Short communication: Factors affecting vitamin B₁₂ concentration in milk of commercial dairy herds: An exploratory study

M. Duplessis,*†¹ D. Pellerin,* R. I. Cue,‡ and C. L. Girard†²

*Département des Sciences Animales, Université Laval, Québec, G1V 0A6, Canada

†Agriculture et Agroalimentaire Canada, Centre de Recherche et Développement de Sherbrooke, Sherbrooke, Québec, J1M 1Z3, Canada

‡Department of Animal Science, McGill University, Ste-Anne-de-Bellevue, Québec, H9X 3V9, Canada

ABSTRACT

Only bacteria can synthesize vitamin B₁₂, and this requires adequate Co supply. The natural source of vitamin B₁₂ in human diets comes from animal products, especially those from ruminants. This study aimed to describe variability regarding vitamin B₁₂ concentration in milk among and within commercial dairy herds in early lactation. A secondary objective was to explore potential causes for this variability such as genetic variation and diet characteristics. In total, 399 dairy cows (135 primiparous and 264 multiparous; 386 Holstein and 13 Jersey cows) in 15 commercial herds were involved. Milk samples were taken at 27.4 ± 4.1 and 55.4 ± 4.1 d in milk. Neither parity (primiparous vs. multiparous) nor sampling time affected milk concentrations of vitamin B₁₂. Nevertheless, vitamin B₁₂ concentration in milk was highly variable among and within dairy herds. The lowest vitamin B₁₂ concentration in milk of cows was observed in the Jersey herd. Among herds, vitamin B₁₂ concentration in milk ranged from 2,309 to 3,878 pg/mL; one glass (250 mL) of milk from those herds would provide between 23 and 40% of the vitamin B₁₂ recommended daily allowance. Among individual cows, however, this provision varied between 16 and 57% of the recommendation. In spite of the limited size of the studied population, the heritability value was 0.23, suggesting that genetic selection could modify milk vitamin B₁₂ concentration. We observed a positive relationship between milk vitamin B₁₂ concentration and dietary acid detergent fiber content and a negative relationship between milk concentration of vitamin B₁₂ and dietary crude protein content.

Key words: dairy cow, milk, vitamin B₁₂, genetic variation, diet characteristic

Short Communication

Only prokaryotes—bacteria and archaeobacteria—are able to synthesize vitamin B₁₂ if Co supply is sufficient (Martens et al., 2002). Consequently, the natural source of vitamin B₁₂ in human diets comes from animal products, especially those from ruminants because of the close link between the animal and prokaryotes dwelling in its rumen.

Since 1998, in Canada and the United States, it has been mandatory to fortify white flour and other grain products with folic acid (Colapinto et al., 2011) to decrease the risk of neural tube defects at birth. A Canadian survey showed that, after years of mandatory folic acid fortification, folate deficiency was virtually nonexistent in the Canadian population, and 40% had a high red blood cell folate concentration (Colapinto et al., 2011), whereas vitamin B₁₂ deficiency was detected in 4.6% of Canadians (MacFarlane et al., 2011). In humans, high folate status could be detrimental to those having a vitamin B₁₂ deficiency. Indeed, low vitamin B₁₂ status was associated with greater prevalence of both anemia and cognitive impairment when serum folate concentration was high (Selhub et al., 2007). Many studies have shown that plasma concentrations of vitamin B₁₂ are more closely related to the vitamin intake from dairy products than from fish and meat products (Vogiatzoglou et al., 2009) or the synthetic form used in supplements (Matte et al., 2012). Based on dietary reference intake for an adult (National Academy of Sciences, 1998) and composition of pasteurized whole milk (USDA, 2012), a 250-mL glass of milk provides 46% of daily vitamin B₁₂ requirements. Moreover, it has been shown that vitamin B₁₂ in pasteurized milk is not significantly destroyed by daylight exposure or refrigerator storage (Scott et al., 1984b), and bioavailability of vitamin B₁₂ does not differ among raw, pasteurized, or micronized milk (Matte et al., 2012). As annual per capita milk consumption averaged 74 L in 2014 in Canada (Canadian Dairy Information Centre, 2015), assuring a stable vitamin B₁₂ concentration in milk of

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¹Current address: Valacta, Ste-Anne-de-Bellevue, Québec, H9X 3R4, Canada

²Corresponding author: Christiane.Girard@agr.gc.ca

dairy cows could be a natural way to provide a reliable excellent source of the vitamin.

