Whole-body propionate and glucose metabolism of multiparous dairy cows receiving folic acid and vitamin B₁₂ supplements

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ABSTRACT

This study was undertaken to evaluate the effect of supplementation of folic acid and vitamin B₁₂ on glucose and propionate metabolism. Twenty-four multiparous cows were assigned according to a complete block design in a 2 × 2 factorial arrangement to one of the following treatments: (1) saline 0.9% NaCl, (2) 320 mg of folic acid, (3) 10 mg of vitamin B₁₂, or (4) 320 mg of folic acid and 10 mg of vitamin B₁₂. Intramuscular injections were given weekly from 3 wk before the expected calving date until 9 wk postpartum. At 63 d in milk, D-[6,6-²H₂]-glucose (16.5 mmol/h; jugular vein) and [1-¹³C]-sodium propionate (13.9 mmol/h; ruminal vein) were simultaneously infused for 4 h; blood samples were collected from 2 to 4 h of the infusion period. Liver biopsies were carried out the following day. Supplements of folic acid and vitamin B₁₂ respectively increased folate and vitamin B₁₂ concentrations, both in milk and liver. Although dry matter intake was unaffected by treatments, milk and milk lactose yields tended to be lower by 5.0 and by 0.25 kg/d, respectively, for cows receiving the folic acid supplement. Plasma β-hydroxybutyrate concentration with the folic acid supplement followed the same tendency. Hepatic gene expression of methylmalonyl-CoA mutase and S-adenosylhomocysteine hydrolase was higher for cows receiving the combined folic acid and vitamin B₁₂ supplement compared with cows receiving only the supplement of folic acid, whereas no treatment effect was noted for cows not receiving the folic acid supplement. Whole-body glucose rate of appearance and the proportion of whole-body glucose rate of appearance secreted in milk lactose decreased by 229 g/d and 5%, respectively, for animals receiving the folic acid supplement, concomitant with the lower milk lactose

synthesis in these cows, indicating that supplementary folic acid may alter energy partitioning in cows. The absence of treatment effect on plasma concentrations of methylmalonic acid as well as on the proportion of glucose synthesized from propionate, averaging 60%, supports the fact that vitamin B₁₂ supply was sufficient in control cows in the current study. Our results suggest that the folic acid supplement reduced glucose-derived lactose synthesis by redirecting glucose for other metabolic activity in the mammary gland or in other tissues. **Key words:** dairy cow, folic acid, vitamin B₁₂, propionate, glucose

INTRODUCTION

The transition from late gestation to early lactation represents a challenge for high-yielding dairy cows. Major metabolic, physiological, and nutritional changes occur at parturition and the onset of lactation (Goff and Horst, 1997). Detrimental health effects may be induced by excessive negative energy balance in dairy cows coping with this challenging period (McArt et al., 2013). Previous studies concluded that a combined supplement of folic acid and vitamin B₁₂ seems to improve the efficiency of energy metabolism in early lactation (Girard and Matte, 2005; Graulet et al., 2007; Preynat et al., 2009b) as suggested by the enhancement of lactational performance without a DMI increase for cows receiving the combined vitamin supplement.

In lactating cows, approximately 20% of glucose supply is provided through glucose absorption into the hepatic portal vein following starch digestion, whereas the remaining originates from gluconeogenesis (Galindo et al., 2011). In fed cows, propionate is the major precursor of glucose and liver removal of propionate contributes up to 60% of glucose hepatic release (Reynolds, 2006; Larsen and Kristensen, 2013). Before entering into the Krebs cycle, propionate needs to be transformed into propionyl-CoA, then into methylmalonyl-CoA, and, finally, into succinyl-CoA (Scott, 1999; Preynat et al., 2010). The latter transformation is vitamin B₁₂-dependent, the vitamin playing a role of coenzyme for

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methylmalonyl-CoA mutase (EC 5.4.99.2). In case of a vitamin B₁₂ deficiency, methylmalonyl-CoA accumulates and is instead converted into methylmalonic acid (Scott, 1999).

In a previous study, increased plasma glucose was observed for multiparous cows in early lactation receiving a combined dietary supplement of folic acid and vitamin B₁₂, whereas no effect was noted for cows receiving only a vitamin B₁₂ supplement (Graulet et al., 2007). Moreover, in multiparous cows at 87 DIM, weekly intramuscular injections of a combined supplement of folic acid and vitamin B₁₂ increased the rate of appearance (**Ra**) of glucose by 160 g/d (Preynat et al., 2009a). The glucose Ra represents the sum of glucose entry rates into the plasma pool from portal absorption, glycogenolysis, and gluconeogenesis. Preynat et al. (2009a) hypothesized that this increase was likely due to an enhanced gluconeogenesis caused by the combined vitamin supplement. Differences in glucose absorption and glycogenolysis were discarded as being the cause of the increased glucose Ra as DMI was similar between treatments and no study reports an effect

of a combined supplement of folic acid and vitamin B₁₂ on glycogen kinetics (Preynat et al., 2009a).

Vitamin B₁₂ also acts as a coenzyme in the methylation cycle (Figure 1) and is closely interrelated with folate metabolism. Indeed, 5-methyl-tetrahydrofolate, the methylated form of folic acid, can give its methyl group to homocysteine (**Hcy**) to form Met using vitamin B₁₂ as a coenzyme of Met synthase (EC 2.1.1.13). Methionine could be used to form proteins or could be transformed into S-adenosylmethionine, which is the major methyl donor in mammals (Scott, 1999). After its demethylation, tetrahydrofolate might be involved in purine and pyrimidine syntheses, both DNA components. Consequently, a lack of folic acid and vitamin B₁₂ impedes cell division (Scott, 1999).

