Prepartum nutritional strategy affects reproductive performance in dairy cows

F. C. Cardoso,* S. J. LeBlanc,† M. R. Murphy,* and J. K. Drackley*¹

*Department of Animal Sciences, University of Illinois, Urbana 61801

†Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada N1G 2W1

ABSTRACT

Negative energy balance during early postpartum is associated with reduced reproductive performance in dairy cows. A pooled statistical analysis of 7 studies completed in our group from 1993 to 2010 was conducted to investigate the association between prepartum energy feeding regimen and reproductive performance. The interval from calving to pregnancy (days to pregnancy, DTP) was the dependent variable to assess reproductive performance. Individual data for 408 cows (354 multiparous and 54 primiparous) were included in the analysis. The net energy for lactation (NE_L) intake was determined from each cow's average dry matter intake and calculated dietary NE_L density. Treatments applied prepartum were classified as either controlled-energy (CE; limited NE_L intake to ≤100% of requirement) or high-energy (HE; cows were allowed to consume >100%) diets fed during the far-off (FO) or close-up (CU) dry periods. Cow was the experimental unit. The Cox proportional hazard model revealed that days to pregnancy was shorter for CE (median = 157 d) than HE (median = 167 d) diets during the CU period [hazard ratio (HR) = 0.70]. Cows fed HE diets during the last 4 wk prepartum lost more body condition score in the first 6 wk postpartum than those fed CE diets (−0.43 and −0.30, respectively). Cows fed CE diets during the FO period had lower nonesterified fatty acids concentrations in wk 1, 2, and 3 of lactation than cows fed HE diets. Higher nonesterified fatty acids concentration in wk 1 postpartum was associated with a greater probability of disease ($n = 251$; odds ratio = 1.18). Cows on the CE regimen during the FO period had greater plasma glucose concentrations during wk 1 and 3 after calving than cows fed the HE regimen. Higher plasma glucose (HG) concentration compared with lower glucose (LG) in wk 3 (HG: $n = 154$; LG: $n = 206$) and wk 4 (HG: $n = 71$; LG: $n = 254$) after calving was associated with shorter days to

pregnancy (wk 3: median = 151 and 171 d for HG and LG, respectively, and HR = 1.3; wk 4: median = 148 and 167 d, respectively, and HR = 1.4). In the first 2 wk after calving, cows that received HE diets in the FO period had higher concentrations of total lipids and triglyceride and greater ratio of triglyceride to glycogen in liver than cows fed CE diets. In conclusion, cows fed CE diets during the CU period had a shorter interval between parturition and conception, which may be explained by increased NE_L intake during the first 4 wk postpartum and lower incidence of periparturient diseases. Lower body condition score loss during the first 6 wk postpartum and slightly higher glucose concentration at wk 3 likely contributed to improved reproductive performance.

Key words: transition diet, energy intake, days to pregnancy, controlled energy

INTRODUCTION

Reproductive performance is a major reason for premature culling of dairy cows, having a great effect on lifetime milk production of individual cows (Beever, 2006). Reproductive inefficiency also reduces the number of calves born, which decreases the number of replacements available (Gröhn and Rajala-Schultz, 2000) and further increases the economic losses caused by infertility. Reproductive efficiency in dairy cattle is commonly well below economic targets not only in the United States but also in Ireland, United Kingdom, and Australia (Lucy, 2001).

An index used to measure infertility in dairy herds is days to pregnancy (DTP), defined as the time in days from calving to the last breeding in which the cow became pregnant. Because conception does not necessarily occur at the first breeding, cows may have to be inseminated more than once. Feed intake and feeding behavior during the transition period may be related to increased risk for uterine diseases in dairy cattle (Urton et al., 2005; Hammon et al., 2006; Huzzey et al., 2007). Hammon et al. (2006) observed that cows developing uterine disease postpartum experienced decreased DMI beginning 1 wk before parturition. In

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¹Corresponding author: drackley@illinois.edu

concordance, cows diagnosed with severe metritis after calving were already consuming less DM 2 wk before calving (Huzzey et al., 2007). Excessive plasma concentrations of NEFA and BHBA, which are higher in cows experiencing stronger negative energy balance (**NEB**), are negatively associated with the developmental capacity of oocytes (Leroy et al., 2005) and pregnancy rates (Walsh et al., 2007). Cows with low BCS at 65 d postpartum are more likely to be anovular (Santos et al., 2008), which can compromise pregnancy success at first postpartum insemination.

Negative energy balance is associated with infertility in dairy cows (Jorritsma et al., 2003). Cows are typically unable to achieve the necessary DMI to maintain energy balance in early lactation (Bauman, 2000). Negative energy balance results from a mismatch between the rapid increase in energy requirements at the onset of lactation and the rate of increase in DMI (Butler, 2000). Negative energy balance suppresses pulsatile LH secretion and reduces ovarian responsiveness to LH stimulation, both of which result in reduced fertility (Butler, 2000). Negative energy balance may begin just before calving, reaches its nadir about 2 wk after calving, and on average lasts until approximately 6 wk postpartum (Butler and Smith, 1989; Bell, 1995; Grummer, 2008). The key determinant of the severity of NEB is DMI in early lactation (Lucy, 2001; NRC, 2001). Feeding strategies in the dry period have as a primary objective to maximize DMI in early lactation.

Based on previous reports (Kunz et al., 1985) and on field observations, our group has been motivated to better understand the possible effects of controlled energy feeding during the transition period. The strategy developed was to formulate and feed diets with relatively low energy density (1.30 to 1.38 Mcal of NE_L/kg of DM) during the entire dry period. The incorporation of low-energy ingredients (straw or low-quality grass hays) allows cows to consume feed for ad libitum intake without exceeding their daily energy requirements (Janovick and Drackley, 2010). Benefits of feeding controlled-energy (**CE**) diets prepartum to dairy cows have been reported (Beever, 2006; Dann et al., 2006; Douglas et al., 2006; Janovick and Drackley, 2010). Recently, Janovick et al. (2011) suggested that cows fed CE diets during the dry period had fewer diseases and disorders than cows fed high-energy (**HE**) diets (>100% of NE_L requirement). Feeding HE diets demonstrated that cows can overconsume energy relative to their energy requirement, independent of diet adjustments (Dann et al., 2006; Janovick and Drackley, 2010).

