Short communication: Casein hydrolysate and whey proteins as excipients for cyanocobalamin to increase intestinal absorption in the lactating dairy cow

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ABSTRACT

Bioavailability of vitamin B₁₂ is low in humans and animals. Improving vitamin B₁₂ absorption is important for optimal performance in dairy cows and for increasing vitamin B₁₂ concentrations in milk for human consumption. However, when supplemented in the diet, 80% of synthetic vitamin B₁₂, cyanocobalamin (CN-CBL), is degraded in the rumen of dairy cows and only 25% of the amount escaping destruction in the rumen disappears from the small intestine between the duodenal and ileal cannulas. In pigs, vitamin B₁₂ from milk is more efficiently absorbed than synthetic CN-CBL. The objective of this study was to determine the efficacy of casein hydrolysate and whey proteins as excipients for CN-CBL to increase portal-drained viscera (PDV) flux of the vitamin in lactating dairy cows. Four multiparous lactating Holstein cows (237 ± 17 DIM) equipped with a rumen cannula and catheters in the portal vein and a mesenteric artery were used in a randomized Youden square design. They were fed every 2 h to maintain steady digesta flow. On experimental days, they received a postruminal bolus of (1) CN-CBL alone (0.1 g), (2) CN-CBL (0.1 g) + casein hydrolysate (10 g), or (3) CN-CBL (0.1 g) + whey proteins (10 g). Starting 30 min after the bolus, blood samples were taken simultaneously from the 2 catheters every 15 min during the first 2 h and then every 2 h until 24 h postbolus. Milk yield, DMI, and vitamin B₁₂ portal-arterial difference and PDV flux were analyzed using the MIXED procedure of SAS. Milk yield and DMI were not affected by treatments. The portal-arterial difference of vitamin B₁₂ during the 24-h period following the bolus of vitamin was greater when the vitamin was given in solution with casein hydrolysate (2.9 ±

4.6 pg/mL) than alone (−17.5 ± 5.2 pg/mL) or with whey protein (−13.4 ± 4.2 pg/mL). The treatment effects were similar for the PDV flux. The present results suggest that CN-CBL given with casein hydrolysate increases vitamin B₁₂ absorption as compared with CN-CBL given alone.

Key words: cow, cyanocobalamin, bioavailability, vitamin B₁₂, casein hydrolysate

Short Communication

Bacteria present in the rumen synthesize vitamin B₁₂, and unless dietary Co supply is inadequate, the amount of vitamin B₁₂ produced in the rumen is sufficient to prevent deficiency symptoms in the lactating dairy cow (NRC, 2001). Despite this, when given to dairy cows in early lactation in combination with folic acid, intramuscular injections of vitamin B₁₂ improved lactational performance and metabolic efficiency, suggesting that rumen microbial synthesis might not always be sufficient to optimize animal performance (Girard and Matte, 2005; Preynat et al., 2009). For practical applications, systematic use of intramuscular injections of the vitamin is not desirable, whereas bioavailability of a dietary supplement of the synthetic form of vitamin B₁₂, cyanocobalamin (**CN-CBL**), not protected from degradation in the rumen is very low (Girard et al., 2009).

Previous studies in pigs have estimated the bioavailability of dietary vitamin B₁₂ using portal-drained viscera (**PDV**) flux (Matte et al., 2010, 2012). The cumulative net PDV fluxes of vitamin B₁₂ after ingestion of meals based on corn, wheat, and soybean meal and supplemented with 44 and 71 µg of CN-CBL were not different from 0 (Matte et al., 2012), suggesting no detectable absorption of vitamin B₁₂. However, when CN-CBL was offered with a semi-synthetic diet consisting of vitamin-free casein hydrolysate, corn starch, and sucrose, the cumulative net PDV fluxes of vitamin B₁₂ during the 24 h following dietary boluses of 25 and

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250 µg of CN-CBL were positive at 2.4 and 5.1 µg, respectively, and differed between doses (Matte et al., 2010). Therefore, combining a supplement of CN-CBL with specific milk proteins or peptides may be an approach to increase the amount of vitamin B₁₂ reaching portal blood.

