

## Net flux of folates and vitamin B<sub>12</sub> through the gastrointestinal tract and the liver of lactating dairy cows

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In study 1, four cows had a ruminal canula, a catheter in the right ruminal vein and an ultrasonic flow probe around the right ruminal artery; a catheter was placed in the auricular artery on experimental days. Blood samples were taken every 10 min from –20 to 60 min after ruminal infusion of 5.79 mmol pteroylmonoglutamic acid and cyanocobalamin. There was a net release of these vitamins across the rumen wall following the infusion ( $P=0.06$ ). In studies 2 and 3, four cows had catheters in the portal, one hepatic and two mesenteric veins and one mesenteric artery. Plasma flow was determined using *p*-aminohippurate. In study 2, blood samples were taken before and every 30 min for 6 h after feeding 0 or 4 mg of pteroylmonoglutamic acid. Flow of folates through the portal-drained viscera (PDV) and the total splanchnic tissues (TSP) tended to increase with the ingestion of pteroylmonoglutamic acid ( $P=0.19$ ). In study 3, blood samples were collected every 30 min for the first 3 h to calculate plasma flow and basal net fluxes of folates and vitamin B<sub>12</sub>. The cows were fed 2.6 g pteroylmonoglutamic acid and 500 mg cyanocobalamin; blood samples were taken every 2 h for 24 h. Vitamin supplements increased the net release of folates and vitamin B<sub>12</sub> from PDV ( $P=0.04$ ) and TSP ( $P=0.13$ ). The present results demonstrate that, in dairy cows, at doses reported to improve animal performance, passage of pteroylmonoglutamic acid to the portal blood appears during the 6 h following its ingestion, whereas for cyanocobalamin, it is a slow process, not yet completed 24 h after its ingestion.

### Dairy cow: Folic acid: Vitamin B<sub>12</sub>: Absorption: Liver

Production of folates and vitamin B<sub>12</sub> by ruminal microflora is generally considered sufficient to avoid deficiency symptoms in ruminants (McDowell, 1989). However, parenteral and oral supplementations in folic acid (Girard *et al.* 1995, 1998; Girard & Matte, 1998) and vitamin B<sub>12</sub> (Girard & Matte, 1997) increased milk production and modified milk composition in dairy cows, suggesting that rumen microbial synthesis might not be sufficient to optimize animal performance. However, such production responses require huge amounts of dietary folic acid and vitamin B<sub>12</sub> in order to increase plasma concentrations of folates and vitamin B<sub>12</sub> at levels similar to those observed after intramuscular injections of these vitamins.

In steers, folic acid and vitamin B<sub>12</sub> given in the diet are extensively destroyed by the ruminal microflora (Zinn *et al.*

1987). In single-stomached species, folic acid is efficiently absorbed (over 50% of the amount ingested) in the distal duodenum and jejunum by both a saturable active process and a non-saturable passive process (diffusion). The absorption by diffusion increases linearly with the concentration of folic acid in the lumen of the intestine (Le Grusse & Watier, 1993; Combs, 1998). At low doses (1–2 µg), vitamin B<sub>12</sub> is absorbed efficiently in the terminal ileum by active saturable transport involving a binding protein, the intrinsic factor. It takes 8–12 h for the complex intrinsic factor–vitamin B<sub>12</sub> fixed on the ileal mucosa to release vitamin B<sub>12</sub> linked to transcobalamin II in the blood circulation (Combs, 1998; Quadros *et al.* 1999). At higher doses, it is absorbed by simple diffusion throughout the small intestine, but the efficiency of this process is low,

**Abbreviations:** PAH, *p*-aminohippurate; PDV, portal-drained viscera; TSP, total splanchnic tissues.

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approximately 1% of the amount ingested (Le Grusse & Watier, 1993; Combs, 1998).

After their absorption into the portal circulation, folic acid and vitamin B<sub>12</sub> flow into the liver. Part of the folates reaching the liver are metabolized into pteroylpolyglutamates and used by the hepatic cells, while the remaining folates are methylated and released into the blood circulation for further utilization by peripheral tissues. Vitamin B<sub>12</sub>, in contrast to the other water-soluble vitamins, is stored in significant amounts in the body with approximately 60% of the total body reserves stored in the liver (Le Grusse & Watier, 1993; Combs, 1998).

All this information was obtained in single-stomached animals, but it has not been confirmed in ruminants. The aim of the present study was therefore to report a series of investigations conducted to elucidate the fate in the gastrointestinal tract and the liver of lactating dairy cows of supplementary pteroylmonoglutamic acid and cyanocobalamin, given in the large quantities used in production trials to improve animal performance. The first experiment (study 1) was designed to determine whether these two vitamins, when infused into the rumen of lactating dairy cows, could reach the blood across the rumen wall. Studies 2 and 3 aimed to define the net transport of folates and vitamin B<sub>12</sub> through the portal and splanchnic vascular routes after oral supplementation of these vitamins at the high doses used in previous production trials.

### Materials and methods

The experimental procedures of the three reported studies were approved by the Institutional Animal Care Committee of the Dairy and Swine Research and Development Centre and cows were cared for according to the guidelines laid down by the Canadian Council on Animal Care (1993).

Throughout the manuscript, means were considered to be significantly different at  $P \leq 0.10$  and to tend to differ at  $0.10 \leq P \leq 0.20$ .

