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Title: FERTILITY IN THE HIGH PRODUCING POSTPARTUM DAIRY COW AS AFFECTED BY DIETARY CRUDE PROTEIN

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Forty-five high-producing dairy cows were assigned randomly in equal numbers and fed one of three isocaloric rations of 12.7% 16.3% or 19.3% crude protein (CP) starting at 4 days postpartum and continuing for 90 days. The 19.3% CP group had fewer days to first observed estrus (27 days) than the 16.3% (45 days) and 12.7% CP groups (36 days; $P < .05$). The 12.7% and 16.3% CP groups had significantly fewer services per conception (1.47 and 1.87, respectively) than the 19.3% CP group (2.47; $P < .05$). The 12.7% CP group had fewer days open than the 16.3% and 19.3% CP groups (69, 96 and 106 days, respectively; $P < .05$). Estrous cycle length did not differ between groups; however, only 24% of the observed estrous cycles were in the range of 18 to 24 days in the 12.7% CP group vs 53% and 62% in the 16.3% and 19.3% CP groups, respectively. A linear relationship between days open and dietary protein intake and services per conception existed in the three groups combined ($R^2 = .68$; $P < .01$).

Average fat corrected milk (AFCM) was not correlated significantly with days open, days to first observed estrus or services per conception. However, in the 19.3% CP group, days to first ovulation were positively correlated with AFCM ($r=.62$) while days to first ovulation were not significantly correlated in the other groups.

Serum LH increased between the first and second weeks of lactation in all groups. The 12.7% CP group had decreased ($P=.02$) basal serum LH levels ($1.1 \pm .04$ ng/ml) as compared to the 16.3% and 19.3% CP groups ($1.3 \pm .03$ ng/ml). A linear decline in serum LH occurred in cows which became pregnant ($\hat{Y}=1.33 - .057X$; $R^2=.04$; $P < .025$). 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 the 12.7% CP group compared to the 16.3% and 19.3% CP groups, particularly on day 2 of the conception cycle ($1.2 \pm .2$ and $1.8 \pm .2$ ng/ml; $P =.06$). Cows fed 12.7% or 16.3% CP had a decreased response to 100μg gonadotropin releasing hormone (GnRH) injected intramuscularly compared to those fed 19.3% CP ($P=.06$). Serum progesterone was significantly higher in the 12.7% CP group than in the 16.3% and 19.3% CP groups on day 14 of the first observed cycle and conception cycle ($P < .05$). An interaction ($P < .05$) between the percent CP fed and the change in progesterone from the first observed cycle to the cycle of conception was observed. A negative correlation between LH and progesterone ($r=-.12$; $P < .01$) existed in the weekly blood samples collected during the 14 weeks of the trial. Serum total protein and serum albumin did not change between groups. Serum albumin increased quadratically over the 14 weeks of the trial ($P < .001$) while serum total protein increased linearly the first 4 weeks ($P < .01$) and then plateaued. Increased crude protein level (within the range tested, 12.7% to 19.3% CP) increased days open and services per conception. Since discontinuous protein levels were used it cannot be ascertained that the optimum crude protein level is between the 12.7% and 16.3% CP rations. Consequently, fertility and milk production should be considered before final recommendations for an optimum crude protein level.

Fertility in the High-Producing Postpartum Dairy Cow as Affected by Dietary Crude Protein

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CHAPTER I

Effect of Crude Protein Level on Reproductive Efficiency, Serum Total Protein and Albumin in the High-Producing Dairy Cow[1]

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Key Words: Dietary Protein, Reproductive Efficiency, Albumin, Total Protein, Postpartum Interval

[1]Technical Paper No. 4867, Oregon Agricultural Experiment Station.

ABSTRACT

Forty-five high-producing dairy cows were assigned randomly in equal numbers and fed one of three isocaloric rations of 12.7%, 16.3% or 19.3% crude protein (CP) starting at 4 days postpartum and continuing for 90 days. The 19.3% CP group had fewer days to first observed estrus (27 days) than the 16.3% and 12.7% CP groups (41 days; $P < .05$). The 12.7% and 16.3% CP groups had significantly fewer services per conception (1.67) than the 19.3% CP group (2.47). The 12.7% CP group had fewer days open than the 16.3% and 19.3% CP groups (69, 96 and 106 days; $P < .05$). Estrous cycle length did not differ between groups; however, only 24% of the observed estrous cycles were in the range of 18 to 24 days in the 12.7% CP group vs 53% and 62% in the 16.3% and 19.3% CP groups, respectively. A linear relationship between days open and dietary protein intake and services per conception existed in the three groups combined (R^2=.68; $P < .01$). Average fat corrected milk (AFCM) was not correlated significantly with days open, days to first observed estrus or services per conception. However, in the 19.3% CP group, days to first ovulation were positively correlated with AFCM (r=.62) while days to first ovulation were not significantly correlated in the other groups. Serum total protein and serum albumin did not differ between groups. Serum albumin increased quadratically over the 14 week trial ($P < .001$)

while serum total protein increased linearly the first 4 weeks ($P < .01$) and then plateaued. Increased crude protein level had a negative influence on the reproductive parameters measured within the range tested (12.7-19.3% CP).

INTRODUCTION

Adequate nutrition is required to permit maximum reproduction as well as milk secretion in the high-producing dairy cow. The current National Research Council (9) recommendation for the dairy cow producing > 30 kg of milk daily is 16% crude protein (CP) on a dry matter (DM) basis. Gould (3) observed that anestrus increased, conception rate decreased and peak milk production decreased when cows were fed more than 1.84 to 1.93 kg of digestible CP/cow/day. However, VanHorn and Jacobson (20) found that increasing CP from 1.96 to 2.75 kg/cow/day resulted in increased milk production. Cows fed 75% of the recommended CP intake had a 46-day interval between calving and first estrus compared to a 35-day interval in cows fed the recommended level of CP (19). The cows on 75% of the recommended CP tended to have a shorter calving interval (20 vs 27.5 wks). Milk production was unaffected (19).

Hypoalbuminaemia occurs in cows with low crude protein intake, while hyperalbuminaemia has been found in herds on high crude protein intake (12). The serum concentration of albumin is inversely related to the number of services required per conception (13).

