Bioavailability of dietary cyanocobalamin (vitamin B₁₂) in growing pigs¹

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ABSTRACT: The present project aimed to estimate bioavailability of dietary vitamin B₁₂, for which little information is available in growing pigs. Two approaches, each using 2 quantities of dietary cyanocobalamin, were compared; the first was based on whole body retention for 8 d and the second was based on nycthemeral portal net flux of vitamin B₁₂. In the first trial, 15 blocks of 3 pigs (31.7 ± 0.5 kg of BW) were formed according to their vitamin B₁₂ status. Within each block, 1 pig (CONT) was killed and tissues were sampled for vitamin B₁₂ determination. The remaining 2 piglets were fed 25 (B₁₂-25) or 250 (B₁₂-250) μg daily of cyanocobalamin for 8 d. Urine was sampled twice daily, and the pigs were killed and sampled as CONT pigs. The total content of vitamin B₁₂ in the carcass, urine, and intestinal tract was affected by the dietary treatments (P < 0.01) but not in the liver (P > 0.019). The whole body retention of vitamin B₁₂ was greater (P = 0.02) in B₁₂-250 than B₁₂-25 pigs, but the corresponding bioavailability was estimated to be 5.3 and 38.2%, respectively. In trial 2, 11 pigs (35.1 ± 4.0 kg of BW and 75.4 ± 5.9 d of age) fed a diet unsupplemented with vitamin B₁₂ from weaning at 28 d of age were surgically equipped with catheters in the portal vein and carotid artery and an ultrasonic flow probe around the portal vein. Each pig received 3 boluses of 0 (B₁₂-0), 25, and 250 μg of dietary vitamin B₁₂ according to a crossover design. Postprandial nycthemeral arterial plasma concentrations of vitamin B₁₂ reached minimum values (P < 0.01) between 15 and 18 h postmeal that were 29.6, 15.6, and 10.0% less than the premeal values for B₁₂-0, B₁₂-25, and B₁₂-250 pigs, respectively (linear, P < 0.01). The cumulative net flux of vitamin B₁₂ for 24 h corresponded to 2.4 and 5.1 μg for B₁₂-25 and B₁₂-250 treatments, respectively, and the corresponding bioavailability was estimated to be 9.7 and 2.0%, respectively. Although bioavailability estimates varied according to approaches, both showed the inverse relationship between dietary vitamin B₁₂ and bioavailability of the vitamin. The dietary supplement of 25 μg was sufficient to maximize hepatic vitamin B₁₂ retention and to attenuate the nycthemeral decrease of arterial plasma concentration of the vitamin.

Key words: cyanocobalamin, intestinal absorption, pig, retention, vitamin B₁₂

INTRODUCTION

Estimates of dietary vitamin B₁₂ requirements in postweaning pigs are based on studies carried out before 1966 (NRC, 1998) and vary from 15 to 20 μg/kg. Typical amounts used by the feed industry range between 20 and 30 μg/kg (BASF, 2001). Recently, House and Fletcher (2003) and Giguère et al. (2008) showed that supplements of 35 and 25 μg/kg of dietary vitamin B₁₂ would be required in weaner or grower-finisher pigs, respectively. In gilts, Simard et al. (2007) showed that when dietary vitamin B₁₂ increased 10 times, from 20 to 200 μg/kg, vitamin B₁₂ in blood plasma was doubled. This indicated that vitamin B₁₂ bioavailability in pigs (defined as the fraction that can be absorbed across the
Bioavailability of vitamin B₁₂ in pigs

Table 1. Ingredients and chemical composition of the basal diet used in trials 1 and 2

<table>
<thead>
<tr>
<th>Item</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient, %</td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>63.85</td>
</tr>
<tr>
<td>Soybean meal, 48% CP</td>
<td>22.51</td>
</tr>
<tr>
<td>Extruded soybean</td>
<td>4.00</td>
</tr>
<tr>
<td>Animal fat</td>
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</tr>
<tr>
<td>Biophos ¹</td>
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</tr>
<tr>
<td>Plasma protein</td>
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</tr>
<tr>
<td>Dried whey</td>
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<tr>
<td>Limestone</td>
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</tr>
<tr>
<td>Ca formate, 98%</td>
<td>0.60</td>
</tr>
<tr>
<td>L-Lys</td>
<td>0.32</td>
</tr>
<tr>
<td>D₃, L-Met</td>
<td>0.12</td>
</tr>
<tr>
<td>L-Thr</td>
<td>0.08</td>
</tr>
<tr>
<td>Mineral and vitamin premix²</td>
<td>1.97</td>
</tr>
</tbody>
</table>

