Effects of concentrations of cyanocobalamin in the gestation diet on some criteria of vitamin B_{12} metabolism in first-parity sows^{1,2}

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ABSTRACT: In swine nutrition, little is known about the role of vitamin B_{12} in the reproductive processes. The current study was undertaken to obtain information on the dose-response pattern of different metabolic criteria related to the homeostasis of vitamin B_{12} and homocysteine in gestating sows receiving various concentrations of dietary vitamin B_{12} (cyanocobalamin). Homocysteine is a detrimental intermediate metabolite of the vitamin B_{12}-dependent remethylation pathway of Met. Forty nulliparous (Large White × Landrace) sows were randomly assigned during gestation to dietary treatments containing 5 concentrations of cyanocobalamin (0, 20, 100, 200, or 400 μg/kg). During lactation, a diet containing 25 μg of cyanocobalamin/kg (as-fed) was given to all sows. During gestation, plasma vitamin B_{12} increased as concentrations of dietary cyanocobalamin increased (linear and quadratic, \( P < 0.01 \)) and the effect persisted during lactation (21 d postpartum) both in plasma (linear and quadratic, \( P < 0.05 \)) and the liver (linear and quadratic, \( P < 0.04 \)). Plasma homocysteine decreased with concentrations of dietary cyanocobalamin provided to sows during gestation (linear, quadratic, and cubic, \( P < 0.01 \)). At parturition, vitamin B_{12} in colostrum increased as concentrations of cyanocobalamin increased (linear and quadratic, \( P < 0.01 \)), but the treatment effect persisted (linear, \( P = 0.01 \)) only up to 1 d postfarrowing. However, in piglets there was no treatment effect (\( P = 0.59 \)) on plasma vitamin B_{12} before colostrum intake, but a linear effect of concentrations of cyanocobalamin (\( P = 0.04 \)) was observed 1 d later. Plasma homocysteine in piglets during lactation decreased with increasing concentrations of cyanocobalamin given to sows in gestation (linear and quadratic, \( P < 0.01 \)). Based on a broken-line regression model, the concentrations of dietary cyanocobalamin that maximized plasma vitamin B_{12} and minimized plasma homocysteine of sows during gestation were estimated to be 164 and 93 μg/kg, respectively. The maximal residual responses in sows and piglets during lactation were observed with treatments of 100 or 200 μg of cyanocobalamin/kg. The dietary cyanocobalamin concentration necessary to optimize the response of these metabolic criteria remains to be refined within lower and narrower ranges of cyanocobalamin concentrations (i.e., <200 mg/kg). Moreover, the biological significance of such concentrations of cyanocobalamin needs to be validated with performance criteria by using greater numbers of animals during several parities.

Key words: gestation, gilt, homocysteine, milk, piglet, vitamin B_{12}


INTRODUCTION

Vitamin B_{12} was the last vitamin to be identified in the late 1940s. It acts primarily through 2 important metabolic pathways: as an enzymatic cofactor in the remethylation of Met from homocysteine and 5-methyltetrahydrofolate, and also in the methylmalonyl-CoA mutase pathway. Vitamin B_{12} is particularly important in tissues with high rates of cell turnover (Le Grusse and Watier, 1993; McDowell, 2000).
In swine nutrition, little is known about the role of vitamin B12 in reproduction. Moreover, the information used to estimate the requirement is outdated (Cunha et al., 1944; Anderson and Hogan, 1950; Teague and Gribo, 1966b). However, it was the only information available to the NRC (1998) and Agricultural Research Council (1981) to estimate the requirements as being 15 μg/kg. In this respect, the statement made by Agricultural Research Council (1981) is quite evocative: “The proposed figure of 15 μg·kg⁻¹·day⁻¹ of dietary DM must be considered very tentative and may need to be increased tenfold or more...” Recently, it was reported that plasma vitamin B12 was approximately 2-fold increased tenfold or more...” Recently, it was reported that plasma vitamin B12 was approximately 2-fold lower in gilts than in multiparous sows (Guay et al., 2002a) and could be a limiting factor for the action of folates on the uterus and embryo during the first pregnancy (Matte et al., 2006a). Moreover, considering that the total vitamin B12 content in uterine horns at pregnancy (Matte et al., 2006a) and could be a limiting factor for the action of folates on the uterus and embryo during the first pregnancy. In addition, Guay et al. (2002b) showed that a supplement of 160 (vs. 20) μg/kg of cyanocobalamin was an effective tool for increasing the plasma vitamin B12 of sows in early pregnancy.

