Effects of cobalt supplementation and vitamin B₁₂ injections on lactation performance and metabolism of Holstein dairy cows

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ABSTRACT

The objective of this study was to determine lactation performance and metabolism of primiparous and multiparous dairy cows fed different levels and sources (inorganic and organic) of Co or given weekly vitamin B₁₂ injections. Forty-five primi- and multiparous cows at 60 d prepartum were blocked by expected calving date, and randomly assigned to 1 of 5 treatments in a randomized complete block design with treatments starting at 60 d prepartum. The 5 treatments were (1) no supplemental dietary Co (control, CON), (2) 25 mg/d of supplemental dietary Co from Co carbonate (CoCarb), (3) 25 mg/d of supplemental dietary Co from Co glucoheptonate (LCoGH), (4) 75 mg/d of supplemental dietary Co from Co glucoheptonate (HCoGH), and (5) CON diet plus weekly 10 mg i.m. of vitamin B₁₂ injections (IB12). Cows remained on their respective treatment until 150 d after calving. Cobalt concentrations (mg/kg of dry matter) in the lactating diets were 1.0, 1.9, 2.3, and 5.1 for CON/IB12, CoCarb, LCoGH, and HCoGH, respectively. Dry matter intake, body weight, and body condition score were not affected by treatment. The LCoGH treatment tended to have greater milk yield than CoCarb, and CON had similar milk yields to the mean of LCoGH and HCoGH. Cobalt supplementation or the use of vitamin B₁₂ injections did not influence plasma or liver measures of energy metabolism. Injections of vitamin B₁₂ increased plasma, liver, and milk vitamin B₁₂ contents. Dietary Co addition did not affect plasma vitamin B₁₂ concentrations; however, it did increase milk vitamin B₁₂ concentrations throughout lactation and liver vitamin B₁₂ at calving with no effect of source or level of Co. Folate status of cows in the study was low and possibly limited the effect of improved vitamin B₁₂ status on lactation performance. Overall, Co supplementation (inorganic and organic) or vitamin B₁₂ injections improved measures

of vitamin B₁₂ status, but not lactation performance compared with CON possibly due to Co being above requirements in the CON diet.

Key words: cobalt, vitamin B₁₂, dairy cow

INTRODUCTION

Vitamin B₁₂ is a water-soluble vitamin produced by rumen microbes for their use and use by the host animal. Two vitamin B₁₂-dependent enzymes exist in dairy cattle: (1) methionine synthase, which transfers a methyl group from 5-methyl tetrahydrofolate to homocysteine to regenerate methionine and tetrahydrofolate (THF), and (2) methylmalonyl-CoA mutase, which transforms methylmalonyl-CoA into succinyl-CoA to enter the Krebs cycle and then gluconeogenesis (McDowell, 2000). Methylmalonyl-CoA is produced mainly from the metabolism of propionate, a major product from rumen fermentation, through a biotin-dependent pathway (McDowell, 2000). In early lactation, dairy cattle fed supplementary folic acid and rumen-protected methionine were observed to have lower-than-optimal levels of vitamin B₁₂, confirmed by reduced serum methylmalonic acid concentrations and increased ECM production of primiparous dairy cows given weekly intramuscular vitamin B₁₂ injections (Girard and Matte, 2005). Early lactation cows have reduced vitamin B₁₂ status due to increased demands of lactation (Girard and Matte, 1999). Kincaid et al. (2003) reported serum vitamin B₁₂ concentrations of 2.4, 2.0, and 1.2 ng/mL at –21, 7, and 120 d relative to parturition, respectively, and the decrease from 21 d prepartum to 7 DIM was greater for primiparous cows. Using multiparous cows, Kincaid and Socha (2007) showed a significant decrease in serum vitamin B₁₂ concentration from 5.7 ng/mL at 55 d prepartum to 2.3, 2.0, and 1.9 ng/mL at –20, 7, and 120 d relative to parturition, respectively.

Cobalt is essential for ruminal synthesis of vitamin B₁₂ as Co resides at the center of the corrin ring of vitamin B₁₂ (McDowell, 2000). The dietary requirement of dairy cattle for Co is 0.11 mg/kg of DM (NRC, 2001). Dietary Co content has been shown to positively influence ruminal vitamin B₁₂ synthesis (Mills, 1981; Kawashima

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et al., 1997; Tiffany and Spears, 2002; Tiffany et al., 2006). Using continuous-flow cultures, Tiffany et al. (2006) reported increased vitamin B₁₂ concentrations as Co increased from 0.1 to 1.0 mg/kg of DM. Stangl et al. (2000) suggested a Co requirement of 0.25 mg/kg of DM to maximize vitamin B₁₂ status using beef steers. Kincaid et al. (2003) found that multiparous cows fed a diet with 1.26 mg of Co/kg of DM had greater milk and 3.5% FCM yields than multiparous cows fed diets with 0.37 or 0.68 mg of Co/kg of DM; however, no effect of dietary Co on milk yield was observed in primiparous cows. Limited research has determined the effect of Co source in dairy cattle diets on lactation performance and metabolism. Furthermore, no known studies have compared Co supplementation to a positive control treatment of vitamin B₁₂ injections. The objective of this study was to determine if Co supplementation (inorganic and organic forms) above NRC (2001) requirements or intramuscular injections of vitamin B₁₂ would influence the vitamin B₁₂ status and milk production of lactating primiparous and multiparous dairy cows. The hypotheses of the study were that dietary Co supplementation would improve the vitamin B₁₂ status and lactation performance of dairy cattle compared with an unsupplemented diet. The response to Co supplementation was hypothesized to be source (inorganic vs. organic) and dose dependent, with organic Co and higher Co doses having greater effects. In addition, it was hypothesized that cows given vitamin B₁₂ injections would have similar vitamin B₁₂ status and lactation responses to cows fed diets supplemented with Co.

MATERIALS AND METHODS

Cows and Treatments

The experimental protocol was approved by the Animal Care and Use Committee of the College of Agriculture and Life Sciences (University of Wisconsin, Madison). Forty-five primiparous and 45 multiparous Holstein dairy cows, 60 d before their expected calving, were used in a randomized complete block design. Animals were blocked on parity (1 or ≥ 2) and expected calving date, and then randomly assigned to 1 of 5 treatments: (1) no dietary Co supplementation (control, **CON**), (2) 25 mg/d of supplemental dietary Co from Co carbonate (**CoCarb**), (3) 25 mg/d of supplemental dietary Co from Co glucoheptonate (**LCoGH**), (4) 75 mg/d of supplemental dietary Co from Co glucoheptonate (**HCoGH**), or (5) CON diet plus weekly 10 mg i.m. of vitamin B₁₂ injections (**IB12**). Previous lactation mature equivalent 305-d milk production was $11,456 \pm 2,600$, $9,544 \pm 1,648$, $10,486 \pm 1,973$, $10,662 \pm 1,533$, and $11,801 \pm 2,136$ kg for multiparous cows

on CON, CoCarb, LCoGH, HCoGH, and IB12 diets, respectively. The average BW at the beginning of the study was 643 ± 95 , 647 ± 91 , 625 ± 57 , 660 ± 88 , and 639 ± 72 kg for CON, CoCarb, LCoGH, HCoGH, and IB12 cows, respectively. Cows were in the study through 150 DIM. Cobalt glucoheptonate was provided by Zinpro Corp. (Eden Prairie, MN). Weekly vitamin B₁₂ (cyanocobalamin; 5 mg/mL; Phoenix Pharmaceutical Inc., St. Joseph, MO) injections (2 mL) were given intramuscularly in the rear leg. Animals were individually fed a TMR for 5% refusals in tiestalls or stanchions, with the amounts fed and refused weighed daily to determine DMI. Diets were mixed once daily in the morning and fed twice daily after each milking. Treatment premixes (Vita Plus Corp., Madison, WI) were added to the TMR at a rate of 100 g/cow per day. In addition, a complete dry or lactating cow trace mineral premix with no supplemental Co was added to the TMR at a rate of 100 g/cow per day. The complete trace mineral premix also contained rumen-protected methionine to provide 15 g/cow per day in dry cow diets and 25 g/cow per day of Smartamine M (Adisseo USA Inc., Alpharetta, GA) and monensin sodium (Rumensin; Elanco Animal Health, Greenfield, IN) in lactating cow diets to provide 250 mg/cow per day. Both far-off (60 to 29 d before expected calving) and close-up (28 d before expected calving to calving date) dry cow diets were fed (Table 1). A lactating cow diet (Table 1) was fed after calving through 150 DIM. Nutrient composition of the diets is presented in Tables 1 and 2. All multiparous cows received bovine somatotropin (Posilac; Elanco Animal Health) injections beside the tailhead every 14 d starting at 63 DIM.

