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The effects of supplementation with 3-omega fatty acids on plasma metabolites and ovarian function of dairy cows.

S. MEIER
Dairying Research Corporation, Private Bag 3123, Hamilton, New Zealand

ABSTRACT

Two- year- old Holstein Friesian dairy cows (n=20) were allocated to receive either a rumen- protected fish oil supplement (omega group, n=10), starting between 5-6 weeks post-partum, or pasture only (control group, n=10). .Serum metabolites, including cholesterol, urea, glucose and beta-hydroxybutyrate (BOH) were measured weekly from calving through to week 11-12 post-partum. Within 2 weeks of the commencement of treatment, serum cholesterol concentrations had increased significantly in the omega group vs the control group, and continued to increase through to weeks 11-12 post-partum. None of the other serum metabolites measured showed any differences with treatment. However, levels of serum urea, BOH and plasma glucose showed significant variations from week to week . The size of the dominant follicles and corpus luteum were measured, using transrectal ultrasonography, on days 8 to 16 of both oestrous cycles. The corpus luteum of the second cycle was significantly larger in the omega group. The second oestrous cycle was interrupted through an oxytocin challenge carried out to measure prostaglandin synthesis, on day 16 of the cycle. After the oxytocin challenge, the omega group exhibited a longer interval to the next observed oestrus. These results suggest that supplementing dairy cows with 3-omega fatty acids will result in changes at the site of the ovary and uterus.

Keywords: Fatty acids; plasma metabolites; reproduction; ovary; cow.

INTRODUCTION

It has been postulated that dietary supplementation with products high in certain fatty acids, will have a positive effect on reproduction. Intravenous infusion of soybean oil (containing 50% linoleic acid) into Holstein heifers resulted in an increase in plasma concentrations of the circulating metabolite of $PGF_{2\alpha}$ (13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$: PGFM; Lucy *et al.*, 1990). Feeding calcium salts of long chain fatty acids to lactating cows increased the number of large follicles and the diameter of the largest follicle (Lucy *et al.*, 1991). High levels of linoleic acid supplementation increased pregnancy rates (Wilkins *et al.*, 1996). These effects on reproductive function have been attributed to the fatty acids themselves and not to any effect on energy balance (Lucy *et al.*, 1993).

The mechanisms underlying the effects of fatty acid supplementation are not well understood. During the oestrous cycle, luteal fatty acid content changes, with a linear increase in n-3 fatty acids. These changes are thought to be due to a change in the ratio between n-6 and n-3 fatty acids, which, in turn, plays a role in the rate of n-6 eicosanoid formation (Lands, 1992). Hinckley *et al.* (1996), suggested that the observed changes in luteal fatty acids may mirror functional changes occuring during the oestrous cycle. They examined the effects of polyunsaturated fatty acids on progesterone and prostaglandin synthesis by dispersed bovine luteal cells. The C20:5n-3, C22:4n-6 and C22:6n-3 fatty acids were potent inhibitors of progesterone and prostaglandin synthesis.

Hawkins *et al.* (1995) found that lipid supplementation increased the lipid content of luteal cells and altered progesterone synthesis and clearance in supplemented animals. A study by Hightshoe *et al.* (1991) showed changes in progesterone synthesis in cows fed salts of fatty acids. Burke *et al.* (1997) also observed altered luteal function in cows fed Menhaden fish meal in early lactation. Another study which fed Menhaden fish meal, containing 17g/ day of eicosapentaenoic acid (EPA) and docasahexaenoic acid (DHA), altered both CL function (increased progesterone secretion) and uterine prostaglandin synthesis (decreased plasma PGFM to oestradiol/oxytocin stimulus; Thatcher, pers. comm). Oxytocin-induced prostaglandin synthesis on day 15 of the oestrous cycle was depressed in lactating cows which had received an infusion containing 17% linoleic acid into the abomasum (Oldick *et al.*, 1997). Fatty acids such as linoleic acid can act as both a substrate and inhibitor to PGF synthesis, which may be determined by the nutritional status of the animal and the presence of other fatty acids that may compete for linoleic and arachidonic acid (Mathews & van Holde, 1990; Oliw *et al.*, 1983; Pace-Ascik & Wolfe, 1968).

