Metabolic Characteristics of Induced Ketosis in Normal and Obese Dairy Cows[1]

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ABSTRACT

Four groups of 6 cows were used to determine the effects of body condition on induction of ketosis. At calving, obese cows were heavier by 108 kg and had a higher body condition score by 0.74 units than did normal cows. Susceptibility to induced ketosis was evaluated by restricting dry matter intake by 20% and feeding 7% 1,3-butanediol from 15 to 49 d in milk (DIM) to one group of obese cows and to one group of normal cows. No normal or obese cows fed the control diet developed ketosis. Two normal and 2 obese cows developed ketonemia because of the induction protocol, and 1 cow in each of the two groups developed clinical ketosis. Obese cows lost 59% more body weight during the first 14 DIM than did normal cows, and cows fed the restricted diet plus 7% 1,3-butanediol lost 15% more body weight than did cows fed the control diet during the induction period. Concentrations of nonesterified fatty acids increased at parturition, peaked at 7 to 14 DIM, and returned to prepartum concentrations by 21 DIM. Plasma β-hydroxybutyrate concentrations increased after calving and was increased additionally by the induction protocol. At the onset of lactation, plasma insulin decreased, plasma glucagon increased, hepatic triacylglycerols increased, and hepatic glycogen decreased. The incidence of ketonemia and clinical ketosis was the same for obese and normal cows, but, on the basis of changes of blood and liver composition, incidence of ketosis would probably increase if obese cows were overfed throughout the entire dry period.

(**Key words**: ketosis, fatty liver, body condition, obese cows)

Abbreviation key: **ACAC** = acetoacetate, **BCS** = body condition score, **BD** = 1,3-butanediol, **EB** = energy balance, **FR** = feed restriction, **GLN** = glucagon, **GLY** = glycogen, **INS** = insulin, **TAG** = triacylglycerol.

INTRODUCTION

An induction protocol has been developed to produce a metabolic ketosis in lactating dairy cows that appears to be very similar to naturally occurring spontaneous ketosis (4, 5, 18, 19). The protocol uses feed restriction (**FR**) starting at 14 DIM to about 80% of previous DMI provided for ad libitum intake and includes 1,3-butanediol (**BD**), a ketone precursor, in the diet at approximately 7% of DMI. When this FR and BD protocol was used, ketosis developed gradually over about 4 wk, and cows became ketotic at a mean of 42 DIM (30).

Development of fatty liver is a common metabolic change associated with ketosis during lactation (9, 11, 15, 17, 25). Elucidation of the etiology of fatty liver and ketosis has been hampered, however, by lack of an experimental model. Use of the FR and BD protocol to induce ketosis has provided data that suggest that the development of ketosis is dependent on prior development of fatty liver (4, 30). Cows with a ratio of hepatic triacylglycerol (**TAG**) to glycogen (**GLY**) below 2:1 at calving seemed to be resistant to induction of ketosis, but ketosis was induced when the ratio was somewhat greater than 2:1 (4). Thus, development of fatty liver appears to precede ketosis and to contribute to the onset of ketosis.

Accumulation of TAG within the liver is normal for dairy cows during early lactation (8, 21, 23). As part of the homeorhetic adaptation to support lactation, NEFA are mobilized from adipose tissue (22) and enter the liver. In the liver, NEFA are either oxidized or reesterified and exported as very low density lipoproteins. During early lactation, however, the liver can be overwhelmed with NEFA, which may accumulate within hepatocytes as TAG droplets.

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Body condition score (**BCS**) at calving is a measure of adipose tissue reserves that can be used during lactation to supply energy and precursors for milk fat. Excess body condition at calving, however, increases losses in BW and body condition during lactation and decreases DMI and milk production (28). In addition, obesity at calving contributes to the development of metabolic diseases such as fat cow syndrome, mastitis, metritis, and fatty liver (10).

We hypothesized that cows that are obese at calving have greater adipose tissue reserves that result in increased mobilization of NEFA (28). Therefore, these cows would develop more severe fatty liver during early lactation. Obese cows would be more susceptible to the induction of ketosis than would cows of normal body condition. The objectives of this study were to determine the effects of BCS at calving on the susceptibility of lactating dairy cows to induced ketosis and to determine differences in changes in blood and liver constituents between cows that are relatively susceptible to ketosis and cows that are relatively resistant to ketosis.

MATERIALS AND METHODS

Cows

Twenty-four multiparous Holstein cows were assigned to a 2 × 2 factorial design; body condition at calving (normal or obese) and diet (ad libitum access to feed or FR and BD) were the two factors evaluated. We recorded BCS, a qualitative estimate of the amount of adipose tissue stored, on a five-point scale [1 = extremely thin to 5 = extremely obese (6)]. The BCS of each cow was recorded weekly after the previous lactation ended and throughout the trial. Cows were divided into two categories late in the previous lactation. Twelve cows with BCS around 4.0 were assigned to the normal category and were fed to maintain their BCS until calving. Twelve cows with BCS >4.0 were assigned to the obese category and were fed to calve at a mean expected BCS of 4.5. To avoid fat cow syndrome, adjustments in BCS were made during the last few weeks of the previous lactation and up to 3 wk into the dry period by providing feed in various quantities from 80 to 120% of NRC recommendations (20). Composition of the basal diet and of the supplement fed during the dry period is shown in Table 1.

Experimental Diets

All cows were fed the control diet (Table 2) for the first 14 DIM. Before calving, both normal and obese cows were assigned randomly so that half would receive the control diet and half would receive the FR diet plus BD after 14 DIM. Thus, four groups of 6 cows were used: normal cows fed the control diet, normal cows fed the FR diet plus BD, obese cows fed the control diet, and obese cows fed the FR diet plus BD. Concentrate and silage were fed as a mixture, and alfalfa hay was offered to each cow at 3.63 kg/d. Cows were fed daily at 0700 and 1400 h. Orts from hay and from the mixture of concentrate and silage were collected separately and weighed before the a.m. feeding. The mixture was sampled monthly, and DM was determined by drying at 60°C for 2 d in a forced-

TABLE 1. Composition of the diet fed during the dry period.

Component	Amount fed	NE_L
		(Mcal/kg of DM)
Basal diet		
Oat hay	Ad libitum	0.8
Corn silage, kg/d	1	1.6
Concentrate,[1] % of concentrate		
Cracked corn	41.7	1.84
Whole oats	41.7	1.77
Molasses	16.7	1.72
Total	100.0	1.77

[1]Fed as required for adjustment of body condition scores during the first 3 wk of the dry period at up to 20% of NRC (20) recommendations for maintenance.

TABLE 2. Composition of the control diet.[1,2]

Ingredient	
	(% of DM)
Concentrate mix[3]	
Cracked corn	45.34
Soybean meal	14.57
Energy Booster® fat[4]	1.94
Sodium bicarbonate	0.65
Yeast culture	0.65
Dicalcium phosphate	0.65
Magnesium oxide	0.32
Sodium chloride	0.32
Calcium carbonate	0.16
Vitamin and mineral premix[5]	0.16
Corn silage[6]	35.23

[1]Alfalfa hay was offered to each cow at 3.63 kg/d.

