

Protein Degradability and Calcium Salts of Long-Chain Fatty Acids in the Diets of Lactating Dairy Cows: Reproductive Responses¹

C. M. GARCIA-BOJALIL,* C. R. STAPLES,^{†,2,3} C. A. RISCO,^{†,4}
J. D. SAVIO,[‡] and W. W. THATCHER^{†,3}

*Centro de Ganaderia, Colegio de Postgraduados,
Montecillo, 56230 Mexico

[†]University of Florida, Gainesville 32611

[‡]Av. De Boetendael 9, 1180 Brussels, Belgium

ABSTRACT

Multiparous Holstein cows (n = 45) were assigned at calving to one of four diets arranged in a 2 × 2 factorial design. The two main factors were dietary concentration (dry matter basis) of 1) degradable intake protein (11.1 or 15.7%) and 2) supplemental fat (Ca salts of long-chain fatty acids; 0 or 2.2%). Soybean meal and urea were replaced with less degradable protein meals (corn gluten meal, meat and bone meal, fish meal, and blood meal). During the first 9 wk postpartum, cows fed diets containing the greater concentration of highly degradable protein demonstrated less follicular development on their ovaries, were delayed in their first luteal activity postpartum (25.2 vs. 38.6 d), accumulated less luteal tissue (<15 vs. >70 mm), and had lower plasma progesterone accumulated over time. The supplementation of Ca salts of long-chain fatty acids to the 15.7% degradable protein diet doubled the number of corpora lutea, reduced time to first rise in progesterone by 6 d, doubled the number of normal luteal phases, and restored the pattern of accumulated plasma progesterone concentrations to a pattern that was similar to that induced by other diets. Cows were synchronized to estrus and inseminated at approximately 65 d postpartum. Pregnancy rate was increased from 52.3 to 86.4% when fat was supplemented. Cows fed fat tended to have more corpora lutea and a larger corpus luteum and accumulated more plasma progesterone than did cows not fed fat. Diets containing excess degradable protein or Ca salts

of long-chain fatty acids influenced ovarian structures and reproductive performance.

(**Key words:** protein degradability, fat, reproduction)

Abbreviation key: CaLCFA = Ca salts of long-chain fatty acids, CL = corpus luteum, DIP = degradable intake protein, P₄ = progesterone, PP = postpartum.

INTRODUCTION

Intake of protein beyond that needed for productive purposes has been reported to affect negatively the reproductive performance of lactating dairy cows (3, 5, 21, 22) and replacement heifers (8). However, the effect of overfeeding protein on fertility has sometimes been innocuous (6, 13, 20). The ruminal degradability of dietary protein can also influence animal response. Excess intake of ruminally degradable intake protein (**DIP**), when diets contained equal concentrations of CP, resulted in reduced conception rates (3). The proportion of fertilized ova recovered during superovulation was decreased in lactating dairy cows (1), but was unchanged in nonlactating dairy cows (15), that were fed a diet containing excess DIP contents. Ferguson and Chalupa (10) stated that the degradability of dietary protein was important in their model, which was designed to explain relationships between protein and fertility.

One mechanism by which excess protein in the diet might negatively impact reproduction is by significantly increasing the amount of energy the animal must expend to detoxify NH₃ at the liver (34). Although fat is often supplemented in the diets of dairy cows in an attempt to increase energy intake during early lactation, its impact on reproductive performance is not well documented (32).

The objective of the present experiment was to evaluate the effect of diets with differing concentrations of DIP and Ca salts of long-chain fatty acids (**CaLCFA**) on the reproductive performance of lac-

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²To whom reprints can be addressed.

³Dairy and Poultry Sciences Department.

⁴Veterinary Medicine, Large Animal Clinical Sciences.

tating Holstein cows during the first 120 d postpartum (PP).

MATERIALS AND METHODS

Cows and Diets

Multiparous Holstein cows beginning their second (n = 25), third (n = 16), or fourth (n = 4) lactation were assigned at calving to a completely randomized design employing a 2 × 2 factorial arrangement of treatments. The dietary proportions of DIP were formulated to meet and exceed NRC (28) recommendations for cows during wk 1 to 3 PP (approximately 11.1 and 15.7% of dietary DM). Soybean meal and urea were the primary N sources for the diets containing 15.7% DIP, and a combination of corn gluten meal, fish meal, blood meal, and meat and bone meal were the protein sources for the diets containing 11.1% DIP. Two concentrations (0 or 2.2% of dietary DM) of supplemental fat made up the other main factor. Fat was supplemented as CaLCFA (Megalac®; Church & Dwight Co., Inc., Princeton, NJ). Detailed information on cow management, dietary composition, and feeding protocols were previously detailed by Garcia-Bojalil et al. (16). Cows were fed the assigned diets from 1 to 120 d PP.

Plasma Progesterone

Blood (10 ml) was collected via coccygeal venipuncture into heparinized tubes three times weekly (Monday, Wednesday, and Friday) following the afternoon milking from calving until diagnosed pregnant or until 120 d PP, whichever occurred first. Samples were placed immediately in ice and centrifuged at 3000 × g for 30 min. Plasma was harvested and stored at -20°C until assayed. Plasma progesterone (P₄) concentrations (24) were determined in all samples by radioimmunoassay. Intraassay and interassay coefficients of variation were 10.6 and 10.3%, respectively.