Also, Beever (2006) and Colman et al. (2011) stated that farmers have repeatedly observed easier calving and greater DMI around parturition when energy intake

is controlled prepartum. Excess energy consumption prepartum also seems to result in a larger decrease in DMI prepartum compared with cows having CE diets prepartum (Janovick et al., 2011). Such steep changes in DMI prepartum have been associated with increased deposition of lipid in liver postpartum (Drackley et al., 2005). Restricted DMI prepartum was associated with a greater rate of increase and higher DMI postpartum (Douglas et al., 2006; Janovick and Drackley, 2010). Nevertheless, the effect of energy intake prepartum on reproductive performance in dairy cows is still to be assessed. Previous experiments did not have the statistical power necessary to explore such relationships.

The objectives of this study were to examine the associations between feeding CE or HE diets during the dry period and the interval to pregnancy in dairy cows. The effects of prepartum dietary energy regimen on BCS, NE_L intake (**NE_LI**), concentrations of glucose, insulin, and NEFA in blood, and concentrations of total lipids, triglyceride (**TG**), and glycogen also were determined. Our hypothesis was that cows fed the CE regimen would have more favorable metabolic health in the transition period and, consequently, shorter DTP. Data from several similar experiments were pooled to investigate these associations.

MATERIALS AND METHODS

Database Construction and Data Collection

The database was developed from 7 experiments completed at the University of Illinois (Urbana) from 1993 to 2010 (Table 1). Individual cow experimental data were obtained from Microsoft Excel (Microsoft Corp., Redmond, WA) files from each experiment. Individual cow data for management, health, and reproduction were obtained from PCDART herd management software (Dairy Records Management Services, Raleigh, NC) or individual cow record cards. A total of 408 cows (354 multiparous and 54 primiparous) were included in the analyses.

Prepartum treatments were defined as follows: (1) HE, where cows were allowed ad libitum access to moderate-energy diets that would allow cows to exceed NRC requirements (NRC, 2001) for NE_L, and (2) CE, where cows either were fed restricted amounts of moderate-energy diets to target NE_L intakes of 80% of NRC requirements or were allowed ad libitum access to high-fiber, low-energy diets to limit NE_L intake to approximately 100% of NRC requirements.

Parity was dichotomized as cows starting first or second lactation in one group (**LAG1**) and cows in the third-or-greater lactation in a second group (**LAG2**). In addition, calving season (winter: December to

Table 1. Experiments from which data were used in a pooled analysis to examine the association of dietary energy density in the dry period with reproductive performance in the following lactation

Experiment	Description ¹	Reference
1	59 ² multiparous Holstein cows receiving a control diet, moderately high-energy diet with nonfiber carbohydrates, and isocaloric, fat-supplemented, low-NFC diet prepartum; FO period from -60 d to -14 d before calving; CU period from -14 d to calving	Douglas et al. (2006)
2	34 ² multiparous Holstein cows receiving restricted (80% of NE _L requirement) or ad libitum dietary treatments prepartum; FO period from -60 d to -21 d before calving; CU from -21 d to calving	Dann et al. (2005)
3	82 ² multiparous Holstein cows receiving restricted (80% of NE _L requirement), control (100% of NE _L requirement) or ad libitum (150% of NE _L requirement) dietary treatments prepartum; FO period from dry-off to -25 d before calving; CU period from -25 d to calving	Dann et al. (2006)
4	29 ² multiparous Holstein cows receiving high-forage, high forage plus fat, or high-grain diets prepartum; FO period from -60 d to -7 d before calving; CU period from -7 d to calving	Grum et al. (1996)
5	52 ² multiparous Holstein cows receiving 1 of 4 amounts of supplemental carnitine from -25 d prepartum to 56 DIM; FO period from dry-off to -25 d before calving; CU period from -25 d to calving	Carlson et al. (2007)
6	48 ² (25 multiparous and 23 primiparous) Holstein cows receiving restricted (80% of NE _L requirement), control (100% of NE _L requirement) or ad libitum (150% of NE _L requirement) dietary treatments prepartum; FO period from -65 d to -21 d before calving; CU period from -21 d to calving	Janovick and Drackley (2010)
7	104 ² (73 multiparous and 31 primiparous) Holstein cows receiving controlled-energy high-fiber diet (CEHF, 1.34 Mcal of NE _L /kg of DM ad libitum), overfed diet (OVRFD, 1.61 Mcal of NE _L /kg of DM ad libitum), or 2-stage treatment (2-stage, CEHF from dry-off until 21 d prepartum, followed by OVRFD until parturition); FO period from -60 d to -21 d before calving; CU period from -21 d to calving	Richards (2011)

¹FO = far-off; CU = close-up.²The total number of animals in each experiment.

February, spring: March to May, summer: June to August, and autumn: September to November), and twin births, dystocia, and clinical disease occurrence (**DISE**) were included in the database. Diseases and disorders included were retained placenta, ketosis, uterine prolapse, digestive problems, displaced abomasum, ovarian cyst, and metritis. Diseases and disorders were diagnosed by the research staff and herd veterinarian. If cows had more than 1 DISE, they were classified as having multiple diseases (**MDISE**). At least 1 occurrence of retained placenta, metritis, or cystic ovary was grouped as the explanatory variable reproductive pathology (**RPAT**).

Body condition score was assigned independently by more than 1 individual weekly using a 5-point scale (Ferguson et al., 1994) and the median score was used for each cow. Weekly BCS was used either as a continuous variable or was classified as thin (BCS ≤ 2.75), moderate (BCS ≥ 3 but ≤ 3.75), or fat (BCS > 3.75).