Sampling and Analyses

Body weight and BCS (1 to 5 in 0.25 increments; Wildman et al., 1982) were measured weekly throughout the trial. Colostrum samples were taken within 12 h of calving and frozen at -20°C until analysis for total protein (Lowry et al., 1951), IgG, IgA, and IgE by single radial immunodiffusion (VMRD Inc., Pullman, WA; Fleenor and Stott, 1981), and vitamin B₁₂ and Co. Individual milk yields were recorded daily. Milk samples were taken at 4 consecutive milkings each week and analyzed for fat, true protein, lactose, SCC, and MUN content by infrared analysis using a Foss FT6000 spectrum analyzer (AgSource Cooperative Services, Menomonie, WI). The log SCC was calculated as $\log_2 (\text{SCC}/100) + 3$ (Shook, 1993). In addition, milk samples were frozen at -20°C for vitamin B₁₂ and Co analysis. Vitamin B₁₂ in milk and colostrum was determined using a radioassay kit designed for human plasma (competitive protein-binding assay using a puri-

Table 1. Ingredient and nutrient composition of diets fed to dry and lactating cows

Item	Diet		
	Far-off dry	Close-up dry	Lactating
Ingredient, % of DM			
Corn silage	49.5	31.3	32.2
Alfalfa silage	28.3	15.6	19.3
Chopped wheat straw	16.5	18.2	—
Chopped hay	—	—	3.1
Concentrate mix ^{1,2,3}	4.2	33.2	35.4
Dried distillers grain	—	—	9.2
Mineral premix ^{4,5}	0.77	0.86	0.40
Treatment premix ^{6,7,8,9}	0.77	0.86	0.40
Nutrient, % of DM ± SD			
CP, %	13.8 ± 0.4	15.1 ± 0.3	18.0 ± 0.5
NDF, %	48.8 ± 1.4	40.2 ± 1.3	32.9 ± 1.8
Ether extract, %	2.5 ± 0.1	2.9 ± 0.1	5.0 ± 0.5
Starch, %	16.4 ± 1.9	24.4 ± 1.3	24.0 ± 1.5
NFC, %	27.8 ± 1.3	35.7 ± 1.2	38.3 ± 1.1
TDN ₁₈ , %	60.3 ± 0.6	66.2 ± 0.8	71.9 ± 1.3
DE ₁₈ , ¹⁰ Mcal/kg of DM	2.71 ± 0.03	2.98 ± 0.03	3.25 ± 0.06

¹Composition of the far-off grain mix on an as-fed basis was 86.0% soybean meal (48% CP), 6.2% magnesium oxide, 3.9% dicalcium phosphate, and 3.9% salt.

²Composition of the close-up grain mix on an as-fed basis was 60.0% ground corn, 17.5% soybean meal (48% CP), 17.5% expeller soybean meal, 2.1% calcium sulfate, 2.1% magnesium sulfate, 0.4% magnesium oxide, and 0.4% dicalcium phosphate.

³Composition of the lactating grain mix on an as-fed basis was 58.5% ground corn, 30.1% soybean meal (48% CP), 4.7% tallow, 2.4% calcium carbonate, 2.4% sodium bicarbonate, 0.96% salt, 0.56% magnesium oxide, and 0.38% magnesium-potassium sulfate.

⁴Composition of the dry cow mineral premix on an as-fed basis was 68.7% wheat middlings, 15% Smartamine M (Adisseo USA Inc., Alpharetta, GA), 3.6% zinc methionine complex (10% Zn and 20% methionine; Zinpro Corp., Eden Prairie, MN), 2.5% manganese methionine complex (8% Mn and 18% methionine, Zinpro Corp.), 2% vitamin E (50%), 1.8% manganese sulfate, 1.5% zinc sulfate, 1.4% Rumensin 80 (Elanco Animal Health, Greenfield, IN), 1.3% mineral oil, 1.3% copper lysine complex (10% copper and 46% lysine, Zinpro Corp.), 0.45% selenium 0.8%, 0.21% copper sulfate, 0.18% iron sulfate, 0.12% vitamin A/D₃ (2,200 KIU of vitamin A and 440 KIU vitamin of D per kilogram), 0.032% vitamin D₃ (1,100 KIU/kg), and 0.021% ethylenediamine dihydroiodide (99%).

⁵Composition of the lactating cow mineral premix on an as-fed basis was 28.0% calcium carbonate, 25.0% wheat middlings, 25.0% Smartamine M (Adisseo USA Inc.), 4.1% zinc methionine complex (10% Zn and 20% methionine, Zinpro Corp.), 3.8% manganese sulfate, 3.4% zinc sulfate, 2.5% manganese methionine complex (8% Mn and 18% methionine, Zinpro Corp.), 2% vitamin E 50%, 1.4% sodium monensin (176 g/kg as fed, Elanco Animal Health, Greenfield, IN), 1.3% mineral oil, 1.3% copper lysine complex (10% copper and 46% lysine; Zinpro Corp.), 0.83% selenium 0.8%, 0.82% copper sulfate, 0.35% iron sulfate, 0.12% vitamin A/D₃ (2,200 KIU of vitamin A and 440 KIU of vitamin D per kilogram), 0.032% vitamin D₃ (1,100 KIU/kg), and 0.032% ethylenediamine dihydroiodide 99%.

⁶Composition of the control and IB12 (no supplemental Co plus weekly 10-mg vitamin B₁₂ injections) treatment premix on an as-fed basis was 99.5% wheat middlings and 0.5% mineral oil.

⁷Composition of the CoCarb (25 mg of supplemental Co/d from Co carbonate) treatment premix on an as-fed basis was 99.4% wheat middlings, 0.5% mineral oil, and 0.055% cobalt carbonate.

⁸Composition of the LCoGH (25 mg of supplemental Co/d from Co glucoheptonate) treatment premix on an as-fed basis was 98.5% wheat middlings, 1.0% cobalt glucoheptonate (2.5% Co, Zinpro Corp.), and 0.5% mineral oil.

⁹Composition of the HCoGH (75 mg of supplemental Co/d from Co glucoheptonate) treatment premix on an as-fed basis was 96.5% wheat middlings, 3.0% cobalt glucoheptonate (2.5% Co, Zinpro Corp.), and 0.5% mineral oil.

¹⁰Digestible energy concentration (Mcal/kg of DM) of the diet fed at maintenance level.

fied form of intrinsic factor specific for true cobalamins; SimulTRAC-S; MP Biomedicals LLC, Solon, OH), as described by Preynat et al. (2009) who validated the kit for bovine milk. The recovery of a known amount of cyanocobalamin was 98.1%, and the interassay coefficient of variation was 5.8%.