Therefore, the current study was undertaken to provide updated information on the response pattern of different criteria related to homeostasis of vitamin B12 and homocysteine in first-parity sows fed various concentrations of cyanocobalamin.

**MATERIALS AND METHODS**

**Animals**

All animals were cared for and slaughtered according to the recommended code of practice of Agriculture Canada (1993). The procedure, which followed the guidelines of the Canadian Council on Animal Care (1993), was approved by the local Institutional Animal Care Committee.

Forty Large White-Landrace prepubertal gilts (average BW, 109.5 ± 1.4 kg) were transported to the experimental unit and grouped 2 to 3 per pen (1.5 × 2.5 m), and pens were located next to a boar to stimulate estrus. Gilts were fed 3.0 kg/d of a diet without any supplemental vitamin B12 (Table 1). Eight blocks of 5 animals were formed according to their total circulating vitamin B12 concentration and an estimated plasma volume of 4% of BW (Matte and Girard, 1996). Estrus was detected twice a day by introducing a boar into the pen between 0800 and 0900 and from 1600 to 1700. At the first pubertal estrus (average BW of 121.8 ± 1.4 kg), gilts were placed in individual stalls (0.6 × 2.2 m) and fed 2.25 kg of the diet/d (Table 1). Sows were randomly assigned within each block to 5 dietary treatments to provide 0, 20, 100, 200, or 400 μg of cyanocobalamin (Sigma-Aldrich Inc., St. Louis, MO) per kilogram of diet (as-fed) by top-dressing with a premix. At the third estrus, gilts were inseminated twice with 85 mL of semen (3 × 10⁹ live sperm cells from pooled semen of 3 Duroc boars) provided by a local AI center (CIPQ Inc., St-Lambert, Quebec, Canada). When estrus was first detected in the morning, gilts were inseminated twice, at 8 and 24 h later, and when estrus was first detected in the afternoon, the 2 inseminations were done 16 and 24 h later. The day of the second insemination was considered as d 0 of gestation.

A week before the expected farrowing, the sows were placed in individual farrowing crates (1.5 × 2.2 m). On d 112 of gestation, all sows received an i.m. (neck) injection of 1.5 mL of cloprostenol (Planate, Schering-Plough Animal Health, Pointe Claire, Quebec, Canada) to induce farrowing, which usually occurred within 20 to 30 h after the injection. During farrowing, piglets were placed in a box outside the crate to prevent access to mammary glands and colostrum intake until blood collection from 3 piglets of average BW and condition (visual assessment) was completed. The number of piglets (total and born alive) was recorded, and they were individually identified (ear tagged). On the day after farrowing (d 1), all litters were standardized to a maximum of 12 piglets per sow, and there was no cross-fostering (range of 9 to 12 piglets per sow). Teeth filing, tail clipping, and iron injection in the neck (1 mL of iron dextran containing 100 mg of Fe/mL, Ironol, P.V.U., Lavaltrie, Quebec, Canada) were also done on d 1. At that time, 3 representative piglets (average litter BW ± 1 SD) were chosen for repeated blood sample collections throughout the lactation period (21 d) for vitamin B12 and homocysteine analyses. Beginning on the day of farrowing, all sows received the same commercial (corn- and soybean-based) lactation diet containing 3,300 kcal.

**Table 1. Centesimal composition of the basal diet fed during gestation, as-fed basis**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>50.0</td>
</tr>
<tr>
<td>Barley</td>
<td>20.0</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>20.0</td>
</tr>
<tr>
<td>Soybean meal (48%)</td>
<td></td>
</tr>
<tr>
<td>Lime</td>
<td>2.5</td>
</tr>
<tr>
<td>Calcium hydrogen phosphate</td>
<td>1.4</td>
</tr>
<tr>
<td>Salt</td>
<td>0.5</td>
</tr>
<tr>
<td>Mineral premix²</td>
<td>0.1</td>
</tr>
<tr>
<td>Vitamin premix³</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*The calculated composition for ME, CP, Lys, Ca, and P of the basal diet were 3,096 kcal/kg, 11.3%, 0.46%, 1.2%, and 0.7%, respectively.
²Supplied, per kg of feed: Mn as manganous oxide, 30 mg; Zn as zinc oxide, 100 mg; Fe as ferrous sulfate, 100 mg; Cu as copper sulfate, 25 mg; I as calcium iodate, 300 μg; and Se as selenite, 300 μg.
³Supplied, per kg of feed: vitamin A as retinyl palmitate and acetate, 10,000 IU; vitamin D₃ as cholecalciferol, 2,000 IU; vitamin E as α-tocopheryl, 35 IU; vitamin K as menadione, 2.2 mg; thiamine as thiamine monohydrate, 2 mg; riboflavin, 5 mg; niacin, 25 mg; pantothenic acid as Ca pantothenate, 16 mg; folic acid, 10 mg; pyridoxine as pyridoxine hydrochloride, 3 mg; biotin, 250 μg; and choline as choline chloride, 300 mg.*
of ME/kg, 17.8% CP, 1.0% Lys, and 25 μg/kg (as fed) of vitamin B₁₂. The allowance was 3.25 kg on d 1 and was increased by 0.5 kg/d until it reached ad libitum intake toward the end of the first week of lactation.