Dry matter intake was recorded daily for individual cows and weekly means by cow were used in the database. Dietary NE_L density varied from 1.21 to 1.73 Mcal/kg of DM across experiments. The NE_{LI} was calculated from the average DMI and the respective dietary NE_L density. The close-up (**CU**) period (Table 1) included a negative DCAD diet that was fed during the last 3 wk prepartum in some treatments in all experiments, except that of Grum et al. (1996).

Blood was sampled at different times prepartum and postpartum among experiments. Weekly means were established for the blood metabolites and its sample size is indicated further in the respective analysis. The blood metabolites analyzed were NEFA (Johnson and Peters, 1993) in all experiments; glucose (Trinder, 1969) by kit no. 315 (Sigma, St. Louis, MO) in experiments 1 and 4 and by glucose/HK kit (Roche Diagnostics Corp., Indianapolis, IN) using the glucose-6-phosphate dehydrogenase reaction (Peterson and Young, 1968) in experiments 2, 3, 5, 6, and 7; and insulin by RIA (Coat-a-Count insulin kit; Diagnostic Products Inc., Los Angeles, CA) as modified by Studer et al. (1993) in experiments 1, 2, 3, 4, 6, and 7. When of interest, the NEFA concentration was dichotomized as high or low using a cut-off value of 700 $\mu\text{Eq/L}$ during wk 1 as defined by Ospina et al. (2010) and glucose concentration was categorized at the median concentration values of 60 mg/dL at wk 3 and 65 mg/dL at wk 4 as high (**HG**) or low (**LG**). Even though dichotomization can reduce total information, this strategy allowed us to explore the nonlinear association of plasma glucose concentrations throughout lactation that have reduced variation in dairy cows (Herbein et al., 1985).

Puncture biopsy of liver was performed under local anesthesia to obtain approximately 3 to 5 g of liver tissue (Hughes, 1962; Drackley et al., 1991; Douglas et al., 2004) at d -65 (experiments 1, 2, 3, and 6),

−30 (experiments 2 and 3), −21 (experiments 1, 4, and 5), and −14 (experiments 2, 3, 6, and 7) relative to expected parturition, and at d 1 (experiment 7), 10 (experiments 5 and 7), 14 (experiments 2, 3, and 6), 21 (experiments 1 and 4), 28 (experiments 2, 3, 5, and 6), 42 (experiments 2, 6, and 7), 49 (experiment 3), 60 (experiment 4), and 65 (experiment 1) after calving. Tissue was frozen immediately in liquid N₂ until analysis for contents of total lipid (Drackley et al., 1991) and glycogen (**GLY**; Lo et al., 1970). Total lipids were extracted from liver tissue by using a hexane and isopropanol (3:2) mixture as described by Hara and Radin (1978). Triglyceride was analyzed in the lipid fraction according to established methods (Fletcher, 1968; Foster and Dunn, 1973). Total lipids and TG were kept as continuous variables after calving due to lack of a considerable range in the values among cows. The ratio between liver TG and GLY concentrations was named TG:GLY.

Animals and Housing

All experimental procedures were conducted according to protocols approved by the University of Illinois Institutional Animal Care and Use Committee. Before calving, cows were housed either in freestalls with individual Calan feed gates (American Calan Inc., Northwood, NH) or in tie-stalls. Approximately 2 d before expected parturition, cows were moved to individual maternity pens until parturition. After parturition, cows were returned to a tie-stall. When housed in tie-stalls, cows were allowed to exercise daily for 2 to 3 h in an outside lot. Cows were milked twice daily. During lactation, all cows received the same diet within experiment (except experiment 5, in which cows were fed different amounts of supplemental carnitine until 56 d postpartum; Table 1). Reproductive management consisted of a voluntary waiting period of 40 d until first insemination in experiments 1 and 4, synchronized based on PGF_{2α} injections. Experiments 2, 3, 5, 6, and 7 had a voluntary waiting period of 60 d. First service was synchronized based on the Presynch protocol (2 doses of PGF_{2α} 14 d apart; Moreira et al., 2001) and Ovsynch protocol (1 dose of GnRH, 7 d later 1 dose of PGF_{2α}, and 2 d later another dose of GnRH; Pursley et al., 1995) and subjected to a timed AI. All cows were bred through AI. Reproductive management remained constant within experiment.

Statistical Analysis

A final data set including all the variables was constructed in SAS (version 9.2; SAS Institute Inc., Cary, NC). Statistical analyses were performed using

the PROC GLIMMIX, MIXED, and PHREG of SAS, considering cow as the experimental unit.

The relationship between prepartum dietary energy content (NE_L) and the interval from calving to first AI was explored as a possible confounding factor for treatment effects. The first outcome of interest was the relationship between prepartum NE_L and the DTP. The secondary outcome of interest was the relationship between prepartum dietary regimen and variables reflecting the physiological status of the animal (e.g., blood metabolites, liver composition, and DISE). The statistical analysis was performed in a hypothesis-driven segmented scheme.

First, a Cox proportional hazard model (PROC PHREG) was used to assess DTP in a survival analysis where experiments were treated as strata to adjust for the random effect of experiment (Gröhn et al., 1998; St-Pierre, 2001; Allison, 2010). Treatment effects [i.e., CE and HE diets during far-off (**FO**) or CU periods] were forced into the model and the interaction of dietary energy with time period was included when statistically significant ($P < 0.1$). When statistically significant, the covariates parity, RPAT, and calving season were included in the model. A manual backward stepwise elimination of these variables and their interactions with diet treatments was used when $P \leq 0.05$. A final model was built for the outcome of interest (DTP) and each predictor of interest and week. Parity, RPAT, and calving season were retained as covariates in separate models for the predictor variables glucose, insulin, liver total lipid, and liver TG concentration. The covariates RPAT and calving season remained in the models for the predictor variables liver total lipid concentration in wk −3 and TG concentration in wk −3. The covariate RPAT was used in the models for the predictor variables liver lipid concentration in wk 4, TG concentration in wk 2 and 4, and TG:GLY throughout. The model considered reproductive data from 10 to 400 d postpartum. The assumption of the proportionality of hazard of the model was assessed graphically by plotting the logarithm of the hazard function by the logarithm of time. Residuals were evaluated for homogeneous distribution.