The present study was conducted to evaluate reproductive efficiency in early postpartum, high-producing dairy cows fed diets of 12.7%, 16.3% or 19.3% CP. Fertility parameters (days open, services per conception and days to first observed estrus) were compared with CP level, milk secretion and levels of serum total protein and serum albumin.

MATERIALS AND METHODS

Selection of 45 high-producing dairy cows was based upon production of 30 kg of milk or more (mature equivalent basis) during the peak period of their previous lactation. On day four following calving, the cows were divided randomly into three groups. The groups were fed ad libitum isocaloric total rations of 12.7%, 16.3% or 19.3% CP (74% TDN) on a DM basis. Cows remained on the experimental rations until day 95 postpartum or until conception occurred. The ration (as fed basis) consisted of 59% corn or grass silage (4.2% CP, 31.4% DM), 10% alfalfa hay (20.4% CP, 86.7% DM) and 31% concentrate (table 1). The concentrate was formulated to meet the protein requirements for each ration but the percent concentrate remained constant within each ration during the experiment. The hay was chopped and the entire ration mixed and fed once daily as a complete ration. The rations were also balanced for minerals, and each group had free access to minerals, TM salt and water. The average age of the cows was approximately 4.5 yr and did not differ between groups.

Heat detection aids and daily visual observations for mounting

TABLE 1. Average composition and analysis of experimental rations.

Component	Experimental group		
	Low CP	Intermediate CP	High CP
	% of ration, DM basis		
Corn or grass silage	28.92	28.83	28.87
Alfalfa hay	17.18	17.12	17.14
Pacific barley	49.18	40.35	29.79
Soybean meal	-----	10.84	21.50
Cane molasses	3.20	1.44	1.44
Iodized salt	.30	.30	.30
Mg oxide	.06	.06	.06
Dicalcium phosphate	.40	.16	----
Limestone	.76	.90	.90
Analysis[a]			
Crude protein	12.7	16.3	19.3
TDN[b]	74.2	73.9	73.7
Ca	.7	.7	.7
P	.4	.4	.4

[a]Dry matter basis.
[b]Calculated (9).

activity, vulval mucus discharge, restlessness and other signs of estrus were used. Cows were artificially inseminated at the first heat period after 45 days postpartum, provided the reproductive tract was normal as determined by rectal palpation. Estrous cycles were estimated using visual observations and progesterone data when the visual observations were not definitive. A cow was assumed to be in estrus if serum progesterone was < 1 ng/ml at the approximate time of ovulation and increased thereafter. Services per conception, days postpartum to first behavioral estrus and days to conception were determined. Days open were assigned a maximum of 150 days if conception had not occurred prior to 150 days postpartum.

Each cow was bled from a coccygeal vessel on the day the cow was started on experiment and every 7 days thereafter until 95 days postpartum. After centrifugation, the serum was removed and stored at -20 C until analyzed by the Lowry protein determination for total protein (7) and by a colorimetric dye-binding method for albumin (Spectru AB2 Albumin Reagent, Pierce Chemical Co.).

Data were analyzed by analysis of variance, Chi-Square, correlation and regression analysis to determine the effect of dietary protein intake on fertility and blood components. Milk production, body weight and certain metabolites in the blood were measured as part of another phase of this experiment (11).

RESULTS

Days to first observed estrus were fewer ($P < .05$) in the 19.3% CP group vs the 16.3% and 12.7% CP groups (table 2). The 12.7% and 16.3% CP groups had fewer estrous cycles preceding the first insemination (62 ± 2.5 days postpartum) than the 19.3% CP group (1.7 ± .1 vs 2.5 ± .3; $P < .01$). The number of days open decreased ($P < .05$) with decreasing CP levels as did the services per conception ($P < .05$). The average number of days open (89 ± 5 days) in the lactation preceding the experiment did not differ between groups.

Neither the observed (25, 25 and 22 days) nor the estimated length of estrous cycle (23, 23 and 22 days) differed between the 12.7%, 16.3% and 19.3% CP groups, respectively. However, in the 12.7% group only 24% of the estrous cycles were in the range of 18 to 24 days compared to 53% and 62% in the 16.3% and 19.3% CP groups, respectively ($P > .10$).

In the three groups combined a linear relationship ($P < .01$) existed between days open and the level of crude protein (table 3, part A). A greater percentage of the variation was accounted for when services per conception, in addition to dietary protein intake, was regressed on days open (table 3, part B). Services per conception accounted for most of the variation when regressed on days open in the 16.3% CP and 19.3% CP groups but not in the 12.7% CP group.

TABLE 2. Effect of dietary protein intake on the reproductive status of high-producing dairy cows

Experimental group	Reproductive parameters[a]			
	Days open	Days to first observed estrus	Services per conception	Days to first ovulation[b]
19.3% CP	106 ± 11[c]	27 ± 3[c]	2.47 ± .34[c]	16 ± 2[c]
16.3% CP	96 ± 9[d]	45 ± 4[d]	1.87 ± .27[d]	28 ± 4[d]
12.7% CP	69 ± 5[e]	36 ± 4[d]	1.47 ± .17[d]	18 ± 1[d]
Overall groups	90 ± 5	36 ± 2	1.93 ± .16	21 ± 2

[a]Mean ± SE.

[b]Estimated by non-definitive visual signs and serum progesterone.

[c, d, e]Differing superscripts within a column indicate $P < .05$.

TABLE 3. Relationship of days open to protein intake and to various other reproductive parameters.

			R^2
A.	Regression of days open on dietary protein intake (overall groups)		
	$\hat{Y} = .62 + 5.57X$	X = level of crude protein Y = days open, all groups	.18**
B.	Regression of days open on dietary protein intake (X_1) and services per conception (X_2) (overall groups)		
	$\hat{Y} = 12.86 + 1.78X_1 + 25.26X_2$	X_1 = level of crude protein X_2 = services per conception Y = days open, all groups	.68**
C.	Regression of days open on services per conception (12.7% CP group)		
	$\hat{Y} = 66.49 + 1.85X$	X = services per conception Y = days open, 12.7% CP group	.004
D.	Regression of days open on services per conception (16.3% CP group)		
	$\hat{Y} = 37.07 + 31.68X$	X = services per conception Y = days open, 16.3% CP group	.85*
E.	Regression of days open on services per conception (19.3% CP group)		
	$\hat{Y} = 38.16 + 27.31X$	X = services per conception Y = days open, 19.3% CP group	.74[a]

[a]$P < .08$. *$P < .05$. **$P < .01$.