Calculated nutrient composition, %

<table>
<thead>
<tr>
<th>Item</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>DE, MJ/kg</td>
<td>14.21</td>
</tr>
<tr>
<td>CP</td>
<td>19.01</td>
</tr>
<tr>
<td>Fat</td>
<td>5.54</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>2.65</td>
</tr>
<tr>
<td>Lys</td>
<td>1.24</td>
</tr>
<tr>
<td>Met</td>
<td>0.42</td>
</tr>
<tr>
<td>Trp</td>
<td>0.21</td>
</tr>
<tr>
<td>Thr</td>
<td>0.78</td>
</tr>
<tr>
<td>Ca</td>
<td>0.85</td>
</tr>
<tr>
<td>P</td>
<td>0.71</td>
</tr>
</tbody>
</table>

¹Biophos (monocalcium phosphate) is an inorganic P source providing 21% available P, 18% Ca, and 0.21% F.
²Supplied per kilogram of feed: Mn as manganous oxide, 61 mg; Zn as zinc oxide, 528 mg; Fe as ferrous sulfate, 375 mg; Cu as copper sulfate, 198 mg; I as calcium iodate, 2 mg; and Se as selenite, 368 μg; vitamin A as retinyl palmitate and acetate, 15,000 IU; vitamin D₃ as cholecalciferol, 1,547 IU; vitamin E as α-tocopherol, 102 IU; vitamin K as menadione, 2.7 mg; thiamine as thiamine monohydrate, 2.8 mg; riboflavin, 9 mg; niacin, 32 mg; pantothenic acid as Ca pantothenate, 22 mg; folic acid, 0.7 mg; pyridoxine as pyridoxine hydrochloride, 2.7 mg; biotin, 102 μg; and choline as choline chloride, 303 mg.
³The dietary allowance at 1.2 kg/d corresponded to approximately 1.3 times the energy maintenance.

MATERIALS AND METHODS

For both trials, the experimental procedures followed the guidelines of the Canadian Council on Animal Care (1993) and were approved by the Institutional Animal Care Committee of the Dairy and Swine Research and Development Centre of Sherbrooke (Québec, Canada). All animals were cared for and slaughtered according to the recommended code of practice of Agriculture Canada (1993).

Trial 1

Animals and Diet. Forty-five castrated male piglets (Yorkshire-Landrace × Duroc) were weaned at 28 d of age and fed ad libitum a diet not supplemented with vitamin B₁₂ (Table 1) for a period of at least 6 wk. At 8 wk of age, a blood sample was collected from each animal in disposable evacuated tubes containing EDTA as the anticoagulant (Becton Dickinson, Franklin Lakes, NJ), centrifuged for 10 min at 4°C (1,800 × g), and the plasma was frozen at −20°C for subsequent vitamin B₁₂ determination. The animals were assigned to 15 blocks (replications) of 3 animals each based on their calculated total amount of vitamin B₁₂ in plasma, which was based on plasma vitamin B₁₂ concentration and an estimated plasma volume (4% of BW) as described by Simard et al. (2007).

Treatments and Tissue Sampling. All piglets were transferred to a metabolic cage and fed 1.2 kg of the basal diet (Table 1) and 2.4 L of water per d for 1 wk. After this adaptation period, 1 pig (CONT) in each block was randomly selected and killed using a captive bolt followed by exsanguination. The liver was collected (gallbladder removed) and weighed; samples of approximately 1 g were collected from the 3 main lobes and frozen at −20°C. The digestive tract (including stomach and small and large intestines) was flushed of its contents and frozen at −20°C. The carcass (including mesenteric tissue and blood) was weighed and also stored at −20°C.

Within each block, the remaining 2 piglets were maintained on the same basal regimen as during the adaptation period for an additional 8 d. During this last period, pigs were offered a 50-g pellet made of the basal diet mixed with unsweetened apple sauce to induce rapid consumption and 25 μg (B₁₂-25) or 250 μg (B₁₂-250) of cyanocobalamin (V-2876, Sigma-Aldrich, St. Louis, MO), corresponding to 20 or 200 μg/kg of diet, before the daily meal. Urine was collected twice daily at 0800 and 1600 h into dark glass bottles (4 L) containing 10 mL of 1% NaCN (S-3296, Sigma-Aldrich) to stabilize all vitamin B₁₂ forms into cyanocobalamin for 2 d before initiation of treatments and during the
treatment period (8 d). At collection time, urine volume was measured and a sample was frozen at −20°C. At the end of the collection period, all pigs were killed and measurements and samplings were done as described for CONT pigs.