Sows were weighed upon their arrival at the Research Centre, at first pubertal estrus (assignment of treatments), insemination, d 110 of gestation, and weaning. Piglets were weighed on the day of farrowing (d 0) and on d 1, 7, 14, and 21 (weaning) of lactation. Before weighing, all piglets were separated from their mother for 1 h, which is slightly more than the normal interval between 2 suckling episodes (Pond and Maner, 1984b), to standardize the effect of previous milk intake on BW and plasma metabolite concentrations. After weaning, during the nursery period, feed and water were removed for 8 h before weighing and blood sampling. Sows were slaughtered at the end of the lactation period for determination of hepatic vitamin B₁₂ concentrations.

**Samplings and Analytical Measurements**

**Blood and Tissue Collection.** Blood was collected in disposable evacuated tubes containing EDTA as the anticoagulant (Becton Dickenson, Franklin Lakes, NJ), centrifuged for 10 min (1,800 × g), and the plasma was frozen at −20°C. After a fasting period of at least 16 h, samplings were done at arrival, when the sows were at the same time of the day (0830 to 1000) and used to assign sows to treatments (first pubertal estrus), at the time of insemination (third postpubertal estrus, d 0 of gestation), and on d 15, 30, 60, 90, and 110 of gestation. During lactation, blood samples were collected from sows and from 3 piglets per litter on d 1, 7, 14, and 21 after farrowing. However, for piglets, the fasting period was 1 h, which corresponded to the time during which all piglets were separated from their mother for the weighing procedure. All blood samples were collected at the same time of the day (0830 to 1000) and used to determine plasma concentrations of vitamin B₁₂, homocysteine, and Cys. Milk samples were also collected on d 0 (colostrum, before the first suckling), 1, 7, 14, and 21 of lactation to measure vitamin B₁₂ concentrations.

Samples were obtained by milking 3 glands on each side of the mammary gland after an i.v. injection of 0.5 mL of oxytocin (20 IU/mL, P.V.U.) in the marginal ear vein (Farmer et al., 1999). For each sampling time, all samples taken from each sow were mixed and stored at −20°C until analysis. After slaughter (weaning), liver samples were collected, frozen immediately on dry ice, and stored at −75°C until analysis for vitamin B₁₂.

**Analytical Measurements.** Concentrations of plasma vitamin B₁₂ were measured by radioassay (Quantaphase II, B₁₂ Radioassay, Bio-Rad Laboratories (Canada) Ltd., Montreal, Québec, Canada) following a procedure validated by Bilodeau et al. (1989) and Guay et al. (2002a); inter- and intraassay CV were ≤6.1%. Concentrations of total plasma homocysteine and Cys were determined by using a method adapted from the methods of Gilfix et al. (1997). A 100-μL quantity of plasma were mixed with 10 μL of 10% Tris (2-carboxymethylphosphine and incubated at room temperature for 30 min. Then, 100 μL of 10% perchloric acid containing 1 mM disodium EDTA was added. The mixture was centrifuged for 10 min at 11,000 × g, and 100 μL of the supernatant was transferred to a tube containing 20 μL of NaOH (1.55 M). The solution was incubated for 1 h at 60°C, and then left at room temperature to cool. A 20-μL quantity of this solution was injected into an HPLC system, using the following components: a solvent delivery system (Model Prostar 210, Varian, Walnut Creek, CA) equipped with a column (Microsorb MV 100-5 C18, 250 × 4.6 mm, Varian), an autosampler with a 20-μL injection loop (model 9300, Varian), and a fluorometric detection system (model LC240, Perkin-Elmer, Woodbridge, Ontario, Canada) adjusted at 750 and 375 nm for emission and excitation. The system was controlled by a computing program (Star Workstation Version 5, Varian). The mobile phase was 6% acetonitrile containing 50 mM H₂SO₄ and the flow rate was adjusted to 1 mL/min. Standards were prepared from stock solutions at 13.5 and 12.1 mg/mL of DL-homocysteine and L-Cys (Sigma-Aldrich), respectively. For homocysteine, the intra- and interassay CV were 0.8% (n = 8) and 6.1% (n = 48), respectively, and the recovery was 99.8% (n = 6). For Cys, the intra- and interassay CV were 2.5% (n = 13) and 5.6% (n = 48), respectively, and the recovery was 99.5% (n = 8).

