Effects of Biotin Supplementation on Peripartum Performance and Metabolites of Holstein Cows*

O. Rosendo,¹ C. R. Staples,¹ L. R. McDowell,¹ R. McMahon,²
L. Badinga,¹ F. G. Martin,³ J. F. Shearer,⁴ W. M. Seymour,⁵
and N. S. Wilkinson¹

¹Department of Animal Sciences,

²Department of Food Science and Human Nutrition,

³Department of Statistics, and

⁴College of Veterinary Medicine, University of Florida, Gainesville 32611

⁵Roche Vitamins Inc., Parsippany, NJ 07054

ABSTRACT

Fifty-two multiparous Holstein cows were randomly assigned to receive 0 or 20 mg of biotin/d starting at an average of 16 d prepartum and then switched to 0 or 30 mg of biotin/d from calving through 70 d postpartum to determine whether supplemental biotin would affect cow performance, hepatic lipidosis, and plasma metabolites. Mean concentration of biotin in plasma sampled weekly was greater in cows fed biotin (4.3 vs. 9.4 nmol/L). Postpartum dry matter intake as a percentage of body weight (3.9% vs. 4.0%), milk production (35.8 vs. 34.8 kg/d), and milk fat concentrations (3.59% vs. 3.69%) were similar between treatment groups. Milk from biotin-supplemented cows tended to have a greater concentration of protein (2.73% vs. 2.83%). Concentrations of plasma nonesterified fatty acids were lower at wk 2 (652 vs. 413 μ Eq/mL) and 4 (381 vs. 196 μ Eq/mL) postpartum in cows fed supplemental biotin. However, mean plasma concentrations of β -hydroxybutyric acid were not affected by biotin supplementation. Mean concentration of plasma glucose was greater for lactating cows fed supplemental biotin (63.4 vs. 66.6 mg/dL). Biopsies of liver were taken at 2, 16, and 30 d postpartum. The triacylglycerol concentration in liver (wet basis) tended to decrease at a faster rate after d 2 postpartum with biotin supplementation compared with control cows. The potential mechanisms that link improved glucose status and decreased lipid mobilization in cows supplemented with biotin warrant further investigation.

(Key words: biotin, energy, liver, milk)

Abbreviation key: TAG = triacylglycerol.

INTRODUCTION

Using in vitro continuous culture techniques, Abel et al. (2001) determined that biotin synthesis by ruminal microorganisms was reduced as the proportion of dietary concentrate increased. Feeding high concentrate diets is common during the periparturient and early lactation periods. Supplemental biotin has exerted beneficial effects on hoof health and integrity (Midla et al., 1998; Bergsten et al., 2003), milk production (Zimmerly and Weiss, 2001; Bergsten et al., 2003; Majee et al., 2003), protein yield (Zimmerly and Weiss, 2001; Majee et al., 2003), lactose yield, and DMI (Majee et al., 2003). The increase in milk production when biotin was supplemented in field trials could possibly be a result of increased DMI caused by improved hoof health (Midla et al., 1998; Bergsten et al., 2003). However, Midla et al. (1998) reasoned that biotin supplementation to dairy cows might lead to increased capacity of biotin-dependent carboxylase enzymes; Zimmerly and Weiss (2001) suggested that biotin may increase milk yield through increased glucose production and/or increased fiber digestibility. However, supplemental biotin had no effect on the molar percentages of ruminal VFA at either 30 or 100 DIM (Zimmerly and Weiss, 2001) nor on the apparent total tract DM, OM, and NDF digestibilities (Majee et al., 2003). Moreover, the actual effects of biotin status on carboxylase activities in ruminants have not been reported.

Biotin serves as an essential coenzyme for 4 carboxylases in mammals, 2 of which catalyze critical steps in the gluconeogenic process. Rate of glucose synthesis increased after parenteral biotin was administered to starved pregnant ewes close to lambing (Kempton et al., 1978). Low energy intake around calving causes the dairy cow to mobilize lipid from its adipose tissue, which

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Corresponding author: L. R. McDowell; e-mail: mcdowell@animal.ufl.edu.

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can result in fatty liver infiltration (Grummer, 1993). During that period and especially during development of fatty liver, control of gluconeogenesis by the hepatic rate-limiting enzymes seems to be altered (Rukkwamsuk et al., 1999). A number of periparturient diseases such as ketosis, retained placenta, metritis, milk fever, mastitis, and laminitis are associated with fatty liver (Breukink and Wensing, 1998). If supplemental biotin improves milk production through either an increased ruminal fiber digestion or hepatic gluconeogenesis in dairy cows (Weiss and Zimmerly, 2000), an additional benefit of improved energy status in dry and periparturient cows may mean a reduction in the occurrence of metabolic disorders such as fatty liver and ketosis. Therefore, the objective of this study was to test the effects of supplemental biotin to cows during the late dry and early lactation periods on performance, hepatic lipidosis, and plasma metabolites.