Second, once associations were established between prepartum dietary treatments and DTP, a linear mixed model (PROC MIXED) was constructed to explore associations between FO and CU feed regimens and BCS, DMI, and plasma NEFA, insulin, and glucose concentrations, and liver total lipid, TG, and glycogen concentrations, and liver TG:GLY. Treatment variables were forced into the model. The covariates parity and calving season, as well as their interactions with treatment, were left in the model when $P \leq 0.05$. Parity was used as a covariate in separate models for the predictor variables NEFA (wk −1 and 1), glucose,

Table 2. Dry period energy intakes and diet particle size distribution from 7 experiments on prepartum dietary energy density and intake from a total of 408 pregnant Holstein cows¹

Item ²	NE _{LI} ³ (Mcal/d; mean $\pm$ SD)	PSSB (% $\pm$ SD) ⁴		
		>19 mm	8–19 mm	<8 mm
FO				
HE	20.2 $\pm$ 6.8			
CE	12.1 $\pm$ 4.2			
CU				
HE	19.8 $\pm$ 6.8	6.44 $\pm$ 1.3	43.08 $\pm$ 1.3	50.48 $\pm$ 3.1
CE	12.1 $\pm$ 4.7	21.8 $\pm$ 2.6	35.2 $\pm$ 1.7	43 $\pm$ 1.4

¹Individual cow DMI was measured daily and diet energy and particle size distribution were measured weekly.

²FO = far-off period; CU = close-up period; HE = high-energy diet; CE = controlled-energy diet.

³Net energy for lactation intake (NE_{LI}; diet NE_L $\times$ DMI) based on the median for each value during the FO and CU segments of the dry period.

⁴Mean and SD of distribution of TMR particle sizes from samples taken weekly, assessed with the Penn State Particle Separator (PSSB; Pennsylvania State University, University Park) from experiments 3, 6, and 7 (see Table 1).

insulin (wk 1 and 2), total lipid (wk 1 and 2), and TG (wk 1 and 2) concentration. Experiment was considered a random effect (St-Pierre, 2001). Degrees of freedom were estimated by using the Kenward-Roger method (Littell et al., 2002) in the model statement. Residual distribution was evaluated for normality and homoscedasticity and variables were transformed if necessary. A logarithmic transformation was used for the variables NEFA, insulin, total lipid, TG, and GLY concentration for better homogeneity of the distribution of residuals. Means shown in tables for these variables are back transformed.

Third, the metabolite and liver variables found to be associated with prepartum dietary treatments in the mixed models were further investigated as predictors of DTP in Cox proportional hazard models as described above.

Finally, multivariable logistic mixed models (PROC GLIMMIX) considering the binary outcome variables twins, dystocia, DISE, and MDISE were constructed. Prepartum dietary treatments were forced into the models. Experiment was considered as a random effect (St-Pierre, 2001). Parity and calving season were included as covariates in the model when $P \leq 0.05$. Parity and calving season were retained as covariates for DISE, MDISE, and twins; only calving season was used for dystocia.

RESULTS

A summary of the prepartum CE and HE dietary treatments fed during the FO and CU periods is shown in Table 2. For all 408 cows across the 7 experiments, mean ($\pm$ SD) values of milk production, 3.5% FCM, and BW at wk 4 postpartum were 34.9 ± 7.6 , 35.9 ± 9.0 , and 607 ± 75 kg, respectively. The Cox proportional hazard model stratified by experiment revealed no sig-

nificant difference in days to first AI between cows ($n = 332$) fed HE compared with CE diets during the FO period (median = 88 and 95 d, respectively; hazard ratio (HR) = 0.959; 95% CI = 0.6 to 1.4; $P = 0.84$) or cows ($n = 332$) fed HE compared with CE diets during the CU period (median = 95 and 86 d, respectively; HR = 1.075; 95% CI = 0.7 to 1.6; $P = 0.72$).

The outcome of interest (DTP) analyzed with the Cox proportional hazard model (Table 3) stratified by experiment revealed no significant difference between cows ($n = 332$) fed HE compared with CE diets during the FO period (median = 164 and 165 d, respectively; HR = 1.229; 95% CI = 0.8 to 1.8; $P = 0.29$). In contrast, cows fed HE rather than CE diets during the CU period had a significantly longer interval to pregnancy (median = 167 and 157 d, respectively; HR = 0.696; 95% CI = 0.5 to 0.9; $P = 0.04$).

Cows ($n = 296$) fed HE diets during the CU period lost more BCS during the first 6 wk postpartum ($P = 0.04$) than cows fed CE diets (Figure 1). Interactions between parity and treatment were not significant. Parity had a significant effect; cows in LAG2 lost more BCS ($P = 0.04$) than cows in LAG1.

Cows classified as thin ($n = 43$) versus those that were fat ($n = 26$) at wk -4 prepartum had a longer median DTP (207 and 116 d, respectively; HR = 0.509; 95% CI = 0.3 to 0.9; $P = 0.02$). At wk 1 postpartum, cows classified as thin ($n = 154$) had a longer median DTP than cows ($n = 160$) in moderate BCS (170 and 148 d, respectively; HR = 0.760; 95% CI = 0.5 to 0.9; $P = 0.05$); no cows were classified as fat at this time.