It was hypothesized that increasing folic acid and vitamin B₁₂ supplies would facilitate propionate entering into the Krebs cycle and then would increase gluconeogenesis. This study was undertaken to evaluate whole-body (**WB**) kinetics of glucose and propionate and the proportion of glucose synthesized from propionate at 9 wk of lactation following a supplementation of

Figure 1. Simplified metabolic pathways involving folic acid and vitamin B₁₂. Enzymes: 1 = methionine adenosyltransferase; 2 = glycine N-methyltransferase; 3 = adenosylhomocysteinase; 4 = betaine homocysteine methyltransferase; 5 = methionine synthase and vitamin B₁₂ as a coenzyme; 6 = 5,10-methylenetetrahydrofolate reductase; 7 = cystathionine β-synthase; and 8 = cystathionine β-lyase (adapted from Preynat et al., 2010). THF = tetrahydrofolate.

folic acid and vitamin B₁₂, alone or combined, given by weekly intramuscular injections from 3 wk prepartum to 9 wk postpartum. This experiment allowed studying the influence of folic acid supplementation on the effect of vitamin B₁₂ supplementation on the above cited propionate metabolic pathway. Moreover, plasma concentrations of AA and metabolites and hepatic gene expression closely related to the methylation cycle and propionate pathway were measured to better characterize the effects of these vitamin supplements in dairy cows.

MATERIALS AND METHODS

Cows and Treatments

Twenty-four multiparous Holstein cows from the dairy herd at the Agriculture and Agri-Food Canada Research Centre (Sherbrooke, QC, Canada) were enrolled in this experiment as previously described by Duplessis et al. (2017). Briefly, animals were kept in a tie-stall barn and milked twice daily in a milking parlor at 12-h intervals under 1830 h of light per d. Cows were assigned to 6 blocks of 4 animals each according to their previous 305-d milk production to one of the following treatments: (1) saline 0.9% NaCl (**B₉-B₁₂-**); (2) 320 mg of folic acid (**B₉+B₁₂-**; pteroylmonoglutamic acid, MP Biomedicals, Solon, OH); (3) 10 mg of vitamin B₁₂ (**B₉-B₁₂+**; cyanocobalamin, 5,000 µg/mL, Vétoquinol, Lavaltrie, QC, Canada); or (4) 320 mg of folic acid and 10 mg of vitamin B₁₂ (**B₉+B₁₂+**). Intramuscular injections of 5 mL were administered weekly from 3 wk before the expected calving date until 9 wk of lactation. The parenteral route was chosen instead of dietary supplementation as it has been shown that the utilization and destruction of these vitamins by the rumen microorganisms are large (Santschi et al., 2005).

Lactational performance and energy status of cows from the current study from 3 wk before calving until 7 wk of lactation have been previously reported (Duplessis et al., 2017). Briefly, cows receiving folic acid supplement produced similar milk total solid yield, had lower plasma nonesterified fatty acid (**NEFA**) concentration and BCS losses, and higher plasma glucose and insulin concentrations than cows that did not, suggesting that supplementary folic acid altered energy partitioning in early lactation. Hepatic and mammary tissue gene expression for these cows at wk 9 of lactation has also been presented elsewhere (Ouattara et al., 2016).

Before parturition, a precalving diet (CP = 14.7% DM; NE_L = 1.51 Mcal/kg of DM; NDF = 39.2% DM; Co = 1.45 mg/kg of DM) was fed to dairy cows and a lactation diet was offered postpartum (CP = 17.3%

DM; NE_L = 1.65 Mcal/kg of DM; NDF = 26.6% DM; Co = 1.05 mg/kg of DM; Duplessis et al., 2017). Daily intake and orts for each cow were weighed and feed offered was adjusted if needed allowing 10% refusals. Cows had free access to water. Six d before the infusions of labeled glucose and propionate, beginning approximately at 8 wk of lactation, 12 equal meals per day were given every 2 h to dairy cows using automated feeders (Ankom, Fairport, NY) to minimize postprandial variations during the measurements of WB glucose and propionate Ra. Care of cows followed the guidelines of the National Farm Animal Care Council (2009). All procedures were approved by the Institutional Committee on Animal Care of the Research Center according to the guidelines of the Canadian Council on Animal Care (2009).

Surgery

A surgery to insert a catheter (Tygon Microbore Tubing, Formula S-54-HL, Saint-Gobain Performance Plastics, Courbevoie, France) into a ruminal vein for performing the infusion of labeled propionate was carried out at 54 ± 2 DIM. The day before the surgery, a catheter was inserted into each jugular vein: one for labeled glucose infusion and one for blood sampling. Two days and 1 d before the surgery, 2/3 and 1/3, respectively, of the TMR intake was offered to the cows and water access was cut off 12 h before the surgery. The night before the surgery, a solution of ultra-pure water (19.0 L), NaCl (90.0 g), and dextrose 50% (1.0 L) was infused in 1 jugular vein. At least 30 min before the surgery, 40 mL of penicillin G procaine (300,000 IU of penicillin G/mL; Zoetis Canada Inc., Kirkland, QC, Canada) and 20 mL of ketoprofen (100 mg of ketoprofen/mL; Merial Canada Inc., Baie-d'Urfé, QC, Canada) were injected i.m.

The surgery was done under paravertebral anesthesia on the left side of the standing cow. A skin incision of 20 to 30 cm was performed on the ventral part of the paralumbar fossa. After incising muscles and peritoneum, the dorsal sack of the rumen was exteriorized to localize 1 ruminal vein. On average, a catheter length of 40 cm was inserted into 1 left ruminal vein. The catheter was then exteriorized between left transverse processes of lumbar vertebrae 3–4 or 4–5. Immediately after the surgery, cows had free access to water and hay was offered. A few hours after the surgery, half of the TMR previous intake was offered. A solution of ultra-pure water (19.0 L), NaCl (110.0 g), KCl (30.0 g), dextrose 50% (0.5 L), and calcium borogluconate 23% (0.5 L) was infused in 1 jugular vein to help the cows recovering from the surgery. During the 2 d following

the surgery, cows received intramuscular injections of 40 mL of procainic penicillin G twice per day and 20 mL of ketoprofen once per day.

