in younger or older cows. Averaged over all cows yield of milk protein was increased (1.37 v 1.34 kg/d; $P<0.11$). Cows allocated to fish meal had slightly poorer body condition immediately after calving and also had a greater loss in body condition, especially during the period 30 to 60 days post-partum. During this critical period leading up to breeding, cows fed fish meal continued to lose body condition whereas control cows were improving in condition. Despite this loss of condition and the known relationship between better body condition and improved fertility which is also evident in the whole data, feeding fish meal improved conception rate (37.5% v 29.1%, $P<0.07$) to insemination at this time (first synchrony), increased conception rate at the second insemination (24.5% v 9.0%) and also a tendency to improved overall pregnancy rate to 120 days (41.3% v 31.9%).

There is a suggestion that this increased pregnancy rate might be attributable to increased survival of the embryo at the time of pregnancy recognition. Levels of plasma progesterone were higher in fish meal fed cows seven days after the initiation of the ovulation cycle during which insemination took place.

The lack of response in either fertility or milk production at Farm A could have arisen because less fish meal was fed (0.5 kg v 0.7 kg per cow per day), cows were lower yielding and fertility was already greater. Also, small differences in milk yield that might have been expected as a result of fish meal feeding may not have been detectable with milk recording on only one day each month.

To give a better understanding of dairy cow reproduction, a brief fact sheet is given after the extended summary.

EXTENDED SUMMARY

Good fertility is essential if cows are to achieve high milk production. Annual production is at a maximum when a cow calves every 355 to 365 days. As pregnancy (gestation) is about 280 days, this leaves approximately 85 days for pregnancy to be established if optimum performance is to be achieved.

Poor fertility results in more non-productive days. For every extra dry day for a cow, there is a loss of $4 to $5.

Dairy farmers should aim to have cows inseminated around eight weeks after calving. Whether they do so will be dependent on how soon the cow starts her oestrus cycle and 'come on heat' or 'into season', and the success rate with which this is detected. The oestrus cycle can be stimulated, or synchronised for a herd by administering a hormone (prostaglandin $F_{2\alpha}$). The success rate having served a cow is expressed as pregnancy rate[1] or conception rate[2]. An acceptable pregnancy rate for first service is 50% to 65%, calving to service interval 60 to 70 days, and calving to conception 80 to 85 days.

Fertility is affected by many factors, including nutrition. It tends to be lower in higher yielding cows, partly because of the greater demand for nutrients. Stresses will reduce fertility, particularly heat stress. In the USA, particularly on the larger units in areas with hot summers, high yielding herds have poor pregnancy rates - sometimes as low as 20%.

Fish meal has been shown to improve fertility. Trials in Israel and Northern Ireland were reported in IFOMA's Fish Meal Flyer number 19[3].

The present trials, undertaken on two large dairy farms in Florida, were designed to examine the effects of fish meal on reproduction of dairy cows in a US situation. Both herds were large - over 2000 cows in each. They were high yielding averaging over 9,000 litres per cow.

The trial involved 341 cows on one farm (Farm A) and 300 on the other (Farm B). Both farms substituted fishmeal for animal proteins -meat and bone meal and blood meal, and lacto-whey (milk protein) in the case of Farm A, or corn-gluten on Farm B (see Table 1). On each farm the diets with (0.5 kg/cow/day on Farm A and 0.7 kg on Farm B) and without fish meal were equated for energy and protein content. The trials were conducted between January and June.

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[1] Pregnancy rate - proportion of hormone treated (oestrus synchronised) cows confirmed pregnant

[2] Conception rate - proportion of cows that were detected in oestrus and inseminated that were pregnant. As cows received oestrus synchronisation regardless of whether they return to oestrus or not, pregnancy rate is lower than conception rate

[3] The Israel trial is similar in that cows were high yielding and fed maize silage or hay plus high concentrates. Unlike fresh grass these ingredients would lack omega-3 fatty acids

On both farms cows were treated by hormone injection to synchronise oestrus. This is normal commercial practice on many farms in the USA. It did, however, preclude differences being found in return to oestrus after calving resulting from fish meal feeding. Milk yield was measured on one day a month on Farm A and daily on Farm B. Milk from Farm B was analysed for fat and protein whereas milk analysis was not possible on Farm A. Blood samples were taken from a representative number of cows on both farms and analysed for blood urea nitrogen. More blood samples were taken on Farm B and additionally the content of the reproductive hormone progesterone was measured. The body condition score of cows was determined.

The Results

On Farm A no differences were seen in either reproductive performance or milk production between cows fed diets with and without fish meal. Because of inaccuracies in feed mixing, slightly less fish meal was fed (0.5 kg rather than 0.7 kg planned). Also, even with the high numbers of cows involved monthly milk recording would have given variable results which may have masked treatment differences.

On Farm B, pregnancy rate at 120 days (the number of cows that were observed in oestrus, were served and found to be pregnant at 120 days as a proportion of all cows given oestrus synchronisation treatment) was significantly higher with fish meal (41.3 v 31.9 - see Table 3). This compares with pregnancy rates of 72% v 65% for diets with and without fish meal in a trial in Israel (see Flyer number 19). There is a suggestion that the increased pregnancy rate in the present trial might be attributable to increased survival of the embryo at the time of pregnancy recognition.

Feeding fish meal affected the pattern of change of plasma progesterone. A greater proportion of cows fed fish meal had plasma progesterone greater than 1 ng/ml at 48 hours after an injection of prostaglandin $F_{2\alpha}$ used to bring about the breakdown of the corpus luteum from the previous ovulation cycle. However, this indication of an interference or slowing down of the regression of the corpus luteum did not affect the number of days from prostaglandin $F_{2\alpha}$ injection to detection of oestrus and insemination (3.12 v 3.06 days). There was also an increase in prostaglandin $F_{2\alpha}$ at 65 days post-partum in the fish meal fed group (2.6 v 1.5 ng/ml; $P<0.01$). This appears to be due to that proportion of cows which failed to respond to synchronisation with injection of prostaglandin $F_{2\alpha}$ having higher progesterone than the control cows. Cows which did respond to synchronisation with the start of a new ovulation cycle would have very low plasma progesterone at this early stage (3 to 4 days) of the cycle, end of maturation of the developing ovum and prior to the formation of the progesterone-producing corpus luteum. However, this effect had disappeared by 72 days post-partum (6.6 v 6.4 ng/ml) when responding cows would be at day 14 of the cycle and the new corpus luteum would also be producing progesterone. There was also a significant positive relationship between plasma progesterone at day 58 post-partum, that is immediately before giving the injection of prostaglandin $F_{2\alpha}$ to synchronise the next cycle, and pregnancy rate at the subsequent synchronised oestrus for fish meal fed cows but not the controls. Progesterone is important in the preparation of the uterus for implantation of the

fertilised ovum but the biological significance of the observed differences in progesterone in the present experiment is not known.

On Farm B, production of fat corrected milk (i.e. equating yield of milk fat) and milk protein yield were slightly higher with fish meal - 42.9 v 41.9 kg (NS) and 1.37 kg v 1.33 kg ($P<0.11$) per cow per day respectively. This response was obtained when fish meal replaced mainly animal proteins (meat and bone and blood) plus some maize gluten. Although the rumen undegraded protein was similar, the better supply of essential amino acids beyond the rumen from fish meal may have accounted for the improved milk production. The response was mainly obtained with cows calving for the second time and not in younger or older cows. Fish meal increased milk yield by 2.3 kg/d in these second parity cows.

There were no differences in blood urea nitrogen from cows on fish meal compared with the control on Farm B. There is evidence that high blood urea levels can be associated with poorer fertility, but no such relationship was seen in the present experiment. Body scores were reduced with fish meal especially in the critical period 30 to 60 days post-partum leading up to breeding time. Cows with lower scores tended to be less fertile.

