Prepartum Intake, Postpartum Induction of Ketosis, and Periparturient Disorders Affect the Metabolic Status of Dairy Cows*

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ABSTRACT

Nutritional management during the dry period may affect susceptibility of cows to metabolic and infectious diseases during the periparturient period. Thirty-five multiparous Holstein cows were used to determine the effect of prepartum intake, postpartum induction of ketosis, and periparturient disorders on metabolic status. Cows were fed a diet from dry-off to parturition at either ad libitum intake or restricted intake [RI; 80% of calculated net energy for lactation (NE_L) requirement]. After parturition, all cows were fed a lactation diet. At 4 d in milk (DIM), cows underwent a physical examination and were classified as healthy or having at least one periparturient disorder (PD). Healthy cows were assigned to the control (n = 6) group or the ketosis induction (KI; n = 9) group. Cows with PD were assigned to the PD control (PDC; n = 17) group. Cows in the control and PDC groups were fed for ad libitum intake. Cows in the KI group were fed at 50% of their intake on 4 DIM from 5 to 14 DIM or until signs of clinical ketosis were observed; then, cows were returned to ad libitum intake. During the dry period, ad libitum cows ate more than RI cows; the difference in intake resulted in ad libitum cows that were in positive energy balance (142% of NE_L requirement) and RI cows that were in negative energy balance (85% of NE_L requirement). Prepartum intake resulted in changes in serum metabolites consistent with plane of nutrition and energy balance. Prepartum intake had no effect on postpartum intake, serum metabolites, or milk yield, but total lipid content of liver at 1 d postpartum was greater for ad libitum cows than for RI cows. The PD cows had lower intake and milk

yield during the first 4 DIM than did healthy cows. During the ketosis induction period, KI cows had lower intake, milk yield, and serum glucose concentration but higher concentrations of nonesterified fatty acids and β -hydroxybutyrate in serum as well as total lipid and triacylglycerol in liver than did control cows. Cows with PD had only modest alterations in metabolic variables in blood and liver compared with healthy cows. The negative effects of PD and KI on metabolic status and milk yield were negligible by 42 DIM, although cows with PD had lower body condition score and BW. Prepartum intake did not affect postpartum metabolic status or milk yield. Periparturient disorders and induction of ketosis negatively affected metabolic status and milk yield during the first 14 DIM.

(**Key words:** prepartum intake, ketosis, periparturient disorder, periparturient cow)

Abbreviation key: AP = alkaline phosphatase, AST = aspartate aminotransferase, GGT = gamma glutamyl transferase, KI = ketosis induction, PD = periparturient disorder, PDC = periparturient disorder control, RI = restricted intake, SDH = sorbitol dehydrogenase.

INTRODUCTION

Nutritional management during the dry period may affect susceptibility of cows to metabolic disorders and infectious diseases during the periparturient period (Grummer, 1995; Drackley, 1999). The current convention is to maximize DMI and energy intake prepartum and minimize the drop in DMI as parturition approaches (Grummer, 1995; Mashek and Grummer, 2003). Douglas (2002) challenged the convention of maximizing DMI and suggested that moderate feed restriction (allowing only 80% of NE_L requirement) during the dry period actually may result in less total lipid and triacylglycerol accumulation in the liver and higher DMI after parturition. Other researchers have evaluated feed and energy restriction resulting in a negative energy balance during the dry period and found no effect on postpartum intake (Boisclair et al., 1986), an increase in postpartum intake (Tesfa et al., 1999), no effect on milk yield (Boisclair et al., 1986), an increase

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in milk yield (Tesfa et al., 1999), no effect on blood metabolites and health (Boisclair et al., 1987), and no effect on liver total lipid (Tesfa et al., 1999) compared with cows fed at or above energy requirement.

Douglas (2002) fed diets that were either high in non-structural carbohydrates or high in fat during the entire dry period. Groups of cows consumed each diet either for ad libitum intake or in amounts restricted to provide 80% of calculated NE_L requirement. During the prepartum period, restricted-fed cows, regardless of diet, had lower concentrations of glucose and insulin and higher concentrations of NEFA in plasma. Postpartum concentrations of total lipid and triacylglycerol in liver were approximately 50% of those in cows that were fed for ad libitum DMI during the dry period. Based on research by Douglas (2002), we speculated that cows that were feed-restricted during the dry period would be more resistant to development of ketosis after parturition. As a first step in testing this hypothesis, the objective of this study was to evaluate the effects of prepartum intake, postpartum health, and postpartum induction of ketosis on the metabolic status of multiparous Holstein cows.

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. Thirty-five multiparous Holstein cows were fed a diet (Table 1) in the form of a TMR from dry-off (approximately -60 d relative to expected parturition) to parturition at either ad libitum intake ($n = 17$) or restricted intake (**RI**; $n = 18$). Intake was restricted to 80% of calculated NE_L requirement (NRC, 1989). A close-up premix (Table 1) and calcium carbonate were added to the prepartum diet beginning -21 d relative to expected parturition; the amount of this TMR offered continued to be restricted to the same amount. After parturition, all cows were fed a lactation diet (Table 1). Alfalfa hay (~2 kg of DM) was top-dressed on the lactation TMR from parturition through 14 DIM.

At 4 DIM, cows underwent a thorough physical examination (described subsequently) and were classified as healthy ($n = 15$) or having at least one periparturient disorder (**PD**; $n = 17$). Healthy cows were assigned to either the control ($n = 6$) group or to the ketosis induction (**KI**; $n = 9$) group. Cows with PD were assigned to the periparturient disorder control (**PDC**; $n = 17$) group; no cows with PD were assigned to the KI group. Three cows were excluded from the postpartum data sets for reasons unrelated to this study. Cows in the control and PDC groups were fed for ad libitum intake. Ketosis induction was by feed restriction (Bahaa et al., 1997).

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

Component	Diet		
	Far-off dry ¹	Close-up dry ²	Lactation ³
	(% of DM)		
Ingredient			
Alfalfa hay	14.30	13.12	—
Alfalfa silage	15.30	14.03	20.00
Corn silage	53.20	48.80	25.00
Cottonseed	2.00	1.83	10.00
Ground shelled corn	5.00	4.59	26.65
Soybean meal	4.50	4.13	13.50
Soy hulls	—	—	1.50
Oat hulls	4.47	4.10	—
Mineral-vitamin mix ⁴	0.20	0.18	0.25
Vitamin E ⁵	0.25	0.23	—
Sodium chloride	0.53	0.49	0.25
Magnesium oxide	0.25	0.23	0.14
Calcium carbonate	—	1.17	—
Close-up premix ⁶	—	7.10	—
Sodium bicarbonate	—	—	1.00
Dicalcium phosphate	—	—	0.45
Limestone	—	—	1.10
Sodium sulfate	—	—	0.16
Chemical ⁷			
n^8	8	8	8
CP	15.2 ± 0.4 ⁹	14.6 ± 0.4	18.7 ± 0.4
ADF	31.8 ± 0.4	29.2 ± 0.4	20.7 ± 0.4
NDF	45.6 ± 0.7	41.8 ± 0.6	29.5 ± 0.4
NE_L , ¹⁰ Mcal/kg	1.60 ± 0.04	1.47 ± 0.03	1.77 ± 0.02
Ca	0.58 ± 0.02	1.44 ± 0.02	0.99 ± 0.05
P	0.38 ± 0.01	0.38 ± 0.01	0.50 ± 0.02
Mg	0.37 ± 0.01	0.52 ± 0.01	0.32 ± 0.01
K	1.56 ± 0.06	1.46 ± 0.05	1.22 ± 0.05
Na	0.30 ± 0.01	0.27 ± 0.01	0.49 ± 0.04

¹Far-off dry period diet was fed from dry-off until -22 d before expected parturition.

²Close-up dry period diet was fed from -21 d before expected parturition.

³Alfalfa hay (~2 kg of DM) was top-dressed on the lactation diet from parturition to 14 DIM.

⁴Contained a minimum of 5% Mg, 10% S, 7.5% K, 2.0% Fe (from iron sulfate), 3.0% Zn (from zinc oxide), 3.0% Mn (from manganous oxide), 5000 mg/kg of Cu (from copper sulfate), 250 mg/kg of I (from calcium iodate), 40 mg/kg of Co (from cobalt carbonate), 150 mg/kg of Se (from sodium selenite), 2200 kIU/kg of vitamin A, 660 kIU/kg of vitamin D₃, and 7700 IU/kg of vitamin E.