Sampling and Measurements

Blood. Blood samples were taken at 61 ± 3 DIM at 1315 h by venipuncture of the coccygeal vein using a vacutainer system (Becton, Dickinson and Co., Franklin Lakes, NJ) and were treated as described by Duplessis et al. (2017). Blood was collected in tubes with EDTA to determine plasma folates, vitamin B₁₂, NEFA, and BHB concentrations and in heparinized tubes for plasma glucose, insulin, methylmalonic acid, urea, and AA concentrations.

BW and BCS. Cows were weighed after the morning milking for 2 consecutive d at 70 and 71 ± 3 DIM. Average BW for those 2 consecutive d was used for statistical analysis. Body condition scores were done by the same individual at 61 ± 3 DIM according to a 1 (very thin) to 5 (very fat) scale using quarter points (Wildman et al., 1982; Ferguson et al., 1994).

Milk. Milk yields were recorded at each milking the week before the tracer infusion (i.e., between 56 and 63 DIM and daily yields were averaged for the whole week). Milk samples were taken from 2 consecutive milkings before performing tracer infusions at 62 ± 3 DIM.

Blood and Milk Analyses

Blood. Plasma concentrations of folates, vitamin B₁₂, glucose, insulin, BHB, NEFA, and urea were analyzed using commercial kits as previously described by Duplessis et al. (2017). Plasma concentrations of methylmalonic acid and AA were analyzed by GC-MS (model CG6890-MS5973, Hewlett Packard Co., Wilmington, DE) in electron ionization mode as described by Duplessis et al. (2017).

Milk. Milk composition (fat, protein, and lactose) was analyzed by Valacta (Dairy Production Center of Expertise, Québec and Atlantic Provinces, Sainte-Anne-de-Bellevue, QC, Canada) using mid-infrared reflectance spectrometry (MilkoScan FT 6000, Foss, Hillerød, Denmark). Milk fat, protein, and lactose yields were computed from average milk yields the week before the tracer infusion multiplied by respective milk component concentrations. Folates and vitamin B₁₂ in milk were analyzed in duplicate by RIA using a commercial kit (SimulTRAC B₁₂/Folate-S, MP Biomedicals) as described previously (Duplessis et al., 2015). The interassay CV were 2.3 and 1.7% for folate and vitamin B₁₂ analyses, respectively.

Tracer Infusions. At 63 ± 3 DIM, D-[6,6-²H₂]-glucose (99 mol percent excess; Cambridge Isotope Laboratories Inc., Andover, MA) at a rate of 16.5 ± 0.4 mmol/h and [1-¹³C]-sodium propionate (99 mol percent excess; Cambridge Isotope Laboratories Inc.) at a rate of 13.9 ± 0.4 mmol/h were simultaneously infused in 1 jugular vein and 1 ruminal vein, respectively, using a syringe pump. Tracers were previously dissolved in sterile saline. Infusions began on average at 0930 h, 90 min after a meal, and lasted 4 h.

Blood Sampling. The day of tracer infusions, 1 blood sample was collected on average 1 h before the initiation of infusions to determine isotopic natural abundance. Two hours following the beginning of the labeled glucose and propionate infusions, blood samples were taken every 15 min from the contralateral jugular catheter for the next 2 h ($n = 9$) and placed in heparinized vacutainer tubes (Becton, Dickinson and Co., Franklin Lakes, NJ). Tubes were kept on ice until centrifugation and were centrifuged within 60 min after collection at $3,000 \times g$ and 4°C for 15 min. Aliquots of plasma were transferred into 2-mL tubes and stored at -80°C until analysis.

Enrichment Analyses. The isotopic enrichment of glucose was performed with GC-MS (model CG6890-MS5973N, Hewlett Packard Co.) as described by Preynat et al. (2009a) in the positive chemical ionization mode. The isotopic enrichment of glucose isotopomers were assessed by monitoring ions at m/z 331, 332, and 333 to quantify isotopic enrichment of $m+1$ and $m+2$.

Propionate isotopic enrichment was measured using a GC-MS (model CG6890-MS5973N, Hewlett Packard Co.) with mass selective detector operating in the positive chemical ionization mode. Plasma (500 μL) was first deproteinized with 0.2 g of 5-sulfosalicylic acid hydrate (Thermo Fisher Scientific, Waltham, MA) vortexed for 1 min and then centrifuged at $16,200 \times g$ for 5 min at 4°C. Supernatants were collected, 1.5 mL of ethyl ether was added into 2-mL Eppendorf tubes, and the mixture was vortexed 5 times before a centrifugation at $16,200 \times g$ for 2 min at 4°C. Supernatants were transferred into a 2-mL Eppendorf tubes with Drierite in the bottom (calcium sulfate, W. A. Hammond Drierite Co. Ltd., Xenia, OH), kept at room temperature for 2 h, and frequently shaken. Two hours later, tubes were centrifuged at $16,200 \times g$ for 2 min at 4°C. A total of 60 μL of acetonitrile mixed with sodium sulfate was pipetted into 2-mL glass vials (reacti-Vial) and a pen mark was made at the level of the sodium sulfate acetonitrile blend on the vial. Supernatants from the last centrifugation were transferred into the glass vials, which were capped and then vortexed. Samples were then evaporated under N using a multineedle manifold

(Reacti-Therm III, Heating module, Pierce, Rockford, IL) until the volume decreased to the pen mark on the glass vials. A total of 10 μ L of *N*-tert-butyldimethylsilyl-*N*-methyltrifluoroacetamide (Sigma-Aldrich, Oakville, ON, Canada) was added and samples were heated at 60°C for 10 min and analyzed for isotopic enrichment by monitoring ions at m/z 131 and 132.