Concentration of milk vitamin B₁₂ was measured by sample preparation, as suggested by Gregory (1954). A 1-mL quantity of milk was mixed with 1 mL of 0.1 M sodium acetate buffer (pH 4.6) and was incubated in a water bath at 60°C for 5 min. One milliliter of a papain (Sigma p-3250, Sigma-Aldrich) solution (50 mg/mL), and 37 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 with ice. The pH was adjusted to 4.6 by using 100 and 200 μL of 0.2 N HCl for colostrum and milk, respectively, and the mixture was centrifuged at 1,800 × g for 11 min. The supernatant was collected, and the pH was adjusted to 6.5 with 300 and 350 μL of 0.1 N NaOH for colostrum and milk, respectively. This hydrolysate was centrifuged again at 11,000 × g for 5 min. Milk hydrolysates were used directly and colostrum hydrolysates were diluted to 1:5 (vol/vol) for the radioassay procedure, as described previously for plasma. The intra- and interassay CV were 3.7% (n = 24) and 2.5% (n = 24), respectively.

Liver concentrations of vitamin B₁₂ were measured by using a sample preparation adapted from that described for milk. A 250-mg quantity of liver was homogenized in a glass grinder tube with 4 mL of sodium acetate buffer (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 (Sigma p-3250, Sigma-Ald-
Vitamin B12 in reproducing swine

RESULTS AND DISCUSSION

Performance

Two sows had to be removed during the experiment because one did not show any signs of estrus and the other developed locomotion problems during the last month of gestation. No treatment effect ($P = 0.28$) was observed on BW changes during gestation or lactation. The average values were $148.2 \pm 1.5$, $208.6 \pm 2.0$, and $173.2 \pm 2.2$ kg at insemination, farrowing, and weaning, respectively.

There was a tendency for a treatment effect (quartic, $P = 0.06$) on litter size and weight at parturition (Table 2). At weaning, there was still a residual effect of the gestation treatments on litter size (quartic, $P = 0.02$) but not for the total litter weight ($P = 0.61$; Table 2). Although some treatment differences were shown to be statistically significant, the biological significance of those results should be interpreted with caution because the number of replications ($n = 7$ or $8$) per treatment in the current study was less than the number required for a reliable interpretation of data on litter size (>30; Aaron and Hays, 2001). In the current study, we aimed to maximize the number of treatments for the best possible determination of a dose-response curve for several metabolic criteria associated with vitamin B12 metabolism in prolific sows. Reproductive performance data were provided as an indication of the prolificacy of the first-litter sows used in the experiment (overall mean of $11.8 \pm 0.4$ total piglets born).