#### Study 1

*Animals, surgery and diets.* Four multiparous lactating dairy cows, between 146 and 171 d after calving, were used in the present study. Mean daily milk production was 21.1 (SE 1.1) kg. The cows had a ruminal canula, an ultrasonic flow probe (8 mm flow probe, R-series; Transonic Systems Inc., Ithaca, NY) installed around the right ruminal artery, approximately 15–20 cm distal to its bifurcation from the coeliac artery, and a catheter fixed in the right ruminal vein with its tip at the level of the probe around the ruminal artery. The operations were performed approximately 4 months before the present study for the purpose of another experiment, according to the method described by Rémond *et al.* (1993) and adapted for the cow.

The cows had free access to feed and water. They were fed seven times per d a total mixed ration containing 35.1% grass–legume silage, 24.7% maize silage, 20.2% barley, 7.4% rapeseed meal, 3.6% distiller's dry grain (maize), 2.9% distiller's dry grain (wheat), 3.6% micronized soyabean, 0.7% limestone, 1.8% mineral and vitamin premix. The premix contained no B-complex vitamins.

Mean daily DM intake was 17.8 (SE 1.0) kg. Cows were kept under 16 h of light per d and they were milked every 12 h.

*Experimental procedures.* The day of the experiment, the feed was removed 6 h before the infusion of vitamins into the rumen and a catheter was installed in the auricular artery approximately 1 h before the beginning of the experimental period. A solution of 5.79 mmol (2.6 g) pteroylmonoglutamic acid (F 7876; Sigma, St Louis, MO, USA) and 5.79 mmol (7.8 g) cyanocobalamin (1111223; Hoffmann-LaRoche, Cambridge, ON, Canada) was infused through the rumen canula in less than 6 min. The amount of pteroylmonoglutamic acid was determined from a previous production study (Girard & Matte, 1998); on a molar basis, the same amount of cyanocobalamin was given. Blood flow in the right ruminal artery was recorded continuously from 25 min before to 65 min after the infusion. Blood samples were taken from the two catheters 20, 10 and 0 min before the infusion to establish the baseline, and then 10, 20, 30, 40, 50 and 60 min after the end of the infusion. Immediately after collection, blood was transferred from the syringes to EDTA-treated tubes. Packed cell volume (PCV) was measured in duplicate on fresh blood by microcentrifugation. An aliquot of blood was immediately frozen for haemoglobin determination according to the method of Drabkin (Manet, 1969). Plasma was collected after centrifugation and frozen for vitamin determination. Folates and vitamin B<sub>12</sub> were determined in duplicate by radioassay with a commercial kit designed for human plasma (Quantaphase Folate II and Quantaphase B<sub>12</sub>; Bio-Rad Laboratories (Canada) Ltd., Mississauga, ON, Canada) and validated for bovine plasma by the conduction of recovery and parallelism tests (Girard & Matte, 1988; Girard *et al.* 1989). Inter-assay CV were 4.2 and 4.1% for folates and vitamin B<sub>12</sub>, respectively.

*Calculations and statistical analysis.* The net flux of folic acid or vitamin B<sub>12</sub> across the rumen wall was calculated according to the following equation:

$$\text{Net flux} = ([X]_v \times PF_v) - ([X]_a \times PF_a),$$

in which  $[X]_v$  and  $[X]_a$  are the concentrations of the studied vitamins in plasma from venous (v) and arterial (a) blood and  $PF_v$  and  $PF_a$  are the plasma flow in the right ruminal vein and artery, respectively. For each sampling time, the plasma flow in the artery was obtained by calculating the mean whole blood flow recorded by the flow probe from 5 min before to 5 min after blood sampling, corrected for the packed cell volume of arterial blood ( $PCV_a$ ) (whole blood flow  $\times (1 - PCV_a)$ ). The plasma flow in the right ruminal vein was calculated from the value of plasma flow in the right ruminal artery, corrected for water exchange across the rumen wall, evaluated by the ratio of haemoglobin in the arterial ( $Hb_a$ ) and venous ( $Hb_v$ ) blood ( $PF_a \times (Hb_a/Hb_v)$ ). For the vitamin flux, a negative value indicates net tissue removal, whereas a positive value denotes net release.

Data were analysed using ANOVA in General Linear Model (Statistical Analysis Systems, 1997) according to the following model:

$$Y_{ij} = \mu + C_i + T_j + \varepsilon_{ij},$$

in which  $Y_{ij}$  was the studied variable (arterial concentration

or net flux across the rumen wall of folates or vitamin B<sub>12</sub>),  $\mu$  was the overall mean,  $C_i$ , the cow effect,  $T_j$ , the time effect and  $\varepsilon_{ij}$ , the residual error. When the time effect reached a level of significance of 90%, a Dunnett's *t* test was conducted to compare each time post-infusion to the baseline level, calculated as the mean of the three samples taken before the infusion of vitamins into the rumen.

### Study 2

**Animals, surgery and diets.** Four multiparous lactating dairy cows, averaging 199 (SE 1) d after calving and 26.7 (SE 1.3) kg/d milk, were used in the present study. Catheters were implanted in the portal vein, in one hepatic vein, in two mesenteric veins and in the caudal aorta via a mesenteric artery. The operations were performed approximately 4 months before the present study for the purpose of another experiment, according to the method described by Huntington *et al.* (1989).

The cows had free access to water and were fed twelve equal meals per day at 95% of their *ad libitum* feed intake, a total mixed ration containing 28.4% legume silage, 27.6% maize silage, 27.5% ground maize grain, 4.0% grass hay, 4.1% soyabean hulls, 2.3% animal fat, 4.0% soyabean meal, 2.1% mineral and vitamin premix. The premix contained no B-complex vitamins. Daily DM intake was 17.8 (SE 1.2) kg. Cows were kept under 16 h of light per d and they were milked every 12 h.