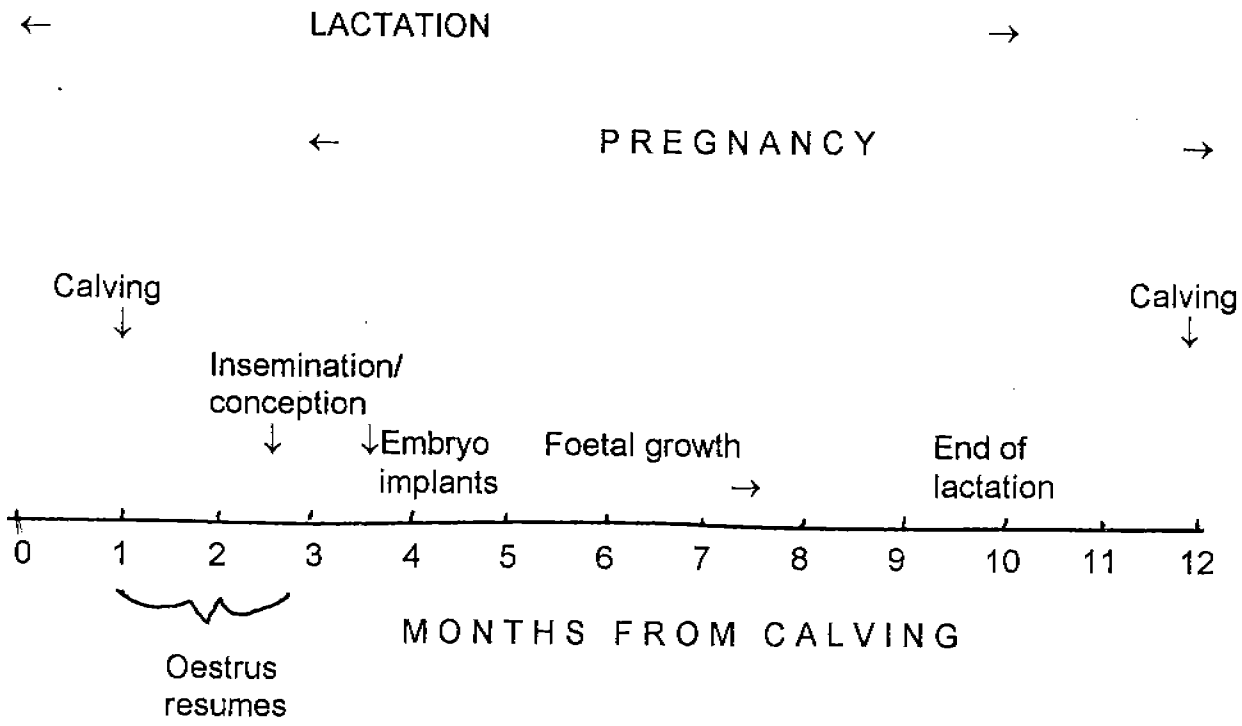
FACT SHEET

REPRODUCTION IN THE DAIRY COW

The reproductive cycle of the dairy cow is shown in Figure 1. Following calving the cow resumes her oestrus cycle at 30 to 60 days with the release of eggs from the ovarian follicles. As soon as oestrus is detected the cow can be inseminated but the conception rate is greatly improved if insemination is delayed until the second or third cycle. Insemination usually takes place eight to ten weeks after calving. Conception is the successful fertilisation of the egg by a sperm - the beginning of pregnancy. From the fertilised egg the embryo develops and this implants in the uterus. As mammalian eggs carry relatively small quantities of nutrients the embryo is dependent on establishing a nutrient supply directly from the dam; this occurs through the placenta. In the cow, implantation occurs at between thirty and thirty-five days after conception. The foetus then develops with nutrients and oxygen being supplied through the placenta. Meanwhile the cow continues to lactate to within six to eight weeks of calving. Pregnancy (gestation) lasts about 280 days.

Reproductive failure can occur at all stages of the reproductive cycle. For example, the cow may fail to resume oestrus cycles or they may be delayed; artificially stimulating resumption of the cycle through hormone injection to try to co-ordinate cycling in the herd - oestrus synchronisation, is widely practised in the USA. The egg may fail to be released or fertilised; the resulting embryo may fail to implant or having implanted it may fail to grow or the resulting foetus may fail and abort. A successful outcome of pregnancy can first be detected by feel (palpation) at around 40 days after conception. If the cow resumes oestrus cycles after service, this may indicate failed pregnancy, but not necessarily. As pregnancy progresses, detection becomes more reliable.

FIGURE 1 REPRODUCTION IN THE DAIRY COW



Note: This represents the target repoductive cycle dairy farmers aim to achieve; in practice, it is longer. The average length in the U.K. is 390 to 395 days.

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Effect of Feeding a Ruminant Grade Menhaden Fish Meal on Reproductive and Productive Performance of Lactating Dairy Cows[1]

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ABSTRACT

Menhaden fish meal, fed at 0.7 kg/d (2.7% of diet DM) replaced a control diet of blood, meat and bone meal (1.8% of diet DM) at Dairy A (n = 341) or the same plus corn gluten meal (1.5% of diet DM) at Dairy B (n = 300) in a corn silage/hominy-based totally mixed ration. During winter, cows were assigned to diets at ~24 d postpartum and continued on the diet for ~85 d. Cows were synchronized for estrus using an injection of GnRH agonist at 51 ± 3 d postpartum followed 7 d later by an injection of $PGF_{2\alpha}$ and inseminated at detected estrus. Cows not detected in estrus were resynchronized once with the same program and inseminated at detected estrus. Diet failed to alter reproductive responses at first synchronized AI for either dairy. Pregnancy rate at 120 d was similar between diets at Dairy A (65.4%, control vs. 60.2%, fish meal), but was improved by fish meal at Dairy B (31.9 vs. 41.3%, dairy by diet interaction). A greater proportion of cows fed fish meal had plasma concentrations of progesterone > 1 ng/ml at 48 h after $PGF_{2\alpha}$ injection (29 vs. 4%; Dairy B only), indicating incomplete CL regression at 48 h in some cows. Diet did not influence milk production at Dairy A. Feeding fish meal was associated with a 2.3 kg/d increase in milk production by second parity cows, but not older cows at Dairy B. At dairy B, production of milk fat and 4% fat-corrected milk were improved when cows at all parities except the fourth consumed fish meal.

(**Key words**: body condition, fish meal, lactation, reproduction, undegradable protein)

Abbreviation key: BCS = body condition score, **CSLCFA** = calcium salts of long chain fatty acids, **DHA** = docosahexaenoic acid, **EPA** = eicosapentaenoic acid, **GnRHa** = GnRH agonist, **PP** = postpartum, **PGFM** = $PGF_{2\alpha}$ metabolite.

INTRODUCTION

Supplementation of fatty acids for metabolism can influence events important to successful reproduction of dairy cows. An emulsion of soybean oil (50% linoleic acid) was infused i.v. into Holstein heifers (13) that resulted in increased plasma concentrations of $PGF_{2\alpha}$ metabolite (**PGFM**) and altered follicular dynamics. The numbers of ovarian follicles were increased and the diameter of the largest follicle was greater. In another, feeding calcium salts of long chain fatty acids (**CSLCFA**) to lactating cows increased the numbers of 3 to 5 mm follicles, 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 (14). This effect of CSLCFA on increased size of the preovulatory follicle was found to be the result of the fatty acids themselves rather than improved energy status of the cows (15). In addition, feeding CSLCFA at the rate of approximately 0.5 kg/d improved conception rates of lactating Holstein cows by 120 d postpartum (**PP**) from 52 to 86% (10).

Supplemental fat also can influence uterine metabolism, as well as that of the ovary. Peak plasma concentration of PGFM in response to a pulse dose of oxytocin given i.v. on d 15 of an estrous cycle was depressed in lactating cows receiving an abomasal infusion of 0.45 kg/d of yellow grease (17% linoleic acid) relative to those infused with water, glucose or tallow (2% linoleic acid; 48 vs. 90 ng PGFM/ml) (18). The dominant follicle of cows receiving yellow grease grew more rapidly than follicles of cows receiving tallow.

Fish meal contains approximately 8% fat of which two-thirds is long chain, polyunsaturated fatty acids, including the unique fatty acids, eicosapentaenoic (20:5n-3; **EPA**) and docosahexaenoic (22:6n-3; **DHA**). Typically, unsaturated fatty acids are biohydrogenated by

ruminal microorganisms. However, EPA and DHA found in fish oil appear to escape biohydrogenation (2, 20). Therefore, feeding fish meal may result in uptake of these fatty acids for metabolism by reproductive tissues of the lactating cow. These fatty acids can inhibit cyclooxygenase activity and, in turn, decrease $PGF_{2\alpha}$ synthesis (26).

Feeding fish meal to lactating dairy cows has improved conception rates, although the mechanisms of action have not been elucidated. Armstrong et al. (1) reported a 20 percentage unit increase in conception rates by partial replacement of soybean meal with fish meal in the diet. Bruckental et al. (4) reported a 20 percentage unit increase in pregnancy rates by supplementing diets with fish meal rather than soybean meal. Whether these responses were due to a reduction in intake of ruminally degradable protein, or an increase in intake of fatty acids, or both is not known.

