Three levels of dietary crude protein (CP), provided by soybean meal, were fed to determine effects of protein intake on progesterone and luteinizing hormone (LH) concentrations in serum of early postpartum high-producing dairy cows. Serum LH increased between the first and second weeks of lactation in all groups. Cows fed 12.7% CP had decreased basal serum LH levels (1.1 ± .04 ng/ml) as compared to those fed 16.3 and 19.3% CP (1.3 ± .03 ng/ml). A linear decline in serum LH occurred in cows which became pregnant, whereas serum LH remained unchanged in non-pregnant cows. Serum LH on day 2 and 14 of the first postpartum estrous cycle, the preconception cycle and the conception cycle tended to be lower in cows fed 12.7% CP compared to those fed 16.3 and 19.3% CP, particularly on day 2 of the conception cycle (1.2 ± .2 and 1.8 ± .2 ng/ml). Cows fed 12.7 or 16.3% CP had a decreased response to 100 µg gonadotropin releasing hormone (GnRH) compared to those fed 19.3% CP (1718 ± 552 and 3,660 ± 543 ng min/ml). Serum progesterone was significantly higher in cows fed 12.7% CP than in those fed 16.3 and 19.3% CP on day 14 of the first observed cycle and conception cycle. An interaction between percent CP fed and the change in progesterone from the first observed cycle to the cycle of conception was observed. Concentrations of LH and progesterone in blood collected during the 14 weeks of the trial were negatively correlated (r = -.12, P<.01).

(Key Words: LH, Progesterone, Postpartum Interval, Dietary Protein, High-Producing Dairy Cows.)

Introduction

Restricting dietary intake in cattle resulted in decreased concentrations of progesterone in plasma (Gombe and Hansel, 1973; Hill et al., 1970; Beal et al., 1978) and either increased (Gombe and Hansel, 1973) or did not affect concentrations of luteinizing hormone (LH) in plasma (Hill et al., 1970). Restricting energy results in increased LH release by exogenous gonadotropin releasing hormone (GnRH) in heifers, but not in cows (Beal et al., 1978).

Protein intake, as well as the energy intake, has been reduced in some of the experiments cited, but the results do not distinguish between the effects of energy and protein. In the present experiment an isocaloric ration was fed at three protein levels to determine if progesterone and LH are affected by protein intake in high-producing dairy cows.

Materials and Methods

Forty-five dairy cows, which had produced >30 kg milk/day (mature equivalent basis) during the peak period of their previous lactation, were assigned randomly in equal numbers, as they became available, to one of three groups at day 4 postpartum. The groups were fed a total ration of 12.7, 16.3 or 19.3% CP (74% TDN) on a dry matter (DM) basis, that was balanced for minerals, as described previously (Jordan and Swanson, 1979). The ration (as fed) consisted of 59% corn or grass silage (4.2% CP, 31.4% DM), 10% chopped alfalfa hay (20.4% CP, 86.7% DM) and 31% concentrate.
The concentrates were formulated to meet protein and energy requirements by adjusting the proportions of Pacific barley, soybean meal and cane molasses. The percentage concentrate remained constant within each ration throughout the experiment. Feedstuffs were mixed and fed once daily as a complete ration. Cows remained on the experiment until day 95 postpartum or until conception (maximum of 150 days). Each group had *ad libitum* access to minerals (Ca, P and Mg), trace mineralized salt and water.

Heat detection aids and daily visual observations for mounting activity, mucus discharge and restlessness were used to determine estrus, which was designated as day 0 of an estrous cycle. Cows were bred by artificial insemination at the first estrus after 45 days postpartum, unless the reproductive tract was abnormal as determined by rectal palpation. Cows with an abnormal reproductive tract at 45 days were re-examined and inseminated at the first estrous cycle after the tract was determined normal.

Each cow was bled from a coccygeal vessel on the day the cow was started on the experiment and every 7 days thereafter until 95 days postpartum. In addition, blood was obtained on day 2 and 14 of each observed estrous cycle. After allowing the clot to form at room temperature, serum was separated by centrifugation and then frozen until analyzed by radioimmunoassay for LH (McCarthy and Swanson, 1976) and progesterone (Koligian and Stormshak, 1977).