In the 12.7% CP group the days to first estrus accounted for the largest amount of variation when regressed on days open ($\hat{Y}$ =53.88 + .43X; R^2 = .14; P > .10). Days open were not significantly correlated with services per conception (r =.07) in the 12.7% CP group while it was (P<.01) in the 16.3% CP group (r=.92) and in the 19.3% CP group (r=.86).

Average fat corrected milk (AFCM) for the first 45 days (range: 20 to 46 kg daily), the second 45 days (range: 16 to 43 kg daily) and the entire 90 days (range: 19 to 43 kg daily) of the experiment (11) was not correlated significantly with days open, days to first observed estrus, or services per conception, either in the three groups combined or in any individual group. Days to first ovulation were positively correlated with AFCM (P < .025) in the 19.3% CP group (r=.62) during the first 45 days of the experimental period, but not during the second 45 day period or the entire 90 day period. All other correlations between AFCM and days to first estimated ovulation were nonsignificant.

Serum total protein (6.22 ± .04 g/100 ml) and serum albumin (3.43 ± .02 g/100 ml) did not differ between groups. Serum albumin increased (P < .001) with time ($\hat{Y}$ = 2.58 + .18X - .007X^2; R^2 = .30) during the 14 weeks (fig. 1). Serum total protein increased linearly (P < .01) during the first 4 weeks of the experiment ($\hat{Y}$ = 5.03 + .34X;

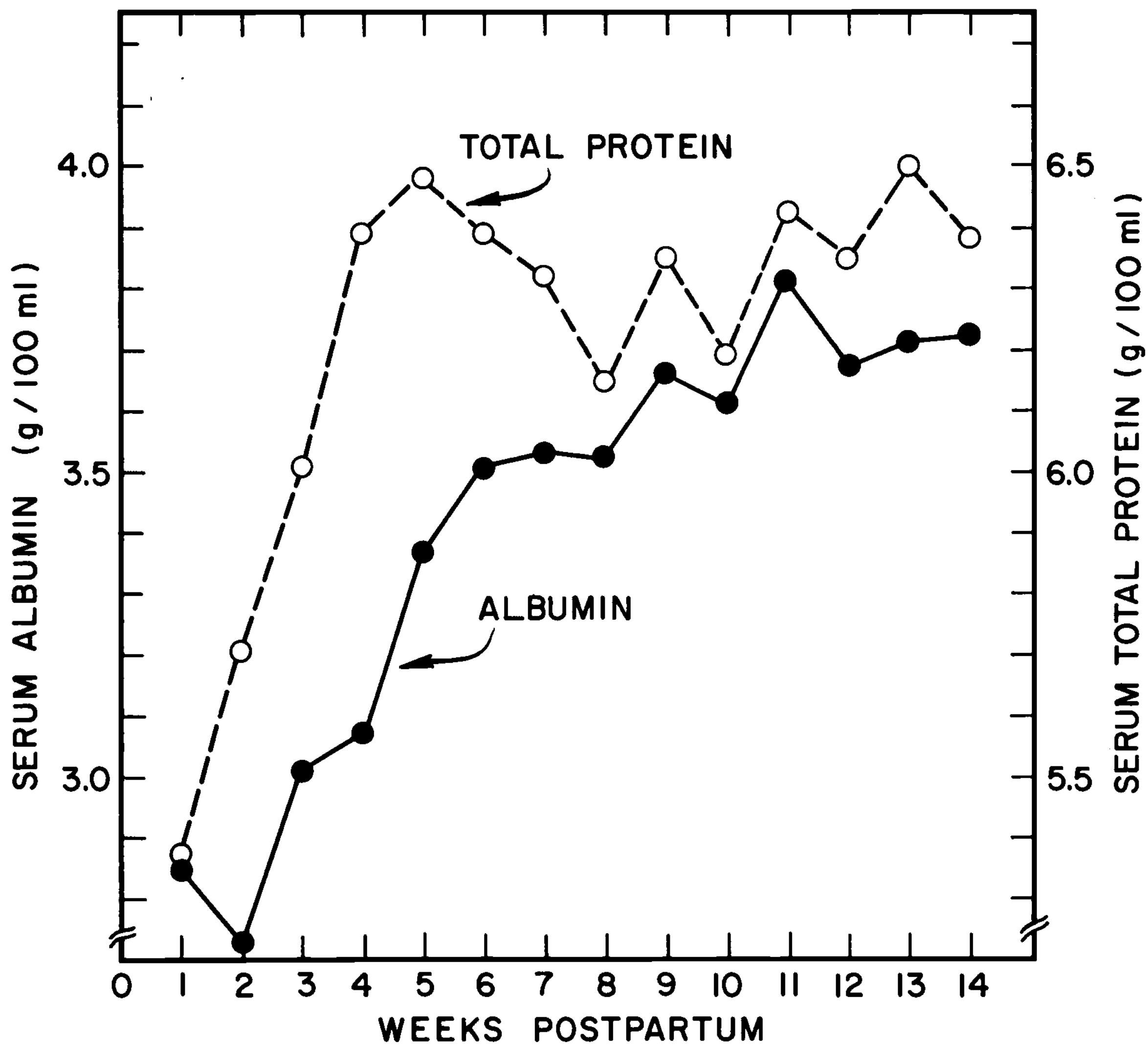

FIG. 1. Mean serum albumin and total protein in early postpartum, high-producing dairy cows fed isocaloric rations containing 12.7%, 16.3% or 19.3% CP. The SE's were proportional to the means and averaged .02 g/100 ml (serum albumin) and .04 g/100 ml (total protein).

$R^2 = .21$), then plateaued the remaining 10 weeks (fig. 1). Total serum protein did not change ($P > .10$) during the 3 weeks preconception and the 3 weeks postconception; however, serum albumin decreased prior to conception relative to postconception ($P < .01$).

DISCUSSION

In contrast to the effects of low energy rations, low protein rations (12.7% CP) apparently do not decrease fertility. Dairy cows fed a high energy ration of hay and free choice concentrates required fewer inseminations per conception and conceived 19 days earlier than cows fed a ration formulated to meet production and maintenance requrements (2). In this study, decreasing protein to 80% of NRC recommendations for cows producing > 30 kg of milk per day increased fertility by decreasing services per conception and days open. Milk production was not diminished (11).