Blood samples from the coccygeal vein were taken between d −63 and −57, between d −7 and −1, on d 1, between d 27 and 33, between d 87 and 93, and between d 147 and 153 relative to calving. Blood samples were taken before the morning feeding. Samples were collected in Vacutainer tubes (Becton Dickinson, Franklin

Table 2. Mineral concentrations (mean $\pm$ SD) of the diets¹

Mineral	Diet		
	Far-off dry	Close-up dry	Lactating
Calcium, %	0.59 $\pm$ 0.045	0.56 $\pm$ 0.040	0.82 $\pm$ 0.051
Phosphorus, %	0.30 $\pm$ 0.013	0.35 $\pm$ 0.011	0.38 $\pm$ 0.015
Magnesium, %	0.34 $\pm$ 0.030	0.36 $\pm$ 0.017	0.37 $\pm$ 0.025
Potassium, %	1.73 $\pm$ 0.112	1.54 $\pm$ 0.081	1.48 $\pm$ 0.116
Sodium, %	0.07 $\pm$ 0.011	0.04 $\pm$ 0.007	0.35 $\pm$ 0.009
Zinc, mg/kg	104 $\pm$ 4.4	116 $\pm$ 4.8	104 $\pm$ 5.9
Manganese, mg/kg	133 $\pm$ 5.8	138 $\pm$ 8.6	103 $\pm$ 9.6
Copper, mg/kg	21 $\pm$ 1.5	23 $\pm$ 1.4	19 $\pm$ 0.8
Iron, mg/kg	163 $\pm$ 20.6	175 $\pm$ 36.4	203 $\pm$ 91.7

¹Diet mineral concentrations calculated from individual feed mineral analysis performed by Dairy One Laboratory, Ithaca, NY.

Lakes, NJ) containing either sodium heparin (for analysis of vitamin B₁₂, folates, and Co) or potassium oxalate and sodium fluoride (for analysis of glucose, NEFA, and BHBA), and then centrifuged at 625 $\times$ g for 15 min. All plasma samples were analyzed for glucose by the glucose oxidase/peroxidase method (Karkalas, 1985), vitamin B₁₂, folates, and cobalt. Vitamin B₁₂ and folates in plasma were determined using a radioassay kit designed for human plasma (competitive protein-binding assay using a purified form of intrinsic factor specific for true cobalamins and a purified folate binder from bovine milk; SimulTRAC-S; MP Biomedicals LLC) and validated for bovine plasma. The plasma folate and vitamin B₁₂ assay had recoveries of 97 and 107% of a known amount of added folate and cyanocobalamin and interassay coefficients of variation of 9.2 and 9.8%, respectively. Plasma samples taken on d -60, between d -7 and -1, and on d 1 and 30 relative to calving were analyzed for BHBA (Gibbard and Watkins, 1968) and NEFA (acetyl-CoA synthase, acyl-CoA oxidase and peroxidase; NEFA HR 2; Wako Diagnostics, Richmond, VA). Percutaneous liver biopsies were taken under local anesthesia (10 mL of lidocaine) on d -60, 1, 30, 90, and 150 relative to calving. Liver samples were immediately rinsed with saline to remove blood, frozen in liquid nitrogen, and stored at -20°C for triglyceride (TG; Vazquez-Añon et al., 1994) and Co analysis, and at -80°C for vitamin B₁₂ analysis using a radioassay kit designed for human plasma (SimulTRAC-S; MP Biomedicals LLC) as described by Graulet et al. (2007) and validated for bovine liver. The liver vitamin B₁₂ assay had a recovery of 101.7% and an interassay coefficient of variation of 7.1%.

Cobalt analysis for plasma, liver, milk, and colostrum was done by flame atomic absorption spectrometry (PerkinElmer Inc., Waltham, MA). Samples of plasma (2 mL), liver (1 g, wet weight), milk (5 mL), and colostrum (5 mL) were added to a 40-mL glass beaker able to withstand 500°C, dried at 100°C for 24 h, and

then dry ashed at 500°C for 16 h. Liver samples were weighed after drying at 100°C for 24 h to determine Co content on a dry basis. After dry ashing, the ash was dissolved in 1 mL of 12 M trace metal-grade HCl and then analyzed using flame atomic absorption spectrometry (PerkinElmer Inc.).

Individual feed samples were taken weekly. All feed samples were dried at 60°C for 48 h in a forced-air oven to determine DM content, ground to pass a 1-mm screen of a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA), and composited before sending to Dairy One Forage Laboratory Services (Ithaca, NY) for wet chemistry analysis. Composites were analyzed for DM and OM (method 942.05; AOAC International, 2006); CP (method 990.03; AOAC International, 2006); NDF (Van Soest et al., 1991), determined using α -amylase and sodium sulfite adapted for an Ankom A200 digestion unit (Ankom Technology, Macedon, NY); neutral detergent insoluble CP (method 990.03; AOAC International, 2006), determined on NDF residue from digestion without sodium sulfite; ADF (method 973.18; AOAC International, 2006), adapted for an Ankom A200 digestion unit (Ankom Technology); acid detergent-insoluble CP (method 990.03; AOAC International, 2006), determined on ADF residue; lignin (ADF residue digested in 72% sulfuric acid for 3 h in an ANKOM Daisy); starch using a YSI 2700 SELECT biochemistry analyzer with YSI application note number 319 (YSI Inc. Life Sciences, Yellow Springs, OH); ether extract (method 2003.05; AOAC International, 2006), and macro and trace minerals, including Co, using an inductively coupled plasma radial spectrometer (Thermo Fisher Scientific Inc., Waltham, MA) after microwave digestion (CEM Corp., Matthews, NC) of the samples in a mixture of nitric and hydrochloric acid. In addition, feed Co was analyzed at the University of Wisconsin Dairy Nutrition Laboratory (Madison, WI) by adding to a glass beaker between 1 and 4 g of sample, depending on the expected cobalt concentra-

tion of the sample, drying for 24 h at 100°C, weighing the dry sample and beaker, dry ashing at 500°C for 16 h, dissolving the ash with 5 mL of 12 M trace metal-grade HCl, diluting the sample with 15 mL of distilled water, and analyzing the sample using a flame atomic absorption spectrometer (PerkinElmer Inc.). Energy balance (**EB**) was calculated weekly based on NRC (2001) equations. Net energy intake (**NE_I**) was calculated by multiplying weekly DMI by the calculated **NE_L** concentration of the diet (NRC, 2001). The **NE_M**, net energy for pregnancy (**NE_P**), and **NE_L** were calculated according to NRC (2001) calculations. Estimated prepartum **EB** was calculated as $EB = NE_I - (NE_M + NE_P)$. The estimated postpartum **EB** was calculated as $EB = NE_I - (NE_M + NE_L)$.

Statistical Analysis

Data were analyzed as a randomized complete block design using PROC MIXED of SAS (SAS Institute 2010). Prepartum data including DMI, BW, EB, and BCS were averaged over 2-wk periods. Lactation data including DMI, BW, EB, BCS, and milk yield and component data were averaged over 3-wk periods. The model for prepartum and lactation data included fixed effects of parity (first lactation or second lactation and greater), treatment, time, and interactions of these effects. Time was included as a repeated measure using the first-order autoregressive covariance structure. Random effects included block and the block by cow interaction. Degrees of freedom were calculated using the Satterthwaite option. Means were determined using the least squares means statement and treatment means were compared using orthogonal contrasts. The model for blood and liver metabolite data was similar to the lactation data except a spatial power covariance structure was used for repeated measures due to the unequal spacing of sampling times during the experiment. Covariate adjustments were made for blood and liver data with the initial sample at -60 d relative to calving used as a covariate. Preplanned orthogonal contrasts used included CON versus mean of LCoGH and HCoGH, CoCarb versus LCoGH, IB12 versus mean of LCoGH and HCoGH, and LCoGH versus HCoGH. Statistical significance and trends were declared at $P \leq 0.05$ and $P \geq 0.06$ to $P \leq 0.10$, respectively.