This study investigated effects of a rumen-protected 3-omega fatty acids, given as a feed supplement, on the size of the dominant follicle and the corpus luteum, plasma progesterone, blood metabolites and the induction of luteolysis in post-partum dairy cows

MATERIALS AND METHODS

Two-year-old Holstein Friesian dairy cows (n=20) were allocated to receive either a rumen-protected fish oil supplement (omega group, n=10), starting between 5-6 weeks post-partum, or pasture only (control group, n=10). Management up to calving was according to standard farm practice pre- and post-calving. Animals were balanced for calving date when allocated to a treatment. All animals were monitored for ovulation by measuring milk progesterone concentrations from samples taken 3 times a week.

Urea, glucose, cholesterol and beta-hydroxybutyrate (BOH) were measured in serum taken weekly from calving through to week 11-12 post-partum. Blood samples were taken from the coccygeal vein into vacutainers and were analysed using a spectrophotomeric auto-analyser Hitachi 717 (Alpha Scientific Ltd, Hamilton, New Zealand) for urea nitrogen (urease method), glucose (hexokinase method),

cholesterol (cholesterol esterase method) and BOH. The inter-assay coefficients of variation (CVs) were 2%, 2%, 2% and 3%, respectively. The intra-assay CVs were 1%, 2%, 2% and 2%, respectively.

On days 26-37 post-partum (average 33.3±0.75 days), the heifers were treated with an intravaginal device containing progesterone for 6 days (CIDR-B,, InterAg, Hamilton, New Zealand) followed by an i.m. injection of oestradiol benzoate (1 mg ODB, Intervet Pty Ltd, Castle Hill, NSW, Australia) at 24hr after CIDR removal. From the day of induced oestrus, the animals in the treatment group (omega group) were given twice daily doses of rumen-protected fish oil. The supplement was made up of MAXEPA, a fish oil containing 30% of EPA/DHA (Scherer RP Holdings, Melbourne, Australia). The total supplement was 220g per day of protected fish oil, equal to 18g EPA/DHA in the form of rumen-protected fish oil.

Ovarian development during the first cycle after the induced oestrus and the following oestrous cycle was monitored by plasma progesterone concentrations measured from samples taken every second day. Concentrations of progesterone in milk and plasma were determined using a commercially available RIA kit (Coat-A-Count™, DPC, California, USA). This assay has been validated for bovine plasma and milk progesterone (Dieleman and Bevers, 1987; McDougall *et al.*, 1995). Sensitivity of the assay was 0.07 ng/ml. The intra- and inter-assay coefficients of variation, for plasma and milk (n=10 assays), at concentrations of 4.5, 3.1 and 0.4 ng/ml are 8.7% and 7%, respectively. Follicle development was monitored using transrectal ultrasonography carried out every second day from day 8 to 16 of each oestrous cycle. The maximum diameter of the dominant follicle , at this time, and the maximum diameter of the CL were monitored, during this period.

Animals that responded to the initial treatment and continued to show cyclic activity underwent an oxytocin challenge. On day 15 of the second oestrous cycle, animals were subjected to an oxytocin challenge consisting of an intravenous injection of 100 IU of synthetic oxytocin (Butocin, Bomac Laboratories Ltd, Manukau City, New Zealand).

The statistical package Data Desk (6.1; Data Description Inc., Ithaca, New York, USA) was used to analyse the results. Differences between the two groups were analysed using ANOVA and a Post-Hoc test. Where appropriate, logarithmic transformation of data was carried out to maintain the homogeneity of the data. All data are expressed as the mean with the standard error of the mean (mean ± SEM). Differences of $P<0.05$ were considered significant unless otherwise noted. The correlation between plasma metabolites was tested using the Pearson product-method.

