Diets During Far-Off and Close-Up Dry Periods Affect Periparturient Metabolism and Lactation in Multiparous Cows¹

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ABSTRACT

The objectives of this study were to determine the effects of far-off and close-up diets on prepartum metabolism, postpartum metabolism, and postpartum performance of multiparous Holstein cows. From dry-off to –25 d relative to expected parturition (far-off dry period), cows were fed a control diet to meet National Research Council (NRC) recommendations for net energy for lactation (NE_L) at ad libitum intake (100NRC; n = 25) or a higher nutrient density diet, which was fed for either ad libitum intake to provide at least 150% of calculated NE_L requirement (150NRC; n = 25) or at restricted intake to provide 80% of calculated NE_L requirements (80NRC; n = 24). From –24 d relative to expected parturition until parturition (close-up period), cows were fed a diet that met or exceeded NRC nutrient recommendations at either ad libitum intake (n = 38) or restricted intake (n = 36) to provide 80% of the calculated NE_L requirement. After parturition, all cows were fed a lactation diet and measurements were made through 56 d in milk (DIM). Prepartum metabolism was consistent with the plane of nutrition. During the first 10 DIM, far-off treatments had significant carryover effects on dry matter intake, energy balance, serum nonesterified fatty acid (NEFA) concentration, and serum β -hydroxybutyrate concentration. Cows with the lower energy balance during the far-off period (100NRC and 80NRC) had higher dry matter intake and energy balance and lower serum NEFA and β -hydroxybutyrate during the first 10 DIM. There were no effects of close-up diet and no interactions of far-off and

close-up treatments. During the first 56 DIM, there were no residual effects of far-off or close-up diets on dry matter intake, milk yield or composition, body weight, body condition score, serum glucose and insulin concentrations, or muscle lipid concentration. Serum NEFA was higher for 150NRC than 80NRC; 100NRC was intermediate. Thus, the effects of far-off and close-up treatments on postpartum variables diminished as lactation progressed. Overfeeding during the far-off period had a greater negative impact on peripartum metabolism than did differences in close-up period nutrition.

Key words: close-up dry period, far-off dry period, prepartum intake, periparturient cow

INTRODUCTION

The nutrition of cows during the dry period has been an active area of research during the past 15 yr. Research has generally focused on dividing the dry period into the early, or “far-off,” period (generally the first 4 to 6 wk) and the “close-up” period (generally the last 3 wk before expected parturition). Numerous studies have been conducted that provide some insight into how to feed dry cows, particularly during the close-up period. In a review, Grummer (1995) suggested that prepartum DMI was positively correlated with postpartum DMI and that prepartum DMI should be maximized to improve postpartum performance and health. Grummer (1995) suggested that increasing the nutrient density of the diet could increase DMI and thus nutrient intake. In response to this review, several researchers focused on maximizing DMI or energy intake during the close-up period (Minor et al., 1998; Dann et al., 1999; VandeHaar et al., 1999; Mashek and Beede, 2000; Doepel et al., 2002; Rabelo et al., 2003). In contrast to this approach, other researchers focused on limiting energy intake during either the entire dry period (Tesfa et al., 1999; Agenäs et al., 2003; Holtenius et al., 2003; Douglas et al., 2006) or the close-up period (Holcomb et al., 2001) to improve postpartum DMI.

The effects of energy intake during the close-up period or the entire dry period on postpartum metabolism and performance have been mixed. Possibly, differences among the studies in postpartum outcomes may be ex-

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Table 1. Ingredient and chemical composition of diets fed to multiparous Holstein cows during the dry and lactating periods

Component	Diet ¹			
	100NRC	80NRC and 150NRC	Close-up	Lactation ²
	% of DM			
Ingredient				
Alfalfa silage	41.68	26.24	25.14	20.10
Alfalfa hay	—	13.97	13.40	—
Corn silage	21.10	25.52	24.49	28.13
Wheat straw	26.15	—	—	—
Cottonseed	—	4.39	4.18	9.65
Ground shelled corn	7.20	17.24	16.31	25.72
Soybean meal	3.05	—	—	5.15
Expeller soybean meal ³	—	1.73	1.62	5.94
Soybean hulls	—	10.24	9.67	1.48
Calcium chloride	—	—	1.10	—
Limestone	—	—	1.29	1.58
Sodium bicarbonate	—	—	—	1.08
Magnesium oxide	—	—	—	0.13
Magnesium sulfate	—	—	1.81	—
Dicalcium phosphate	0.09	—	0.18	0.56
Sodium chloride	0.24	0.18	0.19	0.22
Mineral-vitamin mix ⁴	0.27	0.27	0.28	0.22
Vitamin E ⁵	0.18	0.18	0.28	0.04
Vitamin A and D ⁶	0.04	0.04	0.06	—
Chemical ⁷				
n	13	12	14	15
CP	15.8 ± 0.1	16.5 ± 0.2	15.7 ± 0.1	18.1 ± 0.2
ADF	31.8 ± 0.3	26.1 ± 0.4	25.6 ± 0.4	19.0 ± 0.3
NDF	46.5 ± 0.4	38.1 ± 0.6	36.9 ± 0.5	28.7 ± 0.4
Starch	13.2 ± 0.1	21.4 ± 0.2	20.9 ± 0.4	27.8 ± 0.3
Total fatty acids	1.7 ± 0.03	3.1 ± 0.05	2.9 ± 0.05	4.6 ± 0.1
NE _L , Mcal/kg	1.30 ± 0.01	1.59 ± 0.01	1.61 ± 0.01	1.77 ± 0.01
Ash	10.9 ± 0.2	8.4 ± 0.1	11.2 ± 0.2	9.6 ± 0.2
Ca	0.92 ± 0.02	0.84 ± 0.02	1.85 ± 0.10	1.43 ± 0.03
P	0.27 ± 0.01	0.29 ± 0.01	0.37 ± 0.06	0.47 ± 0.01
Mg	0.21 ± 0.01	0.26 ± 0.02	0.45 ± 0.03	0.31 ± 0.01
K	2.16 ± 0.05	1.82 ± 0.04	2.12 ± 0.34	1.55 ± 0.02
Na	0.13 ± 0.01	0.12 ± 0.01	0.14 ± 0.01	0.52 ± 0.03

¹Diets fed to meet 100% (100NRC), 150% (150NRC), or 80% (80NRC) of NRC requirements for NE_L during the far-off dry period.

²Alfalfa hay (~2 kg DM) was top-dressed on the lactation diet from parturition to 14 DIM; chemical composition reflects only the TMR without hay.

³SoyPLUS (West Central Cooperative, Ralston, IA).

⁴Contained a minimum of 5% Mg, 10% S, 7.5% K, 2.0% Fe, 3.0% Zn, 3.0% Mn, 5,000 mg/kg of Cu, 250 mg/kg of I, 40 mg/kg of Co, 150 mg/kg of Se, 2,200 kIU/kg of vitamin A, 660 kIU/kg of vitamin D₃, and 7,700 IU/kg of vitamin E.

⁵Contained 44,000 IU/kg.

⁶Contained 10,000 kIU/kg of vitamin A and 1,249 kIU/kg of vitamin D.

⁷Calculated from monthly analyses of individual ingredients in diets. Values are presented as mean ± standard error.

plained by the duration or timing of maximizing or restricting energy intake. Moreover, nutrition during the far-off period might influence how cows respond in the close-up and postpartum periods. Unfortunately, few studies focusing on nutrition during the close-up period have reported how cows were fed during the far-off period.

Previously in our research group, Douglas et al. (2006) fed isocaloric diets either high in fat or high in

concentrate throughout the entire dry period. Each diet was fed either ad libitum or in amounts restricted to provide only 80% of the calculated NE_L requirement (NRC, 1989). Diet composition had few effects, but restricted feeding of either diet resulted in lower liver triacylglycerol (TAG) contents at 1 d postpartum and greater increases in DMI postpartum compared with ad libitum feeding of either diet during the dry period. Cows fed for ad libitum DMI overconsumed energy rela-

tive to the NRC requirements. However, no control diet was fed to meet, but not exceed, NRC requirements in that study (Douglas et al., 2006). Moreover, restricted feeding during the dry period is problematic for cows housed and fed in groups.

Because of these issues and the potential differential effects of nutrition during the far-off dry period compared with those during the close-up period, the objectives of this study were to determine the effects of the far-off period diet, the close-up period diet, and their interaction on prepartum metabolism and postpartum metabolism and performance. Our hypothesis was that the diet during the far-off dry period is at least as important as the diet during the close-up period on periparturient metabolism and performance.