RESULTS AND DISCUSSION

Nutrient composition of the diets is presented in Tables 1 and 2. Cobalt concentrations of the ingredients (Table 3) and diets (Table 4) were higher than anticipated, with the CON lactating diet containing 1.0 mg of Co/kg of DM. The NRC (2001) recommended

Co requirement is 0.11 mg of Co/kg (DM basis), so the negative CON diet had 9 times the concentration of Co needed. There were numerical differences for feed Co analysis results from the University of Wisconsin laboratory and the commercial laboratory. These differences may be due to the differences in analytical techniques; however, the use of dry ashing and microwave wet ash digestion has shown excellent recoveries of Co (Soon, 1998). Dry ashing does have the advantage of having a lower solution:sample ratio than does wet ash digestion, but atomic absorption spectrometry and inductively coupled plasma spectrometry are both able to detect Co of samples processed either by dry or wet ashing (Soon, 1998). The recovery of a known amount of Co was 96.5% at the University of Wisconsin laboratory. The detection limits for Co analysis reported from the commercial laboratory was 0.01 mg/kg of DM and from the University of Wisconsin laboratory was 0.025 mg/kg of DM.

Two animals were removed from the trial at calving. A primiparous cow on HCoGH was removed due to major calving difficulty, and a multiparous cow on LCoGH was removed due to delivering twins. Health-related incidence rates (not statistically analyzed) are presented in Table 5. Three cases of displaced abomasum and milk fever occurred for multiparous cows on CoCarb and 7 cows on IB12 had mastitis during the study. These health events were likely unrelated to treatments.

Prepartum Data

Daily DMI (data not presented) was unaffected by treatment ($P = 0.63$), and averaged 11.8 kg of DM/d. Body weight and EB (data not presented) were unaffected by treatment ($P > 0.10$) and averaged 676 kg and 4.3 Mcal/d, respectively. Body condition score (data not presented) during the dry period tended to be lower for cows on IB12 compared with the mean of LCoGH, and HCoGH (3.6 vs. 3.8; $P = 0.10$).

Lactation Performance

Lactation performance data are presented in Table 6. Body weight, BCS, EB, and DMI of lactating cows were unaffected by treatment ($P > 0.10$). Energy balance was negative, with a mean of -3.3 Mcal/d from parturition to 21 wk into lactation. No treatment ($P = 0.15$) or treatment by time interaction ($P = 0.15$) effects were observed on milk yield, but there was a tendency ($P = 0.07$) for greater milk yield for LCoGH (42.9 kg/d) than CoCarb (39.7 kg/d). Contrasts between CON and the mean of LCoGH and HCoGH for all lactation measures were nonsignificant ($P > 0.10$).

Table 3. Cobalt concentrations (mg/kg of DM $\pm$ SD) of ingredients analyzed at 2 laboratories

Ingredient ¹	n ²	Laboratory	
		Dairy One ³	UW ⁴
Corn silage	17	0.70 $\pm$ 0.12	0.76 $\pm$ 0.07
Alfalfa silage	17	0.59 $\pm$ 0.57	2.91 $\pm$ 0.37
Hay	15	1.68 $\pm$ 0.59	2.10 $\pm$ 0.30
Wheat straw	4	0.97 $\pm$ 0.15	1.29 $\pm$ 0.23
Far-off cow grain	4	1.48 $\pm$ 0.40	2.01 $\pm$ 0.12
Close-up cow grain	4	2.55 $\pm$ 1.22	1.13 $\pm$ 0.01
Lactating cow grain	5	0.97 $\pm$ 0.11	2.08 $\pm$ 0.29
Distillers grain	12	0.08 $\pm$ 0.05	0.96 $\pm$ 0.10
Dry cow mineral premix	4	25.7 $\pm$ 5.2	23.6 $\pm$ 0.7
Lactating cow mineral premix	5	45.3 $\pm$ 11.2	34.8 $\pm$ 6.7
Control premix	6	18.6 $\pm$ 14.3	18.0 $\pm$ 11.1
CoCarb premix	6	235 $\pm$ 18.5	269 $\pm$ 18.8
LCoGH premix	6	320 $\pm$ 57.7	330 $\pm$ 29.5
HCoGH premix	6	1,031 $\pm$ 269	972 $\pm$ 34.1

¹Control = no supplemental Co; CoCarb = 25 mg of supplemental Co/d from Co carbonate; LCoGH = 25 mg of supplemental Co/d from Co glucoheptonate (Zinpro Corp., Eden Prairie, MN); HCoGH = 75 mg of supplemental Co/d from Co glucoheptonate.

²Number of samples analyzed of each ingredient.

³Cobalt analysis using microwave wet ash digestion and inductively coupled plasma spectrometry (Dairy One Laboratory, Ithaca, NY).

⁴Cobalt analysis using dry ashing and flame atomic absorption spectrometry [University of Wisconsin (UW) Dairy Nutrition Laboratory, Madison].

The 3.5% FCM yield, ECM yield, fat content, protein content, lactose content, and log SCC were unaffected by treatment and interactions of treatment with parity or time were not detected ($P > 0.10$). Cows administered IB12 tended to have greater milk protein content than the mean of LCoGH and HCoGH (contrast of IB12 vs. mean of LCoGH and HCoGH, $P = 0.06$). Also, log SCC tended to be lower for the mean of LCoGH and HCoGH than CON (contrast of CON vs. mean of LCoGH and HCoGH, $P = 0.09$). Milk urea nitrogen concentration was affected by a treatment by parity

interaction ($P = 0.04$) such that primiparous cows fed CoCarb (13.8 mg/dL) had numerically greater MUN concentrations than the primiparous cows on the other treatments (12.1, 12.2, 11.5, 12.0 mg/dL for CON, LCoGH, HCoGH, and IB12, respectively); however, multiparous cows had similar MUN concentrations across treatments, with a mean of 12.3 mg/dL.

Previous studies have reported that Co and vitamin B₁₂ supplementation affect lactation performance. Kincaid et al. (2003) did not observe effects of Co supplementation on BW or BCS; however, they did observe

Table 4. Cobalt concentrations (mg/kg of DM $\pm$ SD) of treatment diets from 2 laboratories¹

Item	Treatment ²			
	Control/IB12	CoCarb	LCoGH	HCoGH
Dairy One ³				
Far-off dry	1.1 $\pm$ 0.23	2.8 $\pm$ 0.28	3.5 $\pm$ 0.25	9.9 $\pm$ 1.66
Close-up dry	1.7 $\pm$ 0.39	3.6 $\pm$ 0.35	4.5 $\pm$ 0.47	11.2 $\pm$ 2.27
Lactating	1.0 $\pm$ 0.14	1.9 $\pm$ 0.15	2.3 $\pm$ 0.16	5.1 $\pm$ 1.00
UW ⁴				
Far-off dry	1.9 $\pm$ 0.09	3.9 $\pm$ 0.08	4.4 $\pm$ 0.20	9.4 $\pm$ 0.27
Close-up dry	1.8 $\pm$ 0.14	4.0 $\pm$ 0.15	4.5 $\pm$ 0.20	10.0 $\pm$ 0.34
Lactating	1.9 $\pm$ 0.11	2.9 $\pm$ 0.11	3.1 $\pm$ 0.11	5.7 $\pm$ 0.20

¹Diet Co concentrations calculated based on analysis of individual feeds.

²Control = no supplemental Co; IB12 = no supplemental Co plus weekly 10-mL vitamin B₁₂ injections; CoCarb = 25 mg of supplemental Co/d from Co carbonate; LCoGH = 25 mg of supplemental Co/d from Co glucoheptonate (Zinpro Corp., Eden Prairie, MN); HCoGH = 75 mg of supplemental Co/d from Co glucoheptonate.