RESULTS

The serum metabolites urea, glucose, cholesterol and beta-hydroxybutyrate (BOH) were measured weekly from calving through to week 11-12 post-partum. Within 2 weeks of the treatment commencing, serum cholesterol concentrations had increased in the omega group vs the control group, and continued to increase significantly through to weeks 11-12 post-partum (Figure 1). None of the other serum metabolites measured showed any differences with treatment, however, levels of serum urea and glucose varied from week to week. A negative correlation between serum urea and glucose was evident (Figure 2). This correlation was strong enough to be present even when normal trends were removed. The weekly correlation between urea and glucose was -0.22 ± 0.08 (mean ± SEM across all weeks, $P>0.02$, respectively). The mean of this correlation for all the animals was 0.28±0.12, respectively.

FIGURE 1. Changes in mean (± SEM) serum cholesterol concentrations (mmol/l) in post-partum heifers treated with the fish oil supplement (omega group; n=10, ■) or untreated (control; n=10, O), with the start of supplementation occurring on week 5-6. * Statistical significant difference $P<0.06$; ** statistical significant difference $P<0.02$.

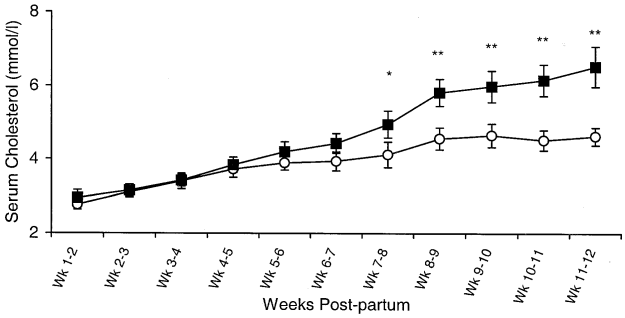
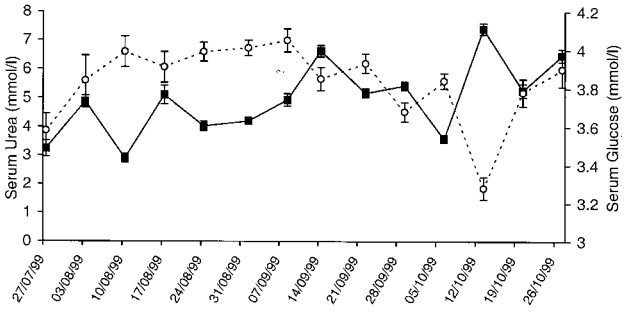


FIGURE 2. Weekly concentrations of serum urea (■) and glucose (O) in 2-year-old lactating dairy cows, from late July to late October 1999. Data represented as the mean ± SEM (n=20).

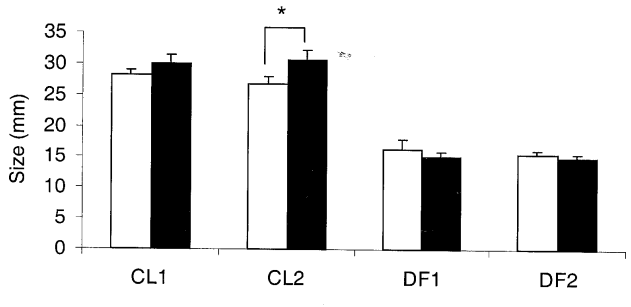


The maximum diameter of the dominant follicle and the corpus luteum were measured using transrectal ultrasonography on days 8 to 16 of both oestrous cycles. The corpus luteum of the second cycle was significantly larger in the omega group (30.5 ± 1.8 vs 26.8 ± 1.2 mm, respectively; $P<0.05$; Figure 3). Maximum progesterone concentrations reached during the first oestrous cycle were similar in the omega group vs the control group (8.3±0.6 vs 7.0±0.6 ng/ml, respectively; $P<0.07$). Again, during the

second oestrous cycle there was no difference between plasma progesterone concentrations in the omega vs the control group (8.7±0.6 vs 9.0±0.5vs 8.7±0.6 ng/ml, respectively).