MATERIALS AND METHODS

Experimental Design and Management of Cows

All procedures were conducted under protocols approved by the University of Illinois Institutional Animal Care and Use Committee. Eighty-six multiparous Holstein cows were enrolled in the study. Twelve cows did not complete the study: 2 cows aborted, 1 cow was found to be nonpregnant, and 9 cows had health problems unrelated to dietary treatments [lung infection (1), tumor in the rectal area (1), duodenal blockage (1), biopsy complications (2), bone infection (1), and mastitis (3)]. Thus, 74 cows completed the experiment and were used in the analyses.

Cows were randomly assigned to 1 of 3 dietary treatments during the far-off dry period from dry-off to -25 d relative to expected parturition (Table 1). The control diet (**100NRC**) was formulated to meet, but not greatly exceed, NRC (2001) recommendations for all nutrients during the far-off dry period when consumed at predicted ad libitum DMI. The higher nutrient density diet exceeded NRC (2001) recommendations for all nutrients during the far-off dry period and was fed either ad libitum to allow at least 150% of NRC recommendations for NE_L (**150NRC**) or in restricted amounts (**80NRC**) to allow only 80% of the calculated NE_L requirement of the cow based on BW at dry-off (NRC, 2001). The higher nutrient density diet was similar in composition to a typical close-up diet but did not contain anionic salts.

At d -24 relative to expected parturition, cows from each far-off treatment were randomly assigned to 1 of 2 close-up period treatments until parturition. The close-up diet (Table 1) met or exceeded NRC (2001) nutrient recommendations and was fed either ad libitum to allow at least 150% of calculated NE_L requirement or at restricted intake to provide 80% of the calculated NE_L requirement of the cow based on BW at dry-

off (NRC, 2001). The close-up diet was similar to the far-off higher nutrient density diet except for the addition of anionic salts (Table 1). Thus, treatments were in a 3×2 factorial arrangement of 3 far-off period treatments (100NRC, $n = 25$; 150NRC, $n = 25$; 80NRC, $n = 24$) and 2 close-up period treatments (ad libitum, $n = 38$; restricted, $n = 36$), resulting in 6 dry period treatment groups (100NRC, ad libitum close-up, $n = 13$; 100NRC, restricted close-up, $n = 12$; 150NRC, ad libitum close-up, $n = 13$; 150NRC, restricted close-up, $n = 12$; 80NRC, ad libitum close-up, $n = 12$; and 80NRC, restricted close-up, $n = 12$). After parturition, all cows were fed a lactation diet (Table 1). All diets were mixed once daily and fed as TMR. Approximately half of each day's TMR was fed in the morning (1100 h) and the remainder was fed in the late afternoon (1700 h). Alfalfa hay (~2 kg DM daily) was top-dressed on the lactation diet until 14 DIM.

Cows were housed in tie stalls throughout the experiment and were allowed to exercise daily in an outside lot for 3 h (0700 to 1000 h). Two weeks before expected parturition, cows were moved to box stalls until parturition. During that time, the cows continued to receive the same close-up diet and maintained the same exercise regimen. After parturition, the cows were returned to tie stalls. Cows were milked twice daily (0300 and 1500 h).

Data Collection, Sampling Procedures, and Analytical Methods

Intake by each cow was measured daily from dry-off to 56 DIM. Samples of feed ingredients and TMR were obtained weekly and analyzed for DM contents (AOAC, 1995). Weekly samples of individual ingredients were frozen at -20°C and were composited monthly. Composite samples were analyzed for contents of DM, CP, NDF, ADF, Ca, P, Mg, and K using wet chemistry methods (Dairy One, Ithaca, NY). Composites of individual ingredients also were analyzed for contents of starch (Thi-vend et al., 1972; Kit 510, Sigma Chemical Co., St. Louis, MO), ash (AOAC, 1995), and total fatty acids (Sukhija and Palmquist, 1988). The fatty acid methyl esters were separated on a fused-silica capillary column (SP-2380, 100 m $\times$ 0.25 mm i.d.; Supelco, Bellefonte, PA) in a gas chromatograph (GC-17A; Shimadzu Corporation, Kyoto, Japan) equipped with an autosampler and flame-ionization detector. Particle size distribution (Lammers et al., 1996) was determined weekly for samples of TMR (Table 2).

Body weight was measured and BCS was assigned in 0.25-unit increments (Ferguson et al., 1994) for each cow weekly from dry-off to 56 DIM. Three individuals

Table 2. Particle size distribution of diets fed to multiparous Holstein cows during the far-off, close-up, and lactating periods

Variable	Diet ¹			
	100NRC	80NRC and 150NRC	Close-up	Lactation ²
	% as fed retained			
n	49	47	52	54
Particle size ³				
>19.0 mm (top)	21.9 ± 1.0	7.0 ± 0.7	5.8 ± 0.3	4.4 ± 0.3
8.0 to 19 mm (middle)	36.7 ± 0.7	40.5 ± 0.7	39.8 ± 0.6	39.3 ± 0.5
<8.0 mm (bottom)	41.4 ± 0.7	52.5 ± 0.9	54.4 ± 0.7	56.3 ± 0.6

¹Diets fed to meet 100% (100NRC), 150% (150NRC), or 80% (80NRC) of NRC requirements for NE_L during the far-off dry period.

²Does not include hay that was top-dressed on the lactation diet until 14 DIM.

³Mean ± standard error.

assigned BCS independently at each time of scoring throughout the experiment.

Milk weights were recorded daily from 1 to 56 DIM and samples were obtained from consecutive a.m. and p.m. milkings weekly from 8 to 56 DIM. Consecutive a.m. and p.m. samples were composited in proportion to milk yield at each sampling and preserved (800 Broad Spectrum Mirotabs II; D&F Control Systems, Inc., San Ramon, CA). Composite samples were analyzed for fat, protein, lactose, urea N, and SCC using midinfrared procedures (AOAC, 1995) at a commercial laboratory (Dairy Lab Services, Dubuque, IA). Milk fatty acid composition was determined (Kelly et al., 1998) for samples collected during wk 2. The fatty acid methyl esters were separated on the same column and gas chromatograph as described for feed samples.

Liver was sampled via puncture biopsy (Hughes, 1962; Veenhuizen et al., 1991) from cows under local anesthesia at approximately 0700 h on d -65 (before dry-off), -30 (to determine effects of far-off diets), -14 (to determine effects of close-up diets), 1 (to determine effects of dry period nutrition on the immediate lipid accumulation following parturition), 14 (approximate time of maximal hepatic lipid accumulation), 28, and 49 (when lipid accumulation was expected to be depleted) relative to parturition. Liver was frozen immediately in liquid N, transferred to a -80°C freezer for storage, and later analyzed for contents of total lipids (Hara and Radin, 1978), TAG (Fletcher, 1968; Foster and Dunn, 1973), and glycogen (Lo et al., 1970).

A biopsy of the middle gluteus muscle was obtained on the same days as liver biopsies. Lidocaine was injected subcutaneously and intramuscularly posterior to the tuber coxae and a 2-cm incision was made with a sterile scalpel. Approximately 50 mg of muscle tissue was removed using a biopsy needle (Bard Magnum, 12 gauge × 16 cm; C. R. Bard, Inc., Murray Hill, NJ). Muscle tissue was frozen immediately and stored in liquid

N until analyses for total lipid and TAG as described for liver.

Blood was sampled from the coccygeal vein or artery weekly from dry-off to -22 d, at -21, -17, -14, and -12 d relative to expected parturition, daily from -10 to 10 d, and at 12, 14, 17, 21, 28, 35, 42, 49, and 56 d relative to actual parturition. Frequency of sampling was greater around parturition because of the greater expected variability in metabolites during that time. Samples were collected before feeding (1000 h) into evacuated serum tubes (SST; Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) containing clot activator. Serum was obtained by centrifugation at 1,300 × g. Aliquots of serum were frozen at -20°C until later analysis for contents of glucose (Glucose/HK kit; Roche Diagnostics Corp., Indianapolis, IN), urea N (urea/BUN kit; Roche Diagnostics Corp.), total protein (total protein kit; Roche Diagnostics Corp.), and BHBA (Ranbut kit; Randox Laboratories Ltd., Oceanside, CA) by automated methods (University of Illinois Clinical Pathology Laboratory, Urbana, IL). Concentrations of NEFA were determined enzymatically using a commercial kit (NEFA C kit; Wako Chemicals USA, Inc., Richmond, VA) as modified by Johnson and Peters (1993). Insulin was measured using a radioimmunoassay kit (Coat-a-Count Insulin kit; Diagnostic Products Corporation, Los Angeles, CA) as modified by Studer et al. (1993). Serum NEFA was analyzed in samples from all available time points. Serum glucose, BHBA, urea N, total protein, and insulin were analyzed only in samples from wk -6, -4, -3, -2, d -10 to 10, and d 14, 21, 28, 42, and 56 relative to actual parturition, which corresponded to key physiological time points of interest.