⁵Contained 44,000 IU/kg.

⁶Contained 10% CP, 1% fat, 3% crude fiber, 6.3% Ca, 0.3% P, 2.5% Mg, 3.2% S, 0.43% K, 11% Cl, 6.6 mg/kg of Se, 110 kIU/kg of vitamin A, 44 kIU/kg of vitamin D₃, 1320 IU/kg of vitamin E, and 13,200 mg/kg of niacin.

⁷Calculated from monthly analyses of individual ingredients in diets.

⁸Number of samples used to determine the chemical composition of the diet.

⁹Mean ± standard error.

¹⁰Calculated by Dairy One (Ithaca, NY) using the NRC (1989) energy equations for concentrates and the Van Soest variable discount method for forages.

Beginning at 5 DIM, cows in the KI group were fed at 50% of their intake at 4 DIM until signs of clinical ketosis (anorexia, ataxia, or abnormal behavior) or until

14 DIM and then were returned to ad libitum intake and treated, if necessary. Health records were maintained for all cows.

Classification as healthy or PD was based on a physical examination at 4 DIM, and the same physical examinations were conducted daily from 4 to 14 DIM to monitor health status. The physical examination included evaluation of the following parameters: attitude (alert or depressed); behavior (normal or abnormal); gait and stance (normal or abnormal), ability to rise (normal, with difficulty, or recumbent); rectal temperature (by digital thermometer); urine ketone concentration (by dipstick test; Labstix; Bayer Corporation, Elkhart, IN); respiratory rate (by observation for 30 s); respiratory effort (normal or increased); breath sounds (by auscultation); heart rate and rhythm (by auscultation for 30 s); rumen fill (by ballottement); rumen contraction rate (by auscultation for 2 min); abdominal pings (by simultaneous auscultation and percussion); mucous membrane color and capillary refill time (assessed at vulva); odor (none or foul) of vaginal discharge; fetal membranes (present or absent); skin tent duration (on lateral neck); eyeball recession into the orbit; fecal score; subjective evidence of blood in the feces (black feces or fresh blood); withers pinch test (ventroflexion, no ventroflexion, grunting, or no grunting); palpation of peripheral lymph nodes (normal or subjectively enlarged); mammary gland size (normal, large, or small) and consistency (normal, firm, or edematous); viscosity, color, and consistency of mammary secretions; and teat lesions.

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. After parturition, cows were returned to tie stalls. Cows were milked twice daily (0300 and 1500 h).

Data Collection, Sampling Procedures, and Analytical Methods

Intake of each cow was measured daily from dry-off to 42 DIM. Samples of feed ingredients and TMR were taken weekly and analyzed for DM content (AOAC, 1995). Weekly samples of individual ingredients were frozen at -20°C and then composited monthly and analyzed for contents of DM, CP, NDF, ADF, Ca, P, Mg, and K by wet chemistry methods (Dairy One, Ithaca, NY).

Body weight and BCS (Wildman et al., 1982) were determined for each cow weekly from dry-off to 42 DIM. Body condition scores were assigned independently by the same 4 individuals at each time of scoring throughout the experiment. The scores of the 4 individuals were averaged at each time of scoring.

Milk weights were recorded daily from 1 to 42 DIM. Milk was sampled from consecutive a.m. and p.m. milkings daily from 5 to 14 DIM and weekly from 15 to 42 DIM. Consecutive a.m. and p.m. samples were composited in proportion to milk yield at each sampling and stored with preservative (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 mid-infrared procedures (AOAC, 1995) on an automated analyzer (Foss 4000; Foss North America, Eden Prairie, MN) by Dairy Lab Services Inc. (Dubuque, IA). Fatty acid composition of milk fat was determined by preparation of methyl esters of fatty acids (Kelly et al., 1998). The fatty acid methyl esters were separated on a gas chromatograph (GC-17A; Shimadzu Corporation, Kyoto, Japan) equipped with an auto sampler, a flame ionization detector, and a fused silica capillary column (SP-2380, 100 m $\times$ 0.25 mm i.d.; Supelco, Bellefonte, PA).

Liver was sampled via puncture biopsy (Hughes, 1962; Veenhuizen et al., 1991) at 0700 h on -65 d (prior to dry-off), -30 d, -15 d, 1 d, at signs of clinical ketosis or 14 d, 28 d, and 42 d relative to parturition. A portion of the liver was frozen immediately in liquid nitrogen and later analyzed for concentrations of total lipid (Hara and Radin, 1978), triacylglycerol (Fletcher, 1968; Foster and Dunn, 1973), and glycogen (Lo et al., 1970).

Blood was sampled from the coccygeal vein or artery weekly from dry-off to -14 d, daily from -14 to 14 d, and weekly from 14 to 42 d relative to parturition. Samples were collected before feeding (1000 h). Blood was collected into evacuated serum tubes (SST; Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) containing clot activator. Serum was obtained by centrifugation at $1300 \times g$. Aliquots of serum were frozen at -20°C until later analysis for concentrations of NEFA (Johnson and Peters, 1993), insulin (Coat-a-Count Insulin kit; Diagnostic Products Corporation, Los Angeles, CA) according to Studer et al. (1993), glucose (Glucose/HK kit; Roche Diagnostics Corp., Indianapolis, IN) using the hexokinase-glucose-6-phosphate dehydrogenase reaction (Peterson and Young, 1968), and BHBA (kit number 310-A; Sigma Chemical Co., St. Louis, MO) using procedures of Williamson and Mellanby (1974). Serum samples from d -14 , d 4, at signs of clinical ketosis or d 14, d 21, and d 28 relative to parturition were analyzed for enzymes indicative of liver function using an autoanalyzer. These enzymes included alkaline phosphatase (**AP**), analyzed using a kit (Alkaline Phosphatase IFCC Liquid kit; Roche Diagnostics Corp.) based on the method of Tietz et al. (1983); aspartate aminotransferase (**AST**), analyzed using a kit (AST kit; Roche Diagnostics Corp.) based on the method of Bergmeyer et al. (1986); gamma glutamyl

transferase (**GGT**), analyzed using a kit (GGT Szasz Liquid kit; Roche Diagnostics Corp.) based on the method of Persijn and van der Slik (1976); and sorbitol dehydrogenase (**SDH**), analyzed using a kit (SDH kit; Sigma Chemical Co., St. Louis, MO) based on the method of Rose and Henderson (1975).

Calculations

Energy balance was calculated (NRC, 2001) individually for each cow. 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 (Dairy One) was used to calculate the mean NE_L content of the diet. Dairy One used the Ohio State/NRC summative energy equation (NRC, 2001) 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 NRC (2001). The NE_L for forages was adjusted by the Van Soest variable discount method (Dairy One).

Net energy required for maintenance (NE_M ; Mcal/d) was calculated as $BW^{0.75} \times 0.08$. Pregnancy requirements (NE_P ; Mcal/d) were calculated as $[(0.00318 \times \text{day of gestation} - 0.0352) \times (\text{calf birth weight}/45)]/0.218$. Lactation requirements (NE_{LAC} ; Mcal/d) were calculated as $(0.0929 \times \text{fat percent} + 0.0563 \times \text{protein percent} + 0.0395 \times \text{lactose percent}) \times \text{milk yield (kg)}$. 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 a percentage of requirement.

Statistical Analyses

Three cows were not included in the postpartum period data sets because one cow had complications associated with surgery for displaced abomasum and was euthanized, one cow had an intestinal volvulus and was euthanized, and one cow was mistakenly allowed ad libitum access to feed during a portion of the KI period. Frequency of health disorders was determined, but no statistical analysis was conducted.

Data from defined portions of the prepartum period (dry-off to parturition; -7 DIM to parturition) and postpartum periods (1 to 4 DIM; 5 DIM to signs of clinical ketosis or 14 DIM; 15 to 42 DIM) were analyzed separately. This approach was used to focus on critical time points for metabolic changes in the cow. For example, changes in DMI and NEFA are most pronounced during the last few days prior to parturition (Grummer, 1995); therefore, we desired to determine responses during the period from -7 DIM to parturition separately from the entire dry period.