The basal P₄ concentration (1.07 ng/ml) was the mean plasma P₄ concentration of all cows during the early anovulatory period (d 3 to 12 PP). Day PP of the first luteal phase was defined as the time when plasma P₄ concentration was >1 ng/ml for at least 2 consecutive sampling d. Length of the first luteal phase was the number of days that plasma P₄ was maintained at >1 ng/ml. Peak P₄ concentration (nanograms per milliliter) was the maximum value during a luteal phase. Plasma P₄ concentration was accumulated over days of sampling PP. Area under the curve was calculated (unadjusted by diet) for

each cow after fitting the curve of a fifth-order polynomial regression equation.

Reproductive Management

Rectal palpation. Beginning at 7 d PP, the size of the uterine horns and cervix and the presence of ovarian structures [follicles and corpora lutea (CL)] were determined weekly by rectal palpation by the same veterinarian until time of estrus synchronization (approximately 50 to 57 d PP). Cervix diameter (millimeter), diameter (millimeter) of each uterine horn at the external bifurcation, ovarian volume (length × width × height of each ovary; cubic millimeter), and diameter (millimeter) of palpable follicles and CL on each ovary were recorded. All follicular structures ≥25 mm in diameter that persisted for at least 10 d in the absence of a CL were classified as ovarian cysts (23). An ovarian cyst was further classified as a follicular cyst if it was thin walled and if the plasma P₄ concentration was ≤0.5 ng/ml or as a luteal cyst if it was thick walled and if the plasma P₄ concentration was between 0.6 and 2 ng/ml.

The previously gravid uterine horn was identified from records of pregnancy determined between 40 and 60 d of gestation and was confirmed by difference in the diameter of the uterine horns at the first rectal examination after parturition. Pregnancy during this experiment was confirmed 40 d after AI without return to estrus by rectal palpation and ultrasonography.

Estrous synchronization and AI. Cows that calved within a 7-d period of one another were grouped together. Each group of cows was subjected to a synchronization protocol after reaching 50 to 57 d PP. At this time, 8 μg of buserelin (Receptal; Hoechst-Roussel Agri-Vet Co., Somerville, NJ), a GnRH agonist, was injected i.m. in a single dose. Seven days after the injection of buserelin, 25 mg of PGF_{2α} (PGF_{2α} tam salt; Lutalyse®; Upjohn Co., Kalamazoo, MI) were injected i.m. in a single dose. At this time, a combination of tail paint and chalk was used to help in estrus detection; overnight activity was determined based on the integrity of the chalk and paint (27). In addition, all cows were placed in a sand lot and were observed for estrus for at least 1 h after each milking. Response to synchronization was considered affirmative if a cow presented visual signs of estrus within 5 d after PGF_{2α} injection.

Six to 8 h after a cow was determined to be in standing estrus, AI was performed by the same technician using semen from a single high fertility bull and batch (bull code number and name, 29H4397

SHOGUN). This procedure for estrus detection was continued until a cow was diagnosed pregnant or until the end of the experimental period. Cows were not bred after 120 d PP. Cows that were bred between 110 and 120 d PP were fed the experimental diets until the cows were confirmed pregnant or nonpregnant from that AI. All cows received one or two AI. Ultrasonography and rectal palpation were used to determine pregnancy. Services per conception included only cows that were confirmed pregnant. Two cows assigned to the 11.1% DIP diet were not inseminated because of a lack of cyclicity; therefore, these cows were not included in the data for service per conception and estrus detection. Only cows that were inseminated during the experiment were included in the statistical analyses of reproductive data that were collected after the synchronization period.

Ultrasonography. Transrectal ultrasonography (Equisonics 300A linear array ultrasound scanner equipped with a 7.5-mHz transducer; Tokyo Keiki Co. Ltd., Tokyo, Japan) was used to reconfirm the presence of any abnormal ovarian structure (i.e., cysts) as determined by rectal palpation (9); this tool enabled the early diagnosis of pregnancy (25 to 30 d post-AI).

Health program. Cows were treated for infectious disease (e.g., mastitis, metritis, pneumonia, or pododermatitis) as needed with commonly used antibiotics as recommended by the veterinarian in charge. Pyometra was treated with a single dose of PGF_{2α} (25 mg, i.m.). Large or luteinized follicles or persistent CL were not treated to correct them specifically until the time of synchronization.

Statistical Analyses

Data were analyzed by least squares analysis of variance using the GLM procedure of SAS (30). The linear model used in the analyses of palpation data and plasma hormones contained treatment, cow within treatment, time PP (week), and the interaction of treatment and time PP. Cow within treatment was used as the error term to test the dietary treatment effects. Remaining terms were tested using residual error. Orthogonal contrasts with a single degree of freedom were used to determine differences among the main effects of protein degradability, fat supplementation, and their interaction. Differences were considered significant at $P < 0.05$, and a tendency toward significance was determined when $0.05 \geq P \geq 0.10$.

Tests of homogeneity of regression were performed to compare changes in variables over time by fitting a single polynomial regression curve and testing the

gain in fit (difference) from fitting individual regressions for each of the diets as described by Wilcox et al. (35). The model included the effects of treatment, cow within treatment, time PP (day or week), and the interactions of treatment and time PP. For analyses of the palpation data, the model also included the effects of status of the uterine horn based on previous gestation (gravid or nongravid), time PP, and the interactions of treatment and uterine horn status; treatment and time PP; uterine horn status and time PP; and treatment, uterine horn status, and time PP. All palpation data, excluding measurements of the uterine horns and cervix, were accumulated in time before analyses. Curves were considered different at $P < 0.05$.