**Sample Preparation and Vitamin B₁₂ Analysis.** The frozen carcasses were first coarsely cut into about 10 cm slices, which were ground together and passed 3 times through a meat grinder (model Autio-1101, Autio Company Inc., Astoria, OR) equipped with a circular plate measuring 25 cm in diameter with 6-mm diameter openings. A representative sample of each carcass (approximately 1 kg) was then collected and placed in molds of 1 cm³ and refrozen at −20°C. These samples were further ground to ≤1 mm in a centrifugal grinder (model ZM-1, Sybron Brinkman Instruments Canada Ltd., Mississauga, Ontario, Canada), which was located in a freezer, and liquid nitrogen was used to keep the sample frozen. The same procedure was used for the digestive tract.

For vitamin B₁₂ determination, carcass (500 mg), digestive tract (500 mg), and liver (250 mg) samples were homogenized in a glass grinder tube with 4 mL of sodium acetate buffer (0.1 M, pH 4.6). One milliliter of this homogenate was incubated in a water bath at 60°C for 5 min.

One milliliter of a papain (P-3250, Sigma-Aldrich) solution (50 mg/mL) and 75 μL of 1% NaCN were added and incubated in a water bath at 60°C for 1 h. The mixture was then autoclaved at 121°C for 10 min and cooled in an ice-cold water bath. The pH was adjusted to 4.6 with approximately 100 μL of 0.2 N HCl, and the mixture was centrifuged at 1,800 × g for 13 min at 4°C. The supernatant was collected, with the pH adjusted to 6.5 with approximately 300 μL of 0.1 N NaOH, and centrifuged again at 11,000 × g for 5 min at 4°C. Two homogenates were prepared for each tissue sample and vitamin B₁₂ was determined in duplicate for each homogenate (interassays), with 200 μL of this supernatant diluted [1.8 (vol/vol) in water for liver] or undiluted (carcass and digestive tract), using a radioassay procedure (Quantaphase II B₁₂, Bio-Rad Diagnostics Group, Hercules, CA), for which pig intrinsic factor as the vitamin B₁₂ binding protein. For urine, a 200-μL sample was used directly in the radioassay procedure, and vitamin B₁₂ was also determined in duplicate. Intra- and interassay CV, respectively, were 4.3 (n = 30) and 2.1% (n = 30) for liver, 3.5 (n = 13) and 2.2% (n = 11) for carcass, 6.0 (n = 13) and 5.8% (n = 16) for digestive tract, and 4.1 (n = 15) and 2.6% (n = 18) for urine.

**Calculations and Statistical Analysis.** The liver, digestive tract, and carcass contents of vitamin B₁₂ (total fresh weight × sample concentration) were calculated for each pig. For each B₁₂-25 or B₁₂-250 pig, urinary excretion of vitamin B₁₂ was calculated as the amount excreted (vitamin concentration × urine volume) during the experimental period of 8 d minus the average daily excretion measured 2 d before the initiation of treatments. Similarly, vitamin B₁₂ retention in each body portion of B₁₂-25 and B₁₂-250 pigs was calculated as the amount measured in these animals minus the amount present in tissues of the CONT pig within the same block. The total amount of vitamin B₁₂ absorbed corresponded to the summation of retention and urinary excretion. The bioavailability of dietary cyano- and urinary excretion. The bioavailability of dietary cyanocobalamin was expressed in percentage as follows: [(vitamin B₁₂ retention + vitamin B₁₂ urinary excretion)/vitamin B₁₂ intake] × 100.

All variables were analyzed as a randomized complete block design, with 2 treatments in 15 blocks, using the MIXED procedure (SAS Inst. Inc., Cary, NC). Results were reported as means and SE. The significance level was defined at P ≤ 0.05, and trends toward significance were considered at 0.05 < P ≤ 0.10.

**Trial 2**

**Animals, Surgery, and Diet.** After weaning at 28 d of age, 11 female piglets (Yorkshire-Landrace × Duroc) were fed ad libitum a diet not supplemented with vitamin B₁₂ (Table 1) for approximately 47 d before surgical modification. Average BW and age at surgery were 35.1 ± 4.0 kg and 75.4 ± 5.9 d, respectively. The surgical procedure has been described by Hoopa et al. (2009). Briefly, the animals were equipped with an ultrasonic flow probe (14 mm flow probe, SB-series, Transonic Systems Inc., Ithaca, NY) around the portal vein and a catheter inside the portal vein at 3.5 and 2.5 cm from the liver, respectively. Another catheter was inserted through the carotid artery up to the junction between carotid and subclavian arteries.