Treatment CE versus HE diet during the CU period tended ($P = 0.10$) to have a positive effect on DMI in the first 4 wk postpartum (LSM $\pm$ SEM = 16.5 ± 0.98 and 15.4 ± 0.93 kg for CE and HE diets, respectively), with calving season explaining part of the variability in the model ($P < 0.01$). Cows calving during the summer

Table 3. Final Cox proportional hazard model of the associations of dietary energy intake in the dry period with time from calving to pregnancy in 332 Holstein cows in 7 studies, accounting for experiment as a cluster effect

Variable ¹	Level	Coefficient	SEM	Hazard ratio	95% CI	P-value
FO	HE ²	0.2061	0.19	1.229	0.84–1.80	0.29
CU	HE	−0.3626	0.18	0.696	0.49–0.99	0.04
Season of calving ³						
Spring		0.1414	0.28	1.152	0.66–1.99	0.61
Summer		0.6807	0.26	1.975	1.18–3.31	<0.01
Autumn		0.5582	0.28	1.748	1.01–3.02	0.04
RPAT		−0.4411	0.16	0.643	0.47–0.88	<0.01

¹FO = far-off period; CU = close-up period; RPAT = reproductive pathology (at least 1 of metritis, ovarian cyst, or retained placenta), relative to cows without any of these conditions.

²HE = high-energy diet compared with controlled-energy (CE) diet (see Table 2).

³Winter is the referent.

had lower DMI in the first 4 wk postpartum than cows calving in the other seasons of the year ($P < 0.01$). Interactions between calving season and treatment were not significant. Additionally, CE diet during the FO period had a positive association ($P = 0.01$) with mean NE_{LI} in the first 4 wk postpartum compared with HE diet during the FO period (Figure 2).

Dry period dietary energy regimen was not associated with a greater probability of cows having twins or

dystocia ($P > 0.4$). Not surprisingly, cows with twins ($n = 36$), compared with single-calving cows, had lower HR for DTP ($n = 253$; median = 229 and 174 d, respectively; HR = 0.650; 95% CI = 0.4 to 1, $P = 0.06$). Days to pregnancy was increased in cows experiencing dystocia ($n = 41$) compared with non-dystocia cows ($n = 163$; median = 217 and 170 d, respectively; HR = 0.662; 95% CI = 0.4 to 1; $P = 0.07$). Dietary NE_{LI} was not associated with DISE ($P > 0.8$) or MDISE ($P >$

Figure 1. Least squares means and SE for BCS (1 to 5 scale) loss from wk 1 to wk 6 postpartum for cows receiving different dietary treatments prepartum. CU = close-up period; FO = far-off period; HE = high-energy diet; CE = controlled-energy diet (see Table 2). * $P = 0.04$.

Figure 2. Least squares means and SE for NE_L intake (Mcal/d) during the first 4 wk postpartum for cows fed different dietary treatments prepartum. CU = close-up period; FO = far-off period; HE = high-energy diet; CE = controlled-energy diet (see Table 2). ** $P = 0.01$.

0.2). As expected, DISE and MDISE were associated with lower HR for DTP ($P = 0.01$ and $P = 0.02$). An interaction between DISE and MDISE with parity was present ($P < 0.05$), as cows in LAG2 were more likely to have disease ($P < 0.01$).

Cows fed CE diets during the FO period had lower NEFA concentrations during wk 1, 2, and 3 ($P < 0.01$). However, cows fed CE diets during the CU period had higher ($P < 0.01$) NEFA concentrations at wk -2 and -1 before calving (Table 4). At wk -1 and wk 1 postpartum, cows in LAG2 had higher concentrations of NEFA than those in LAG1, but no interactions ($P > 0.1$) were observed of prepartum diet with parity. Higher NEFA concentrations at wk 1 were associated with a greater probability of DISE ($n = 251$; odds ratio = 1.176; 95% CI = 1 to 1.5; $P < 0.03$).

Cows fed HE diets during the CU period had greater insulin concentrations at wk -2 and -1 ($P < 0.01$) and wk 1 and 2 after calving ($P < 0.05$) compared with cows receiving CE diets during the same period (Table 4). In contrast, cows fed CE diets during the FO period had higher insulin concentrations at wk 1 and 2 ($P < 0.01$) than did HE. Parity remained in the model ($P = 0.07$), showing that cows in LAG1 had higher insulin

concentrations than those in LAG2. Higher concentrations of insulin at wk 2 after calving were associated with longer DTP ($P = 0.01$; Table 5).

Cows fed HE diets during the CU period had higher glucose concentrations at wk -1 and -2 than cows fed CE diets ($P < 0.01$). Cows fed CE diets during the FO period had higher glucose concentration when compared with those fed HE diets at wk 1 ($P = 0.07$) and wk 3 ($P = 0.02$) after calving (Table 4). Both prepartum and postpartum, LAG1 cows had higher blood glucose concentrations than LAG2 cows ($P < 0.05$). Higher glucose versus LG in wk 3 (HG $n = 154$; LG $n = 206$) or 4 (HG $n = 71$; LG $n = 254$) after calving was associated with a shorter interval to pregnancy (wk 3: median = 151 and 171 d, respectively; HR = 1.334; 95% CI = 1 to 1.7; $P = 0.04$; wk 4: median = 148 and 167 d, respectively; HR = 1.394; 95% CI = 1 to 1.9; $P = 0.04$) (Table 5).