Three cows were selected from each group during the experiment and were given 100 μg GnRH in 2 ml saline intramuscularly. One cow in the group fed 16.3% CP was anestrus. She was removed from the analysis due to high preinjection levels of LH and a subsequent estrus 21 days later. The others were between day 3 and 11 of an estrous cycle, 37 to 48 days postpartum. Blood was taken from a coccygeal vessel at −30, 0, 30, 60, 90, 120, 150, 180, 210, 240, 300, and 360 minutes. Time 0 immediately preceded GnRH. All samples were processed as outlined above and analyzed for LH. In addition, the −30 and 0 min samples were analyzed for progesterone.

Body weight changes and general reproductive parameters such as days open, services per conception, days to first observed estrus, and days to first estimated ovulation were reported previously (Jordan and Swanson, 1979). Milk production and feed consumption were measured as part of another phase of this experiment (Pangborn, 1978). Data were analyzed by analysis of variance and correlation and regression analysis to determine the effect of dietary protein intake on hormonal components of blood.

**Results and Discussion**

In all groups LH increased in serum between the first and second weeks postpartum (figure 1; P<.01). Serum LH in nonpregnant cows plateaued after the initial rise. LH was lower in serum of cows fed 12.7% CP (P = .02) compared to those fed 16.3 and 19.3% CP (1.1 ± .04 vs 1.3 ± .03 ng/ml). Erb *et al.* (1971) reported that plasma LH increased from .5 to 8 days postpartum with no subsequent changes related to time, corresponding to the initial postpartum rise in LH observed in this experiment. Holstein heifers restricted to 60% of recommended TDN had decreased LH in serum and corpora lutea weights relative to heifers fed the recommended level of TDN in one study (Apgar *et al.*, 1975), while others have found that restricting energy to 62% of recommended TDN increased LH (Gombe and Hansel, 1973). On the other hand, serum LH levels in crossbred beef heifers were not affected by restricting only energy to 1/3 of NRC recommendations compared to heifers fed all nutrients at recommended levels (Spitzer *et al.*, 1978).

In cows fed 12.7% CP, LH levels in serum on day 2 and 14 of the first estrous cycle, the estrous cycle preceding conception and the estrous cycle of conception tended to be lower than in those fed 16.3 and 19.3% CP, particularly on day 2 of the cycle of conception (table 1; P = .06). (Not all cows could be used in this analysis due to missing observations caused by conception occurring at the first observed cycle, conception occurring after removal from the experiment, or a missed sample.) A subsample of fifteen cows in which a complete set of samples from all three cycles could be compared indicated no significant change from the first observed cycle to the cycle of conception in serum LH concentration at day 2 or 14 of the estrous cycle. Christensen *et al.* (1974) reported serum LH values ranging from .6 to 1.8 ng/ml during the estrous cycle, comparable to values reported herein.

Progesterone in serum samples taken on day 2 of an estrous cycle did not differ among groups (table 2). Progesterone was higher...
Figure 1. Mean serum LH in early postpartum cows fed varying levels of dietary protein and split into pregnant and non-pregnant groups after week 6. The pooled SE is .33. Number in parentheses indicates the number of observations in each group. The mean square error for protein level was 3.335 (df = 2; P = .06) and the mean square error for time was .6415 (df = 13; P < .05).

(P < .05) in the cows fed 12.7% CP on day 14 of the first observed cycle and the conception cycle, although progesterone did not differ during the preconception cycle. Saitok and Takahashi (1977) found that ovaries from rats at day seven of pregnancy fed 30.0% CP (4.9 kcal/g DE) produced four times as much progesterone as rats fed 39.9% CP (4.9 kcal/g DE). Conversely, cows and heifers fed low energy rations tended to have lower serum progesterone (Beal et al., 1978). Spitzer et al. (1978) reported heifers fed 1/3 of recommended energy had progesterone levels comparable to controls.

In the subsample of 15 cows with observations at all three cycles (first observed cycle, preconception cycle and conception cycle), serum progesterone concentrations on day 2 of the estrous cycle were low and did not change with time; however, on day 14 an interaction (P < .05) was observed between the three cycles and treatment. Holstein heifers fed 62% of recommended TDN had slightly higher plasma progesterone than heifers fed recommended levels during the first estrous cycle of experimentation but progesterone was lower in subsequent cycles (Gombe and Hansel, 1973).