**Experimental procedures.** The experiment had a cross-over design in which the cows, at 2 d intervals, received 500 g total mixed ration mixed with 0 or 4 g pteroylmonoglutamic acid (Sigma), the highest dose used in a previous production study (Girard *et al.* 1998). The supplemented meal was given at 08.00 hours (time 0) and cows ate it in less than 5 min. Thirteen sets of arterial, portal and hepatic blood were simultaneously collected every 30 min for 6 h, the first sample being collected just before the supplemented meal. Plasma flows in the portal and hepatic veins were determined by downstream dilution of sodium *p*-aminohippurate (PAH; 100 g/l) infused continuously (14.4 g/h) into the distal mesenteric catheter, following a priming dose of 2 g. The PAH infusion was initiated at least 40 min before the collection of the first set of samples. Immediately after collection, blood was transferred from the syringes to EDTA-treated tubes. Harvested plasma was stored at -20°C until analysed. Plasma samples were analysed for PAH using an automatic analyser (Technicon Autoanalyzer II; Technicon Instruments, Tarrytown, NY, USA) according to the method described by Huntington (1984). Folates were measured in duplicate as described for study 1.

**Calculations and statistical analysis.** Plasma flows from the portal-drained viscera (PDV), the liver and total splanchnic tissues (TSP) were calculated from downstream dilution of PAH, according to the method of Katz & Bergman (1969). Net fluxes were obtained by multiplying the venous-arterial difference by the corresponding plasma flow.

Mean arterial concentrations and net fluxes of folates reaching, extracted by or leaving the liver were analysed using ANOVA in General Linear Model (Statistical

Analysis Systems, 1997) according to the following model:

$$Y_{ijk} = \mu + C_i + P_j + S_k + \varepsilon_{ijk},$$

in which  $Y_{ijk}$  is the studied variable (arterial concentrations or net flux of folates),  $\mu$  is the overall mean,  $C_i$  is the cow effect,  $P_j$  is the effect of period,  $S_k$  is the effect of pteroylmonoglutamic acid supplements and  $\varepsilon_{ijk}$  is the residual error.

Net amounts of folates reaching or leaving the liver after supplementation were calculated as follows:

$$\Sigma[(F_{ts} - F_{t0}) \times 0.5 \text{ h}],$$

in which  $F_t$  is the net flux from PDV or TSP at a given time (s) after ingestion of the supplement and  $F_{t0}$  is the value before ingestion of supplements.

### Study 3

**Animals, surgery and diets.** Four multiparous lactating dairy cows, averaging 151 (SE 8) d after calving and a daily milk production of 30.6 (SE 2.2) kg, were used in the present study, conducted approximately 3 months after surgery. Operations, animal care, milking and feeding management were similar to those in study 2. Composition of the total mixed ration was 19.0% legume silage, 25.1% maize silage, 32.1% ground maize grain, 11.0% soyabean meal, 4.3% cracked maize grain, 4.7% whole micronized soyabean, 1.1% molasses, 2.2% vitamin and mineral premix. The premix contained no B-complex vitamins. Mean daily intake of DM was 21.2 (SE 1.4) kg. The day of the experiment, the cows were kept under 24 h of light.

**Experimental procedures.** The experimental period was divided into two parts. During the first part, six sets of arterial, portal and hepatic blood were collected simultaneously every 30 min for 3 h. Plasma flows in the portal and hepatic veins were determined by downstream dilution of sodium PAH as described for study 2. Samples collected during this period were used to estimate plasma flow and establish the baseline for net fluxes of folates and vitamin B<sub>12</sub> before dietary supplementation of these vitamins. At the beginning of the second part, the cows were fed a dietary supplement containing 2.6 g pteroylmonoglutamic acid (Sigma) and 500 mg cyanocobalamin (Hoffmann-LaRoche) mixed with 500 g total mixed ration. The quantity of pteroylmonoglutamic acid was similar to that used in study 1. The dose of cyanocobalamin was chosen according to previous dose-response curves (response of jugular plasma concentrations to dietary supplements) conducted with dairy cows (CL Girard and A Desrochers, unpublished results). Sets of arterial, portal and hepatic blood samples were collected simultaneously every 2 h for the next 24 h. Immediately after collection, blood was transferred from the syringes to EDTA-treated tubes. Harvested plasma was stored at -20°C until analyses. PAH, folates and vitamin B<sub>12</sub> were measured in duplicate as described for studies 1 and 2. Throughout the first and second parts of the study, cows ate each meal completely before the following meal was delivered, so there were no refusals.

**Calculations and statistical analysis.** Calculations were performed as described for study 2. Mean arterial

concentrations and net total fluxes of folates and vitamin B<sub>12</sub> across the splanchnic tissues were analysed using the repeated-measures ANOVA in General Linear Model (Statistical Analysis Systems, 1997) according to the following model:

$$Y_{ij} = \mu + C_i + T_j + \varepsilon_{ij},$$

in which  $Y_{ij}$  is the studied variable (arterial concentrations or net flux of folates or vitamin B<sub>12</sub>),  $\mu$  is the overall mean,  $C_i$  is the cow effect,  $T_j$  is the effect of time before or after ingestion of the dietary supplement of pteroylmonoglutamic acid and cyanocobalamin and  $\varepsilon_{ij}$  is the residual error.