[2]The total mixed diet was a combination of the concentrate mix and corn silage (21 and 79%, as fed, respectively).

[3]Whole cottonseed and soybean meal were substituted in the concentrate mix (up to 4.2 and 8.9% of the ration, as fed, respectively) when required.

[4]Milk Specialities Co. (Dundee, IL).

[5]West Central Coop. (Ralston, IA).

[6]Alfalfa haylage was substituted for up to 35% of corn silage when available.

air oven. The diet was 62% DM and was calculated to contain 1.8 Mcal of NE_L/kg, 14.9% CP, 4.78% ether extract, 22% NDF, and 12.7% ADF.

Daily DMI was measured beginning at 10 DIM. After 14 DIM, normal and obese cows fed the control diet continued to receive the control diet for ad libitum intake. Normal and obese cows fed the FR diet, however, were restricted to 80% of their previous DMI and were also given BD (donated by Celanese Chemical Co., Dallas, TX) in their diet. The BD was increased gradually over 5 d to 7% of DMI by 18 DIM. The FR diet plus BD was continued from 15 to 49 DIM (the ketosis induction period) or until cows became ketotic.

Subclinical ketosis was determined by daily milk production records and visual observation of the cows; the condition was confirmed by blood ketone analysis using nitroprusside powder (Ketonate®; Labanco Inc., Addison, IL). Cows that had slightly increased plasma ketone concentrations plus slight decreases in milk production and feed intake were considered to be subclinically ketotic and were observed closely for more severe signs. Cows with severely elevated ketones and severely decreased appetite and milk production were considered clinically ketotic; liver biopsy samples were taken, and the FR and BD protocol was discontinued. Cows with clinical ketosis were then fed the control diet for a recovery period of 14 d. After 49 DIM, any cow fed the FR diet plus BD that had not developed signs of clinical ketosis was also returned to the control diet for a 14-d recovery period.

Samples

Liver biopsies (14, 30) were taken at 10 d prepartum and at 7, 14, 21, 28, 35, 42, 49, and 63 DIM. A biopsy site between the 11th and 12th ribs was shaved, scrubbed with Betadine® (Povidone-iodine, 7.5%; Purdue Frederick Co., Norwalk, CT), and anesthetized with Lidocaine® (lidocaine hydrochloride, 2%; Med Tech Inc., Elkwood, KS). An incision of about 1 cm was made through the skin, and the biopsy needle was used to pierce the intercostal muscles and peritoneum. The liver was located, and a 5- to 7-g sample was removed. A portion of the sample was frozen quickly in liquid nitrogen and stored at –80°C for later analyses of TAG and GLY contents. The incision site was sutured and treated topically with Furacin® powder (Durvet, Inc., Blue Springs, MO).

On d –11, 13, 27, and 41 DIM, one day preceding the corresponding liver biopsies, hourly blood samples were collected during a 10-h window to establish concentrations of insulin (**INS**) and glucagon (**GLN**). Plasma concentrations of immunoreactive INS and GLN were measured by radioimmunoassay (1, 7, 13).

Plasma was isolated from blood samples that were collected just before each liver biopsy, and aliquots of plasma were used to determine metabolite concentrations. Concentrations of acetoacetate (**ACAC**) and BHBA (31) were determined on protein-free filtrates (27) that were prepared from aliquots of blood plasma. Glucose concentrations were determined using glucose oxidase (Sigma Trinder kit number 315; Sigma Chemical Co., St. Louis, MO), and concentrations of NEFA were determined using a kit (NEFA-C; Wako Chemicals, Dallas, TX) with the following modifications (16): 16 ml of water were added to reagent A and its diluent, and 33 ml of water were added to reagent B and its diluent. To 25 μl of sample or standard, 0.4 ml of reagent A and 0.8 ml of reagent B were added. The reaction was linear through 2000 μeq/L. Plasma processed for GLN assays contained 2500 IU/ml of added aprotinin (Trasyolol®; Mobay Chemical Corp., FBA Pharmaceuticals, New York, NY).

TABLE 3. Prepartum characteristics of cows completing the study.[1]

	Normal cows		Obese cows		
Variable	Control	FR plus BD[2]	Control	FR plus BD	SE[3]
Cows, no.	5	6	4	4	
Parity	2.8	2.7	2.5	3.0	0.4
Dry period, d	67	65	78	66	2.9
BW, kg	658	673	759	789	28.1
Body condition score[4]	3.5	3.5	4.2	4.3	0.17

[1]Least squares means.

[2]FR = Feed restriction; BD = 1,3-butanediol.

[3]Mean standard error pooled by group.

[4]Scored on a five-point scale where 1 = extremely thin to 5 = extremely obese (6).

Energy Balance Calculations

Intakes of NE_L were calculated from daily intakes of the total mixed diet and hay and from as-fed composition (Table 2). Milk energy content was calculated by inserting fat and protein percentages into a prediction equation (29): milk energy content (megacalories per kilogram) = (226.09 + 89.5 × percentage of fat + 49.83 × percentage of protein)/1000. Lactation energy (LE) was calculated according to the following equation: LE = milk production (kilograms) × milk energy content. Net energy requirements for maintenance (NE_m) were calculated from BW (20) according to the equation $NE_m = 0.080 \times BW^{0.75}$. Energy balances (**EB**) were calculated weekly during the induction and recovery periods using the equation $EB = NE_L - LE - NE_m$.

Statistical Analysis

Data were evaluated as a split plot in time design using the general linear models procedure of SAS (26). Whole-plot effects (body condition, diet, and their interaction) were tested using cow within group as the error term. Subplot effects (sampling time and time × treatment interactions) were tested against residual error. Values presented in the text, tables, and figures are least squares means ± standard errors. Least squares means also were used to determine the significance of differences between means in preplanned comparisons. Significance was declared at $P < 0.10$.

RESULTS

Experimental Cows

Prepartum characteristics of the 19 cows that completed the study are summarized in Table 3. There were no significant differences in parity or days in the dry period among groups. Five cows did not complete the study and were not used in the statistical analysis. Three cows were removed after irregular feed intake and subsequent diagnosis and treatment for displaced abomasum. One cow died after the hepatic artery was perforated at the prepartum biopsy. This fatality was the only serious problem during all of the biopsies. One cow was removed after displaying signs of fat cow syndrome.

At calving, obese cows averaged 108 kg more of BW ($P = 0.002$) and 0.74 units more of BCS ($P < 0.001$) than normal cows. Normal and obese cows were below the BCS specified in the experimental design; however, obese cows still had significantly higher BCS at calving than did normal cows.

None of the normal or obese cows fed the control diet developed ketosis. At the completion of the study, 6 of the 10 cows fed the FR diet plus BD showed some degree of ketosis. Four cows (2 normal and 2 obese cows fed the FR diet plus BD) were observed to have subclinical ketosis at a mean of 33 DIM, and 2 cows (1 normal cow and 1 obese cow fed the FR diet plus BD) developed clinical signs of ketosis at a mean of 36 DIM.

