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Blood immunometabolic indices and polymorphonuclear neutrophil function in peripartum dairy cows are altered by level of dietary energy prepartum[1]

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ABSTRACT

Cows experience some degree of negative energy balance and immunosuppression around parturition, making them vulnerable to metabolic and infectious diseases. The effect of prepartum feeding of diets to meet (control, 1.34 Mcal/kg of dry matter) or exceed (overfed, 1.62 Mcal/kg of dry matter) dietary energy requirements was evaluated during the entire dry period (~45 d) on blood polymorphonuclear neutrophil function, blood metabolic and inflammatory indices, and milk production in Holstein cows. By design, dry matter intake in the overfed group (n = 9) exceeded energy requirements during the prepartum period (−4 to −1 wk relative to parturition), resulting in greater energy balance when compared with the control group (n = 10). Overfed cows were in more negative energy balance during wk 1 after calving than controls. No differences were observed in dry matter intake, milk yield, and milk composition between diets. Although nonesterified fatty acid concentration pre- (0.138 mEq/L) and postpartum (0.421 mEq/L) was not different between diets, blood insulin concentration was greater in overfed cows prepartum (16.7 μIU/mL) compared with controls pre- and postpartum (~3.25 μIU/mL). Among metabolic indicators, concentrations of urea (4.63 vs. 6.38 mmol/L), creatinine (100 vs. 118 μmol/L), and triacylglycerol (4.0 vs. 8.57 mg/dL) in overfed cows were lower prepartum than controls. Glucose was greater pre- (4.24 vs. 4.00 mmol/L) and postpartum (3.49 vs. 3.30 mmol/L) compared with control cows. Among liver function indicators, the concentration of bilirubin increased by 2 to 6 fold postpartum in control and overfed cows. Phagocytosis capacity of polymorphonuclear neutrophils was lower prepartum in overfed cows (32.7% vs. 46.5%); phagocytosis in the control group remained constant postpartum (50%) but it increased at d 7 in the overfed group to levels similar to controls (48.4%). Regardless of prepartum diet, parturition was characterized by an increase in nonesterified fatty acid and liver triacylglycerol, as well as blood indices of inflammation (ceruloplasmin and haptoglobin), oxidative stress (reactive oxygen metabolites), and liver injury (glutamic oxaloacetic transaminase). Concentrations of the antioxidant and anti-inflammatory compounds vitamin A, vitamin E, and β-carotene decreased after calving. For vitamin A, the decrease was observed in overfed cows (47.3 vs. 27.5 μg/100 mL). Overall, overfeeding energy and higher energy status prepartum led to the surge of insulin and had a transient effect on metabolism postpartum.

Key words: dairy cow, inflammation, phagocytosis, dry period

INTRODUCTION

Peripartum cows experience some degree of negative energy balance (**NEB**) and immunosuppression (Goff, 2006). The latter is characterized by an impairment of neutrophil trafficking, phagocytosis, and killing capacity (Kehrli et al., 1989). Lymphocyte numbers decrease around parturition as a function of decreased proliferation. In addition, the cytokine and hormonal changes around parturition are closely related to neutrophil development and immunity-related activities, although these changes are inadequately understood (Burton et al., 2005). It is not entirely clear why most cows experience immunosuppression around the time of calving, but it is apparent that metabolic challenges associated with the onset of lactation are among the different factors capable of affecting immune function (Goff, 2006).

The NEB associated with parturition leads to extensive mobilization of FA stored in adipose tissue, therefore causing marked elevations in blood NEFA

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and BHBA concentrations (Dann et al., 2006; Goff, 2006). Prepartum dietary energy intake can potentially affect adipose tissue deposition and the amount of NEFA released into blood and available for metabolism in liver (Janovick et al., 2011). Elevated blood NEFA and BHBA as well as decreased concentrations of antigen-binding antibodies (van Knegsel et al., 2007) during peripartum NEB all can contribute to immunosuppression (Goff, 2006). Ketone body concentrations similar to those observed around parturition impair the phagocytic and bactericidal capacity of polymorphonuclear neutrophils (**PMN**) in vitro (Suriyasathaporn et al., 1999). Similarly, high concentrations of NEFA negatively affect bovine PMN viability in vitro (Scalia et al., 2006).

The efficacy of current NRC (NRC, 2001) feeding practices for pregnant nonlactating cows has been called into question because increasing amounts of moderate-to-high-energy diets (i.e., those more similar to lactation diets in the content of energy) during the last 3 wk prepartum have largely failed to overcome peripartum health problems, excessive body condition loss after calving, or decreasing fertility (Beever, 2006). Current prepartum feeding practices can lead to elevated intakes of energy, which can increase fat deposition in the viscera and upon parturition lead to compromised liver metabolism (Beever, 2006; Dann et al., 2006). In addition, prepartum feeding practices can affect indices of metabolism and inflammation, which can potentially influence immature immune cells in the peripheral circulation.

