Effect of Cobalt Supplementation During Late Gestation and Early Lactation on Milk and Serum Measures¹

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ABSTRACT

Thirty-six multiparous cows were assigned to a study to determine the effects of dietary Co supplementation during late gestation and early lactation on concentrations of Co in serum and liver, vitamin B₁₂ concentrations in serum and milk, and milk yield. Nonlactating cows received diets containing 0.15, 0.89, or 1.71 mg/kg of Co (dry matter basis) from 55 d before parturition, and lactating cows received diets containing 0.19, 0.57, or 0.93 mg/kg of Co (dry matter basis) from parturition through 120 d postpartum. Serum vitamin B₁₂ concentrations declined sharply in all cows between 55 and 20 d prepartum. Dietary Co supplementation tended to cause an increase in the concentration of vitamin B₁₂ in colostrum and milk. Cobalt intake did not affect concentrations of Co in liver or serum, but increased the Co concentration of milk (0.089, 0.120, and 0.130 µg of Co/mL) at 120 days in milk. There was no effect of Co supplementation on dry matter intake or yield of milk and milk components. In conclusion, serum concentrations of vitamin B₁₂ are reduced in the early dry period, and added dietary Co may increase ruminal synthesis of vitamin B₁₂ as indicated by a tendency for increased vitamin B₁₂ concentrations in colostrum and milk of cows supplemented with dietary Co.

Key words: cobalt, vitamin B₁₂, cattle

INTRODUCTION

Ruminants require dietary Co for synthesis of vitamin B₁₂ in the rumen. The dietary requirement of dairy cows for Co is 0.11 mg/kg (NRC, 2001). However, ruminal synthesis of vitamin B₁₂ is increased by higher concentrations of dietary Co (Mills, 1981). Using closed-

system fermentors, Tiffany et al. (2006) recorded increased synthesis of vitamin B₁₂ as the Co concentration of the diet increased from 0.1 to 1.0 mg/kg. Based upon performance and blood measures, the Co requirement for fattening cattle may be between 0.15 and 0.25 mg/kg (Stangl et al., 2000a,b; Tiffany et al., 2003). However, ruminal synthesis of vitamin B₁₂ was increased with dietary levels of 1.0 mg/kg of Co (Tiffany and Spears, 2002). In addition, a lower ratio of forage to concentrate in the diet is known to reduce ruminal synthesis of vitamin B₁₂ (Walker and Elliot, 1972). More recently, Tiffany and Spears (2005) used fattening cattle to show an effect of grain source in the diet on ruminal synthesis of vitamin B₁₂ with greater response to Co supplementation when corn was the grain source compared with barley.

Secretion of B₁₂ into milk is a drain on maternal reserves of B₁₂; consequently, serum and liver B₁₂ concentrations are reduced in early lactation (Elliot et al., 1965; Wilson et al., 1967). Girard and Matte (2005) reported that cows given weekly i.m. injections of 10 mg of vitamin B₁₂ had increased secretion of ECM. Thus, the objective of this study was to determine if Co supplementation during the dry period and early lactation of dairy cows would affect serum B₁₂ concentrations and milk production.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee of Washington State University approved the protocol.

Multiparous Holstein cows (n = 36), 55 d before their expected calving date, were assigned to prepartum diets of 70% grass hay, 22% concentrate, and 8% alfalfa haylage (DM basis). Cows were individually fed via Calan feeding gates (American Calan, Inc., Northwood, NH). The concentrate was composed of 82.25% ground corn, 7.9% soybean meal (44% CP), 4% molasses, 1.9% ammonia chloride, 1.5% iodized salt, 1.0% trace mineral premix (Table 1), 0.5% limestone, 0.20% Mg oxide, 0.4% Se premix (200 mg/kg of Se as Na selenite), 0.1% vitamin A premix (30,000 IU/g), 0.1% vitamin D premix (8,810 IU/g), 0.1% vitamin E premix (500 IU/g), and 0.05%

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Table 1. Trace mineral premixes for dry cow concentrates

Ingredient	Low Co	Medium Co	High Co
	% of salt mix		
Iodized salt	72.29	70.79	69.29
Zinc methionine ¹	10.90	10.90	10.90
Zinc sulfate ²	3.67	3.67	3.67
Copper lysine ³	3.79	3.79	3.79
Copper sulfate ⁴	0.30	0.30	0.30
Manganese methionine ⁵	7.58	7.58	7.58
Manganese oxide ⁶	1.47	1.47	1.47
Cobalt glucoheptanate ⁷	0	1.5	3.0
Total	100	100	100