Feed Intake

Feed consumption by normal and obese cows after calving was not different (data not shown); therefore, feed intake data were combined (Table 4). During the induction period (d 15 to 49), intake of the concentrate and silage mixture was restricted for cows fed the FR diet plus BD and averaged 9.0 kg/d less than that of cows fed the control diet ($P = 0.002$). Energy intake of cows fed the FR diet plus BD was increased by the addition of BD to the diet and by a slight increase of hay consumption (0.55 kg/d; $P = 0.22$) during the induction period, but NE_L intake of cows fed the FR diet plus BD still averaged 12.6% less than that of cows fed the control diet ($P = 0.13$). There was, however, a significant diet × week interaction ($P = 0.028$) in which NE_L intake continued to increase in cows fed the control diet during the induction period but remained relatively stable in cows fed the FR diet plus BD. After termination of the FR and BD protocol, the mean increase in consumption of the concentrate and silage mixture by cows from the two treatment groups was 46%.

TABLE 4. Feed intake during ketosis induction (15 to 49 DIM) and recovery (50 to 63 DIM) in lactating dairy cows fed the control diet or the feed restricted (FR) diet plus 1,3-butanediol (BD).[1]

	Diet			
Period and variable	Control	FR plus BD	SE[2]	$P > F$
Induction				
Concentrate and silage mixture, kg/d	28.8	19.8	0.53	0.002
BD, kg/d	0	0.85	0.07	0.0001
Hay, kg/d	2.4	2.9	0.10	0.22
NE_L, Mcal/d	34.8	30.4	0.64	0.13
Recovery				
Concentrate and silage mixture, kg/d	34.8	28.9	1.6	0.011
Hay, kg/d	2.3	2.6	0.33	0.52
NE_L, Mcal/d	41.4	35.2	2.0	0.024

[1]Least squares means.

[2]Mean standard error pooled by group.

We combined both groups receiving the FR diet plus BD; these cows were then subdivided into a susceptible group, which developed ketosis (6 cows), and a resistant group, which did not develop ketosis (4 cows). We were then able to perform additional statistical analyses to examine possible differences between susceptible and resistant cows.

Cows from the combined group that were fed the FR diet plus BD and that were susceptible to ketosis had lower hay intakes during the 1st wk of lactation ($P = 0.056$) than did cows that were resistant to ketosis (0.3 ± 1.1 and 3.3 ± 0.57 kg/d, respectively; data not shown); this decreased intake might have been a factor that caused ketosis. Hay consumption did not differ between susceptible and resistant cows during wk 2 of lactation; however, during the induction period, cows that were susceptible to ketosis averaged 37% greater hay intake ($P = 0.0006$) than did cows that were resistant to ketosis (3.14 ± 0.13 and 2.30 ± 0.18 kg/d, respectively). The increased forage intake of cows that were susceptible to ketosis was maintained throughout the induction period and was not an artifact of decreased intake at the onset of ketosis. Consumption of the concentrate and silage mixture was similar in both cows that were susceptible or resistant to ketosis throughout the study.

Milk Production

Milk production and composition (data not shown) were not different across the four groups during the adjustment period (1 to 14 DIM); however, to account for individual cow variation, subsequent data were corrected for these covariates. During wk 5 postpartum, milk production peaked at 38 kg/d in normal and obese cows fed the control diet and remained relatively stable thereafter. The FR and BD protocol decreased milk production by a mean of 15% ($P = 0.0068$) throughout the entire induction period. There was no effect of BCS on milk production. Milk fat and protein percentages were not affected by treatments, but milk fat percentage was numerically higher, and protein percentage was numerically lower, for cows fed the FR diet plus BD throughout the induction period. Effects of the FR and BD protocol on milk production and composition were similar to results from another report from our laboratory (5).

Effects of Liver Biopsies

Data from 132 liver biopsies were used to evaluate the effects of biopsies on feed intake and milk production. There were no differences among groups in response to biopsies; combined data are shown in Figure 1. Measurements taken on both days preceding each biopsy were averaged and served as the baseline measurements. Concentrate and silage intake averaged 1.3 ± 0.46 kg below the baseline values on d 0 when biopsies were taken ($P = 0.049$). Hay intake and milk production were lowest on the day of the biopsy; however, neither differed significantly from the baseline values. Concentrate and silage intake and milk production both returned to baseline values or greater within 2 d. The increase in milk production was not unexpected for early lactation cows. Thus, only the intake of concentrate and silage was decreased significantly on the day of biopsy, but the decrease was neither dramatic nor sustained.

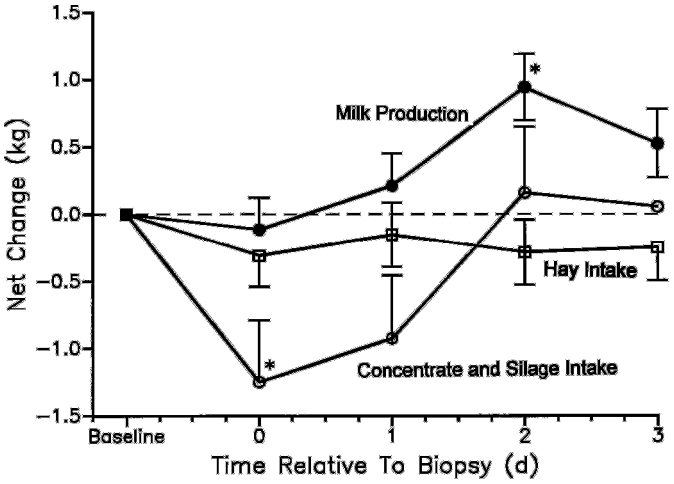

Figure 1. Effect of liver biopsies on daily milk production and intakes of hay and concentrate and silage (i.e., grain) of lactating dairy cows. Baseline observations were averaged for the 2 d preceding each biopsy. The standard errors, pooled by group at each sample, averaged ±0.24 kg of milk production, ±0.23 kg of hay intake, and ±0.46 kg of concentrate and silage intake. Asterisks indicate significance ($P < 0.1$) from baseline values.

BW and BCS

Prepartum differences in BW and BCS between obese and normal cows have been discussed. Body weight (Figure 2A) and BCS (Figure 2B) decreased for all cows after parturition. Loss of BW during wk 1 postpartum included the weight of the fetus, which caused loss of BCS to lag behind loss of BW. Changes in BW and BCS, however, were highly correlated from wk 2 through 7 (R = 0.96).

