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# *Short communication:* Relationships among plasma and milk vitamin $B_{12}$, plasma free fatty acids, and blood β-hydroxybutyrate concentrations in early lactation dairy cows

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## ABSTRACT

This study was undertaken to evaluate the relationship between plasma and milk concentrations of vitamin $B_{12}$ as well as the relationship between plasma or milk concentrations of vitamin $B_{12}$ and plasma concentration of free fatty acids (FFA) or blood concentration of β-hydroxybutyrate (BHB) of early lactating Ayrshire (AY) and Holstein (HO) cows. A total of 44 dairy herds (7 AY and 37 HO herds) and 62 AY (21 in first, 19 in second, and 22 in third and more lactations) and 228 HO (51 in first, 74 in second, and 103 in third and more lactation) cows between 3 and 40 d in milk were involved in the study. Hand-stripped milk samples and blood samples were taken 6 h after the morning milking. Milk and plasma samples were analyzed for vitamin $B_{12}$ concentration and plasma samples were analyzed for FFA concentration. A handheld device was used for blood BHB concentration determination. Thresholds for elevated plasma FFA concentration and hyperketonemia were set at ≥0.70 and ≥1.2 mmol/L, respectively. Vitamin $B_{12}$ concentration in milk of AY primiparous cows [2,557 (1,995–3,276) pg/mL] was lower than in milk from HO primiparous cows [3,876 (3,356–4,478) pg/mL], whereas no difference was observed among other parities and breeds. Regardless of breeds, plasma concentration of vitamin $B_{12}$ of first and second parities was lower than plasma concentration of third and more lactation cows. Milk vitamin $B_{12}$ concentration was positively correlated with plasma vitamin $B_{12}$ concentration, but the relationship was stronger for AY (ρ averaging 0.63) than for HO cows (ρ averaging 0.36). For AY and HO breeds, a significant relationship between milk or plasma vitamin $B_{12}$ concentrations and plasma FFA concentration (ρ between 0.29 and 0.59) was observed. Moreover, cows with elevated plasma FFA concentration had greater milk and plasma vitamin $B_{12}$ concentrations than cows with normal plasma FFA concentration. No relationship between vitamin $B_{12}$ concentration in milk or plasma and blood BHB concentration and hyperketonemia was noted. In summary, milk is not well correlated with plasma vitamin $B_{12}$ concentration for HO. It could be hypothesized that elevated plasma concentration of FFA could have a negative effect on the use of vitamin $B_{12}$ by cow cells, which increases the concentration of the vitamin in plasma and its secretion in milk.

**Key words:** dairy cow, vitamin $B_{12}$, milk, free fatty acid, β-hydroxybutyrate

## Short Communication

In ruminants, vitamin $B_{12}$ acts as a coenzyme for methylmalonyl-CoA mutase (Enzyme Commission number = 5.4.99.2), which transforms methylmalonyl-CoA into succinyl-CoA (Scott, 1999), a step allowing propionate to enter the Krebs cycle. In dairy cows, propionate is the precursor for up to 60% of glucose released from the liver (Larsen and Kristensen, 2013; Duplessis et al., 2017a). In early lactation, when energy supply is lower than energy demand for milk production, dairy cows enter into a state of negative energy balance. They adapt by mobilizing body fat reserve (Goff and Horst, 1997), increasing plasma concentration of free fatty acids (**FFA**). When FFA oxidation by the Krebs cycle is incomplete, plasma concentration of ketone bodies such as BHB will increase as a consequence (Goff and Horst, 1997). Several studies reported that a combined supplement of vitamin $B_{12}$ and folic acid altered energy partitioning in early lactation (Graulet et al., 2007; Preynat et al., 2009; Duplessis et al., 2017b) either by increasing milk production without affecting

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plasma FFA concentration or by decreasing plasma FFA concentration with no effect on milk production. Nevertheless, under the current state of knowledge, it is not known if a relationship exists between plasma or milk vitamin $B_{12}$ concentrations and plasma FFA or blood BHB concentrations of cows without vitamin supplementation. However, older studies reported lower plasma concentration of vitamin $B_{12}$ for cows with elevated concentration of ketones in urine and blood (Corse and Elliot, 1970; Korpela and Mykkänen, 1983).