An LH peak was seen at the 8th week postpartum in pregnant cows (figure 1). Because 6 of the 7 cows had ovulated during the three days prior to collection of the blood sample, this elevation can probably be attributed to the LH surge associated with ovulation. Adjustment of the LH data from pregnant cows to weeks postconception instead of weeks postpartum indicated that a rise in LH levels above those of the nonpregnant cows did not occur. When standardized to weeks postconception, LH in serum of pregnant cows declined linearly (P < .025) from 1.33 ng/ml at the first sample postconception to .84 ng/ml at the seventh week postconception (Y = 1.33 - .057X; R² = .04). Randel and Erb (1971) reported a significant decline in plasma LH from day 0 to 7 postconception, but not after this time interval. LH was reported by Wtemman and Hafs (1973) to be lower in serum of pregnant (1.0 ng/ml) than nonpregnant (1.2

![LH Levels](image)

**TABLE 1. SERUM LH (NG/ML) ON DAYS 2 AND 14 OF THE ESTROUS CYCLE (FIRST OBSERVED CYCLE, PRECONCEPTION CYCLE AND CONCEPTION CYCLE) IN COWS Fed DIFFERENT LEVELS OF CRUDE PROTEIN**

<table>
<thead>
<tr>
<th>Stage of estrous cycle</th>
<th>Dietary protein</th>
<th>12.7%</th>
<th>16.3%</th>
<th>19.3%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First observed cycle</td>
<td>1.1 ± .1(10)</td>
<td>1.6 ± .3(11)</td>
<td>1.5 ± .2(13)</td>
<td></td>
</tr>
<tr>
<td>Preconception cycle</td>
<td>1.2 ± .1(10)</td>
<td>1.3 ± .2(4)</td>
<td>1.3 ± .2(10)</td>
<td></td>
</tr>
<tr>
<td>Conception cycle</td>
<td>1.2 ± .2(12)b</td>
<td>1.8 ± .3(9)c</td>
<td>1.8 ± .2(8)c</td>
<td></td>
</tr>
<tr>
<td><strong>Day 14</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First observed cycle</td>
<td>1.1 ± .2(10)</td>
<td>1.3 ± .2(11)</td>
<td>1.2 ± .4(13)</td>
<td></td>
</tr>
<tr>
<td>Preconception cycle</td>
<td>1.4 ± .2(10)</td>
<td>1.5 ± .5(4)</td>
<td>1.6 ± .2(10)</td>
<td></td>
</tr>
<tr>
<td>Conception cycle</td>
<td>1.2 ± .1(12)</td>
<td>1.6 ± .2(9)</td>
<td>1.2 ± .2(8)</td>
<td></td>
</tr>
</tbody>
</table>

*aMean ± SE. Numbers in parentheses indicate the number of observations.

b, c Differing superscripts within a row indicate P = .06.
TABLE 2. SERUM PROGESTERONE (NG/ML) ON DAYS 2 AND 14 OF THE ESTROUS CYCLE (FIRST OBSERVED CYCLE, PRECONCEPTION CYCLE AND CONCEPTION CYCLE) IN COWS FED DIFFERENT LEVELS OF CRUDE PROTEIN

<table>
<thead>
<tr>
<th>Stage of estrous cycle</th>
<th>Dietary proteina</th>
<th>12.7%</th>
<th>16.3%</th>
<th>19.3%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12.7%</td>
<td>16.3%</td>
<td>19.3%</td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First observed cycle</td>
<td>.4 ± .1(10)</td>
<td>.5 ± .1(11)</td>
<td>.5 ± .1(13)</td>
<td></td>
</tr>
<tr>
<td>Preconception cycle</td>
<td>.4 ± .1(10)</td>
<td>.5 ± .1(4)</td>
<td>.6 ± .2(10)</td>
<td></td>
</tr>
<tr>
<td>Conception cycle</td>
<td>.4 ± .1(12)</td>
<td>.6 ± .2(9)</td>
<td>.5 ± .1(8)</td>
<td></td>
</tr>
<tr>
<td>Day 14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First observed cycle</td>
<td>4.9 ± .5(10)c</td>
<td>3.5 ± .3(10)b,d</td>
<td>3.5 ± .6(13)d</td>
<td></td>
</tr>
<tr>
<td>Preconception cycle</td>
<td>3.9 ± .7(10)</td>
<td>4.1 ± 2.1(4)</td>
<td>4.2 ± .4(10)</td>
<td></td>
</tr>
<tr>
<td>Conception cycle</td>
<td>4.5 ± .4(12)c</td>
<td>3.4 ± .5(9)d</td>
<td>3.4 ± .3(8)d</td>
<td></td>
</tr>
</tbody>
</table>

aMean ± SE. Numbers in parentheses indicate the number of observations.
bOne observation, greater than two standard deviations above the mean, was dropped.
c,dDiffering superscripts within a row indicate P<.05.