Total amounts of vitamins reaching, taken by or leaving the liver were calculated as follows:

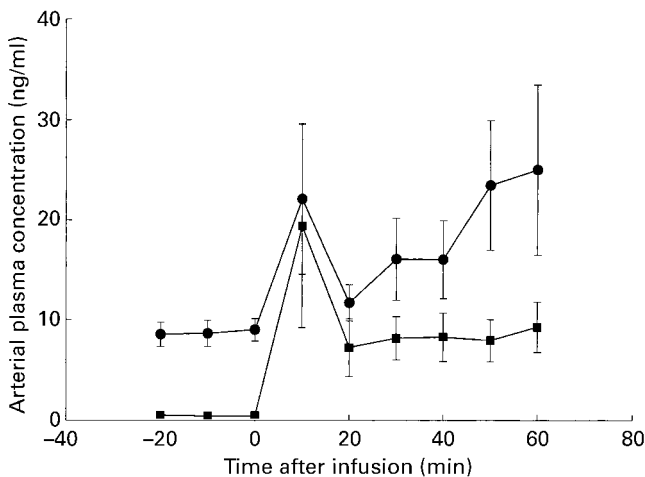
$$\Sigma[(F_{ts} - F_{t0}) \times 2 \text{ h}],$$

in which  $F_t$  is the net flux from PDV, TSP or liver at a given time (s) after ingestion of the supplement and  $F_{t0}$  is the value before ingestion.

## Results

### Study 1

Arterial plasma concentrations of folates (time effect,  $P=0.02$ ) and vitamin B<sub>12</sub> (time effect,  $P=0.05$ ) were increased by the infusion of vitamins into the rumen (Fig. 1). There was a net release of folates (time effect,  $P=0.06$ ) and vitamin B<sub>12</sub> (time effect,  $P=0.004$ ) across the rumen wall during the 60 min following the infusion of vitamins (Fig. 2). The net release of folates was different from the pre-infusion level only for the first 20 min after the infusion of vitamins, whereas a significant difference remained during the 60 min studied for vitamin B<sub>12</sub>. During the 60 min post-infusion, 189.4  $\mu\text{g}$  folates and 164.5 ng vitamin B<sub>12</sub> were released into the blood circulation from the portion of rumen wall drained by the right ruminal vein. The mean blood flow in the right ruminal artery was 1.17 l/min (SE 0.12). If it were



**Fig. 1.** Arterial plasma concentrations of folates (●) and vitamin B<sub>12</sub> (■) during the hour following the infusion of a solution of 5.79 mmol pteroylmonoglutamic acid and cyanocobalamin into the rumen of lactating dairy cows. Values are means for four cows, with standard errors represented by vertical bars.

hypothesized that the areas drained by the right and the left ruminal veins are similar, 0.015 % and  $4 \times 10^{-6}$  % of the quantities of infused pteroylmonoglutamic acid and cyanocobalamin, respectively, would reach the blood circulation across the rumen wall during the hour following the infusion.

### Study 2

During the 6 h period following its ingestion, the dietary supplement of 4 g pteroylmonoglutamic acid increased the average arterial plasma concentration of folates from 15.5 (SE 2.4) to 82.2 (SE 22.5) ng/ml ( $P=0.05$ ) and tended to increase the net flux of folates across PDV ( $P=0.19$ ) and TSP ( $P=0.08$ ), as compared to the unsupplemented diet (Table 1). The rate of removal of folates by the liver was numerically increased with supplementation but this effect was not significant ( $P=0.31$ ; Table 1). The changes in arterial plasma concentrations of folates during the 6 h after ingestion of the dietary supplements are shown in Fig. 3 and the net fluxes of folates across the PDV are illustrated in Fig. 4. Portal and splanchnic plasma flows averaged 1310.6 (SE 67.9) and 1592.8 (SE 61.1) l/h, respectively, and they were not affected by the ingestion of vitamins ( $P>0.6$ ).

After ingestion of the unsupplemented diet, there was no detectable net flux of folates across the PDV and the liver during the 6 h studied. During the first 30 min after the ingestion of 4 g pteroylmonoglutamic acid, 379  $\mu\text{g}$  folates reached the portal circulation. This amount is the same as that calculated for the whole rumen in study 1 during the first 20 min after the ruminal infusion of vitamins, in spite of a difference in the quantity of pteroylmonoglutamic acid used (4 g in study 2 v. 2.6 g in study 1). During the 6 h following the ingestion of 4 g pteroylmonoglutamic acid, 100.7 mg folates reached the portal blood, representing 2.5 % of the amount ingested. Part of those folates, 73.5 % (74.0 mg) were extracted by the liver, leaving 26.7 mg for the peripheral tissues. However, in the present study involving only four animals, one cow responded differently from the others. The calculations made without this cow completely reversed the percentage of folates taken by the liver (27.2) and that released towards peripheral tissues (72.8). Study 3 was undertaken partially in an attempt to solve this contradiction.

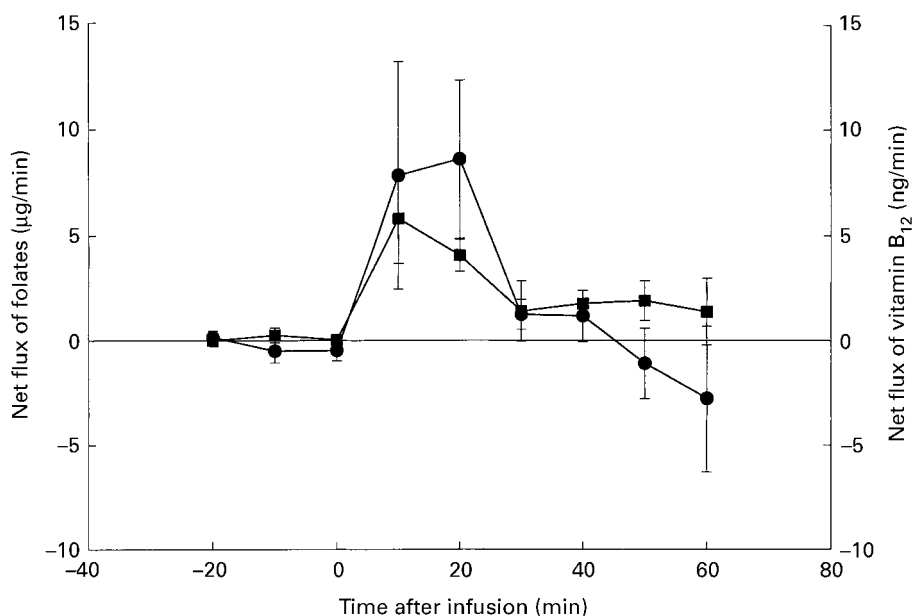
**Table 1.** Splanchnic net flux of folates (mg/h) in lactating dairy cows during the 6 h following the ingestion of 0 or 4 g pteroylmonoglutamic acid