Response to synchronization, conception rate, and pregnancy rate data were analyzed using the CATMOD procedure of SAS (30).

RESULTS AND DISCUSSION

Palpation Results

Cervix and uterine horns. The pattern of cervix regression over the first 8 wk PP was not affected by diet (mean diameter = 40.6 ± 0.6 mm). As expected, the open uterine horn involuted faster ($P = 0.01$) than did the previously pregnant uterine horn of pregnant cows across diets. Rate of involution of uterine horns was influenced by diets. The diet containing proteins of lower degradability (11.1% DIP) plus CaLCFA resulted in a slower regression of the uterus compared with the other three diets (interaction of DIP and fat, $P = 0.039$) (Figure 1). Guilbault et al. (18) reported that a faster rate of uterine involution may be associated with a greater secretion of PGF_{2α} by the uterus as determined by greater detectable concentrations of prostaglandin metabolites. Those researchers (18) concluded that, although the mechanisms by which PGF_{2α} alter uterine involution are unknown, a plausible factor could be the acceleration of muscle protein turnover and contraction. During early lactation, most tissues can contribute nutrients to meet the substrate needs of the mammary gland. When protein degradability of the diet was reduced from 15.7 to 11.1% DIP, cows experienced a reduction in tissue mobilization as was reflected by less BW loss (16). The hydrolysis of labile protein reserves, which includes smooth muscle such as that present in the intestine and the reproductive tract, likely was diminished.

Ovaries. Ovarian activity was greater on the contralateral side as expected, exhibiting an inhibitory effect of the previous pregnant horn of gestation on

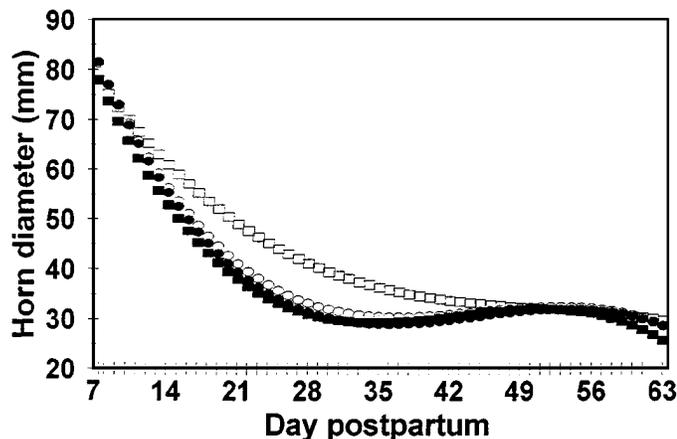


Figure 1. Third-order polynomial regression curves for the diameter (millimeters) of open and pregnant uterine horns of lactating Holstein cows fed diets containing 11% degradable intake protein (DIP) and 0% Ca salts of long-chain fatty acids (CaLCFA) (□), 15.7% DIP and 0% CaLCFA (●), 11.1% DIP and 2.2% CaLCFA (○), and 15.7% DIP and 2.2% CaLCFA (■). The interactions of DIP and CaLCFA differed ($P = 0.039$) among dietary treatments. The standard error of the mean was 0.4.

PP ovarian function of the ipsilateral ovary (Table 1). Lewis et al. (25) found that the mean ovarian volume and the percentage of ovaries with CL were reduced in ovaries that were ipsilateral to the previously gravid uterine horn and that the negative associations between the condition of the uterus and ovarian activity probably were due to negative effects

of the uterus on the ovaries, resulting in some local control over ovarian recrudescence.

Ovarian volume, calculated from estimates of width, height, and length of the ovary via palpation, reflects the presence of normal and pathological structures on the ovary. The diet containing more degradable protein and the supplementation of CaLCFA as main effects did not affect ovarian volume (Table 1). However, the volume of the ovary associated with the contralateral uterine horn decreased when CaLCFA were added to the 11.1% DIP diet (76,500 vs. 49,400 mm³), but ovarian volume increased when CaLCFA were added to the 15.7% DIP diet (47,400 vs. 64,000 mm³); the inhibited ipsilateral ovary was not affected by diet (interaction of DIP, fat, and side, $P = 0.001$). This three-way interaction effect on ovarian volume was driven by the greater follicular area on the contralateral side in cows fed the 11.1% DIP diet without supplemental CaLCFA (interaction of DIP, fat, and side for follicular area, $P = 0.003$) as well as a tendency toward greater luteal development (interaction of DIP, fat, and side for number of CL, $P = 0.085$) (Table 1).

An increase in the degradability of the dietary protein resulted in less intense ovarian activity. The mean number of follicles (2.1 vs. 1.7; $P = 0.062$), size of the largest follicle (24.8 vs. 18.9 mm; $P = 0.037$), and total area of all follicles (27.9 vs. 21.3 mm; $P = 0.052$) were greater or tended to be greater in cows

TABLE 1. Mean cumulative responses over the first 50 to 57 d postpartum for size and number of ovarian structures as determined by weekly rectal palpation of lactating Holstein cows fed diets differing in concentrations of degradable intake protein (DIP) and supplemental fat (0 or 2.2% Ca salts of long-chain fatty acids).