Obese cows lost 59% more BW during the first 14 DIM than did normal cows ($P = 0.023$). Increased BW loss occurred primarily during wk 1 postpartum and might have been related to greater losses associated with calving. These results agree with those

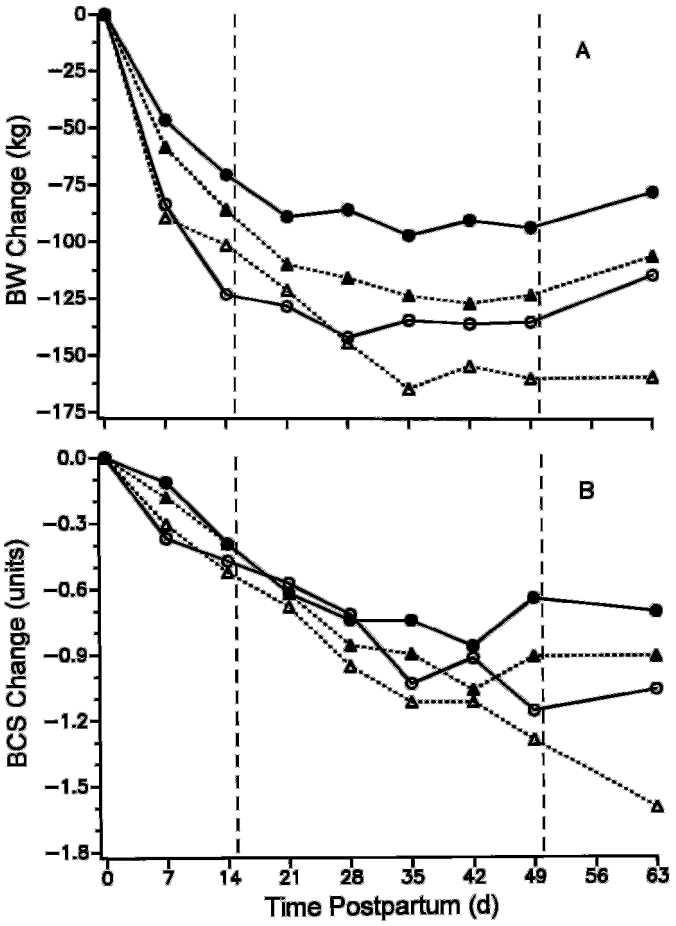

Figure 2. Effects of body condition at calving and effects of feed restriction (FR) plus dietary 1,3-butanediol (BD) on changes in BW (A) and body condition score (BCS) (B) in dairy cows postpartum. The standard errors, pooled by group at each sample, averaged ±8.1 kg of BW change and ±0.12 units of change in BCS. The dashed vertical lines at 14 and 50 d indicate the onset and conclusion of the ketosis induction period, respectively. Legend: ● = normal cows fed the control diet, ○ = obese cows fed the control diet, ▲ = normal cows fed the FR diet plus BD, and △ = obese cows fed the FR diet plus BD.

of Treacher et al. (28), who reported decreased DMI and an increased loss of BW and BCS for cows that were fatter at calving. Despite increased BW loss during the first 14 DIM, obese cows weighed 79 kg more than did normal cows at the start of the ketosis induction period, and this difference persisted throughout the period.

Loss of BW continued into the induction period for cows in all groups, reaching a nadir at about 35 DIM. Obese and normal cows fed the FR diet plus BD lost 167% more BW during the induction period (diet × week interaction; $P = 0.031$) than did obese and normal cows fed the control diet (48 ± 7.4 and 18 ± 7.0 kg, respectively). During the 2-wk recovery period, BW gain averaged 16 ± 9.1 kg and was not different among groups. Body condition at calving had no significant effect on BW changes during the induction or recovery periods; however, cumulative BW loss during the experiment for obese cows fed the FR diet plus BD was double that for normal cows fed the control diet ($P < 0.0001$).

Decreases in BCS generally paralleled losses of BW during early lactation (Figure 2B), but measurements of BCS were less precise and thus showed less statistical significance than did BW. There were no treatment differences in BCS at any stage of the study, but the decrease in BCS over the entire study averaged 52% more for obese cows (category × week interaction; $P = 0.007$) than for normal cows.

For all cows fed the FR diet plus BD, BW loss through 35 DIM for cows that were susceptible to ketosis was 61% greater ($P = 0.006$) than that for cows that were resistant to ketosis (156 ± 8.6 and 96 ± 16.8 kg, respectively; data not shown). There were no differences in BCS losses between susceptible and resistant cows for the entire induction period. However, BCS losses during the recovery period were actually greater for resistant cows ($P = 0.068$) than for susceptible cows (–0.58 ± 0.19 and –0.04 ± 0.12 units, respectively).

EB

All cows were in negative EB entering the induction period with a mean of –4.3 Mcal/d at 14 DIM (Figure 3). Despite a greater requirement for net energy for maintenance that was based on BW of obese cows, BCS at calving did not significantly affect EB; therefore, the two control groups and the two groups fed the FR diet plus BD were pooled into two groups for presentation of EB data. During the ketosis induction period, the EB of cows fed the FR diet plus BD averaged 1.0 Mcal/d less than the EB of cows fed the control diet; however, there was a significant diet × week interaction ($P = 0.07$) in which the EB of cows fed the control diet steadily improved, reflecting increases in DMI. Cows fed the control diet attained positive EB at about 42 DIM. Conversely, the initial response of cows to the FR and BD protocol was to limit milk production, which, in combination with additional energy from BD, elevated the EB of cows fed the FR diet plus BD above that of cows fed the control diet. As the induction of ketosis progressed, however, cows fed the FR diet plus BD limited their intake to less than the amount of the FR diet plus BD that was actually offered, but milk production remained relatively stable; therefore, by the end of the

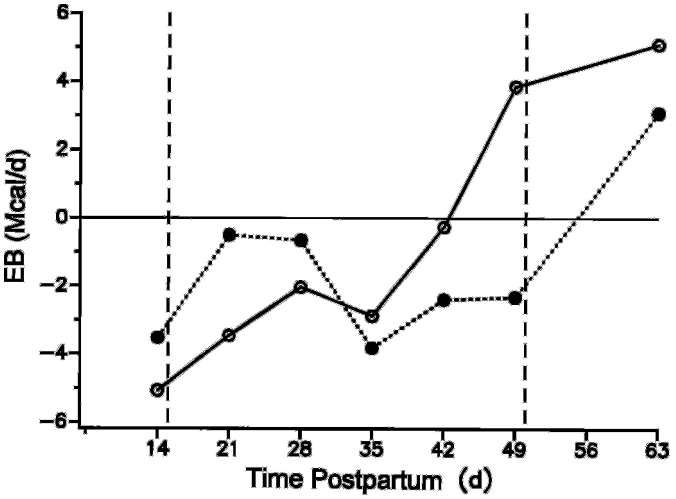

Figure 3. Calculated energy balance (EB) for cows fed the control diet (○) versus cows subjected to feed restriction plus dietary 1,3-butanediol (●) from 15 to 49 DIM. The standard errors, pooled by diet at each sample, averaged ±1.7 Mcal/d. The dashed vertical lines at 14 and 50 d indicate the onset and conclusion of the ketosis induction period, respectively.

induction period, the EB of cows fed the FR diet plus BD averaged 6.2 Mcal/d below that of cows fed the control diet (P = 0.022). Cows fed the FR diet plus BD did not attain a positive EB until after the protocol was terminated at 49 DIM. For cows fed the FR diet plus BD, there were no significant differences in EB between those that were susceptible or resistant to ketosis.