**Treatments and Collection of Samples.** After surgery, the animals were penned (1 × 1.8 m) individually and fed a single daily meal of 1.2 kg of the diet (Table 1). During the whole experimental period, catheters were rinsed 3 times per week with heparinized saline (200 IU/mL) complemented with sodium penicillin (5,000 IU/mL). Ten to fifteen days after the surgery, or when the animals had recovered full appetite and normal growth rate, they were adapted to the metabolic cage (with free access to water) and consumption of a semi-purified diet (1.2 kg/d) containing 67.48% cornstarch, 16.20% vitamin-free casein (C-3400, Sigma-Aldrich), 10.77% sucrose, 4.30% dicalcium phosphate, 0.51% salt, and 0.72% limestone. The semi-purified diet was used only for experimental meals in an attempt to uniformize and minimize the duration of ingestion and its impact on the nystomeral profiles. Each animal received the 3 experimental treatments according to a crossover design. The treatments were given as a 50-g pellet as described for trial 1, providing 0 (B₁₂-0), 25 (B₁₂-25), or 250 μg (B₁₂-250) of dietary cyanocobalamin (V-2876, Sigma-Aldrich) just before the meal (1.2 kg of the semi-purified diet). On the experimental day, blood samples (4 mL) were collected simultaneously from the 2 catheters 15 and 5 min before the meal, every 45 min for the first 3 h postfeeding, and every hour for the next 21 h. Portal blood flow was recorded continuously for...
24 h (WinDaq software, Dataqa Instruments Inc., Akron, OH). Between experimental days (n = 3 for each pig), the animals were moved back to their pen for 3 to 4 d and fed the basal diet (Table 1).

Sample Preparation and Analysis. Immediately after sampling, blood was transferred from the syringes to EDTA-treated tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ). Plasma was collected after centrifugation at 1,800 × g for 10 min at 4°C and frozen at −20°C for further analysis. Plasma concentration of vitamin B12 was measured by radioassay in duplicate with 200 μL as described by Simard et al. (2007). Packed cell volume (PCV) was measured in duplicate on fresh blood by microcentrifugation, whereas an aliquot of blood was immediately frozen for hemoglobin determination according to the method of Drabkin (Manet, 1969). Blood hemoglobin concentrations and PCV values were used for calculation of venous and arterial plasma flows from the recorded blood flow as described by Hooda et al. (2009).

Calculations and Statistical Analysis. The net flux of vitamin B12 across the portal-drained viscera was calculated as described by Girard et al. (2001) for each sampling time. A positive net flux indicates a release of a nutrient from the portal-drained viscera, whereas a negative flux indicates an uptake. Arterial concentrations and net flux of vitamin B12 across the portal-drained viscera recorded 15 and 5 min before the meal were averaged and used as time 0. Arterial concentrations and net flux of vitamin B12 across the portal-drained viscera were analyzed using the MIXED procedure of SAS according to crossover design, and pigs, periods, and treatments (unequally spaced) were included in the model along with repeated measures in time. Because the time intervals were unequally spaced, the following covariance structures were compared: space(power), space(Gaussian), space(exponential), space(linear), space(linear logarithmic), and space(spherical).

For each variable, the statistical analysis retained was with the best fit statistic value. Results are reported as least squares means and SEM. Values were assumed to be different at P ≤ 0.05 and tended to differ at P ≤ 0.10.

RESULTS AND DISCUSSION

Trial 1

In carcass, vitamin B12 concentration and total content increased (P < 0.01) with the dietary intake of vitamin B12 (Table 2). Giguère et al. (2008) reported that vitamin B12 concentrations in LM of 10-wk-old pigs were 3.7 and 4.1 μg/g after a long-term (8 wk) dietary supplementation of cyanocobalamin at 25 and 150 μg/kg, respectively. The present carcass values included mainly muscles but also other metabolic pools such as blood, bone marrow, and other organs that might have contributed to greater vitamin B12 concentrations compared with Giguère et al. (2008) because, according to Scheid and Schweigert (1954), concentrations of vitamin B12 in kidneys, pancreas, spleen, heart, brain, and lungs of pigs vary between 21 and 165 ng/g.