Ovarian measurement	11.1% DIP		15.7% DIP		Contrast ¹											
	0% Fat	2.2% Fat	0% Fat	2.2% Fat	SEM	P	F	P × F	S	P × S	F × S	P × F × S				
Ovarian volume, ³ mm ³ × 100	765	437	494	435	474	294	640	466	69	0.501	0.905	0.125	0.162	0.979	0.001	0.001
Follicles, no.	2.8	1.9	1.7	1.9	2.1	1.4	1.8	1.3	0.2	0.062	0.111	0.444	0.002	0.026	0.242	0.111
Largest follicle, mm	32.7	21.0	20.8	24.7	20.0	17.4	21.8	16.4	2.5	0.037	0.505	0.416	0.026	0.069	0.201	0.010
Follicular area, ⁴ mm	39.4	23.8	21.8	26.5	23.0	19.7	25.2	17.4	2.9	0.052	0.260	0.266	0.009	0.060	0.074	0.003
Corpus luteum (CL), no.	1.6	0.7	1.4	0.8	0.4	0.7	1.0	1.0	0.1	0.008	0.074	0.076	0.003	0.977	0.021	0.085
Largest CL, mm	23.6	10.3	25.2	11.5	4.2	10.9	14.6	17.6	2.5	0.049	0.088	0.225	0.017	0.563	0.053	0.641
Luteal area, ⁴ mm	24.3	11.6	26.2	11.5	4.4	11.3	14.7	18.3	2.7	0.035	0.101	0.182	0.026	0.480	0.043	0.866
Follicular cyst area, ⁴ mm	6.4	0	0	0	0	2.6	11.7	0	1.1	0.454	0.828	0.134	0.001	0.005	0.001	0.001
Luteal cyst area, ⁴ mm	0	0	0	0	0	3.9	4.3	0.2	0.7	0.083	0.909	0.909	0.947	0.001	0.764	0.001

¹Orthogonal contrasts of the main effects of protein degradability (P), fat (Ca salts of long-chain fatty acids) (F), side of the ovary with respect to previous gravid uterine horn (S), and their interactions.

²Location of the ovary with respect to the previously gravid uterine horn: IPL = ipsilateral (same side) and CTL = contralateral (opposite side).

³Width × height × length.

⁴Sum of the diameters of all palpable follicular and luteal structures on an ovary.

fed the low DIP diets, principally because of the ovary that was situated contralateral to the previous pregnant horn (interaction of DIP and side, $P \leq 0.069$) (Table 1). Supplemental CaLCFA did not influence the size of the largest follicle as determined by rectal palpation weekly. Using ultrasonography, Lucy et al. (26) reported an increase in the size of the largest and second largest follicles in a postsynchronized cycle when lactating cows were fed supplemental CaLCFA.

As in the case of follicular development, luteal development also was suppressed when 15.7% DIP diets were fed (Table 1). The number of CL were reduced from 1.1 to 0.7 ($P = 0.008$), the size of the largest CL was reduced from 17.6 to 11.8 mm ($P = 0.049$), and the total luteal area was reduced from 18.4 to 12.2 mm ($P = 0.035$) by replacing animal byproduct protein sources with soybean meal and urea. Dietary CaLCFA tended to increase the number of CL (0.8 vs. 1.0; $P = 0.074$), the largest CL (12.2 vs. 17.2 mm; $P = 0.088$), and the total luteal area (12.9 vs. 17.7 mm; $P = 0.101$). This positive effect of dietary CaLCFA on numbers of CL tended to be ($P = 0.076$) most beneficial when CaLCFA were added to the 15.7% DIP diet (interaction of DIP and fat). The number of CL doubled from 0.5 to 1.0 when CaLCFA increased from 0 to 2.2% of dietary DM in diets containing 15.7% DIP, but CL numbers that were elevated in cows fed the 11.1% DIP diet were not further stimulated by CaLCFA supplementation (1.1 vs. 1.1).

The ovary on the contralateral side demonstrated more ($P = 0.001$) follicular cyst area than did the

ovary on the ipsilateral side (4.5 vs. 0.6 mm). Addition of CaLCFA to the 15.7% DIP diet increased the presence of follicular cysts on the contralateral side (0 vs. 11.7 mm), but supplementation of CaLCFA to the 11.1% DIP diet led to a reduction in follicular cysts (6.4 vs. 0 mm) (interaction of fat and side, $P = 0.0001$). The ipsilateral side was virtually free of follicular cysts (side, $P = 0.001$).

Protein degradability influenced the total number of follicles accumulated over the first 63 d PP (Figure 2). Cows fed the 11.1% DIP diets had a greater rate of increase ($P = 0.018$) in the accumulation of ovarian follicles than did cows fed the highly degradable protein diets (peak of 5.0 to 5.5 vs. 4.0 to 4.5 at 63 d PP).

The pattern of luteal tissue development over time was greater for cows fed the low DIP diets. The amount of total luteal tissue accumulated by 63 d PP was >70 mm for cows fed the 11.1% DIP diets but was <15 mm for cows fed the 15.7% DIP diets (DIP, $P = 0.004$) (data not shown). The number of CL accumulated on the contralateral ovary by 63 d PP was lower in cows fed the 15.7% DIP diets (Figure 3a) but not on the ipsilateral ovary (Figure 3b). The addition of CaLCFA to the high DIP diet increased the number of accumulated CL on both ovaries but had no effect when added to the low DIP diet (Figure 3, a and b) (interaction of DIP, fat, and side, $P = 0.01$). This stimulation of the number of CL by supplemental CaLCFA fed with the high DIP diet was supported by independent measurements of accumulated P_4 (interaction of DIP and fat; Figure 4). Accumulated P_4 was decreased in cows fed the 15.7% DIP diet, but this suppression was reversed when CaLCFA were fed.