¹10% Zn (Zinpro Corp., Eden Prairie, MN).²36.4% Zn.³10% Cu (Zinpro Corp.).⁴25% Cu.⁵8% Mn (Zinpro Corp.).⁶72% Mn.⁷2.5% Co (Zinpro Corp.).

pellet binder. The concentrations of Co (DM basis) in the treatment dry cow diets were 0.15, 0.89, and 1.71 ppm (Table 2).

Subsequent to parturition, cows remained assigned to their respective treatments of low, medium, and high Co treatments until 120 DIM. The concentrations of Co for the 3 postpartum dietary treatments were 0.19, 0.57, and 0.93 mg/kg of Co, respectively. The lactating cows were individually fed a TMR (Table 3) via Calan feeding gates, and daily feed intakes were measured. The chemical composition of the TMR is given in Table 4.

Daily DMI was recorded for all cows, and samples of the TMR were taken weekly and composited by month. Cow BW was measured on d -55, -20, 7, and 120 relative to parturition. A sample of colostrum was collected from a nonsuckled teat after the cow was observed to have calved. Milk yields were recorded daily, and samples of a.m. and p.m. milk were taken monthly for analysis via an infrared spectrophotometer (Bentley 2000;

Table 2. Chemical composition of TMR fed to prepartum cows¹

Composition	Treatment		
	Low Co	Medium Co	High Co
CP, %	15.0	14.2	14.8
NDF, %	47	49	49
ADF, %	27	28	28
Ca, %	0.68	0.65	0.64
Co, mg/kg	0.15	0.89	1.71
Zn, mg/kg	73	62	68
Cu, mg/kg	15	11	13
Fe, mg/kg	185	208	206
Mn, mg/kg	68	80	60

¹By analysis, DM basis.**Table 3.** Composition of TMR for lactating cows

Ingredient	%, DM basis
Alfalfa haylage	25.62
Alfalfa hay	22.28
Concentrate ¹	35.65
Wheat mill run	6.57
Cottonseeds, whole	9.88

¹The ingredient composition of the concentrate was 44.13% corn, 30% barley, 8.0% peas, 4.5% soybean meal (44% CP), 4.0% corn gluten meal, 2.5% molasses, 0.5% animal fat, 0.8% limestone, 1.5% iodized salt, 2.5% sodium bicarbonate, 0.6% trace mineral premix (the trace mineral premix contained 0, 0.92, and 1.84% Co glucoheptanate; other ingredients were 1.5% Cu sulfate, 2.35% Cu lysine, 2.33% Mn methionine, 2.75% Mn oxide, 6.60% Zn sulfate, 6.65% Zn methionine, and the remainder was iodized salt, 75.98 to 77.82%), 0.4% Mg oxide, 0.4% Se premix (200 mg/kg Se), 0.05% vitamin A premix (30,000 IU/g), 0.05% vitamin D premix (8,810 IU/g), 0.02% vitamin E premix (500 IU/g), and 0.05% pellet binder.

Bentley Instruments, Chaska, MN; AOAC, 1990) of major components by the regional DHIA laboratory (Burlington, WA). Individual milk samples for Co and vitamin B₁₂ analyses were collected on d 120. Blood samples were collected on d -55, -20, 7, and 120 relative to parturition. Samples of serum, colostrum, and milk were frozen until analysis. Percutaneous liver biopsies were obtained under local anesthesia (5 mg of lidocaine and 50 mg of flunixin meglumine) at -55, 7, and 120 DIM between the 9th and 10th intercostal space using a 15-cm Tru-Cut biopsy needle (Tranvenol Labs, Deerfield, IL). Biopsy specimens were transferred to a tared vial and reweighed. The samples were stored at -30°C until analysis.