Blood Constituents

Plasma BHBA (Figure 4A) and ACAC (Figure 4B) concentrations were not statistically different among groups prepartum, and there were no significant treatment effects on concentrations of these two plasma ketones during the first 14 DIM. Plasma BHBA concentrations increased for all groups with the onset of lactation and increased further when the FR and BD protocol began. By wk 2 of the induction period, BHBA concentrations in cows fed the FR diet plus BD averaged 234% (P = 0.0025) of those in cows fed the control diet (37.8 ± 4.9 and 16.2 ± 5.6 mg/dl, respectively). Concentrations of BHBA remained elevated for cows fed the FR diet plus BD over the entire induction period; however, the difference in BHBA concentrations between these cows and cows fed the control diet was not significant (P = 0.23). Similarly, during the induction period, plasma ACAC concentrations were numerically greater for cows fed the FR diet plus BD than for cows fed the control diet,

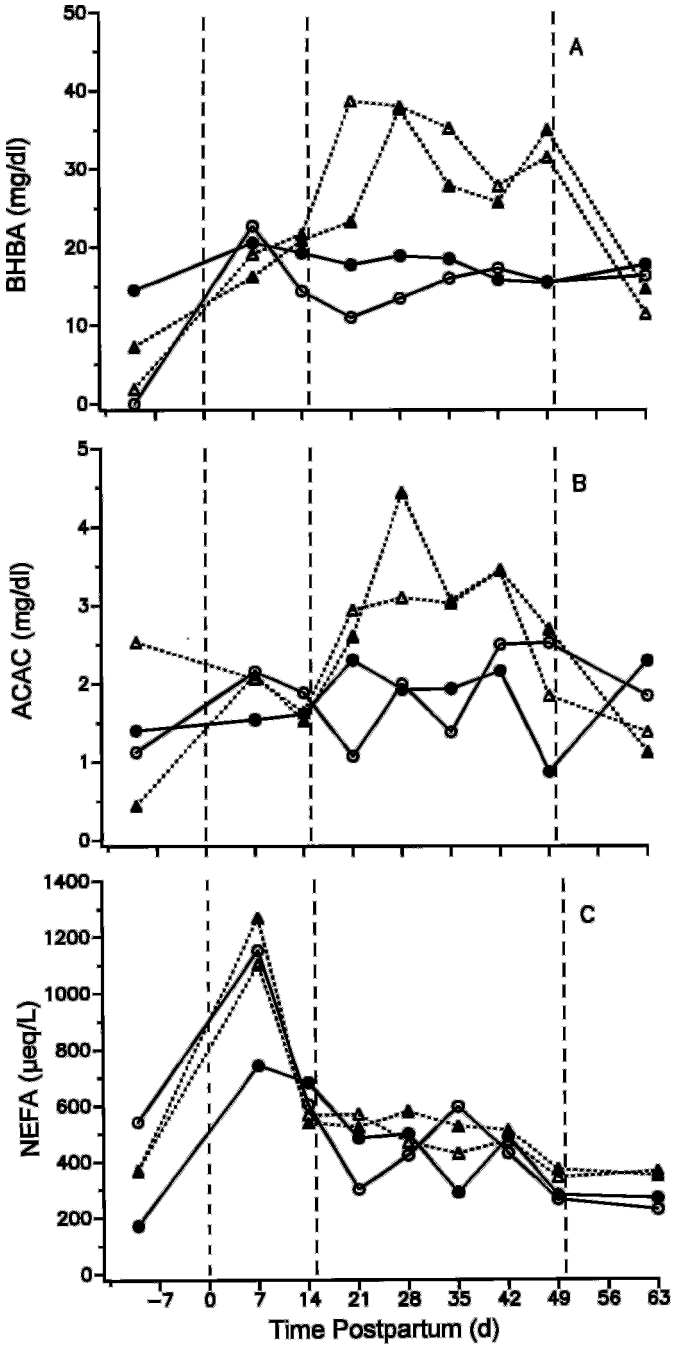

Figure 4. Effects of body condition at calving and effects of feed restriction (FR) plus dietary 1,3-butanediol (BD) on plasma concentrations of BHBA (A), acetoacetate (ACAC) (B), and NEFA (C) in dairy cows postpartum. The standard errors, pooled by group at each sample, averaged ±6.4 mg/dl of BHBA, ±0.97 mg/dl of ACAC, and ±101 meq/L of NEFA. Dashed vertical lines at d 0, 14, and 50 indicate parturition and the onset and conclusion of the ketosis induction protocol, respectively. Legend: ● = normal cows fed the control diet, ○ = obese cows fed the control diet, ▲ = normal cows fed the FR diet plus BD, and △ = obese cows fed the FR diet plus BD.

but the differences were not significant. Thus, ketone concentrations of cows fed the FR diet plus BD were increased as early as 3 wk before signs of ketosis developed. This observation agrees with results of a previous report (30). After termination of the FR and BD protocol, ketone concentrations in cows previously fed the FR diet plus BD declined and were similar to those of cows fed the control diet by 63 DIM. There were no significant effects of BCS on concentrations of ketone bodies.

Plasma BHBA concentrations averaged about 10-fold more than those of ACAC; however, the ratio of BHBA to ACAC was variable throughout the study (data not shown). Obese cows fed either diet tended to have greater ratios of BHBA to ACAC during the early portion of the induction period (body condition × week interaction; $P = 0.13$). A greater ratio of BHBA to ACAC might have been caused by hepatic mitochondria that were more reduced (decreased ratio of NAD to NADH), suggesting that hepatic fatty acid oxidation was increased in obese cows. The FR and BD protocol to induce ketosis had no significant effect on the ratio of BHBA to ACAC.

When obese and normal cows fed the FR diet plus BD were compared, no differences in concentrations of ketone bodies were found between cows that were susceptible or resistant to ketosis at any stage of the experiment (data not shown). Plasma BHBA concentrations during the first 14 DIM were numerically greater in susceptible cows than in resistant cows (30.1 ± 8.9 and 12.5 ± 10.7 mg/dl, respectively; $P > 0.2$), and this relationship persisted into the induction period (42.5 ± 7.4 and 24.8 ± 10.8 mg/dl, respectively; $P = 0.19$). However, the differences were not significant. Similarly, during the induction period, concentrations of ACAC tended to be greater ($P = 0.17$) for cows that were susceptible to ketosis than for cows that were resistant to ketosis (3.6 ± 0.7 and 2.1 ± 0.9 mg/dl, respectively).

Plasma NEFA concentrations increased from prepartum concentrations, peaked at 7 DIM, and then returned to prepartum values by 21 DIM (Figure 4C). No significant differences were found in NEFA concentrations among groups prepartum or during the first 14 DIM. Plasma NEFA concentrations of cows fed the FR diet plus BD were numerically higher than those of cows fed the control diet during the induction period (475 ± 47 and 415 ± 53 μeq/L, respectively); however, there were no significant treatment effects. These data are consistent with other reports (2, 12) of increasing NEFA concentrations during early lactation and with the observations of Veenhuizen et al. (30), who noted increased NEFA