Data measured over time (DMI, milk yield and components, BCS, BW, energy balance, and serum components) within the period of interest were subjected to ANOVA by using the REPEATED statement in the MIXED procedure of SAS (Littell et al., 1996; SAS Inst. Inc., Cary, NC; Release 8.2). For each variable analyzed, 4 covariance structures were evaluated: compound symmetry, autoregressive order 1, autoregressive heterogeneous order 1, and unstructured covariance. The covariance structure that resulted in the Akaike's information criterion closest to zero was used (Littell et al., 1996). Data not analyzed over time (BCS change, BW change, liver composition, and serum enzymes) were subjected to ANOVA by using the MIXED procedure of SAS (Littell et al., 1996). The Kenward-Roger degrees of freedom method was used with the MIXED procedure.

Prepartum data were analyzed as a randomized design. The model contained the effects of prepartum intake (ad libitum or RI) and time, when appropriate. Cow was designated as a random effect. Postpartum data before the start of KI (1 to 4 DIM) were analyzed using MIXED procedures with a 2×2 factorial arrangement of main effects. The model contained the effects of prepartum intake (ad libitum or RI), postpartum health status (healthy or PD), and the interaction of prepartum intake and postpartum health status. Time was included in the model when appropriate. Cow was designated as a random effect. Prepartum BW and BCS data were adjusted by analysis of covariance using the dry-off value. The number of cows expected to be assigned to the healthy or PD groups within ad libitum and RI prepartum intake groups was expected to be similar. To test that assumption, an odds ratio test was conducted using the LOGISTIC procedure of SAS.

Postpartum data obtained during the KI period (5 DIM to signs of clinical ketosis or 14 DIM) and after return of all cows to ad libitum intake (15 to 42 DIM) were analyzed as an incomplete $2 \times 2 \times 2$ factorial arrangement of main effects (prepartum intake $\times$ postpartum health status $\times$ KI status) with KI randomized within healthy cows. The model contained the effects of prepartum intake (ad libitum or RI), postpartum health status (healthy or PD), KI status (control, KI, or PDC), interaction of prepartum intake and postpartum health status, and interaction of prepartum intake and KI status.

Least squares means are reported. Significance was declared at $P < 0.10$. Tukey's procedure for multiple means comparisons was used to separate treatment means for postpartum analyses after 5 DIM (control vs. KI vs. PDC).

RESULTS AND DISCUSSION

Prepartum (Dry-Off to Parturition)

At the start of the experiment, there were no differences between prepartum intake groups for lactation

Table 2. Body condition score, BW, DMI, energy balance, and serum components from dry-off to parturition for multiparous Holstein cows with different prepartum intake.

Variable	Prepartum intake (PRE)		SE	<i>P</i>		
	Ad libitum	RI ¹		PRE	Time	PRE × time
BCS ²	3.16	3.05	0.04	0.05	<0.001	0.03
BCS change ³	−0.03	−0.20	0.08	0.12	—	—
BW, ² kg	752	719	7	0.001	<0.001	0.06
BW change, ³ kg	31	−2	10	0.02	—	—
DMI, kg/d	13.31	8.16	0.42	<0.001	<0.001	<0.001
DMI, % of BW/d	1.82	1.12	0.05	<0.001	<0.001	<0.001
Energy balance, ⁴ %	142	85	4	<0.001	<0.001	<0.001
Serum						
BHBA, mg/dL	3.5	4.2	0.4	0.13	0.004	0.67
Glucose, mg/dL	61	58	1	0.01	<0.001	0.47
Insulin, μ IU/mL	8.19	4.84	0.50	<0.001	<0.001	0.16
NEFA, μ Eq/L	167	292	21	<0.001	<0.001	0.40

¹RI = Restricted intake.²Adjusted by analysis of covariance with dry-off value.³Last value before parturition minus dry-off value.⁴Percentage of NE_L requirement.

number for the upcoming lactation (mean $\pm$ SE = 2.8 ± 0.3); BW (727 ± 14 kg); BCS (3.08 ± 0.09); and previous lactation 305-d mature equivalent yields of milk ($11,243 \pm 247$ kg), fat (407 ± 10 kg), and protein (365 ± 8 kg). Cows were dry for 56 ± 1 d and were fed the far-off diet for 38 ± 1 d and the close-up diet for 19 ± 1 d.

During the dry period, ad libitum cows ate more ($P < 0.001$) than RI cows as planned (Table 2). The difference in DMI resulted in ad libitum cows that were in positive energy balance (142% of NE_L requirement) and RI cows that were in negative energy balance (85% of NE_L requirement). Concentrations of glucose and insulin in serum were higher, and serum NEFA were lower, in ad libitum cows than in RI cows (Table 2). These results are similar to those of Douglas (2002); cows that were allowed ad libitum access to feed during the dry period had higher plasma concentrations of glucose and insulin and a lower concentration of NEFA compared with restricted-fed cows. The difference in energy balance resulted in greater BW gain in ad libitum cows than in RI cows; RI cows actually lost BW during the dry period (Table 2). Boisclair et al. (1986) found that cows restricted to 70% of their energy requirement during the dry period gained less BW and lost more BCS than did cows fed to requirement. According to research on fetal development (Becker et al., 1950; Bereskin and Touchberry, 1967), BW gain from an increase in weight of fetus, fluids, fetal membranes, and uterus can be 55 to 68 kg for Holstein cows of this BW. Cows in our study probably mobilized maternal tissues to support fetal development (Bell and Ehrhardt, 2000), which is indicated in part by a loss in BCS (Table 2). Calf birth weight averaged 49.2 ± 2.2 kg and did not differ between ad libitum and RI cows ($P = 0.21$). Similarly, Douglas

(2002) and Tesfa et al. (1999) found no difference in calf birth weight from cows that received diets containing an adequate or deficient amount of energy during the dry period.

Concentrations of total lipid and triacylglycerol in liver at −30 and −15 DIM were not affected ($P > 0.38$; Table 3) by prepartum intake. Tesfa et al. (1999) observed no difference in liver total lipid at −6 and −1 wk before parturition in cows receiving 75, 100, or 125% of their metabolizable energy requirement during the dry period. Liver integrity and function at −14 d before expected parturition was not affected ($P > 0.41$) by prepartum intake, as indicated by serum activities of AST, SDH, AP, and GGT. Activities (mean $\pm$ standard error) of AST, SDH, AP, and GGT were 83 ± 7 , 17.9 ± 2.7 , 39 ± 2 , and 21 ± 1 U/L, respectively, which all were within the normal (healthy) reference range for each enzyme (Aiello, 1998). Positive correlations between the concentration of lipid in liver and serum activities of AST ($r = 0.38$) and SDH ($r = 0.47$) have been observed (Gröhn et al., 1983). We expected that there would be no effect of prepartum intake on activities of AST, SDH, AP, and GGT at −14 DIM because there was no effect of prepartum intake on concentrations of total lipid and triacylglycerol in liver at −30 and −15 DIM.

Prepartum (−7 d Before Expected Parturition to Parturition)

A treatment $\times$ time interaction occurred ($P = 0.04$) for DMI during the last 7 d of gestation (Table 4). Cows fed ad libitum decreased DMI by $\sim 31\%$, whereas RI cows decreased DMI by $\sim 7\%$. Although the ad libitum cows had a greater change in DMI, they did not reach

Table 3. Concentrations of total lipid, triacylglycerol, and glycogen in liver from multiparous Holstein cows with different prepartum intake, postpartum health status, and ketosis induction status.