During the transition period, the first week of lactation is of great importance because of a significant risk for development of mastitis infections (Hogan et al., 1989) in addition to metabolic diseases (e.g., fatty liver). Although different studies have evaluated effects of prepartum manipulation of BCS, overfeeding or underfeeding energy, and lipid supplementation on metabolism and performance (Dann et al., 2005, 2006), the present study sought to expand on the available body of knowledge accumulated regarding the concept of "feed to fill" (Dann et al., 2006; Janovick and Drackley, 2010). That approach has shown that overfeeding moderate-energy diets results in greater metabolic stress and incidence of disorders postpartum (Janovick et al., 2011).

Our general hypothesis was that overfeeding dietary energy during the dry period would be accompanied by greater blood NEFA and markers of oxidative stress, which would lead to impaired immune function early postpartum. The main objective was to evaluate the effect of level of dietary energy prepartum on peripartum PMN phagocytosis, chemotaxis, and blood indices of metabolism and inflammation during the first week after parturition.

MATERIALS AND METHODS

Animals and Diets

All procedures were conducted under protocols approved by the University of Illinois Institutional Animal Care and Use Committee (Urbana; Protocol # 06145). Forty Holstein cows entering their second or greater lactation were enrolled in the study. Eleven of the original 40 cows were removed from statistical analysis due to lameness or bacteriologically positive quarters after parturition. The average composite SCC was approximately 128,000 ± 108,000/mL during the previous lactation. Cows were assigned randomly (n = 20 per diet) to a control diet (controlled energy and high fiber), which was fed for ad libitum intake to provide approximately 100% of calculated NE_L (1.34 Mcal/kg of diet DM, control group, n = 10), or were fed a diet to provide at least 150% of calculated NE_L requirements (1.62 Mcal/kg of DM, overfed group, n = 9) during the entire 45-d dry period (NRC, 2001). Ingredient composition of the diets is in Table 1. Samples of feed ingredients and TMR were obtained weekly and analyzed for DM content to maintain desired ingredient ratios. Weekly samples of individual ingredients were frozen at −20°C and were composited monthly. Composite samples were sent to a commercial laboratory (Dairy One Cooperative Inc., Ithaca, NY) for analysis of DM, CP, NDF, ADF, Ca, P, Mg, and K contents (Table 1). Diets were fed as TMR once daily (0600 h) using an individual gate feeding system (American Calan Inc., Northwood, NH).

Cows were housed in a ventilated enclosed barn during the dry period (photoperiod 8 h light:16 h dark) and had access to sand-bedded freestalls until 5 d before expected calving date, when they were moved to an individual maternity pen bedded with straw. After parturition, cows were moved to a tie-stall barn where the photoperiod was 16 h light:8 h dark (established management strategy for the University of Illinois dairy farm) and were fed a common lactation diet (NE_L = 1.69 Mcal/kg of DM) as TMR once daily (0600 h). Cows were milked twice daily (0400 and 1600 h). Diets were mixed in a Keenan Klassik 140 mixer wagon (Richard Keenan & Co. Ltd., Borris, Ireland) equipped with knives and serrated paddles; straw in large square bales was chopped directly by the mixer without preprocessing.

Weekly BW and BCS were measured for each cow. Milk weights were recorded daily and samples were

Table 1. Ingredients and chemical composition of experimental diets

Composition	Prepartum		Lactation
	Overfed	Control	
Ingredient, % of DM			
Wheat straw	—	41.9	—
Corn silage	50.3	29.3	30.0
Alfalfa silage	18.0	10.0	15.0
Soybean meal	3.50	9.60	2.40
Ground shelled corn	14.0	3.60	—
Alfalfa hay	6.10	3.40	5.60
Magnesium sulfate	0.63	0.64	—
Magnesium oxide	0.43	0.44	0.10
Vitamin E	0.24	0.30	—
Mineral mix[1]	0.20	0.18	0.22
Magnesium chloride	0.40	0.17	0.00
Urea	—	0.17	0.13
Salt	0.24	0.15	0.13
Sodium phosphate	—	0.13	—
Vitamin A	0.01	0.01	—
Vitamin D	0.01	0.01	—
Whole cottonseed	5.03	—	5.60
Calcium carbonate	0.91	—	0.56
Corn ground	—	—	20.3
Wet brewers grain	—	—	13.0
Soybean hulls	—	—	5.55
Sodium bicarbonate	—	—	0.83
Dicalcium phosphate	—	—	0.54
Vitamin H	—	—	0.28
Chemical composition			
DM, %	50.0	51.9	60.5
NE_L, Mcal/kg of DM	1.62	1.34	1.69
CP, % DM	15.0	12.0	17.4
Acid detergent-insoluble CP, % DM	0.73	0.70	5.53
NDF, % DM	36.6	53.4	34.1
ADF, % DM	25.7	36.6	21.8
Ca, % DM	0.73	0.67	0.80
P, % DM	0.31	0.24	0.43
Mg, % DM	0.57	0.50	0.33
K, % DM	1.28	1.45	1.16
S, % DM	0.25	0.21	0.21
Na, % DM	0.09	0.07	0.29
Fe, mg/kg	339	305	203
Zn, mg/kg	80.0	66.6	65.8
Cu, mg/kg	14.6	13.0	10.9
Mn, mg/kg	70.3	72.0	67.0