MATERIALS AND METHODS

Cows, Diets, and Experimental Design

Fifty-two nonlactating, pregnant multiparous cows that were due to calve from September 14 to October 6, 2000 were moved into shaded pens and trained to use electronic feed gates (Calan gates; American Calan Inc., Northwood, NH) at approximately 4 wk prior to expected calving date (range = 20 to 30 d) at the University of Florida Dairy Research Unit (Hague). Cows were blocked according to expected calving date, previous lactation milk yield, and BCS and were randomly assigned within block to 1 of 2 diets (0 or 20 mg/d of supplemental biotin [Rovimix-H 100, Roche Vitamins Inc., Parsippany, NJ]). Treatment diets were intended to be fed from 20 d before expected date of calving until the day of calving, but because of differences between actual and estimated calving dates, some cows calved earlier than expected. Seven cows were excluded from the experiment because they consumed their assigned diets for <10 d before calving. After calving, 3 cows were removed from the experiment because they did not retrain fully to the Calan gates in the free stall barn. In addition, 4 other cows were removed for the following health reasons: one cow had ketosis and displaced abomasum, one cow had metritis and was in very poor condition, one cow had laminitis, and one cow had severe mastitis. Therefore, data from 14 cows were not included in analyses of performance and metabolites. The University of Florida Animal Care and Use Committee approved the handling and sampling of animals in this experiment.

Cows calved between September 1 and October 21 and continued in the experiment until 70 DIM. All non-

Table 1. Ingredient composition of diets fed during the close-up and lactating periods.

Ingredient	Close-up ¹	Lactating ²
	(% of DM)	
Bermudagrass hay	13.6	...
Corn silage	38.4	26.6
Alfalfa hay	...	10.0
Cottonseed hulls	...	5.1
Citrus pulp	3.8	9.5
Prolak ³	...	1.4
Distillers grains	3.5	...
Corn meal	22.4	21.3
Soybean meal	3.6	11.4
Whole cottonseed	7.6	9.8
Mineral and vitamin mix ⁴	...	4.7
Mineral and vitamin mix ⁵	6.7	...
Biophos ⁶	...	0.2
Trace mineral salt ⁷	0.5	...

¹Diets for close-up dry cows were formulated to contain 0 or 0.00017% supplemental biotin (Rovimix H-100; 0.022% biotin; Roche Vitamins Inc., Parsippany, NJ) (DM basis).

²Diets for lactating cows were formulated to contain 0 or 0.00011% supplemental biotin (Rovimix H-100; 0.022% biotin, Roche Vitamins Inc.) (DM basis).

³H. J. Baker and Bro., Inc. (Atlanta, GA).

⁴Contained (DM basis) 10.2% Ca, 0.90% P, 3.1% Mg, 1.5% S, 5.1% K, 8.6% Na, 1698 mg/kg of Zn, 512 mg/kg of Cu, 339 mg/kg of Fe, 2231 mg/kg of Mn, 31 mg/kg of Co, 26 mg/kg of I, 7.9 mg/kg of Se, 147,756 IU vitamin A/kg, 43,750 IU of vitamin D/kg, and 787 IU of vitamin E/kg.

⁵Contained (DM basis) 22.9% Ca, 0.2% P, 0.2% K, 2.8% Mg, 0.7% Na, 2.4% S, 8% Cl, 147 mg/kg of Mn, 27 mg/kg of Fe, 112 mg/kg of Cu, 95 mg/kg of Zn, 7 mg/kg of Se, 8 mg/kg of I, 268,130 IU vitamin A/kg, 40,000 IU/kg of vitamin D, and 1129 IU/kg of vitamin E.

⁶Contained (DM basis) 16% Ca, 21.2% P, 0.04% K, 0.70% Mg, 1% S, and 0.08% Na.

⁷Contained (DM basis) 40% Na, 55% Cl, 0.25% Mn, 0.2% Fe, 0.033% Cu, 0.007% I, 0.005% Zn, and 0.0025% Co.

lactating cows were fed their assigned diet (Tables 1 and 2) twice daily (0900 and 1500 h) in ad libitum amounts until calving. The concentrate portion of the TMR was identical except for the inclusion of supplemental biotin (Rovimix-H 100) at 0 and 0.02% for the 0- and 20-mg/d treatments, which replaced corn meal.

After calving, all cows were moved to the same free stall barn with electronic feed gates, where they remained until 70 d postpartum. Diets were fed twice daily as TMR in ad libitum amounts to ensure approximately 10% orts (as-fed basis). The 2 concentrate mixes were identical except for the inclusion of supplemental biotin at 0 and 0.04% (DM basis) for the 0- and 30-mg/d treatments, which replaced corn meal.

Measurements, Sampling, and Chemical Analyses

Amount of feed offered and orts removed for each cow was recorded daily. Corn silage samples were obtained weekly and dried at 55°C for 48 h to determine DM. Results were used to adjust the forage-to-concentrate

Table 2. Chemical composition of diets fed during the close-up and lactating periods.

Chemical analysis	Close-up ¹	Lactating ²
	(Mcal/kg) ³	
NE _L	1.53	1.63
	(% of DM)	
CP	13.4	17.3
ADF	21.2	23.0
NDF	36.1	34.3
Ca	2.32	1.15
P	0.34	0.39
Mg	0.42	0.34
K	1.08	1.30
Na	0.30	0.42
S	0.27	0.22
Cl	1.10	0.28
	(mg/kg of DM)	
Fe	310	270
Zn	102	96
Cu	24	28
Mn	50	73

¹Diets for close-up dry cows were formulated to contain 0 or 0.00017% supplemental biotin (Rovimix H-100: 0.022% biotin; Roche Vitamins Inc., Parsippany, NJ) (DM basis).

²Diets for lactating cows were formulated to contain 0 or 0.00011% supplemental biotin (Rovimix H-100: 0.022% biotin; Roche Vitamins Inc.) (DM basis).