A clinical chemistry profile was determined for serum samples from d -10 relative to expected parturition and d 10 after parturition (large animal liver panel; University of Illinois Clinical Pathology Laboratory). Sampling times were selected to represent dry period

nutritional effects at defined times before and after parturition but to avoid possible changes associated with liver or muscle biopsies. Commercially available kits were used in an autoanalyzer to determine alkaline phosphatase (**AP**; Alkaline Phosphatase IFCC Liquid kit; Roche Diagnostics Corp.), aspartate aminotransferase (**AST**; AST kit; Roche Diagnostics Corp.), γ -glutamyl transferase (**GGT**; GGT Szasz Liquid kit; Roche Diagnostics Corp.), sorbitol dehydrogenase (**SDH**; SDH kit; Diagnostic Chemicals Limited, Charlottetown, Prince Edward Island, Canada), total cholesterol (Cholesterol/HP kit, Roche Diagnostics Corp.), albumin (Albumin Plus kit, Roche Diagnostics Corp.), and total bilirubin (Total Bilirubin kit; Roche Diagnostics Corp.).

In a posthoc analysis, serum collected from cows at -8 d relative to parturition was analyzed for Se (Animal Health Diagnostic Laboratory, Lansing, MI) in an attempt to explain the high occurrence of retained placenta in this study.

Calculations and Estimates

Energy balance was calculated (NRC, 2001) individually for each cow. All equations used units of megacalories per kilogram. Net energy intake (NE_I) was determined by multiplying DMI by the calculated mean NE_L density of the diet. The NE_L value of each individual feed (Dairy One) was used to calculate the mean NE_L content of the diet. Dairy One used the Ohio State-NRC summative energy equation for predicting total digestible nutrients at maintenance intake ($1\times$). The NE_L at $3\times$ maintenance was predicted from total digestible nutrients according to the NRC (2001). The NE_L for forages was adjusted with the Van Soest variable discount method (Dairy One, 1999).

Net energy required for maintenance (NE_M) was calculated as $BW^{0.75} \times 0.08$. Net energy requirements for pregnancy (NE_P) were calculated as $[(0.00318 \times \text{day of gestation} - 0.0352) \times (\text{calf birth weight}/45)]/0.218$. Milk net energy requirements (NE_{LAC}) were calculated as $(0.0929 \times \text{fat}\% + 0.0563 \times \text{protein}\% + 0.0395 \times \text{lactose}\%) \times \text{milk}$. The equation used to calculate prepartum energy balance (EB_{PRE}) was $EB_{PRE} = NE_I - (NE_M + NE_P)$. The equation used to calculate postpartum energy balance (EB_{POST}) was $EB_{POST} = NE_I - (NE_M + NE_{LAC})$. Energy balance was expressed as percentage of the requirement.

Metabolizable protein supply and balance were estimated with the NRC model (2001; Table 3). Default model chemical compositions were used for grains, minerals, and vitamins. Default model chemical compositions of alfalfa silage, alfalfa hay, corn silage, wheat straw, and cottonseed were adjusted to reflect the average monthly composite analyses (Dairy One). Actual

dietary treatment means were used as inputs for prepartum DMI and BW. Inputs of postpartum DMI, BW, milk yield, and milk composition were the means across dietary treatments.

Statistical Analyses

Statistical computations were performed using SAS (Release 8.2; SAS Institute Inc., Cary, NC). Data were subjected to ANOVA using the MIXED procedure of SAS (Littell et al., 1996). Data from the far-off period (dry-off to -25 d relative to expected parturition), close-up period (-24 d relative to expected parturition to parturition; -7 d relative to parturition to parturition), and postpartum period (1 to 10 DIM; 1 to 56 DIM) were analyzed separately. As described elsewhere (Dann et al., 2005), we analyzed the periods of -7 to -1 d prepartum and 1 to 10 DIM separately to focus on critical times when metabolic changes were most evident.

Data measured over time were subjected to ANOVA by using the REPEATED statement in the MIXED procedure of SAS (Littell et al., 1996). For each variable analyzed, cow nested within treatment was subjected to 3 covariance structures: compound symmetry, autoregressive order 1, and unstructured covariance. The covariance structure that resulted in the Akaike information criterion closest to zero was used (Littell et al., 1996). Data for BW and BCS were adjusted by analysis of covariance using the respective measurements obtained at dry-off. Data not analyzed over time were subjected to ANOVA by using the MIXED procedure of SAS (Littell et al., 1996).

Data for the far-off period were analyzed as a randomized design. The model for far-off period data contained the effects of far-off treatment (100NRC, 150NRC, and 80NRC). Time and time $\times$ treatment interactions were included in the model when appropriate. Cow nested within far-off treatment was designated as a random effect and was used as the error term to test the effects of far-off treatment. Least squares means for far-off treatment effects were separated by use of the PDIF statement when the overall F -test was $P < 0.05$. Trends were indicated when $P < 0.10$.

Data for the close-up period and the postpartum period were analyzed as a randomized design with a 3×2 factorial arrangement of treatments (far-off treatments $\times$ close-up treatments). The model for close-up period data and postpartum data contained the effects of far-off treatment (100NRC, 150NRC, and 80NRC), close-up treatment (ad libitum or restricted intake), and the interaction of far-off and close-up treatments. Time and interactions of time with far-off or close-up treatment were included in the model when appropriate. Cow nested within far-off and close-up treatments was

Table 3. Inputs used to estimate MP in the NRC (2001) model and estimates of MP supply and balance for multiparous Holstein cows given different far-off and close-up diets

Variable	Diet ¹					Lactation
	100NRC	150NRC	80NRC	Ad libitum close-up	Restricted close-up	
Inputs						
DMI, kg	10.4	14.4	6.6	12.4	7.2	21.3
BW, kg	702	711	681	727	713	629
Day of gestation	237	237	237	267	267	—
DIM	—	—	—	—	—	28
Milk, kg	—	—	—	—	—	37.8
Milk fat, %	—	—	—	—	—	3.65
Milk protein, %	—	—	—	—	—	2.82
Milk lactose, %	—	—	—	—	—	4.84
Estimates						
MP supply, g/d	875	1,320	596	1,086	622	2,422
MP balance, g/d	163	492	8	257	−49	72

¹Diets fed to meet 100% (100NRC), 150% (150NRC), or 80% (80NRC) of NRC requirements for NE_L during the far-off dry period.

designated as a random effect and was used as the error term to test the effects of far-off and close-up treatments. Least squares means were reported for far-off and close-up treatment effects. Significance was declared at $P < 0.05$ and trends were indicated at $P < 0.10$. Far-off treatment effects were separated by use of the PDIFF statement as described previously. Health data obtained from farm records were analyzed by the Fisher's exact test (Hatcher and Stepanski, 1994).

RESULTS AND DISCUSSION

There were no differences among treatment groups at the start of the experiment for lactation number for the upcoming lactation (mean $\pm$ SEM = 2.9 ± 0.2); previous lactation 305-d mature equivalent for milk ($11,242 \pm 163$ kg), fat (412 ± 7 kg), and protein (347 ± 5 kg); dry-off BW (688 ± 10 kg); dry-off BCS (2.96 ± 0.06); dry-off concentrations of glucose, NEFA, BHBA, urea N, total protein, and insulin in serum; contents of total lipids, TAG, and glycogen in liver; or contents of total lipids and TAG in muscle. The number of days on dietary treatments did not differ among groups. Cows were dry for 62.0 ± 1.4 d and averaged 40.6 ± 1.3 d on the far-off treatments and 21.3 ± 0.5 d on the close-up treatments.

Dietary treatments were formulated on the basis of calculated NE_L supply with all nutrients in appropriate balance. Because total DMI were different by design, intakes of other nutrients also were different in addition to NE_L intakes. As shown in Table 3, estimated MP balances were positive or near zero for all dietary groups, which confirms that relative differences in NE_L balance were likely more substantial than the supply of other nutrients. Therefore, differences are discussed primarily in the context of differences in NE_L intake;

potential effects attributable to differences in supply of MP or other nutrient fractions are embedded in differences in NE_L intake and cannot be differentiated within this experimental design.