In liver, vitamin B12 concentration and total content did not respond (P > 0.19) to dietary cyanocobalamin supplementation. The result contrasted with Catron et al. (1952) who found that the concentration of vitamin B12 in liver increased from 49 to 135 ng/g with dietary vitamin B12 supplementation at 0 and 13.2 μg/kg. The hepatic concentration of 150 ng/g in CONT pigs was possibly at the saturation threshold for vitamin B12 content in liver. It seems that the postweaning period, during which the animals were fed the diet unsupplemented with vitamin B12 (approximately 8 wk), was not sufficient to deplete liver reserves. Body reserves of vitamin B12 are used up slowly (several months) in most species (Le Grusse and Watier, 1993; Combs, 1998), including pigs where a slow hepatic release and metabolic utilization of vitamin B12 have been reported (Frederick and Brisson, 1961). The hepatic reserves in CONT pigs were likely built up through the pre- and postnatal transfer of vitamin B12 (Simard et al. 2007) from their dam fed gestation and lactation diets containing 25 μg of cyanocobalamin/kg. In research by Catron et al. (1952), pigs were from litters of lactating dams fed a plant protein diet supplemented only with vitamins A and D3, and after weaning, these pigs showed symptoms of vitamin B12 deficiency (growth stasis, in particular) when they were fed a nonsupplemented diet. This apparent saturation of the hepatic pool for vitamin B12 is further emphasized by the observation that the total amount of vitamin B12 in liver was maximized at approximately the same amount as that reported by Catron et al. (1952) who observed total contents of 111 and 114 μg of vitamin B12 after dietary supplementation of 8.8 and 13.2 μg/kg of vitamin B12, respectively. After saturation of hepatic tissue, vitamin B12 reaching the liver would be either redirected toward other tissues or excreted in the bile, and then, entering into the enteric or hepatic cycle, as observed in sheep (Smith and Marston, 1970). In the present experiment, the determination of vitamin B12 in bile from a limited number of pigs showed mean values of 827.5 ± 61.1 (n = 3) and 1,436.9 ± 382.7 (n = 3) pg/mL for B12-25 and B12-250 pigs, respectively. The basal value for CONT pigs was 653.1 ± 89.6 (n = 2). Assuming an average daily bile volume of 2.25 L in pigs of similar BW (Laplace and Ouaisi, 1977; Juste et al., 1979; Sambrook, 1981), the daily biliary release of vitamin B12 in B12-250 pigs can be estimated to be 3.2 μg as compared with 1.9 μg for B12-25 pigs, which are likely of biological importance compared with the estimated daily vitamin B12 deposition in liver (approximately 2.7 and 2.2 μg/d for B12-25 and B12-250 pigs, respectively). Bile is considered the second (after the nonabsorbed fecal pool) major route of vitamin B12 excretion (Hall, 1964).

The present hepatic responses are also in accordance with findings in other species showing a lack of [56Co]vitamin B12 accumulation in liver after supplementation.
with greater than 41 μg of vitamin B12/kg, indicating the presence of a regulation mechanism for hepatic vitamin B12 in rats (Birn et al., 2003). Taking into account that negligible amounts of vitamin B12 are free in mammalian organs (Kim et al., 2008), it can be hypothesized that, for pigs, this regulation proceeds through a saturable binding protein system involving transcobalamin II and haptocorrin, similar for the rat (Li et al., 1994; Birn et al., 2003). In liver, the vitamin is also bound to the 2 intracellular vitamin B12-dependent enzymes, cytoplasmic methionine synthase and mitochondrial methylmalonyl-CoA mutase (Combs, 1998).

The present results on carcass and liver challenge concepts that are related to vitamin B12 partitioning in the body. Although liver is a major pool of body vitamin B12 in pigs as reported by Trugo et al. (1985), hepatic vitamin B12 represented a maximum of 33 to 40% of the total amount of vitamin B12 in whole body. In human adults, vitamin B12 is mainly stored in the liver (about 60% of the total body store) and muscles (about 30% of the total; Le Grusse and Watier, 1993). The ratio of liver to carcass (including muscle, kidney, and blood) in the present study is different than in adult human and could be interpreted as species differences. However, this apparent species difference may be confounded with age because values in reproducing sows of approximately 1 yr of age (300 ng/g; Simard et al., 2007) were approximately 2-fold greater than those observed in the present research.

For the digestive tract, vitamin B12 supplementation increased concentration and total content even though the latter represented less than 5% of total body vitamin B12. It cannot be ruled out that the vitamin B12 content in digestive tract tissue was influenced in part by the last experimental meal. Indeed, although pigs were fasted 24 h before slaughter, the kinetics of portoarterial differences in trial 2 showed that the duration of absorption process might be longer than 15 h as reported by Chanarin et al. (1978) and Combs (1998). Therefore, it seems possible that some vitamin B12 from the previous meal was still sequestered by enterocytes (Seetharam and Yammani, 2003).