³NE_L calculated according to NRC (2001) NE_L values of individual feed ingredients at 3× maintenance.

ratio. Dried corn silage samples were composited over 4-wk periods. Bermudagrass hay, alfalfa hay, and concentrate samples were obtained weekly, stored frozen, and then composited over 4-wk periods and dried at 55°C for 48 h. All composited and dried feed samples were ground through the 1-mm screen of a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA) and sent to a DHIA Forage Testing Laboratory (Dairy One, Ithaca, NY), where feed samples were analyzed for CP, NDF, ADF, DM, Ca, P, Mg, K, Na, Zn, Cu, Mn, S, Fe, and Cl contents.

Body weight was determined on approximately d 20 (pretreatment) before expected calving date and on the same day each week throughout the trial after the morning milking. Body condition score (one evaluator) was determined on d 20 (pretreatment) before expected calving date, at week of calving, and at wk 2 and 4 postpartum.

Energy balance postpartum was calculated weekly as the difference between energy consumed and required, where NE_L required postpartum = NE_L for maintenance plus NE_L for milk. The calculation of the NE_L content of the diet was based on NRC (2001) NE_L values of individual feed ingredients at 3 × maintenance. Energy required for maintenance (Mcal/d) = $0.08 \times \text{kg BW}^{0.75}$, and for lactation = $\text{kg of milk} \times [(0.0929 \times \text{milk fat } \%) + (0.0547 \times \text{milk protein } \%) + 0.192]$ (NRC, 2001).

Cows were milked at 0100, 0900, and 1700 h. Daily milk yield was electronically recorded. Milk samples (40-mL vial with preservative) were taken weekly on consecutive morning and afternoon milkings and were analyzed for CP, fat, and MUN by a DHI milk testing laboratory (McDonough, GA). Milk samples (no preservative) taken at 28 DIM were stored frozen at -20°C and then centrifuged at $14,000 \times g$ for 15 min in a refrigerated centrifuge (Sorvall RC-5 Superspeed Refrigerated Centrifuge; DuPont Instruments, Wilmington, DE) to remove the fat layer and obtain an aliquot of the skim fraction for analysis of biotin (see subsequently).

Blood samples were collected via the coccygeal arterio-vein at 5 h after the morning feeding each week using evacuated tubes containing sodium heparin (Vacutainer; Becton Dickinson, Franklin Lakes, NJ). Tubes were immediately placed on ice and centrifuged within 4 h at 4°C for 30 min at $3000 \times g$. Plasma was harvested and stored at -20°C until analyzed.

Biotin concentrations in plasma (wk -2 to 10) and skim milk (28 DIM) were measured as avidin-binding substances by a competitive binding assay of biotin (Lewis et al., 2001) without HPLC separation for biotin and its metabolites. This avidin-binding assay measures the ability of biotin to compete with biotinylated protein adsorbed to plastic for the binding site of avidin linked to horseradish peroxidase. A 1:20 dilution was used for plasma, and a 1:800 or a 1:1600 dilution was used for skim milk.

Plasma NEFA were determined weekly using the NEFA-C kit (Wako Chemicals USA Inc., Richmond, VA) with the modifications of Johnson and Peters (1993). Plasma BHBA was determined weekly using the BHBA kit (Sigma Chemical Co., St. Louis, MO). Plasma glucose was analyzed (wk -2 to 10) in an automated analyzer (Bran + Luebbe, model II; Bran + Luebbe Analyzing Technologies, Elmsford, NY) following the colorimetric procedure of Gochman and Schmitz (1972).

Liver samples (mean = 2.5 g wet weight, SD = 1.5) were obtained by biopsy on average at d 16 (10 to 30 d) before calving date and on d 2 (1 to 4), 16 (14 to 18 d), and 30 (27 to 34 d) postpartum. Upon collection, the liver tissue was rinsed with saline to removed excess blood and immediately placed into liquid nitrogen. After freezing, samples were placed on dry ice for up to 2 h. Samples were stored at -70°C until analyzed for total lipid, triacylglycerol (TAG), protein, glycogen, and DM content. Liver lipids were extracted by the method of Drackley et al. (1992). Liver TAG content was determined by the method of Foster and Dunn (1973). Liver glycogen was determined by the method of Lo et al. (1970). Liver total protein was measured by the method of Lowry et al. (1951).

Statistical Analysis

Measurements of DMI postpartum, milk yield, and milk composition were reduced to weekly means before statistical analysis. Analyses were performed using PROC MIXED of SAS (2001).

Data for DMI, BW, BCS, milk yield, milk composition, energy balance, plasma metabolites, and liver metabolites were analyzed according to the following model:

$$Y_{ijk} = \mu + T_i + C_{ji} + D_k + TD_{ik} + e_{ijk}$$

where

Y_{ijk} = dependent variable,
 μ = overall mean of the population,
 T_i = effect of treatment i ,
 C_{ji} = effect of cow j nested within treatment i ,
 D_k = effect of time period k of sampling relative to parturition,
 TD_{ik} = interaction effect of treatment i by time period k relative to parturition, and
 e_{ijk} = unexplained residual assumed to be independent and identically distributed normally ($0, \sigma^2$).

Cow nested within treatment was used in the random statement as the error term to test for treatment effects. Prepartum and postpartum data were analyzed separately for DMI measurements. Time period represented daily measurements for DMI during the prepartum period but represented weekly means for DMI as a percentage of BW prepartum and all DMI measurements postpartum. Week 0 represents the week of calving.