Of the available feedstuffs high in undegradable protein, fish meal fed singly often is effective in improving milk production. In a summary of eight studies in which corn silage was the main dietary forage source, feeding of fish meal increased milk production by an average of 1.6 kg/d per cow (21). Milk protein content tended to be increased or unchanged; whereas, milk fat content 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 intake of fish oil depresses fiber digestion via toxicity to ruminal microbes, which may account for the lowered fat content of milk. Feeding less than 0.75 kg/d of fish meal is not likely to depress milk fat content. In addition, fish meal provides greater concentrations of the essential and often limiting amino acids, lysine and methionine, than other typical concentrated protein feedstuffs.

Objective of this study was to determine the effect of dietary fish meal on reproductive

performance, milk production and composition, body condition score (BCS), and blood urea nitrogen (BUN) of Holstein cows at two dairy farms in Florida.

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. Cows were housed in an open-sided, free-stall barn with a concrete floor and self-locking stanchions with sand used as bedding. Cows were assigned randomly to a control diet (n = 166) containing blood meal plus meat and bone meal as ruminally undegradable protein sources or to the experimental diet containing a ruminant grade Menhaden fish meal (n = 175; Sea-Lac®, Zapata Haynie Corp., Hammond, LA). Composition of diets is listed in Table 1. Targeted intake of fish meal was 0.7 kg/d per cow. Cows were fed the diets starting at 25 ± 0.5 d 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 analyzed for CP, soluble CP, ether extract, NDF, ADF, Ca, P, K, and Mg (Northeast DHIA Forage Testing Laboratory, Ithaca, NY).

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 was 60 d PP. At 30 ± 3 d PP, cows were injected with $PGF_{2\alpha}$ (25 mg i.m. Lutalyse®; The Upjohn Co., Kalamazoo, MI) to regress any existing corpus luteum and potentially increase the number of estrous cycles prior to first AI. In addition, subsequent dilation of the cervix associated with estrus contributes to optimizing the

uterine environment by reducing the occurrence of metritis or pyometra (22). Cows were synchronized for estrus with an injection of GnRH agonist (**GnRHa**; 8 µg i.m. Buserelin; Hoechst-Roussel Agri-Vet, Somerville, NJ) at 51 ± a range of 3 d PP, followed 7 d later with an injection of $PGF_{2\alpha}$ (Table 2) (3, 24, 28). This program synchronizes both follicle development and regression of the corpus luteum (16, 27). The method of estrus detection used was visual observations 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® Paintstik®, LA-CO Industries, Inc./Markal Co., 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 were considered responders to first synchronization if signs of estrus were displayed within 7 d from injection of $PGF_{2\alpha}$. Cows which did not show signs of estrus at the first synchrony were resynchronized 7 d later by an injection of GnRHa at approximately 65 d PP followed by an injection of $PGF_{2\alpha}$ 7 d later and bred to detected estrus. Any cows not exhibiting estrus after two synchrony attempts were allowed to undergo spontaneous cycles and bred upon observation of standing estrus. Cows which were bred at synchrony and did not conceive were rebred upon returning to estrus. Palpation of the uterus and its contents per rectum at ≥ 42 d post-AI was used to diagnose pregnancy. Pregnancy rate was defined as the proportion of treated cows that were pregnant. Conception rate was the proportion of cows that were detected in estrus and inseminated that were pregnant.

Dairy B: The experiment was conducted on a Florida dairy herd from January to June 1995 using 300 multiparous, lactating Holstein cows. Cows were housed in an opened-sided,

concrete floor barn with old hay and recycled newspapers used as bedding. In addition, cows had 24-h access to a sandlot and cooling pond. Control cows (146 cows with even-numbered ear tags) were assigned to a diet containing a mixture of four ruminally undegradable protein feedstuffs (Table 1). Cows with odd-numbered ear tags were assigned to the fish meal diet (n = 154; Table 1). Feed samples were collected and analyzed chemically 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 wk per cow. Milk was measured biweekly for fat and protein content. Somatic cell counts were measured monthly.

The reproductive management program for the cows synchronized during the first eight wk of the study (Period 1; n = 225) was similar to that used at Dairy A. A timed AI program was utilized for the cows that were synchronized during the last 6 wk (Period 2; n = 75). This change in breeding program was implemented because of a low estrus detection rate at first synchrony (50%; Table 3). Previous research from our laboratory indicated that pregnancy and conception rates using timed AI in primiparous and multiparous cows were comparable to the program followed in Period 1 (5). The timed AI program requires an additional injection of GnRHa 48 h after the $PGF_{2\alpha}$ injection given at 58 d PP and cows were inseminated 16 h later without regard to estrus detection. In Period 2, a second synchronization was not necessary because all cows were inseminated. The method of estrus detection was a combination of visual observation and pedometer readings using the Afikim system. Inseminations (using a total of 80 bulls) were performed by one of 12 technicians at approximately 8 h after the activity (steps per h)

of the cow reached two standard deviations above individual average activity. Semen sources were used randomly across treatments. Pregnancy diagnosis per rectal palpation occurred ≥ 45 d post-AI.

Determination of BCS, progesterone, and BUN. Blood samples were collected from the coccygeal vessel immediately prior to injecting GnRHa or $PGF_{2\alpha}$. In Period 2, samples were collected from 56 cows at Dairy B before the second injection of GnRHa to estimate proportion of cows responding to injection of $PGF_{2\alpha}$. Samples were held constantly in ice, centrifuged (3,000 x *g* for 20 min at 4°C) within 16 h of collection, and plasma collected. Plasma was stored at -20°C until analyzed for progesterone (12) and BUN (17). Inter- and intraassay coefficients of variation for progesterone assay were 9.3% and 12.9%, respectively, and 8.7% and 1.6%, respectively for BUN. Cows were considered to have undergone luteolysis when plasma concentrations of progesterone declined to less than 1 ng/ml.

Cows were scored for body condition (five-point scale; 1 = thin to 5 = fat) using a quarter point system (11) between 0 and 10 d, at 30 ± 3 d, and 58 ± 3 d PP for Dairies A and B. A final score was recorded at 104 ± 1 d PP for Dairy A and 96 ± 2 d PP for Dairy B.

Statistical Analyses. Data were analyzed using the GLM procedure of SAS (23). The mathematical model used to analyze estrus detection and pregnancy rates at the synchronized estrus for both dairies included dietary treatment, parity, month of synchronization (January to May), and synchrony (first or second). Period was included in the model for Dairy B. For analysis of conception rate, inseminator also was included in the model. For Dairy B, involving 12 inseminators, eight inseminators were pooled together because each inseminated less than four cows.

Other reproductive responses evaluated included number of days to first AI (for all cows inseminated by 120 d PP), number of AI per conception, overall pregnancy and conception rates by 120 d PP, and number of days open for cows that conceived by 120 d PP. These analyses examined effects of diet, and at Dairy B, period (Period 1 = breed at detected estrus, Period 2 = timed AI). Dairy by diet interactions were calculated and reported when significant. In addition, the interval between the injection of $PGF_{2\alpha}$ at 58 ± 3 d PP and AI within 7 d was compared between dietary groups.

Plasma concentrations of progesterone at the time of the GnRHa and $PGF_{2\alpha}$ injections were analyzed using GLM with dietary treatment in the model. A chi square analysis was used to determine whether the distribution of 56 cows with plasma concentrations of progesterone greater than versus less than 1 ng/ml at 48 h after the injection of $PGF_{2\alpha}$ were different between dietary treatments at Dairy B. The analyses of BUN included dietary treatment, parity, and the interaction in the mathematical model using GLM.

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

Regression analyses (23) were used to evaluate the relationship between plasma concentrations of progesterone or BCS at 58 d PP and estrus detection, pregnancy rate, and conception rate as dependent variables adjusted for the appropriate independent variables. For BCS analyses, synchronization was included in the mathematical model; cows synchronized only

once were compared with those cows not detected in estrus and resynchronized. Regression analyses also were used to examine the relationship between change in BCS and estrus detection, pregnancy, and conception rates with dairy and dietary treatment included as independent variables in the mathematical model. 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. The order of regression for each was tested.

Data for milk production and composition were analyzed using GLM procedure (23). The mathematical model included diet, cow within diet by parity, DIM, and interactions. Orthogonal contrasts were used to determine effect of parity, diet, and diet by parity interactions. Contrasts for parity were 1) second vs. older, 2) third vs older, and 3) fourth vs older. Analyses were performed that utilized the mature equivalent milk production from previous lactations as a covariable. The response variable was the least squares mean for milk production for the experimental period adjusted for DIM.