Sommer (17) hypothesized than an excessive intake of protein causes ruminal alkalosis and increased rumen ammonia, resulting in energy deficits and liver cell disorder. Feeding increased dietary protein resulted in increased plasma levels of liver glutamic dehydrogenase and ornithine carbamyl transferase in dairy cows (18). Cellular damage may result in an increased systemic level of these liver enzymes (18). Thus, feeding dietary protein in excess of that required by tissues may cause cellular damage throughout the body, resulting in a tolerable, but not optimal, uterine or ovarian environment, thereby reducing reproductive efficiency.

Increasing protein intake also results in increased serum urea (12). Pangborn (11) observed that plasma urea nitrogen increased significantly from the 12.7% and 16.3% CP groups to the 19.3% CP group (9.08 vs 18.25 mg/100 ml, respectively). Blood ammonia tended to increase from 468 μg/100 ml in the 12.7% and 16.3% CP groups to 517 μg/100 ml in the 19.3% CP group ($P > .10$). Increases in urea nitrogen and ammonia could increase blood pH in the reproductive tract, thus reducing fertility, as sperm are most active and survive for the longest time at a neutral pH (21).

The percent CP in the ration did not affect the level of total serum protein or serum albumin. Similar findings were reported by Treacher et al. (19). Since albumin increased throughout the 90-day trial and an interaction ($P = .05$) between time and treatment occurred, the three groups were apparently increasing at different rates, masking any differences between the three protein levels.

Since total serum protein remained constant after the fourth week in all groups, it appears that the 12.7% CP ration supplied precursors adequate for milk production and body maintenance. Pangborn (11) reported no difference between the three groups in AFCM.

Olds and Seath (10) reported a significant and positive correlation between milk production and the length of the postpartum interval to first estrus. Increased milk production was significantly correlated with a decrease in the interval to uterine involution, but not with conception rate (8). When average daily milk production was correlated in this experiment with days open, days to first observed estrus, or services per conception, no impairment of fertility was associated with increased production. At the highest level of protein (19.3% CP) there was a positive correlation between days to first estimated ovulation and AFCM for the first 45 days of lactation, similar to the observations of Olds and Seath (10).

If the reproductive parameters had been compared to milk production at a later stage of lactation, the correlations may have been significant. Holtz (5) suggested that a mature cow produces 4.5 kg less milk per day after conception than a cow which has not conceived. Schaeffer and Henderson (14) and Smith and Legates (16) have computed correction factors for 305-day milk yield based on the number of days open. The AFCM 305-day ME lactation records for the 12.7%, 16.3% and 19.3% CP groups (7790, 8199 and 8474 kg/cow, respectively) did not differ significantly (11). Utilizing the Dairy Herd Improvement Association projected actual milk yield (uncorrected for fat) at 90 days, the 12.7%, 16.3% and 19.3% CP groups would have produced 7209 ± 190, 8278 ± 211, and 8030 ± 196 kg (P=.08) of milk, respectively. Correcting the latter lactation records for days open using the method of Schaeffer and Henderson (14), the projected average 305-day ME lactation records for the 12.7%, 16.3% and 19.3% CP groups were not different (7732 ± 216, 8469 ± 209, and 8186 ± 251, respectively; P > .10).

Services per conception and interval from calving to first service are important factors affecting calving interval (15). The increase in services per conception in the 19.3% CP group as compared to the other groups indicates that high levels of crude protein intake decreased fertility, possibly by causing a suboptimum environment in the reproductive tract.

Bodyweight changes over the 90 days did not differ between the 12.7%, 16.3% and 19.3% CP groups; however, slight positive correlations (P > .10) were found between days open and bodyweight changes. Downie (1) observed that cows with declining bodyweight and rising plasma glucose were fertile, but if bodyweight and plasma glucose were both declining, the cows tended to be infertile.

Since estrous cycle lengths were not different among the three groups, the effect of protein on days open does not appear to be due to an alteration in length of the cycle. However, there was more variability in cycle length in the 12.7% CP group than in the other groups. Hill (4) found that undernourishing beef heifers by feeding 85% of the control rations caused an increase in the variation of estrous cycle length. Apparently, dairy cows fed 12.7% CP do not have impaired fertility due to variability in cycle length. But it may contribute to increased difficulty in detection of estrus.

Maximum lifetime production can be obtained only when the number of days open is kept to a minimum (6). It appears from this study that feeding > 12.7% CP increased the number of days open in the high-producing cow. This may be caused by ruminal dysfunction, resulting in energy deficits and liver cell disorder, depressing gluconeogenesis, globulins and cholesterol and causing a suboptimal uterine environment. Feeding excess protein appears to be wasteful in that it is expensive and impairs reproductive efficiency without resulting in significant increases in milk production.

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CHAPTER II

SERUM PROGESTERONE AND LUTEINIZING HORMONE IN DAIRY CATTLE FED VARYING LEVELS OF CRUDE PROTEIN[1, 2, 3]

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[1]Technical Paper No. 4900, Oregon Agricultural Experiment Station.
[2]GnRH was supplied by Dr. Ray Rippel, Abbott Laboratory, North Chicago, IL and the LH used for iodination (LER 1072-2) and for standards (NIH-LH-B7) was supplied by the Endocrine Study Section, NIH.
[3]Anti-progesterone-11-BSA serum (#337) was provided by Dr. G. D. Niswender, Colorado State University, Fort Collins, CO.
[4]Department of Animal Science.

Summary

Three levels of dietary crude protein (CP), provided by soybean meal, were fed to determine the effects of protein intake on serum progesterone and luteinizing hormone (LH) in the early-postpartum high-producing dairy cow. Serum LH increased between the first and second weeks of lactation in all groups. The 12.7% CP group had decreased ($P = .02$) basal serum LH levels (1.1 ± .04 ng/ml) as compared to the 16.3% and 19.3% CP groups (1.3 ± .03 ng/ml). A linear decline in serum LH occurred in cows which became pregnant ($\hat{Y} = 1.33 - .057X$; $R^2 = .04$; $P < .025$) whereas serum LH remained unchanged in the nonpregnant 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 the 12.7% CP group compared to the 16.3% and 19.3% CP groups, particularly on day 2 of the conception cycle (1.2 ± .2 and 1.8 ± .2 ng/ml; $P = .06$). Cows fed 12.7% or 16.3% CP had a decreased response to 100 µg gonadotropin releasing hormone (GnRH) injected intramuscularly compared to those fed 19.3% CP (1717 ± 552 and 3660 ± 543 ng min/ml; $P = .06$). Serum progesterone was significantly higher in the 12.7% CP group than in the 16.3% and 19.3% CP groups on day 14 of the first observed cycle and conception cycle ($P < .05$). An interaction ($P < .05$) between the percent CP fed and the change in progesterone from the first observed

cycle to the cycle of conception was observed. A negative correlation between LH and progesterone ($r = -.12$; $P < .01$) existed in the blood samples collected during the 14 weeks of the trial.