g/ml) heifers from days 2 through 11 after insemination. Randel and Erb (1971) found an average LH in serum of .4 ng/ml from 0 to 260 days of gestation and progesterone in serum varied from 9 to 26 ng/ml after day 7 gestation. Serum LH may be reduced in pregnant cows due to negative feedback at the pituitary or hypothalamus caused by the maintenance of progesterone during pregnancy at levels comparable to those during the luteal phase of an estrous cycle. Randel and Erb (1971) reported that plasma LH levels were decreased in those animals having increased plasma progesterone.

As reported previously (Jordan and Swanson, 1979), cows in the 12.7% group had fewer days open than cows in the 16.3 and 19.3% CP groups. Folman et al. (1973) hypothesized that a concentration of plasma progesterone >4 ng/ml at the peak of the luteal phase, at least one estrous cycle preceding insemination, is positively associated with conception. Cows in the 12.7% CP group had higher progesterone levels at the first observed estrous cycle postpartum and fewer days open, supporting this hypothesis.

LH and progesterone from the weekly data in the combined groups were negatively correlated (r = -.12, P<.01), indicating that progesterone may act via a negative feedback on LH release from the pituitary as reported by others (Randel and Erb, 1971; Swanson et al., 1972).

After injection of 100 μg GnRH a greater release of LH was observed during the 6-hr sampling period in cows fed 19.3% CP than in those fed 12.7 or 16.3% CP (3,660 ± 543 vs 1,718 ± 552 ng min/ml; P = .06; figure 2). Peak LH levels of 25.6 ng/ml at 90 min in the 19.3% CP group, 10.6 ng/ml at 90 min in the 16.3% CP group and 11.7 ng/ml at 120 min in the

![Figure 2](image_url). Mean response to GnRH injection (100 μg intramuscular) in lactating dairy cows fed three levels (12.7%, 16.3% or 19.3%) of dietary crude protein (CP). Each line represents the mean of 3 cows with the exception that the line representing the 16.3% CP group only includes 2 cows. The SE's were proportional to the means and ranged from .13 ng/ml to 8.05 ng/ml.
12.7% CP group occurred after the GnRH injection. Kesler et al. (1977) reported similar peak levels of serum LH (13.6 ng/ml at .5 hr) in postpartum cows after intramuscular injection of 100 μg GnRH. A second peak occurred at 2 to 3 hr post-injection (Kesler et al., 1977). In contrast to low CP, restricted dietary energy did not alter LH release after GnRH injection in intact cows, but increased LH release in intact heifers and spayed cows (Beal et al., 1978). These authors hypothesized that restricted energy intake increased LH both indirectly, since lower concentrations of progesterone were observed, and directly by increasing pituitary responsiveness to GnRH. Progesterone immediately prior to the GnRH injection was not correlated significantly with quantity of LH released in the present experiment. Since the group fed 19.3% CP had an increased release compared to the groups fed 16.3% and 12.7% CP, excess dietary protein may act directly on the pituitary to increase the responsiveness to GnRH. These results, however, must be interpreted as preliminary observations since only eight animals were used.

By decreasing hatchability and embryo survival, high levels of CP have also been found detrimental to reproduction in other animals such as the chicken, rat and mouse (Patel and McGinnis, 1977; Saitoh and Takahashi, 1977; and Knapka et al., 1977). These results indicate high levels of dietary CP supplied by soybean meal may be detrimental to fertility in early lactation of dairy cows. Feeding lower levels of CP may cause a decrease in LH and an increase in progesterone. Thorough hormonal studies, coupled with monitoring of ruminal and liver function, are needed to determine the mechanism by which high dietary CP may impair fertility.

**Literature Cited**