For urine, samples were collected only from pigs receiving vitamin B12 supplementation, and the net excretion was twice greater in B12-250 than in B12-25 pigs. Such response was consistent with Catron et al. (1952) in pigs and Birn et al. (2003) in rats, the latter showing a numerical increase in urinary [57Co]B12 after supplementation with 41 μg of vitamin B12/kg. In fact, Birn et al. (2003) proposed a model showing the critical role of kidney for vitamin B12 homeostasis through a glomerular filtration of the complex transcobalamin-vitamin B12, followed by renal reuptake of the vitamin. This would explain the large accumulation of vitamin B12 in kidneys after a vitamin B12 load in rats. In the present experiment, kidneys were not separated from the carcass.

The whole body retention of vitamin B12 was greater (P < 0.02) in B12-250 than in B12-25 pigs. However, for

### Table 2. Distribution of vitamin B12 in carcass, liver, digestive tract, and urine of pigs after 8 d of a daily dietary supplementation of 25 (B12-25) or 250 (B12-250) μg of cyanocobalamin (trial 1)

<table>
<thead>
<tr>
<th>Item</th>
<th>Carcass1</th>
<th>Digestive tract1</th>
<th>Liver1</th>
<th>Urine net excretion2</th>
<th>Whole body3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item</td>
<td>CON, WW, TC, RET1, g (μg)</td>
<td>CON, WW, TC, RET1, μg</td>
<td>CON, WW, TC, RET1, g (μg)</td>
<td>CON, WW, TC, RET1, μg</td>
<td>CON, WW, TC, RET1, g (μg)</td>
</tr>
<tr>
<td>Basal B12 content6</td>
<td>4.22 (31.44)</td>
<td>122.38 (96.15)</td>
<td>4.73 (39.34)</td>
<td>181.24 (104.17)</td>
<td>76.70 (41.02)</td>
</tr>
<tr>
<td>B12-257</td>
<td>4.73 (38.33)</td>
<td>148.68 (118.65)</td>
<td>5.58 (37.51)</td>
<td>209.08 (114.18)</td>
<td>80.17 (42.94)</td>
</tr>
<tr>
<td>SEM</td>
<td>0.13</td>
<td>0.59</td>
<td>0.41</td>
<td>0.34</td>
<td>0.34</td>
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<tr>
<td>P-value</td>
<td>&lt;0.01</td>
<td>0.34</td>
<td>0.47</td>
<td>&lt;0.01</td>
<td>0.02</td>
</tr>
</tbody>
</table>

1 Tissue vitamin B12 concentration (CON), wet weight (WW), total content (TC), and retention (RET).
2 Urinary net excretion of vitamin B12 was calculated as the amount excreted (vitamin concentration × urine volume) during the experimental period of 8 d minus the average daily excretion.
3 Summation of whole body retention and urinary net excretion.
4 Vitamin B12 retention in each body portion of B12-25 and B12-250 pig was calculated as the amount measured in these animals minus the amount present in organs of the control pig in the same block.
5 Summation of whole body retention and urinary net excretion.
6 Values are means of 15 pigs slaughtered (control pigs) before initiation of B12-25 or B12-250 treatments for 8 d.
7 Basal B12 content measured 2 d before the initiation of treatments (3.72 ± 0.29 and 3.38 ± 0.29 μg/d for B12-25 and B12-250, respectively).
the 8 d of experimentation, the efficiency of intestinal absorption (based on body retention and urine excretion) was estimated to be 38.2 and 5.3% for B12-25 and B12-250 pigs, respectively.

**Trial 2**

**Arterial Plasma Concentrations of Vitamin B12.** Changes in arterial plasma concentrations of vitamin B12 are illustrated in Figure 1. Premeal values (time = 0) were not different (P = 0.46) among treatments. After meal, plasma vitamin B12 decreased (P < 0.01) to reach minimum values between 15 and 18 h postmeal. These minimum values were 29.6, 15.6, and 10.0% less than the premeal values for B12-0, B12-25, and B12-250 pigs, respectively (linear, P < 0.01; cyanocobalamin × time postmeal, P = 0.08). Profiles of vitamin B12 in peripheral venous blood were reported in humans (von Castel-Roberts et al., 2007) and showed a continuous increase from 1.5 to 6 h postmeal and then a plateau up to 12 h postmeal. It is noteworthy to mention that although the daily dietary B12 provision was comparable with the present B12-25 treatment, it was administered in 3 consecutive 9-μg doses at 6-h intervals. In another report in humans, vitamin B12 was measured in blood plasma collected from the femoral artery after ingestion of a supplement of 1,000 μg of cyanocobalamin, but the postprandial period was limited to 2 h (Baker et al., 1994). Thus, those results cannot be compared with the present 24-h profiles. In both studies in humans, profiles were reported in absence of information relative to control diet without vitamin B12. To the best of our knowledge, the peculiar feature of the postfeeding arterial responses of vitamin B12, especially in the B12-0 treatment, has never been reported before and likely reflects that the systemic vitamin input from diet or recirculation was less than its output counterpart (i.e., tissue utilization or excretion).