TABLE 2. Least squares means for luteal activity within the first 50 d postpartum of all lactating dairy cows fed diets differing in concentrations of degradable intake protein (DIP) and supplemental fat (0 or 2.2% Ca salts of long-chain fatty acids).

Measurement	11.1% DIP		15.7% DIP		SEM	Contrast		
	0% Fat	2.2% Fat	0% Fat	2.2% Fat		DIP	Fat	DIP × Fat
Cows, no.	13	11	10	11				
Anestrus cows, no.	1	1	4	1				
Cystic cows, no.	2	0	2	3				
	P							
Days to first rise in P_4^1 (>1 ng/ml)	25.2	25.2	41.8	35.4	3.0	0.002	0.074	0.110
Length of first luteal phase, d	16.4	19.8	7.5	13.4	2.8	0.009	0.105	0.667
Peak plasma P_4 , ng/ml	8.1	8.3	3.9	7.8	1.3	0.079	0.125	0.169
Short luteal phases, ² no.	0.2	0.2	0.3	0.4	0.1	0.282	0.818	0.818
Normal luteal phases, ² no.	1.8	1.5	0.7	1.5	0.2	0.007	0.267	0.021
Accumulated plasma P_4 concentration, ³ ng	848	1007	371	702	99	0.001	0.019	0.397

¹Progesterone.

²Luteal phases were considered to be normal or short if plasma P_4 concentrations >1 ng/ml lasted for more or less than 12 d, respectively. Data are presented on a per cow basis.

³Area under the curve calculated after fitting a fifth-order polynomial equation for the entire period.

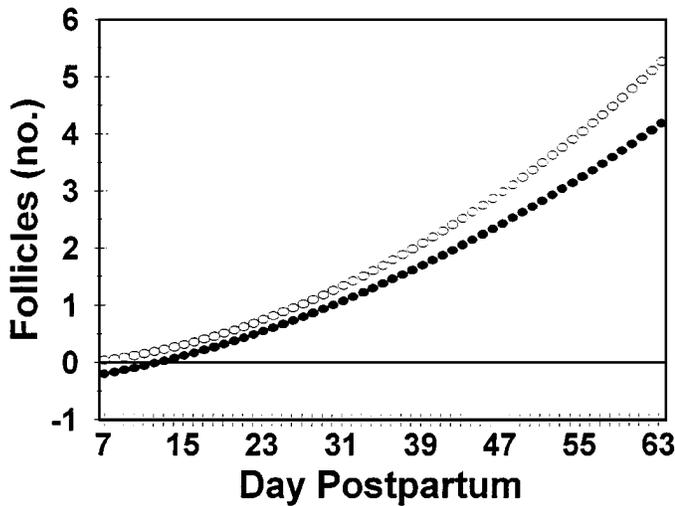


Figure 2. Second-order polynomial regression curves of the accumulated number of follicles from lactating Holstein cows fed diets containing 11.1% (○) or 15.7% (●) degradable intake protein (DIP). Curves differed ($P = 0.018$) between dietary treatments. The standard error of the mean was 0.2.

The combined results from rectal palpation data, ultrasonography records, and plasma P_4 profiles during the first 50 d of lactation are presented in Tables 2 and 3. Results shown in Table 2 include all cows used in the experiment; Table 3 contains data only from those cows that showed estrous activity. Four of the 10 cows fed the 15.7% DIP diet without supplemental CaLCFA were anestrus during this period, which was confirmed by the low number and size of CL and the total luteal area present when compared with cows fed the other diets (Table 1). Four of the 5 cystic cows that were fed the 15.7% DIP diet demonstrated a pathological structure on the ovary contralateral to the previous gravid uterine horn. The