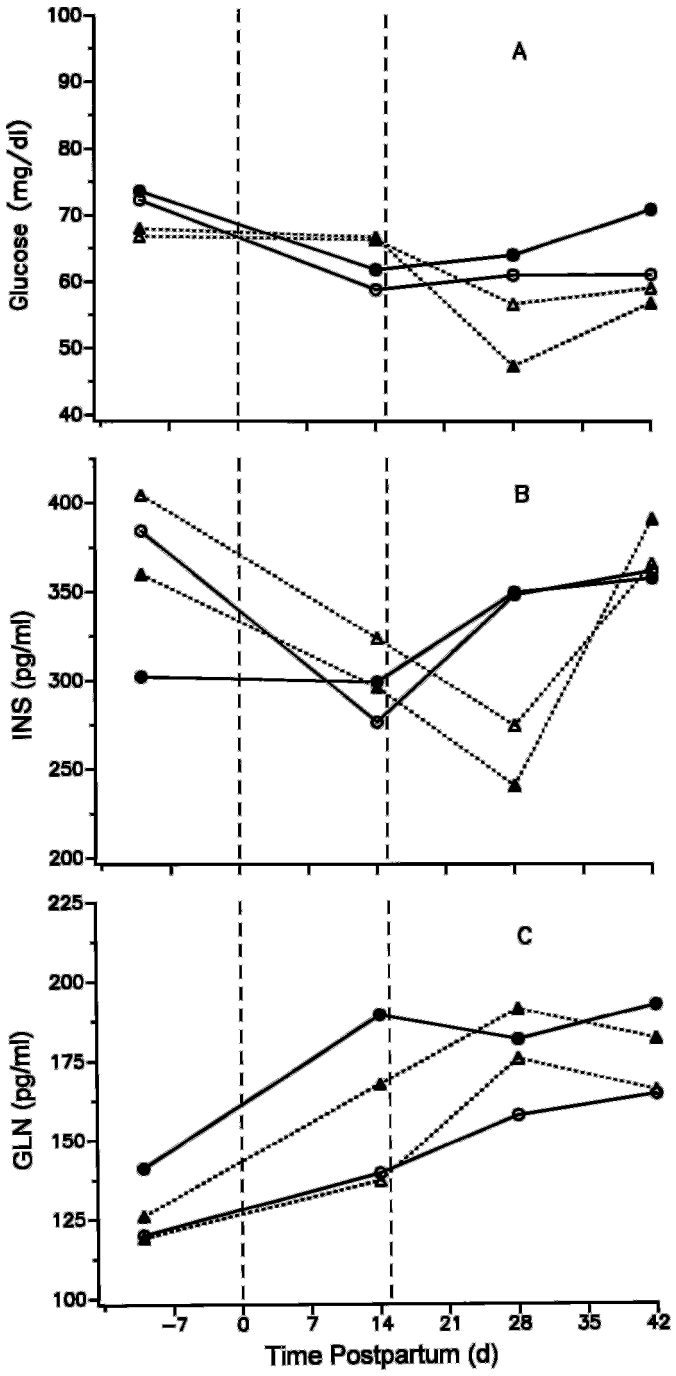

Figure 5. Effects of body condition at calving and effects of feed restriction (FR) plus dietary 1,3-butanediol (BD) on plasma concentrations of glucose (A), insulin (INS) (B), and glucagon (GLN) (C) in dairy cows postpartum. The standard errors, pooled by group at each sample, averaged ±4.2 mg/dl of glucose, ±20 pg/ml of INS, and ±6.8 pg/ml of GLN. Dashed vertical lines at d 0 and 14 indicate parturition and the onset of the induction protocol, respectively. Data do not extend over the entire experiment because only four window samplings were completed for hormones. Legend: ● = normal cows fed the control diet, ○ = obese cows fed the control diet, ▲ = normal cows fed the FR diet plus BD, and △ = obese cows fed the FR diet plus BD.

concentrations in response to the FR and BD protocol. There also were no significant differences in plasma NEFA concentrations during the recovery period.

Elevated NEFA concentrations during lactation suggest increased mobilization of lipids from adipose tissue. Obese cows, which lost more body condition than did normal cows, had concentrations of NEFA that were similar to those of normal cows, suggesting that obese cows were able to utilize the additional NEFA. The modest increase in concentrations of NEFA in cows fed the FR diet plus BD suggests that the induction protocol limited carbohydrate availability. This suggestion is supported by decreased concentrations of plasma glucose (Figure 5A) and by decreased liver GLY (Figure 6B).

Plasma NEFA concentrations of all cows were similar prepartum and through 14 DIM (data not shown). Plasma NEFA concentrations increased for both susceptible and resistant cows fed the FR diet plus BD, but NEFA concentrations of susceptible cows were numerically greater by wk 1 of treatment (592 ± 98 and 470 ± 178 μeq/L, respectively; $P > 0.2$), and, by wk 2, NEFA concentrations were more than twice those of resistant cows (687 ± 107 and 322 ± 128 μeq/L, respectively; $P = 0.038$). Over the entire induction period, NEFA concentrations averaged 41% greater for susceptible cows than for resistant cows (549 ± 46 and 389 ± 66 μeq/L, respectively; $P = 0.059$).

Plasma glucose concentrations were similar for all cows through 14 DIM (Figure 5A). For cows fed the control diet, plasma glucose concentrations were lowest at 14 DIM and then increased slowly as lactation progressed; however, for cows fed the FR diet plus BD, plasma glucose concentrations continued to decrease after 14 DIM, and, at 28 DIM, the plasma glucose concentrations of cows fed the FR diet plus BD averaged 17% less than those of cows fed the control diet ($P = 0.050$). The trend toward decreased plasma glucose concentrations because of the FR and BD protocol has been noted previously (4, 30) and is consistent with metabolic demands of lactation and with the influence of the induction protocol as cows progress toward ketosis. No effects of BCS on plasma glucose concentrations were detected.

Prepartum plasma INS concentrations were numerically higher in obese cows (Figure 5B); however, they were not different for any group at 14 DIM. Plasma INS values decreased with the onset of lactation, were lowest at 14 DIM, and then increased through the remainder of the study (quadratic effect of week, $P < 0.0001$). At 28 DIM, plasma INS concentrations in cows fed the FR diet plus BD were 26% less than those in cows fed the control diet (257 ± 16