Far-Off Dry Period (Dry-Off to −25 d Relative to Expected Parturition)

During the far-off period, DMI and energy balance were different ($P = 0.001$; Table 4) among far-off treatments, as expected. Measured energy intakes of 77, 95, and 160% of the NE_L requirement for cows fed 80NRC, 100NRC, 150NRC, respectively (Table 4), were close to the target values of 80, 100, and 150%. The far-off period BW change, BCS change, and concentrations of NEFA and insulin in serum were consistent with the plane of nutrition (Table 4). Despite similar glucose concentrations in serum, the insulin concentration for cows fed 150NRC was more than twice that of cows fed 80NRC or 100NRC. Similarly, Holtenius et al. (2003) found higher plasma insulin concentrations in cows that received 178% of their energy requirement during the dry period compared with cows that received 75 or 110% of their energy requirement.

Concentrations of total lipid, TAG, and glycogen in liver at −30 d relative to expected parturition were not affected by far-off treatment (Table 4). Concentrations of total lipid and TAG in skeletal muscle also did not differ among treatment groups during the far-off dry period (Table 4).

Close-Up Dry Period (−24 d Relative to Expected Parturition)

Cows were switched to the close-up diet at −24 d relative to expected parturition. During the close-up

Table 4. Least squares means for BCS, BW, DMI, energy balance, and serum components from dry-off to -25 d relative to expected parturition, and liver and muscle composition at -30 d relative to expected parturition for multiparous Holstein cows given different far-off (F) diets

Variable	Far-off treatment ¹			SEM	P-value	
	100NRC	150NRC	80NRC		F	Time
BCS	3.04 ^b	3.16 ^a	2.94 ^b	0.04	0.003*	0.004
BCS change ²	0.14 ^b	0.38 ^a	0.02 ^b	0.06	0.001	—
BW, kg	702 ^a	711 ^a	681 ^b	4	0.001*	0.001
BW change, ² kg	28 ^b	50 ^a	2 ^c	5	0.001	—
DMI, % BW	1.49 ^b	2.03 ^a	0.98 ^c	0.07	0.001*	0.001
DMI, kg	10.4 ^b	14.4 ^a	6.6 ^c	0.5	0.001*	0.001
Energy balance, ³ %	95 ^b	160 ^a	77 ^c	5	0.001*	0.001
Serum						
BHBA, mg/dL	4.04	4.31	4.52	0.25	0.40	0.001
Glucose, mg/dL	55.9	57.4	55.2	1.2	0.39	0.43
Insulin, μ IU/mL	3.40 ^b	7.45 ^a	2.75 ^b	0.89	0.001	0.19
NEFA, μ Eq/L	174 ^b	123 ^c	218 ^a	16	0.001	0.001
Total protein, g/dL	7.53	7.95	7.83	0.13	0.07	0.006
Urea N, mg/dL	14.68	14.07	13.33	0.43	0.08	0.15
Liver (-30 d)						
Total lipids, ⁴ %	4.15	4.11	4.00	0.08	0.40	—
Triacylglycerols, ⁴ %	0.25	0.25	0.27	0.03	0.87	—
Glycogen, ⁴ %	4.23	4.53	4.45	0.15	0.33	—
Triacylglycerols:glycogen	0.06	0.06	0.06	0.01	0.75	—
Skeletal muscle (-30 d)						
Total lipids, ⁴ %	4.9	4.5	5.4	0.6	0.51	—
Triacylglycerols, ⁴ %	4.4	4.3	4.4	0.6	0.34	—

^{a-c}Subcolumn means within the row and treatment category with different superscripts differ ($P < 0.05$).

¹Diets fed to meet 100% (100NRC), 150% (150NRC), or 80% (80NRC) of NRC requirements for NE_L during the far-off dry period.

²Last value before switched to close-up treatments minus the dry-off value.

³Percentage of NE_L requirement.

⁴Percent wet weight.

*Treatment $\times$ time was significant ($P < 0.05$).

period, cows fed the close-up diet ad libitum ate more (12.4 kg DMI; $P = 0.001$) than cows fed in restricted amounts (7.2 kg DMI) as planned (Table 5). The difference in DMI resulted in cows fed ad libitum being in positive energy balance (136% of NE_L requirement), whereas cows on restricted diets were in negative energy balance (78% of NE_L requirement). Compared with cows fed restricted diets, ad libitum-fed cows had greater BW gain (9 vs. -3 kg), higher concentrations of glucose (56.6 vs. 53.4 mg/dL) and insulin (3.63 vs. 1.79 μ IU/mL) in serum, and a lower concentration of NEFA (183 vs. 352 μ Eq/L) in serum (Table 5). Serum NEFA was 92% higher for restricted cows, which corresponded to greater liver concentrations of total lipids (4.69 vs. 4.17%) and TAG (0.68 vs. 0.36%) at -14 d relative to expected parturition (Table 5). Despite higher NEFA concentrations, however, concentrations of total lipids (4.5%) and TAG (3.5%) in the skeletal muscle did not differ significantly (Table 5).

An indirect assessment of hepatic function was made at -10 d relative to expected parturition by measuring the concentrations of albumin, cholesterol, and total bilirubin and the activities of AST, SDH, AP, and GGT

in serum (Table 6). Far-off treatment did not affect concentrations of albumin, cholesterol, and total bilirubin or activities of SDH, AP, and GGT. The activity of AST was higher for cows fed 150NRC (57 U/L) than for cows fed 100NRC (48 U/L) or 80NRC (50 U/L), but the magnitude of increase was not likely to be of clinical relevance. Close-up treatment did not affect concentrations of albumin and cholesterol or activities of AP and GGT. Cows fed ad libitum had higher activities of AST (54 vs. 49 U/L and SDH (5.9 vs. 4.3 U/L) and a lower concentration of total bilirubin (0.2 vs. 0.3 mg/dL) than did restricted cows, but the differences are not likely to be of clinical importance (Aiello, 1998). Gröhn et al. (1983) found positive correlations between the concentration of fat in liver and serum activities of AST ($r = 0.377$) and SDH ($r = 0.466$). In contrast, in this study, cows fed ad libitum, which had lower hepatic contents of total lipids and TAG at -14 d relative to expected parturition, had higher activities of AST and SDH at -10 d relative to expected parturition. Activities of AST and SDH were low compared with reference ranges (University of Illinois Clinical Pathology Laboratory; Aiello, 1998) and may therefore represent statistical

Table 5. Least squares means for BCS, BW, DMI, energy balance, and serum components from -24 d relative to expected parturition to parturition and liver and muscle composition at -14 d relative to expected parturition for multiparous Holstein cows given different far-off (F) and close-up (C) diets¹

Variable	Far-off dry period treatment						SEM	<i>P</i> -value			
	100NRC		150NRC		80NRC			F	C	F × C	Time
	Ad libitum close-up	Restricted close-up	Ad libitum close-up	Restricted close-up	Ad libitum close-up	Restricted close-up					
BCS	3.19 ^b	2.90 ^b	3.29 ^a	3.20 ^a	2.96 ^b	2.86 ^b	0.08	0.001	0.02	0.41	0.001
BCS change ²	0.05	−0.21	−0.07	−0.12	−0.12	−0.09	0.09	0.95	0.18	0.18	—
BW, kg	721 ^b	706 ^b	749 ^a	730 ^a	711 ^b	703 ^b	7	0.001	0.02	0.72	0.40
BW change, ² kg	8 ^{ab}	−6 ^{ab}	1 ^b	−7 ^b	19 ^a	2 ^a	5	0.04	0.004	0.67	—
DMI, % BW	1.91	1.02	1.69	0.92	1.66	1.05	0.09	0.17*	0.001	0.28*	0.06
DMI, kg	13.6	7.3	12.4	6.8	11.1	7.4	0.5	0.06*	0.001	0.04*	0.04
Energy balance, ³ %	147	80	135	72	126	83	7	0.26*	0.001	0.19*	0.04
Serum											
BHBA, mg/dL	4.21 ^b	4.88 ^b	5.43 ^a	6.02 ^a	5.09 ^{ab}	4.84 ^{ab}	0.50	0.04	0.36	0.52*	0.009
Glucose, mg/dL	57.4	54.5	58.0	52.6	54.6	53.2	1.7	0.34	0.009	0.42	0.03
Insulin, μIU/mL	3.78	1.01	3.78	2.26	3.34	2.11	0.82	0.68	0.002	0.46	0.002
NEFA, μEq/L	159 ^b	304 ^b	191 ^a	406 ^a	196 ^{ab}	288 ^{ab}	46	0.04	0.001	0.08	0.001
Total protein, g/dL	7.28 ^b	7.12 ^b	7.51 ^a	7.85 ^a	7.44 ^{ab}	7.42 ^{ab}	0.20	0.03	0.71	0.35	0.001
Urea N, mg/dL	11.68	12.57	11.77	14.26	12.00	11.59	0.67	0.15*	0.06*	0.08	0.001
Liver (−14 d)											
Total lipids, ⁴ %	4.22	4.42	4.01	5.36	4.28	4.27	0.26	0.23	0.02	0.02	—
Triacylglycerols, ⁴ %	0.43	0.49	0.27	1.05	0.38	0.48	0.17	0.34	0.03	0.06	—
Glycogen, ⁴ %	3.77	3.50	3.51	2.81	3.68	3.32	0.40	0.47	0.17	0.84	—
Triacylglycerols:glycogen	0.21	0.18	0.10	0.86	0.14	0.17	0.25	0.38	0.22	0.22	—
Skeletal muscle (−14 d)											
Total lipids, ⁴ %	4.52	4.80	3.13	4.48	4.50	3.63	0.77	0.49	0.66	0.27	—
Triacylglycerols, ⁴ %	4.05	3.54	2.36	3.66	3.17	4.04	0.87	0.52	0.36	0.47	—