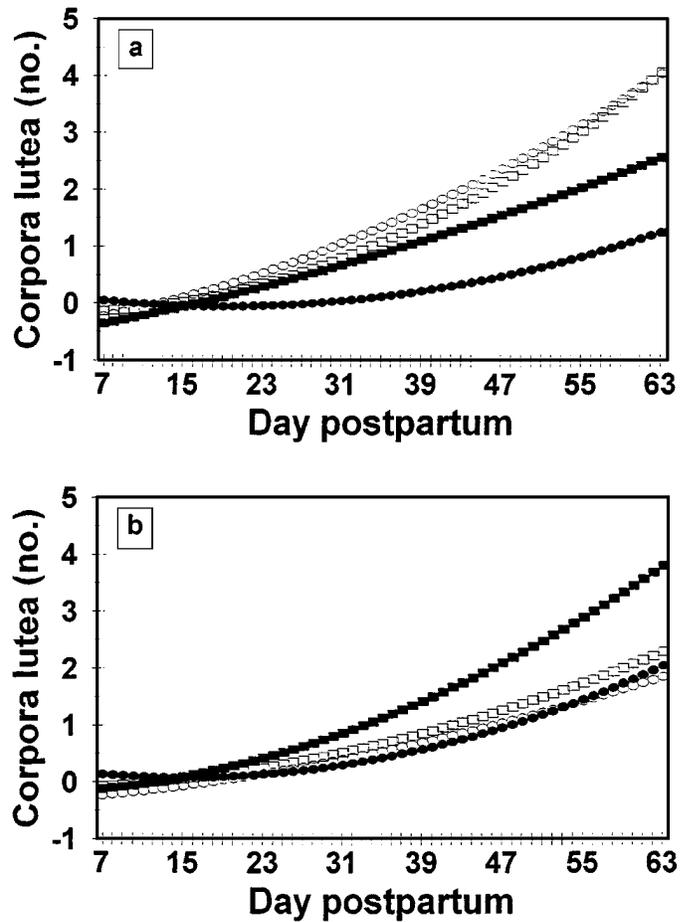


Figure 3. Second-order polynomial regression curves of the number of corpora lutea (CL) from the contralateral (a) and ipsilateral (b) sides of the uterus (location of the ovary with respect to previously gravid uterine horn) from lactating Holstein cows fed diets containing 11.1% degradable intake protein (DIP) and 0% Ca salts of long-chain fatty acids (CaLCFA) (□), 15.7% DIP and 0% CaLCFA (●), 11.1% DIP and 2.2% CaLCFA (○), and 15.7% DIP and 2.2% CaLCFA (■). The interactions of DIP, CaLCFA, and side differed among dietary treatments ($P = 0.01$). The standard error of the mean was 0.1.

TABLE 3. Least squares means for luteal activity within the first 50 d postpartum of cycling lactating dairy cows fed diets differing in concentrations of degradable intake protein (DIP) and supplemental fat (0 or 2.2% Ca salts of long-chain fatty acids).

Measurement	11.1% DIP		15.7% DIP		SEM	Contrast		
	0% Fat	2.2% Fat	0% Fat	2.2% Fat		DIP	Fat	DIP × Fat
Cows showing luteal activity								
no.	12	10	6	10				
%	92	91	60	91				
	P							
Days to first rise in P_4^1 (>1 ng/ml)	23.7	22.7	34.2	30.8	2.5	0.001	0.408	0.663
Length of luteal phase, d	17.7	21.8	15.7	17.0	2.4	0.179	0.291	0.592
Peak plasma P_4 , ng/ml	8.8	9.2	6.7	9.7	1.1	0.478	0.154	0.267
Accumulated plasma P_4 concentration, ng	896	1065	429	735	106	0.001	0.003	0.536

¹Progesterone.

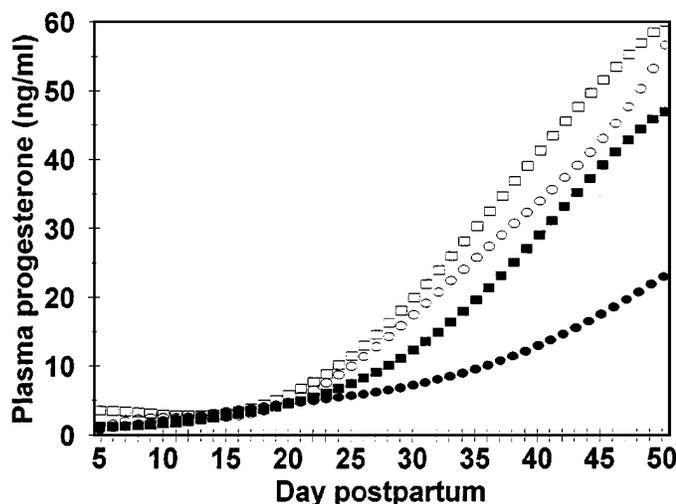


Figure 4. Fifth-order polynomial regression curves of accumulated plasma progesterone concentrations (nanograms per milliliter) from lactating Holstein cows fed diets containing 11.1% degradable intake protein (DIP) and 0% Ca salts of long-chain fatty acids (CaLCFA) (\square), 15.7% DIP and 0% CaLCFA (\bullet), 11.1% DIP and 2.2% CaLCFA (\circ), and 15.7% DIP and 2.2% CaLCFA (\blacksquare). The interaction of DIP and CaLCFA differed among dietary treatments ($P = 0.0001$). The standard error of the mean was 0.9.

implications of this occurrence may be that as PP ovarian activity began with the contralateral ovary, it was interrupted by a cystic condition that resulted in the absence of luteal tissue formation, therefore delaying the luteal phase.

The initiation of luteal activity (based on individual P_4 profiles) was delayed by 13.4 d (38.6 vs. 25.2 d; $P = 0.002$) when the percentage of DIP was increased. The addition of CaLCFA to the 15.7% DIP diet tended to reduce the number of days to the first luteal phase PP from 42 to 35; no improvement was detected when CaLCFA were added to the 11.1% DIP diet (interaction of DIP and fat, $P = 0.110$). Cows fed highly degradable protein had a shorter first luteal phase (10.4 vs. 18.1 d; $P = 0.009$), tended to have a lower maximum concentration of P_4 (5.8 vs. 8.2 ng/ml; $P = 0.079$), and had fewer numbers of normal luteal phases (1.2 vs. 1.6; $P = 0.007$) during the first 50 d PP than did cows fed the 11.1% DIP diets. Dietary fat tended to increase the length of the first luteal phase by 4.7 d (16.6 vs. 11.9 d; $P = 0.105$). The supplementation of CaLCFA to diets with a high DIP content returned the number of normal luteal phases to a number that was similar to that for cows fed the other diets (interaction of DIP and fat, $P = 0.021$). Accumulative plasma P_4 concentrations, estimated from the area under the curve after fitting a fifth-order polynomial regression equation for the entire experimental period, confirmed that lower protein

degradability (537 vs. 927 ng/ml; $P = 0.001$) as well as supplemental CaLCFA (609 vs. 854 ng/ml; $P = 0.019$) improved P_4 concentrations during the first 50 d of lactation. Although no interaction was detected for mean accumulated P_4 concentrations, curves of accumulated P_4 concentrations demonstrated an interaction of DIP and fat (Figure 4). Patterns of P_4 accumulations indicated that the detrimental effect of 15.7% DIP diets was alleviated markedly by supplementation of CaLCFA ($P = 0.001$), but supplementation of CaLCFA to the 11.1% DIP diet was not stimulatory.