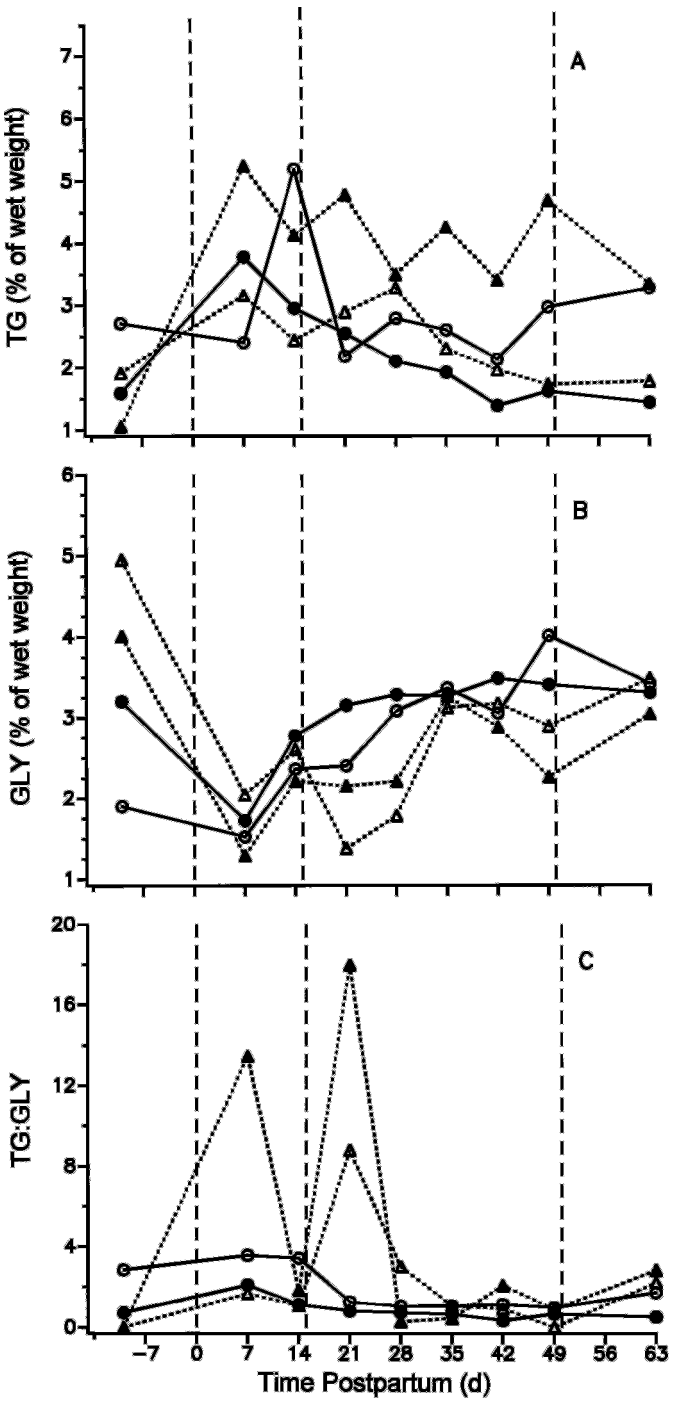

Figure 6. Effects of body condition at calving and effects of feed restriction (FR) plus dietary 1,3-butanediol (BD) on contents of liver triacylglycerol (TAG) (A) and glycogen (GLY) (B) and on the ratio of TAG to GLY (C) in dairy cows postpartum. The standard errors, pooled by group at each sample, averaged ±0.86% TAG, ±0.43% GLY, and ±3.8 units for the ratio of TAG to GLY. Dashed vertical lines at d 0, 14, and 50 indicate parturition and the onset and conclusion of the induction protocol, respectively. Legend: ● = normal cows fed the control diet, ○ = obese cows fed the control diet, ▲ = normal cows fed the FR diet plus BD, and △ = obese cows fed the FR diet plus BD.

and 349 ± 13.8 pg/ml, respectively; $P < 0.0001$); however, this difference had disappeared by 42 DIM. Plasma INS concentrations were not affected by BCS at any stage. Previous reports indicated that INS concentrations increased in obese cows (10, 24) and obese heifers (32) before calving.

Changes in plasma GLN concentrations (Figure 5C) were generally opposite those of INS. Plasma GLN concentrations increased with the onset of lactation, peaked at about 28 DIM, and remained stable thereafter (quadratic effect of week, $P < 0.0001$). After calving, concentrations of GLN in obese cows averaged 15% below those in cows of normal body condition ($P = 0.091$). The FR and BD protocol for induction of ketosis had no significant effects on concentrations of GLN.

The ratio of INS to GLN was not different for any group prepartum or at 14 DIM (data not shown). There was a small but nonsignificant decrease for cows in all groups with the onset of lactation. At 35 DIM, the ratio of INS to GLN in cows fed the FR diet plus BD averaged 34% less than that in cows fed the control diet ($P < 0.0001$), reflecting the decrease in plasma INS discussed previously; however, as with INS, the difference in the ratio of INS to GLN had disappeared by 42 DIM. The ratio of INS to GLN in obese cows averaged 13% greater than that in normal cows throughout the experiment; however, the difference was never significant.

When obese and normal cows fed the FR diet plus BD were compared, prepartum concentrations of INS were lower ($P = 0.10$) in cows that were susceptible to ketosis than in cows that were resistant to ketosis (356 ± 20 and 422 ± 35 pg/ml, respectively; data not shown). This difference persisted at 14 and 28 DIM, but, at 42 DIM, concentrations of INS in susceptible cows averaged 17% less ($P = 0.042$) than those in resistant cows (326 ± 22 and 392 ± 24 pg/ml, respectively). At all stages of the study, plasma glucose concentrations were similar between cows that were either susceptible or resistant to ketosis and that were fed the FR diet plus BD. Prepartum GLN concentrations were similar between susceptible and resistant cows, but concentrations of GLN increased in susceptible cows with the onset of lactation and, at 14 DIM, averaged 47% above ($P = 0.0005$) those in resistant cows (174 ± 7.6 and 118 ± 12.6 pg/ml, respectively). Glucagon concentrations remained elevated in susceptible cows through 28 DIM, then decreased, and were not different between the two groups at 42 DIM (data not shown). The ratio of INS to GLN was lower in susceptible cows than in resistant cows prepartum (2.72 ± 0.16 and 3.42 ± 0.26, respectively; $P = 0.032$) but declined in both groups with the onset of lactation and was not different between the two groups at 14 DIM. During the induction period, the ratio of INS to GLN in susceptible cows again averaged 30% less than that in resistant cows (1.78 ± 0.13 and 2.59 ± 0.15, respectively; $P = 0.0002$). The inability to adjust the ratio of INS to GLN properly may be one key factor that contributes to the onset of lactation ketosis.

Liver Composition

Hepatic TAG was not different among groups prepartum or through 14 DIM (Figure 6A). In normal cows fed the control diet, the hepatic TAG content increased with the onset of lactation, peaked at about 7 DIM at a mean of 237% of prepartum concentrations ($P = 0.10$), and then gradually declined thereafter. The decline seemed slower for cows fed the FR diet plus BD, and the overall mean values were 47% greater for cows fed the FR diet plus BD than for controls during the induction period; however, the difference was not significant.

Prepartum hepatic GLY contents (Figure 6B) were greater in cows fed the FR diet plus BD than in cows fed the control diet ($P = 0.037$); however, there were no subsequent differences between groups during the first 14 DIM. Hepatic GLY contents decreased in all groups with the onset of lactation and reached a minimum at 7 DIM, which was 56% below prepartum concentrations ($P < 0.0001$). Hepatic GLY then began to increase, and, by 35 DIM, the concentration of hepatic GLY was not different from the prepartum value in cows fed the control diet. However, in cows fed the FR diet plus BD, hepatic GLY was decreased by the induction protocol and, at 28 DIM, averaged 37% less than that of cows fed the control diet ($P = 0.026$). The hepatic GLY content in cows fed the FR diet plus BD then rebounded but averaged less than that of control cows throughout the remainder of the induction period.