RESULTS

Reproductive Responses

For Dairy A reproductive responses were not different between cows fed experimental diets nor between the two synchronies (Table 3). Estrus detection rate to synchronized estrus declined ($P < 0.005$) from January (72.8 ± 5.6%) to April (46.0 ± 6.8%) for Dairy A. For Dairy B estrus detection rate of cows fed fish meal tended to increase from first to second synchronization (treatment by synchronization interaction, $P < 0.11$; Table 3). Pregnancy and conception rate at each synchronized estrus were similar between dietary treatments (Table 3).

However, pregnancy rate by 120 d PP tended to be improved ($P < 0.06$; diet by dairy interaction) when cows were fed fish meal at Dairy B (31.9 vs. 41.3%). Pregnancy and conception rates by 120 d PP declined with month of first synchronization (pregnancy rate was 82.6 ± 6.2% in January and 48.7 ± 8.2% in April, $P < 0.001$; conception rate was 86.7 ± 6.2% in January and 50.8 ± 8.2% in April, $P < 0.001$). Number of days to first AI increased with month at Dairy A (66.3 ± 2.4 d in January and 76.0 ± 3.1 d in April, $P < 0.02$). Other reproductive responses including number of days open, number of AI per conception, number of days to first AI, interval from $PGF_{2\alpha}$ injection to AI, and number of observed heats prior to AI were unaffected by diet at Dairy B.

Pregnancy rate at the synchronized estrus was not different between cows inseminated in Period 1 and Period 2 at Dairy B (16.2 ± 2.9% vs. 15.3 ± 4.6%); however, conception rate tended to be lower in Period 2 (33.1 ± 5.3% vs.16.3 ± 8.1%, $P < 0.07$). A reduced conception rate for cows managed in a timed AI program was expected because all cows were inseminated, including cows not responding to synchronization. Number of days to first AI at Dairy B was reduced in Period 2 (60.5 ± 1.5 d vs. 68.9 ± 1 d, $P < 0.001$) because all cows were inseminated 3 d after the injection of $PGF_{2\alpha}$, compared with approximately 50% of cows in Period 1 that were detected in estrus by this time. The interval between injection of $PGF_{2\alpha}$ and AI was reduced ($P < 0.04$) in Period 2 relative to Period 1 (2.9 ± 0.1 d vs. 3.2 ± 0.1 d), because all cows were inseminated 3 d after the $PGF_{2\alpha}$ injection, compared with a range of 0 to 7 d for cows in Period 1.

At Dairy A the proportion of cows detected in estrus prior to synchronization at 48 d PP was 41.8 ± 3.7 and did not differ between dietary treatments. The proportion of cows with

plasma concentrations of progesterone greater than 1 ng/ml just before the injection of GnRHa on 51 d PP were 74.0 and 72.6% for Dairy A and 58.9 and 55.7% for Dairy B for control and fish meal fed cows, respectively. One week following the injection of GnRHa and at the time of $PGF_{2\alpha}$ injection, cows with plasma concentrations of progesterone greater than 1 ng/ml were 78.3 and 82.3% for Dairy A, and 78.1 and 80.2% for Dairy B for control and fish meal fed cows, respectively. Thus a majority of cows were cycling at 51 d PP at Dairy A and, in response to the injection of GnRHa, a majority of cows were cycling by the time of the injection of $PGF_{2\alpha}$ at Dairy B.

Efficacy of corpus luteum regression by $PGF_{2\alpha}$ injection was examined. Blood samples collected from cows at Dairy B at 2 d after injection of $PGF_{2\alpha}$ revealed that a greater proportion of cows consuming fish meal had plasma concentrations of progesterone > 1 ng/ml compared with the control group (29 vs. 4%, $P < 0.025$; Figure 1). In addition, cows consuming the fish meal diet that failed to respond to synchronization treatment at Dairy B, had greater ($P < 0.01$) concentrations of progesterone at 65 d PP compared to those consuming the control diet (Table 3). Plasma concentration of progesterone was related positively to pregnancy rate at synchronized estrus for cows fed fish meal compared to those fed the control diet at Dairy B, but not Dairy A ($P < 0.025$). Pregnancy rate improved 3.2% for every 1 ng/ml increase in plasma concentration of progesterone at 58 d PP for cows fed fish meal compared with a 0.3% increase in cows consuming a control diet [$y_{control} = 14.71 + 0.34x$; $y_{fish\ meal} = -0.37 + 3.20x$, where y = pregnancy rate (%) and x = plasma concentration of progesterone (ng/ml); $P < 0.002$; $R^2_{model} = 0.06$; $R^2_{reg} = 0.05$].

Body Condition Scores

Dietary Effects. At dairy A, mean BCS of cows at calving was 3.4. Cows lost 0.4 BCS units by 30 d PP and had returned to their starting body condition by 104 d PP (Table 4). Dietary treatment did not affect BCS at any time during the experiment at Dairy A (Table 4). Parity influenced body condition as measured at 0 to 10 d PP ($P < 0.02$), 30 d PP ($P < 0.02$), 58 d PP ($P < 0.008$), and at 104 d PP ($P < 0.003$).

At Dairy B, consumption of fish meal reduced ($P < 0.03$) BCS at 58 d PP by 0.2 units compared with cows consuming the control diet (Table 4). Body condition of cows was similar among parities at each day of measurement. Cows in Dairy B had a greater decrease in BCS and had not returned to initial body condition at calving by 96 d PP.

Influence on Milk Production. Body condition at 0 to 10 d PP positively influenced milk production of cows at Dairy B, but not Dairy A. For every 1 unit increase in BCS at this time, mean milk production increased 2.7 kg over the experimental period ($y = 37.0 + 2.7x$, where y = mean milk production and x = BCS; $P < 0.01$; $R^2_{model} = 0.19$; $R^2_{reg} = 0.04$). Body condition score at 58 d PP was related quadratically to milk production. Milk production was maximized (46.6 kg/d) by cows scoring 2.5 at 58 d PP ($y = 32.9 + 10.95x + -2.21x^2$; $P < 0.05$; $R^2_{model} = 0.18$; $R^2_{reg} = 0.03$; Figure 2).

Influence on Reproductive Responses. At Dairy A, the BCS of cows requiring only one synchronization to initiate estrus behavior was greater at 30 ($P < 0.04$), 58 ($P < 0.001$), and 104 d PP ($P < 0.005$) compared with cows failing to show signs of estrus at the first synchrony and thus requiring a second synchrony (Figure 3). Body condition at 58 d PP was related positively ($P < 0.005$) to estrus detection rate for cows synchronized a second time ($y_{sync2} = -$

14.1 + 23.8x, where y = estrus detection rate and x = BCS; $P < 0.01$; $R^2_{model} = 0.37$; $R^2_{reg} = 0.05$), whereas BCS had little relationship to a successful first synchrony ($y_{sync1} = 98.9 + 1.4x$). For every 0.5 BCS unit increase at 58 d PP for cows synchronized twice, estrus detection rate increased 11.9% compared with a 0.7% increase in cows synchronized once. A similar positive relationship existed between BCS at 58 d PP and pregnancy rate ($y_{sync2} = -53.9 + 26.4x$, $y_{sync1} = 46.7 + 1.4x$; $P < 0.01$; $R^2_{model} = 0.08$; $R^2_{reg} = 0.04$), as well as conception rate ($y_{sync2} = -54.6 + 29.2x$, $y_{sync1} = 39.0 - 1.5x$; $P < 0.05$; $R^2_{model} = 0.04$; $R^2_{reg} = 0.04$) for cows synchronized twice versus only once. For every 0.5 BCS unit increase at 58 d PP for cows synchronized twice, pregnancy rate increased 13.2% and conception rate increased 14.6%.

At Dairy B the relationship of BCS to estrus detection, pregnancy, or conception rate was not different between synchronies. For all cows at Dairy B, for every 0.5 BCS unit increase at 58 d PP, pregnancy rate increased by 7.0% [y = -17.9 + 14.0x, where y = pregnancy rate (%) and x = BCS; $P < 0.03$; $R^2_{model} = 0.08$; $R^2_{reg} = 0.03$]. Similarly, BCS at 58 d PP tended to be related positively to estrus detection rate ($y = 28.1 + 11.0x$; $P < 0.10$; $R^2_{model} = 0.10$; $R^2_{reg} = 0.01$) as well as to conception rate ($y = -13.5 + 13.8x$; $P < 0.09$; $R^2_{model} = 0.07$; $R^2_{reg} = 0.02$).

Across both dairies change in BCS from calving to 30 d PP and from calving to 58 d PP was related negatively to estrus detection, pregnancy and conception rates at first synchronization (Table 5).