Variable	Prepartum intake (PRE)			Postpartum health status (POST)			Ketosis induction status (IND)			SE	<i>P</i>				
	Ad libitum	RI ¹	SE	Healthy	PD ²	SE	Healthy		PD		PRE	POST	IND	PRE × POST	PRE × IND
							Control	KI ³	PDC ³						
Total lipid, % wet wt.															
–65 d	4.52	4.25	0.17	—	—	—	—	—	—	—	0.25	—	—	—	—
–30 d	4.23	4.44	0.17	—	—	—	—	—	—	—	0.38	—	—	—	—
–15 d	4.55	4.58	0.20	—	—	—	—	—	—	—	0.90	—	—	—	—
1 d	7.71	6.01	0.48	6.40	7.32	0.48	—	—	—	—	0.02	0.18	—	0.12	—
14 d or ketosis	8.46	7.18	0.86	9.50	6.15	0.87	5.46 ^b	13.54 ^a	6.15 ^b	1.28	0.27	0.01	<0.001	0.61	0.25
28 d	5.88	4.83	0.58	5.32	5.40	0.61	4.34	6.30	5.40	0.91	0.21	0.93	0.13	0.76	0.35
42 d	4.50	4.56	0.28	4.78	4.28	0.29	4.43	5.14	4.28	0.43	0.97	0.22	0.23	0.17	0.23
Triacylglycerol, % wet wt.															
–65 d	0.20	0.24	0.02	—	—	—	—	—	—	—	0.31	—	—	—	—
–30 d	0.28	0.25	0.05	—	—	—	—	—	—	—	0.64	—	—	—	—
–15 d	0.44	0.41	0.10	—	—	—	—	—	—	—	0.82	—	—	—	—
1 d	1.87	1.56	0.25	1.56	1.88	0.25	—	—	—	—	0.38	0.36	—	0.12	—
14 d or ketosis	2.44	2.06	0.43	3.18	1.32	0.43	1.24 ^b	5.11 ^a	1.32 ^b	0.64	0.49	0.005	<0.001	0.62	0.17
28 d	1.17	0.83	0.28	0.99	1.01	0.30	0.47	1.50	1.01	0.44	0.41	0.94	0.10	0.96	0.36
42 d	0.66	0.58	0.15	0.80	0.45	0.15	0.54	1.05	0.45	0.22	0.65	0.10	0.10	0.61	0.37
Glycogen, % wet wt.															
–65 d	3.94	3.47	0.32	—	—	—	—	—	—	—	0.31	—	—	—	—
–30 d	4.48	3.54	0.34	—	—	—	—	—	—	—	0.06	—	—	—	—
–15 d	3.86	3.33	0.35	—	—	—	—	—	—	—	0.29	—	—	—	—
1 d	0.91	1.62	0.36	1.63	0.91	0.36	—	—	—	—	0.17	0.17	—	0.87	—
14 d or ketosis	2.07	2.85	0.44	2.34	2.58	0.45	3.23	1.44	2.58	0.65	0.20	0.70	0.05	0.78	0.91
28 d	3.30	4.13	0.43	4.26	3.17	0.46	4.12	4.40	3.17	0.67	0.13	0.08	0.76	0.14	0.43
42 d	3.78	3.94	0.35	4.01	3.71	0.36	3.53	4.49	3.71	0.53	0.67	0.54	0.19	0.42	0.69

^{a,b}Subcolumn means within row and treatment category with different superscripts differ ($P < 0.05$).¹RI = Restricted intake.²PD = Periparturient disorder.³KI = Ketosis induction and PDC = periparturient disorder control.

Table 4. Dry matter intake, energy balance, and serum components from -7 to -1 d before parturition for multiparous Holstein cows with different prepartum intake.

Variable	Prepartum intake (PRE)		SE	P		
	Ad libitum	RI ¹		PRE	Time	PRE × time
DMI, kg/d	10.67	8.10	0.67	0.009	<0.001	0.04
DMI, % of BW/d	1.44	1.10	0.08	0.006	<0.001	0.04
Energy balance, ² %	103	76	6	0.004	<0.001	0.03
Serum						
BHBA, mg/dL	6.0	5.7	0.7	0.82	0.23	0.04
Glucose, mg/dL	54	54	1	0.82	0.18	0.22
Insulin, μ IU/mL	4.11	2.56	0.48	0.03	0.03	0.75
NEFA, μ Eq/L	452	520	48	0.33	<0.001	<0.001

¹RI = Restricted intake.²Percentage of NE_L requirement.

negative energy balance (~81% of NE_L requirement) until 1 d before parturition. The large decrease in DMI before parturition by ad libitum cows was consistent with previous data (Grummer, 1995; Ingvarlsen and Andersen, 2000). Interestingly, RI cows maintained DMI until 1 d before parturition.

Prepartum intake did not affect serum concentrations of glucose, BHBA, or NEFA during the last 7 d of gestation (Table 4). Serum insulin concentration (Table 4) remained lower for RI cows than for ad libitum cows during the last 7 d before parturition, but the average concentration for both groups was lower than earlier in the dry period. Serum insulin concentration decreased during the last 7 d of gestation, which is consistent with previous observations (Kunz et al., 1985; Grum et al., 1996).

Serum NEFA concentration increased during the last 7 d of gestation, as expected (Vazquez-Añón et al., 1994). Interestingly, serum NEFA concentration increased from 260 to 762 μ Eq/L (193%) for ad libitum cows and increased from 445 to 633 μ Eq/L (42%) for RI cows during the last 7 d of gestation (treatment × time interaction, $P < 0.001$). At 1 DIM, ad libitum cows had more lipid accumulation in the liver than did RI cows (Table 3). Adipose lipid mobilization leads to increased serum NEFA, increased uptake of NEFA by the liver, and increased triacylglycerol accumulation in the liver (Drackley, 1999). Dyk (1995) found that higher prepartum plasma NEFA concentrations were associated with greater incidences of dystocia, retained placenta, ketosis, displaced abomasum, and mastitis, but not hypocalcemia.

Postpartum Health

Postpartum health data should be interpreted with caution because of the small number of cows used in the study. The frequency of postpartum health disorders was not different (odds ratio = 1.6; $P = 0.49$) for

ad libitum and RI cows (Table 5). By design, cows assigned to the PD group had more health disorders than cows assigned to the healthy group (Table 5). Of the 17 PD cows, 16 had a retained placenta (failure to expel the placenta within 24 h of parturition) with some degree of metritis, and one had subclinical ketosis at the physical examination conducted at 4 DIM. Twelve PD cows had subclinical ketosis between 4 and 14 DIM based on a positive urine ketone test, but only 8 PD cows were treated for subclinical ketosis. The PD cows were treated for subclinical ketosis and mastitis more than were healthy cows, as expected.

The KI cows with subclinical ketosis (positive urine ketone test) were not treated so that clinical ketosis could develop between 4 to 14 DIM. Four of 9 KI cows showed signs of clinical ketosis and were treated with 500 mL of 50% dextrose (intravenous) and 500 mL of propylene glycol (per os). The cows showed signs of clinical ketosis at 9 DIM (2 cows), 10 DIM (one cow), and 14 DIM (one cow). Five of 9 KI cows had subclinical ketosis based on a positive urine ketone test, but did not require treatment. Three of the 4 cows that showed clinical signs of ketosis had been fed ad libitum during the dry period. One KI cow developed a displaced abomasum after the KI period.

The occurrence of retained placenta was high (50%; Table 5) in this study. The occurrence was similar between ad libitum cows (47%) and RI cows (53%). Factors that have been associated with retained placenta are age, gestation length, number of calves, species, heredity, environment, hormones, immune function, and nutrition (Julien et al., 1976; Laven and Peters, 1996; Kimura et al., 2002). However, the exact cause and mechanism are still unknown. In this study, cows with retained placenta calved 4 d before expected parturition, and cows without retained placenta calved 1 d before expected parturition. Deficiency of Se has been linked to the occurrence of retained placenta in dairy cows (Julien et al., 1976). In serum collected from cows

Table 5. Frequency¹ of health disorders and twins for cows used in the postpartum data set with different prepartum intake, (RI = restricted intake), postpartum health status (PD = periparturient disorder), and ketosis induction status (KI = ketosis induction and PDC = periparturient disorder control).

Variable	Ad libitum			RI		
	Healthy		PD	Healthy		PD
	Control	KI	PDC	Control	KI	PDC
Cows, no.	3	5	7	3	4	10
Displaced abomasum	0	0	0	0	1	1
Foot and leg problems ²	1	2	3	1	1	6
Ketosis ³						
Subclinical, ⁴ not treated	2	2	2	2	3	2
Subclinical, ³ treated ⁵	0	0	2	0	0	6
Clinical ⁶	0	3	0	0	1	0
Mastitis	0	0	3	0	0	4
Metritis	0	0	7	0	2	9
Hypocalcemia	0	0	0	0	0	0
Retained placenta	0	0	7	0	0	9
Twins	1	0	1	0	0	4

¹Number of cows with at least one incidence.