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

Introduction

Restricting dietary intake to 60% of the recommended TDN or 85% of controls in cattle resulted in decreased plasma concentrations of progesterone (Gombe and Hansel, 1973; Hill et al., 1970; Beal et al., 1978) and either increased (Gombe and Hansel, 1973) or did not affect plasma luteinizing hormone (LH) levels (Hill et al., 1970). Restricting energy results in an increased LH release after an injection of gonadotropin releasing hormone (GnRH) in heifers, but not in cows (Beal et al., 1978).

The 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 as described previously (Jordan and Swanson, submitted). Cows remained on the experiment until day 95 postpartum or until conception (maximum of 150 days) occurred. Each group had *ad libitum* access to minerals (Ca, P and Mg), TM salt and water.

Heat detection aids and daily visual observations for mounting activity, mucus discharge, restlessness and other signs of estrus were used to determine estrus, which was designated as day 0 of the estrous cycle. Artificial insemination began 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 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 samples were obtained on day 2 and 14 of each observed estrous cycle. After allowing the clot to form at room temperature, the 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, either during anestrus or between day 3 and 11 of the estrous cycle, 37 to 48 days postpartum. Blood samples were taken from the coccygeal vessel at -30, 0, 30, 60, 90, 120, 150, 180, 210, 240, 300 and 360 minutes. Time 0 immediately preceded the injection. The samples were processed as outlined above but were analyzed only for LH with the exception that the -30 and 0 min samples were also 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, submitted). Milk production and feed consumption were measured as part of another phase of this experiment (Pangborn, 1978). Data were analyzed by analysis of variance, correlation and regression analysis to determine the effect of dietary protein intake on hormonal components of the blood.

Results and Discussion

In all groups serum LH increased betweeen the first and second weeks postpartum (figure 1; $P < .01$). Serum LH in the nonpregnant cows plateaued after the initial rise. Serum LH levels were lower in the 12.7% CP group ($P = .02$) compared to the 16.3% and 19.3%

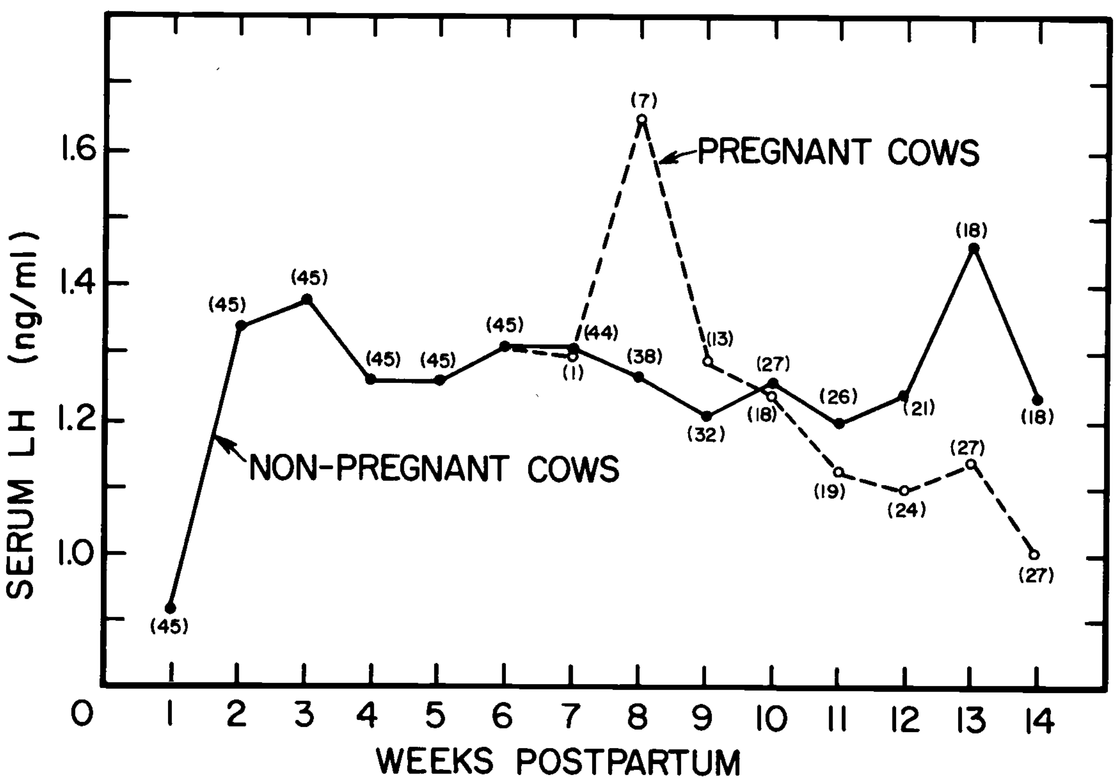

Figure 1. Mean serum LH in early postpartum cows fed varying levels of dietary protein and split into pregnant and non-pregnant groups after week 6. The pooled SE is .33. Number in parentheses indicates the number of observations in each group.

CP groups (1.1 ± .04 vs 1.3 ± .03 ng/ml) during the experiment. 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 significantly decreased LH levels 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 significantly affected by restricting only energy to 1/3 of NRC recommendations as compared to heifers fed all nutrients at the recommended level (Spitzer et al., 1978).