Analyses

Composite feed samples were dried (60°C for 48 h), ground to pass a 2-mm screen (Wiley mill; Arthur H. Thomas Co., Philadelphia, PA), subsampled, then re-ground to pass through a 1-mm screen, subsampled

Table 4. Chemical composition of TMR for lactating cows^{1,2}

Composition	Treatment		
	Low Co	Medium Co	High Co
CP, %	19.7	19.8	19.6
NDF, %	34.9	35.3	35.2
ADF, %	21.2	21.1	21.2
Ca, %	0.74	0.66	0.74
Co, mg/kg	0.19	0.57	0.93
Zn, mg/kg	99	85	93
Cu, mg/kg	13.6	13.8	13.7
Fe, mg/kg	120	150	150
Mn, mg/kg	58	76	74

¹By analysis, DM basis.²NE_L = 1.572 Mcal/kg (calculated from NRC, 2001).

Table 5. Effect (least squares means) of dietary Co supplementation on BW, feed intake, and yield of milk and milk components in lactating dairy cows

Measure	Treatment (Trt)			SEM	Type II SS $P > F$ for main comparison and interaction		
	Low Co	Medium Co	High Co		Trt	Time	Trt $\times$ time
DMI, kg/d	27.1	27.4	25.1	0.90	NS	0.01	0.01
Milk yield, kg/d	42.9	43.6	43.6	0.30	NS	0.05	0.01
3.5% FCM, kg/d	44.7	43.8	46.4	1.00	NS	0.01	0.01
ECM, kg/d	43.6	43.5	45.2	1.13	NS	0.01	0.01
Fat, kg/d	1.61	1.54	1.68	0.05	NS	0.01	0.01
Protein, kg/d	1.20	1.29	1.25	0.025	NS	0.01	0.01
Fat, %	3.81	3.54	3.91	0.12	NS	0.01	0.01
Protein, %	2.82	2.97	2.90	0.15	NS	NS	0.06
FCM/DMI	1.81	1.78	1.92	0.123	NS	0.01	0.01
ECM/DMI	1.76	1.76	1.87	0.116	NS	0.01	0.01
ME/IE ¹	0.766	0.766	0.813	0.051	NS	0.01	0.01

¹ME/IE = milk energy (Mcal) divided by intake energy (Mcal of NE_L).

again, and stored at -20°C until further analysis. Feed analysis consisted of DM at 100°C (AOAC, 1990), CP by an automated nitrogen analyzer (Leco FP-528, Leco Corp., St. Joseph, MI), and NDF and ADF using an Ankom 200 Fiber Analyzer (Ankom Technologies, Fairfield, NY). Blood, liver, colostrum, and milk samples were analyzed for Co by neutron activation analysis (^{60}Co with a half-life of 5.27 yr using a multichannel analyzer; Ametek Inc., Paoli, PA) at the Washington State University's Nuclear Reactor Center. Copper, Ca, Zn, Cu, Mn, and Fe, and Co in feeds were determined by atomic absorption spectrophotometry (Robinson, 1975). Nonesterified fatty acids were determined using a commercial kit (NEFA-C, Wako Chemical GmbH, Neuss, Germany). Vitamin B₁₂ analyses were performed by RIA (Dualcount, Diagnostic Products Co., Los Angeles, CA), which was modified so proteins were denatured by heating samples in a boiling water bath for 15 min instead of incubating at 37°C for 30 min.

Statistical Analysis

Data were analyzed by GLM of PROC MIXED procedures for a completely randomized design with repeated measures (SAS Institute, 2001). The cows were blocked according to expecting calving date, and treatment assignments were rotated among cows within a block. The analysis of all time series data was performed using the model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \delta_k (\alpha_i\beta_j) + e_{ijk}$$

where Y_{ijk} = dependent variable, μ = dependent variable mean, α_i = effect of treatment i ($i = 1$ to 3), β_j = effect of sampling at week j ($j = 1$ to 17), $(\alpha\beta)_{ij}$ = interaction effect between treatment and sampling week, $\delta_k (\alpha_i\beta_j) =$

random effect of cow, and e_{ijk} = residual error term. Statistical significance was declared at $P < 0.05$, and trends for treatment effects were declared at $P > 0.05$ and $P < 0.15$.

RESULTS AND DISCUSSION

Effects on Performance

The initial average BW of cows during the study was 677 ± 70 kg. Because initial BW was not considered when assigning cows to treatment, cows assigned to the medium Co treatment weighed an average of 36 kg less. During the dry period, initial DMI were less than expected primarily because cows were adapting to the Calan feeding gates. Mean DMI was 16.0, 15.2, and 14.3 kg/d (SE = 0.93), respectively, for the low, medium, and high Co diets. There was no effect ($P > 0.15$) of treatment on prepartum DMI.