(Mean values with their standard errors for four cows)

Tissue	Dietary supplements of pteroylmonoglutamic acid (g)				$P^*$
	0		4		
	Mean	SE	Mean	SE	
PDV	0.58	0.19	15.98	6.98	0.19
Liver	-1.03	0.19	-11.11	6.27	0.31
TSP	-0.45	0.10	4.87	1.46	0.08

PDV, portal-drained viscera; TSP, total splanchnic tissue.

\* Difference between means (ANOVA).



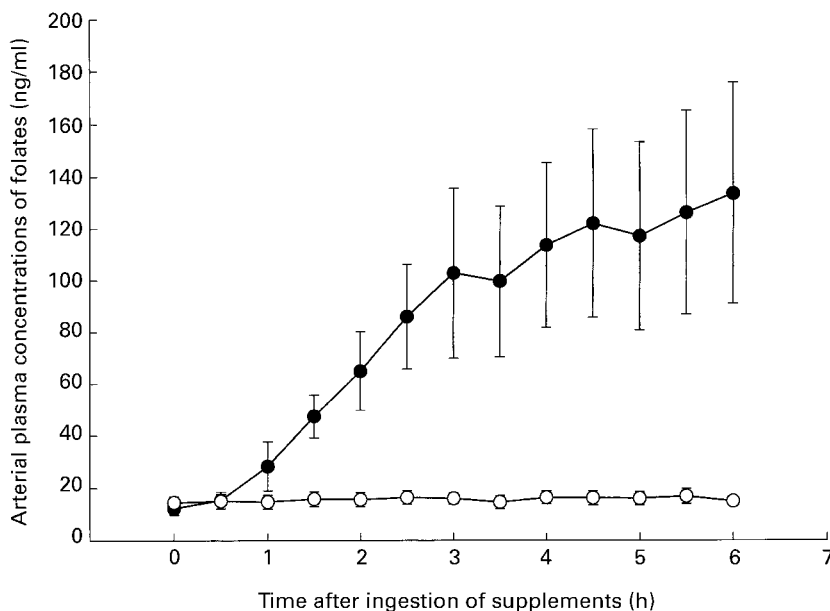
**Fig. 2.** Net flux of folates (●) and vitamin B<sub>12</sub> (■) across the portion of rumen wall drained by the right ruminal vein during the hour following the infusion of a solution of 5.79 mmol pteroylmonoglutamic acid and cyanocobalamin into the rumen of lactating dairy cows. Values are means for four cows, with standard errors represented by vertical bars. The net flux of folates differs from the baseline 10 and 20 min post-infusion whereas the net flux of vitamin B<sub>12</sub> differs from the baseline 10, 20, 30, 40 and 50 min post-infusion ( $P \leq 0.10$ ).

### Study 3

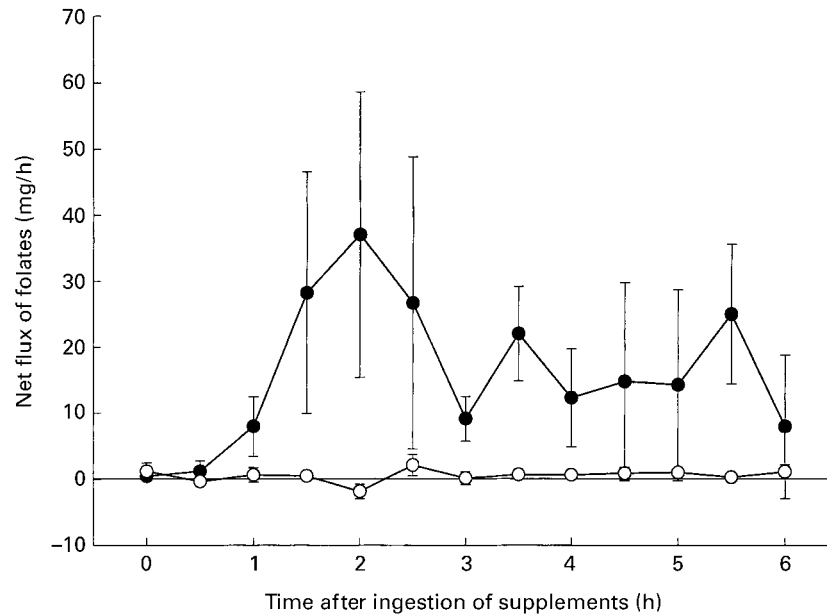
The ingestion of the vitamin supplements increased the net flux of folates and vitamin B<sub>12</sub> from PDV ( $P \leq 0.04$ ) and TSP ( $P \leq 0.13$ ) (Table 2). The amount of folates taken by the liver was not significantly increased ( $P = 0.26$ ) by the dietary supplement, whereas that of vitamin B<sub>12</sub> increased ( $P = 0.1$ ). On average, the arterial plasma concentrations of folates ( $P = 0.03$ ) and vitamin B<sub>12</sub> ( $P = 0.003$ ) increased after the

ingestion of dietary supplements of vitamins from 11.75 (SE 2.06) ng/ml and 144.67 (SE 23.60) pg/ml to 52.33 (SE 11.56) ng/ml and 326.15 (SE 30.82) pg/ml for folates and vitamin B<sub>12</sub>, respectively. Changes in arterial plasma concentrations of the two studied vitamins are shown in Fig. 5. Portal and splanchnic plasma flows averaged 1538.8 (SE 74.9) and 1675.5 (SE 70.1) l/h.