^{a,b}Main effects of far-off treatments with different superscripts differ ($P < 0.05$).

¹Diets fed to meet 100% (100NRC), 150% (150NRC), or 80% (80NRC) of NRC requirements for NE_L during the far-off dry period.

²Last value before parturition minus first value when on the close-up treatment.

³Percentage of NE_L requirement.

⁴Percent wet weight.

*Treatment × time was significant ($P < 0.05$).

Table 6. Least squares means for the serum clinical chemistry profile at -10 d relative to expected parturition and 10 d after parturition for multiparous Holstein cows given different far-off (F) and close-up (C) diets¹

Variable	Far-off dry period treatment						SEM	<i>P</i> -value		
	100NRC		150NRC		80NRC					
	Ad libitum close-up	Restricted close-up	Ad libitum close-up	Restricted close-up	Ad libitum close-up	Restricted close-up		F	C	F × C
−10 d										
Albumin, g/dL	3.3	3.3	3.3	3.4	3.3	3.0	0.1	0.30	0.37	0.24
Alkaline phosphatase, U/L	32	27	31	31	28	23	3	0.16	0.17	0.66
Aspartate aminotransferase, U/L	53 ^b	43 ^b	56 ^a	58 ^a	54 ^b	45 ^b	4	0.02	0.04	0.11
Cholesterol, mg/dL	89	101	105	99	93	84	8	0.18	0.90	0.34
γ-Glutamyl transferase, U/L	22	18	22	20	21	21	2	0.78	0.18	0.63
Sorbitol dehydrogenase, U/L	6.1	3.7	6.0	4.9	5.8	4.3	1.1	0.85	0.05	0.80
Total bilirubin, mg/dL	0.2	0.3	0.2	0.3	0.2	0.2	0.1	0.57	0.001	0.53
10 d										
Albumin, g/dL	3.3	3.1	3.1	2.9	3.1	3.1	0.1	0.24	0.23	0.42
Alkaline phosphatase, U/L	29	25	28	27	24	25	2	0.35	0.43	0.47
Aspartate aminotransferase, U/L	87	80	92	83	84	79	6	0.64	0.16	0.95
Cholesterol, mg/dL	93	83	87	75	89	85	7	0.52	0.13	0.83
γ-Glutamyl transferase, U/L	21	21	25	22	21	22	2	0.36	0.60	0.60
Sorbitol dehydrogenase, U/L	9.7	6.1	8.0	6.2	5.8	5.4	1.3	0.20	0.07	0.49
Total bilirubin, mg/dL	0.3	0.2	0.4	0.3	0.3	0.2	0.1	0.16	0.22	0.84

^{a,b}Main effect of far-off treatments with different superscripts differ ($P < 0.05$).

¹Diets fed to meet 100% (100NRC), 150% (150NRC), or 80% (80NRC) of NRC requirements for NE_L during the far-off dry period.

differences of minor physiological importance. Boisclair et al. (1987) found that prepartum energy intake (102, 131, and 162% of the requirement) did not affect the serum activity of AST or concentrations of albumin or total bilirubin 1 wk before parturition.

The far-off treatment had significant carryover effects on variables measured during the close-up period regardless of the close-up treatment. Cows fed 150NRC had the highest serum concentrations of total protein, NEFA, and BHBA and lost the most BW during the close-up period (Table 5). It is interesting to note that 150NRC cows, which had the highest serum insulin concentration during the far-off period (Table 4), also had the highest serum NEFA concentration during the close-up period (Table 5) regardless of the close-up treatment. These data are suggestive of the development of insulin resistance in adipose tissue of cows overfed during the far-off period, which could result in increased lipolysis, greater BW loss, and higher serum NEFA concentrations during the close-up period. A follow-up experiment utilizing a glucose tolerance test (Holtenius et al., 2003) or other more direct measure of insulin resistance should be conducted to test this hypothesis.

Because an increased TAG concentration in muscle is a hallmark of obesity- and diabetes-related insulin resistance in humans and laboratory animals (Kelley, 2002), we postulated that total lipid and TAG concentrations in muscle would be greater in cows that overconsumed energy (150NRC). However, no differences among treatments were observed for either variable at -14 d. More direct measurements of insulin resistance and sensitivity will be needed to address effects of energy overconsumption in cows.

Interactions of far-off and close-up treatments were detected for serum NEFA concentrations ($P = 0.08$; Table 5), liver total lipid contents ($P = 0.02$; Table 5), and liver TAG contents ($P = 0.06$; Table 5). The serum NEFA concentration averaged 159, 301, 191, 454, 199, and 299 $\mu\text{Eq/L}$ (SEM = 39) for treatment combinations 100NRC + ad libitum close-up, 100NRC + restricted close-up, 150NRC + ad libitum close-up, 150NRC + restricted close-up, 80NRC + ad libitum close-up, and 80NRC + restricted close-up, respectively. Thus, the serum NEFA concentration during the close-up period was highest for cows fed the combination of 150NRC and restricted close-up diet. A higher serum NEFA concentration was associated with increased total lipid and TAG contents in liver at -14 d relative to expected parturition for those cows. Total lipid concentrations in liver averaged 4.22, 4.42, 4.01, 5.36, 4.28, and 4.27% of wet weight (SEM = 0.26), and TAG concentrations in liver averaged 0.43, 0.49, 0.27, 1.05, 0.38, and 0.48% of wet weight (SEM = 0.17) for treatment combinations

100NRC + ad libitum close-up, 100NRC + restricted close-up, 150NRC + ad libitum close-up, 150NRC + restricted close-up, 80NRC + ad libitum close-up, and 80NRC + restricted close-up, respectively. Thus, the treatment combination 150NRC + restricted close-up had higher ($P < 0.05$) total lipid and TAG contents in the liver compared with all other treatment combinations, which did not differ from one another ($P > 0.10$). Overfeeding during the far-off period combined with underfeeding during the close-up period resulted in detrimental effects on prepartum energy metabolism.

Close-Up Dry Period (-7 d Relative to Parturition)

The effect of close-up treatment on metabolism was evident during the last 7 d before parturition (Table 7), which is the time of most rapid adaptive changes prepartum (Grummer, 1995). Cows fed ad libitum had higher concentrations of glucose (55.7 vs. 51.4 mg/dL) and insulin (3.82 vs. 1.20 $\mu\text{IU/mL}$) and a lower concentration of NEFA (270 vs. 449 $\mu\text{Eq/L}$) in serum than did cows fed restricted amounts. As expected (Grummer, 1995; Ingvarsen and Andersen, 2000), DMI decreased before parturition. The interaction of close-up treatment and time ($P = 0.02$) for DMI during the last 7 d of gestation indicated that cows fed ad libitum decreased DMI more (~18%) than did cows fed restricted amounts (~7%). Although ad libitum-fed cows had a greater change in DMI, they did not pass into negative energy balance before parturition. Holcomb et al. (2001) observed similar changes in DMI during the last 7 d of gestation for cows that were fed a low-forage diet at either ad libitum intake or restricted intake (~8 of kg DM). Cows with restricted intake in that study (Holcomb et al., 2001) consumed enough DM to meet NE_L requirements, which is in contrast to cows on restricted diets in our study, which consumed enough feed to meet only ~80% of the NE_L requirement.