[1]Mineral mix: Zn = 60 mg/kg, Cu = 15 mg/kg, Mn = 60 mg/kg, Se 0.3 mg/kg, I = 0.6 mg/kg, Fe = 50 mg/kg, and Co = 0.2 mg/kg. Rumensin (Elanco Animal Health, Greenfield, IN) 12 g/ton: 360 mg/d in the lactation diet.

obtained once weekly from consecutive a.m. and p.m. milkings. Composite milk samples were in proportion to milk yield at each sampling and preserved (800 Broad Spectrum Microtabs II; D & F Control Systems Inc., San Ramon, CA). Composite samples were analyzed for fat, protein, lactose, urea N, and SCC content using mid-infrared procedures (AOAC, 1995) at a commercial laboratory (Dairy One Cooperative Inc.).

Energy Balance Calculations and Estimates

Energy balance was calculated individually for each cow using equations described previously (NRC, 2001). Briefly, net energy intake (NE_I; Mcal/d) was determined by multiplying DMI by the calculated mean NE_L density of the diet. The NE_L value of each individual feed, provided by Dairy One was used to calculate the mean NE_L content of the diet. The NE_M was calculated as $BW^{0.75} \times 0.08$. The net energy requirement for pregnancy (NE_P; Mcal/d) was calculated as $[(0.00318 \times \text{day of gestation} - 0.0352) \times (\text{calf birth weight}/45)]/0.218$. The milk net energy requirement (NE_{MILK}; Mcal/kg) was calculated as $(0.0929 \times \text{fat\%} + 0.0563 \times \text{protein\%} + 0.0395 \times \text{lactose \%}) \times \text{milk yield}$. The equation used to calculate prepartum energy balance (EB_{PRE}; Mcal/kg) was $EB_{PRE} = NE_I - (NE_M + NE_P)$. The equation used to calculate postpartum energy balance (EB_{POST}; Mcal/d) was $EB_{POST} = NE_I - (NE_M + NE_{MILK})$.

Blood Metabolites

Blood was sampled from the coccygeal vein or artery at −14 ± 3 d and 7 d relative to parturition. Samples were collected at 1200 h into evacuated tubes (Becton Dickinson Vacutainer Systems; Becton Dickinson and Co., Franklin Lakes, NJ) containing either EDTA or lithium heparin for plasma and a clot activator for serum. After blood collection, tubes with EDTA and lithium heparin were placed on ice and tubes with clot activator were kept at 21°C until centrifugation (~30 min). Serum and plasma were obtained by centrifugation at 1,900 × g for 15 min. Aliquots of serum and plasma were frozen (−20°C) until further analysis. Measurements of NEFA and BHBA were performed using commercial kits in an autoanalyzer at the University of Illinois Veterinary Diagnostic Laboratory (Urbana). Other traits were measured in lithium heparin samples at the Istituto di Zootecnica at the Università Cattolica del Sacro Cuore in Piacenza, Italy. Glucose, albumin, cholesterol, bilirubin, creatinine, urea, and glutamic-oxalacetic transaminase were determined using kits purchased from Instrumentation Laboratory Co. (Lexington, MA; IL Test) following the procedures described previously (Bionaz et al., 2007) in a clinical autoanalyzer (ILAB 600; Instrumentation Laboratory Co.). Triacylglycerol (**TAG**) was measured using a commercial kit (LabAssay Triglyceride; Wako Chemicals Inc., Richmond, VA). Haptoglobin and ceruloplasmin were analyzed using methods described previously (Bertoni et al., 2008) adapted to the ILAB 600 conditions. Plasma vitamin A, vitamin E, and β-carotene were extracted with hexane and analyzed by reverse-phase HPLC using an Allsphere ODS-2 column (3 μm, 150 × 4.6 mm; Grace Davison Discovery Sciences, Deerfield, IL), a UV detector set at 325 nm (for vitamin A), 290 nm (for vitamin E), or 460 nm (for β-carotene), and 80:20 methanol:tetrahydrofurane as the mobile phase. Total plasma reactive oxygen metabolites were measured using the analytical method patented by Diacron International s.r.l. (Grosseto, Italy; Bertoni et al., 2008). Plasma insulin concentrations were measured by a double antibody RIA, using a kit for human insulin following the procedures from the vendor (Diagnostic Systems Laboratories Inc., Webster, TX). The detection limit of the assay was 1.3 mU/mL; the coefficients of variation averaged 7.5% within assay and 9.5% between assays.

Liver Tissue Composition

Liver was sampled via puncture biopsy (Dann et al., 2006) from cows under local anesthesia at approximately 0730 h on d −14 ± 3 and 7 relative to parturition. Liver was frozen immediately in liquid nitrogen and stored until further analysis for contents of total lipids and TAG (Dann et al., 2006).