Given that ruminal synthesis of vitamin B₁₂ is affected by diet composition (Castagnino et al., 2016) and that the vitamin is partially secreted in milk (Ferlay et al., 2013), differences in diet composition and management among dairy herds are likely to affect vitamin B₁₂ concentration in milk. Moreover, Rutten et al. (2013) showed that vitamin B₁₂ concentration variability among cows could be explained in part by genetic factors. The purpose of this work was to describe the variability regarding milk vitamin B₁₂ concentration in early lactation among herds and individuals in commercial dairy herds in Québec, Canada. Even though this trial was not primarily designed to study the effect of diet characteristics and genetic variation on milk vitamin B₁₂ concentration, these 2 analyses were performed to target future research work on factors responsible for differences in milk concentrations of vitamin B₁₂ among herds and cows.

All procedures of this experiment were approved by the animal care committee from Université Laval, Québec, Canada, following the guidelines of the Canadian Council on Animal Care (2009). Herds and cows were previously described by Duplessis et al. (2014a). Briefly, the study used 15 commercial dairy herds located around Québec City (Québec, Canada). The size of dairy herds ranged from 25 to 120 cows, and cows were housed in tiestall barns and milked twice daily. Diet management differed among herds; 7 herds used a TMR and 8 herds had individual concentrate feeding systems. Data on culling rate, diseases, reproduction, lactational performance, and economic impact following a combined supplement of folic acid and vitamin B₁₂ were presented elsewhere (Duplessis et al., 2014a,b,c). As stated by Duplessis et al. (2014c), dairy herds involved in this experiment were representative of Québec dairy production in 2010. For the purpose of the present study, only vitamin B₁₂ concentration in milk of control cows (i.e., those not receiving any B-vitamin supplements) will be presented (399 dairy cows; 135 primiparous and 264 multiparous; 386 Holstein and 13 Jersey cows). The study lasted 14 mo from February 2010 to April 2011. During this period, each herd was visited every 2 wk on the same schedule.

To participate to the study, dairy herds were required to record milk production and fat and protein contents monthly through the DHI agency (Valacta, Ste-Anne-de-Bellevue, QC, Canada), to be visited at least once a month by a veterinarian, to have a preventive medicine program, and to not routinely use timed AI. Dairy producers that fulfilled the above conditions were contacted by veterinarians working close to Québec city (QC, Canada) and participation was voluntary.

Milk samples for determination of vitamin B₁₂ concentration were taken at 27.4 ± 4.1 (time 1) and 55.4 ± 4.1 (time 2) DIM. After collection, milk samples were delicately shaken to obtain a homogeneous blend, poured into 15-mL conical tubes, and put on ice for transportation. Upon arrival at the laboratory, they were frozen at -20°C and stored until analysis. Vitamin B₁₂ in milk was analyzed in duplicate by radioassay using a commercial kit (SimulTRAC B₁₂/Folate-S, MP Biomedicals, Solon, OH) as previously described by Duplessis et al. (2015). The interassay CV was 2.2%.

Early-lactation rations (ingredients and nutrient composition) from calving to 60 to 100 DIM were obtained from herd nutritionists throughout the study when ingredient changes were made. Forage samples were taken and analyzed throughout the study when switching over one forage cut to the other, and diet formulation was then based on these analyses. On average, 7 (from 3 to 10) early-lactation rations per herd were collected during the 14-mo trial. Average (minimum–maximum) nutrient and major ingredient composition was as follows: CP: 17.1% (15.5–19.8%), fat: 3.9% (3.0–5.2%), ADF: 20.5% (16.1–26.3%), NDF: 33.5% (22.1–40.6%), NFC: 38.8% (32.4–44.9%), DCAD: 258.1 mEq/kg (102.7–449.5 mEq/kg), Co: 0.55 mg/kg (0.19–1.27 mg/kg), NE_L: 1.63 Mcal/kg (1.54–1.77 Mcal/kg), concentrate: 40.6% (29.8–53.0%), corn silage: 18.6% (0.0–44.1%), haylage: 33.4% (12.3–58.7%), and hay: 6.3% (0.0–24.3%). Haylage was defined as forage having less than 70% DM and hay as having more than 70% DM. Among herds, rations were computed to meet or exceed Co requirements (0.11 mg/kg of DM; NRC, 2001). For each Holstein dairy cow, based on DIM during which each early-lactation ration was fed from calving to 8 wk of lactation, weighted average of CP, fat, ADF, NDF, and NFC (%) and weighted average of DCAD (mEq/kg), Co (mg/kg), and NE_L (Mcal/kg) on a DM basis were calculated. The same calculation was done for percentages of concentrate, corn silage, haylage, and hay on a DM basis.