The effects of far-off treatments on close-up variables were diminished during the last 7 d of gestation (Table 7) compared with the last 4 wk of gestation (Table 5). Cows previously fed 150NRC had higher total protein and BHBA in serum than did cows fed 100NRC or 80NRC (Table 7). The higher total protein with similar albumin concentration (Table 6) for cows fed 150NRC suggests that those cows had greater globulin concentrations. Interestingly, the concentration of NEFA in serum did not differ among far-off treatments (Table 7), but the interaction of far-off treatment and time approached significance ($P < 0.10$). During the last 7 d of gestation, serum concentrations of NEFA increased 153, 52, and 58% for cows fed 100NRC, 150NRC, and 80NRC, respectively.

Table 7. Least squares means for DMI, energy balance, and serum components from -7 d relative to parturition to parturition for multiparous Holstein cows given different far-off (F) and close-up (C) diets¹

Variable	Far-off dry period treatment						SEM	<i>P</i> -value			
	100NRC		150NRC		80NRC			F	C	F × C	Time
	Ad libitum close-up	Restricted close-up	Ad libitum close-up	Restricted close-up	Ad libitum close-up	Restricted close-up					
DMI, % BW	1.83	1.01	1.60	0.92	1.81	1.01	0.11	0.26	0.001*	0.78*	0.001
DMI, kg	12.9	7.2	11.7	6.7	12.3	7.1	0.7	0.42	0.001*	0.84*	0.001
Energy balance, ² %	139	78	126	71	137	79	9	0.41	0.001*	0.94*	0.001
Serum											
BHBA, mg/dL	4.73 ^b	5.36 ^b	5.49 ^a	6.97 ^a	5.16 ^b	4.84 ^b	0.53	0.03	0.16	0.23	0.001
Glucose, mg/dL	56.1	51.1	57.3	51.5	53.7	51.5	1.7	0.56	0.003*	0.53	0.89
Insulin, μIU/mL	3.46	0.89	5.06	1.29	2.94	1.42	0.81	0.36	0.001	0.38	0.16
NEFA, μEq/L	270	417	288	527	253	402	50	0.23	0.001	0.56	0.001
Total protein, g/dL	6.79 ^b	6.48 ^b	7.15 ^a	7.17 ^a	6.85 ^b	6.84 ^b	0.16	0.004	0.40	0.49	0.001
Urea N, mg/dL	11.73	11.72	10.60	11.99	12.80	10.83	0.71	0.73	0.73	0.06	0.37

^{a,b}Main effect of far-off treatments with different superscripts differ ($P < 0.05$).

¹Diets fed to meet 100% (100NRC), 150% (150NRC), or 80% (80NRC) of NRC requirements for NE_L during the far-off dry period.

²Percentage of NE_L requirement.

*Treatment × time was significant ($P < 0.05$).

Postpartum Period (1 to 10 DIM)

Despite the difference in nutrient intake during the far-off and close-up periods, calf birth weight did not differ among far-off treatments ($P = 0.46$), between close-up treatments ($P = 0.56$), or among the interaction of far-off and close-up treatments ($P = 0.83$) and averaged 45.2 ± 1.2 kg. Similarly, Douglas et al. (2006) and Tesfa et al. (1999) found no difference in calf birth weight from cows that received diets containing either adequate or deficient amounts of energy during the dry period.

Surprisingly, DMI ($P = 0.07$), energy balance ($P = 0.03$), and concentrations of NEFA ($P = 0.07$) and BHBA ($P = 0.03$) in serum during the first 10 DIM were affected by far-off treatment but not by close-up treatment (Table 8). Cows with a lower energy balance (100NRC and 80NRC) during the far-off period tended to have higher DMI, had a higher energy balance, tended to have lower serum NEFA, and had lower BHBA concentrations during the first 10 DIM (Table 8). The interaction of far-off treatment × close-up treatment × day was significant for serum BHBA (Table 8; Figure 1) and NEFA (not shown). Cows fed the dietary combinations of 100NRC and ad libitum close-up, 150NRC and ad libitum close-up, or 150NRC and restricted close-up had higher serum BHBA and NEFA concentrations for a longer period postpartum.

Postpartum Period (1 to 56 DIM)

Fewer significant effects of the far-off treatment on postpartum metabolism were present for data averaged across the first 56 DIM compared with the first 10 DIM,

indicating that the carryover effects of dry-period feeding decreased as lactation progressed. There were no effects of far-off or close-up treatments on DMI, milk yield, BW, BCS, or concentrations of glucose and insulin in serum when data were averaged over the first 56 DIM (Table 9). Other researchers have found no effect of prepartum intake (ad libitum vs. restricted) on postpartum DMI (Holcomb et al., 2001; Agenäs et al., 2003), milk yield (Holcomb et al., 2001; Agenäs et al., 2003; Douglas et al., 2006), BW (Holcomb et al., 2001; Agenäs et al., 2003), BCS (Holcomb et al., 2001), serum glucose concentration (Holcomb et al., 2001; Douglas et al., 2006), or serum insulin concentration (Holcomb et al., 2001; Douglas et al., 2006). In contrast, Douglas et al. (2006) found that DMI during the first 15 wk of lactation was greater for cows in which intake was restricted during the entire dry period by the same amount as in our study. Although not significant, cows in our study fed 80NRC followed by restricted close-up intake ate approximately 1 kg more DM per day during the first 56 DIM than did cows fed 150NRC and ad libitum during the close-up period.

The serum NEFA concentration tended ($P = 0.08$) to be higher for cows previously fed 150NRC (376 μ Eq/L) than for those fed 80NRC (296 μ Eq/L), whereas 100NRC (336 μ Eq/L) was intermediate; serum BHBA tended ($P = 0.11$) to follow a pattern similar to serum NEFA. Close-up treatments did not affect serum NEFA and BHBA concentrations. Compared with cows fed restricted amounts in the close-up period, cows fed ad libitum tended to have a lower energy balance (102 vs. 107%), had greater BCS loss (-0.35 vs. -0.13), and tended to have a higher milk fat content (3.73 vs. 3.57%)

Table 8. Least squares means for DMI, energy balance, milk yield, and serum components from 1 to 10 DIM and concentrations of total lipids, triacylglycerols, and glycogen in liver and concentrations of total lipids and triacylglycerols in skeletal muscle at 1 DIM for multiparous Holstein cows given different far-off (F) and close-up (C) diets¹

Variable	Far-off dry period treatment						SEM	<i>P</i> -value			
	100NRC		150NRC		80NRC			F	C	F × C	Time
	Ad libitum close-up	Restricted close-up	Ad libitum close-up	Restricted close-up	Ad libitum close-up	Restricted close-up					
DMI, % BW	2.47	2.45	2.18	2.14	2.47	2.53	0.16	0.07	0.98	0.95	0.001
DMI, kg	16.0	15.8	14.2	14.0	15.4	16.2	1.0	0.14	0.91	0.85	0.001
Energy balance, ² %	86 ^{ab}	91 ^{ab}	78 ^b	82 ^b	90 ^a	96 ^a	5	0.03	0.21	0.95	0.001
Milk, kg	30.0	29.4	26.5	25.6	26.7	26.1	2.0	0.12	0.66	0.99	0.001
Serum											
BHBA, mg/dL	8.61 ^{ab}	7.65 ^{ab}	8.73 ^a	9.37 ^a	6.85 ^b	6.37 ^b	0.90	0.03	0.71	0.65*	0.001
Glucose, mg/dL	48.6	47.7	51.1	48.0	51.0	50.6	1.8	0.35	0.32	0.72*	0.001
Insulin, μIU/mL	1.49	1.17	1.71	1.35	1.89	1.58	0.30	0.40	0.18	0.99	0.01
NEFA, μEq/L	870	703	793	792	686	568	79	0.07	0.14	0.55	0.001
Total protein, g/dL	6.99	6.51	7.10	6.87	6.95	6.85	0.17	0.40	0.06	0.53	0.31
Urea N, mg/dL	14.88 ^a	15.23 ^a	12.74 ^b	13.99 ^b	15.33 ^{ab}	13.98 ^{ab}	0.67	0.03	0.88	0.15	0.001
Liver											
Total lipids, ³ %	7.85	5.89	7.46	6.63	5.67	5.30	0.85	0.08	0.09	0.56	—
Triacylglycerols, ³ %	3.48	1.47	2.78	2.40	1.42	1.31	0.77	0.14	0.13	0.33	—
Glycogen, ³ %	1.45	1.07	1.67	1.62	1.73	1.83	0.32	0.19	0.63	0.69	—
Triacylglycerols:glycogen	3.96	4.69	5.44	2.83	0.99	1.29	2.15	0.19	0.74	0.62	—
Muscle											
Total lipids, ³ %	4.35	4.14	3.15	3.68	5.18	3.71	0.72	0.30	0.53	0.34	—
Triacylglycerols, ³ %	3.19	3.56	2.44	3.27	3.67	3.24	0.91	0.61	0.65	0.60	—

^{a,b}Main effect of far-off treatments with different superscripts differ ($P < 0.05$).