When only those cows that demonstrated estrous activity (as determined by P_4 profiles) before d 50 PP were considered, the high DIP diets still delayed return to first luteal activity by 9 d ($P = 0.001$; Table 3). Neither length of the first luteal phase nor peak plasma P_4 concentration was influenced by diet. However, as is shown in Table 2, accumulated plasma concentrations of P_4 during the first 50 d PP were depressed ($P = 0.001$) by the highly degradable protein (582 vs. 980 ng/ml). Dietary CaLCFA improved ($P = 0.003$) circulating concentrations of P_4 across both DIP diets (662 vs. 900 g/ml). No interaction was detected for any of the variables.

A delay PP to first CL activity caused by a diet containing highly degradable protein was reported by Figueroa et al. (12). Lactating dairy cows fed isonitrogenous diets (20% CP) with low (60% of dietary CP) or high (65% of dietary CP) DIP concentrations required 34 and 50 d to exhibit first CL activity after calving, respectively. This 16-d increase in the time from parturition to first luteal activity when protein degradability was increased was comparable with the 13- and 9-d increases found in the present experiment when all cows or only cycling cows were included in the analysis, respectively (Tables 2 and 3). The effectiveness of the synchronization treatment to restart ovarian activity in anestrus cows was 85.7% (6 of 7 cows), similar to results reported by Twagiramungu et al. (33).

A mean of 70% of the cows showed signs of estrus upon synchronization; no effects were due to diet (Table 4). Behavioral estrus was observed usually within 2 to 3 d of injection of $\text{PGF}_{2\alpha}$. Mean conception rate at synchronized estrus was 30% and did not differ among diets. Although mean conception rates at first AI were not different among cows fed the four diets, conception rates at the second AI were improved ($P = 0.030$) from 31 to 75% when cows were fed CaLCFA. This improvement carried over to pregnancy rate at the end of the experiment (52.2 vs. 86.3%; $P = 0.021$). The number of AI per conception (range, 1.2 to 1.5), the number of days open (range,

73 to 86 d), the total number of AI (range, 15 to 17 per group), and the efficiency of estrus detection (range, 84 to 88%) were not different because of diet. All cows had the same opportunity to be detected in estrus and to be inseminated. Potential differences caused by sire and technician were eliminated because a single batch of semen from a single bull was used and the same technician performed all AI.

Although the effect of the supplementation of CaLCFA on reproductive performance has been inconsistent, supplemental fat (CaLCFA and tallow) has previously improved conception and pregnancy rates (32). Fat supplementation may impact reproduction responses in the dairy cow by providing precursors for synthesis of hormones that are important to the reproductive process (e.g., cholesterol for progesterone and linoleic acid for PGF_{2α}). Linoleic acid also may act to inhibit the release of PGF_{2α} from the uterus, which may increase the lifespan of the CL on the ovary (32).

Fat supplementation usually results in an increase in the plasma concentration of cholesterol (17). The

plasma concentration of P₄ also is increased often by fat supplementation (32). Elevated concentrations of plasma P₄ have been related to improved fertility (14). Supplemental CaLCFA in the current study tended to increase the number of CL and the size of the largest CL (Table 1), which helped contribute to greater accumulation of plasma P₄ concentrations within the first 50 d PP (Table 2).

The effect of a diet with elevated amounts of degradable protein on recrudescence of ovarian activity is similar to the effects reported for negative energy status as experienced by lactating dairy cows in early PP. Extent and duration of negative energy status is an important factor that affects the return to normal ovarian activity after calving. First ovulation was delayed a mean of 2.75 d for every 1 Mcal of negative energy status experienced during the first 20 d PP (4). Can excess protein in the diet result in significant energy costs to the cow or compromise normal energy-yielding pathways, which would uncouple energy metabolism?

TABLE 4. Response to synchronization (d 50 to 57 postpartum), conception rate at first and second AI, services per conception (S/C), days open, estrus detection rate, and pregnancy rate of lactating Holstein cows fed diets differing in concentrations of degradable intake protein (DIP) and supplemental fat (0 or 2.2% Ca salts of long-chain fatty acids).

Measurement	11.1% DIP		15.7% DIP		Contrast		
	0% Fat	2.2% Fat	0% Fat	2.2% Fat	DIP	Fat	DIP × Fat
	————— P —————						
Synchronization response							
no./no.	6/11	8/11	7/10	9/11			
%	54.5	72.7	70.0	81.8	0.390	0.293	0.918
Estrus, ¹ d	2.7	2.1	2.1	2.5	0.832	0.906	0.086
Conception							
no./no.	1/6	2/8	4/7	3/9			
%	16.7	25.0	57.1	33.3	0.180	0.784	0.385
Conception							
At first AI							
no./no.	3/11	5/11	4/10	5/11			
%	27.3	45.4	40.0	45.5	0.650	0.420	0.650
At second AI							
no./no.	3/8	4/6	1/5	5/6			
%	37.5	66.7	20.0	83.3	0.983	0.030	0.354
Total AI, no.	17	15	15	17			
S/C, ² no.	1.5	1.4	1.2	1.5	0.541	0.541	0.376
Days open ²	80	83	73	86	0.742	0.247	0.454
Estrus detection rate ³	85	84	88	85			
Pregnancy rate ⁴							
no./no.	6/11	9/11	5/10	10/11	0.695	0.021	0.533
%	54.5	81.8	50.0	90.9			

¹Signs of behavioral estrus within 5 d of PGF_{2α} injection.