Blood Urea Nitrogen

At the time of first estrus synchronization, BUN values were similar between cows fed the

two experimental diets at Dairies A and B (Table 4). At 104 d PP, concentration of BUN tended to be lower in cows consuming fish meal compared with those consuming a control diet (16.4 ± 0.2 vs. 17.0 ± 0.2 mg/100 ml; $P < 0.07$), whereas values were similar between dietary treatments at Dairy B. Cows at Dairy B appeared to have consistently greater concentrations of BUN compared with cows at Dairy A (21.3 vs. 17.2 mg/100 ml). The relationship of BUN to milk production was not significant ($P > 0.10$) at either dairy farm. In addition, mean BUN concentrations at 104 or 96 d PP at Dairies A or B were not different between pregnant and nonpregnant cows (17.3 ± 0.19 vs. 17.4 ± 0.25 mg/100 ml, Dairy A; 21.2 ± 0.27 vs. 21.5 ± 0.34 mg/100 ml, Dairy B, $P > 0.10$). Regression analyses indicated that BUN at 58 d PP was unrelated to pregnancy or conception rates.

Feed Intake and Milk Production and Composition

Feed intake was 23.6 and 24.0 kg/d DM at Dairy A and 24.7 and 25.0 kg/d DM at Dairy B for cows fed control or fish meal supplemented diets, respectively. Because cows were fed in groups and not individually, DMI data could not be analyzed statistically.

Mean milk production was 42.3 and 46.4 kg/d per cow at Dairies A and B, respectively, over the duration of the experiment (Table 6). As expected, cows of different parities differed in milk produced ($P < 0.002$), 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, $P < 0.04$). When fish meal was included in the diet, cows in their second lactation produced 2.3 kg/d more milk (diet by second vs. ≥ third parity interaction, $P < 0.01$) and cows in their fourth lactation

produced 3.1 kg/d less milk (diet by fourth vs. ≥ fifth parity interaction, $P < 0.07$) compared with the control cows (Table 6).

When milk production was adjusted using mature equivalent milk weight as a covariable, dietary effects on milk production remained the same (Table 7). Milk production was unaffected by feeding fish meal at Dairy A, whereas milk production by second parity cows was stimulated by 2.3 kg/d per cow at Dairy B. Cows in their third parity or greater did not respond to inclusion of fish meal in the diet (diet by second vs. ≥ third parity interaction, $P < 0.02$).

Only Dairy B analyzed milk for fat, protein and SCC content. 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 percent when fed fish meal (Table 8). This effect contributed to improved daily production of fat by all parities of cows except those in their fourth lactation (diet by fourth vs. ≥ fifth parity interaction, $P < 0.01$; Table 8). Only cows in their fourth lactation failed to produce more 4% fat-corrected milk when fed fish meal (diet by fourth vs. ≥ fifth parity interaction, $P < 0.01$; Table 9).

Milk protein concentration was not different due to dietary treatment. However, production of milk protein tended to increase ($P < 0.11$) across all parities when fish meal replaced corn gluten meal, blood meal, and meat and bone meal in the diet (Table 10).

Somatic cell scores were greater ($P < 0.01$) for cows consuming the fish meal diet (3.54 ± 0.17) compared with control cows (2.88 ± 0.19). A score of 3.58 represents a SCC of 150000 and a score of 2.85 represents a value of 90000. Both scores indicate that milk was of high quality.

DISCUSSION

The injection of $PGF_{2\alpha}$ initiates regression of the corpus luteum. This regression assists with the final development of the follicle that was recruited after the injection of GnRHa. The long chain fatty acids, EPA and DHA, have been shown to inhibit prostaglandin synthesis in ram seminal vesicles (9, 26). 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 because uterine endogenous secretion of $PGF_{2\alpha}$ may be reduced and luteal regression would be more dependent on the exogenous $PGF_{2\alpha}$. Indeed, 2 d after injection of $PGF_{2\alpha}$, the proportion of cows with plasma concentrations of progesterone greater than 1 ng/ml was greater when fish meal was fed compared with the control diet (29 vs. 4%), suggesting that fish meal altered the dynamics of corpus luteum regression induced by the injection of $PGF_{2\alpha}$. In support of this, cows supplemented with fish meal that failed to respond to synchronization treatment, had a greater concentration of progesterone 7 d following the luteolytic dose of $PGF_{2\alpha}$, compared to cows consuming the control diet. All cows consuming the fish meal diet did not completely regress the corpus luteum by this time, compared to the cows consuming the control diet that had sub-luteal levels of progesterone. Regression of the corpus luteum was completed eventually based upon similarities in estrus detection rates between diets, as well as number of days from $PGF_{2\alpha}$ injection to AI.

At Dairy B, where conception and pregnancy rates were low, pregnancy rate at first synchrony was related more strongly to plasma concentrations of progesterone in cows consuming fish meal compared with a control diet. Pregnancy rate at 120 d PP tended to be greater in cows consuming fish meal. Perhaps the inclusion of fish meal in the diet altered the

response of the uterus to progesterone produced from the corpus luteum.

The synchronization program using an injection of GnRHa to recruit a new follicle followed 7 d later by an injection of $PGF_{2\alpha}$ to regress an existing or induced CL was effective at both Dairies A and B. Fifty to 65% of the cows were detected in estrus following synchronization that was initiated at 51 d PP. Based on the number of cows at this time with plasma concentrations of progesterone > 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%, and at Dairy B was 81%. In response to the injection of GnRHa at 51 d PP, the number of cows having plasma concentrations of progesterone > 1 ng/ml increased such that the proportion of cows estimated to be cycling by 58 d PP was nearly 100% for both Dairies A and B. Therefore, the synchronization program used was effective to initiate ovarian activity for those anestrus cows at Dairy B. Cows which did not show signs of estrus at first synchronization were resynchronized, resulting in a range of 49 to 72% of cows exhibiting signs of estrus across both dairies. Therefore, approximately 85 and 80% of the cows fed the control and fish meal diets at Dairy A and 74 and 90% of the cows fed the control and fish meal diets at Dairy B were inseminated between 55 and 78 d PP using the GnRHa, $PGF_{2\alpha}$, and bred to standing heat system, as well as the timed AI program.

First service estrus detection rate declined from January to April at Dairy A, perhaps due to a change in environmental temperature, management or both. Pregnancy and conception rates at the synchronized estrus were similar over time, indicative that fertility was not affected by environmental temperature. However, at Dairy A overall pregnancy and conception rate by 120 d PP declined and number of days to first AI increased over month of first synchronization, possibly

due to fluctuations in management towards the end of the study.

The timed AI management system implemented at Dairy B (Period 2) resulted in pregnancy rates at the synchronized estrus that were comparable to that of Period 1. However, conception rate at this time was decreased because all cows were inseminated in Period 2, including cows not responding to synchronization. Also, environmental temperature was greater during months of timed AI management, which may have contributed to lower fertility of cows in Period 2 at Dairy B. Days to first AI declined in Period 2 due to all cows being inseminated at 3 d after the injection of $PGF_{2\alpha}$, compared with approximately 50% of cows in Period 1 that were detected in estrus by this time. The interval between injection of $PGF_{2\alpha}$ and AI was reduced in Period 2 because all cows were inseminated 3 d after injection, compared with a range of 0 to 7 d for cows in Period 1.

Body condition was estimated four times throughout the study. At Dairy A, second parity cows were among the thinnest throughout the study. These cows were among the least productive during the trial. Their requirement for growth plus a lack of energy reserves likely contributed to a lower milk production. At the time of synchronization, thinner cows (1.7 BCS) and more conditioned cows (3.3 BCS) produced less milk. These results would support the idea that over conditioned cows as well as under conditioned cows at this time result in lowered milk production. 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 cows at Dairy A. At 96 d PP, cows were still 0.3 to 0.6 score units below their score at calving. Less body condition during breeding may have contributed to the lower pregnancy and conception rates at Dairy B.

Two populations of cows were evident at Dairy A, those synchronized only once with a

mean BCS of 3.3 and those not detected in estrus to first synchrony with a mean BCS of 3.1 at 58 d PP. A differential relationship of BCS at 58 d PP to estrus detection, pregnancy, and conception rate existed among the two groups of cows. The cows at second synchrony responded with a positive linear relationship between BCS and the reproductive response, whereas no such relationship existed in the cows at first synchrony. This suggests that among cows that failed to respond to first synchrony, those having a lower BCS at 58 d PP likely were meeting metabolic demands for lactation prior to that for reproduction. Cows having a lower BCS were less likely to express behavioral estrus when synchronized and perhaps fertility was impaired. On the other hand, at Dairy B, only one population of leaner cows was apparent. Body condition played a positive role in reproductive responses examined in cows synchronized either once or twice. Again, these cows, which produced about 4 kg/d per cow more milk than cows in Dairy A, were likely directing metabolic signals prioritizing lactation over reproduction. Relationships between the change in body score between calving and days 30 or 58 with rates of estrus detection, conception and pregnancy at day 58 were significant (Table 5) and are presented for the total population of cows in which effect of dairies has been adjusted. No such relationships were detected for change in body condition between days 30 and 58. Thus, the degree of loss in body condition from calving appears to be associated with reproductive responses.