Liver total lipid concentrations were higher in cows on the HE regimen compared with those on the CE regimen in either segment of the dry period (Table 4). In the first 2 wk after calving, cows receiving HE diets in the FO period had greater concentrations of total lipid and TG and a greater TG:GLY in the liver com-

Table 4. Least squares means of blood metabolites and liver composition from Holstein cows fed diets with different energy densities prepartum¹

Variable	Week ²	n	FO ³			CU ⁴			
			HE ⁵	CE ⁶	<i>P</i> -value	HE	CE	SEM	<i>P</i> -value
Blood									
NEFA (μEq/L)	−2	351	251.74	224.30	0.15	181.09	311.81	1.12	<0.01
	−1	349	456.05	408.10	0.14	362.60	513.27	1.12	<0.01
	1	346	816.56	667.14	<0.01	739.96	736.20	1.17	0.93
	2	309	617.95	508.11	0.01	597.05	525.89	1.21	0.09
	3	331	345.64	271.84	<0.01	330.56	284.21	1.20	0.05
Glucose (mg/dL)	−2	349	62.35	61.15	0.22	63.15	60.35	2.37	<0.01
	−1	337	59.67	59.13	0.61	61.85	56.95	1.66	<0.01
	1	343	55.12	57.35	0.08	57.06	55.41	1.90	0.19
	3	331	58.17	60.44	0.03	59.54	59.07	2.00	0.64
Insulin (μIU/mL)	−2	312	4.43	5.15	0.15	6.92	3.30	1.25	<0.01
	−1	217	2.85	3.30	0.21	3.96	2.37	1.33	<0.01 ⁷
	1	315	2.79	4.10	<0.01	3.94	2.90	1.25	<0.01
	2	293	2.73	3.89	<0.01	3.70	2.87	1.29	0.02
Liver									
Lipids (% wet wt)	−2	180	4.47	4.29	0.20	4.24	4.52	1.02	0.04 ⁸
	1	181	6.48	5.43	<0.01	6.17	5.70	1.07	0.17
	2	195	8.22	6.51	0.01	7.63	7.00	1.12	0.32
TG (% wet wt)	−2	175	0.34	0.31	0.18	0.27	0.39	1.08	<0.01 ⁹
	1	181	1.90	1.26	0.02	1.66	1.44	1.31	0.38
	2	196	2.45	1.42	<0.01	1.93	1.80	1.20	0.70
GLY (% wet wt)	−2	159	12.91	15.52	0.38	17.04	11.77	2.74	0.06
	2	179	7.96	9.60	0.24	7.36	10.39	1.66	0.03
TG:GLY	−2	150	0.16	0.14	0.34	0.12	0.20	1.73	<0.01
	1	125	1.20	0.61	0.03	0.85	0.86	1.46	0.98
	2	176	1.45	0.82	0.02	1.16	1.02	1.58	0.56

¹The data are pooled from 7 studies on dietary energy density and intake in the dry period. Least squares means and SEM were back transformed from the natural logarithm for the variables NEFA, insulin, lipids, triglyceride (TG), glycogen (GLY), and TG:GLY.

²Weeks relative to calving.

³Far-off period.

⁴Close-up period.

⁵HE = high-energy diet (see Table 2).

⁶CE = controlled-energy diet (see Table 2).

⁷Interaction ($P = 0.07$); cows fed CE during FO and CU had lower insulin concentration than cows fed HE during FO and CU.

⁸Interaction ($P < 0.01$); cows fed CE during FO and CU had lower liver fat concentration than cows fed HE during FO and fed CE during CU.

⁹Interaction ($P = 0.04$); cows fed HE during FO and CU had lower liver TG concentration than cows fed CE during FO and CU.

pared with those receiving CE diets (Table 4). At wk 1, cows in LAG2 had higher total lipid and TG concentrations and higher TG:GLY than cows in LAG1. At wk −3 before calving, higher concentrations of total lipid and TG were associated with a longer DTP (total lipid: median = 230 and 132 d, respectively; HR = 0.468; 95% CI = 0.2 to 0.9; $P = 0.02$; TG: median = 208 and 147 d, respectively; HR = 0.485; 95% CI = 0.2 to 1.1; $P = 0.11$; Table 5). However, after calving, total lipid and TG concentrations and TG:GLY were associated with higher pregnancy rate (Table 5). Cows fed CE diets during the CU period had a greater concentration of GLY in the liver postpartum than cows fed HE diets ($P = 0.03$; Table 4).

DISCUSSION

Cows receiving CE diets in the CU period had a shorter DTP than cows that consumed energy in excess

of requirements in this period (Table 3). Use of CE diets has been a strategy investigated by our group to decrease the overconsumption of energy by formulating rations of relatively low energy density (1.30 to 1.38 Mcal of NE_L/kg of DM) that cows can consume free choice without greatly exceeding their daily energy requirements. The objective is to feed cows a bulky diet that will just meet their requirements when cows consume feed to rumen fill (Drackley et al., 2007).

An HR >1 indicated that cows fed CE diets had a higher pregnancy rate (i.e., the time after calving which 50% of the cows were pregnant was lower) than cows fed HE diets. Parr et al. (1993) found that sheep fed high dietary energy had increased metabolic clearance of progesterone from blood by the liver. Plasma progesterone concentrations were about 25% lower in heifers fed a high-energy diet compared with those fed a low-energy diet, perhaps because of greater progesterone clearance (Nolan et al., 1998). Butler (2000) observed

Table 5. Final Cox proportional hazard model of the associations of blood metabolites and liver composition with days to pregnancy (DTP) in Holstein cows in 7 studies on diet energy density and intake in the dry period, accounting for experiment as a cluster effect¹

Variable	Week ²	n	Level	Coefficient	SEM	Hazard ratio	95% CI	P-value
Blood								
Glucose (mg/dL)	3	360	H	0.2884	0.14	1.334	1.0–1.77	0.05
	4	325	H	0.3323	0.16	1.394	1.01–1.92	0.04
Insulin (μIU/mL)	2	323		−0.0453	0.02	0.956	0.92–0.99	0.01
Liver								
Total lipid (% wet wt)	−3	131	H	−0.7603	0.33	0.468	0.24–0.90	0.02
			M	−0.1560	0.22	0.855	0.55–1.33	0.49
	4	166	—	0.0891	0.03	1.093	1.03–1.16	<0.01
TG (% wet wt)	−3	131	H	−0.7244	0.45	0.485	0.20–1.18	0.11
			M	0.4504	0.36	1.569	0.77–3.17	0.21
	2	226	—	0.0645	0.02	1.067	1.01–1.12	0.01
	4	167	—	0.1300	0.03	1.139	1.06–1.22	<0.01
TG:GLY	2	206	H	0.4642	0.22	1.591	1.03–2.45	0.04
			M	0.5053	0.18	1.657	1.15–2.38	0.01
	4	167	H	0.4665	0.23	1.594	1.01–2.50	0.04
			M	−0.0232	0.21	0.977	0.63–1.50	0.92