The second oestrous cycle was interrupted by the oxytocin challenge carried out to measure prostaglandin synthesis on day 16 of the cycle. Following the challenge, the omega group exhibited a longer interval between oxytocin injection and luteal regression (4.5 +/- 0.62 vs 3.1 +/- 0.34 days, respectively; P<0.04).

FIGURE 3. Difference in the maximum diameter (mean ± SEM) of the corpus luteum (CL) and the dominant follicle during the first (DF1) and second oestrous cycle (DF2) in the control group (open column) and omega group (closed column). * P<0.05



DISCUSSION

The reproductive effects of supplementing cows with fats or fatty acid derivatives have recently been reviewed (Staples *et al.*, 1998). The effects of the rumen-protected fish oils on reproductive function may be widespread, with reports of effects on ovarian, uterine and metabolic function (Staples *et al.*, 1998). However, the majority, if not all, of these studies, involved controlled ration diets. Due to the reported wide-ranging effects associated with changes in lipids and fatty acids within tissues, this study examined some of the parameters that may play a role in a successful pregnancy establishment.

The effect of fatty acids on ovarian function is varied (Staples *et al.*, 1998). Changes in luteal fatty acids have been associated with different functional states, with the elevation in luteal progesterone production being associated with an increase in luteal lipid content (Hawkins *et al.*, 1995). The weekly variation in glucose concentrations, during the latter stages of the experiment, is interesting as glucose concentrations are correlated with energy balance (Canfield and Butler, 1990) and, are thought to play an important role as an energy source for the ovary (Rabiee *et al.*, 1999). Whether fluctuations in plasma glucose, of the magnitude seen in the current experiment, have functional implications for the ovary or for the establishment of pregnancy is uncertain.

It has previously been reported that feeding calcium salts of fatty acids will alter the number and size of follicles (Lammoglia *et al.*, 1997; Lucy *et al.*, 1993, 1991, 1990; Thomas *et al.*, 1997). In the present study, there were no differences in the sizes of the dominant follicles between days 8 to 16 of the 2 oestrous cycles examined. No attempt was made to identify all follicles within an ovary at these times, so populations of follicles of different sizes cannot be compared. An effect of lipid supplementation on the size of the corpus luteum was evident during the second oestrous cycle but not the first. However, this difference in size between treatments was not reflected in the peripheral concentrations of progesterone, which were similar. Hawkins *et al.* (1995) suggested that changes in serum lipids affected the metabolism of progesterone and, therefore, increased plasma progesterone concentrations. In the current study no significant difference in plasma progesterone was evident, even though plasma cholesterol increased significantly with treatment.

Another role of fatty acids is to act as precursors for the synthesis of prostaglandins (Lands, 1992). A number of fatty acids are important within the reproductive cycle, including arachidonic acid, which is the precursor for $PGF_2\alpha$. Linoleic acid has been shown to inhibit $PGF_2\alpha$ synthesis during early pregnancy (Danet-Desnoyers *et al.*, 1993). The 3-omega fatty acids, derived from fish oil, exhibit an inhibitory effect on prostaglandin synthesis and it is upon this property that the current study has been focused. During early pregnancy, it is essential that endogenous $PGF_2\alpha$ synthesis be inhibited for pregnancy to succeed (Thatcher *et al.*, 1995). With this in mind, it is hypothesised that embryos will have an advantage if $PGF_2\alpha$ synthesis is inhibited. In the present study, oxytocin-induced $PGF_2\alpha$ synthesis was altered by the fish oil supplement, as seen through the delayed onset of oestrus after the oxytocin challenge. This suggests that this type of supplementation could confer advantages as a product for the enhancement of pregnancy. The need remains, to examine the effects of manipulating endogenous $PGF_2\alpha$ synthesis during luteolysis and early pregnancy, and to establish any direct effects of the fatty acid supplement on the conceptus.

These results suggest that supplementing dairy cows with 3-omega fatty acids can influence reproductive function in a manner that may be harnessed to increase reproductive efficiency in pasture-grazed cows.

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