PMN Isolation

Samples of blood (20 mL/tube) were collected at approximately 0700 h (before biopsies to avoid additional stress reflected in the immune cells due to biopsy procedures) from the coccygeal vein or artery in evacuated tubes containing EDTA for chemotaxis and sodium heparin for phagocytosis at −14 ± 3 d and 7 d relative to parturition. After blood collection, tubes were placed on ice (~30 min) until isolation (Salak et al., 1993; Moyes et al., 2009). Samples were centrifuged at 600 × g for 15 min at 4°C. The buffy coat and approximately one-fourth of red blood cells were removed and discarded. The remaining sample was poured into a 50-mL tube. Twenty milliliters of deionized water at 4°C was added to lyse red blood cells, followed by addition of 5 mL of 5× PBS at 4°C to restore an iso-osmotic environment. Samples were centrifuged at 200 × g for 10 min at 4°C. Three subsequent washings using 1× PBS at 4°C were performed with samples centrifuged at 500 × g for 3 min at 4°C. Isolated PMN were resuspended in 1 mL of 1× PBS at 4°C and kept on ice. Cells were counted using a Beckman Coulter Counter after addition of Zap-OGlobin II Lytic Reagent (catalog no. 13020; Beckman Coulter Inc., Indianapolis, IN) to lyse any remaining red blood cells. A total of 3×10^6 cells/mL of RPMI 1640 medium with 5% fetal bovine serum was used for chemotaxis and 2×10^6 cells/mL of RPMI for phagocytosis.

Chemotaxis

Chemotaxis was assessed using a method described previously (Salak et al., 1993) with modifications (Moyes et al., 2009). The assay was conducted in a 48-well Micro AP48 Chemotaxis Chamber (P48AP30; Neuro Probe Inc., Gaithersburg, MD). Thirty microliters of RPMI 1640 (without fetal bovine serum) containing 100 ng/mL of human IL-8 (I1645; Sigma, St. Louis, MO), 10^{-8} M human complement C5a (C5788; Sigma), or without chemoattractants (control) was added to each of 4 wells per sample (quadruplicate). A polyvinyl pyrolidone-free filter (5-μm pore size, 25 × 88 mm; catalog no. 416306; Neuro Probe Inc.) was mounted in each chamber. The chamber was incubated in 5% CO_2, 95% humidity at 37°C for 10 min for equilibration. Fifty microliters of 3×10^6 cells/mL was added in each chamber and incubated in quadruplicate in 5% CO_2, 95% humidity at 37°C for 1 h. The membrane was then removed using forceps. To remove nonmigrated

cells, the side of the membrane in contact with the original cell suspension was carefully dipped in PBS solution (i.e., the other surface was not allowed to get in contact with the PBS) and the cells removed by scraping against a sharp plastic surface. The removal of nonmigrating cells was repeated 3 times. After cleaning, the membrane was allowed to dry and then was fixed with Hema 3 Hematology Staining Methanol Fixative (122–929; Fisher Scientific Inc., Middletown, VA). Cell membranes were stained with Hema 3 Hematology Staining Solution I (122–937; Fisher Scientific Inc.) and then cell nuclei were stained with Hema 3 Hematology Staining Solution II (122–952; Fisher Scientific Inc.). The number of cells in each well was counted using an inverted microscope. Cell counts were corrected based on viability (see below) and background (i.e., control or cell migrated with only RPMI).

Phagocytosis

Phagocytosis was conducted in quadruplicate in 1 mL of RPMI 1640 medium following addition of a 1:10 ratio of Fluoresbrite latex Carboxy Yellow-Green 1.75-μm Microspheres (2.5%, #17687; Polysciences Inc., Warrington, PA). Samples were then incubated for 2 h in 5% CO_2, 95% humidity at 37°C. A control sample was incubated for 2 h at 4°C. After incubation, cells were rinsed twice with 1× PBS (via centrifugation at 1,000 × g for 5 min at 4°C), fixed with 150 μL of 4% paraformaldehyde (P6148; Sigma), and preserved at 4°C until reading using flow cytometry.

Cell Viability and Differential Counts

Aliquots (20 μL) of the cell suspension from each sample for chemotaxis and phagocytosis assays was used to determine viability using a Burke chamber after 2-min incubation with a solution of Trypan blue. The percentage of viable PMN was 71.7 ± 7.8; viability data were used to correct data on chemotaxis and phagocytosis. Aliquots (50 μL) of cell suspension from the samples used for the chemotaxis assay were fixed in a microscope slide to determine cell differentials. Overall, the percentage of PMN in the differential was 56.0 ± 5.5.