Vitamin B12 Homeostasis and Homocysteine in Sows

Plasma vitamin B$_{12}$ before initiation of treatments, 42 d before insemination, was similar ($84.9 \pm 3.6$ pg/mL, $P = 0.34$) among treatments. In nonsupplemented sows, plasma concentrations of vitamin B$_{12}$ decreased by approximately 33% to reach a minimum at 30 d of gestation ($57.6 \pm 5.1$ pg/mL), and returned to the initial concentration ($90.1 \pm 10.3$ pg/mL) at the end of gestation. For sows that received supplements, plasma concentrations of vitamin B$_{12}$ increased rapidly between the initiation of treatment and insemination ($126.2 \pm 12.1, 170.2 \pm 16.1, 243.1 \pm 10.6, 234.6 \pm 15.4$ pg/mL in sows fed 20, 100, 200, or 400 µg/kg, respectively) and remained stable thereafter throughout gestation (treatment × stage of gestation, $P = 0.01$). Taking into account the average values for the whole gestation period, the response to vitamin B$_{12}$ supplementation was linear and quadratic for both plasma vitamin B$_{12}$ and homocysteine ($P < 0.01$; Figure 1), and the inflection point was estimated to be 164 µg/kg of cyanocobalamine. Plasma vitamin B$_{12}$ is recognized and often used as a reliable indicator of the overall vitamin B$_{12}$ status of the animal (Le Grusse et Watier, 1993; Combs, 1998). This is all the more likely in the present experiment because of the uniformity of the feeding regimen, timing of the blood sampling procedure, and fasting period. During lactation (although all sows received the same lactation diet with 25 µg of cyanocobalamine/kg), the effects of supplementation on plasma vitamin B$_{12}$ persisted (linear and quadratic, $P = 0.05$ and 0.01, respectively; Table 3). At the hepatic level (d 21 of lactation), the residual effects of the gestation treatments were similar to those observed for plasma values during lactation (linear and quadratic, $P = 0.04$ and 0.01, respectively; Table 3). This residual response is probably because the liver has the ability to store vitamin B$_{12}$. It is recognized as a reliable indicator of vitamin B$_{12}$ status and holds more than 50% of the body reserves of the vitamin, which are used up slowly (<1 yr) in most species (Le Grusse and Watier, 1993; Combs, 1998). In pigs, a slow hepatic release and metabolic utilization of vitamin B$_{12}$ have been also reported, specifically in reproducing sows (Frederick and Brisson, 1961). Nevertheless, recent data from our laboratory (Matte et al., 2006b) using growing pigs indicated that the hepatic content might be lower in swine (i.e., approximately 30% of the overall body content of vitamin B$_{12}$).

With regard to plasma homocysteine, the mean overall values were affected by the stage of gestation ($P =$
Table 2. Litter size and litter weight at birth and at weaning according to the dietary concentrations of cyanocobalamin fed to primiparous sows from 42 d before gestation until the end of gestation\textsuperscript{1,2}

<table>
<thead>
<tr>
<th>Item</th>
<th>Dietary cyanocobalamin concentration, µg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Sows, n</td>
<td>8</td>
</tr>
<tr>
<td>Litter size total at parturition\textsuperscript{3}</td>
<td>11.9 ± 1.0</td>
</tr>
<tr>
<td>Litter size alive at parturition\textsuperscript{3}</td>
<td>11.4 ± 1.0</td>
</tr>
<tr>
<td>Litter weight alive at parturition, kg\textsuperscript{3}</td>
<td>16.5 ± 1.5</td>
</tr>
<tr>
<td>Litter size at weaning\textsuperscript{4}</td>
<td>10.3 ± 0.8</td>
</tr>
<tr>
<td>Litter weight at weaning, kg</td>
<td>65.9 ± 4.4</td>
</tr>
</tbody>
</table>

\textsuperscript{1}On the day after farrowing (d 1), all litters were standardized to a maximum of 12 piglets/sow. There was no cross-fostering between litters.

\textsuperscript{2}Values are arithmetic means ± SEM.

\textsuperscript{3}Effect of cyanocobalamin concentrations (quartic, $P = 0.06$).

\textsuperscript{4}Effect of cyanocobalamin concentrations (quartic, $P = 0.02$).

Values decreased from 18.3 ± 0.5 µM at the time of insemination to 16.5 ± 0.3 µM between 30 and 110 d of gestation and increased to 23.7 ± 0.6 µM on the day after parturition. These effects of the stage of gestation contrasted with those reported in humans, for whom homocysteine decreased (Murphy et al., 2002; Holmes et al., 2005) or was constant (Cikot et al., 2001) as gestation progressed, at least during the first 2 trimesters of pregnancy. In humans, the effect of hemodilution, changes in serum albumin, or folic acid supplementation have been ruled out to explain those variations in homocysteine (Murphy et al., 2002). In the current study, hemodilution and serum albumin are also unlikely to be important factors because both blood volume (Matte and Girard, 1996) and serum albumin (Robert et al., 1996) in sows change only slightly (i.e., an increase of less than 5% during the first 60 d of gestation). However, variations in the folate status could have an effect on homocysteine metabolism in midgestation as compared with early gestation, because plasma folates decreased by approximately 24% (stage of lactation, $P = 0.01$; from 63.4 ± 2.14 to 48.3 ± 1.66 ng/mL, data not shown) between 15 and 60 d of gestation in spite of an adequate (Matte and Girard, 1999) dietary supplement of folic acid given to those animals. As in humans, folate status is a major determinant of homocysteine concentration.