Cows at Dairy B appeared to have consistently greater concentrations of BUN compared with cows at Dairy A. Greater concentrations of BUN at Dairy B may have resulted from feeding diets of greater concentration of crude protein and soluble protein (Table 1).

The relationship between BUN and reproductive responses were examined. Plasma concentrations of BUN at 58 d PP were similar between cows conceiving or not conceiving to a

synchronized estrus. A lack of difference in BUN concentrations also was noted between pregnant and nonpregnant cows by 120 d PP at either Dairies A or B. Though no differences were detected within a Dairy, the apparently greater concentration of BUN in cows at Dairy B may have contributed to a lower overall conception rate by 120 d PP relative to cows at Dairy A. Alternatively, other factors such as management, greater milk production, and lower BCS, may have contributed to the poorer reproductive responses observed at Dairy B. This differs from Canfield et al. (7) who reported that plasma urea nitrogen concentrations were lower in cows conceiving to the first service than those not conceiving. Also, Butler et al. (6) found that milk urea nitrogen concentrations were lower in cows that conceived compared to those that did not during a 5-d period after AI. These researchers reported that milk urea nitrogen concentrations were lower in pregnant compared with nonpregnant cows.

Milk production was similar between dietary treatments at Dairy A. However, at Dairy B, milk production was greater in second parity cows and milk fat and 4% fat-corrected milk production was greater in all but fourth parity cows when fish meal was included in the diet. There were fewer estimates of milk production in Dairy A which may have reduced sensitivity to detect a response to the fish meal compared with Dairy B. Reports in the literature on the effects of replacing soybean meal with fish meal in the diet on milk production have not been consistent. Several studies report no effect of fish meal on milk production in early lactation (4, 8, 25, 29). Cows fed low-concentrate diets supplemented with fish meal produced 1.5 kg/d more milk compared to cows supplemented with groundnut meal in early lactation (19). However, there was no positive response when fish meal was supplemented in a high-concentrate diet. When corn silage was the main dietary forage source, cows supplemented with fish meal improved milk

production by 1.6 kg/d per cow (21).

CONCLUSIONS

Inclusion of fish meal (oil) in the diet resulted in an alteration in regression dynamics of the corpus luteum as evidenced by a greater proportion of cows having elevated plasma concentrations of progesterone at 48 h after injection of $PGF_{2\alpha}$.

Lowered fertility at Dairy B may have resulted from greater duration of lower body condition. Body condition of cows synchronized twice at Dairy A was related positively to estrus detection, pregnancy and conception rates. Body condition of all cows at Dairy B was related positively to pregnancy rate. A greater percent of cows became pregnant by 120 d PP when fed Menhaden fish meal.

Replacement of typical undegradable protein sources with a ruminant grade Menhaden fish meal, fed at approximately 0.7 kg/d DM, resulted in positive benefits to animal performance. Cows fed fish meal at Dairy B produced a greater amount of fat, fat-corrected milk, and protein daily over the period of study. Daily production of uncorrected milk was improved only by cows in their second lactation (38% of cows on trial). Fish meal provided no benefit to milk production at Dairy A; milk composition was not determined at this farm. Differences for this response between dairies may be due to differences in milk production (10% more milk produced at Dairy B, resulting in a greater amino acid requirement), inability of cows to regain lost body condition at the end of the study at Dairy B (resulting in a greater reliance on dietary amino acids and less on tissue amino acids), or the replacement of a low lysine (corn gluten meal) with a high lysine (fish meal) protein source at Dairy B.

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Figure 1. Plasma concentrations of progesterone (ng/ml) at 48 h after injection of $PGF_{2\alpha}$ for cows consuming the control diet (□; n = 25) or fish meal (■; n = 31). There was a greater proportion of cows consuming fish meal that had concentrations > 1 ng/ml ($P < 0.025$).

Figure 2. The relationship between BCS at 58 d PP and mean milk production of cows at Dairy B ($y = 32.9 + 10.95x + -2.21x^2$; $P < 0.05$; $R^2_{model} = 0.18$; $R^2_{reg} = 0.03$).

Figure 3. Mean BCS at 0 to 10 d, 30 ± 3 d, 58 ± 3 d, and 104 d PP for cows undergoing one synchronization (●; n = 108) or two synchronizations (■; n = 133) at Dairy A.

Table 1. Ingredient and chemical composition of experimental diets.

	Dairy A		Dairy B	
Ingredient	Control	Fish meal	Control	Fish meal
Corn silage	21.5	21.5	22.4	22.4
Alfalfa hay	7.2	7.2	5.3	5.3
Cottonseed hulls	-	-	5.7	5.7
Bermudagrass hay	4.7	4.7	-	-
Hominy	30.9	30.9	30.9	30.5
Whole cottonseed	12.6	12.6	9.0	9.0
Wet brewers grains	5.4	5.4	11.0	11.0
Lacto-whey	-	-	7.0	7.0
Fish meal	-	2.7	-	2.8
Soybean meal	7.7	7.7	1.3	2.4
Meat and bone meal	.9	-	0.7	-
Blood meal	.9	-	1.0	-
Corn gluten meal	-	-	1.5	-
Minerals & Vitamins	5.0	4.1	4.2	3.8
Chemical composition				
DM, %	58.1	58.1	52.9	52.9
NE_L, Mcal/kg	1.71	1.71	1.71	1.71
CP, % DM	18.1	18.1	19.8	19.5
Soluble protein, % CP	25.4	24.6	35.2	33.7
UIP, % DM	7.0	7.1	6.9	6.9
Ether extract, % DM	5.6	5.7	6.7	6.7
NDF, % DM	28.9	28.8	35.8	35.1
ADF, % DM	18.3	18.3	18.9	18.4
Ca, % DM	.98	1.01	1.05	1.00
P, % DM	0.52	0.57	0.52	0.52
K, % DM	1.4	1.4	1.6	1.5
Mg, % DM	0.37	0.34	0.37	0.38

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

	Period 1		Period 2	
Days PP	Inject	Time	Inject	Time
30 ± 3[1]	$PGF_{2\alpha}$	1200 h	$PGF_{2\alpha}$	1200 h
51 ± 3	GnRHa	1200 h	GnRHa	1200 h
58	$PGF_{2\alpha}$	1200 h	$PGF_{2\alpha}$	1200 h
60			GnRHa	1200 h
61	AI at Detected Estrus		Timed AI	0600 h
65[2]	GnRHa	1200 h		
72	$PGF_{2\alpha}$	1200 h		
	AI at Detected Estrus			

[1]Represents a range of days.
[2]If cow was not detected in estrus then resynchronizion occurred at 65 d PP, Period 1.

Table 3. Effect of feeding fish meal on estrus detection, pregnancy, and conception rates, and other reproductive responses by lactating cows at two Florida dairy farms. Least square means are adjusted for appropriate effects in model.

	Dairy A					Dairy B				
	Control		Fish meal			Control		Fish meal		
Response	n	x̄	n	x̄	SE	n	x̄	n	x̄	SE
Number of cows	166		175			146		154		
Estrus detection rate[1], %										
First Synchrony	166	65.3	175	57.1	4.8	109	50.0	116	51.4	5.6
Second Synchrony	57	58.7	76	53.3	6.7	34	49.2	47	71.6	8.6
Pregnancy rate[1], %										
First Synchrony	166	28.7	175	20.7	4.2	109	14.4	116	18.4	4.0
Second Synchrony	56	23.5	76	24.1	6.0	40	10.2	58	18.4	4.9
Conception rate[1], %										
First Synchrony	109	40.1	99	32.9	6.6	56	29.1	50	37.5	7.9[2]
Second Synchrony	31	36.4	39	38.5	9.8	17	9.0	29	24.5	11.1
Overall pregnancy rate to 120 d PP[3], %	161	65.4	174	60.2	3.7	144	31.9	150	41.3	4.0
Overall conception rate to 120 d PP, %	155	66.5	159	70.1	5.9	133	33.4	141	41.3	6.4
Conception rate at first AI[4], %	159	42.3	160	40.9	6.4	133	19.7	150	22.1	5.0
Number of days open to 120 d PP (for cows that conceived), d	106	78.0	105	79.5	2.7	47	74.0	62	77.2	4.0

Table 3. Effect of feeding fish meal on estrus detection, pregnancy, and conception rates, and other reproductive responses by lactating cows at two Florida dairy farms. Least square means are adjusted for appropriate effects in model.