In addition to this analysis, a second analysis was conducted that considered day as a regression variable testing for linear and quadratic effects and interactions with treatment using just postpartum values for liver lipid and TG concentrations.

Data from a liver biopsy obtained at -16 d relative to calving from a control cow were removed from the statistical analysis because total lipids and TAG concentrations were extremely high. Total lipid from this cow was 23.7% (wet weight) and was 3.5 standard deviations from the mean (6.8%). After these data were excluded, mean, standard deviation, and range, respectively, for hepatic total lipids at d -16 were 6.35, 2.35, and 2.9 to 14.4% (wet weight). Liver TAG concentration from this cow was 16.7% (wet weight), also 3.5 standard deviations from the mean (2.9%). After these data were excluded, mean, standard deviation, and range, respectively, for TAG were 2.5, 1.9, and 0.73 to 9.2% (wet weight).

For each analyzed variable, cow nested within treatment was subjected to 4 covariance structures: com-

Table 3. Postpartum diseases of multiparous Holstein cows.

Disorder	Treatment ¹	
	Control (n =18)	Biotin (n = 20)
	(Number of cases)	
Retained fetal membrane	2	3
Metritis	5	9
Mastitis	3	6
Foot problems	4	1
Digestive upset	1	0
Unexplained	1	0

¹Control = 0 mg biotin/d pre- and postpartum; Biotin = 20 mg of biotin/d prepartum and 30 mg biotin/d postpartum.

pound symmetric, heterogeneous compound symmetry, autoregressive order one, and unstructured variance components. The covariance structure that yielded the better fit statistics criteria was considered to be the most desirable analysis. Heterogeneous compound symmetry was used for liver lipids (total lipids and TAG concentrations) analysis. Treatment differences or treatment by time interactions were considered significant at $P < 0.05$, to have a tendency toward significant at $P = 0.05$ to $P < 0.15$, and to be nonsignificant at $P \geq 0.15$. When treatment by time interactions were significant or tended to be significant, the slice option for the LSMEANS statement in PROC MIXED was used to determine which time period means were different.

Postpartum disease incidence rates were analyzed using Fisher's exact test (Agresti, 1996), which is more sensitive for small sample sizes than the χ^2 test. The Odds Ratio test also was used.

RESULTS

Production Data

A total of 38 cows ($n = 20$ for biotin treatment and $n = 18$ for control) completed the experiment. For cows that completed the experiment, time on treatment prepartum after cows were trained to Calan gates ranged from 10 to 27 d, with an average of 16 d for the control cows. Cows fed biotin were on treatment from 10 to 25 d, with an average of 17 d. The overall frequency of postpartum diseases (Table 3) was quite high, especially for metritis. However, incidence rate did not differ between treatment groups for any disorder measured.

Performance parameters are depicted in Table 4. Cows fed supplemental biotin consumed similar amounts of DM daily as control cows when expressed as kilograms per day and as a percentage of BW (Table 4) during both the prepartum and postpartum periods. Biotin supplementation had no effect on BW pre- or postpartum (Table 4). Body weight increased after calv-

Table 4. Effect of biotin supplementation on prepartum and postpartum performances of multiparous Holstein cows.

Measurement	Treatment ¹		SEM	<i>P</i>	
	Control (n = 18)	Biotin (n = 20)		Treatment	Treatment by time
Close-up period					
DMI, kg/d	8.6	10.2	0.8	0.15	0.17
BW, kg	647.6	653.8	17.1	0.80	0.56
DMI, % of BW	1.3	1.6	0.1	0.19	0.61
Lactating period					
DMI, kg/d	22.1	23.6	0.7	0.15	0.18
DMI, % of BW	3.9	4.0	0.1	0.55	0.17
BW, kg	570.1	587.6	14.4	0.39	0.34
BCS ²	2.7	2.9	0.1	0.20	0.80
BCS change ³	−0.46	−0.45	0.11	0.94	...
Energy balance, Mcal/d	2.6	4.8	1.2	0.18	0.76
Milk, kg/d	35.8	34.8	1.3	0.61	0.73
Milk fat					
%	3.59	3.69	0.08	0.33	0.86
kg/d	1.34	1.34	0.05	0.96	0.72
Milk protein					
%	2.73	2.83	0.05	0.12	0.98
kg/d	1.02	1.02	0.04	0.91	0.89
MUN, %	12.8	11.8	0.6	0.25	0.10
4% FCM, ⁴ kg/d	35.0	34.7	1.2	0.84	0.76

¹Control = 0 mg of biotin/d pre- and postpartum; Biotin = 20 mg of biotin/d prepartum and 30 mg biotin/d postpartum.

²Measured from week of calving to 4 wk postpartum, 1 = thin to 5 = fat.

³Change from 3 wk prepartum to 4 wk postpartum.

⁴Calculated as follows: $([0.4 \times \text{milk } \{\text{kg/d}\}] + [15 \times \text{fat } \{\text{kg/d}\}])$.

ing (time effect, $P < 0.0001$), but the treatment by time interaction was not significant for either the pre- or postpartum periods. Body condition score or BCS change was not affected by supplemental biotin.

Mean or weekly pattern of milk yield (Figure 1), 4% FCM yield, milk fat percentage, milk fat yield, and

Figure 1. Milk yield of cows fed 0 (control group; n = 18; $\diamond$) or 30 mg/d of supplemental biotin (biotin group; n = 20; $\blacksquare$) during the first 10 wk of lactation. Values represent least square means. Effect of time was significant ($P < 0.05$).