²Only pregnant cows were included in these analyses.

³Percentage of cows detected in estrus according to plasma progesterone concentrations.

⁴Cows that conceived within 120 d after calving/number of cows serviced.

The energy costs associated with the detoxification of large amounts of NH_3 to urea by the liver may aggravate an energy shortage for lactation, which may divert metabolic attention away from ovarian activity. Cows fed the 15.7% DIP diet lost about 30 kg more BW than did cows fed the 11.1% DIP diet in the first 4 wk PP (16) and experienced a 13.4-d delay to the first rise in P_4 PP. Similarly, cows that lost greater amounts of body condition experienced a greater delay to first ovulation PP (2).

The metabolic hormone insulin is a powerful stimulator of follicular cell development (31). Concentrations of plasma insulin were reduced in cows fed the 15.7% DIP diets (16). The PP development of luteal tissue in cows fed the 15.7% DIP diets might have been slowed because of a reduction in circulating concentrations of insulin.

Another potential mode of action involves the impairment of intermediary metabolism because of an extensive metabolic effort to detoxify excess N. Once NH_3 diffuses through the ruminal wall and is absorbed by the tissues, the acidic intracellular condition causes NH_3 to be ionized (NH_4^+) and thus trapped inside the cell. Once ionized, NH_4 leaves the cell through some of the known N carrier compounds, such as glutamine, alanine, or glutamate. Hanssinger, as cited by Elrod and Butler (8), reported that NH_3 , which escapes detoxification by the liver through the urea cycle system, is prevented from entering peripheral circulation usually by a very active, high affinity perivenous glutamine synthetase system. Therefore, NH_4 ion has to be incorporated into α -ketoglutarate to synthesize glutamate by the enzyme glutamic dehydrogenase, a step requiring energy as NADH^+ . Glutamine, a N carrier, would then be used to transport NH_3 out of the cell. Glutamine is synthesized from glutamate by glutamine synthetase, a step that requires 1 mol of ATP. Under the condition in which large amounts of NH_3 accumulate in tissues, a great deal of α -ketoglutarate would be taken out of the tricarboxylic acid cycle and possibly lessen ATP production. The utilization of these intermediates and precursors of the tricarboxylic cycle can interfere with intermediary metabolism (29). The urea cycle and the tricarboxylic acid cycle are interconnected because they share common intermediates (e.g., fumarate and α -ketoglutarate). An inhibition of gluconeogenesis can occur independent of the rate of urea synthesis but dependent on the presence of high amounts of NH_4 (19). Cells exposed to high concentrations of NH_3 can become internally energy deficient (ATP deficiency) because of use by the urea cycle or the export from the cell (to the

liver) of α -ketoglutarate molecules. This derangement in intermediary metabolism and the energetic cost are potential mechanisms of how high DIP diets might affect productive and reproductive performance of lactating cows.

Excess protein or highly degradable forms of protein in the diet have interfered with intermediary metabolism of ruminants. In experiments in which animals were induced toward hyperammonemia, certain plasma and liver concentrations of the Krebs cycle intermediates and enzymes important in AA catabolism were changed. Lambs fed urea at 4.2% of dietary DM had lower plasma concentrations of α -ketoglutarate, isocitrate dehydrogenase, lactate dehydrogenase, glutamic oxaloacetic transaminase, and glutamic pyruvic transaminase than did lambs fed soybean meal (29). Plasma concentrations of α -ketoglutarate and pyruvate also were increased in ponies dosed with 454 g of urea and in rats injected with crystalline jackbean urease (7). Liver concentrations of glucose and nicotinamide adenine dinucleotide were elevated in lambs fed urea compared with those fed soybean meal (29). These changes have contributed toward elevated blood glucose concentrations in rats (7), lambs (7), and steers (11). Another indication that high amounts of dietary CP can affect energy metabolism is the greater incidence of ketosis (75% vs. 50% vs. 10%) in lactating cows fed diets with 30, 20, or 14% CP [DM basis; (19)].

CONCLUSIONS

Diets containing two concentrations of both degradable protein and CaLCFA affected reproductive performance of lactating Holstein cows during the first 120 d of lactation. Ruminally degradable protein fed in marked excess of recommendations decreased the amount of luteal tissue present in the ovaries of early PP lactating cows. The decrease resulted primarily because of reduced activity on the ovary located contralateral to the previous gravid horn. The addition of CaLCFA to the highly degradable protein diet augmented the development of total luteal tissue in the contralateral ovary. Cows in the present study fed 15.7% DIP diets showed signs of an energy shortage. The supplementation of CaLCFA to the 15.7% DIP diet doubled the number of CL, reduced time to first rise in P_4 by 6 d, doubled the number of normal luteal phases, and restored the pattern of accumulated plasma P_4 concentrations to a pattern that was similar to that induced by the other diets. Partial reversal of the slowed recovery of reproductive tissues PP by

the supplementation of additional energy in the form of CaLCFA suggests that the negative effects of excess dietary DIP was at least partially of energy origin.

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