¹Diets fed to meet 100% (100NRC), 150% (150NRC), or 80% (80NRC) of NRC requirements for NE_L during the far-off dry period.

²Percentage of NE_L requirement.

³Percent wet weight.

*Treatment × time was significant ($P < 0.05$).

Figure 1. Least squares means for serum BHBA from 1 to 10 DIM for multiparous Holstein cows fed diets to meet 100% (100NRC), 150% (150NRC), or 80% (80NRC) of NRC requirements for NE_L during the far-off dry period and a close-up diet at either ad libitum (CA) or restricted (CR) intake during the close-up dry period. For clarity, standard error bars have been omitted. The largest standard error for any treatment and day mean was 1.26 mg/dL.

in the first 56 DIM (Table 9). Holcomb et al. (2001) found that cows fed ad libitum during the close-up period had a higher milk fat content during the first 40 DIM than cows with restricted intake. The higher milk fat content during the early postpartum period reflects greater body fat mobilization (Palmquist et al., 1993).

Cows that had the highest serum insulin concentrations during the far-off period (150NRC cows) or the close-up period (ad libitum-fed cows) had the lowest energy balance, the greatest BCS loss, and the highest serum NEFA concentration during the first 56 DIM. Holtenius et al. (2003) conducted glucose tolerance tests at 3 wk postpartum on cows that were fed 75, 110, or 178% of their energy requirement during the entire dry period. They found that the clearance rate of glucose was ~20% lower in cows that had been fed 178% of their energy requirement than in cows previously fed 75 or 110% of their energy requirement. Holtenius et al. (2003) suggested that the lower glucose clearance rate reflected a greater degree of insulin resistance, which contributed to greater lipolysis in adipocytes, greater BCS loss, and higher concentrations of NEFA in serum during the postpartum period. Insulin resistance may explain the negative metabolic effects observed postpartum for cows that were fed 150NRC in the far-off dry

Table 9. Least squares means for BCS, BW, DMI, energy balance, milk yield and components, and serum components from parturition to wk 8 of lactation for multiparous Holstein cows given different far-off (F) and close-up (C) diets¹

Variable	Far-off dry period treatment						SEM	<i>P</i> -value			
	100NRC		150NRC		80NRC			F	C	F × C	Time
	Ad libitum close-up	Restricted close-up	Ad libitum close-up	Restricted close-up	Ad libitum close-up	Restricted close-up					
BCS	2.46	2.21	2.53	2.36	2.22	2.36	0.08	0.15	0.16	0.04	0.001
BCS change ²	−0.41	−0.06	−0.35	−0.31	−0.29	−0.02	0.13	0.40	0.04	0.46	—
BW, kg	626	630	645	627	621	624	11	0.42*	0.67	0.51	0.001
BW change, ² kg	−22	−13	−20	−23	−21	−6	13	0.84	0.51	0.78	—
DMI, % BW	3.46	3.48	3.22	3.29	3.51	3.47	0.14	0.21	0.89	0.95	0.001
DMI, kg	21.7	21.9	20.6	20.5	21.1	21.6	0.8	0.29	0.72	0.90	0.001
Energy balance, ³ %	102	108	99	104	106	109	3	0.26	0.09	0.87	0.001
Milk											
Milk, kg	39.7	39.2	37.1	36.7	37.1	36.8	1.9	0.29	0.78	0.99	0.001
Fat, %	3.74	3.44	3.90	3.64	3.54	3.63	0.12	0.19	0.09	0.20	0.001
Protein, %	2.83	2.73	2.87	2.78	2.87	2.85	0.06	0.38	0.12	0.78	0.001
Lactose, %	4.88	4.73	4.92	4.85	4.83	4.85	0.06	0.34	0.16	0.29	0.001
Urea N, mg/dL	14.92	15.02	14.43	14.54	14.75	12.74	0.74	0.25	0.31	0.26	0.47
SCC	2.52	3.66	3.04	2.86	2.86	2.97	0.50	0.93	0.38*	0.37	0.001
Serum											
BHBA, mg/dL	6.34	5.27	5.67	5.98	4.87	5.07	0.46	0.11	0.61	0.24	0.001
Glucose, mg/dL	53.8	52.4	56.4	52.5	53.2	53.4	1.4	0.56	0.12	0.32	0.001
Insulin, μIU/mL	2.28	2.15	2.59	2.03	1.93	1.81	0.36	0.44	0.35	0.77	0.001
NEFA, μEq/L	379	293	382	370	308	285	35	0.08	0.15	0.51	0.001
Total protein, g/dL	7.60	7.24	7.65	7.64	7.50	7.51	0.12	0.20	0.23	0.26	0.001
Urea N, mg/dL	15.34	15.98	13.88	15.12	15.60	14.33	0.59	0.14	0.67*	0.09	0.001

¹Diets fed to meet 100% (100NRC), 150% (150NRC), or 80% (80NRC) of NRC requirements for NE_L during the far-off dry period.²Value at wk 8 minus value at wk 1.³Percentage of NE_L requirement.*Treatment × time was significant ($P < 0.05$).

period or ad libitum in the close-up period prepartum, but verification of this hypothesis requires more direct measurements of insulin responsiveness.

Samples of milk fat from wk 2 were analyzed for fatty acid composition (data not shown). Close-up treatment did not affect ($P > 0.05$) milk fat composition. In contrast, cows previously fed 150NRC generally had lower ($P < 0.05$) contents of short-chain and medium-chain fatty acids (6:0, 8:0, 10:0, 12:0, 14:0) and higher contents ($P < 0.05$) of 18:0 and 18:1 *cis*-9 than cows fed 100NRC or 80NRC. Energy balance at wk 2 was 93, 86, and 95% of the NE_L requirement for 100NRC, 150NRC, and 80NRC, respectively. Cows in a negative energy balance mobilize adipose tissue TAG, and the resulting long-chain fatty acids are incorporated into milk fat (Palmquist et al., 1993). The uptake of large quantities of long-chain fatty acids inhibits de novo synthesis of short-chain fatty acids by the mammary gland (Palmquist et al., 1993). Thus, changes in milk fat composition reflected differences in energy balance resulting from nutrition during the far-off dry period.

In our study, liver concentrations of total lipids and TAG increased and glycogen decreased from prepartum to 1 DIM (Tables 5, 8) as expected (Vazquez-Añon et al., 1994). Liver content of total lipids at 1 DIM tended

to be higher for cows with ad libitum intakes than for cows with restricted intakes during the far-off or close-up periods (Table 8). Concentrations of TAG followed the same pattern, but differences among means did not achieve statistical significance. Lipid mobilization leads to increased serum NEFA concentrations, increased uptake of NEFA by the liver, and increased TAG accumulation in the liver (Drackley, 1999). There were no effects of far-off or close-up treatments on liver total lipid and TAG contents at 14, 28, or 49 DIM. Total lipid concentrations averaged 7.1, 6.1, and 4.7% and TAG averaged 3.6, 2.0, and 0.7% at 14, 28, and 49 DIM, respectively. Similar to our data, Douglas et al. (2006) found that cows restricted in DMI during the entire dry period had lower contents of total lipids and TAG in the liver at 1 DIM. In contrast, Tesfa et al. (1999) found no effect of prepartum energy feeding on the liver total lipid content at 1 or 4 wk postpartum.