³Cobalt analysis using microwave wet ash digestion and inductively coupled plasma spectrometry (Dairy One Laboratory, Ithaca, NY).

⁴Cobalt analysis using dry ashing and flame atomic absorption spectrometry [University of Wisconsin (UW) Dairy Nutrition Laboratory, Madison].

Table 5. Incidence of health events¹

Item	Treatment ²				
	Control	CoCarb	LCoGH	HCoGH	IB12
Primiparous cows, no.	9	9	9	8	9
Ketosis ³	1	1	1	2	4
Displaced abomasum ⁴	1	1	0	0	1
Milk fever ⁵	0	0	0	0	0
Retained placenta ⁶	1	1	0	0	0
Mastitis ⁷	4	0	2	2	2
Multiparous cows, no.	9	9	8	9	9
Ketosis	2	1	2	2	1
Displaced abomasum	1	3	0	1	1
Milk fever	0	3	1	0	1
Retained placenta	0	1	0	0	0
Mastitis	4	3	1	4	7

¹Incidence is the number of cows on each treatment that had the event occur.

²Control = no supplemental Co; CoCarb = 25 mg of supplemental Co/d from Co carbonate; LCoGH = 25 mg of supplemental Co/d from Co glucoheptonate (Zinpro Corp., Eden Prairie, MN); HCoGH = 75 mg of supplemental Co/d from Co glucoheptonate; IB12 = no supplemental Co plus weekly 10-mL vitamin B₁₂ injections.

³Ketosis recorded when treatment given after confirmed with urine ketone strip being moderate or greater.

⁴Displaced abomasum recorded after diagnosis by a veterinarian and the cow underwent surgical treatment.

⁵Milk fever recorded when cow was treated for 1 or more of the following symptoms: cold ears, trembling, head bobbing, or inability to rise.

⁶Retained placenta recorded when cow unable to expel placenta within 12 h of parturition.

⁷Mastitis recorded when cow given treatment for mastitis confirmed by a California Mastitis Test (CMT).

Table 6. Effect of treatments on least squares means of lactation performance measures in lactating dairy cows¹

Item	Treatment ²					Effect, ³ <i>P</i> -value						
	Control	CoCarb	LCoGH	HCoGH	IB12	SEM	Trt	Par	Trt × par	Trt × time	Par × time	Trt × par × time
BW, kg	610	602	599	612	600	12	0.91	<0.01	0.98	0.99	<0.01	0.53
BCS	2.9	2.8	2.9	2.9	2.8	0.1	0.85	<0.01	0.87	0.99	<0.01	0.96
DMI, kg/d	21.6	20.8	21.6	21.1	20.9	0.5	0.68	<0.01	0.44	0.96	0.18	0.72
Milk yield, ⁴ kg/d	43.2	39.7	42.9	42.6	40.4	1.2	0.15	<0.01	0.68	0.15	<0.01	0.44
Milk/DMI, kg/kg	2.00	1.93	2.00	2.03	1.95	0.05	0.65	<0.01	0.99	0.46	<0.01	0.56
FCM, kg/d	40.2	38.8	40.8	39.3	38.0	1.1	0.42	<0.01	0.31	0.62	0.12	0.85
FCM/DMI, kg/kg	1.89	1.91	1.92	1.90	1.84	0.06	0.87	<0.01	0.95	0.75	0.05	0.63
ECM, kg/d	40.1	38.2	40.4	39.2	38.0	1.1	0.46	<0.01	0.38	0.71	0.10	0.85
ECM/DMI, kg/kg	1.88	1.90	1.90	1.90	1.85	0.05	0.96	<0.01	0.94	0.66	0.02	0.65
Fat, %	3.17	3.46	3.27	3.10	3.16	0.10	0.14	0.18	0.63	0.62	<0.01	0.42
Fat, kg/d	1.33	1.33	1.37	1.29	1.26	0.05	0.54	<0.01	0.24	0.77	0.01	0.85
Protein, ⁵ %	2.99	2.97	2.96	2.99	3.08	0.05	0.37	<0.01	0.64	0.67	<0.01	0.65
Protein, kg/d	1.28	1.17	1.25	1.26	1.23	0.04	0.27	<0.01	0.81	0.79	<0.01	0.89
Lactose, %	4.96	5.00	4.93	5.02	5.01	0.05	0.72	0.02	0.92	0.89	0.03	0.72
Lactose, kg/d	2.14	1.99	2.11	2.14	2.02	0.06	0.27	<0.01	0.77	0.56	0.04	0.70
Log SCC ^{6,7}	3.02	2.76	2.69	1.96	2.82	0.33	0.23	0.25	0.21	0.45	0.07	0.21
MUN, mg/dL	12.2	13.0	12.4	11.7	12.2	0.4	0.06	0.81	0.04	0.70	0.02	0.90
EB, ⁸ Mcal/d	-3.4	-3.2	-3.5	-3.5	-2.9	0.7	0.97	<0.01	0.84	0.60	0.02	0.35

¹Significant time effect for all measures ($P < 0.01$).

²Control = no supplemental Co; CoCarb = 25 mg of supplemental Co/d from Co carbonate; LCoGH = 25 mg of supplemental Co/d from Co glucoheptonate (Zinpro Corp., Eden Prairie, MN); HCoGH = 75 mg of supplemental Co/d from Co glucoheptonate; IB12 = no supplemental Co plus weekly 10-mg vitamin B₁₂ injections.

³Trt = treatment and Par = parity.

⁴Tendency for significant contrast of CoCarb versus LCoGH ($P = 0.07$).

⁵Tendency for significant contrast of IB12 versus mean of LCoGH and HCoGH ($P = 0.06$).

⁶Tendency for significant contrast of control versus mean of LCoGH and HCoGH ($P = 0.09$).

⁷Log₂(SCC/100) + 3.

⁸EB = energy balance calculated according to NRC (2001) equations.

a treatment by parity by time interaction for EB. In addition, Kincaid et al. (2003) reported a treatment by parity by time interaction, where multiparous cows fed a diet with 1.26 mg of Co/kg of DM had increased milk and 3.5% FCM yields compared with cows fed diets with 0.37 or 0.68 mg of Co/kg of DM, and primiparous cows had similar yields across treatments. In another study, Kincaid and Socha (2007) did not observe an effect of Co supplementation on milk yields or milk composition; however, DMI was lower during the first 5 wk of lactation for cows fed diets with Co concentrations of 0.93 mg/kg of DM compared with 0.19 or 0.57 mg/kg of DM. The Co levels in the control diets of Kincaid et al. (2003) and Kincaid and Socha (2007) were 0.37 and 0.19 mg/kg of DM or 37 and 19%, respectively, of the Co level in the CON lactation diet fed during the current study. A lack of lactation response to supplemental Co in the current study may be due to the CON lactation diet having a Co level 9 times the NRC (2001) requirements.

Vitamin B₁₂ injections also did not elicit a lactation response as previously observed by Girard and Matte (2005). Girard and Matte (2005) fed folic acid and rumen-protected methionine to primiparous cows during early lactation and observed an increase in ECM yield when cows were given weekly 10 mg of vitamin B₁₂ injections. However, cows fed vitamin B₁₂ alone had lower milk production and reached a plateau sooner than cows fed vitamin B₁₂ with folic acid (Graulet et al., 2007). The cows in the present study had lower folate status (mean of 14.1 ng/mL during lactation) compared with the study by Girard and Matte (2005) in which cows were supplemented with 4 mg of folic acid per kilogram of BW and had plasma folate levels of 19.5 ng/mL during early and mid lactation. Methionine supply was likely sufficient to fulfill the needs for methyl groups needed for methylation reactions with supplemental rumen-protected methionine included in the diet, but folic acid is also needed for nucleotide biosynthesis. Girard and Matte (2005) concluded that with low folate supply, the impact of additional vitamin B₁₂ would be marginally beneficial due to a limited THF flux. Tetrahydrofolate is needed for both the formation of methyl donors, principally S-adenosylmethionine, and in nucleotide synthesis. One-carbon groups are added to THF to form 5,10-methylene-THF or 10-formyl-THF, which are needed for nucleotide synthesis. Under low THF supply, the formation of 5,10-methylene-THF, 10-formyl-THF, and thus nucleotides needed for tissue proliferation may be reduced. Thus, additional folate supply may be needed for a lactation response to increases in vitamin B₁₂ supply to be observed.