MUN concentration were not affected by treatment (Table 4). Biotin supplementation tended ($P = 0.12$) to increase mean milk protein percentage, but milk protein yield was not different ($P = 0.91$) (Table 4). Highly significant time effects ($P < 0.0001$) were detected for DMI, milk yield, 4% FCM yield, milk protein percentage, and milk protein yield, and a tendency ($P = 0.09$) was observed for milk fat percentage but not for MUN. The MUN concentrations at wk 2 and 7 tended to be lower for cows fed biotin (treatment by time interaction, $P = 0.10$). No interactions were detected for the other production variables. Net energy balance during lactation (Table 4 and Figure 2) was not affected by biotin supplementation but increased weekly postpartum as expected.

Plasma Parameters and Milk Biotin Concentrations

Mean plasma biotin concentration was increased ($P = 0.0001$) by supplemental biotin (Table 5). This increase was dramatic within the first week of supplementation (Figure 3). Cows were fed supplemental biotin, on average, for between 3 and 9 d before the wk -2 blood sample was collected. Therefore, the increase in plasma biotin occurred very quickly. Upon calving, biotin concentration decreased to a greater degree in cows given supplemental biotin (treatment by time interaction, $P = 0.017$)

Figure 2. Net energy balance of cows fed 0 (control group; $n = 18$; $\diamond$) or 30 mg of supplemental biotin/d (biotin group; $n = 20$; $\blacksquare$) during the first 10 wk of lactation. Values represent least square means. Effect of time was significant ($P < 0.05$).

Figure 3. Plasma biotin concentrations of cows fed 0 (control group; $n = 18$; $\diamond$) or 20 and 30 mg/d (biotin group; $n = 20$; $\blacksquare$) of supplemental biotin/d prepartum and postpartum, respectively. Values represent least square means. Treatments differed at week designated by * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$. Effect of time was significant ($P < 0.05$).

compared with control cows. In addition, plasma biotin concentrations during lactation were steady in cows supplemented with biotin, but control cows experienced a continuous decline. By 3 wk postpartum, milk biotin concentrations were at least twice as high in cows fed supplemental biotin ($P < 0.01$) compared with control cows.

Biotin supplementation increased mean plasma glucose concentration (Table 5) with the increase being very consistent across weeks postpartum, as values were similar only at wk 5, 6, and 7 postpartum. Neither mean concentration of plasma BHBA (Table 5) nor mea-

surements made weekly were affected by biotin feeding. Cows supplemented with biotin tended to have lower ($P = 0.09$) mean plasma NEFA concentrations (Table 4). Plasma NEFA concentrations were highest immediately postpartum and declined as lactation progressed (Figure 4). A tendency for a time by treatment interaction occurred for plasma NEFA ($P = 0.10$) because of the higher concentrations at wk 2 and 4 for control cows. Concentrations reached values comparable to prepartum (wk 2 before calving) at wk 5 postpartum

Table 5. Effect of biotin supplementation on concentrations of selected compounds in plasma, milk, and liver of multiparous Holstein cows.

Measurement	Treatment ¹		SEM	<i>P</i>	
	Control	Biotin		Treatment	Treatment by time
Plasma ²					
Biotin, nmol/L	4.3	9.4	0.5	0.0001	0.02
Glucose, mg/dL	63.4	66.6	0.8	0.008	0.31
NEFA, μ eq/mL	297.2	239.4	26.5	0.13	0.12
BHBA, mg/dL	5.4	4.8	0.3	0.19	0.83
Milk biotin (28 DIM), nmol/L	386	1061	54	0.0001	...
Liver, % (wet basis) ³					
Total lipids ⁴	6.1	5.6	0.4	0.37	0.10
Triacylglycerols ⁵	2.4	2.0	0.4	0.41	0.10
Glycogen	1.5	1.8	0.2	0.22	0.40
Glycogen:triacylglycerol	1.0	1.2	0.2	0.34	0.42
Total proteins	17.6	17.6	0.4	0.89	0.22

¹Control = 0 mg of biotin/d pre and postpartum; Biotin = 20 mg of biotin/d prepartum and 30 mg biotin/d postpartum.

²Control ($n = 20$); Biotin ($n = 18$).

³Control ($n = 18$); Biotin ($n = 17$).

⁴Linear decrease by time effect ($P = 0.10$).

⁵Linear decrease by time effect ($P = 0.05$).

Figure 4. Plasma NEFA concentrations of cows fed 0 (control group; $n = 18$; $\diamond$) or 20 and 30 mg/d (biotin group; $n = 20$; $\blacksquare$) of supplemental biotin/d prepartum and postpartum, respectively. Values represent least square means. Treatments differed at week designated by $**P < 0.01$ and $***P < 0.001$. Effect of time was significant ($P < 0.05$).

(Figure 4). A time effect ($P = 0.0001$) was observed for plasma concentrations of glucose, NEFA, and BHBA.

Liver Composition

No liver biopsy samples were obtained from one control cow and one cow on biotin treatment. Data from one control cow was dropped because it was considered an outlier as previously noted. Numbers of liver samples were as followed: $n = 17$ for control and $n = 18$ for biotin treatment at 2 DIM, $n = 17$ for both control and for biotin treatment at 16 DIM, and $n = 14$ for control and $n = 16$ for biotin treatment at 30 DIM.