	Dairy A					Dairy B				
	Control		Fish meal			Control		Fish meal		
Number of AI per conception to 120 d PP	106	1.5	105	1.4	0.10	46	1.4	62	1.4	0.11
Time to first AI[5], d	155	68.0	159	69.8	1.8	133	64.8	141	64.6	1.4
Interval from $PGF_{2\alpha}$ to AI, d	103	3.2	97	3.2	0.13	88	3.06	87	3.12	0.14
Number of heats before AI	166	0.53	174	0.43	0.05		NE[6]		NE[6]	
Cows detected in estrus before 48 d PP, %	166	40.9	176	42.7	3.7		NE[6]		NE[6]	
Progesterone 51 d PP (ng/ml)	162	5.6	170	5.4	0.35	137	4.5	141	4.7	0.38
Progesterone 58 d PP (ng/ml)	153	4.5	166	4.4	0.29	136	5.2	147	4.5	0.34
Progesterone 65 d PP (ng/ml)[7]	58	2.3	77	1.9	0.27	42	1.5	53	2.6	0.30
Progesterone 72 d PP (ng/ml)	56	5.9	70	5.8	0.49	38	6.4	55	6.6	0.68
Progesterone 2 d post-$PGF_{2\alpha}$, ng/ml		NE[6]		NE[6]		25	0.57	31	1.26	0.30

[1]Cows in Period 2 in Dairy B were not represented in these analyses because these cows were synchronized only once.
[2]Column means within Dairy B differ for response ($P < 0.07$).
[3]Diet x dairy interaction ($P < 0.06$).

[4]Includes synchronized and unsynchronized inseminations.
[5]Includes all cows that were inseminated by 120 d PP.
[6]Not estimated.
[7]Row means within Dairy B differ for response ($P < 0.01$).

Table 4. Body condition scores (BCS) and blood urea nitrogen (BUN) concentrations (mg/100 ml of plasma) of cows consuming control or fish meal diets at two Florida dairy farms. Standard errors follow means. Least square means are adjusted for appropriate effects in model.

	Dairy A					Dairy B				
	Control		Fish Meal			Control		Fish Meal		
Response	n	x̄	n	x̄	SE	n	x̄	n	x̄	SE
BCS, 0 - 10 d PP	69	3.4	81	3.4	0.07	79	3.4	90	3.2	0.07
BCS, 30 d PP	164	3.0	169	3.0	0.05	79	2.8	90	2.8	0.06
BCS, 58 d PP[1]	142	3.1	158	3.1	0.05	124	2.9	134	2.7	0.06
BCS, Final[2]	142	3.3	140	3.4	0.06	138	2.8	145	2.9	0.06
BCS change, 0 - 30 d PP	67	0.39	79	0.35	0.06	50	0.43	62	0.47	0.06
BCS change, 0 - 60 d PP	51	0.39	69	0.29	0.08	55	0.42	67	0.51	0.06
BCS change, 60 - 30 d PP[1]	140	0.08	152	0.09	0.04	95	0.01	108	-0.11	0.04
BUN, 58 d PP	162	17.2	167	17.3	0.2	124	21.4	134	21.3	0.4
BUN, Final[2,3]	140	17.0	142	16.4	0.2	138	21.5	145	21.3	0.5

[1]Row means within Dairy B differ ($P < 0.03$).
[2]Final BCS taken at 104 ± 1 d at Dairy A and 96 ± 2 d at Dairy B.
[3]Row means within Dairy A differ ($P < 0.07$).

Table 5. Relationship between change in body condition score (BCS) and estrus detection, pregnancy, or conception rates.

y	x	Equation	R_{model}^2	R_{reg}^2	*P*
Estrus detection rate (%)	0 - 30 d PP	y = 59.2 - 18.5x	0.11	0.03	0.008
Estrus detection rate (%)	0 - 58 d PP	y = 57.7 - 18.0x	0.09	0.03	0.009
Pregnancy rate (%)	0 - 30 d PP	y = 27.0 - 16.8x	0.08	0.03	0.005
Pregnancy rate (%)	0 - 58 d PP	y = 25.9 - 15.6x	0.08	0.04	0.005
Conception rate (%)	0 - 30 d PP	y = 38.5 - 19.7x	0.04	0.04	0.02
Conception rate (%)	0 - 58 d PP	y = 35.3 - 23.2x	0.07	0.06	0.004

Table 6. Effect of feeding fish meal on uncorrected milk production (kg/d) by lactating dairy cows at two Florida dairy farms.

	Dairy A					Dairy B				
	Control		Fish meal			Control		Fish meal		
Parity	n	x̄	n	x̄	SE	n	x̄	n	x̄	SE
All[1,2]	162	42.3	174	42.5	1.2	147	46.4	156	46.2	1.1
Second[3]	55	41.9	85	40.6	1.6	61	42.6	54	44.9	1.6
Third	46	44.1	28	44.1	2.3	34	47.0	50	46.3	2.0
Fourth[4]	26	42.8	30	44.1	2.7	24	49.9	25	46.8	2.5
≥ Fifth	35	40.6	31	41.0	2.4	28	46.0	27	46.7	2.5

[1]Column means differ for parity (Dairy A, $P < 0.002$; Dairy B, $P < 0.001$).
[2]Diet by parity interaction for Dairy B ($P < 0.03$).
[3]Diet by second vs. ≥ third parity interaction ($P < 0.01$).
[4]Diet by fourth vs. ≥ fifth parity interaction ($P < 0.07$).

Table 7. Effect of feeding fish meal on milk production (kg/d; adjusted with mature equivalent milk weight as a covariable) by lactating dairy cows at two Florida dairy farms.

	Dairy A					Dairy B				
	Control		Fish meal			Control		Fish meal		
Parity	n	x̄	n	x̄	SE	n	x̄	n	x̄	SE
All	160	42.2	174	42.6	1.0	143	46.1	153	46.2	0.9
Second[1,2,3]	55	41.8	85	41.1	1.5	57	43.0	51	45.3	1.5
Third	46	42.5	28	44.0	2.1	34	46.7	50	46.0	1.7
Fourth	26	42.6	30	42.9	2.3	24	48.7	25	47.0	2.2
≥ Fifth	33	41.7	31	42.4	2.2	28	46.2	27	46.5	2.0

[1]Second vs. ≥ third parity were different, Dairy A ($P < 0.05$).
[2]Second vs. ≥ third parity were different, Dairy B ($P < 0.001$).
[3]Diet by second vs. ≥ third parity interaction, Dairy B ($P < 0.01$).

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

	Control		Fish meal		
Measurement	n	x̄	n	x̄	SE
Milk fat (%)					
All lactations	133	3.19	149	3.27	0.05
Second	58	3.20	51	3.28	0.08
Third	28	3.20	48	3.40	0.09
Fourth	23	3.25	24	3.12	0.12
≥ Fifth	24	3.10	26	3.28	0.12
Milk fat (kg/d)					
All lactations[1,2]	133	1.52	148	1.57	0.06
Second	58	1.41	50	1.52	0.09
Third	28	1.52	48	1.62	0.11
Fourth[3]	23	1.68	24	1.52	0.15
≥ Fifth	24	1.47	26	1.63	0.15

[1]Parity ($P < 0.04$).
[2]Diet by parity interaction ($P < 0.06$).
[3]Diet by fourth vs. ≥ fifth parity interaction ($P < 0.01$).

Table 9. Effect of feeding fish meal on 4% fat-corrected milk production (kg/d) by lactating dairy cows at Dairy B.

	Control		Fish meal		
Parity	n	x̄	n	x̄	SE
All parities[1]	133	41.9	148	42.9	1.3
Second	58	38.7	50	41.5	2.0
Third	28	42.0	48	43.3	2.3
Fourth[2]	23	45.9	24	42.5	3.0
≥ Fifth	24	41.3	26	44.4	3.1

[1]Parity ($P < 0.002$).
[2]Diet by fourth vs. ≥ fifth parity interaction ($P < 0.01$).

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

	Control		Fish meal		
Measurement	n	x̄	n	x̄	SE
Milk protein (%)					
All parities[1]	133	2.80	149	2.85	0.02
Second	58	2.83	51	2.88	0.03
Third	28	2.87	48	2.87	0.04
Fourth[2]	23	2.73	24	2.88	0.05
≤ Fifth	24	2.78	26	2.76	0.05
Milk protein (kg/d)					
All parities[3,4]	133	1.33	148	1.37	0.04
Second[5]	58	1.24	50	1.34	0.05
Third	28	1.37	48	1.36	0.06
Fourth	23	1.40	24	1.41	0.08
≤ Fifth	24	1.33	26	1.36	0.08

[1]Parity ($P < 0.05$).
[2]Diet by fourth vs. ≥ fifth parity interaction ($P < 0.04$).
[3]Control vs. fish meal ($P < 0.11$).
[4]Parity ($P < 0.001$).
[5]Diet by second vs. ≥ third parity interaction ($P < 0.04$).