Statistical Analysis

A total of 8 to 10 cows from each prepartum dietary group with the most complete data set including production, blood indices, and PMN function were used for the present report. Each variable of interest was evaluated for normal distribution using the Shapiro-Wilk test (SAS Institute Inc., Cary, NC) and normalized by natural logarithm transformation when necessary before statistical analysis. The MIXED procedure of SAS (SAS Institute Inc.) was used for statistical analysis. The fixed effects included diet (control or overfed energy), time (−14 and 7 d relative to parturition), and the interaction of diet by time. The random effect was cow within diet. A repeated measures analysis using an autoregressive (1) structure was used. For items that required log-transformation before analysis, the actual log-transformed least squares means and standard error of the means are shown in tables. The back-transformed values using the original least squares means are included in parenthesis. All least squares means were compared using the PDIFF statement of SAS (SAS Institute Inc.). Significant differences were declared at $P < 0.10$.

RESULTS AND DISCUSSION

In the present study, we attempted to integrate blood PMN function with whole-animal performance, blood metabolic indicators of metabolism, inflammation, oxidative stress, and liver function in cows that were fed to meet or exceed energy requirements during the dry period. The latter previously increased blood concentrations of NEFA and BHBA (Dann et al., 2006; Janovick et al., 2011), both of which could potentially impair immune cell function (Scalia et al., 2006).

Table 2 reports the DMI, NE_L intake, BW, BCS, calf weight at parturition, milk yield and composition, and energy balance. No differences ($P = 0.17$) in BW between the 2 groups were observed at the beginning of the experimental period (Table 2), supporting (~30 kg) those observed by our group (Janovick and Drackley, 2010). We observed a trend for greater (diet, $P = 0.07$) DMI in kg/d from d −14 to the day of parturition in overfed compared with control cows. The overfed group had greater NE_L intake prepartum (diet, $P < 0.05$). During the first week after calving, NE_L intake was similar in both groups. In a similar fashion, BW was greater (diet, $P < 0.05$) during the last 2 wk prepartum and a tendency for being greater during the first wk postpartum (diet, $P = 0.07$) in the overfed group, which agrees with previous work with similar treatments (Dann et al., 2006; Janovick and Drackley, 2010). The overfed group also had greater BCS from −4 wk until calving (diet, $P < 0.05$) and a tendency for being greater during the first week postcalving (diet, $P = 0.06$). Calf birth weight did not differ between treatment groups.

Dry matter intake in the overfed group exceeded energy requirements during the prepartum period (−4 to −1 wk relative to parturition), resulting in more positive (diet $P < 0.01$) energy balance when compared

Table 2. Performance data prepartum (−28 d to calving) and early postpartum (calving to 7 d) in cows fed a control diet (n = 10; 1.34 Mcal/kg of DM) or a moderate-energy diet (overfed, n = 9; 1.62 Mcal/kg of DM) during the entire dry period

	Prepartum energy			*P*-value		
Measure	Overfed	Control	SEM[1]	Diet	Time	Diet × time
DMI						
% BW						
−28 to −14 DIM[2]	0.59 (1.82)	0.53 (1.70)	0.09	0.59	0.93	0.68
−14 to 0 DIM	2.03	1.66	0.22	0.21	0.40	0.98
−7 to 0 DIM	2.10	1.72	0.22	0.21	0.40	0.98
1 to 7 DIM	2.01	2.07	0.16	0.78	0.01	0.84
kg/d						
−28 to −14 DIM[2]	2.65 (14.2)	2.49 (12.1)	0.08	0.14	0.91	0.73
−14 to 0 DIM	15.3	11.7	1.5	0.07	0.98	0.90
−7 to 0 DIM	15.4	11.9	1.4	0.07	0.79	0.76
1 to 7 DIM[2]	2.57 (14.0)	2.63 (13.1)	0.06	0.41	0.01	0.31
NE_L intake, Mcal						
−28 to −14 DIM	24.6	16.6	2.0	0.01	0.94	0.69
−14 to 0 DIM	24.6	15.9	2.0	0.01	0.99	0.88
−7 to 0 DIM	24.3	15.9	2.0	0.01	0.91	0.90
1 to 7 DIM	24.3	22.8	1.5	0.44	0.01	0.24
BW, kg						
−6 wk	803	742	30	0.17	—	—
−4 to −2 wk	795	730	29	0.09	0.04	0.43
−2 to 0 wk	790	710	26	0.02	0.01	0.73
−1 to 0 wk	781	697	25	0.01	0.01	0.60
wk 1	709	648	25	0.07	—	—
BCS						
−4 to −2 wk	3.43	3.17	0.08	0.03	0.35	0.33
−2 to 0 wk	3.35	3.01	0.08	0.01	0.01	0.27
−1 to 0 wk	3.30	2.96	0.09	0.01	0.01	0.08
wk 1	3.01	2.72	0.11	0.06	—	—
Calf BW, kg	47	46	2	0.70	—	—
Milk yield (1 to 7 d),[2,3] kg	3.31 (27.3)	3.23 (25.3)	0.12	0.61	0.01	0.97
Fat (wk 1), %	3.60	3.95	0.25	0.32	—	—
Protein (wk 1), %	3.58	2.98	0.34	0.23	—	—
Lactose (wk 1), %	4.82	4.91	0.08	0.45	—	—
Energy balance (EB)						
Prepartum						
−4 to −1 wk						
Mcal/d	7.04	−0.04	1.52	0.01	0.46	0.55
% of requirements	146	102	10	0.01	0.47	0.59
−1 wk						
Mcal/d	5.74	0.03	1.97	0.04	—	—
% of requirements	139	102	13	0.04	—	—
Postpartum, wk 1						
Mcal/d	−9.50	−5.99	1.53	0.10	—	—
% of requirements	71.1	80.9	4.6	0.12	—	—
EB difference,[3] Mcal/d	15.2	6.07	2.10	0.01	—	—

[1]Largest standard error of the means is shown.