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**Figure 1.** Average plasma vitamin B\textsubscript{12} [effect of cyanocobalamin concentrations (linear and quadratic, $P < 0.01$)] and homocysteine concentrations [effect of cyanocobalamin concentrations (linear, quadratic, and cubic, $P < 0.01$)] for overall gestation (0, 15, 30, 60, and 110 d) according to dietary concentrations of cyanocobalamin fed to primiparous sows from 42 d before gestation until the end of gestation. Values are arithmetic means ± SEM; n = 7 or 8.
cysteine homeostasis in pigs (Barkow et al., 2001a; Guay et al., 2002a,b).

The average concentration of plasma homocysteine for the overall gestation period decreased with an increasing concentration of vitamin B12 (linear, quadratic, and cubic, \( P < 0.01 \); Figure 1); the inflection point was estimated to be 93 \( \mu \)g/kg of cyanocobalamin. Homocysteine is an intermediary AA known for its deleterious effects on several aspects of metabolism, including abnormal embryonic and fetal development (Pietrzik and Herrmann, 2005). According to responses of both plasma homocysteine and vitamin B12, the vitamin B12 supplementation seems to have stimulated the vitamin B12-dependent remethylation pathway of Met, which regenerates Met from homocysteine by using methyl groups provided by folic acid metabolism (Bassler, 1997). As in the present experiment, folic acid was incorporated into the feed at a concentration of 10 mg/kg, which was shown as the optimum to meet the metabolic needs of gestating sows (Matte and Girard, 1999); thus, it was unlikely a limiting factor for the potential action of vitamin B12. The present hypothesis relating to the remethylation pathway of Met was further supported by the fact that plasma Cys concentrations of sows during gestation were not affected (\( P = 0.23 \)) by cyanocobalamin concentrations. Cysteine can also be synthesized from homocysteine through the transsulfuration pathway (Le Grusse et Watier, 1993; Combs, 1998). The average plasma Cys concentrations declined (stage of gestation, \( P < 0.01 \)) from 249.8 ± 3.9 ng/mL on d 0 to 235.9 ± 3.0, 237.7 ± 3.2, and 210.5 ± 3.5 \( \mu \)M on d 30, 90, and 110 of gestation, respectively. During lactation, no treatment effect of the dietary cyanocobalamin supplementation on plasma homocysteine was observed (\( P = 0.660 \)), down from 23.8 ± 0.6 \( \mu \)M on d 1 to 12.6 ± 0.4 \( \mu \)M and 12.5 ± 0.6 \( \mu \)M on d 14 and 21, respectively (stage of lactation, \( P < 0.01 \)). For plasma Cys, there was no treatment effect (\( P = 0.60 \)) during lactation, but the average overall values decreased (stage of lactation, \( P < 0.01 \)) from 261.1 ± 4.2 at parturition to 253.3 ± 4.7, 230.7 ± 4.4, and 219.4 ± 5.3 \( \mu \)M on d 7, 14, and 21 of lactation, respectively. The significance of those decreases of both homocysteine and Cys during lactation remains to be further investigated.

Transfer of Vitamin B12 from Dam to Piglets

The vitamin B12 concentration in colostrum was dependent (linear and quadratic, \( P < 0.01 \)) on the concentrations of dietary cyanocobalamin given during gestation (Table 3). The maximum response observed at 200 \( \mu \)g/kg of cyanocobalamin was 114 and 69% greater than for sows in the 0 and 20 \( \mu \)g/kg treatments, respectively. By d 1 of lactation, the vitamin B12 content in milk, compared with colostrum, had dropped by approximately 40% in all treatments (stage of lactation, \( P = 0.01 \)), but a residual effect of the gestation treatments persisted (linear, \( P < 0.01 \)). As lactation progressed, the residual effects of the gestation treatments disappeared (\( P = 0.34 \)), and the overall vitamin B12 concentrations in milk were 6.28 ± 0.47, 5.73 ± 0.38, and 5.79 ± 0.31 ng/mL at d 7, 14, and 21, respectively.