Vitamin B₁₂ concentration in milk was analyzed with the MIXED procedure of SAS (version 9.4, SAS Institute, 2012) with repeated measures. Parity, sampling month, herd, and time, as well as parity $\times$ time and herd $\times$ time interactions were fixed effects. Parity referred to primiparous and multiparous cows after calving. Time referred to sampling time; that is, 27.4 ± 4.1 (time 1) and 55.4 ± 4.1 (time 2) DIM. Seven different covariance structures were tested (compound symmetry, heterogeneous compound symmetry, first-order autoregressive, heterogeneous first-order autoregressive, Toeplitz, heterogeneous Toeplitz, and unstructured); heterogeneous first-order autoregressive was chosen because fit statistics were the smallest. Results were

considered significant when $P \leq 0.05$ and as a tendency at $0.05 < P \leq 0.10$.

For the genetic analysis, data from Holstein cows were analyzed using the WOMBAT program (Meyer, 2007) according to the following statistical model:

$$Y_{ijkmn} = \mu + herd_i + DIM_j + Pa_k + YS_m + a_n + pe_n + \varepsilon_{ijkmn},$$

in which Y_{ijkmn} is the milk vitamin B₁₂ concentration from the n th cow in the i th herd, on the j th DIM, on the k th parity, in the m th year-season, μ is the overall mean, $herd_i$ is the random effect of the i th herd ($i = 1, \dots, 14$; the Jersey herd was removed from the analysis), DIM_j is the fixed classification effect of the j th DIM ($j = 21, \dots, 63$), Pa is the fixed classification effect of the k th parity class ($k = 1 = \text{primiparous}$, $k = 2 = \text{multiparous}$), YS_m is the fixed classification effect of the m th year-season (year-seasons were formed as March–August, and September–February), a_n is the additive genetic (random) effect of the n th animal ($n = 1, \dots, 1,894$), pe_n is the permanent random effect of the n th cow, and ε_{ijkmn} is the residual error. Additive genetic relationships among the levels of a were incorporated using Henderson's rules for the calculation of $\mathbf{A}^{-1}$. Significance of the random effects (additive genetic effect and permanent environment cow effect) was tested by fitting the above model and then alternately dropping either the additive genetic effect (a) or the permanent environment cow effect (pe), and comparing the computed Bayesian information criterion (BIC) test statistics. The heritability (h^2) was computed as

$$h^2 = \sigma_{animal}^2 / (\sigma_{animal}^2 + \sigma_{herd}^2 + \sigma_{pe}^2 + \sigma_{\varepsilon}^2).$$

where σ_{animal}^2 was the genetic variation, σ_{herd}^2 was the herd variation, σ_{pe}^2 was the permanent environment variation, and σ_{ε}^2 was the residual variation (Poulsen et al., 2015).

The following requirements were imposed for data being a part of the genetic analysis: herd-sampling dates had to have more than 1 record; cows had to have 2 milk sample records; only Holstein cows were retained (there were insufficient animals of Jersey breed to consider analyzing them); parity had to be less than 9; cows had to have both sire and dam correctly identified (via the Holstein breed pedigree files maintained by the Canadian Dairy Network); and records where the indicated milk vitamin B₁₂ concentration was abnormally high were eliminated (i.e., >10,000 pg/mL). After these sequential editing constraints were imposed, from the

originally 399 participating control cows, 343 cows remained, with 645 milk vitamin B₁₂ concentration records in 199 herd-sampling date groups. Pedigrees were traced back 3 generations; the generated pedigree included 1,894 animals. Milk concentrations of vitamin B₁₂ were scaled by dividing by 1,000 to facilitate numerical stability (from pg/mL to ng/mL).

For the effect of diet characteristic analysis, data from Jersey cows were excluded to remove variability due to breed; one herd was not included as its main breed was Jersey. A second herd was excluded from the ingredient analysis as important information on the rations provided was missing.

Simple correlations using Proc CORR of SAS were performed by time among diet nutrients and ingredients to identify those with a high correlation ($r > 0.75$) to avoid placing them in the same models due to collinearity (Godden et al., 2001). No variable was correlated ($R < 0.75$). Univariate linear regression model analyses were performed separately for each sampling time using the Proc MIXED of SAS to assess relationship between milk vitamin B₁₂ concentrations (dependent variable) and ration nutrient composition and ingredients (independent variables). As independent nutrient composition and ingredient variables, CP, fat, ADF, NDF, and NFC (% of DM), DCAD (mEq/kg of DM), Co (mg/kg of DM), and NE_L (Mcal/kg of DM) were tested in the model as well as ration percentages of concentrate, corn silage, haylage, and hay on a DM basis.