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

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

⁴Cow had at least 1 d with ≥ 5 mg of acetoacetic acid/dL of urine (Labstix; Bayer Corporation, Elkhart, IN).

⁵Propylene glycol given per os or dextrose given intravenously.

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

in this study, at -8 d before parturition, Se averaged 61.1 ± 0.7 ng/mL (Animal Health Diagnostic Laboratory, Lansing, MI), which was considered marginally low. However, there was no difference in serum Se concentration between cows with and cows without retained placenta. The reason for the high occurrence of retained placenta in this study is unknown.

Postpartum (1 to 4 DIM)

Prepartum intake did not affect ($P > 0.20$; Table 6) postpartum DMI, energy balance, milk production, or serum concentrations of BHBA, glucose, insulin, and NEFA during 1 to 4 DIM. At 1 DIM, ad libitum cows had greater total lipid concentrations in liver than did RI cows ($P = 0.02$; Table 3). However, differences in concentrations of triacylglycerol and glycogen in liver at 1 DIM did not reach statistical significance ($P > 0.17$; Table 3). Douglas (2002) found that cows that were feed-restricted during the dry period had lower concentrations of total lipid and triacylglycerol in the liver at 1 DIM than cows fed ad libitum during the dry period. In contrast, Tesfa et al. (1999) found no effect of prepartum energy intake on liver total lipid concentration at 1 or 4 wk postpartum. Increased total lipid or triacylglycerol concentrations in liver of periparturient cows have been linked to greater risk for health problems (Bobe et al., 2004); therefore, although the increased total lipid content for ad libitum cows likely was not clinically rele-

vant in itself, the ad libitum group might be more susceptible to encountering other health problems. Concentrations of triacylglycerol usually increase in parallel with those of total lipid (Grum et al., 1996); we have no explanation for the smaller increases in triacylglycerol in this study.

In our study, liver total lipid and triacylglycerol concentrations increased, and glycogen concentration decreased, from prepartum to 1 DIM (Table 3), as expected (Vazquez-Añon et al., 1994). Fatty infiltration did not cause liver damage as indicated by liver-specific enzymes (AST, SDH, AP, and GGT) measured in serum at 4 DIM. Neither prepartum intake ($P > 0.17$) nor postpartum health status ($P > 0.10$) affected activities of AP (45 ± 4 U/L) and GGT (23 ± 2 U/L). Activity of SDH was higher ($P = 0.08$) for cows fed ad libitum than for RI cows (13.3 vs. 8.8 U/L; SE = 1.8), but was similar ($P > 0.62$) between healthy and PD cows (10.8 ± 1.2 U/L). Cows that were fed ad libitum during the dry period had a higher ($P = 0.02$) activity of AST than did RI cows (134 vs. 100 U/L; SE = 10). Activity of AST also was higher ($P > 0.02$) for PD cows than for healthy cows (134 vs. 99 U/L; SE = 10). Steen et al. (1997) found that AST activity was greater in cows with ketosis (115 U/L) and hepatic lipidosis (252 U/L) than in cows that were healthy (70 U/L).

During the first 4 DIM (Table 6), PD cows had lower DMI ($P = 0.002$) and milk yield ($P = 0.01$) than did healthy cows. Deluyker et al. (1991) reported a 2.4-kg/

Table 6. Dry matter intake, energy balance, milk yield, and serum components from 1 to 4 DIM for multiparous Holstein cows with different prepartum intake and postpartum health status.

Variable	Prepartum intake (PRE)		SE	Postpartum health status (POST)		SE	P			
	Ad libitum	RI ¹		Healthy	PD ²		PRE	POST	PRE × POST	Time
DMI, kg/d	11.39	12.61	0.75	13.71	10.28	0.74	0.25	0.002	0.47	<0.001
DMI, % of BW/d	1.72	1.91	0.10	2.06	1.57	0.10	0.20	0.002	0.82	0.005
Energy balance, ³ %	78	90	7	89	80	7	0.24	0.38	0.78	0.13
Milk, kg/d	18.37	19.20	1.47	21.44	16.14	1.41	0.68	0.01*	0.22	<0.001
Serum										
BHBA, mg/dL	8.9	9.1	0.9	7.9	10.1	0.9	0.83	0.08	0.28†	0.19
Glucose, mg/dL	52.9	52.3	1.8	51.6	53.5	1.8	0.82	0.45	0.87	0.59
Insulin, μ IU/mL	2.76	2.52	0.34	3.22	2.07	0.34	0.61	0.02	0.75	0.008
NEFA, μ Eq/L	1118	1091	104	1097	1112	104	0.85	0.92	0.56	0.004

¹RI = Restricted intake.²PD = Periparturient disorder.³Percentage of NE_L requirement.*Treatment × time was significant ($P < 0.05$).†Treatment × time was significant ($P < 0.10$).

d decrease in milk yield during the first 5 DIM for cows with a retained placenta. Energy balance and concentrations of serum NEFA and glucose did not differ ($P > 0.38$) between healthy and PD cows, but serum BHBA was higher ($P = 0.08$) for PD cows than for healthy cows.

Postpartum (5 DIM to Signs of Clinical Ketosis or 14 DIM)

Prepartum intake had no residual effect on variables during the KI period, except that RI cows had lower contents of fat and protein in milk than did ad libitum cows. Douglas (2002) also observed lower milk fat percentage during the immediate postpartum period in cows that were feed-restricted while dry.

During the KI period, KI cows had an energy balance of 53% of NE_L requirement because of the imposed feed restriction (Table 7). In adaptation to this severe negative energy balance, KI cows decreased milk production from ~30 kg at 5 DIM to ~25 kg at 14 DIM. During this same time period, the control and PDC cows increased milk production by ~9 kg. The KI cows mobilized adipose lipid reserves to support the severe negative energy balance and had elevated concentrations of BHBA and NEFA in serum (nearly twice those of control and PDC cows) and had lower concentrations of glucose and insulin. Because of the large flux in serum NEFA, liver total lipid and triacylglycerol concentrations were greater ($P < 0.001$; Table 3) in KI cows than in control and PDC cows. However, concentrations of total lipid and triacylglycerol in liver remained lower than those likely to be clinically relevant (Bobe et al., 2004). The concentration of BHBA in urine collected at signs of clinical ketosis or 14 DIM was higher ($P = 0.008$) in KI

cows (92.1 mg/dL) than in control (2.7 mg/dL) or PDC (1.1 mg/dL) cows. Metabolic and production changes associated with KI were consistent with data from other researchers (Gröhn et al., 1983; Veenhuizen et al., 1991; Drackley et al., 1992).

An assessment of hepatobiliary status was made at signs of clinical ketosis or 14 DIM by measuring the activities of AST, SDH, AP, and GGT in serum. There was no effect of KI status ($P > 0.23$) on activities of SDH (21.3 ± 2.1 U/L), AP (36 ± 2 U/L), or GGT (23 ± 1 U/L). Activity of AST was higher ($P = 0.07$) for KI cows (134 U/L) than for control (99 U/L) or PDC cows (110 U/L). Steen et al. (1997) evaluated hepatobiliary enzymes (AST, AP, and GGT) in groups of healthy, ketotic, and hepatic lipidosis cows. In agreement with our data, activity of AST was higher in both the ketotic and hepatic lipidosis groups than in the healthy group (Steen et al., 1997). Activities of AP and GGT were higher in the hepatic lipidosis group than in the healthy and ketotic groups. In contrast, Gröhn et al. (1983) evaluated the effect of severity of ketosis on activities of AST, SDH, and GGT. They found no effect of severity of ketosis on AST and GGT activities, but the activity of SDH increased with severity of ketosis.