Calculations

Glucose and propionate isotopic enrichment are expressed as mole percent excess. Whole-body glucose and propionate Ra were computed as follows: Ra (mmol/h) = (infusion rate/isotopic enrichment) – infusion rate where infusion rate is expressed in millimoles per hour. For WB glucose Ra, the calculation was made using the isotopic enrichment of the infused molecule (i.e., $m+2$ glucose isotopomer). The proportion of glucose synthesized from propionate was obtained as follows: % of glucose synthesized from propionate = $(m + 1 \text{ glucose isotopic enrichment} / \text{propionate isotopic enrichment}) \times 2$. This factor 2 was used to take into account that half of the labeled C from $[1-^{13}\text{C}]$ -sodium propionate would be lost as CO_2 out of the mitochondria during the transformation of malate into oxaloacetate, then into phosphoenolpyruvate to form glucose (Mayes, 1981). Glucose flux from propionate (mmol/h) was calculated by multiplying the percentage of glucose synthesized from propionate by WB glucose Ra.

Liver Biopsies and Analyses

Liver biopsies were performed at 64 ± 3 DIM according to the procedure previously described by Graulet et al. (2007). Briefly, liver biopsies were done at the level of the 10th intercostal space under local anesthesia by a veterinarian under ultrasound guidance. On average, $1,658 \pm 338$ mg of liver tissue was taken off. The samples were blotted to remove excess blood and connective tissue before being frozen in liquid N and stored at -80°C until analysis.

Folates and Vitamin B_{12} . Methods for folate and vitamin B_{12} analyses in liver were previously described by Graulet et al. (2007). About 40 and 30 mg of frozen liver samples were used for folate and vitamin B_{12} quantifications, respectively. Commercial kits designed for human plasma were used to determine hepatic concentrations of folic acid and vitamin B_{12} by RIA (SimulTRAC B_{12} /Folate-S, MP Biomedicals).

Total RNA Isolation and Purification. Total RNA was extracted from hepatic tissue by using a QIAzol Lysis Reagent (Qiagen, Toronto, ON, Canada) following the original manufacturer protocol with slight modifications. Briefly, frozen samples (100 mg of liver

tissue) were homogenized in 2 mL of QIAzol Lysis Reagent on ice using a tissue-tearor homogenizer. A volume of 600 μ L of QIAzol Lysis Reagent was added to 400 μ L of homogenate; the mixture was vigorously vortexed and kept at room temperature for 5 min to promote dissociation of nucleoprotein complexes. A volume of 200 μ L of chloroform was added; the mixture was shaken and left at room temperature for 3 min followed by a centrifugation at $12,000 \times g$ for 15 min at 4°C to remove lipids. After centrifugation, the aqueous fraction which contained RNA was carefully pipetted, and RNA was precipitated by adding an equal volume of 70% ethanol and mixed thoroughly by vortexing. The RNA was then purified using RNeasy Mini Kit (Qiagen), including on-column DNase digestion.

Quantitative Real-Time PCR. Quantifications by real-time PCR following reverse transcription were performed as previously described by Lévesque-Sergerie et al. (2007) with minor modifications (Dudemaine et al., 2014). Table 1 provides primer sequences and optimal conditions for PCR of studied genes. The real time-PCR reactions (10 μ L, final volume) were performed on 96-well plates using Fast SYBR Green PCR Master Mix (Life Technologies, Burlington, ON, Canada) in a 7500 Fast Real Time-PCR System (Life Technologies). The expression of 5 reference genes, namely β -actin (*ACTB*), peptidylprolyl isomerase A (*PPIA*), *GAPDH*, ubiquitously expressed transcript (*UXT*), tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide (*YWHAZ*), was determined for all samples. The geometrical mean of *ACTB* and *PPIA* were selected using NormFinder (Andersen et al., 2004) for normalization as their stability variances presented the lowest values.

Methylmalonyl-CoA Mutase Activity. Determination of hepatic total activity of methylmalonyl-CoA mutase and holomutase were performed as described by Ouattara et al. (2013) with a reversed-phase HPLC method.

Statistical Analysis

Two levels of folic acid (0 or 320 mg/wk) and 2 levels of vitamin B_{12} (0 or 10 mg/wk) were tested in a 2×2 factorial arrangement in a 6-randomized block design. Daily milk yield and DMI were averaged for the week before tracer infusions. All variables were analyzed using the MIXED procedure of SAS (version 9.4 SAS Institute Inc., 2012). Fixed effects were folic acid, vitamin B_{12} , blocks, as well as folic acid $\times$ vitamin B_{12} interaction. Results were considered significant when $P \leq 0.05$ and as a tendency at $0.05 < P \leq 0.10$. When the interaction folic acid $\times$ vitamin B_{12} was significant or a tendency, the SLICE option in the LSMEANS

Table 1. Primer sequences for PCR analysis of studied genes of dairy cows

Primer ¹		Primer sequences (5'–3')	Primer position, ² nt	GenBank accession no.	Length, bp
<i>ACTB</i>	Forward	TGGCACCCAGCACAATGA	1,051–1,068	NM_173979	123
	Reverse	CCTGCTTGCTGATCCACATCT	1,053–1,173		
<i>AHCY</i>	Forward	GTGGATCGCTACTTGTTGAAGAAC	1,064–1,087	NM_001034315	114
	Reverse	GAAGGAGTTGCTCATCACAAAGC	1,155–1,177		
<i>BHMT</i>	Forward	GTGACCCCTGGCGAGTGT	640–657	NM_001011679	67
	Reverse	GGCAGTTTACCCCCACGAT	688–706		
<i>CBS</i>	Forward	GGGCAAGCTCTCGCACAT	1,593–1,610	NM_001102000	114
	Reverse	ACGACCCCGAACACCATCT	1,688–1,706		
<i>GAPDH</i>	Forward	GCCTCCTGCACCACCAACT	513–531	NM_001034034	112
	Reverse	TCTTCTGGGTGGCAGTGATG	605–625		
<i>GNMT</i>	Forward	TGAACAACAAGGCCACATG	647–666	NM_001206116	117
	Reverse	CCAGGCAGTGAGGGTAGTAGGA	742–763		
<i>MTHFR</i>	Forward	CCCAACCAGAATGGCTACAAA	1,515–1,535	NM_001011685	150
	Reverse	CGGCTTCCCGTTGATGTT	1,647–1,664		
<i>MUT</i>	Forward	CCCTTGGACGGCCAGATATT	2,257–2,276	NM_173939	84
	Reverse	AAACACCAACTTCAAACAGAAATTCA	2,315–2,340		
<i>PPIA</i>	Forward	ATGCTGGCCCCAACACAA	317–334	NM_178320	101
	Reverse	CCCTCTTTCACCTTGCCAAA	398–417		
<i>UXT</i>	Forward	TGGCAGAAGCTCTCAAGTTCATT	339–361	NM_001037471	105
	Reverse	CATGTGGATATGGGCCTTGAT	423–443		
<i>YWHAZ</i>	Forward	AATGCAACCAACACATCCTATCAG	530–553	NM_174814	131
	Reverse	GTTCAGCAATGGCTTCATCAAAT	638–660		