Changes in BW of the lactating cows were not affected ($P > 0.15$) by dietary treatment. Similarly, DMI of the lactating cows was not affected ($P > 0.15$) by Co intake (Table 5). There was a week by treatment interaction for DMI during lactation whereby cows fed the high Co diet consumed less DM in the first 5 wk postpartum than cows fed the medium Co diet (Figure 1). Daily intakes of Co increased as daily DMI increased with time. For example, Co intakes of the lactating cows were 2.7, 8.3, and 10.3 mg of Co/d for wk 1 postpartum, but 4.9, 15.8, and 25.6 mg of Co/d for wk 18 postpartum for the low, medium, and high Co treatments, respectively.

There was no effect of treatment ($P > 0.15$) on milk composition or yields of milk, milk components, ECM, 3.5% FCM, or feed efficiency (Table 5). Efficiency of milk production (ECM/DMI) decreased with the pro-

Figure 1. Effect of dietary Co on DMI of lactating cows. ^{a,b}Weekly treatment means with different letters differ, $P < 0.05$.

gression of lactation (week by treatment interaction, $P > 0.05$; Table 5; Figure 2) presumably due to increased BW gain. As expected, the trends (not shown) for FCM per DMI and ME per energy intake were similar to that for ECM per DMI.

Girard and Matte (2005) injected cows with 10 mg of vitamin B₁₂ every week and obtained increased yield of milk components and ECM. Croom et al. (1981) did not find an effect on milk fat percentage when cows were injected weekly with 150 mg of vitamin B₁₂; however, that study was only for 21 d of early lactation.

Previously, Kincaid et al. (2003) reported a 3-way interaction of time $\times$ treatment $\times$ parity with higher milk response to Co supplementation of multiparous cows.

Effects on Tissue Co and B₁₂

Concentrations of Co in serum were not affected by Co intake or week of parity (Tables 6 and 7). Although concentrations of vitamin B₁₂ in serum were not affected by Co intake (Table 6), there was a large decrease in serum B₁₂ from 55 to 20 d prepartum, and a much

Figure 2. Effect of Co intake on efficiency of milk production (ECM/DMI). ^{a,b}Weekly treatment means with different letters differ, $P < 0.05$. Standard errors were 0.0966, 0.1008, and 0.1116 for low, medium, and high levels of Co supplementation, respectively.

Table 6. Effect of dietary Co supplementation on selected measures in serum and liver of lactating cows

Measure	Treatment (Trt)			SEM	Type II SS $P > F$ for main comparison and interaction		
	Low Co	Medium Co	High Co		Trt	Week	Trt × week
Serum							
Co, mg/kg	0.207	0.210	0.196	0.023	NS	NS	0.03
Cu, mg/kg	0.63	0.64	0.62	0.05	NS	0.01	NS
Zn, mg/kg	1.25	1.29	1.16	0.12	NS	0.01	NS
B ₁₂ , pg/mL	327	275	304	55	NS	0.01	NS
NEFA, mEq/L	286	219	268	7.7	NS	0.01	NS
IgG, mg/dL	2,882	2,986	3,104	288	NS	0.05	NS
IgM, mg/dL	208	193	176	31	NS	NS	NS
Liver							
Co, mg/kg	1.90	1.94	2.00	0.19	NS	NS	NS

smaller decrease from 7 d postpartum until 120 d postpartum (Table 7; Figure 3). To our knowledge, this is the first report of serum B₁₂ concentrations declining during late pregnancy in cows. A combination of lowered DMI in the early dry period and increased transfer of B₁₂ to the growing fetus may account for the lower serum B₁₂ at 20 d prepartum compared with 55 d prepartum. Although we did not measure DMI of cows before the start of the study, total ruminal synthesis of B₁₂ probably was greater before dry-off because of larger DMI in cows during late lactation compared with the nonlactating, late gestation period. Some of the decline in maternal serum B₁₂ concentration during late pregnancy undoubtedly reflects maternal transfer of B₁₂ to the rapidly growing fetus. Elliot et al. (1965) reported that blood B₁₂ concentrations during early lactation were 23% below those at mid lactation in dairy cows. Girard and Matte (1999) also found serum B₁₂ to be lowest during the first 2 mo of lactation. Judson et al. (1997) found that Co supplemented as an intraruminal pellet increased B₁₂ concentrations in plasma, liver, and milk. They suggested that milk B₁₂ concentration might be a useful indicator of effectiveness of Co supplementa-