Milk fat percentage (Table 7) was higher for KI cows than for control and PDC cows. Cows that underwent KI had lower concentrations of short- and medium-chain fatty acids (C_{6:0}, C_{8:0}, C_{10:0}, C_{12:0}, C_{14:0}) and a higher concentration of C_{18:1cis-9} than did control and PDC cows (Table 8). Other researchers have observed similar changes in milk fatty acid profile in starved or ketotic cows (Luick and Smith, 1963; Brumby et al., 1975). Cows in negative energy balance mobilize adipose lipid reserves, and the resulting long-chain fatty acids are

Table 7. Dry matter intake, energy balance, milk yield and components, and serum components from 5 DIM to signs of clinical ketosis or 14 DIM for multiparous Holstein cows with different prepartum intake, postpartum health status, and ketosis induction status.

Variable	Prepartum intake (PRE)			Postpartum health status (POST)			Ketosis induction status (IND)			P						
	Ad libitum	RI ¹	SE	Healthy	PD ²	SE	Healthy		PD	SE	PRE	POST	IND	PRE × POST	PRE × IND	Time
							Control	KI ³								
DMI, kg/d	13.5	14.8	1.0	14.2	14.1	1.0	18.9 ^a	9.6 ^c	14.1 ^b	1.6	0.45	0.91*	<0.001*	0.44	0.86	<0.001
DMI, % of BW/d	2.16	2.35	0.13	2.18	2.33	0.13	2.90 ^{a,x}	1.45 ^{b,z}	2.33 ^{a,y}	0.20	0.32	0.41*	<0.001*	0.88	0.58	<0.001
Energy balance, ⁴ %	77	84	7	73	88	7	93 ^a	53 ^b	88 ^a	11	0.54	0.14†	0.01†	0.87	0.85	0.01
Milk																
Milk, kg/d	25.8	28.7	1.7	30.5	24.0	1.7	34.2 ^{a,x}	26.8 ^{ab,y}	24.0 ^{b,y}	2.6	0.27	0.008*	0.04*	0.50	0.99	<0.001
Fat, %	4.71	4.19	0.14	4.47	4.43	0.14	4.05 ^{b,y}	4.90 ^{a,x}	4.43 ^{ab,y}	0.21	0.006	0.80*	0.004†	0.13	0.28	<0.001
Protein, %	3.50	3.15	0.10	3.27	3.39	0.10	3.24	3.30	3.39	0.15	0.02	0.38	0.76	0.38	0.87	<0.001
Urea N, mg/dL	17.28	16.48	1.26	16.68	17.09	1.24	16.55	16.80	17.09	1.91	0.49	0.81	0.92	0.10	0.45	0.66
SCS ⁵	3.96	3.62	0.43	3.59	3.99	0.43	2.94	4.25	3.99	0.66	0.57	0.50	0.14	0.96	0.96	0.003
Serum																
BHBA, mg/dL	11.9	9.5	1.4	12.2	9.2	1.4	6.8 ^a	17.6 ^b	9.2 ^a	2.1	0.17	0.12	<0.001	0.41	0.36	0.40
Glucose, mg/dL	48	51	2	46	52	2	54 ^a	39 ^b	52 ^a	3	0.23	0.02*	<0.001*	0.53	0.18	0.08
Insulin, µIU/mL	2.66	3.23	0.35	2.88	3.02	0.35	3.68 ^x	2.07 ^y	3.02 ^{xy}	0.53	0.22	0.77	0.03	0.59	0.36	0.31
NEFA, µEq/L	1038	827	107	1139	725	108	710 ^a	1569 ^b	725 ^a	140	0.14	0.009†	<0.001*	0.43	0.72	0.09

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

^{x,y}Subcolumn means within row and treatment category with different superscripts differ ($P < 0.10$).

¹RI = Restricted intake.

²PD = Periparturient disorder.

³KI = Ketosis induction and PDC = periparturient disorder control.

⁴Percentage of NE_L requirement.

⁵SCS = Somatic cell score calculated with the equation: $\{[\log_{10}(\text{SCC}/1000)-2]/\log_{10}(2)\} + 3$.

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

†Treatment × time was significant ($P < 0.10$).

Table 8. Milk fatty acid composition from 5 DIM to signs of clinical ketosis or 14 DIM for multiparous Holstein cows with different prepartum intake, postpartum health status, and ketosis induction status.

Fatty acid, g/100 g	Prepartum intake (PRE)			Postpartum health status (POST)			Ketosis induction status (IND)				P						
	Ad libitum	RI ¹	SE				Healthy		PD	PRE	POST	IND	PRE × POST	PRE × IND	Time		
				Control	KI ³	PDC ³											
C _{4:0}	4.95	5.02	0.15	5.02	4.96	0.14	5.25	4.78	4.96	0.22	0.73	0.76*	0.12*	0.98	0.24	0.08	
C _{6:0}	1.76	1.84	0.12	1.79	1.81	0.12	2.06 ^x	1.52 ^y	1.81 ^{xy}	0.18	0.63	0.91*	0.03	0.91	0.22	<0.001	
C _{8:0}	0.77	0.79	0.08	0.76	0.80	0.08	0.93	0.60	0.80	0.13	0.80	0.77*	0.06*	0.77	0.40	<0.001	
C _{10:0}	1.40	1.42	0.18	1.35	1.47	0.18	1.69	1.01	1.47	0.28	0.89	0.61*	0.07*	0.73*	0.48	0.005	
C _{12:0}	1.47	1.49	0.17	1.41	1.55	0.18	1.74	1.08	1.55	0.27	0.88	0.55*	0.07*	0.70	0.49	0.001	
C _{14:0}	5.55	5.98	0.38	5.53	6.00	0.38	6.47 ^{a,x}	4.58 ^{b,y}	6.00 ^{ab,x}	0.58	0.40	0.37	0.02	0.73	0.48	0.003	
C _{14:1}	0.39	0.40	0.03	0.42	0.37	0.03	0.44	0.41	0.37	0.04	0.72	0.14	0.66	0.80	0.14	0.04	
C _{15:0}	0.51	0.56	0.04	0.52	0.55	0.04	0.60	0.43	0.55	0.06	0.27	0.54*	0.05*	0.52	0.96	0.004	
C _{16:0}	24.81	25.35	0.37	24.48	25.68	0.37	24.63	24.33	25.68	0.57	0.33	0.03	0.69	0.66	0.70	0.08	
C _{16:1 trans}	0.72	0.74	0.05	0.70	0.77	0.05	0.72	0.68	0.77	0.07	0.73	0.30	0.66	0.78†	0.40	0.77	
C _{16:1}	2.08	2.19	0.15	2.28	2.00	0.15	2.06	2.50	2.00	0.23	0.65	0.17*	0.14*	0.63	0.84	0.91	
C _{17:0}	0.75	0.67	0.01	0.69	0.73	0.01	0.64 ^b	0.74 ^a	0.73 ^a	0.02	0.001	0.08	0.003	0.24	0.07	0.03	
C _{17:1}	0.38	0.36	0.02	0.39	0.36	0.02	0.35	0.42	0.36	0.03	0.50	0.33*	0.15*	0.74	0.56	0.001	
C _{18:0}	13.83	13.39	0.49	13.56	13.66	0.48	13.48	13.65	13.66	0.74	0.51	0.89	0.86	0.91	0.35	0.09	
C _{18:1 trans}	2.12	2.03	0.29	1.76	2.39	0.29	1.95	1.57	2.39	0.44	0.95	0.13†	0.51	0.30	0.99	0.23	
C _{18:1 cis-9}	28.99	28.39	1.42	30.03	27.36	1.42	27.45	32.60	27.36	2.19	0.70	0.18	0.08	0.55	0.67	0.02	
C _{18:1 cis-11}	1.14	1.02	0.12	1.01	1.15	0.11	0.98	1.05	1.15	0.17	0.53	0.41	0.73	0.39	0.81	0.05	
C _{18:2n6}	3.50	3.49	0.10	3.37	3.61	0.10	3.31	3.44	3.61	0.15	0.86	0.09	0.50	0.07	0.67	0.82	
C _{18:3n3}	0.35	0.35	0.02	0.34	0.36	0.02	0.34	0.33	0.36	0.03	0.80	0.28†	0.63	0.75	0.19	0.03	
CLA ⁴ cis-9, trans-11	0.26	0.78	0.01	0.28	0.26	0.01	0.29	0.26	0.26	0.02	0.19	0.49	0.30	0.05	0.89	0.02	
Unidentified <C _{17:0}	1.07	1.13	0.07	1.09	1.11	0.07	1.23	0.95	1.11	0.10	0.49	0.80†	0.04*	0.54	0.56	<0.001	
Unidentified >C _{17:0}	3.23	3.17	0.09	3.23	3.16	0.09	3.40	3.07	3.16	0.13	0.87	0.55	0.06†	0.02	0.81	0.23	

^{a,b}Subcolumn means within row and treatment category with different superscripts differ ($P < 0.05$).^{x,y}Subcolumn means within row and treatment category with different superscripts differ ($P < 0.10$).¹RI = Restricted intake.²PD = Periparturient disorder.³KI = Ketosis induction and PDC = periparturient disorder control.⁴CLA = Conjugated linoleic acid.*Treatment × time was significant ($P < 0.05$).†Treatment × time was significant ($P < 0.10$).