During the 24 h following the ingestion of the vitamin supplement, 110.9 mg folates and 1328.1 µg vitamin B<sub>12</sub>,



**Fig. 3.** Arterial plasma concentration of folates of lactating dairy cows during the 6 h following the ingestion of 0 (○) or 4 (●) g pteroylmonoglutamic acid. Values are means for four cows, with standard errors represented by vertical bars.



**Fig. 4.** Net flux of folates from the portal-drained viscera of lactating dairy cows during the 6 h following the ingestion of 0 (○) or 4 (●) g pteroylmonoglutamic acid. Values are means for four cows, with standard errors represented by vertical bars.

that is 4.3 and 0.27 % of the ingested doses, respectively, reached the portal blood. The liver extracted 28.7 % (31.8 mg) folates and 46 % (611.3 µg) vitamin B<sub>12</sub> reaching the portal blood, the splanchnic tissues releasing 79.1 mg folates and 716.8 µg vitamin B<sub>12</sub> towards the peripheral tissues.

The net flux of the two vitamins from the PDV is shown in Fig. 6. During the first 6 h after ingestion of the vitamin supplement, 108.7 mg folates reached the portal blood. Out of those, 21.5 mg were extracted by the liver and 87.2 mg were available for the other tissues. Folates appeared in portal blood quickly after ingestion; 98 % of the amount reaching the portal blood during the 24 h period arrived during the first 6 h. In contrast, the appearance of vitamin B<sub>12</sub> in the portal blood followed a biphasic pattern. Only 1.4 % of the total amount of vitamin B<sub>12</sub> reaching the portal blood within 24 h appeared during the first 2 h, 49.3 % was released during 4–10 h post-ingestion and 34.9 % 20–24 h post-ingestion. At 24 h after the ingestion of 500 mg cyanocobalamin, the net flux of this vitamin across the PDV was still positive.

## Discussion

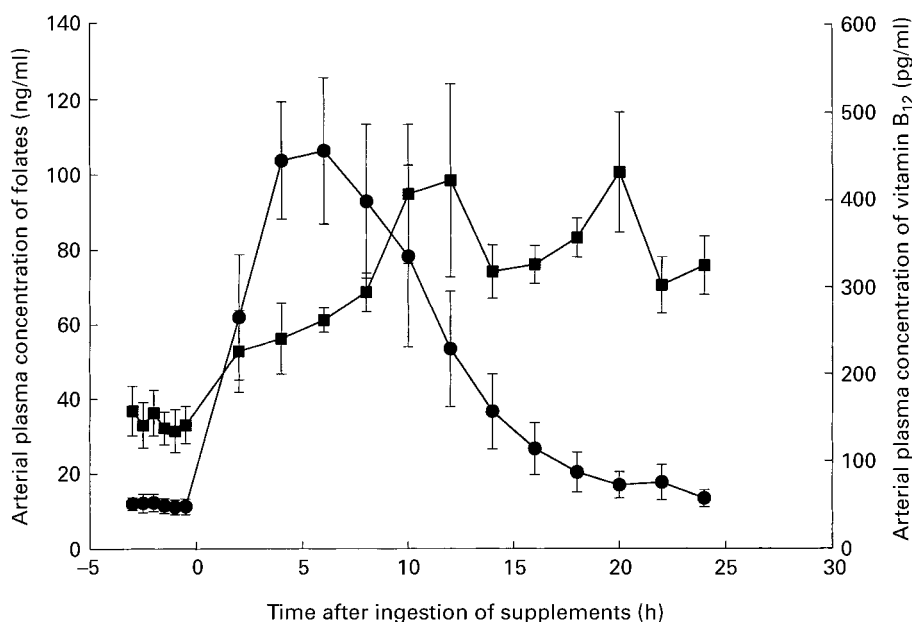
Rérat *et al.* (1958a) did not succeed in demonstrating absorption of vitamin B<sub>12</sub> through the rumen wall of fed sheep. However, when the vitamin was infused into the empty rumen of sheep, it reached the blood circulation across the rumen wall. Moreover, the rumen wall played a regulatory role in vitamin absorption, retaining the vitamins for a time, before releasing them into the blood and/or the rumen cavity (Rérat *et al.* 1958b). Smith & Marston (1970) did not succeed in demonstrating the absorption of vitamin B<sub>12</sub> from the rumen of sheep, but they detected relatively high amounts of vitamin B<sub>12</sub> in the rumen wall. The absorption of B-complex vitamins through the rumen wall had never been clearly demonstrated, perhaps owing to the low sensitivity of the methods used, as most studies were conducted many years ago.