¹Glucose classes at wk 3 were based on median values: low: <60 mg/dL (L; referent) and high: >60 mg/dL (H). Glucose classes at wk 4 were based on median values: low: <65 mg/dL (L; referent) and high: >65 mg/dL (H). Total lipid at wk −3 classes were based on tertiles: low: <4% (L; referent), moderate: 4 to 5% (M), and high: >5% (H); total lipid at wk 4 was used as a continuous variable. Triglyceride (TG) at wk −3 classes were based on tertiles: low: <0.5% (L; referent), moderate: 0.5 to 1% (M), and high: 1% (H); TG at wk 2 and 4 was used as a continuous variable. The TG:glycogen (GLY) ratio at wk 2 classes were based on tertiles: low: <0.8 (L; referent), moderate: 0.8 to 2 (M), and high: >2 (H). The TG:GLY ratio at wk 4 classes were based on tertiles: low: <0.1 (L; referent), moderate: 0.1 to 0.6 (M), and high: >0.6 (H).

²Weeks relative to calving.

increased clearance of progesterone and a carryover effect of NEB that resulted in lower plasma progesterone concentration that led to reduced fertility. Lower levels of circulating progesterone around AI (either before or after) were associated with reduced fertility (Sangsritavong et al., 2002). Unfortunately, plasma progesterone concentrations were not analyzed in the current study, but its association with prepartum dietary treatments is worth exploring.

Previous research, besides the experiments included in the current study, has found positive associations between the CE strategy and a smoother transition period. Such improvement would be reflected in better DMI postpartum (Drackley et al., 2005; Beever, 2006) and better health status of cows (Drackley et al., 2005; Drackley et al., 2007; Litherland et al., 2011). In contrast to other studies in which CE dietary management in the FO period had greater postpartum benefits than its effect in the CU period (Dann et al., 2006; Richards, 2011), in the present analysis of 7 similar studies, limitation of energy intake through CE dietary management in approximately the last 3 wk before calving, but not in the FO period, was associated with improved reproductive performance.

Successful implementation of the CE diets is critical (Drackley et al., 2007). The CE diets had longer particle size, which reflects the high-bulk strategy to reduce the dietary energy density in some experiments. In diets with NDF >50% of total DM, excessive particle

size can lead to intake depression or to sorting against longer forage particles (NRC, 2001), which could accentuate excessive energy intake in some animals and leave inadequate energy, starch, or NFC for others in a group. In the studies used here using tie-stalls or feeding gates, there was no competition for feed access.

The mechanisms that may underlie the association between CE diets during the CU period and better reproductive performance are inconclusive. In the present analysis, it was the CE diet fed during the FO period rather than the CU period that was associated with greater NE_{LI} in the first 4 wk postpartum (24.1 vs. 21.1 Mcal/d). Feeding the CE diet during the FO (but not CU) period was associated with modestly increased plasma glucose concentration at wk 3 postpartum (Table 4), at which time above-median glucose concentration was associated with increased pregnancy rate.

Perhaps the effects of preventing excessive nutrient intake during the FO period are diminished until early postpartum (first 10 DIM), when most of the metabolic changes are seen (Dann et al., 2006). Therefore, the effect of CE diets during the CU period would be responsible for achieving an effect later in lactation, when reproductive performance would be measured.

Holcomb et al. (2001) studied different amount of forages in ad libitum and restricted prepartum regimens for Holstein cows and found that restricted feeding resulted in greater DMI postpartum compared with free-choice feeding prepartum. Higher energy intake in the

first 4 wk postpartum may result in less NEB, as the relationship between milk production and NE_{LI} is alleviated in this time period when cows are able to have higher DMI compared with previous weeks (Gerloff, 2000; Hayirli et al., 2002). Time to the NEB nadir has been positively associated with time to first ovulation (Beam and Butler, 1999; Butler, 2000). A shorter delay to first ovulation may be positively related to higher conception rates (Butler, 2000). In addition, cows fed CE diets during the FO period had less BCS loss in the first 6 wk postpartum (Figure 1). Villa-Godoy et al. (1988) suggested that energy intake is the main factor reflecting the degree of body energy loss in early lactation. Butler and Smith (1989) showed that cows losing <1 BCS unit between calving and first insemination had a mean probability of pregnancy at first AI of 53% compared with 17% for cows losing >1 BCS unit (on a 1 = thin to 5 = obese scale). In Ireland, a pasture-based system with a fixed breeding calendar, it was found that BCS loss should be limited to 0.5 units to avoid detrimental effects on reproductive performance (Buckley et al., 2003).

Cows consuming HE diets had greater concentrations of NEFA in wk 1 postpartum (Table 4). Higher NEFA concentrations postpartum were associated with increased risk of cows becoming ill (Cameron et al., 1998; LeBlanc et al., 2005; Ospina et al., 2010). Not surprisingly, cows with 1 or more clinical disease occurrences had poorer reproductive success in our study, similar to research by others (Curtis et al., 1985; Halpern et al., 1985; Sheldon et al., 2006). Cows receiving CE diets during the CU period had higher NEFA concentrations than those receiving HE diets, although NEFA concentrations were not associated with pregnancy rate in the present analysis. However, cows fed CE diets could be better adapted to use NEFA as a metabolic fuel after parturition (Friggens et al., 2004; Janovick et al., 2011).

Higher concentrations of glucose at wk 3 for the group fed CE diets during the FO period, although modest, were correlated with shorter time to pregnancy (Table 4). Higher blood glucose concentration has been associated with improved fertility in Holstein cows (Plym Forshell et al., 1991). Higher blood glucose concentration may reflect greater energy intake in early lactation by cows previously fed CE diets during the FO period (Figure 2). Cows that received HE diets during the CU period had higher blood glucose concentrations prepartum, as expected from the higher energy intake regimen (Table 4).