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 and medium-chain fatty acids by the mammary gland (Palmquist et al., 1993).

The PDC cows had DMI that was intermediate to control and KI cows and had milk production similar to KI cows, which resulted in an energy balance of 88% of requirement that was similar to control cows. The similar energy balance translated into similar concentrations of BHBA, glucose, insulin, and NEFA in serum (Table 7) and similar concentrations of total lipid and triacylglycerol in liver (Table 3).

The efficacy of the KI protocol to induce clinical ketosis seemed to be lower for this study (4 of 9 cows) compared with an earlier study that used a similar protocol, in which 8 of 10 cows showed signs of clinical ketosis (Bahaa et al., 1997). In that study, Bahaa et al. (1997) restricted feed by 50 or 75%, but incidence or severity of ketosis did not differ between the degrees of feed restriction. Differences between studies may be a function of body condition, negative energy balance, and BW loss of cows during the periparturient period. Bahaa et al. (1997) did not report the BCS of cows used in their study. Cows in our study were not overconditioned (BCS at dry-off = 3.08) and may not have been predisposed to develop ketosis because of less severe negative energy balance and BW loss immediately after parturition. Bahaa et al. (1997) suggested an odds ratio of 1.25, which indicated that the odds of developing clinical ketosis tended to increase by 25% for each additional percentage unit loss of total body energy. Thus, a cow losing 11.9% of total body energy at 5 d postpartum had a 50% chance of developing clinical ketosis (Bahaa et al., 1997).

Drackley et al. (1992) suggested that a liver triacylglycerol to glycogen ratio >1.5 to 2 during early lactation might indicate a greater susceptibility to clinical ketosis and hepatic lipidosis. In our study, KI cows had a triacylglycerol to glycogen ratio at 1 DIM of 2.9 ± 1.3 , and there was no difference ($P = 0.40$) between cows that showed signs of clinical ketosis and those that did not during the KI period. The triacylglycerol to glycogen ratio at 1 DIM does not explain the susceptibility to clinical ketosis in this study. At signs of clinical ketosis or 14 DIM, the triacylglycerol to glycogen ratio was 23.1 and 9.4 ($P = 0.15$) for KI cows with signs of clinical ketosis and KI cows without signs of clinical ketosis, respectively.

Postpartum (15 to 42 DIM)

During the postpartum period of 15 to 42 DIM, residual effects of prepartum intake did not achieve signifi-

cance ($P > 0.23$) for any postpartum variable (Tables 3 and 9), although milk fat content tended ($P = 0.11$) to remain lower, and glucose in serum tended ($P = 0.11$) to be higher, for cows that were previously feed-restricted. Differences among KI status for DMI, energy balance, BW, milk yield, and serum metabolites were non-existent ($P > 0.18$; Table 9) during the period from 15 to 42 DIM. Body condition score was ~ 0.6 units lower in PD cows than in healthy cows ($P < 0.001$), and milk yield tended ($P = 0.12$) to remain lower for PD cows (Table 9). Liver total lipid and triacylglycerol concentrations decreased, and liver glycogen concentration increased, from 14 to 28 and 42 DIM (Table 3). Concentrations of total lipid, triacylglycerol, and glycogen in liver were not different ($P > 0.10$; Table 3) among KI status groups at 28 and 42 DIM.

An assessment of hepatobiliary status was made at 21 and 28 DIM by measuring activities of AST, SDH, AP, and GGT in serum. At 21 DIM, there were no effects of prepartum intake ($P > 0.18$), postpartum health status ($P > 0.18$), or KI status ($P > 0.12$) on activities of AST (89 ± 5 U/L), SDH (14.9 ± 2.5 U/L), AP (35 ± 2 U/L), and GGT (26 ± 1 U/L). At 28 DIM, there were no effects of prepartum intake ($P > 0.25$), postpartum health status ($P > 0.14$), or ketosis induction status ($P > 0.30$) on activities of AST (86 ± 3 U/L), SDH (23.3 ± 2.3 U/L), AP (34 ± 2 U/L), and GGT (27 ± 2 U/L).

Milk fatty acid composition during wk 3 to 6 of lactation was affected by prepartum intake (Table 10). The content of C16:0 was higher, and that of C18:0 was lower, for RI cows than for ad libitum cows. Although not statistically significant, most of the other short- and medium-chain fatty acids synthesized de novo within the mammary gland also were slightly higher for RI cows than for ad libitum cows, and most of the long-chain fatty acids transferred to milk fat from blood were slightly lower for RI cows than for ad libitum cows. The physiological significance of these small changes is unclear, but seems to indicate a diversion of long-chain fatty acids elsewhere, perhaps to restoration of body lipid pools. Milk fatty acid composition was not affected during wk 3 to 6 by previous KI status (Table 10). Stage of lactation had a significant effect on fatty acid composition (Tables 8 and 10), as expected (Palmquist et al., 1993). The concentrations of short-chain and medium-chain fatty acids increased, and the concentrations of C_{16:1} and C_{18:1cis-9} decreased as body lipid mobilization decreased.

Ketosis induction and PD had a short-term negative impact on milk yield (Tables 6 and 7), which was no longer statistically significant by 42 DIM (Table 9). In a review, Fourichon et al. (1999) summarized data from studies conducted after 1965 that provided quantitative estimates of milk losses as a result of disease. Cows

Table 9. Body condition score, BW, DMI, energy balance, milk yield and components, and serum components from wk 3 to 6 of lactation for multiparous Holstein cows with different prepartum intake, postpartum health status, and ketosis induction status.

Variable	Prepartum intake (PRE)			Postpartum health status (POST)			Ketosis induction status (IND)				<i>P</i>					
							Healthy		PD							
	Ad libitum	RI ¹	SE	Healthy	PD ²	SE	Control	KI ³	PDC ³	SE	PRE	POST	IND	PRE × POST	PRE × IND	Time
BCS	2.33	2.36	0.12	2.66	2.02	0.12	2.68	2.65	2.02	0.19	0.92	<0.001	0.90	0.56	0.61	0.43
BCS change ⁴	−0.06	−0.09	0.07	−0.12	−0.03	0.07	−0.18	−0.07	−0.03	0.11	0.94	0.34	0.47	0.21	0.56	—
BW, kg	623	630	17	648	604	18	640	655	604	27	0.73	0.09	0.67	0.70	0.70	0.84
BW change, ⁴ kg	2	4	6	3	4	7	0	6	4	10	0.62	0.94	0.68	0.08	0.59	—
DMI, kg/d	19.9	19.7	1.0	19.9	19.6	1.0	21.3	18.5	19.6	1.5	0.84	0.85	0.18	0.71	0.63	<0.001
DMI, % of BW/d	3.22	3.26	0.1	3.23	3.25	0.1	3.37	3.09	3.25	0.2	0.79	0.91	0.24	0.93	0.65	<0.001
Energy balance, ⁵ %	105	106	4	103	108	4	103	102	108	6	0.69	0.34	0.94 [†]	0.45	0.41	<0.001
Milk																
Milk, kg/d	35.3	36.7	1.6	37.8	34.1	1.7	38.6	37.1	34.1	2.5	0.55 [†]	0.12	0.64*	0.85	0.66	<0.001
Fat, %	3.54	3.36	0.08	3.43	3.47	0.09	3.57	3.29	3.47	0.13	0.11	0.71	0.12	0.25	0.87	0.02
Protein, %	2.84	2.76	0.04	2.79	2.81	0.05	2.80	2.78	2.81	0.07	0.33	0.74	0.88	0.22	0.35	0.002
Urea N, mg/dL	15.50	14.91	0.73	14.88	15.53	0.76	15.83	13.93	15.53	1.13	0.39	0.53	0.22	0.04	0.87	0.79
SCS ⁶	2.89	2.59	0.44	2.65	2.83	0.46	2.51	2.80	2.83	0.69	0.63	0.78	0.76	0.86	0.13	0.22
Serum																
BHBA, mg/dL	4.7	4.2	0.3	4.4	4.5	0.3	4.2	4.6	4.5	0.5	0.23	0.78	0.51	0.77	0.34	0.61
Glucose, mg/dL	56	59	1	58	57	1	57	59	57	2	0.11	0.70	0.37	0.62	0.25	0.66
Insulin, μ IU/mL	5.28	4.36	0.75	5.11	4.53	0.76	5.56	4.67	4.53	1.17	0.39	0.58	0.56	0.99	0.79 [†]	0.02
NEFA, μ Eq/L	307	263	35	289	280	36	321	258	280	54	0.39	0.86	0.39*	0.95	0.87	<0.001