Vitamin $B_{12}$ concentrations in plasma and milk have been shown to vary greatly among dairy cows and among studies (Graulet et al., 2007; Preynat et al., 2009; Duplessis et al., 2016, 2017a). However, to our knowledge, the relationship between vitamin $B_{12}$ concentrations in milk and plasma has never been established in samples collected simultaneously from cows receiving no vitamin $B_{12}$ supplement. Thus, the first objective of this study was to evaluate if vitamin $B_{12}$ concentration in milk could be correlated with vitamin $B_{12}$ concentration in plasma of Ayrshire (**AY**) and Holstein (**HO**) cows in early lactation under commercial conditions. Another aim was to determine if a relationship existed between plasma or milk concentration of vitamin $B_{12}$ and plasma concentration of FFA or blood concentration of BHB. Additionally, the experiment determined plasma and milk concentrations of vitamin $B_{12}$ across 2 breeds, AY and HO. Vitamin $B_{12}$ concentration in AY milk and plasma is not well documented.

All procedures of this experiment were approved by the Animal Care Committee from McGill University, Sainte-Anne-de-Bellevue, QC, Canada, following the guidelines of the Canadian Council on Animal Care (2009). A total of 44 dairy herds (7 AY and 37 HO herds) and 62 AY (21 in first, 19 in second, and 22 in third and more lactations) and 228 HO (51 in first, 74 in second, and 103 in third and more lactations) cows between 3 and 40 DIM were involved in the study. All cows were milked twice daily and housed in tiestall barns, except for one herd that housed cows in a freestall barn. To participate, herds had to record milk production through the DHIA and to have AY or HO cows as main breed. Producers were contacted by phone and participation was on a voluntary basis.

Herds were visited once between May and August 2016. Estimated BW was taken by measuring heart girth circumference using a calibrated tape (Yan et al., 2009). A milk sample with bronopol was taken during the morning milking using an in-line milk meter to obtain its composition and milk yield was recorded. Hand-stripped milk samples were collected 6 h after milking, transported on ice, and stored at −20°C until analysis of vitamin $B_{12}$ concentration. Blood samples from the tail vein were immediately taken after hand-stripped milk sample collection using both EDTA as anticoagulant and serum separator Vacutainer tubes (Becton Dickinson and Company, Franklin Lakes, NJ). A handheld device (FreeStyle Precision Neo, Abbott Diabetes Care Inc., Mississauga, ON, Canada) was used to determine blood BHB concentration immediately after blood collection with tubes without anticoagulant. Blood samples taken with EDTA tubes were put on ice for transportation and were centrifuged within 2 h after collection for 12 min at 3,000 × $g$ at 4°C. Plasma samples were then frozen at −20°C until analysis.

Milk composition (fat, protein, and lactose) was analyzed using mid-infrared reflectance spectrometry (MilkoScan FT 6000, Foss, Hillerød, Denmark) by Valacta (Dairy Production Center of Expertise, Québec and Atlantic Provinces, Sainte-Anne-de-Bellevue, QC, Canada). A commercial kit was used for analysis of plasma concentration of FFA [HR Series NEFA-HR(2), Wako Chemicals USA Inc., Richmond, VA] in duplicate. Plasma and milk concentrations of vitamin $B_{12}$ were analyzed in duplicate by radioassay as previously described by Duplessis et al. (2015) using a commercial kit (SimulTRAC $B_{12}$/Folate-S, MP Biomedicals, Santa Ana, CA). The interassay CV were 4.3 and 2.7% for plasma and milk vitamin $B_{12}$ concentration analyses, respectively.

For analysis purpose, cows were divided according to parity as follows: (1) first, (2) second, and (3) third parity and greater, and DIM were divided into 4 categories: (1) below 10 DIM, (2) between 11 and 20 DIM, (3) between 21 and 30 DIM, and (4) between 31 and 40 DIM. Proc MIXED of SAS (version 9.4, SAS Institute Inc., Cary, NC) was used to analyze estimated BW, morning milk yield and components, plasma and milk vitamin $B_{12}$ concentrations, plasma FFA concentration, and blood BHB concentration according to parity, breed, DIM, as well as parity × breed interaction as fixed effects. Residuals were studied for normality. Plasma and milk vitamin $B_{12}$ concentrations, plasma FFA concentration, and blood BHB concentration violated this assumption, and data were then log-transformed. Least squares means and 95% confidence interval results from log-transformed dependent variable models were back-transformed and presented afterward as geometric means and 95% confidence interval. Results from untransformed data were reported as least squares means and 95% confidence interval. The threshold for elevated plasma concentration of FFA was set at 0.70 mmol/L as previously determined by Ospina et al. (2013). Hyperketonemia (**HYK**) was defined as plasma BHB concentration ≥1.2 mmol/L (Macmillan et al., 2017). Proc MIXED of SAS was used to evaluate vitamin $B_{12}$ concentrations in plasma and milk according to the threshold of elevated plasma concentrations of FFA or