Fatty infiltration evidently did not cause severe liver damage based on concentrations of albumin, cholesterol, and total bilirubin or activities of AST, AP, GGT, and SDH measured in serum at 10 DIM (Table 6). All were within the normal (healthy) reference range (Aiello, 1998). Activity of SDH tended to be higher for cows previously fed ad libitum during the close-up pe-

Table 10. Frequency of health disorders and twins for multiparous Holstein cows given different far-off (F) and close-up (C) diets¹

Variable	Far-off dry period treatment						<i>P</i> -value		
	100NRC		150NRC		80NRC				
	Ad libitum close-up	Restricted close-up	Ad libitum close-up	Restricted close-up	Ad libitum close-up	Restricted close-up	F	C	F × C
Displaced abomasum	1	0	1	1	1	0	1.00	0.62	0.99
Foot and leg problems ²	5	7	7	6	6	6	1.00	0.82	0.97
Ketosis ³									
Subclinical, ⁴ not treated	0	0	0	0	1	0	0.32	1.00	0.65
Subclinical, ⁴ treated ⁵	2	1	5	4	2	2	0.13	0.78	0.47
Clinical ⁶	0	0	0	0	0	0	—	—	—
Ketonemia ⁷	3	3	5	5	3	1	0.20	0.80	0.50
Mastitis	1	2	4	2	3	2	0.57	0.77	0.79
Metritis	1	3	1	4	1	2	0.92	0.06	0.48
Hypocalcemia	0	0	2	1	0	0	0.10	1.00	0.39
Retained placenta	2	4	6	7	4	7	0.11	0.16	0.21
Total health disorders ⁸	12	17	26	25	18	19	—	—	—
Twins	1	1	2	2	1	0	0.49	1.00	0.91

¹Number of cows with at least one incidence. Diets fed to meet 100% (100NRC), 150% (150NRC), or 80% (80NRC) of NRC requirements for NE_L during the far-off dry period.

²Includes abscess, pododermatitis circumscripta (ulcer), interdigital hyperplasia (corn), interdigital phlegmon (foot rot), digital dermatitis, and white line disease.

³The most severe ketosis incident was recorded for each cow.

⁴Cow had ketosuria at least one day (determined by veterinarian using a reagent strip for urinalysis).

⁵Propylene glycol given per os or dextrose given intravenously.

⁶Cow had ketosuria and showed signs of anorexia, ataxia, or abnormal behavior.

⁷Cow had serum BHBA ≥ 1400 μmol/L (15 mg/dL) at least one day during 1 to 10 DIM.

⁸Summation of the frequencies of displaced abomasum, foot and leg problems, subclinical ketosis (not treated and treated), clinical ketosis, mastitis, metritis, hypocalcemia, and retained placenta.

riod than for restricted cows. The higher activity of SDH likely is related to the higher liver total lipid content (Gröhn et al., 1983) and increased hepatocyte membrane permeability. However, by 14 DIM there were no differences between close-up treatments for contents of liver total lipids (7.1%) or TAG (3.6%).

Drackley et al. (1992) suggested that a liver TAG-to-glycogen ratio greater than 1.5 to 2 during early lactation (10 to 14 DIM) might indicate a greater susceptibility to clinical ketosis and hepatic lipidosis. Cows in this study had an average ratio of 3.20 at 1 DIM and 1.77 at 14 DIM. No cows showed signs (anorexia, ataxia, or abnormal behavior) of primary clinical ketosis. However, ~27% of cows had subclinical ketosis in the first 10 DIM based on cows having at least 1 d of serum BHBA ≥ 15 mg/dL (Duffield, 2000; Table 10). As expected, the average liver TAG-to-glycogen ratio decreased with greater DIM; the liver TAG-to-glycogen ratio averaged 0.74 and 0.18 at 28 and 49 DIM, respectively.

Muscle concentrations of total lipids and TAG might be expected to be higher under conditions of insulin resistance induced by overfeeding, in which the oxidation of fatty acids is inhibited (Kelley, 2002). On the other hand, higher intramuscular lipid deposition also occurs under conditions of rapid body fat mobilization

and increased muscle uptake of NEFA (Roberts et al., 1981, 1983). In our study, postpartum concentrations of total lipids and TAG in muscle were not affected by far-off or close-up diets despite differences in serum NEFA, with the exception of TAG at d 28 postpartum. Concentrations of TAG at d 28 were higher ($P = 0.02$) for cows previously fed 80NRC (3.8%) than for those fed 100NRC (2.2%), with cows fed 150NRC intermediate (2.5%). The possible cause or biological significance of these changes at d 28 relative to d 14 or 49 cannot be determined. Muscle total lipids averaged 4.0, 4.1, 3.8, and 3.5% and TAG averaged 3.3, 3.3, 2.9, and 2.6% at 1, 14, 28, and 49 DIM, respectively. Across all time points in our study, concentrations of total lipids and TAG in muscle were highly correlated ($r = 0.92$; $P < 0.001$). The regression equation relating total lipids (Y) and TAG (X) was $Y = 0.86X + 1.34$.

Postpartum Health

The frequency of individual health disorders was not affected significantly by far-off or close-up treatments (Table 10). The frequency of individual health disorders was not different ($P > 0.10$) among the 6 treatment combinations. However, health data from this study

should be interpreted with caution because of the small number of cows used.

Periods of extreme negative energy balance during early lactation are known to be associated with increased digestive, locomotive, and reproductive problems postpartum (Collard et al., 2000). Thus, prepartum nutritional management that causes a shorter, less severe period of negative energy balance should result in improved health. Cows overfed during the far-off dry period had a greater negative energy balance during the first 10 DIM and may thus have been at risk for more health problems. Total health disorders were 29, 51, and 37 for 100NRC, 150NRC, and 80NRC, respectively. To address these tendencies, additional research with more cows is needed to allow for greater statistical power.

The occurrence of retained placenta was high (41%) in this study. Factors that have been associated with retained placenta are age, length of gestation, number of calves, species, heredity, environment, hormones, immune function, and nutrition (Muller and Owens, 1974; Julien et al., 1976; Kimura et al., 2002). However, the exact cause and mechanism are still unknown. Deficiency of Se has been linked to the occurrence of retained placenta in dairy cows (Julien et al., 1976). In serum collected from cows in this study at 8 d before parturition, Se averaged 57.3 ± 0.6 ng/mL, which was considered marginally low (normal reference range = 60 to 90 ng/mL; T. H. Herdt, Michigan State Univ., East Lansing, personal communication). There was no difference in serum Se concentrations between cows with and cows without a retained placenta. The effect of season on the occurrence of a retained placenta was minimal because the retained placenta occurred throughout the 11-mo calving period of this study. The reason for the high occurrence of retained placentas in this study is unknown.

CONCLUSIONS

This study demonstrated that plane of nutrition during the far-off dry period plays a significant role in periparturient metabolism, regardless of the close-up feeding. Cows that were overfed (150NRC) during the far-off period had higher serum concentrations of NEFA and BHBA and lost more BW during the close-up period, and they had the highest serum concentrations of NEFA and BHBA and the lowest DMI and energy balance during the first 10 DIM. The carryover effect of far-off period feeding on the postpartum DMI, energy balance, and serum BHBA concentration diminished as lactation progressed. Milk yield was not affected significantly by the far-off period feeding, although the

mean milk yield was approximately 2.5 kg/d greater for cows fed 100NRC than for those fed 150NRC or 80NRC.

Close-up period feeding affected prepartum metabolism but minimally affected postpartum metabolism. Cows that were restricted in DMI during the close-up period had a lower energy balance, lower serum concentrations of glucose and insulin, and a higher serum concentration of NEFA during the close-up period but had a higher energy balance during the first 56 DIM. Milk yield was not affected by the close-up period feeding.

The interaction of far-off and close-up treatments was not significant for most variables that were analyzed. However, interactions of far-off and close-up treatments were detected for serum NEFA concentration during the close-up period and for liver total lipid and TAG contents at 14 d before expected parturition. The serum NEFA concentration and liver total lipid and TAG concentrations were highest for cows fed the combination of 150NRC and restricted close-up. Overfeeding during the far-off period combined with underfeeding during the close-up period has a negative effect on prepartum energy metabolism. These findings may be applicable to situations in which DMI in close-up groups is decreased by overcrowding or other management limitations.

Preventing excessive nutrient intake in the far-off or close-up periods may improve DMI and energy status in early lactation, but the effects diminish as lactation progresses. Because of limitations in the size of the experiment and available resources, a close-up treatment of 100NRC could not be included in this experiment. However, given the general lack of effect of the close-up diet in our study, it would be of interest to determine the effects of feeding a diet similar to 100NRC during the entire dry period, rather than using a separate close-up diet.

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