¹RI = Restricted intake.²PD = Periparturient disorder.³KI = Ketosis induction and PDC = periparturient disorder control.⁴Value at 6 wk – wk 3 BW.⁵Percentage of NE_L requirement.⁶SCS = Somatic cell score calculated with the equation: $\{\log_{10}(\text{SCC}/1000) - 2/\log_{10}(2)\} + 3$.*Treatment × time was significant ($P < 0.05$).[†]Treatment × time was significant ($P < 0.10$).

Table 10. Milk fatty acid composition from wk 3 to 6 of lactation for multiparous Holstein cows with different prepartum intake, postpartum health status, and ketosis induction status.

Fatty acid, g/100 g	Prepartum intake (PRE)			Postpartum health status (POST)			Ketosis induction status (IND)				P					
	Ad libitum	RI ¹	SE				Healthy		PD	PRE	POST	IND	PRE × POST	PRE × IND	Time	
				Control	KI ³	PDC ³										
C _{4:0}	4.89	4.78	0.10	4.71	4.96	0.10	4.78	4.65	4.96	0.15	0.40	0.08	0.50	0.62	0.28	0.002
C _{6:0}	2.32	2.37	0.07	2.37	2.32	0.07	2.35	2.39	2.32	0.11	0.68	0.64	0.77	0.90	0.07	0.01
C _{8:0}	1.18	1.24	0.05	1.25	1.17	0.05	1.23	1.27	1.17	0.08	0.34	0.28	0.73	0.63	0.14	<0.001
C _{10:0}	2.34	2.52	0.16	2.55	2.31	0.17	2.49	2.61	2.31	0.25	0.38	0.29	0.72	0.43	0.25	<0.001
C _{12:0}	2.46	2.71	0.17	2.70	2.48	0.18	2.62	2.77	2.48	0.27	0.26	0.37	0.68	0.41	0.26	<0.001
C _{14:0}	8.04	8.81	0.36	8.71	8.14	0.37	8.55	8.87	8.14	0.56	0.13	0.27	0.68	0.53	0.33	<0.001
C _{14:1}	0.53	0.58	0.03	0.62	0.49	0.03	0.58	0.66	0.49	0.05	0.21	0.005	0.25	0.45	0.11	<0.001
C _{15:0}	0.75	0.83	0.05	0.80	0.78	0.05	0.81	0.80	0.78	0.08	0.19	0.79	0.92	0.17	0.79	<0.001
C _{16:0}	25.13	26.41	0.45	25.93	25.61	0.46	26.02	25.84	25.61	0.69	0.05	0.62	0.85 [†]	0.60	0.72	0.09
C _{16:1 trans}	0.77	0.73	0.03	0.73	0.77	0.04	0.70	0.77	0.77	0.05	0.48	0.52	0.37	0.41 [†]	0.70	0.53
C _{16:1}	1.67	1.66	0.07	1.78	1.55	0.07	1.82	1.74	1.55	0.11	0.74	0.03	0.58	0.20	0.53	<0.001
C _{17:0}	0.60	0.58	0.01	0.58	0.60	0.01	0.58	0.58	0.60	0.02	0.48*	0.32	0.89	0.07	0.57	0.003
C _{17:1}	0.25	0.23	0.01	0.24	0.23	0.01	0.25	0.24	0.23	0.02	0.21	0.57	0.64	0.30	0.32	<0.001
C _{18:0}	13.23	12.56	0.30	12.39	13.40	0.31	12.69	12.10	13.40	0.46	0.10	0.02	0.35	0.44	0.54	0.06
C _{18:1 trans}	2.40	2.39	0.15	2.34	2.44	0.16	2.32	2.37	2.44	0.24	0.87	0.65	0.88	0.11	0.39	0.05
C _{18:1 cis-9}	23.75	22.09	1.06	22.77	23.07	1.10	22.76	22.78	23.07	1.64	0.23	0.84	0.99	0.30	0.65	<0.001
C _{18:1 cis-11}	0.74	0.73	0.03	0.76	0.71	0.03	0.77	0.76	0.71	0.05	0.68	0.19	0.92	0.14	0.48	<0.001
C _{18:2n6}	3.18	2.96	0.10	2.97	3.16	0.10	2.96	2.98	3.16	0.16	0.17	0.18	0.94	0.54	0.59	0.10
C _{18:3n3}	0.34	0.32	0.01	0.32	0.35	0.01	0.32	0.32	0.35	0.02	0.25	0.15	0.94	0.55	0.56	0.001
CLA ⁴	0.31	0.32	0.01	0.32	0.32	0.01	0.32	0.32	0.32	0.02	0.35	0.84	0.76 [†]	0.02	0.81	0.08
Unidentified <C _{17:0}	1.64	1.69	0.09	1.73	1.61	0.09	1.65	1.80	1.61	0.14	0.67	0.34	0.44	0.50	0.60	<0.001
Unidentified >C _{17:0}	3.47	3.49	0.09	3.41	3.54	0.10	3.41	3.41	3.54	0.15	0.61	0.34	0.99	0.01	0.71	0.39

¹RI = Restricted intake.

²PD = Periparturient disorder.

³KI = Ketosis induction and PDC = periparturient disorder control.

⁴CLA = Conjugated linoleic acid.

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

†Treatment × time was significant ($P < 0.10$).

with retained placenta had a small short-term loss of milk (from 0.3 to 0.7 kg/d during the first month or less) but no long-term loss. Cows with chronic metritis had no loss of milk, but those with acute metritis had moderate to large short- or mid-term losses (from 2 to 2.5 kg/d during the first month, decreasing to 0.7 to 1.3 kg/d the following month) and no long-term loss of milk. Cows with clinical ketosis had milk losses of 2.5 to 3.5 kg/d in the month following diagnosis and 0.3 to 0.7 kg/d during the following month, but no long-term loss of milk.

CONCLUSIONS

Others have suggested (Grummer, 1995; Drackley, 1999) that nutritional management during the dry period affects susceptibility of cows to metabolic disorders and infectious diseases during the periparturient period. Our study did not have the power (i.e., small number of cows) to address that statement effectively by evaluating incidence of metabolic disorders and infectious diseases. However, our study did provide information about the effect of prepartum feed intake on the metabolic status of cows during the periparturient period that can be related to the probability that a metabolic disorder might occur. Cows fed ad libitum generally had indices of greater body fat mobilization and had greater lipid accumulation in liver at 1 d after calving than RI cows.

The utility of the feed restriction model of primary ketosis (Bahaa et al., 1997) was confirmed in this study. In addition, this study showed how the metabolic status of cows differed among cows that were healthy, cows that were ketotic, and cows that had PD (mostly retained placenta). Metabolic alterations in cows with PD in general were much less pronounced than for those in cows with induced ketosis and were not greatly different from healthy cows.

Prepartum intake resulted in changes in serum metabolites consistent with plane of nutrition and energy balance. Prepartum intake had few effects on postpartum intake, serum metabolites, liver composition, or milk yield. Periparturient disorders and induction of ketosis negatively affected metabolic status and milk yield during the first 14 DIM. By 42 DIM, however, negative effects of PD and KI on metabolic status and milk yield could not be detected.

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