An LH peak was seen at the 8th week postpartum in the pregnant cows. The elevation was caused by 6 of the 7 cows having been bred during the three days prior to the sample. A rise in hormone levels above those of the nonpregnant cows did not occur when the LH data of pregnant cows were adjusted to weeks postconception. When standardized, serum LH postconception decreased linearly ($P < .025$) from 1.33 ng/ml at the first sample postconception to .84 ng/ml at the seventh week postconception ($\hat{Y} = 1.33 - .057X$; $R^2 = .04$). Randel and Erb (1971) reported a significant decline in plasma LH from day 0 to 7 postconception, but not after this time interval. Serum LH was lower in pregnant (1.0 ng/ml) than nonpregnant (1.2 ng/ml) heifers from days 2 through 11 after insemination (Wettemann and Hafs, 1973). Randel and Erb (1971) found an average serum LH level of .4 ng/ml from 0 to 260 days of gestation and progesterone levels varied from 9 to 26 ng/ml after day 7 of gestation. Serum LH may be reduced in pregnant cows due to negative feedback at the pituitary caused by the maintenance of luteal phase progesterone levels during pregnancy. Randel and Erb (1971) reported that plasma LH levels were decreased in those animals having increased plasma progesterone.

Serum LH levels in the 12.7% CP group 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 the 16.3% and 19.3% CP groups, 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.) These results correspond to the lower LH levels seen throughout the experiment in the 12.7% CP group. A subsample of fifteen cows in which all three cycles could be compared indicated no significant change from the first observed

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.

Stage of estrous cycle	Dietary Protein[a]		
	12.7%	16.3%	19.3%
Day 2			
First observed cycle	1.1 ± .1(10)	1.6 ± .3(11)	1.5 ± .2(13)
Preconception cycle	1.2 ± .1(10)	1.3 ± .2(4)	1.3 ± .2(10)
Conception cycle	1.2 ± .2(12)[b]	1.8 ± .3(9)[c]	1.8 ± .2(8)[c]
Day 14			
First observed cycle	1.1 ± .2(10)	1.3 ± .2(11)	1.2 ± .4(13)
Preconception cycle	1.4 ± .2(10)	1.5 ± .5(4)	1.6 ± .2(10)
Conception cycle	1.2 ± .1(12)	1.6 ± .2(9)	1.2 ± .2(8)

[a]Mean ± SE. Numbers in parentheses indicate the number of observations.

[b, c]Differing superscripts within a row indicate P=.06.

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 the estrous cycle did not differ among groups (table 2). Progesterone was significantly higher in the 12.7% CP group on day 14 of the first observed cycle and the conception cycle, although progesterone did not differ during the preconception cycle. 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, a significant interaction was observed between the three cycles and treatment on day 14. 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).

As reported previously (Jordan and Swanson, _submitted_), cows

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.

Stage of estrous cycle	Dietary Protein[a]		
	12.7%	16.3%	19.3%
Day 2			
First observed cycle	.4 ± .1(10)	.5 ± .1(11)	.5 ± .1(13)
Preconception cycle	.4 ± .1(10)	.5 ± .1(4)	.6 ± .2(10)
Conception cycle	.4 ± .1(12)	.6 ± .2(9)	.5 ± .1(8)
Day 14			
First observed cycle	4.9 ± .5(10)[c]	3.5 ± .3(10)[b, d]	3.5 ± .6(13)[d]
Preconception cycle	3.9 ± .7(10)	4.1 ± 2.1(4)	4.2 ± .4(10)
Conception cycle	4.5 ± .4(12)[c]	3.4 ± .5(9)[d]	3.4 ± .3(8)[d]

[a]Mean ± SE. Numbers in parentheses indicate the number of observations.

[b]One observation, greater than two standard deviations above the mean, was dropped.

[c, d]Differing superscripts within a row indicate $P < .05$.

in the 12.7% CP 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. The 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. Other researchers have also reported that elevated progesterone may cause a negative feedback on LH release (Randel and Erb, 1971; Swanson _et al_., 1972).

Injection of 100 μg GnRH resulted in decreased total LH release during the 6 hr sampling period in the 12.7% CP and 16.3% CP groups compared to the 19.3% CP group (1718 ± 552 vs 3660 ± 543 ng min/ml; $P = .06$; figure 2). One of the cows from the 16.3% CP group was removed from the analysis due to very high preinjection levels of LH, indicating a concurrent preovulatory LH release. This cow was in heat 21 days later, further indicating that the first postpartum ovulation had occurred. Peak 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

