J. Dairy Sci. 98:1–5
http://dx.doi.org/10.3168/jds.2015-9678

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

V. M. Artegoitia,*† M. J. de Veth,†[1] F. Harte,‡ D. R. Ouellet,§ and C. L. Girard§[2]
*Department of Food Science and Technology, and
†Department of Animal Science, University of Tennessee, Knoxville 37996
‡Department of Food Science, Pennsylvania State University, University Park 16802
§Agriculture and Agri-Food Canada, Sherbrooke, Québec, Canada

ABSTRACT

Bioavailability of vitamin B_{12} is low in humans and animals. Improving vitamin B_{12} absorption is important for optimal performance in dairy cows and for increasing vitamin B_{12} concentrations in milk for human consumption. However, when supplemented in the diet, 80% of synthetic vitamin B_{12}, 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 B_{12} 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 B_{12} 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 B_{12} 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 B_{12} absorption as compared with CN-CBL given alone.
Key words: cow, cyanocobalamin, bioavailability, vitamin B_{12}, casein hydrolysate

Short Communication

Bacteria present in the rumen synthesize vitamin B_{12}, and unless dietary Co supply is inadequate, the amount of vitamin B_{12} 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 B_{12} 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 B_{12}, 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 B_{12} using portal-drained viscera (**PDV**) flux (Matte et al., 2010, 2012).The cumulative net PDV fluxes of vitamin B_{12} 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 B_{12}. However, when CN-CBL was offered with a semi-synthetic diet consist-

Received April 7, 2015.
Accepted August 1, 2015.
[1]Present address: BioNarus LLC, Cary, NC 27512.
[2]Corresponding author: christiane.girard@agr.gc.ca

ing of vitamin-free casein hydrolysate, corn starch, and sucrose, the cumulative net PDV fluxes of vitamin B_{12} during the 24 h following dietary boluses of 25 and 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 B_{12} 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 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 B_{12} in plasma was analyzed in duplicate by radioassay with a commercial kit designed for human plasma (SimulTRAC-S Radioassay kit, vitamin B_{12} (^{57}Co)/folate (^{125}I), MP Biomedicals, Diagnostics Division, Orangeburg, NY).

Portal-arterial difference and net flux of vitamin B_{12} across PDV were calculated as described by Girard

Table 1. Ingredient and chemical composition of the TMR[1]

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

[1]All 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.

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

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

[4]Contained 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.

[5]Arm & Hammer Animal Nutrition, Princeton, NJ.