Figure 1

BLOOD PROGESTERONE LEVELS - COWS ON FARM B

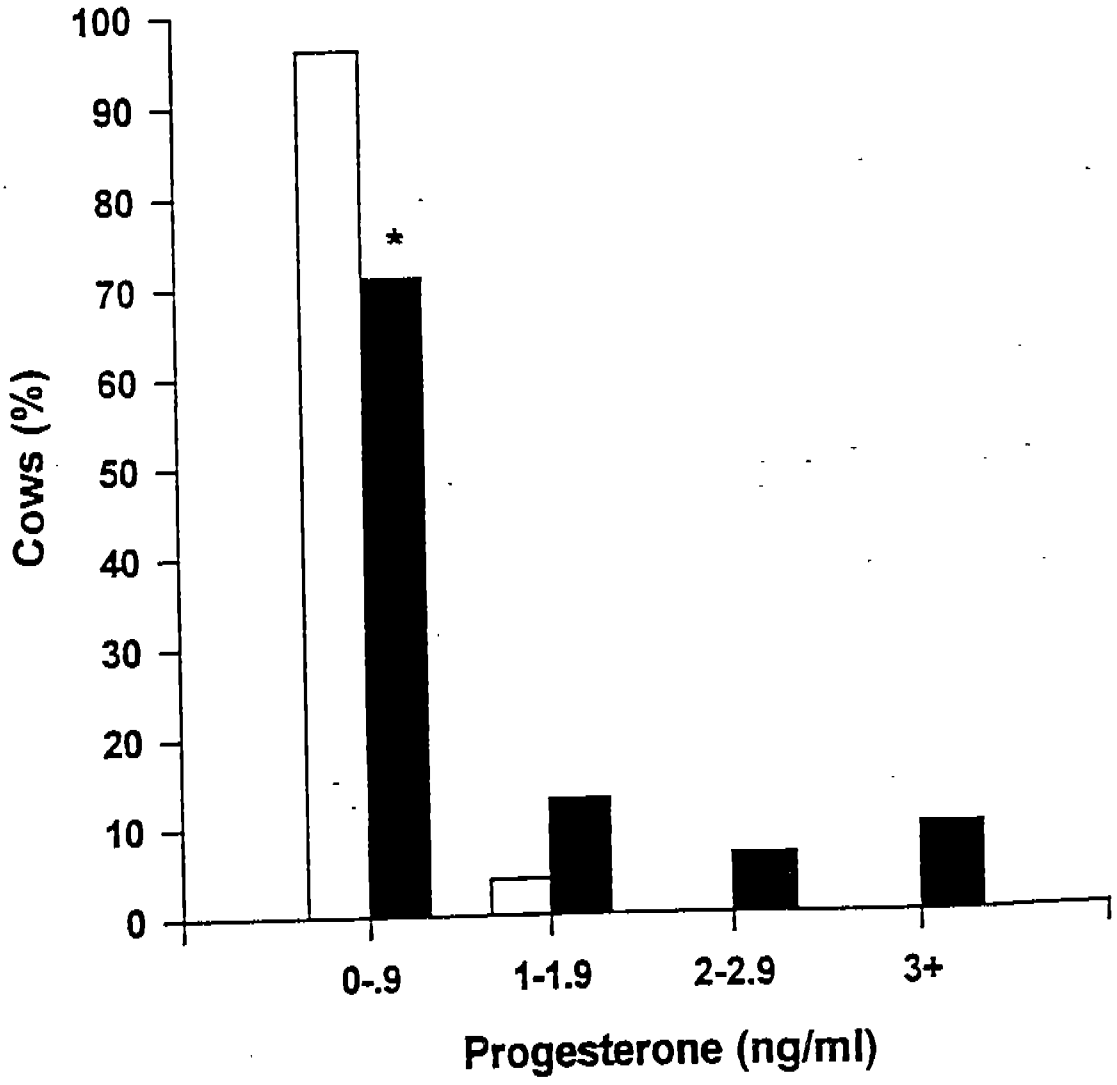

Figure 2

RELATIONSHIP BETWEEN MILK PRODUCTION AND BODY CONDITION SCORE (ON SCALE 0 TO 5)

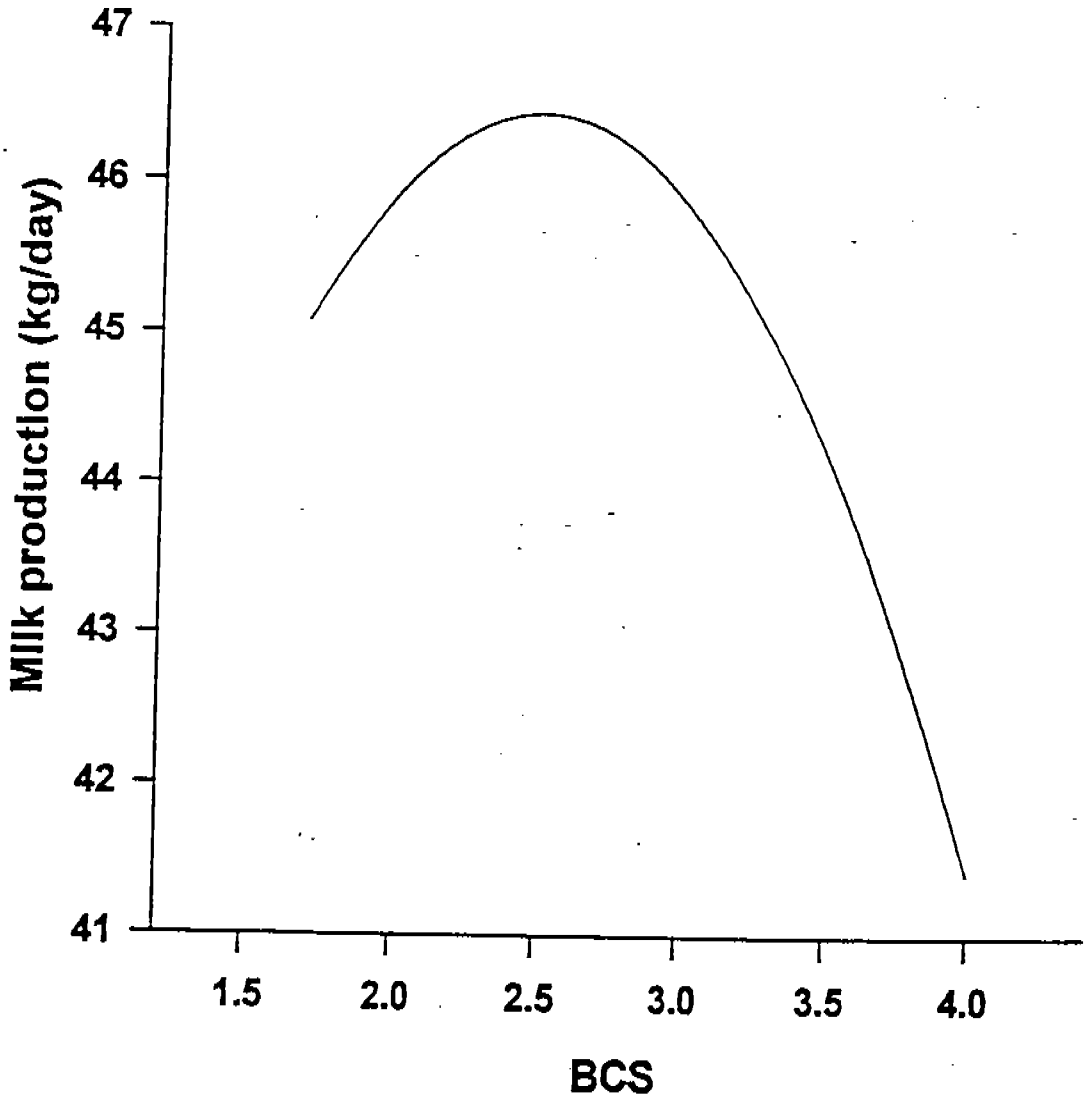

Figure 3

BODY CONDITION SCORE OF COWS REQUIRING ONLY ONE SYNCHRONISATION (●) V THOSE FAILING TO SHOW OESTRUS (■) (FARM A)