Plasma vitamin B12 in piglets before ingestion of colostrum was not affected (\( P = 0.59 \)) by treatments given to sows, and the mean value was 0.59 ± 0.05 ng/mL. Vitamin B12 was measured in liver from a limited number of stillborn (d 0) piglets, which had no access to colostrum. Average concentration of liver vitamin B12 was approximately 2-fold greater (209.7 ± 2.7 ng/g) for piglets from sows fed 200 (\( n = 2 \)) or 400 \( \mu \)g/kg (\( n = 1 \)) compared with those from sows fed 0 (81.3 ± 8.7 ng/g; \( n = 3 \)) or 20 \( \mu \)g/kg of cyanocobalamin (162.1 ± 9.0 ng/g; \( n = 5 \)) during gestation. According to the mean BW of those stillborn piglets at 1.2 kg and a liver weight of 3.1% of BW (Pond and Maner, 1984a), the total amount of vitamin B12 in the liver of those piglets could be estimated to be 3, 6, and 7.8 \( \mu \)g with the 0, 20, and 200 to 400 \( \mu \)g/kg treatments, respectively. Those values are

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Table 3. Plasma, milk, and liver vitamin B12 concentration in sows and piglets (plasma) during lactation according to dietary concentrations of cyanocobalamin fed to primiparous sows from 42 d before gestation up to the end of gestation.

<table>
<thead>
<tr>
<th>Item</th>
<th>Dietary cyanocobalamin concentration, ( \mu )g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td><strong>Sows, n</strong></td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Average (d 1 to 21) plasma vitamin B12 of sows</strong></td>
<td>133.1 ± 16.9</td>
</tr>
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<td></td>
<td></td>
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<tr>
<td><strong>Colostrum (d 0) vitamin B12</strong></td>
<td>4.78 ± 0.70</td>
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<tr>
<td><strong>Milk (d 1) vitamin B12</strong></td>
<td>2.64 ± 0.45</td>
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<tr>
<td><strong>Liver (d 21) vitamin B12</strong></td>
<td>148.7 ± 13.2</td>
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</tr>
<tr>
<td><strong>Plasma (d 1) vitamin B12 of piglets</strong></td>
<td>0.93 ± 0.12</td>
</tr>
</tbody>
</table>

1 Values are arithmetic means ± SEM.
2 Effect of cyanocobalamin concentrations (linear and quadratic, \( P = 0.05 \) and 0.01, respectively).
3 Effect of cyanocobalamin concentrations (linear and quadratic, \( P = 0.01 \)).
4 Effect of cyanocobalamin concentrations (linear, \( P = 0.01 \)).
5 Effect of cyanocobalamin concentrations (linear and quadratic, \( P = 0.04 \) and 0.01, respectively).
6 Effect of cyanocobalamin concentrations (linear, \( P = 0.04 \)).
comparable with the 4.2-μg amount reported by Ford et al. (1975). The corresponding estimation for the total amount of vitamin B₁₂ in plasma of live newborn piglets (mean BW of 1.45 kg and plasma volume at 4% of BW) is negligible, at 0.035 μg. Therefore, although the liver values must be interpreted with caution, it is likely that a substantial amount of vitamin B₁₂ was transferred in utero and increased with the amount of dietary cyanocobalamin given to the sow during gestation. Such treatment responses are in agreement with those reported by Teague and Grifo (1966a), who used concentrations of cyanocobalamin at 0, 110, and 1,100 μg/kg of sow feed.

On d 1, after the ingestion of colostrum, the plasma vitamin B₁₂ concentrations in piglets increased substantially ($P = 0.01$) and were influenced by the amount of vitamin B₁₂ ingested by the sow during gestation (linear, $P = 0.04$; Table 3). From d 7 and as lactation progressed, the residual treatment effects disappeared and the mean plasma concentrations of vitamin B₁₂ decreased rapidly (stage of lactation, $P = 0.01$), with overall values of $0.74 ± 0.08$, $0.38 ± 0.03$, and $0.32 ± 0.02$ ng/mL at d 7, 14, and 21, respectively.

These results indicate that colostrum is one of the important routes of vitamin B₁₂ transfer from the sow to newborn piglets, and it can be modulated by the dietary concentrations of cyanocobalamin given during gestation. In fact, assuming a colostrum intake per piglet of approximately 430 g (Devillers et al., 2004), the vitamin B₁₂ provision would vary between 3 and 4 μg during the day after parturition, and this vitamin B₁₂ source is likely to be highly bioavailable according to Ford et al. (1975) and Trugo et al. (1985). Such provision corresponds to 50 to 100% of the total amount of vitamin B₁₂ stored in the liver at birth, as estimated previously. Although, to our knowledge, such information on vitamin B₁₂ has never been reported for pigs, the importance of the colostral route of transfer from mother to offspring is not unique to vitamin B₁₂, because a similar process would occur for other vitamins, including folic acid (Barkow et al., 2001b) and vitamins A and E (Håkansson et al., 2001).