The objective of this study was to determine the efficacy of casein hydrolysate or whey proteins as excipients for CN-CBL to increase PDV flux of the vitamin in lactating dairy cows.

Animals were cared for according to the recommended Code of Practice for the Care and Handling of Dairy Cattle (2009) and the guidelines of the Canadian Council on Animal Care (CCAC, 2009). Four multiparous lactating Holstein cows (237 ± 17 DIM and averaging 708 kg ± 89 kg of BW), which had been equipped with a rumen cannula and chronic indwelling catheters in the portal and 2 mesenteric veins as well as in a mesenteric artery (Huntington et al., 1989) at least 5 mo before the start of the experiment, were used. The catheters were exteriorized individually over the paralumbar shelf and spine. The right carotid artery was surgically raised to a subcutaneous position to allow access to arterial blood if the catheter placed in a mesenteric artery failed. In cases where both arterial catheters failed, a catheter was installed in an auricular artery to collect blood. Cows were housed in a tie-stall barn with free access to water under 17 h of light per day (0500 to 2200 h) and were fed daily in the morning 1 kg of long hay. To ensure rapid and complete ingestion of each meal, a TMR (Table 1) was served in 12 equal meals (2-h intervals) by automatic feeders (Ankom, Fairport, NY) at 95% of the voluntary feed intake measured the week before the start of the study. Feed intake and refusals, if present, were weighed and recorded daily. Cows were milked twice a day (0700 and 1900 h).

The cows were randomly assigned to 1 of 3 treatments in a Youden square design according to Cochran and Cox (1957) with a minimum of 24 h between periods. The treatments were a postruminal bolus infusion (Clark, 1975) of 60 mL of the following solutions: (1) CN-CBL alone (0.1 g), (2) CN-CBL (0.1 g) + 10 g of casein hydrolysate (casein acid hydrolysate vitamin free, Sigma, St. Louis, MO), and (3) CN-CBL (0.1 g) + 10 g of milk whey protein (Hillmar 9400 whey protein isolate, Canada Colors and Chemicals Ltd., St-Laurent, QC, Canada). All products were dissolved in water. To ensure maximal potential linkage with CN-CBL, 10 g of casein hydrolysate or whey protein isolate was used. The postruminal bolus infusion was given in less than 60 s and was followed by a 60-mL bolus of water.

All feed ingredients were sampled at the beginning of the study and frozen at -20°C until analysis. Samples

Table 1. Ingredient and chemical composition of the TMR¹

Item	%
Ingredient	
Soybean hulls	5.8
Corn silage ²	29.9
Grass silage ³	18.2
Corn	18.1
Canola meal	21.9
Beet pulp	2.0
Mineral and vitamin supplement ⁴	1.8
Calcium carbonate	0.3
Urea	0.2
Megalac ⁵	1.7
Nutrient composition	
DM (%)	50.0
CP (% of DM)	15.1
RDP (% of CP)	49.4
Soluble protein (% of CP)	31.1
ADF (% of DM)	18.9
NDF (% of DM)	32.6
NE _L (Mcal/d)	1.59
Co (mg/kg)	0.40

¹All cows were served daily 1 kg of long hay (85% DM) on a DM basis, 10.5% CP; 2.5% soluble protein; 30.6% ADF; 54.4% NDF.

²On a DM basis, 8.6% CP; 3.4% soluble protein; 21.7% ADF; 40.0% NDF.

³On a DM basis, 16.7% CP; 7.7% soluble protein; 31.2% ADF; 47.6% NDF.

⁴Contained per kilogram: 91.8 g of Ca; 47.9 g of P; 47.9 g of Mg; 11.9 g of K; 80.8 g of Cl; 136.8 g of Na; 15 g of S; 1,946 mg of Fe; 2,656 mg of Zn; 440 mg of Cu; 1,798 mg of Mn; 23 mg of I; 19.50 mg of Se; 441.6 kIU of vitamin A; 56.6 kIU of vitamin D; and 2.63 kIU of vitamin E.