Total protein in colostrum was not affected by treatment ($P = 0.28$), with a mean of 235.4 g of protein/L of colostrum (Table 7). Colostrum IgG and IgM concentrations were not affected by treatment ($P > 0.10$); however, IgA tended to be influenced by treatment ($P = 0.09$). Cows on IB12 tended to have greater IgA concentration than the mean of LCoGH and HCoGH ($P = 0.10$). Colostrum Co content was not affected by treatment ($P = 0.57$), but vitamin B₁₂ content was increased by vitamin B₁₂ injections, with cows on IB12 having 2-fold higher colostrum vitamin B₁₂ content than the mean of LCoGH and HCoGH ($P < 0.01$). Kincaid and Socha (2007) observed a tendency for increased colostrum vitamin B₁₂ content when cows were supplemented with Co at 0.89 and 1.71 versus 0.15 mg/kg of DM during the prepartum period. The current study contrasts these data, but Kincaid and Socha (2007) had lower Co content in the control diet. Similar to the current study, Kincaid et al. (2003) and Kincaid and Socha (2007) did not observe an effect of Co on colostrum Co content. These studies had similar concentrations of colostrum Co as the current study, with values ranging from 0.095 to 0.144 µg of Co/mL of colostrum. Cobalt supplementation numerically increased colostrum IgG from 3,725 to 5,795 mg/dL and IgM from 139 to 185 mg/dL with Co supplementation (Kincaid and Socha, 2007). No studies were found that reported on the effect of vitamin B₁₂ injections on colostrum composition for dairy cattle.

Milk Co content (Table 7) was not affected by treatment ($P = 0.50$), but Co yield tended to be affected by treatment ($P = 0.06$). Cows on the LCoGH diet secreted more milk Co than those on the CoCarb diet (contrast of CoCarb vs. LCoGH, $P < 0.01$) and cows that were administered IB12 secreted less milk Co than the mean of LCoGH and HCoGH ($P = 0.02$). These differences are due to LCoGH cows tending to have greater milk yield than CoCarb cows and the mean of LCoGH and HCoGH cows having numerically higher milk production than IB12 cows.

Vitamin B₁₂ concentrations in milk were affected by treatment (Table 7; $P < 0.01$) and a treatment by parity by time interaction (Table 8; $P = 0.03$). Significant contrasts existed for milk vitamin B₁₂ concentrations, with the mean of LCoGH and HCoGH milks having greater concentrations than CON milk ($P = 0.01$). In addition, injections of vitamin B₁₂ increased milk vitamin B₁₂ concentrations compared with the mean of LCoGH and HCoGH treatments ($P < 0.01$). Primiparous cows supplemented with any Co source had numerically greater milk vitamin B₁₂ concentrations than CON throughout lactation, with the greatest difference occurring during mo 1 of lactation. The numerical improvement in

Table 7. Effects of treatments on least squares means of selected measures in colostrum and milk¹

Item	Treatment ²						Effect, ³ <i>P</i> -value ³					
	Control	CoCarb	LCoGH	HCoGH	IB12	SEM	Trt	Par	Trt × par	Trt × time	Par × time	Trt × par × time
Colostrum												
Protein, g/L	213	253	238	216	256	19	0.28	0.21	0.24	—	—	—
IgG, mg/dL	7,745	8,213	7,003	7,726	8,478	728	0.62	<0.01	0.68	—	—	—
IgM, mg/dL	464	579	492	541	564	41	0.21	0.35	0.65	—	—	—
IgA, ⁴ mg/dL	200	307	242	219	302	36	0.09	<0.01	0.13	—	—	—
Cobalt, µg/mL	0.148	0.151	0.153	0.150	0.148	0.002	0.57	0.83	0.22	—	—	—
Vitamin B ₁₂ , ⁵ ng/mL	12.8	15.7	16.3	13.6	33.4	1.8	<0.01	<0.01	0.51	—	—	—
Milk												
Cobalt, µg/mL	0.107	0.105	0.107	0.107	0.106	0.001	0.50	0.84	0.96	0.24	<0.01	0.69
Cobalt, ^{6,7} mg/d	4.60	4.15	4.62	4.55	4.27	0.14	0.06	<0.01	0.59	0.32	0.03	0.33
Vitamin B ₁₂ , ^{8,9} pg/mL	1,575	2,012	2,070	2,032	4,431	184	<0.01	0.69	0.22	<0.01	0.41	0.03
Vitamin B ₁₂ , ^{8,9} µg/d	68.6	79.3	86.6	83.9	172.5	7.1	<0.01	0.10	0.59	<0.01	0.01	0.21

¹Significant effect of time for all milk measures ($P < 0.01$).²Control = no supplemental Co; CoCarb = 25 mg of supplemental Co/d from Co carbonate; LCoGH = 25 mg of supplemental Co/d from Co glucoheptonate (Zinpro Corp., Eden Prairie, MN); HCoGH = 75 mg of supplemental Co/d from Co glucoheptonate; IB12 = no supplemental Co plus weekly 10-mg vitamin B₁₂ injections.³Trt = treatment and Par = parity.⁴Tendency for significant contrast of IB12 versus mean of LCoGH and HCoGH ($P = 0.10$).⁵Significant contrast of IB12 versus mean of LCoGH and HCoGH ($P < 0.01$).⁶Significant contrast of CoCarb vs. LCoGH ($P = 0.02$).⁷Tendency for significant contrast of IB12 vs. mean of LCoGH and HCoGH ($P = 0.07$).⁸Significant contrast of control versus mean of LCoGH and HCoGH ($P \leq 0.03$).⁹Significant contrast of IB12 versus mean of LCoGH and HCoGH ($P < 0.01$).

milk vitamin B₁₂ concentrations for primiparous cows was influenced by source and level of Co, with HCoGH having the greatest numerical increase, LCoGH being intermediate to HCoGH and CoCarb, and CoCarb having the lowest numerical improvement compared with CON. Multiparous cows on CoCarb and LCoGH had numerically greater milk vitamin B₁₂ concentrations than CON throughout lactation. However, multiparous cows on HCoGH had numerically lower milk vitamin B₁₂ concentrations than those on CoCarb and LCoGH, but numerically greater than CON throughout lactation. Similar to the primiparous cows the largest difference between Co supplemented and CON was during mo 1 of lactation. Primiparous and multiparous cows given vitamin B₁₂ injections had greater milk vitamin B₁₂ concentrations than other treatments throughout lactation.

Daily yield of milk vitamin B₁₂ was influenced by a treatment ($P < 0.01$) and a treatment by time interaction (Figure 1, $P < 0.01$). Cows on IB12 decreased milk vitamin B₁₂ yield steadily from mo 1 (260 µg/d) to mo 3 (135 µg/d) and yield was unchanged during mo 4 and 5, whereas the other treatments had only small changes in vitamin B₁₂ yield during lactation. The mean milk vitamin B₁₂ yield of cows on LCoGH and HCoGH was greater than CON ($P = 0.03$). Also, cows on IB12 had

about 2 times the milk vitamin B₁₂ yield of the mean of LCoGH and HCoGH ($P < 0.01$).