¹*ACTB* = β -actin; *AHCY* = S-adenosylhomocysteine hydrolase; *BHMT* = betaine homocysteine methyltransferase; *CBS* = cystathionine β -synthase; *GNMT* = glycine N-methyltransferase; *MTHFR* = 5,10-methylene-tetrahydrofolate reductase; *MUT* = methylmalonyl-CoA mutase; *PPIA* = peptidylprolyl isomerase A; *UXT* = ubiquitously expressed transcript; *YWHAZ* = tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide.

²Positions refer to the GenBank accession number.

statement of SAS was used to help interpret results. The insertion of the catheter into the ruminal vein was not possible for 1 cow due to a problem with the ruminal vein conformation and the ruminal catheter was not working for 1 cow the day of infusion. For these 2 cows (B₉+B₁₂+), only data from labeled glucose infusion were used in the analysis. One cow (B₉+B₁₂-) was not included in the statistical analyses for WB kinetics because her DMI the day of tracer infusions was 5.8 kg, which was on average lower by 15.0 kg than the DMI of other cows the day of infusions. Liver sample from 1 control cow (B₉-B₁₂-) was not sufficient to perform folate and vitamin B₁₂ analyses.

RESULTS AND DISCUSSION

Production Data

Dry matter intake, BW, and BCS were not different among treatments at wk 9 postpartum ($P \geq 0.30$; Table 2) as previously observed (Girard and Matte, 2005; Graulet et al., 2007; Preynat et al., 2009a,b) with supplementation of these B vitamins. At wk 9 of lactation, milk yield tended to be lower by 5.0 kg/d for cows receiving the folic acid supplement compared with cows that did not receive it ($P = 0.10$; Table 2). In contrast, Preynat et al. (2009a) reported that, at wk 12 postpar-

tum, a supplementation of folic acid plus vitamin B₁₂ increased milk yield by 12%. Moreover, supplementary folic acid increased milk production by 3.4 kg/d during the first 8 wk of lactation in a previous experiment (Graulet et al., 2007). No treatment effects were noted on milk concentrations of fat, protein, lactose, and TS and milk yields of fat, protein, and TS ($P \geq 0.11$; Table 2). The tendency for a decrease in milk lactose yield by 0.25 kg/d for cows receiving the folic acid supplement ($P = 0.07$; Table 2) could explain the decrease in milk production at wk 9.

The supplement of folic acid increased milk concentration of folates ($P = 0.01$; Table 2) but did not increase the amounts of folates secreted daily in milk ($P = 0.36$; Table 2). Graulet et al. (2007) reported an increase of both milk concentration and yield of folates, whereas Preynat et al. (2009b) only observed an augmentation of the amounts of folates secreted daily in milk following supplementary folic acid. Milk concentration and yield of vitamin B₁₂ were increased by the vitamin B₁₂ supplement ($P < 0.0001$; Table 2) as frequently reported in the literature (Girard and Matte, 2005; Graulet et al., 2007; Akins et al., 2013). In addition, in the current study, vitamin B₁₂ concentration of milk was greater by 1,814 pg/mL for cows receiving the folic acid supplement compared with cows that did not ($P = 0.05$; Table 2).

Plasma Variables

An interaction folic acid $\times$ vitamin B₁₂ was observed on plasma concentration of folates ($P = 0.05$; Table 3). However, results from the SLICE option of SAS indicated no significant effect of folic acid within each level of vitamin B₁₂ supplementation ($P \geq 0.11$). At wk 9 postpartum, plasma concentration of folates was unaffected by folic acid supplement ($P = 0.92$; Table 3) and averaged 18.4 ± 1.6 ng/mL, in line with observations made in the same cows from wk 4 to 7 of lactation (Duplessis et al., 2017) and at wk 12 of lactation in another study (Preynat et al., 2009a). Plasma concentration of vitamin B₁₂ was higher ($P = 0.04$) for cows receiving the vitamin B₁₂ supplement than for cows that did not (417 vs. 320 ± 30 pg/mL; Table 3) as similarly reported by Graulet et al. (2007), Preynat et al. (2009b), and Akins et al. (2013).

Plasma concentrations of glucose, insulin, NEFA, methylmalonic acid, urea, Ala, Glu, Gly, Ile, Leu, Lys, Met, Phe, Pro, Ser, Trp, Tyr, Val, branched-chain AA, EAA, and NEAA, and total AA were unaffected by treatments at wk 9 postpartum ($P \geq 0.11$; Table 3). Plasma concentration of methylmalonic acid was lower, even in control cows, than reported by Girard and Matte (2005), which suggests that the vitamin B₁₂ supply was sufficient to avoid a negative effect on methylmalonyl-CoA mutase activity. Plasma concentration of BHB tended to decrease from 0.76 to 0.61

± 0.06 mM with the folic acid supplement ($P = 0.09$; Table 3). The supplement of vitamin B₁₂ tended to increase plasma concentrations of Asn and Gln ($P \leq 0.08$; Table 3). Plasma concentration of Asp decreased ($P = 0.006$), whereas plasma concentrations of His and Hcy increased with the folic acid supplement ($P = 0.04$; Table 3). The highest plasma concentrations of Cys and Thr were observed for cows receiving the combined supplement of folic acid and vitamin B₁₂ ($P \leq 0.10$). Regardless of treatments, plasma concentration of Met was higher than in the trials of Graulet et al. (2007) and Preynat et al. (2009a).