[2]Data were log-transformed before statistics. Back-transformed least squares means data are shown in parentheses. The standard error of the means associated with log-transformed data are log scale.

[3]Milk yield average of d 1 to 7.

with the control group (Table 2). Both groups were in NEB during wk 1 after calving and overfed cows experienced a more dramatic decrease in energy balance between the pre- and postpartum period compared with control cows ($P < 0.05$). These data confirmed a previous study from our group (Janovick and Drackley, 2010) where cows fed to meet or exceed (100% or 150% of NE_L) prepartum energy requirements experienced a decrease in energy balance from positive at wk −3 relative to parturition to negative during the first week of lactation. Cows fed diets to meet 80% of NE_L requirements throughout the dry period were able to adapt better after parturition and were in more positive energy balance than cows fed to exceed ca. 150% of NE_L requirements (Dann et al., 2006). No differences were observed in DMI and milk yield in the present study during the first week of lactation.

In this study, cows in both groups were in NEB during the first week after parturition during which intake of DM only met 71.0 and 80.9% of NE_L in overfed and control cows, respectively (Table 2). To meet the energy requirements during the period of NEB, dairy

cattle rely on mobilization of adipose TAG and muscle tissue (Drackley, 1999). Concentrations of NEFA in the blood are a consequence of hydrolyzed adipose tissue TAG and can be taken up by liver and oxidized to produce ketone bodies (BHBA). In this study, despite differences in energy balance but consistent with the comparable milk production and similar DMI (Table 2), no differences were observed in NEFA or BHBA concentrations between diets (Table 3). Furthermore, the observed concentrations of NEFA were within a normal range (Bertoni et al., 2008) and not suggestive of a ketotic state (BHBA not over 1,000 mmol/L). The blood NEFA pattern around parturition is dynamic and it is likely, based on previous data with similar dietary treatments (Dann et al., 2006; Janovick et al., 2011), that treatment differences would have been more apparent if additional times would have been sampled.

Regardless of the common indicators of lipid mobilization (NEFA and BHBA), blood insulin concentration was greater (diet × time, $P < 0.01$) prepartum in cows overfed energy but decreased sharply by wk 1 postpartum in this group (Table 3). In addition, blood glucose pre- and postpartum was greater ($P < 0.04$) in the overfed cows (Table 3). Together, the prepartum data are consistent with a greater potential for insulin-driven fat deposition. Body weight and BCS data 2 wk before parturition support the idea of greater fat deposition in the overfed group. In the latter, the treatment differences were quite noticeable from 2 wk before parturition through parturition (Table 2). Nikkhah et al. (2008) showed that overfeeding dry nonpregnant cows with a similar energy density as in the present study (i.e., NE_L 1.61 Mcal/kg of DM) for 8 wk increased the deposition of visceral fat by 70% compared with cows fed a controlled-energy/high-fiber diet similar to the present study.

Urea concentration was lower (diet × time, $P < 0.01$) in overfed than in control cows before parturi-

Table 3. Prepartum (−14 d) and early postpartum (7 d) blood and liver measures in cows fed a control diet (1.34 Mcal/kg of DM) or a moderate-energy diet (overfed; 1.62 Mcal/kg of DM) during the entire dry period