tion—a better indicator than plasma B₁₂. The concentrations of vitamin B₁₂ in colostrum and milk tended ($P = 0.11$ and 0.16 , respectively) to be increased by dietary Co supplementation (Table 8). Thus, dietary Co supplementation likely increased ruminal synthesis of vitamin B₁₂ even though serum B₁₂ concentrations were not affected. Possibly, the maternal transfer of B₁₂ from blood to the fetus, colostrum, and milk prevented increased ruminal B₁₂ synthesis from being reflected in serum B₁₂ concentrations in cows.

Despite liver having the highest Co concentration and being the main storage site for Co (Underwood and Suttle, 1999), liver Co concentration was not affected by either Co intake or day of sampling (–55 and 120 d postpartum; Tables 6 and 7). Similarly, Kincaid et al. (2003) previously reported that supplementing diets of lactating and nonlactating cows with Co did not increase liver Co concentrations.

Although the concentration of Co in colostrum was not affected by Co intake, the concentration of Co in milk was increased by Co supplementation (Table 8). The elevated concentration of Co in milk of Co-supplemented cows indicates that intestinal Co absorption

Table 7. Effect of DIM on selected measures in serum and liver of lactating cows

	DIM (relative to parturition)					
Measure	−55	−20	7	120	SEM	<i>P</i> <
Serum						
NEFA, mEq/L	173	118	619	121	7.7	0.01
Co, μg/mL	0.196	0.205	0.210	0.208	0.023	NS
B ₁₂ , pg/mL	576	230	205	196	55	0.001
Zn, μg/mL	1.39	1.20	1.13	1.20	0.12	0.01
Cu, μg/mL	0.68	0.55	0.70	0.59	0.05	0.01
IgG, mg/dL	2,970	2,872	2,805	3,314	288	0.05
IgM, mg/dL	200	205	170	194	31	NS
Liver						
Co, μg/g	1.84	NA ¹	2.19	1.83	0.19	NS

¹NA = not analyzed.

Table 8. Effect of dietary Co supplementation on selected measures in colostrum and milk

Measure	Treatment			SEM	<i>P</i> Treatment
	Low Co	Medium Co	High Co		
Colostrum					
B ₁₂ , pg/mL	30,167	40,722	38,324	3,492	0.11
Co, µg/mL	0.128	0.144	0.095	0.055	NS
IgG, mg/dL	3,725	5,937	5,655	490	NS
IgM, mg/dL	139	188	182	16	NS
Milk					
B ₁₂ , pg/mL	3,369	4,497	4,224	464	0.16
Co, µg/mL	0.089	0.120	0.130	0.0069	0.038

likely was greater in these cows even though Co concentrations in serum and liver were not affected. The concentrations of Cu, Zn, NEFA, IgG, and IgM in serum were not affected by Co intakes; however, concentrations of Cu, Zn, NEFA, and IgG in serum were affected by week of sampling (Table 7). Other measures affected by week of lactation were concentrations of NEFA and IgG in serum (Table 7). In colostrum, neither IgG nor IgM concentration was affected by Co intake of cows.

CONCLUSIONS

Fetal growth during late gestation and milk synthesis are major drains on maternal reserves of vitamin B₁₂. Likewise, lowered DMI of nonlactating cows compared with lactating cows probably results in reduced ruminal synthesis of vitamin B₁₂. Accordingly, serum B₁₂ concentrations declined sharply between d 55 and 20 prepartum, and remained low during early lactation. Although serum B₁₂ was unchanged, added dietary Co caused an increase in milk Co concentration and tended to increase vitamin B₁₂ in colostrum and milk. Numerous factors such as grain source, forage-to-concentrate

ratio, Co intake, and total DMI may affect ruminal synthesis of vitamin B₁₂.

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Figure 3. Changes in serum vitamin B₁₂ concentrations during the dry period and early lactation. Concentrations of B₁₂ in serum declined significantly ($P < 0.001$) with time. Standard errors were 27, 26, 26, and 26 for d -55, -20, 7, and 120, respectively.

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