Liver Variables

B Vitamins. Liver concentration of folates was increased by 13.9 μ g/g of fresh tissue by the folic acid supplement ($P < 0.0001$; Table 4). Graulet et al. (2007) and Preynat et al. (2010) also reported that a folic acid supplement increased hepatic concentration of folates. Compared with the study of Preynat et al. (2010), liver concentrations of folates in the current experiment were greater by 202% for cows receiving the folic acid supplement. However, it is noteworthy that, in the present study, the weekly dose of the folic acid supplement was twice (320 mg) the amount used in Preynat et al. (2010; 160 mg). Intramuscular injections of vitamin B₁₂ increased liver concentration of vitamin B₁₂ ($P = 0.007$;

Table 2. Effects of intramuscular injections of folic acid and vitamin B₁₂ on DMI, BW, BCS, and milk yield and components at wk 9 of lactation¹

Item	Treatment ²				SEM	P-value		
	B ₉ -B ₁₂ -	B ₉ +B ₁₂ -	B ₉ -B ₁₂ +	B ₉ +B ₁₂ +		B ₉	B ₁₂	B ₉ $\times$ B ₁₂
DMI, kg/d	18.1	19.0	18.5	19.4	1.9	0.65	0.85	0.99
BW, ³ kg	606	640	616	621	22	0.39	0.85	0.53
BCS ⁴	2.21	2.42	2.38	2.42	0.12	0.30	0.49	0.49
Milk yield, kg/d	36.4	30.0	38.1	34.4	2.9	0.10	0.31	0.64
Milk composition ⁵								
Fat, g/kg	33.7	38.8	36.0	35.4	2.0	0.30	0.79	0.18
Protein, g/kg	28.6	30.7	29.3	32.2	1.4	0.11	0.44	0.77
Lactose, g/kg	47.2	46.1	46.9	47.2	6.0	0.51	0.57	0.27
TS, g/kg	109.6	115.6	112.3	114.8	2.7	0.14	0.73	0.53
Folates, ng/mL	76.5	102.0	78.8	103.3	8.5	0.01	0.84	0.96
Vitamin B ₁₂ , pg/mL	2,271	3,711	6,720	8,908	843	0.05	<0.0001	0.66
Milk yield								
Fat, kg/d	1.25	1.17	1.38	1.21	0.13	0.34	0.53	0.74
Protein, kg/d	1.04	0.92	1.12	1.09	0.08	0.39	0.14	0.56
Lactose, kg/d	1.72	1.38	1.79	1.62	0.13	0.07	0.26	0.51
TS, kg/d	4.01	3.46	4.28	3.92	0.33	0.19	0.28	0.78
Folate, mg/d	2.97	3.24	3.05	3.58	0.43	0.36	0.63	0.76
Vitamin B ₁₂ , μ g/d	91.5	114.2	258.3	300.3	29.5	0.29	<0.0001	0.75

¹LSM with SEM, $n = 6$.

²B₉-B₁₂- = no vitamin supplement; B₉+B₁₂- = 320 mg of folic acid; B₉-B₁₂+ = 10 mg of vitamin B₁₂; B₉+B₁₂+ = 320 mg of folic acid and 10 mg of vitamin B₁₂ given weekly from 3 wk before the expected calving date until 9 wk postpartum.

³Average BW at 70 and 71 $\pm$ 3 DIM.

⁴Data on BCS were measured at 61 $\pm$ 3 DIM according to a 1 to 5 scale with quarter points (Wildman et al., 1982; Ferguson et al., 1994).

⁵Data averaged from 2 consecutive milkings before tracer infusions.

Table 4) as previously reported (Preynat et al., 2010; Akins et al., 2013).

Gene Expression. No treatment effect was observed on the hepatic gene expression of betaine homocysteine methyltransferase (*BHMT*), cystathionine β -synthase (*CBS*), glycine N-methyltransferase (*GNMT*), and 5,10-methylene-tetrahydrofolate reductase (*MTHFR*; $P \geq 0.23$; Table 4). A folic acid $\times$ vitamin B₁₂ interaction was observed on gene expression of S-adenosylhomocysteine hydrolase (*AHCY*; $P = 0.004$; Table 4); the highest *AHCY* expression was observed for cows receiving the combined supplement of folic acid and vitamin B₁₂ compared with folic acid or vitamin B₁₂ supplementation alone ($P \leq 0.009$). In the methyla-

tion cycle (Figure 1), *AHCY* is required to transform S-adenosylhomocysteine to Hcy (Finkelstein, 1990). The greatest *AHCY* expression for cows receiving the combined vitamin supplement is in accordance with the highest plasma concentration of Hcy and Cys observed in these cows at wk 9 of lactation but also within the first 7 wk postpartum (Duplessis et al., 2017). A tendency for a folic acid $\times$ vitamin B₁₂ interaction on methylmalonyl-CoA mutase (*MUT*) expression was noted ($P = 0.09$; Table 4). Supplementary vitamin B₁₂ increased *MUT* expression when cows received the folic acid supplement ($P = 0.04$), whereas it had no effect when cows did not receive the folic acid supplement ($P = 0.75$). Indeed, the highest *MUT* expression

Table 3. Effects of intramuscular injections of folic acid and vitamin B₁₂ on plasma concentrations of B vitamins, glucose, insulin, BHB, nonesterified fatty acids (NEFA), methylmalonic acid, urea, and AA at 61 $\pm$ 3 DIM¹