Ratios of hepatic TAG to GLY were similar for all groups prepartum (Figure 6C). The ratio increased with the onset of lactation and, at 7 DIM, averaged more than seven times the prepartum ratio, primarily because of a major increase in the normal cows fed the FR diet plus BD. The ratio returned to prepartum values by 14 DIM but increased again in both obese and normal cows fed the FR diet plus BD; at 21 DIM, the ratio of hepatic TAG to GLY in cows fed the FR diet plus BD averaged more than 12 times that in cows fed the control diet (13.4 ± 2.7 and 1.0 ± 2.7, respectively; $P = 0.0023$). There were no significant

effects of BCS on hepatic TAG, GLY, or the ratio of TAG to GLY. Reid et al. (24) reported increased fatty liver in overconditioned cows at calving. The lack of a similar finding in the present study is consistent with the similarity in NEFA responses between obese and normal cows (Figure 4C).

Liver composition was not different between cows that were susceptible or resistant to ketosis, both obese and normal, that were fed the FR diet plus BD (data not shown) either prepartum or during the first 14 DIM. However, hepatic TAG increased in susceptible cows in response to the FR and BD protocol, and, at 21 DIM, the hepatic TAG content averaged three times greater than that in resistant cows (7.9 ± 1.6 and 2.7 ± 2.9%, respectively; $P = 0.018$). Hepatic TAG concentrations after 21 DIM were not different between susceptible and resistant cows until the onset of ketosis. At the time of clinical ketosis, hepatic TAG concentrations in cows that were susceptible to ketosis averaged about 10 times greater than those in cows that were resistant to ketosis (7.5 ± 1.7 and 0.7 ± 1.7%, respectively; $P = 0.011$). Thus, the response of hepatic TAG to the FR and BD protocol differed for cows that were susceptible or resistant to ketosis even before outward signs of the disease were displayed.

Similarly, effects of the FR and BD protocol on hepatic GLY content were greater in cows that were susceptible to ketosis. The minimum hepatic GLY content, which occurred at 7 DIM, was lower in susceptible cows than in resistant cows (1.2 ± 0.56 and 2.9 ± 0.72%, respectively; $P = 0.081$). However, there was no significant difference in hepatic GLY between susceptible and resistant cows at the onset of ketosis.

TABLE 5. Ratios of hepatic triacylglycerol (TAG) to glycogen (GLY) in cows fed a restricted diet plus 1,3-butanediol. Cows were susceptible (i.e., became ketotic) or were resistant to ketosis.[1]

Time peripartum	Susceptible cows	Resistant cows	SE[2]
(d)	(TAG:GLY)		
–10	0.6	0.3	4.2
7	12.8[a]	3.0[b]	2.6
14	1.8	1.1	2.6
21	23.4[a]	1.3[b]	2.8
28	3.7	1.3	3.5
35	2.2	1.4	2.8
42	1.9	1.4	2.6
49	3.7	0.2	4.3
56	0.6	1.4	3.3

[a,b]Means in the same row with different subscripts differ ($P < 0.01$).

[1]Values for susceptible and resistant cows represent least squares means of six and four observations, respectively.

[2]Pooled standard error.

Prepartum hepatic ratios of TAG to GLY were similar in cows that were susceptible or resistant to ketosis (Table 5); however, at 7 DIM, the ratio of TAG to GLY in susceptible cows averaged about four times greater than that in resistant cows ($P = 0.018$). In addition, the ratio of TAG to GLY was increased only in susceptible cows in response to the FR and BD protocol, and, at 21 DIM, the ratio of TAG to GLY in susceptible cows averaged 18 times greater than that in resistant cows. Previous studies with the FR and BD protocol have suggested that the ratio of TAG to GLY is very important to the development of ketosis. Drackley et al. (4) concluded that a postpartum hepatic ratio of TAG to GLY >2 was necessary for induction of ketosis. The precise ratio of TAG to GLY that indicates susceptibility to ketosis remains undefined, but in the current study susceptible cows had a mean ratio of TAG to GLY well in excess of 2 at 7 d postpartum. Furthermore, our data suggest that the ratio of hepatic TAG to GLY at 7 d postpartum might be used to predict susceptibility to ketosis. Although invasive biopsies have limited on-farm practicality, the TAG to GLY index could provide a useful tool for ketosis research, and a noninvasive technique for obtaining the percentage of fat in liver could have great benefits for dairy farmers.

DISCUSSION

The FR and BD protocol for inducing ketosis resulted in ketosis or ketonemia in 60% of the cows that received the FR diet plus BD. Obese cows were chosen from cows with a propensity for greater body condition, and we were able to obtain obese cows that averaged >90 kg of BW and 0.6 BCS units above control herdmates. Loss of BCS after calving was greater for obese cows, but feed intake, milk production, EB, plasma NEFA, and liver composition of obese cows were not different from those of normal cows. In addition, the proportion of cows that developed clinical or subclinical ketosis was the same for both obese and normal cows. Our data suggest that, for this population of cows, greater fatty acid mobilization, which resulted from increased lipid availability in the obese cows, was not large enough to overload mechanisms for the disposal of NEFA.

One possible explanation for why obesity at calving did not increase the incidence of fatty liver and ketosis in the current study is that the differences in BCS between obese and normal cows were not great enough to permit measurable differences. Differences might have been detectable if the mean difference in BCS had been greater (>1). One major difference

between our cows and those of Mills et al. (18, 19) was that our cows were overfed mostly in late lactation and during the early dry period; however, cows in the study of Mills et al. (18, 19) were overfed during the entire dry period. A study completed recently indicated that overfeeding cows continually throughout the entire dry period seriously increases the risk of development of fatty liver and clinical ketosis (A. R. Hippen, P. She, G. L. Lindberg, D. C. Beitz, and J. W. Young, 1997, unpublished data), even with groups of cows that have about the same differences in BCS as those in the current report. Differences in the timing of body condition gain and in the magnitude of gain and their effects on susceptibility to fatty liver and ketosis require further investigation.

Tracing the fate of NEFA was beyond the scope of this project; however, maximal rates of gluconeogenesis are dependent on fatty acid oxidation (3). In addition, our results suggest that obese cows rely on increased oxidation of fatty acids to dispose of NEFA. Oxidation of NEFA has the added effect of increasing the redox potential in hepatocyte mitochondria from the production of NADH. Ketogenesis, an incomplete oxidation of NEFA, may represent a mechanism to decrease the hepatic production of reducing equivalents by shifting the final stages of fatty acid oxidation to extrahepatic tissues.

Reliance on hepatic oxidation to remove excess NEFA may support increased gluconeogenesis; however, it also results in a deleterious increase in the flux of ketones. Our results are not in agreement with our initial hypothesis that increased lipolysis in obese cows would increase the ratio of TAG to GLY and ketone concentrations. However, the results do suggest that obese cows are in a delicate metabolic balance during early lactation and are highly susceptible to ketosis caused by any metabolic disturbance, including the FR and BD protocol.

CONCLUSIONS

This report adds to the general base of knowledge about fatty liver and lactation ketosis in high producing dairy cows. The results show that the incidences of ketonemia and clinical ketosis were about the same for normal and obese cows when the obese cows were overfed late in the previous lactation or early in the dry period. There were major differences in the general metabolic responses of both obese and normal cows that received the FR and BD protocol and that were susceptible to ketonemia or ketosis compared with responses of cows from the same categories that were resistant to ketosis. Evidence is accumulating that obese cows that are overfed throughout the entire dry period are generally very susceptible to the development of severe hepatic lipidosis and subsequent cases of clinical ketosis.

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