Modestly higher insulin concentrations postpartum for cows receiving CE diets during the FO period may be associated with better glucose uptake and, again, a reflection of higher NE_{LI} postpartum. Yet, increased insulin concentration at wk 2 was associated with

slightly longer DTP. Other studies have shown that greater NEB during early lactation was associated with a lower concentration of plasma insulin in early lactation, and prolonged intervals from calving to first ovulation (Beam and Butler, 1997; Beam and Butler, 1999). Gong et al. (2002) showed that dietary induction of increased insulin concentration reduced time to first ovulation. Nevertheless, subsequent fertility parameters, including pregnancy to first service and number of services required per pregnancy, were not affected by diet; further work was suggested to determine the effects of the dietary treatment on fertility. Whether the large increase of plasma NEFA concentrations in HE-fed cows around calving was simply a consequence of greater body fat storage in the dry period or an effect of different insulin sensitivity and responsiveness in muscle and adipose tissues between cows with different BCS cannot be answered by the present work and requires further investigation.

Despite a reduced blood glucose concentration, Radcliff et al. (2006) observed that cows feed restricted during the early postpartum period did not have reduced blood insulin concentration. This profile can be compared with that of type 2 diabetes in humans, where there is no absolute insulin deficiency. Instead, impaired insulin action is thought to be the primary event. Schoenberg et al. (2012) found similar results when comparing dry cows fed at high or low energy intakes. After glucose tolerance tests and hyperinsulinemic-euglycemic clamps, cows fed below energy requirements had greater reduction in plasma NEFA concentration, greater NEFA clearance rate, and greater area under the curve during glucose tolerance tests, indicating that those cows had lower insulin resistance related to lipid metabolism. In addition, Kerestes et al. (2009) showed that severe inflammatory diseases, such as puerperal metritis, with intensive release of proinflammatory cytokines potentially depress insulin secretion and decrease whole-body insulin responsiveness in dairy cows, with long-term effects on metabolism and reproduction. Cows receiving HE diets during the CU period had lower concentrations of glucose and insulin at wk 1 compared with cows receiving CE diets (Table 4). Whether cows receiving HE diets would have greater insulin resistance during the transition period is yet to be determined.

Higher hepatic total lipid concentration at wk -3 was associated with a lower daily probability of pregnancy (Table 5). Cows receiving HE diets during the FO and CU periods versus CE diets for the same periods had higher total lipid concentrations in the liver. Drackley et al. (1991) suggested that an excess of energy consumption prepartum could later lead to increased lipid accumulation in the liver as a result of inability either

to oxidize or export the increased mobilized NEFA. The association of liver lipid accumulation and infertility has been explored before (Wensing et al., 1997). An *in vitro* approach showed that oocytes harvested between 80 and 140 d postpartum had a decreased development capacity in cows with induced hepatic lipidosis postpartum.

Interestingly, higher total lipid concentration and higher TG:GLY in liver after calving were weakly associated with better reproductive performance. One explanation could be that, in the present study, the maximum total liver lipid concentration was 12% (wet basis) during the transition period. According to Gaal et al. (1983), cows could be biochemically classified as having mild fatty liver with total lipid concentrations from 8 to 13%, and more than 13% as moderate, with the latter representing damage to liver cells. In our data, cows classified as having high values for liver lipid might reflect cows with greater intake and greater capacity to metabolize circulating fuels because the hepatic lipid infiltration was mild. Epidemiological research, using different locations (herds), with a larger variation in values for liver lipid and TG [such as the one performed by Jorritsma et al. (2000)] would be better suited to investigate the associations between reproductive success and liver lipid infiltration. In that study, the authors concluded that differences in NEB or the accumulation of TG in the liver of postpartum dairy cows affects reproductive performance.

Another factor to be considered is the fact that cows have an individual variation in liver size that can be misleading when liver biopsy is used to determine the physiological capacity of the liver (Haudum et al., 2011). Rukkwamsuk et al. (1999) was unable to show a statistical difference in time to first ovulation after calving between cows treated with high-energy diet or a control diet to meet energy requirements. However, when the data were pooled and separated by liver TG content, a positive correlation was observed between TG and days to first ovulation. The mechanism by which TG could have affected reproduction remained unclear. Cows that maintained low TG concentrations between 6 to 17 d postpartum and produced more milk had better or equal fertility results than cows with comparable levels of TG producing less milk (Jorritsma et al., 2000). Those results could suggest that individual cow variation regarding liver oxidative, storage, and export capacity has an important role in liver fat measurement as a reflection of a cow's adaptation to the onset of lactation.

Cows fed CE diets had greater hepatic glycogen concentrations at wk 2, similar to findings by Van den Top et al. (1996). In the present study, we did not find any association between glycogen and DTP. Harrison et al.

(1990) found that high-producing cows (10,814 kg, 305-d mature equivalent), in contrast to average producing cows (6,912 kg), had lower hepatic glycogen content and suggested that it was involved in regulating time to first detected estrus and to pregnancy, which both occurred much later in the high-production group. Higher liver glycogen possibly was associated with better DMI (Grum et al., 2002) and a more available source of energy for reproductive function (Harrison et al., 1990).

CONCLUSIONS

Cows that received CE diets during the last 3 wk prepartum had shorter DTP than cows that consumed HE diets in this time period, which may be attributable in part to increased NE_{LI} in the first 4 wk postpartum for cows that received CE diets in the CU period. In addition, lower BCS loss in the first 6 wk and slightly greater glucose concentrations at wk 3 may have contributed to improved reproductive performance. Energy-limited cows had lower liver TG concentrations at wk -2, which led to fewer DTP. A strategy of CE prepartum may have a favorable impact on both health and reproductive performance. Research evaluating the effect of CE diets prepartum on more specific reproduction variables, such as progesterone concentrations, ovarian function, time to first ovulation, and embryonic death, is needed.

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