Item	Treatment ²				SEM	P-value		
	B ₉ -B ₁₂ -	B ₉ +B ₁₂ -	B ₉ -B ₁₂ +	B ₉ +B ₁₂ +		B ₉	B ₁₂	B ₉ $\times$ B ₁₂
Folates, ng/mL	20.5	16.7	16.5	19.9	1.6	0.92	0.81	0.05
Vitamin B ₁₂ , pg/mL	298.9	340.8	385.4	447.9	42.9	0.24	0.04	0.81
Glucose, mM	3.45	3.70	3.61	3.55	0.13	0.48	0.99	0.25
Insulin, μ U/mL	6.9	12.4	10.3	10.4	2.4	0.27	0.78	0.29
BHB, mM	0.68	0.62	0.84	0.61	0.08	0.09	0.39	0.31
NEFA, μ M	189.9	145.5	203.6	157.1	39.6	0.27	0.75	0.98
Methylmalonic acid, μ M	0.37	0.31	0.29	0.38	0.04	0.69	0.94	0.11
Urea, mM	5.46	5.65	5.04	5.51	0.45	0.47	0.54	0.76
AA, μ M								
Ala	241.8	237.7	234.0	231.1	16.0	0.83	0.66	0.97
Asn	48.5	49.4	53.9	58.2	3.4	0.46	0.06	0.64
Asp	45.7	35.1	49.4	29.1	4.6	0.006	0.80	0.32
Cys	111.0	114.8	113.2	126.5	4.0	0.05	0.10	0.25
Glu	45.1	45.9	48.4	44.6	3.4	0.67	0.76	0.51
Gln	264.4	271.7	285.3	304.9	14.3	0.36	0.08	0.68
Gly	253.1	262.8	272.1	261.5	14.1	0.97	0.54	0.48
His	53.8	59.2	55.6	64.3	3.1	0.04	0.28	0.61
Hcy ³	4.86	5.08	4.93	5.75	0.25	0.04	0.13	0.21
Ile	135.0	130.7	170.2	143.0	19.9	0.44	0.25	0.57
Leu	181.2	180.7	210.2	183.7	20.7	0.52	0.45	0.54
Lys	89.7	85.7	78.0	85.6	7.7	0.82	0.45	0.46
Met	28.2	28.3	28.6	29.0	1.6	0.88	0.75	0.91
Phe	58.4	61.0	58.6	60.8	2.9	0.41	1.0	0.93
Pro	83.7	97.7	87.4	92.5	8.2	0.22	0.92	0.57
Ser	75.9	81.6	80.4	79.3	5.5	0.68	0.85	0.54
Thr	92.9	104.7	106.0	114.5	5.3	0.08	0.05	0.76
Trp	49.8	48.6	48.1	50.9	2.1	0.72	0.88	0.36
Tyr	62.5	60.8	64.8	70.3	4.2	0.66	0.18	0.40
Val	238.8	243.3	267.4	241.5	22.2	0.64	0.55	0.50
BCAA ⁴	555.0	554.6	647.7	568.2	61.8	0.53	0.40	0.53
EAA ⁵	927.8	942.1	1,022.6	979.2	73.8	0.85	0.40	0.71
NEAA ⁶	1,231.6	1,257.4	1,288.9	1,331.4	47.5	0.50	0.20	0.87
TAA ⁷	2,164	2,205	2,316	2,238	102	0.86	0.38	0.57

¹LSM with SEM, n = 6.

²B₉-B₁₂- = no vitamin supplement; B₉+B₁₂- = 320 mg of folic acid; B₉-B₁₂ + = 10 mg of vitamin B₁₂; B₉+B₁₂ + = 320 mg of folic acid and 10 mg of vitamin B₁₂ given weekly from 3 wk before the expected calving date until 9 wk postpartum.

³Hcy = homocysteine.

⁴BCAA = branched-chain AA = Ile + Leu + Val.

⁵EAA = His + Ile + Leu + Lys + Met + Phe + Thr + Trp + Val.

⁶NEAA = Ala + Asn + Asp + Cys + Gln + Glu + Gly + Pro + Ser + Tyr.

⁷TAA = total AA.

was obtained with the combined supplement of folic acid and vitamin B₁₂ as in the study of Preynat et al. (2010). However, Graulet et al. (2007) did not report any treatment effect in early lactation on *MUT* expression for cows receiving dietary folic acid and vitamin B₁₂ supplement. The lowest hepatic concentration of vitamin B₁₂ and *MUT* expression were observed in cows receiving the folic acid supplement alone. In rat liver, Nakao et al. (2009) noted that mRNA level of *MUT* was decreased by a vitamin B₁₂-deficient diet compared with a diet providing sufficient amount of vitamin B₁₂. In the current study, even though vitamin B₁₂ supply was probably not deficient for dairy cows receiving the folic acid supplement alone, it might explain the lower *MUT* expression observed for these cows as vitamin B₁₂ concentration in liver was also lower than in other treatment groups.

Methylmalonyl-CoA Mutase Activity. As reported by Graulet et al. (2007), total methylmalonyl-CoA mutase specific activity was unaffected by supplementation of folic acid and vitamin B₁₂ and averaged 14.39 ± 0.71 nmol/mg of protein per min ($P \geq 0.25$; Table 4). Holomutase activity, which represents the activity of the enzyme bound to its coenzyme, was increased by 0.13 nmol/mg of protein per min by the vitamin B₁₂ supplement ($P = 0.05$; Table 4). Nevertheless, the percentage of holomutase activity from total methylmalonyl-CoA mutase specific activity was not different among treatments ($P \geq 0.16$; Table 4) and

was less than 5% as reported by Nakao et al. (2009) in rat liver.

Whole-Body Glucose and Propionate Kinetics.