Figure 5. Total lipids concentration of liver (% wet weight) at 2 d ($\square$; $n = 17$)($\blacksquare$; $n = 18$), 16 d ($\square$; $n = 17$)($\blacksquare$; $n = 17$), and 30 d ($\square$; $n = 16$)($\blacksquare$; $n = 14$) after calving from cows fed 0 (control group) or 20 and 30 mg/d of supplemental biotin/d (biotin group) prepartum and postpartum, respectively. Values represent least square means. Test of effects of treatment by day interaction was $P = 0.10$, of linear decrease of treatment by day interaction was $P = 0.05$, and of time was $P < 0.05$.

Figure 6. Triacylglycerol concentrations of liver (% wet weight) at 2 d ($\square$; $n = 17$)($\blacksquare$; $n = 18$), 16 d ($\square$; $n = 17$)($\blacksquare$; $n = 17$), and 30 d ($\square$; $n = 16$)($\blacksquare$; $n = 14$) after calving from cows fed 0 (control group) or 20 and 30 mg/d of supplemental biotin/d (biotin group) prepartum and postpartum, respectively. Values represent least square means. Test of effects of treatment by day interaction was $P = 0.10$, of linear decrease of treatment by day interaction was $P = 0.05$, and of time was $P < 0.05$.

When the 4 biopsy times were included in the model, both liver lipid and liver TAG concentrations were similar between the 2 treatment groups at each sampling time. When the prepartum (-16 d relative to calving) sample was dropped from the analysis, a tendency ($P = 0.10$) for a treatment by time interaction was detected for both hepatic total lipids concentration (percentage, wet basis) and hepatic TAG concentration (Table 5). Liver lipid concentration tended to decrease at a faster rate over time for cows supplemented with biotin compared with control cows (linear decrease by treatment interaction, $P = 0.10$). Means were 6.1%, 7.0%, and 5.4% for controls vs. 6.3%, 5.8%, and 4.7% for biotin-supplemented cows at 2, 16, and 30 DIM, respectively (Figure 5). Liver TAG concentration tended to decrease at a faster rate over time for cows supplemented with biotin compared with control cows (linear decrease by treatment interaction, $P = 0.05$). Means were 2.4%, 2.9%, and 1.9% for controls vs. 2.5%, 2.2%, and 1.2% for biotin-supplemented cows at 2, 16, and 30 DIM, respectively (Figure 6). Biotin supplementation had no effect on mean hepatic concentrations of glycogen, glycogen to TAG ratio, and percentage of total protein (wet basis; Table 5).

DISCUSSION

Biotin supplementation did not affect DMI when expressed as a percentage of BW during the prepartum (20 mg of biotin/d) nor postpartum (30 mg biotin/d) periods. Data on DMI was not reported in studies on supplemental biotin at commercial herds (Midla et al., 1998; Bergsten et al., 2003). In 2 of 3 control-type studies, biotin supplementation to lactating dairy cows during the first 100 d of lactation did not affect DMI (Zim-

merly and Weiss, 2001). Majee et al. (2003) recently reported that DMI was 0.7 kg/d higher for cows between 46 to 160 DIM that were supplemented with 20 mg/d of dietary biotin when compared with the DMI of control cows. These differences across studies do not seem to be related to diet composition.

Mean milk production was similar between cows with and without biotin supplementation. Mature equivalent, 305-d milk production was 878 kg greater in 46 cows supplemented with 20 mg of biotin/d than in 48 control cows (Bergsten et al., 2003). The 305-d mature equivalent milk production of first lactation heifers taken from monthly DHIA records was greater for the supplemented biotin group than for the control group ($12,110 \pm 2500$ vs. $11,790 \pm 4520$ kg) (Midla et al., 1998). However, actual daily milk production was not measured in these studies. Recent controlled studies also have reported that biotin supplementation increased milk production in dairy cows. Zimmerly and Weiss (2001) reported that milk yield was increased linearly by 0.9 and 2.8 kg/d above control (no supplemental biotin) for cows consuming 10 and 20 mg/d of supplemental biotin, respectively, during the first 100 DIM with no significant change in DMI. Majee et al. (2003) reported that milk yield was 1.7 kg/d greater for cows supplemented with 20 mg/d of dietary biotin compared with control cows between 46 to 162 DIM. In a second trial, milk yield from cows fed 40 mg/d of biotin was similar to that of cows fed 20 mg/d of supplemental biotin or a B-vitamin blend (Majee et al., 2003). Why biotin did not stimulate milk production in the current study is unclear.

The mean concentration of milk fat was not affected by biotin supplementation. Similar results have been found by others (Zimmerly and Weiss, 2001; Majee et al., 2003). If supplemental biotin does improve energy status as suggested by Zimmerly and Weiss (2001), a resulting decrease of lipolysis in adipose tissue around parturition may produce a shortage of endogenous fatty acids for milk fat synthesis in the mammary gland. However, activity and expression of acetyl CoA carboxylase in the mammary gland of lactating ewes was increased during the periparturient period (Travers and Barber, 2001). Therefore, supplemental biotin may maintain milk fat by promoting a greater acetyl CoA carboxylase activity in the mammary gland as suggested by Steinberg et al. (1995).