Using Proc MIXED of SAS, multivariate linear regression models for each sampling time were composed of independent variables that were significant in univariate models ($P < 0.20$) to describe individual milk concentration of vitamin B₁₂. Independent variables that were no longer significant in the final multivariate model ($P < 0.10$) were removed by backward elimination, as described by Godden et al. (2001).

Herd was first fitted as a random effect in each model. The BIC fit statistic value was compared for the same model with and without herd as a random effect. If the BIC difference was <3, the random effect was considered to have no strong effect. There was no strong evidence for a herd random effect in the 2 final multivariate models and it was thus removed.

In the current study, vitamin B₁₂ concentration in milk of cows was not affected by sampling time ($P = 0.36$), as previously reported within the first 8 wk of lactation (Graulet et al., 2007), or parity ($P = 0.46$), as shown by Akins et al. (2013). Concentration of vitamin B₁₂ in milk differed among herds ($P < 0.0001$; Table 1); indeed, vitamin B₁₂ concentration in milk ranged from 2,309 to 3,878 pg/mL. Among herds, a glass of milk (250 mL) would provide only 51 to 88% of the

Table 1. Mean, SD (coefficient of variation in parentheses), and minimum, and maximum vitamin B₁₂ concentration in milk from early lactating cows

Herd	Cows (no.)	Vitamin B ₁₂ concentration in milk (pg/mL)			
		Mean	SD (CV)	Minimum	Maximum
1	13	2,615	531 (20)	1,795	3,333
2	25	3,170	910 (29)	1,857	5,017
3	39	3,671	1,057 (29)	1,671	6,121
4	23	3,313	848 (26)	1,878	5,040
5	61	3,177	581 (18)	1,710	4,589
6	22	3,153	914 (27)	2,057	5,012
7	22	3,049	678 (20)	1,972	5,116
8	21	3,438	908 (27)	1,874	5,236
9	21	2,827	1,163 (37)	1,213	6,702
10	27	2,776	711 (26)	1,754	5,454
11	13	3,520	751 (21)	2,256	4,730
12	16	3,878	1,067 (28)	2,509	6,755
13	35	3,135	850 (27)	1,741	5,787
14	7	2,309	607 (27)	1,626	3,292
15	40	2,937	910 (31)	1,553	5,668

value reported in nutritional tables for whole pasteurized milk (USDA, 2012). According to the literature, the average vitamin B₁₂ concentration in milk of cows is highly variable in early lactation; vitamin B₁₂ concentrations in milk ranging from 1,575 to 4,781 pg/mL have been reported (Preynat et al., 2009; Akins et al., 2013; Rutten et al., 2013).

The lowest milk concentration of vitamin B₁₂ (Table 1) was observed in the Jersey herd. Previous studies also reported that concentration of vitamin B₁₂ in milk from Jersey cows was lower than that in milk from Holstein cows (Anthony et al., 1951; Miller et al., 1966). However, because there was only one Jersey herd involved

in this study, diet and management factors could not be discarded as an explanation for this difference. Vitamin B₁₂ concentration in milk varied among sampling months ($P = 0.01$); it was greater during spring and fall months than during winter and summer months (Figure 1). Scott et al. (1984a) observed a similar pattern. Milk concentration of vitamin B₁₂ was highly variable among individuals within each herd (Table 1), as similarly reported by Collins et al. (1951), Gregory et al. (1958), and Rutten et al. (2013).

Rutten et al. (2013) showed that genotype of the cow affects vitamin B₁₂ concentration in milk. Poulsen et al. (2015) showed similar results regarding milk

Figure 1. Vitamin B₁₂ concentration in milk of cows among sampling months (month effect, $P = 0.01$). Milk samples were taken at 27.4 ± 4.1 and 55.4 ± 4.1 DIM. Error bars indicate $\pm$ SE.

Table 2. Variance components and heritability (h^2) of milk vitamin B₁₂ concentration of 343 Holstein cows

Source of variation ¹	Vitamin B ₁₂ ² (ng/mL)
σ_{animal}^2	0.24 ± 0.22
σ_{pe}^2	0.21 ± 0.21
σ_{herd}^2	0.12 ± 0.03
σ_{ε}^2	0.61 ± 0.06
h^2	0.23 ± 0.20

¹pe = permanent environment of the cow; ε = residual error;

$h^2 = \sigma_{animal}^2 / (\sigma_{animal}^2 + \sigma_{herd}^2 + \sigma_{pe}^2 + \sigma_{\varepsilon}^2)$.