⁵Arm & Hammer Animal Nutrition, Princeton, NJ.

were dried, ground, and analyzed for DM, CP (AOAC International, 2000), ADF, and NDF (Ankom200 fiber analyzer, Ankom Technology Corp., Fairport, NY), and cobalt (inductively coupled plasma emission spectrometry; Agri-Food Laboratories, Guelph, ON, Canada).

Starting 30 min after the postruminal infusion, blood samples (6 mL) were simultaneously taken from the portal vein and an artery every 15 min for the first 2 h, and then, every 2 h until 24 h after the postruminal bolus. Immediately after collection, blood samples were put in Vacutainer tubes with EDTA (Becton Dickinson Inc., Franklin Lakes, NJ), the tubes were then placed on ice until centrifuged (2,000 × *g* for 10 min at -4°C) and plasma was stored at -20°C. Vitamin B₁₂ in plasma was analyzed in duplicate by radioassay with a commercial kit designed for human plasma (SimulTRAC-S Radioassay kit, vitamin B₁₂ (⁵⁷Co)/folate (¹²⁵I), MP Biomedicals, Diagnostics Division, Orangeburg, NY).

Portal-arterial difference and net flux of vitamin B₁₂ across PDV were calculated as described by Girard et al. (2001) for each sampling time. However, portal blood flow was estimated according to the following equation ($r^2 = 0.98$):

$$\gamma = 400.38 + 47.351 \times \text{DMI},$$

Table 2. Arterial plasma concentration, portal-arterial difference, and portal-drained viscera (PDV) flux of vitamin B₁₂ during the 24 h following a postruminal bolus of cyanocobalamin alone (CN-CBL), with casein hydrolysate (CN-CBL + C) or milk whey protein isolate (CN-CBL + WP)¹

Item	Treatment			P-value		
	CN-CBL	CN-CBL + C	CN-CBL + WP	Treatment	Time	Treatment × time
n	3	4	4			
Arterial plasma concentration of vitamin B ₁₂ (pg/mL)	323 ± 23 ^b	403 ± 19 ^a	413 ± 19 ^a	0.05	0.21	0.41
Portal-arterial difference of vitamin B ₁₂ (pg/mL)	-17.5 ± 5.2 ^b	2.9 ± 4.6 ^a	-13.4 ± 4.2 ^b	0.05	0.0003	0.34
Net PDV flux of vitamin B ₁₂ (µg/h)	-25.4 ± 7.4 ^b	3.9 ± 6.6 ^a	-19.8 ± 6.0 ^b	0.05	0.0003	0.39

^{a,b}Means within a row with different superscripts were considered different ($P \leq 0.05$).

¹Least squares means with SE.

where γ is the average portal blood flow (L/h) and DMI is dry matter intake (kg/d) based on values obtained from the cows used in the present study and fed the same diet in a previous study (G. Maxin, Institut de la recherche agronomique, St-Genès-Champagnelle, France; personal communication), where plasma flow in the portal vein was calculated by downstream dilution of *p*-aminohippurate (Katz and Bergman, 1969). A positive net flux indicates a release by the PDV tissues into the portal vein, whereas a negative flux indicates an uptake by these tissues.

All variables were analyzed as a Youden square design using the MIXED procedure (SAS version 9.3, SAS Institute Inc., Cary, NC) according to the following model:

$$Y_{ijkl} = \mu + C_k + P_i + B_j + d_{ijk} + T_l + BT_{jl} + e_{ijkl},$$

where Y_{ijkl} indicates the dependent variable, μ is the overall mean, C_k is the effect of cow, P_i is the effect of period, B_j is the effect of treatment, and T_l is the effect of time. The error terms are d_{ijk} (error a) and e_{ijkl} , the residual error. Fixed effects were cows, periods, and treatments. As the time intervals (repeated measure) between samples were not always the same, the following covariance structures were compared: SP(POW), SP(GAU), SP(EXP), SP(LIN), SP(LINL), SP(SPH), ANTE(1), and UN. For each variable, the covariance structure retained was the one that provided the smallest fit statistic values from Akaike information criteria. Results are reported as least squares means and standard errors. Data from one cow are missing because of the failure of the portal catheter during the last period of sampling. Means were assumed to be different at $P \leq 0.05$; they were compared using an adjusted Tukey's test.