As in our study, milk protein percentage, but not milk protein yield, tended to be improved by biotin (Zimmerly and Weiss, 2001; Majee et al., 2003). Positive milk protein responses to biotin supplementation may be partially due to increased microbial growth. Both cellulolytic and saccharolytic microbes may require biotin to digest cellulose or to produce propionate (Bal-

dwin, 1995). Biotin supplementation potentially may increase propionate synthesis in the rumen, which might also have a positive effect on the net yield of ATP available for microbial growth and microbial protein synthesis. If propionate production were increased by biotin supplementation, it would also explain increases in milk lactose. In those studies that have shown positive effects of supplemental biotin on milk yield (Zimmerly and Weiss, 2001), it is unknown if either cellulose or fiber digestion was improved. Total tract DM, OM, or NDF digestibilities were not affected by the inclusion of 20 mg/d of supplemental biotin in the diet (Majee et al., 2003). In vitro data suggest that ruminal basal concentrations of biotin for lactating dairy cows exceeded requirements for microbial growth and that 20 and 40 mg of supplemental biotin/d increased the basal concentration up to 180 and 370 times without improvement in ruminal forage fiber digestibility (Rosendo et al., 2003). The results from this in vitro study may explain why the results of Majee et al. (2003) did not support the suggestion of Zimmerly and Weiss (2001) that biotin increases milk yield partially through increased fiber digestibility. More studies that specifically address the possible effects of supplemental biotin on rumen function are needed.

Similar to Zimmerly and Weiss (2001), a greater concentration of plasma biotin was observed at 2 wk before parturition than during lactation. However, plasma biotin concentrations at 2 wk before calving in the current study were more comparable with those reported by Steinberg et al. (1995). They observed a mean concentration of plasma biotin of 14.85 nmol/L in non-lactating cows receiving 20 mg of biotin/d.

For the lactating period, concentrations of biotin in plasma for control cows and cows fed 30 mg of supplemental biotin/d were slightly higher than previously reported (Midla et al., 1998; Zimmerly and Weiss, 2001). Cows supplemented with 20 mg of biotin/d had 3.07, 7.12, and 7.04 nmol/L serum biotin at 25, 108, and 293 DIM, respectively (Midla et al., 1998). Recently, Zimmerly and Weiss (2001) observed mean plasma biotin of 2.70 nmol/L in control cows as compared with 4.3 nmol/L in the present study. Cows fed 10 or 20 mg of biotin/d from 14 d before calving had mean plasma biotin concentrations of 9.41 and 18.54 nmol/L, respectively, at calving. Mean plasma biotin concentrations from 30 to 100 DIM were 3.40 and 4.99 nmol/L for cows supplemented with 10 and 20 mg of biotin/d (Zimmerly and Weiss, 2001) as compared with 9.4 nmol/L in cows fed 30 mg/d of biotin in the present study.

Zimmerly and Weiss (2001) observed an increase in both plasma and milk (colostrum) biotin concentrations at calving (1 DIM) for cows fed 10 and 20 mg of supplemental biotin/d during 14 d prepartum. In the current

study, biotin was not assayed in colostrum. However, we did not find an increase in plasma biotin at week of calving (Figure 3) in cows fed 20 mg of biotin/d as reported by Zimmerly and Weiss (2001). Those researchers suggested that events associated with parturition altered plasma biotin concentrations in biotin-supplemented cows. However, the avidin-binding assays used to measure biotin concentrations are not specific for biotin. Significant quantities of avidin-binding substances other than biotin (e.g., bisnorbiotin, biotin sulfide) are present in human plasma. Data on the presence of avidin-binding substances other than biotin in cattle serum or milk have not been published.

Similar to previous reports, lactation reduced plasma biotin concentrations as compared with the dry period. At 28 DIM, milk biotin concentrations in cows fed supplemental biotin were greater than those reported previously (Midla et al., 1998; Zimmerly and Weiss, 2001), but closer to those reported by Steinberg et al. (1995). Steinberg et al. (1995) found an increase in milk biotin concentration from an average of 112.0 to 607.5 nmol/L after supplementing with 20 mg biotin/d.

Zimmerly and Weiss (2001) calculated that circulating biotin in plasma was 0.04 mg for cows fed 20 mg of biotin/d with 2.8 mg of biotin/d excreted in milk, which calculates a turnover of 70 (2.8/0.04) times circulating plasma biotin level.

Plasma glucose concentration was increased by supplemental biotin in this experiment. Rats supplemented with biotin had greater blood glucose as compared with nonsupplemented animals (Marshall et al., 1985). Holstein cows fed either 10 or 20 mg/d of supplemental biotin from 14 d before expected calving date to 100 DIM had similar concentrations of plasma glucose at 1, 30, 60, and 100 DIM (Zimmerly and Weiss, 2001). Early lactating Holstein cows fed 20 mg/d of supplemental biotin alone, 20 mg/d plus a B-vitamin blend, or 40 mg/d plus a B-vitamin blend experienced no change in concentration of plasma glucose (Majee et al., 2003). A daily intramuscular injection of 500 μ g of biotin into fasted pregnant ewes increased glucose synthesis rates up to 38% (Kempton et al., 1978). Increased plasma glucose concentration could be a result of increased gluconeogenesis, glycogenolysis, or both. Both in vivo and in vitro rat and chicken models of biotin depletion-repletion have shown an effect on gluconeogenic biotin-dependent carboxylase activities (pyruvate carboxylase and propionyl CoA carboxylase) (Bannister et al., 1983). However, the response may vary depending upon the content of the biotinylated pool of enzymes (Lewis et al., 2001).