	Prepartum energy							
	Overfed		Control			*P*-value		
Measure	−14	7	−14	7	SEM[1]	Diet	Time	Diet × time
n	10	10	9	9				
Metabolism								
NEFA,[2] mEq/L	−1.96 (0.140)	−0.65 (0.521)	−1.99 (0.136)	−1.14 (0.320)	0.23	0.25	0.01	0.30
BHBA,[2] mmol/L	−0.66 (0.514)	−0.46 (0.631)	−0.59 (0.554)	−0.64 (0.528)	0.11	0.61	0.45	0.23
Glucose,[2] mmol/L	1.44 (4.24)	1.25 (3.49)	1.39 (4.00)	1.19 (3.30)	0.03	0.04	0.01	0.95
Insulin,[2] μIU/mL	2.82 (16.7)[a]	1.31 (3.70)[c]	1.37 (3.94)[b]	0.84 (2.31)[d]	0.15	0.01	0.01	0.01
Glucose:insulin[2]	−1.37 (0.25)[c]	−0.06 (0.94)[b]	0.02 (1.02)[b]	0.36 (1.43)[a]	0.14	0.01	0.01	0.01
Urea, mmol/L	4.63[b]	5.53[a]	6.38[a]	5.49[a]	0.49	0.15	0.98	0.01
Creatinine,[2] μmol/L	4.61 (100)[b]	4.66 (106)[b]	4.77 (118)[a]	4.62 (102)[b]	0.03	0.09	0.04	0.01
Triacylglycerol,[2] mg/dL	1.39 (4.00)[b]	1.53 (4.64)[b]	2.15 (8.57)[a]	3.86 (1.35)[b]	1.02	0.03	0.01	0.01
Liver function								
Bilirubin, μmol/L	0.76[b]	4.67[a]	1.71[b]	3.61[a]	0.50	0.94	0.01	0.02
Albumin, g/L	36.6	36.2	37.0	37.2	0.60	0.31	0.74	0.51
Cholesterol,[2] mmol/L	1.10 (3.02)	2.54 (0.93)	1.04 (2.83)	0.92 (2.52)	0.04	0.46	0.01	0.51
Acute-phase proteins								
Ceruloplasmin, μmol/L	2.44	3.16	2.24	2.94	0.13	0.20	0.01	0.81
Haptoglobin,[2] g/L	−1.12 (0.33)	−0.45 (0.64)	−1.41 (0.24)	−0.71 (0.49)	0.18	0.14	0.01	0.91
Oxidative stress								
ROM,[3] mg of H_2O_2/100 mL	11.3	14.1	10.5	13.3	0.6	0.25	0.01	0.90
Liver injury								
GOT,[2,4] U/L	4.30 (73.8)	4.78 (119)	4.42 (82.8)	4.83 (125)	0.07	0.29	0.01	0.66
Antioxidants–anti-inflammation								
Vitamin A, μg/100 mL	47.3[a]	27.5[b]	36.4[b]	32.4[b]	4.1	0.53	0.01	0.02
Vitamin E, μg/mL[2]	1.57 (4.78)	1.01 (2.75)	1.35 (3.88)	0.88 (2.41)	0.08	0.09	0.01	0.57
β-carotene, mg/100 mL	0.20	0.12	0.17	0.12	0.01	0.31	0.01	0.14
Liver tissue, % wet weight								
Lipid	4.52	5.98	4.21	5.21	0.42	0.23	0.01	0.55
Triacylglycerol[2]	−0.70 (0.49)	0.59 (1.80)	−0.74 (0.48)	−0.14 (0.87)	0.41	0.35	0.03	0.40

[a–d]Means within a row with different superscripts differ (diet × time $P < 0.05$).

[1]Largest standard error of the means is shown.

[2]Data were log-transformed before statistics. Back-transformed least squares means data are shown in parentheses. The standard error of the means associated with log-transformed data are log scale.

[3]Reactive oxygen metabolites.

[4]Glutamic-oxalacetic transaminase.

tion (Table 3). This might be explained by several factors, including insulin effects on skeletal muscle protein synthesis, insulin insensitivity (e.g., greater ratio of insulin to glucose), liver function, or ruminal dietary AA metabolism, and intestinal absorption. By design, prepartum intake of dietary CP (and total N) was greater in overfed than in control cows, and only a minor fraction of dietary N was apparently unavailable in the rumen (i.e., low ADICP %; Table 1). Therefore, assuming that overfed cows absorbed greater amounts of AA, the lower blood urea prepartum might have been due to greater skeletal muscle uptake and utilization. This is suggested by the hyperinsulinemia observed in overfed cows. Insulin is known to stimulate protein synthesis in skeletal muscle (Bolster et al., 2004). The blood concentration of creatinine, an index of muscle mass (Baxmann et al., 2008), during the prepartum was lower (diet × time, $P < 0.01$) in overfed compared with control cows (Table 3). The very high insulin concentration observed in overfed cows before calving might have reduced muscle protein catabolism and enhanced AA utilization (Bolster et al., 2004). The lower urea prepartum in overfed cows was likely not due to a decrease in hepatic function. This is supported by the lack of difference in blood bilirubin concentration prepartum in overfed cows compared with control cows (Table 3). An increase in bilirubinemia is a consequence of reduced liver function (Bionaz et al., 2007; Bertoni et al., 2008).

Measures of liver function (i.e., serum bilirubin, albumin, and cholesterol), acute-phase proteins (i.e., ceruloplasmin and haptoglobin), oxidative stress (i.e., reactive oxygen metabolites), and glutamic-oxalacetic transaminase were not different between groups (Table 3). Furthermore, all those measures indicated normal and satisfactory liver activity during the peripartum period (Bertoni et al., 2008). Vitamin A concentration was greater (diet × time, $P = 0.02$) prepartum in overfed than in control cows (Table 3). Vitamin A is absorbed in the intestine and stored mainly in the liver, and plays important roles in mucosal immunity and immune tolerance (Kim, 2011). Greater vitamin A concentration together with no differences in other indices of liver activity indicated that overfeeding cows did not result in impairment of liver function prepartum, at least through the first week postpartum.