**Homocysteine Homeostasis in Piglets**

There was a marked residual effect of the treatments given to dams during gestation on plasma homocysteine concentrations of the piglets (linear and quadratic, $P < 0.01$), which persisted throughout lactation (Figure 2). Most of the homocysteine reduction was seen among the cyanocobalamin treatments of 0, 20, or 100 μg/kg. At 35 d of age, there was no more residual effect of treatments ($P = 0.31$) on plasma homocysteine concentrations ($13.0 ± 0.5 \mu M$; $n = 19$). Those residual treatment effects on plasma homocysteine of piglets during lactation indicate that the pre- and postnatal transfer of vitamin B₁₂, although not apparent (except for d 1) in the plasma concentrations of vitamin B₁₂ of piglets, were important enough to influence the remethylation pathway of Met during early lactation. This result is further supported by the fact that plasma Cys concentrations in piglets, which are related to the transsulfuration pathway for disposal of homocysteine, were not affected ($P = 0.10$) by a residual effect of the dietary cyanocobalamin treatments to dams during gestation. Aside from those treatment effects, the overall variations of the general profile of both plasma homocysteine and Cys in piglets deserve to be mentioned. Indeed, concentrations of homocysteine at birth were approximately 10 times lower than the corresponding values for the dam, and they increased sharply, on average from $2.6 ± 0.1 \mu M$ at d 0 to $28.8 ± 1.1 \mu M$ at d 21 (stage of lactation, $P = 0.01$). This homocysteine is likely to originate from the metabolism of the piglet, because measurements of this metabolite in milk samples collected throughout lactation revealed that it was absent from the mammary secretions. For Cys, there was also an overall marked increase (stage of lactation, $P < 0.01$) during the same period, with values of $54.0 ± 2.1$, $74.9 ± 2.6$, $133.6 ± 2.7$, $181.7 ± 2.6$, and $186.8 ± 2.6 \mu M$ at d 0, 1, 7, 14, and 21, respectively. These results are in agreement with recent data from Ballance and House (2005), who also showed similar changes in plasma homocysteine and Cys along with a substantial increase in the transsulfuration pathway enzyme activities (cystathione-β synthase and cystathionine-γ-lyase) and a drastic decrease in Met synthase activity between birth and 26 d of age. This early increase in efficiency of the transsulfuration pathway may not be sufficient to deal with the huge and sudden production of homocysteine from the piglet metabolism. The transmethylation pathway (Met synthase), although declining with age, will have a considerable impact on homocysteine disposal as long as the enzyme is fully functional, with adequate provision of folic acid and vitamin B₁₂. In the current study, although the maximum residual effect of the gestation treatments (≥100 μg/kg) represented a decrease in plasma homocysteine of at least 34% (8 μM) and 21% (3 μM) as compared with piglets from sows fed 0 and 20 μg/kg of cyanocobalamin, respectively, those minimal values of homocysteine remained high as compared with values in other species (<10 μM) such as dairy cows (Girard et al., 2005), rats (Sarwar et al., 2000), mice (Hofmann et al., 2001), cats (Ruaux et al., 2001), and humans (Pietrzik and Brönnstrup, 1997). These differences among species deserved to be better understood.

In conclusion, the dose-response approach used in the present experiment provided new information to better understand the metabolic changes related to dietary provision of cyanocobalamin for sows. The concentrations of cyanocobalamin that maximized plasma vitamin B₁₂ and minimized plasma homocysteine of sows during gestation were estimated to be 164 and 93 μg/kg, respectively. The maximal residual responses in sows and piglets during lactation were observed with treatments of 100 or 200 μg of cyanocobalamin/kg. The dietary cyanocobalamin necessary to optimize the re-
Figure 2. Change in plasma homocysteine concentrations [effect of cyanocobalamin concentrations (linear and quadratic, \( P \leq 0.01 \)] according to age of piglet and to dietary concentrations of cyanocobalamin fed to their mothers from 42 d before gestation until the end of gestation. Values are arithmetic means per litter ± SEM; \( n = 7, \) or \( 8. \)

sponse of those metabolic criteria remains to be refined within lower and narrower ranges of cyanocobalamin concentrations (<200 mg/kg). Moreover, the biological significance of such concentrations of cyanocobalamin needs to be validated with performance criteria by using large numbers of animals during several parities. The present experiment also showed that the supplementation of vitamin \( B_{12} \) during gestation affected the transfer of this vitamin to the piglets, which occurred both in utero and through colostrum intake, and it prevented accumulation of homocysteine in piglets during lactation.

**LITERATURE CITED**