Additionally, biotin affects the amounts (protein mass) and the genetic expression of holocarboxylase synthetase (catalyzes the formation of carboxylases

from inactive apocarboxylases) among other enzymes in rat models (Rodriguez-Melendez et al., 2001). It is not known if the same may occur in ruminants. Alternatively, biotin supplementation potentially increases propionate synthesis in the rumen. The availability of propionate is the major factor determining the rate of gluconeogenesis (Drackley et al., 2001). In the current study, a greater plasma glucose concentration also might have contributed to the tendency for a greater milk protein percentage in cows fed biotin because of less oxidation of AA by nonmammary tissues. It may be speculated also that plasma glucose was higher in biotin-supplemented cows in this study because biotin-supplemented cows did not convert the additional glucose into milk, whereas in previous studies milk yield, but not plasma glucose, was increased by supplemental biotin.

As previously described (Grum et al., 1996), a sharp increase in plasma NEFA at calving was detected. Decreased NEFA concentrations in plasma during biotin supplementation have not been observed previously (Zimmerly and Weiss, 2001; Majee et al., 2003). In this study, plasma NEFA concentrations tended to be decreased by biotin supplementation, most likely because of inhibition of lipolysis by elevated plasma glucose, despite the fact that BCS change in the present study was not affected by biotin supplementation.

Although plasma NEFA concentrations tended to be decreased by biotin supplementation, a decrease in plasma BHBA was not observed. The BHBA plasma values followed the same pattern that plasma NEFA followed up to wk 4 of lactation. These concentrations were lower than what was reported in some studies (Grum et al., 1996). The BHBA plasma values increased slightly for the remaining 6 wk of lactation in both biotin-supplemented and control cows, which suggests that alimentary ketogenesis was promoted as intake increased.

Mean concentrations of liver TAG (Figures 5 and 6, respectively) were comparable with values reported by Bremner et al. (2000) but were much lower than those in other studies with (Rukkwamsuk et al., 1999) or without (Grum et al., 1996) a fatty liver-inducing feeding regimen, more likely because of differences in prepartum BCS. In the present study, mean BCS at approximately 20 d before calving was 3.1 for the control cows and 3.3 for the biotin-supplemented cows without statistical differences ($P = 0.26$). Holstein cows that had greater body condition than those in our study had extremely high liver TAG concentrations (>10%, wet weight) at 1 and 21 DIM. Mean liver TAG values found in the present study are indicative of a mild fatty liver (Gerloff et al., 1986). Mean concentrations of total lipid and TAG at approximately 16 d prior to calving and

just prior to being assigned to experimental diets were 7.0% vs. 5.7% and 3.0% vs. 2.0% for the control and biotin-fed groups, respectively, and were not different ($P > 0.15$). The addition of biotin to the diet tended to decrease total hepatic lipids at a faster rate from 2 to 30 DIM. Total liver lipid concentrations were 17 and 13% lower in biotin-supplemented cows at 16 and 30 d postpartum. Hepatic TAG followed the same pattern of total lipids as previously reported (Skaar et al., 1989) with 24 and 37% lower concentrations than controls at 16 and 30 d postpartum, respectively. The tendency for a decrease in both hepatic lipid and TAG contents in cows receiving biotin might have been a result of decreased plasma NEFA concentration. The difference in liver total lipid and TAG concentrations at d 16 postpartum accompanied a difference in plasma NEFA concentrations during the same time (wk 2 postpartum).

Neither liver glycogen nor liver glycogen-to-TAG ratio concentration were affected by biotin supplementation. The inverse relationship, the TAG-to-glycogen ratio, has been used as an indicator of a cow's susceptibility to ketosis (Drackley et al., 1992). Liver glycogen is extremely low at calving relative to other times. In this study, mean liver glycogen was 1.5% (wet weight basis) at calving. Because liver glycogen has a fast turnover, a ratio of glycogen to TAG concentration could be used to indicate the glycogenic capacity of the liver as affected by fat infiltration during the periparturient period and could be a more sensitive index for fatty liver evaluation than either liver glycogen or TAG alone (Greenfield et al., 2000). The lack of differences in glycogen-to-TAG ratio could explain the absence of hyperketonemia in the present study.

Although changes in lipid infiltration in the liver and reduced glycogen storage around parturition were observed, mean liver protein concentration was constant across time. Similar values to those observed here (17.6%, wet basis) have been reported for clinically ketotic cows (Ford and Boyd, 1960) and lactating cows (17.3 to 19.1%, wet basis) with different degrees of fatty liver (3.9 to 9.6% TAG, wet basis) (Gruffat et al., 1997). In healthy lactating cows between 119 and 123 DIM (1.2% total liver lipid, wet basis), lower total protein concentrations in liver (approximately 14%) were observed (Pocius and Herbein, 1986). Although biotin is involved in protein synthesis through increasing guanylate cyclase activity and RNA synthesis, no difference was observed between treatments.

CONCLUSIONS

Feeding supplemental biotin at 20 g/d during the last 16 d postpartum and at 30 g/d from calving through 70 d postpartum elevated concentrations of biotin in

plasma and milk compared with cows fed 0 mg/d of supplemental biotin. Supplemental biotin elevated plasma glucose and lowered NEFA. These positive changes likely explained the trends for decreased TAG concentrations in liver by 24 and 37% at 16 and 30 d postpartum, respectively, although milk yield was not increased by biotin supplementation. It is likely that metabolic responses to supplemental biotin are involved with hepatic gluconeogenesis.

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