The increase in milk vitamin B₁₂ concentration and yield for cows on IB12 is similar to that in other studies giving supplemental vitamin B₁₂ via injection (Girard and Matte, 2005) or diet (Graulet et al., 2007). Cobalt supplementation improved supply of vitamin B₁₂, as indicated by increased milk vitamin B₁₂ concentration and yield, likely due to increased ruminal vitamin B₁₂ synthesis. Ruminal vitamin B₁₂ synthesis was increased by Co supplementation (Stemme et al., 2008); however, that study used dietary Co concentrations of 0.17 and 0.29 mg/kg of DM, which are considerably less than in the present study. In vitro fermentation systems have been used to evaluate higher Co concentrations. Tiffany et al. (2006) used continuous flow-through fermentors and observed that increasing Co concentrations from 0.05 to 1.05 mg/kg of DM increased vitamin B₁₂ concentrations.

No effects of treatment or treatment by time interaction were observed for blood glucose, NEFA, or BHBA concentrations (Table 9; $P > 0.10$). Glucose, NEFA, and BHBA averaged 60.4 mg/dL, 440 mmol/L, and 8.3 mg/dL, respectively. Liver TG were unaffected by treatment ($P = 0.34$), but a parity by time interaction ($P < 0.01$) was observed. Primiparous cows had

Figure 1. Effect of treatment by time interaction on least squares means of milk vitamin B₁₂ yield (µg/d). Control = no supplemental Co; CoCarb = 25 mg of supplemental Co/d from Co carbonate; LCoGH = 25 mg of supplemental Co/d from Co glucoheptonate (Zinpro Corp., Eden Prairie, MN); HCoGH = 75 mg of supplemental Co/d from Co glucoheptonate; IB12 = no supplemental Co plus weekly 10-mg vitamin B₁₂ injections. Treatment × time: *P* < 0.01.

liver TG of 1.58, 5.72, and 5.72% DM at d −60, 1, and 30 relative to calving. Multiparous cows had liver TG of 1.49, 10.37, and 14.85% DM at −60, 1, and 30 relative to calving. The difference in liver TG between primiparous and multiparous cows has been previously reported by Petit et al. (2007) and may be due to greater milk production and more severe negative energy balance for multiparous cows leading to greater mobilization of body tissues and liver TG accumulation. In a previous experiment (Graulet et al., 2007), plasma glucose and NEFA concentrations were also unaffected by additional vitamin B₁₂. However, when folate was

given in combination with vitamin B₁₂, plasma glucose concentrations were increased (Graulet et al., 2007).

Plasma Co concentrations were influenced by a treatment by time interaction (*P* = 0.05); LCoGH had higher concentrations at 30 d postpartum than other treatments, with no apparent differences at other sampling times. Also, LCoGH tended to have greater plasma Co than CoCarb (contrast of CoCarb vs. LCoGH, *P* = 0.08). Vitamin B₁₂ concentrations in plasma were influenced by a treatment by time interaction (Figure 2; *P* < 0.01). Injections of vitamin B₁₂ increased plasma vitamin B₁₂ approximately 2-fold more than other treatments throughout the study. During the prepartum period, plasma vitamin B₁₂ concentrations increased for all treatments until calving, with IB12 having a larger increase than other treatments. After parturition, plasma vitamin B₁₂ concentrations of all treatments decreased to a nadir at 30 d postpartum and then increased to a plateau at 90 d postpartum, with IB12 having greater levels throughout; however, IB12 did have a larger decrease from d 1 to d 30 of lactation than the other treatments. In addition, cows on IB12 had increased plasma vitamin B₁₂ concentrations compared with the mean of LCoGH and HCoGH (*P* < 0.01) and cows fed LCoGH tended to have greater plasma vitamin B₁₂ concentration than those fed HCoGH (*P* = 0.07). However, the plasma vitamin B₁₂ concentration of cows on IB12 at d 1 of lactation was inflated by cows calving and sampling occurring throughout the 7 d between injections, leading to cows having higher plasma vitamin B₁₂ concentrations the closer parturition was to the day of injection (Figure 3). Plasma folate concentration was not affected by treatment (*P* > 0.10), but tended to be influenced by a parity by time interaction, with plasma folates being lower for multiparous cows

Table 8. Effect of treatment by parity by time on milk vitamin B₁₂ concentration (pg/mL)¹

	Month of lactation					
Treatment ²	1	2	3	4	5	SEM
Primiparous						
Control	1,314	1,641	1,186	1,443	1,668	306
CoCarb	2,266	1,769	1,594	1,650	1,745	300
LCoGH	2,409	1,895	1,721	1,741	1,842	305
HCoGH	2,883	1,962	1,793	1,827	2,167	316
IB12	7,904	4,519	3,222	3,672	3,814	290
Multiparous						
Control	2,099	1,427	1,472	1,844	1,659	306
CoCarb	2,773	2,258	1,852	2,084	2,138	299
LCoGH	3,106	1,930	1,814	2,023	2,254	327
HCoGH	2,241	1,738	1,537	1,900	2,273	300
IB12	6,891	3,894	3,353	3,432	3,614	289

¹Significant treatment by parity by time interaction (*P* = 0.03).

²Control = no supplemental Co; CoCarb = 25 mg of supplemental Co/d from Co carbonate; LCoGH = 25 mg of supplemental Co/d from Co glucoheptonate (Zinpro Corp., Eden Prairie, MN); HCoGH = 75 mg of supplemental Co/d from Co glucoheptonate; IB12 = no supplemental Co plus weekly 10-mg vitamin B₁₂ injections.

Table 9. Effects of treatments on least squares means of plasma and liver parameters¹

Item	Treatment ²						Effect, ³ <i>P</i> -value					
	Control	CoCarb	LCoGH	HCoGH	IB12	SEM	Trt	Par	Trt × par	Trt × time	Par × time	Trt × par × time
Plasma												
Glucose, mg/dL	60.5	59.5	61.0	60.4	60.6	1.15	0.71	<0.01	0.46	0.21	<0.01	0.36
NEFA, mmol/L	448	423	438	468	421	34	0.80	0.12	0.32	0.99	<0.01	0.95
BHBA, mg/dL	8.3	8.1	9.1	8.5	7.3	0.97	0.75	0.34	0.70	0.12	0.79	0.50
Cobalt, ⁴ µg/mL	0.148	0.144	0.149	0.145	0.150	0.002	0.22	0.40	0.43	0.05	0.46	0.99
Vitamin B ₁₂ , ^{5,6} pg/mL	245	250	262	231	396	11.6	<0.01	0.51	0.43	<0.01	0.61	0.73
Folates, ng/mL	12.2	11.7	11.9	12.1	12.0	0.41	0.92	0.33	0.85	0.90	0.06	0.91
Liver												
Triglycerides, ⁶ % of DM	5.9	7.3	8.2	5.7	6.0	0.98	0.34	<0.01	0.54	0.60	<0.01	0.36
Vitamin B ₁₂ , ⁷ µg/g of DM	3.39	3.60	3.45	3.45	4.14	0.13	<0.01	0.63	0.48	0.03	0.37	0.37
Cobalt, ^{7,8,9} µg/g of DM	1.31	1.36	1.39	1.56	1.34	0.02	<0.01	0.02	0.34	<0.01	<0.01	0.22

¹Significant time effect for all parameters ($P < 0.01$).²Control = no supplemental Co; CoCarb = 25 mg of supplemental Co/d from Co carbonate; LCoGH = 25 mg of supplemental Co/d from Co glucoheptonate (Zinpro Corp., Eden Prairie, MN); HCoGH = 75 mg of supplemental Co/d from Co glucoheptonate; IB12 = no supplemental Co plus weekly 10-mL vitamin B₁₂ injections.³Trt = treatment and Par = parity.⁴Tendency for significant contrast of CoCarb versus LCoGH ($P = 0.08$).⁵Significant contrast of IB12 versus mean of LCoGH and HCoGH ($P < 0.01$).⁶Tendency for significant contrast of LCoGH and HCoGH ($P \leq 0.08$).⁷Significant contrast of IB12 versus mean of LCoGH and HCoGH ($P < 0.01$).⁸Significant contrast of control versus mean of LCoGH and HCoGH ($P < 0.01$).⁹Significant contrast of LCoGH versus HCoGH ($P < 0.01$).