Dry matter intake (19.6 ± 1.0 kg/d) and milk production (33 ± 2.5 kg/d) were similar ($P \geq 0.6$) among treatments. Arterial plasma concentrations of

vitamin B₁₂ were greater ($P = 0.05$) when CN-CBL was infused with either of the milk proteins (Table 2). Portal-arterial difference and PDV flux of vitamin B₁₂ differed ($P = 0.05$) among treatments, being greater when CN-CBL was given with casein hydrolysate than alone or with milk whey protein (Table 2; Figure 1). Across the 3 treatments, portal-arterial differences of vitamin B₁₂ changed over time ($P = 0.003$; Table 2; Figure 1), being positive during the first 90 min following the postruminal bolus; the maximal value was already reached 30 min postbolus and rapidly decreased thereafter. From 120 min until 24 h after the bolus, average portal-arterial differences of vitamin B₁₂ were negative or not different from 0 (data not shown).

In contrast with our present findings, the net PDV flux of vitamin B₁₂ in mid-lactation dairy cows was still positive 24 h after ingestion of a supplement of 500 mg of CN-CBL with a net portal balance over the 24-h period of 0.27% of the dose ingested (Girard et al., 2001). The postruminal bolus in the present study represented 20% of the level provided in the diet by Girard et al. (2001) because it was previously observed that 80% of orally ingested CN-CBL never arrived to the duodenum (Girard et al., 2009). In the present study, the maximal PDV flux was already reached at the first blood sample (30 min) following the postruminal bolus of CN-CBL, whereas Girard et al. (2001) observed the highest PDV flux (20 µg/h) at 10 h after ingestion of the CN-CBL supplement. The greatest PDV flux (88 µg/h) was observed 30 min after the postruminal bolus of CN-CBL and casein hydrolysate, and the net portal balance of vitamin B₁₂ for this treatment represented 0.1% of the dose infused. This difference in the peak and the overall net PDV flux between the 2 studies is likely to be due to the mode of administration of the vitamin supplement. In the study by Girard et al. (2001), the CN-CBL was probably released at a lower but more constant rate over the 24-h sampling period, due to the effect of rumen passage, compared with the postrumi-

Figure 1. Portal-arterial differences of vitamin B₁₂ during the 24 h following a postruminal bolus of cyanocobalamin alone (—; SE 29), with casein hydrolysate (- - -; SE 25) or milk whey protein isolate (.....; SE 25; interaction treatment × time, $P = 0.39$).

nal bolus of CN-CBL in the current study. Smith and Marston (1970) observed an accumulation of vitamin B₁₂ in gastrointestinal walls after an intravenous injection of labeled vitamin B₁₂. This organ regulates vitamin absorption, retaining the vitamin and releasing it toward the blood or the gastrointestinal cavity (Rérat et al., 1958). Consequently, as portal-arterial difference and PDV flux estimated the amount of vitamin reaching the portal blood during a finite period, it could lead to underestimation of the amount of vitamin absorbed.

In pigs, PDV flux of vitamin B₁₂ was positive when dietary CN-CBL was given with a semi-purified diet containing vitamin-free casein (Matte et al., 2010), but it was negative or not different from 0 when CN-CBL was given in a meal based on corn, wheat, and soybean meal (Matte et al., 2012). Indeed, in pig, PDV flux of vitamin B₁₂ was greater after ingestion of milk than the synthetic form of the vitamin, CN-CBL (Matte et al., 2012). In milk, most vitamin B₁₂ (~95%) is bound to proteins (Kim et al., 1965) and the vitamin binds to all milk protein fractions (Kim et al., 1965; Gizis et al., 1965). Milk seems to have a strong potential as a carrier for vitamin B₁₂, but little is known about the efficacy of milk protein as well as differences on the effect of casein hydrolysate vs. whey proteins on the intestinal absorption of CN-CBL. Moreover, a differential effect of peptides vs. proteins as excipient for CN-CBL cannot be ruled out. Results from the present study show that, although the daily balance was still very low, postru-

mental bolus of CN-CBL given with casein hydrolysate increased the amount of vitamin B₁₂ reaching portal blood as compared with CN-CBL given alone.

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