Vitamin E is an important antioxidant that binds free radicals and prevents lipid peroxidation, which may have importance to the immune response (Bendich, 1993). In this study, vitamin E concentration was not greater (diet, $P = 0.09$) in the overfed cows both pre- and postcalving (Table 3), but an overall decrease (time, $P < 0.05$) in concentration occurred, regardless of diet after calving, supporting Hogan et al. (1990) and Weiss et al. (1990). Higher vitamin E concentration in blood has been associated with lower incidence of clinical mastitis during the first week of lactation (Weiss et al., 1997), and lower concentrations seem to hamper immune function (Grasso et al., 1990). Our results suggested that serum vitamin E concentration soon after calving was not related to phagocytic capacity (Table 3); for overfed cows an increase in PMN phagocytosis occurred between −14 and 7 DIM (diet × time, $P < 0.05$).

In the present study, concentration of β-carotene decreased over time ($P = 0.01$) in both groups. β-Carotene is the major precursor of vitamin A and can affect the regeneration of mucosal barriers and function of immune cells (Stephensen, 2001). In addition, it can react with oxygen, serving as an antioxidant preventing host tissue damage (Chew and Park, 2004). Our data partly support previous findings because a decrease in chemotaxis occurred in response to IL-8 (Table 4), but no decrease in chemotaxis occurred with complement C5a or phagocytosis (Table 4).

In this study, no differences in chemotaxis were observed between prepartum treatments, but a decrease (time, $P = 0.02$) in chemotaxis in response to IL-8 was observed after parturition, regardless of diet (Table 4). The transition period is accompanied by decreased immunological capacity from 2 to 3 wk before parturition (Mallard et al., 1998; Goff, 2006). After parturition, dramatic changes in patterns of leukocyte trafficking occur, including increased cell counts and an increased number of immature cells; at the same time, chemotaxis and the oxidative burst are impaired (Burvenich et al., 2003). These changes can lead to impairment of several immunological measures including chemotaxis and phagocytosis around parturition (Waller, 2000).

Phagocytosis capacity of PMN was greater (diet × time, $P < 0.01$) prepartum in the control group than in the overfed group; after parturition phagocytosis capacity in the control group remained constant, whereas in the overfed group, phagocytosis increased to levels similar to the control. In healthy cows, PMN function (e.g., phagocytosis, superoxide anion generation, and chemotaxis) declined gradually as parturition approached (Gilbert et al., 1993; Llamas Moya et al., 2008). The lowest PMN function is often reached soon after parturition and continues through 15 d postpartum (Gilbert et al., 1993), after which PMN function increases through at least 6 wk postpartum (Gilbert et al., 1993; Llamas Moya et al., 2008). The increase in phagocytosis capacity in the overfed group from −14 to 7 d might be explained by the greater overall glucose concentration. Glucose rather than AA, ketone bodies,

Table 4. Prepartum (−14 d) and early postpartum (7 d) polymorphonuclear neutrophil (PMN) chemotaxis and phagocytosis in cows fed a control diet (1.34 Mcal/kg of DM) or a moderate-energy diet (overfed; 1.62 Mcal/kg of DM) during the entire dry period

	Prepartum energy							
	Overfed		Control			*P*-value		
Measure	−14	7	−14	7	SEM[1]	Diet	Time	Diet × time
n	9	9	10	8				
Chemotaxis, cells/cm^2								
Complement C5a	23.9	65.4	27.6	71.4	36.4	0.89	0.22	0.97
IL-8	144.0	80.8	149.0	40.3	37.0	0.60	0.02	0.50
Phagocytosis, %	32.7^b	48.4^a	46.5^a	50.0^a	4.66	0.20	0.01	0.01

[a,b]Means within a row with different superscripts differ (diet × time $P < 0.05$).

[1]Largest standard error of the means is shown.

or FA were the preferred metabolic fuel for immune cells (Pithon-Curi et al., 2004) and glucose concentration was greater in overfed cows during the whole study.

It was noteworthy that at −14 d, the concentrations of glucose and insulin were greater in the overfed group, but the phagocytosis capacity was lower compared with control cows. We speculate that the high insulin concentration prepartum decreased immune function in those cows, at least in part, by increasing the length of time that immune cells must spend to complete the process of phagocytosis.

Overall, the more positive energy status prepartum and the ensuing surge of insulin had a transient effect on metabolism. Indirect evidence suggests that greater insulin concentration may have decreased muscle protein turnover and perhaps increased the circulating TAG uptake by adipose tissue before parturition. Elevated insulin concentration could have impaired phagocytic capacity of the PMN before calving. The underlying mechanisms for insulin's impairment of PMN function is not evident and warrants further studies. The effect of overfeeding dietary energy prepartum might be more noticeable in the long term as the demands for milk production and reliance on adipose mobilization continue to increase through at least the first 30 d postpartum.

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