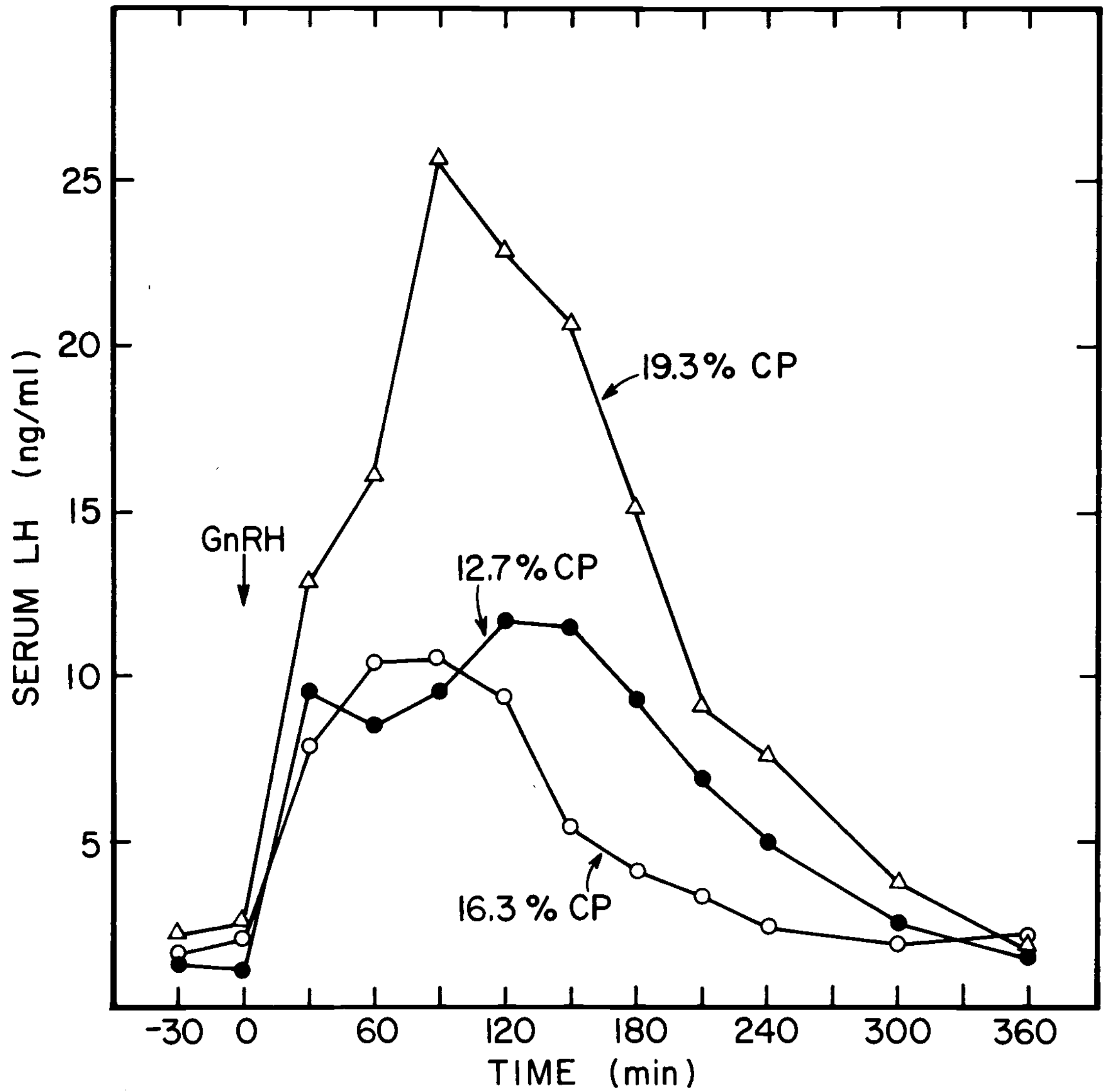

Figure 2. Mean response to GnRH injection (100 μg intramuscular) in lactating dairy cows fed three levels (12.7%, 16.3% or 19.3%) of dietary crude protein (CP). Each line represents the mean of 3 cows with the exception that the line representing the 16.3% CP group only includes 2 cows. The SE's were proportional to the means and ranged from .13 ng/ml to 8.05 ng/ml.

at 120 min in the 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 to increase pituitary responsiveness to GnRH. Progesterone immediately prior to the GnRH injection was not correlated significantly with the quantity of LH released in the present experiment, suggesting that restricted dietary protein acted directly on the pituitary to decrease the responsiveness to GnRH.

Sommer (1975) hypothesized that excess protein intake causes ruminal alkalosis and lesions in the ruminal epithelium. The ruminal disorders then cause liver cell disorders, possibly reducing synthetic efficiency. Subsequently, steroid synthesis, i.e., progesterone, may be reduced as a result of decreased cholesterol precursor.

Protein deprivation in the rat causes an increase in cyclic adenosine monophosphate (cAMP) in the β lymphocytes (Spach and

Aschkenasy, 1977). If cAMP was elevated in the corpora lutea of the 12.7% CP group, LH stimulation may have induced a greater amount of progesterone synthesis as seen in the present study.

These results indicate high levels of dietary CP supplied by soybean meal may be detrimental to fertility in early lactation. 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.

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APPENDIX

APPENDIX TABLE I: Estrous cycle length and services per conception for individual cows.

Cow No.	CP Group	Cycle Length as Observed[a]	Estimated Cycle Length using Progesterone Data[a,b]	Services per Conception[c]
1287	12.7%	28	30, 20, 20, 28	1
1175	12.7%	--	21, 22	1
1160	12.7%	15, 21	21, 15, 21	2
1188	12.7%	--	31	1
1132	12.7%	31	21, 26	2
1314	12.7%	26	22, 21	1
1321	12.7%	17, 24	17, 24	1
P-19	12.7%	26	21, 26	1
1317	12.7%	10, 40	20, 40	1
1186	12.7%	33	28, 33	2
1256	12.7%	26, 27	21, 26, 27	2
1299	12.7%	17	21, 27	3
1232	12.7%	23	23	1
1195	12.7%	23	21, 21, 23	2
1334	12.7%	16	19, 16	2
P-16	16.3%	27	21, 22, 27	2
1231	16.3%	--	23	1
1079	16.3%	24, 23, 23	24, 24, 23, 23	3
1134	16.3%	--	21, 21	1
1304	16.3%	--	24	1
1295	16.3%	20, 25, 21	20, 25, 21	1
1227	16.3%	--	21, 20	1
1200	16.3%	21, 29	21, 21, 21, 29, 21, 24	2
1282	16.3%	13	22, 22, 21	4
1315	16.3%	--	19, 22, 21	1
1047	16.3%	41	41	1
1276	16.3%	15	21, 17, 15	2
P-28	16.3%	21, 22	21, 22	3
1154	16.3%	21	21	1
1308	16.3%	33, 35	25, 33, 35	2
1176	19.3%	27, 22, 26, 25, 20, 23	27, 22, 26, 25, 20, 23	5
1266	19.3%	18, 23, 14, 21, 23, 26	18, 23, 14, 21, 23, 26	4
1209	19.3%	21, 32	21, 21, 32	3
P-26	19.3%	20, 20	21, 20, 21, 20, 20	2
P-21	19.3%	19	21, 19	1
1194	19.3%	25	25, 16	1
1300	19.3%	29, 24	22, 19, 29, 24	3
1237	19.3%	13, 20	13, 20	1
1193	19.3%	15, 6, 19	21, 19	1
1068	19.3%	20	20, 26, 20	4
1339	19.3%	21, 20	21, 21, 20, 21, 20	3
1248	19.3%	21, 23, 24	25, 21, 23, 20	3
1251	19.3%	25	26, 23, 18, 25, 25	3
1192	19.3%	--	24	1
P-20	19.3%	21, 27	21, 21, 23, 23, 18, 27	2

[a]Cycles are listed from first observed cycle to last observed cycle.
[b]Estimated by non-definitive visuals signs and serum progesterone.
[c]Up to a maximum of 150 days postpartum.

APPENDIX TABLE II: Mean serum total protein (g/100 ml) and albumin (g/100 ml) in early post-partum, high-producing dairy cows fed isocaloric rations containing 12.7%, 16.3% or 19.3% CP on a DM basis.