**Vitamin B12 Net Flux.** Mean portal net fluxes of vitamin B12 for 24 h postmeal were 1.02 ± 0.68, 1.83 ± 0.63, and 3.53 ± 0.63 ng/min for B12-0, B12-25, and B12-250 treatments (linear, P < 0.01). For the B12-0 treatment, mean portal net flux of vitamin B12 for 24 h after the meal was not different from 0 (P > 0.15), whereas it was greater than 0 (P < 0.01) for B12-25 and B12-250 treatments. Throughout the postprandial period, portal net fluxes of vitamin B12 varied with time (P = 0.05; Figure 2), but there was no interaction with treatment (P = 0.98). Positive portal net fluxes different from 0 were observed from 7 to 10 h (P ≤ 0.04), at 13 and 15 h (P = 0.03), and finally 24 h after the meal (P < 0.01). This indicates the presence of at least 2 distinct periods of intestinal absorption, from 7 to 10 h and 13 to 15 h after the meal. Moreover, the fact that the portal net flux was positive (P < 0.04) 24 h after the meal indicates the initiation of a third period of intestinal absorption. The first period of absorption coincided with the postprandial maximal appearance of 14C in peripheral venous plasma after dietary intake of 14C-vitamin B12 in humans (Carkeet et al., 2006) and in

![Figure 1. Nycthemeral profile of arterial concentrations of plasma vitamin B12 after a meal containing 0 (B12-0), 25 (B12-25), or 250 (B12-250) μg of cyanocobalamin (trial 2). Values are least squares means of 11 pigs per treatment (cyanocobalamin: linear, P < 0.01; time postmeal, P < 0.01; and cyanocobalamin × time postmeal, P = 0.08).](image-url)
rabbits using $^{56}\text{Co}$ cyanocobalamin (Simnet and Spray, 1965). Unfortunately, in those 2 last studies, the lack of repeated blood samples beyond 12 h after the meal did not allow for comparisons with the present study. In lactating dairy cows, Girard et al. (2001) indicated that, 24 h after an oral bolus of 0.5 g of cyanocobalamin, the net portal flux of vitamin B$_{12}$ showed a biphasic pattern with peaks at 4 to 10 h and 20 to 24 h post-meal. These authors explained that the second peak was the result of the active enterohepatic recirculation, which is known to play a major role in vitamin B$_{12}$ homeostasis in ruminants (Smith and Marston, 1970). If enterohepatic recirculation has the same importance in pigs, it would be compatible with a second and possibly a third episode of intestinal absorption of vitamin B$_{12}$, as observed in the present experiment. This role of biliary recirculation of vitamin B$_{12}$ in pigs is further supported by the numerical differences among treatments reported in trial 1 for concentrations of biliary vitamin B$_{12}$.

The cumulative net flux of vitamin B$_{12}$ for 24 h corresponded to 2.4 and 5.1 μg for B$_{12}$-25 and B$_{12}$-250 treatments, respectively. Therefore, the efficiency of intestinal absorption was estimated to be 9.7 and 2.0% for B$_{12}$-25 and B$_{12}$-250 treatments, respectively.

**Comparison Between the 2 Methods**

The estimations of vitamin B$_{12}$ absorption based on body retention and urine excretion in trial 1 are consistent with the estimation of intestinal capacity for the vitamin B$_{12}$ absorption reported in pigs by Ford et al. (1975), which was approximately 10 μg per d. The corresponding values for B$_{12}$-25 and B$_{12}$-250 pigs in the present study were 9.6 and 13.3 μg per d, respectively. The inverse relationship between absorption efficiency of vitamin B$_{12}$ and dietary cyanocobalamin was also reported for human by Herbert (1987) with absorption efficiencies of 46 and 6% after dietary vitamin B$_{12}$ intake of 2 and 20 μg, respectively. In fact, this was also apparent in trial 2, although absolute values were different.