Net flux of vitamins from the rumen is the difference between the amount absorbed from the lumen and the amount used (or stored) in cells of the rumen wall. Results of the present experiment clearly demonstrate that there was

**Table 2.** Splanchnic net flux of folates and vitamin B<sub>12</sub> in lactating dairy cows before and during the 24 h following the ingestion of 2.6 g pteroylmonoglutamic acid and 500 mg cyanocobalamin (Mean values with their standard errors for four cows)

Tissue	Folates (mg/h)				P*	Vitamin B <sub>12</sub> (µg/h)				P*
	Before		After			Before		After		
	Mean	SE	Mean	SE		Mean	SE	Mean	SE	
PDV	0.38	0.29	4.99	1.16	0.04	16.75	2.90	72.09	7.33	0.02
Liver	-0.19	0.41	-1.51	1.00	0.26	-4.84	3.50	-30.3	6.24	0.10
TSP	0.19	0.38	3.49	1.49	0.13	11.92	3.84	41.79	5.84	0.07

PDV, portal-drained viscera; TSP, total splanchnic tissue.  
\* Difference between means (repeated-measures ANOVA).

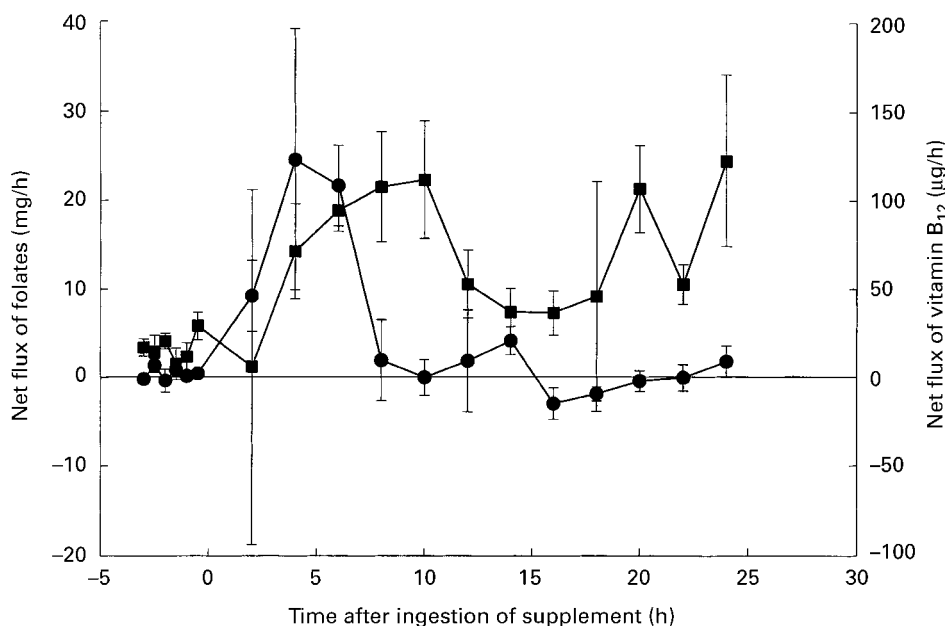


**Fig. 5.** Arterial plasma concentration of folates (●) and vitamin B<sub>12</sub> (■) of lactating dairy cows during the 24 h following the ingestion of 2.6 g pteroylmonoglutamic acid and 500 mg cyanocobalamin. The point 0 is the mean of six samples collected at 30 min intervals prior to the ingestion of vitamin supplements. Values are means for four cows, with standard errors represented by vertical bars.

no detectable net flux of folates and vitamin B<sub>12</sub> across the rumen wall before the infusion of vitamins. However, folates and vitamin B<sub>12</sub> reached the blood circulation across the rumen wall when high doses of the vitamins were infused in the rumen of fed cows, but the efficiency of this process was very low, especially for vitamin B<sub>12</sub>.

In rats, at physiological concentrations, folate uptake in the small intestine occurs by a saturable process and, at higher concentrations, by a non-saturable process (Dhar

*et al.* 1977). In neonatal goats, the transport of pteroylmonoglutamic acid in the small intestine involves a saturable process; no passive uptake at higher concentrations has been found (Blakeborough & Salter, 1988). When comparing the three present studies, the quantities of folates reaching ruminal and portal blood during similar periods of time were very close, in spite of differences in the amount of pteroylmonoglutamic acid ingested. Although the methods used in the present experiment did not allow



**Fig. 6.** Net flux of folates (●) and vitamin B<sub>12</sub> (■) from the portal-drained viscera of lactating dairy cows during the 24 h following the ingestion of 2.6 g pteroylmonoglutamic acid and 500 mg cyanocobalamin. The point 0 is the mean of six samples collected at 30 min intervals prior to the ingestion of vitamin supplements. Values are means for four cows, with standard errors represented by vertical bars.

determination of vitamin concentrations at the mucosal absorptive surface, the present results would suggest that, in dairy cows, pteroylmonoglutamic acid is absorbed from the gastrointestinal tract mainly through a saturable active process. This process seems to be already saturated by a dietary supplement of less than 2.6 g pteroylmonoglutamic acid. The reduced importance of passive absorption as well as the destruction of pteroylmonoglutamic acid by ruminal microflora could explain the lower efficiency of absorption of folic acid (less than 5%) as compared to that of single-stomached animals (40–90%; Zettner *et al.* 1981; Steinberg *et al.* 1982).