Weeks Postpartum	Dietary Crude Protein[a] (%, DM basis)					
	12.7		16.3		19.3	
	Serum Total Protein	Serum Albumin	Serum Total Protein	Serum Albumin	Serum Total Protein	Serum Albumin
1	5.35 ± .17	2.82 ± .14	5.15 ± .17	2.66 ± .12	5.63 ± .17	3.07 ± .11
2	5.46 ± .21	2.76 ± .12	5.85 ± .21	2.49 ± .13	5.81 ± .22	2.93 ± .10
3	5.90 ± .22	3.17 ± .15	6.21 ± .17	2.91 ± .13	6.23 ± .15	3.26 ± .09
4	6.34 ± .19	3.11 ± .11	6.55 ± .23	2.98 ± .14	6.27 ± .16	3.43 ± .09
5	6.37 ± .15	3.20 ± .16	6.65 ± .19	3.26 ± .14	6.40 ± .21	3.64 ± .09
6	6.40 ± .16	3.48 ± .12	6.53 ± .21	3.45 ± .15	6.24 ± .15	3.59 ± .10
7	6.17 ± .21	3.47 ± .12	6.60 ± .27	3.58 ± .11	6.23 ± .20	3.53 ± .11
8	5.90 ± .24	3.38 ± .13	6.42 ± .17	3.51 ± .12	6.12 ± .22	3.67 ± .10
9	6.10 ± .25	3.52 ± .17	6.18 ± .19	3.55 ± .12	6.77 ± .28	3.92 ± .08
10	5.80 ± .28	3.50 ± .13	6.42 ± .33	3.50 ± .11	6.35 ± .29	3.82 ± .12
11	6.45 ± .27	3.85 ± .15	6.20 ± .28	3.70 ± .12	6.63 ± .29	3.89 ± .11
12	6.14 ± .28	3.56 ± .13	6.60 ± .21	3.79 ± .13	6.31 ± .27	3.65 ± .10
13	5.99 ± .20	3.68 ± .14	6.56 ± .18	3.82 ± .14	6.94 ± .25	3.80 ± .11
14	6.10 ± .17	3.80 ± .18	6.61 ± .27	3.67 ± .13	6.45 ± .32	3.85 ± .15

[a]Mean ± SE.

APPENDIX TABLE III: Weekly Serum LH (ng/ml) levels in early postpartum, high-producing dairy cows fed varying levels of dietary crude protein.

Weeks Postpartum	Dietary Crude Protein[a] (%, DM basis)		
	12.7	16.3	19.3
1	.84 ± .11	.98 ± .10	.94 ± .09
2	1.22 ± .18	1.50 ± .15	1.33 ± .12
3	1.24 ± .20	1.46 ± .14	1.47 ± .17
4	1.11 ± .11	1.48 ± .15	1.19 ± .08
5	1.03 ± .13	1.36 ± .15	1.42 ± .24
6	1.23 ± .15	1.46 ± .14	1.27 ± .10
7	1.15 ± .16	1.38 ± .15	1.43 ± .20
8	1.07 ± .10	1.45 ± .10	1.46 ± .13
9	1.08 ± .16	1.21 ± .18	1.45 ± .15
10	1.01 ± .11	1.30 ± .13	1.46 ± .09
11	1.11 ± .12	1.04 ± .12	1.37 ± .16
12	1.13 ± .15	1.25 ± .12	1.14 ± .12
13	1.12 ± .12	1.39 ± .09	1.30 ± .15
14	1.01 ± .16	1.07 ± .12	1.23 ± .15
Overall Mean	1.10 ± .04	1.32 ± .03	1.31 ± .03

[a]Mean ± SE. Fifteen cows per group.

APPENDIX TABLE IV: Mean serum LH (ng/ml) in early postpartum cows (pregnant and nonpregnant) fed varying levels of dietary protein.

Weeks Postpartum	Reproductive Status[a, b]	
	Nonpregnant	Pregnant
1	.92 ± .06	----------
2	1.34 ± .08	----------
3	1.38 ± .10	----------
4	1.26 ± .07	----------
5	1.26 ± .10	----------
6	1.31 ± .07	----------
7	1.31 ± .10 (44)	1.30 ± 0 (1)
8	1.27 ± .08 (38)	1.66 ± .24 (7)
9	1.21 ± .12 (32)	1.29 ± .15 (13)
10	1.26 ± .08 (27)	1.24 ± .13 (18)
11	1.20 ± .09 (26)	1.13 ± .09 (19)
12	1.24 ± .12 (21)	1.09 ± .10 (24)
13	1.46 ± .12 (18)	1.14 ± .08 (27)
14	1.24 ± .16 (18)	1.01 ± .08 (27)

[a]Mean ± SE.

[b]Parentheses indicate the number of observations. All other data include 45 observations.

APPENDIX TABLE V: Serum LH levels (ng/ml) standardized to time following conception.

Weeks Postconception	Number of Observations	Serum LH[a]
1	26	1.33 ± .13
2	26	1.11 ± .10
3	25	1.19 ± .08
4	20	1.13 ± .09
5	19	1.02 ± .08
6	13	1.06 ± .11
7	7	.84 ± .18

[a]Mean ± SE.

APPENDIX TABLE VI: Serum LH (ng/ml) response to intramuscular injection of GnRH (100 μg) administered during postpartum anestrus or the luteal phase of the estrous cycle.

Time, Min	Dietary Crude Protein[a] (%, DM basis)		
	12.7[b]	16.3[c]	19.3[b]
-30	1.37 ± .30	1.63 ± .61	2.21 ± .42
0[d]	1.16 ± .24	2.06 ± .65	2.55 ± .18
30	9.57 ± 3.20	7.96 ± 2.65	12.92 ± 1.76
60	8.46 ± 3.03	10.41 ± 1.88	16.14 ± 2.71
90	9.53 ± 2.72	10.58 ± 1.09	25.65 ± 8.05
120	11.68 ± 4.16	9.38 ± 1.55	22.89 ± 2.56
150	11.44 ± 4.84	6.43 ± .36	21.66 ± 2.39
180	9.25 ± 4.60	4.09 ± 1.18	15.13 ± 1.21
210	6.88 ± 3.83	3.37 ± .66	9.15 ± 1.07
240	5.00 ± 2.79	2.40 ± .56	7.60 ± .73
300	2.52 ± 1.02	1.87 ± .43	3.78 ± .46
360	1.56 ± .27	2.11 ± .13	1.89 ± .48

[a]Mean ± SE.
[b]Three observations.
[c]Two observations.
[d]Time 0 is the time of the GnRH injection.