²All milk concentrations of vitamin B₁₂ were scaled by dividing by 1,000 to facilitate numerical stability.

vitamin B₂ (riboflavin) concentration. In the present study, the heritability (i.e., the proportion of variation due to genetic variation) was 0.23 ± 0.20 , suggesting that genetic selection could modify milk vitamin B₁₂ concentration (Table 2). Rutten et al. (2013) reported a heritability value of 0.37 for milk concentration of vitamin B₁₂ of 544 first-lactation Dutch Holstein Friesian cows. It is important to note that the current study was not primarily designed to estimate genetic variability and hence the sampling cannot be considered optimal for genetic parameter estimation.

It is worth noting that the current study is an exploratory analysis that relies on the accuracy of diet formulation based on forage analyses to estimate what dairy cows ingested during the experimental period. Commercial concentrate blends were used in many herds, and the design of the current trial did not allow us to measure the actual quantity of each ingredient ingested by each cow at sampling days.

For both sampling times, independent variables that partially explained differences in milk vitamin B₁₂ concentration in the univariate models ($P < 0.20$) were percentage of CP and percentage of ADF, which had negative and positive relationships, respectively, with the dependent variable. These 2 variables remained in the final multivariate linear model ($P \leq 0.10$). No other nutrient or ingredient was associated with changes in milk vitamin B₁₂ concentration according to the current analysis.

Very few studies have been published on the influence of dietary management on milk vitamin B₁₂ concentration (Graulet, 2014). To our knowledge, the relationship between vitamin B₁₂ concentration in milk and ration nutrient composition has never been studied before. Different species of microorganisms in the rumen are involved in the degradation of forage and concentrate (Petri et al., 2012), and Dryden et al. (1962) showed

that some strains of ruminal bacteria could synthesize a greater amount of vitamin B₁₂ than others. As vitamin B₁₂ is synthesized by ruminal microbes and a proportion of the synthesized vitamin is secreted into milk, we could reasonably hypothesize that what affects apparent vitamin B₁₂ ruminal synthesis would also affect milk vitamin B₁₂ concentration. Apparent ruminal synthesis of vitamin B₁₂ was positively correlated with dietary NDF and sugar contents and negatively correlated with NFC content in the trial of Schwab et al. (2006). Santschi et al. (2005) observed that apparent ruminal synthesis of biologically active vitamin B₁₂ was greater with a 60:40 forage-to-concentrate ratio diet than with a 40:60 forage-to-concentrate ratio diet. Similarly, apparent ruminal synthesis was about 3-fold greater for cows receiving a high-fiber diet compared with cows receiving a high-starch diet (Beaudet et al., 2016). In the current study, even if no relationship was observed between vitamin B₁₂ concentration and NDF content, ADF content was positively associated with milk vitamin B₁₂ concentration.

In the current trial, a negative relationship was observed between vitamin B₁₂ concentration in milk and ration CP. Beaudet et al. (2016) concluded that apparent ruminal synthesis of vitamin B₁₂ was not affected by 2 levels of CP in the ration: 11.1% and 14.3% CP on a DM basis. However, the latter comparison was done with lower CP content than in the current study, in which CP content averaged 17.1% DM among herds. To our knowledge, no other studies have measured the effect of CP content of diet on milk concentration of vitamin B₁₂.

In conclusion, vitamin B₁₂ concentration in milk was highly variable among dairy herds and cows. Among herds, one glass (250 mL) of milk would have provided between 23 and 40% of the recommended daily allowance of vitamin B₁₂. Under the studied conditions, concentrations of vitamin B₁₂ in milk of cows were lower than the values used to calculate the daily provision of the vitamin in human nutrition. Among individual cows, however, this provision varied between 16 and 57% of the recommendation. Despite the limited sample size, the heritability value was 0.23 ± 0.20 , suggesting that genetic selection could be used to modify milk vitamin B₁₂ concentration. A positive relationship between vitamin B₁₂ concentration in milk and ADF content and a negative relationship between milk concentration of vitamin B₁₂ and CP content of the diet on a DM basis were observed. This suggests that vitamin B₁₂ concentration of milk could be modified by ration composition. Our results will need to be confirmed but they open new research avenues on factors affecting the vitamin B₁₂ concentration in milk.

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