The biphasic pattern of absorption of cyanocobalamin could be explained by the presence of two different transport mechanisms (Le Grusse & Watier, 1993; Combs, 1998). Passive absorption, which is simple diffusion through the walls of the gastrointestinal tract at high doses of vitamin, could explain the increased net flux from the ruminal vein and the low efficiency of the process. It could also explain the increase of vitamin B<sub>12</sub> net flux across the PDV during the first hours following supplementation. The second increase of vitamin B<sub>12</sub> net flux across the PDV, 20–24 h after dietary supplementation, is in accordance with an active saturable transport process, an efficient but slow process demonstrated in the ileum of other species and involving successive linkage of vitamin B<sub>12</sub> with intrinsic factor and transcobalamin II (Le Grusse & Watier, 1993; Combs, 1998; Quadros *et al.* 1999). Smith & Marston (1970) calculated that, in sheep fed 500 µg cyanocobalamin during 4 months, 1–3% of the dose is absorbed. These percentages are higher than that calculated in study 3, in which only 0.27% of the single dose ingested reached the portal blood in 24 h. The difference could be species-related but it is more likely to be due to the reabsorption of vitamin B<sub>12</sub> secreted in the bile and reabsorbed in the small intestine (Smith & Marston, 1970; Steinberg *et al.* 1979), the extent of the recycling through the enterohepatic cycle increasing with the duration of the supplementation.

Calculations by Cooper (1977) showed that, during the 2 h following the ingestion of a solution of pteroylmonoglutamic acid, 37% of the folates released into the portal blood were absorbed by the liver of rats. Steinberg *et al.* (1979) demonstrated that, in rats, during the first hour following pteroylmonoglutamic acid supplementation, 25% of folates from the portal blood were absorbed by the liver, hepatic retention representing 12% of this amount while the other 13% was released into the bile. In the dairy cow, the ratio of liver extraction on portal release of folates was similar to those values, 27% in study 2 (with only three cows) and 28% in study 3.

The liver of mature non-gestating ewes retained 16.5% of a dose of [<sup>58</sup>Co]cyanocobalamin given by subcutaneous injection (Smith & Marston, 1970). In study 3, the liver of lactating dairy cows extracted 46% of the vitamin B<sub>12</sub> reaching the portal blood. This difference could be species-related. Vitamin B<sub>12</sub> is an essential coenzyme for one of the reactions in the metabolic pathway for entry of propionate into the Krebs cycle (McDowell, 1989). In sheep, the activity of enzymes involved in propionate metabolism is similar in the liver and in the rumen epithelium, whereas in the bovine, their activity in the liver is three to four times higher than in

the epithelium (Elliot, 1979). It is likely that increased propionate metabolism augments the demand for coenzymes, such as vitamin B<sub>12</sub>, involved in these reactions. Some of the differences between the two experiments could also be due to the physiological state of the animals, the demand being greater for lactation than for maintenance only.

The rate of extraction of folates and vitamin B<sub>12</sub> by the liver of dairy cows has not been previously reported; the present study was conducted on cows in late lactation. Vitamin B<sub>12</sub> is metabolically active as a cofactor for two enzymes in mammals: methionine synthase and methylmalonyl-CoA mutase. The former is essential for the transfer of one C-unit from methyltetrahydrofolate to homocysteine, producing methionine and biologically active folates. The latter plays a part in the conversion of propionate into succinyl-CoA on which depends its use as a gluconeogenic substrate (McDowell, 1989; Le Grusse & Watier, 1993). This enzyme is active in the liver of high-yield dairy cows in early lactation, owing to: the huge needs in energy for milk production; the dominating role of gluconeogenesis in the production of glucose, the precursor of milk lactose; the preferential utilization of propionate as a substrate for gluconeogenesis (60% of the glucose is derived from propionate in dairy cows); the huge amount of absorbed propionate due to diets rich in concentrates given to high-yield dairy cows to compensate for their negative energy balance after calving (Elliot, 1979; Overton, 1998). Folates are involved in DNA, RNA and protein synthesis (McDowell, 1989; Le Grusse & Watier, 1993). Given the metabolic roles of these two vitamins, it is likely that the demand for folates and vitamin B<sub>12</sub> could be higher in early lactation than in late lactation and then, could modify liver extraction of these vitamins.

### Conclusion

The ruminant is generally considered independent of an exogenous supply of B-complex vitamins. However, after many decades of certainty about the adequacy for ruminant requirements of B-complex vitamins of the supply of these vitamins by the diet and the ruminal micro-organisms, new questions are being raised. These studies dealt with the appearance of deficiency symptoms as a criterion for dietary needs; few tried to define the requirements for optimal production. Previous production trials (Girard *et al.* 1995, 1998; Girard & Matte, 1997, 1998, 1999) have indicated that, under some conditions, folic acid and vitamin B<sub>12</sub> could be limiting for dairy cows. Destruction by the ruminal microflora of these vitamins given in dietary supplements, although not measured in the present experiment, is suspected of being extensive. Therefore, high quantities of these vitamins were given orally to overcome this problem, but this increased supplementation costs. Results from the present experiment demonstrated that, in dairy cows, net release of pteroylmonoglutamic acid into portal blood appeared during the first 6 h after ingestion of a dietary supplement by a saturable process. Passage of ingested cyanocobalamin into the portal blood was a very slow process still in progress 24 h after the ingestion of a dietary supplement of 500 mg cyanocobalamin. Only 2.5 to 4.5% of pteroylmonoglutamic acid and 0.27% of cyanocobalamin



ingested were released into portal blood. The extraction by the liver represented 28 % of the folates and 46 % of vitamin B<sub>12</sub> released into portal blood in dairy cows in late lactation. However, the ratio of extraction by the liver could change according to the stage of lactation. Therefore, it appears that, when given in large amounts, dietary supplements of folic acid and vitamin B<sub>12</sub>, in spite of extensive losses in the gastrointestinal tract, still provided significant amounts of these vitamins to the liver and post-splanchnic tissues, such as mammary glands, and this could explain the changes in production observed in previous experiments. Understanding of the fate of ingested folic acid and vitamin B<sub>12</sub> will help design further studies on metabolism of these two vitamins in lactating dairy cows.

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