than primiparous cows at -7 d before calving and d 1 of lactation (Figure 4; $P = 0.06$). Furthermore, plasma folates were affected by a time effect ($P < 0.01$), with folates decreasing before calving to a nadir at -7 d relative to calving, and then increased thereafter. Liver Co and vitamin B₁₂ were both affected by a treatment by time interaction ($P \leq 0.03$). Cows on HCoGH had increased liver Co concentrations compared with cows receiving the other treatments throughout the study (Figure 5; $P < 0.01$). Liver vitamin B₁₂ concentration was increased at calving for CoCarb, LCoGH, HCoGH, and IB12 treatments compared with CON; however, the vitamin B₁₂ concentration for LCoGH, HCoGH, and CoCarb decreased at d 30 and was similar to CON for the remainder of the study (Figure 6; $P = 0.03$). The liver vitamin B₁₂ concentration of cows on IB12 remained higher than other treatments until 150 d relative to calving.

A reduction in vitamin B₁₂ after parturition is due to increased demand from secretion of vitamin B₁₂ in milk and increased metabolism during lactation. A vitamin B₁₂ deficiency is considered at plasma levels of 200 pg/mL (Suttle, 2010). Vitamin B₁₂ status was adequate for all treatments during lactation except at 30 d postpartum when cows on HCoGH had a plasma vitamin B₁₂ concentration of 192 pg/mL. Also, at 30 d postpartum, cows on CON, CoCarb, and LCoGH diets had plasma vitamin B₁₂ concentrations of 217, 225, and 248 pg/mL, respectively. Plasma vitamin B₁₂ concentrations

increased for all treatments from 30 to 90 d postpartum. Girard and Matte (1999) observed a similar decrease in plasma vitamin B₁₂ concentration during early lactation, where vitamin B₁₂ reached a nadir at 4 to 8 wk postpartum and then increased to a plateau at 16

Figure 2. Effect of treatment and time on least squares means of plasma vitamin B₁₂ concentration (pg/mL). Control = no supplemental Co; CoCarb = 25 mg of supplemental Co/d from Co carbonate; LCoGH = 25 mg of supplemental Co/d from Co glucoheptonate (Zinpro Corp., Eden Prairie, MN); HCoGH = 75 mg of supplemental Co/d from Co glucoheptonate; IB12 = no supplemental Co plus weekly 10-mg vitamin B₁₂ injections. Treatment × time: $P < 0.01$.

Figure 3. Effect of the number of days after injection of vitamin B₁₂ on plasma vitamin B₁₂ concentrations (raw means) of cows with no supplemental Co plus weekly 10-mg vitamin B₁₂ injections (IB12) on d 1 of lactation.

wk postpartum. The increase in plasma vitamin B₁₂ concentration due to injections of vitamin B₁₂ was comparable to the increase observed by Girard and Matte (2005). Kincaid and Socha (2007) observed a reduction in serum vitamin B₁₂ concentration at 20 d before calving, which is different from the present study. A reduction in serum vitamin B₁₂ may be due to decreased DMI during the prepartum period causing decreased ruminal vitamin B₁₂ synthesis. Also, increased maternal transfer of vitamin B₁₂ may decrease serum vitamin B₁₂ concentration, especially during late pregnancy.

Figure 4. Effect of parity and time on least squares means of plasma folates (ng/mL). Control = no supplemental Co; CoCarb = 25 mg of supplemental Co/d from Co carbonate; LCoGH = 25 mg of supplemental Co/d from Co glucoheptonate (Zinpro Corp., Eden Prairie, MN); HCoGH = 75 mg of supplemental Co/d from Co glucoheptonate; IB12 = no supplemental Co plus weekly 10-mg vitamin B₁₂ injections. Parity $\times$ time; $P = 0.06$.

Figure 5. Effect of treatment and time on least squares means of liver cobalt ($\mu\text{g/g}$ of DM). Control = no supplemental Co; CoCarb = 25 mg of supplemental Co/d from Co carbonate; LCoGH = 25 mg of supplemental Co/d from Co glucoheptonate (Zinpro Corp., Eden Prairie, MN); HCoGH = 75 mg of supplemental Co/d from Co glucoheptonate; IB12 = no supplemental Co plus weekly 10-mg vitamin B₁₂ injections. Treatment $\times$ time: $P < 0.01$.

Mean plasma folate levels during lactation in the current study were lower (14.1 vs. 19.5 ng/mL) than those observed by Girard and Matte (2005) where folic acid and rumen-protected methionine were supplemented. Girard and Matte (2005) observed an increase in ECM yield when cows were given injections of vitamin B₁₂, which differs from the current study. Also, only when cows had serum vitamin B₁₂ levels above 200 pg/mL

Figure 6. Effect of treatment and time on least squares means of liver vitamin B₁₂ ($\mu\text{g/g}$ of DM). Control = no supplemental Co; CoCarb = 25 mg supplemental Co/d from Co carbonate; LCoGH = 25 mg of supplemental Co/d from Co glucoheptonate (Zinpro Corp., Eden Prairie, MN); HCoGH = 75 mg of supplemental Co/d from Co glucoheptonate; IB12 = no supplemental Co plus weekly 10-mg vitamin B₁₂ injections. Treatment $\times$ time: $P = 0.03$.

did supplemental folic acid increase milk yield, protein content, and protein yield (Girard and Matte, 1998). The present study further demonstrates that supplementation with rumen-protected methionine may fulfill the need for methyl groups, but supplemental folates may be needed for optimal DNA precursor biosynthesis and cell growth (Girard and Matte, 2005).

An increase in liver vitamin B₁₂ concentration with Co supplementation has been observed with beef cattle when determining Co requirements. Stangl et al. (2000) observed an increase in liver vitamin B₁₂ concentration with increasing dietary Co to a maximum at 0.2 mg Co/kg of DM. The increase in liver vitamin B₁₂ concentration at calving suggests improvement of vitamin B₁₂ status with Co supplementation; however, the decrease after calving is likely due to increased vitamin B₁₂ demand and secretion in milk. Liver Co concentration was increased only for HCoGH and may be due to the high Co concentration or the Co source, but this cannot be elucidated because an inorganic source was not fed at the same concentration as HCoGH. However, no metabolic role for Co has been found in mammals so the importance of liver Co levels is uncertain. The increase in liver Co has not been demonstrated previously (Kincaid et al., 2003; Kincaid and Socha, 2007).

CONCLUSIONS

Supplementation with Co glucoheptonate or Co carbonate above NRC (2001) requirements and injections of vitamin B₁₂ did not affect milk yield or component yield compared with CON. Cows fed LCoGH had a trend toward improved lactation performance compared with cows fed CoCarb. However, the level of Co glucoheptonate did not affect lactation performance. Vitamin B₁₂ injections did not affect lactation performance compared with the Co glucoheptonate treatments. Cobalt supplementation or vitamin B₁₂ injections did not affect energy metabolite measures. However, Co supplementation, regardless of source or level, increased milk vitamin B₁₂ levels and liver vitamin B₁₂ at calving, but plasma vitamin B₁₂ concentration was not changed. Injections of vitamin B₁₂ increased all measures of vitamin B₁₂ as expected. Plasma folate levels were lower than those in previous studies, which observed an effect of vitamin B₁₂ injections, so folate status may have influenced the results due to reduced supply of precursors for DNA synthesis. Future research is warranted to understand the effect of cobalt supplementation when folate supplements are provided.

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