Vitamin B$_{12}$ absorption in humans occurs through an active process mediated by ileal intrinsic factor and enterocyte transcobalamin at reduced dietary content (Nicolas and Guéant, 1994), whereas, at greater dietary content of vitamin B$_{12}$, after saturation with the active absorption process, the excess vitamin would be absorbed by passive intestinal diffusion (Herbert, 1987; Combs, 1998). If the same explanation applies to pigs, it can be hypothesized that the active absorption was the most important process in B$_{12}$-25 pigs, and consequently, most of the difference of absorption between B$_{12}$-250 and B$_{12}$-25 (13.3 – 9.6 = 3.7 μg/d in trial 1) would be due to passive intestinal diffusion. The corresponding calculation for trial 2 was 2.7 μg per d (5.1 to 2.4). In term of percentage of the supplementary vitamin B$_{12}$ (250 – 25 = 225 μg), the passive intestinal diffusion would represent approximately 1.6% (3.7/225) and 1.2% (2.7/225) for trials 1 and 2, respectively, which are comparable with the range of 1 to 3% reported for humans (Herbert, 1987). It seems, therefore, that most of the differences in estimations of bioavailability of dietary vitamin B$_{12}$ between the 2 trials were due to the active intestinal absorption process.
Both approaches in trials 1 and 2 give an indirect estimation of bioavailability of cyanocobalamin. The retention (trial 1) approach underestimated, in theory, intestinal absorption because part of the vitamin B_{12} absorbed during the experimental period of 8 d was used for metabolism and, therefore, catabolized. On the other hand, the portal appearance approach (trial 2) also underestimated intestinal absorption because part of the vitamin B_{12} absorbed after the meal is retained or retained and used directly by the intestinal tissue before reaching the portal vein. In the portal appearance approach, it can be estimated, from results in trial 1, that at least 6 and 9% of the absorbed vitamin B_{12} is retained and used directly by the intestinal tissue after reaching the portal vein. In the portal appearance approach, it can be estimated, from results in trial 1, that at least 6 and 9% of the absorbed vitamin B_{12} is retained in the digestive tract for B_{12}-25 and B_{12}-250, respectively. This retention represented, therefore, the minimal amount of absorbed vitamin B_{12}, which is not accounted for by the portal appearance approach in trial 2.

Other aspects of the digestive physiology relative to vitamin B_{12} might be involved in the differences between the 2 approaches. Some vitamin B_{12} can be detected in the lymph of rats (Taylor and French, 1960; Boass and Wilson, 1964) and dogs (Reizenstein et al., 1960) after an oral bolus of vitamin B_{12}. The lymph carries vitamin B_{12} coming directly from the intestinal lumen and from blood circulation; the former would represent between 3 to 9% of the intestinal absorption of this vitamin (Boass and Wilson, 1964). If the situation is similar in pigs, it can be hypothesized that a small quantity of vitamin B_{12} absorbed through the lymphatic system in trial 2 was not accounted for in the calculation of bioavailability by portal-arterial differences. This could be another contributing factor, although small, for the discrepancy between the 2 approaches for estimation of bioavailability.

In the retention approach in trial 1, each pig received a daily supplement of vitamin B_{12} for 8 consecutive days, whereas in trial 2, each pig received each level of supplement only once according to a crossover design. In trial 1, the repeated bolus of supplemental cyanocobalamin along with its successive waves of biliary vitamin B_{12} recycled from the enterohepatic cycle could have gradually enriched the intestinal content in intrinsic factor-bound vitamin B_{12}, as reported by Kanazawa et al. (1985). Moreover, vitamin B_{12} from this endogenous source was possibly more bioavailable than the dietary source because, in other species such as baboons, cobalamin from bile is better absorbed and retained than cyanocobalamin (Green et al., 1982). Although all the aforementioned factors related to digestive physiology of vitamin B_{12} can contribute to differences of bioavailability measurements between the 2 trials, their relative importance cannot be determined with the present available information.

In summary, although the estimations of bioavailability varied according to the approach chosen, it was nevertheless possible to differentiate the impact of dietary vitamin B_{12} on its bioavailability as previously reported for humans. A daily dietary supplementation of 25 μg, which is greater than NRC (1998) recommendation (15 μg/kg) but similar to the level commonly used by the industry (BASF, 2001), seemed adequate to maximize vitamin B_{12} in liver (trial 1) and to attenuate the nycthemeral decrease in arterial plasma concentrations of vitamin B_{12} (trial 2). Although the bioavailability of vitamin B_{12} was apparently substantially decreased with a daily dietary supplementation with 250 μg, such a supplement could build up whole body reserves (carcass and digestive tract) by up to 40% (trial 1) or doubled the cumulative net flux of the vitamin across portal-drained visera (trial 2) over a supplementation with 25 μg.

LITERATURE CITED