Plasma concentration of glucose during the tracer infusions was unaffected by treatments and averaged 3.51 ± 0.13 mM ($P \geq 0.66$; Table 5). Net portal appearance of propionate in early lactating cows reported by Reynolds et al. (2003) and Raun and Kristensen (2011) was in the same range as the WB propionate Ra in the current experiment. Whole-body glucose Ra in the current study was larger than previously reported in some studies infusing D-[6,6-²H₂]-glucose to dairy cows at later stages of lactation, but the ratio of milk lactose to WB glucose Ra was in a similar range (Lemosquet et al., 2009a; Galindo et al., 2011; Maxin et al., 2013).

The WB Ra of propionate was not affected by treatments ($P \geq 0.46$; Table 5) in line with no effect of the supplements of vitamins on DMI. Propionate is the major glucogenic precursor in fed ruminants, although lactate and glucogenic AA can also contribute to a lesser extent to gluconeogenesis (Reynolds, 2006; Larsen and Kristensen, 2013). To enter into the Krebs cycle, propionate has to be successively transformed (Scott, 1999). One reaction involves methylmalonyl-CoA mutase and vitamin B₁₂ as a coenzyme; a lack of vitamin B₁₂ impedes this reaction (Scott, 1999). In lactating ewes, gluconeogenesis from propionate was improved when using intramuscular injections of vitamin B₁₂ (Peters and Elliot, 1983). Accordingly, Preynat

Table 4. Effects of intramuscular injections of folic acid and vitamin B₁₂ on hepatic concentrations of folates and vitamin B₁₂, expression of selected genes, and methylmalonyl-CoA mutase activity at 64 ± 3 DIM¹

Item	Treatment ²				SEM	P-value		
	B ₉ -B ₁₂ -	B ₉ +B ₁₂ -	B ₉ -B ₁₂ +	B ₉ +B ₁₂ +		B ₉	B ₁₂	B ₉ × B ₁₂
Vitamin, µg/g of fresh tissue								
Folate	17.3	30.4	17.6	32.4	2.1	<0.0001	0.57	0.68
Vitamin B ₁₂	0.78	0.63	0.89	0.97	0.08	0.65	0.007	0.11
Gene expression ³								
<i>AHCY</i>	1.64	1.22	1.41	2.03	0.16	0.51	0.07	0.004
<i>BHMT</i>	1.09	0.72	0.92	0.90	0.22	0.35	0.97	0.41
<i>CBS</i>	1.38	1.25	1.24	1.29	0.24	0.85	0.82	0.64
<i>GNMT</i>	0.86	0.94	0.72	0.55	0.27	0.84	0.24	0.57
<i>MTHFR</i>	0.98	0.73	1.06	0.94	0.15	0.23	0.32	0.67
<i>MUT</i>	1.18	0.80	1.10	1.37	0.20	0.77	0.21	0.09
Methylmalonyl-CoA mutase enzyme activity, nmol/mg of protein per min								
Total	13.84	14.11	14.97	14.65	0.71	0.97	0.25	0.68
Holomutase	0.51	0.58	0.62	0.72	0.06	0.17	0.05	0.81
Holomutase/total, %	3.73	4.13	4.15	4.87	0.39	0.18	0.16	0.65

¹LSM with SEM, n = 6 except for vitamin analyses, where n = 5 because the amount of liver sample from 1 cow was not sufficient; the largest SEM is given.

²B₉-B₁₂- = no vitamin supplement; B₉+B₁₂- = 320 mg of folic acid; B₉-B₁₂ = 10 mg of vitamin B₁₂; B₉+B₁₂ = 320 mg of folic acid and 10 mg of vitamin B₁₂ given weekly from 3 wk before the expected calving date until 9 wk postpartum.

³*AHCY* = S-adenosylhomocysteine hydrolase; *BHMT* = betaine homocysteine methyltransferase; *CBS* = cystathionine β-synthase; *GNMT* = glycine N-methyltransferase; *MTHFR* = 5,10-methylene-tetrahydrofolate reductase; *MUT* = methylmalonyl-CoA mutase.

Table 5. Effects of intramuscular injections of folic acid and vitamin B₁₂ on whole-body (WB) glucose and propionate kinetics at 63 ± 3 DIM¹

Item	Treatment ²				SEM	P-value		
	B ₉ -B ₁₂ -	B ₉ +B ₁₂ -	B ₉ -B ₁₂ +	B ₉ +B ₁₂ +		B ₉	B ₁₂	B ₉ × B ₁₂
Cows, n	6	5	6	6				
Glucose								
Plasma glucose, mM	3.52	3.48	3.48	3.55	0.11	0.92	0.93	0.66
WB glucose Ra, ³ mmol/h	848	772	810	780	32	0.10	0.64	0.45
Milk lactose/WB glucose Ra, ⁴ %	50.8	44.7	53.7	49.6	3.1	0.09	0.19	0.73
Propionate								
WB propionate Ra, ³ mmol/h	1,132	964	930	994	180	0.75	0.58	0.46
Glucose Ra from propionate, %	64.6	61.3	58.3	54.7	8.5	0.65	0.39	0.98
Glucose Ra from propionate, mmol/h	545	467	473	437	78	0.42	0.45	0.75

¹LSM with SEM, n is as given except for propionate results, treatment B₉+B₁₂+, where n = 4 because 2 cows were discarded due to a problem with ruminal catheter. The largest SEM is given.

²B₉-B₁₂- = no vitamin supplement; B₉+B₁₂- = 320 mg of folic acid; B₉-B₁₂+ = 10 mg of vitamin B₁₂; B₉+B₁₂+ = 320 mg of folic acid and 10 mg of vitamin B₁₂ given weekly from 3 wk before the expected calving date until 9 wk postpartum.

³Whole-body glucose and propionate rate of appearance (Ra) measured using D-[6,6-²H₂]-glucose and [1-¹³C]-sodium propionate continuous and simultaneous infusions.

⁴Ratio calculated from lactose expressed in glucose equivalent and WB Ra of glucose.

et al. (2009a) observed that a combined supplement of folic acid and vitamin B₁₂ increased WB glucose Ra at wk 12 of lactation and hypothesized that this effect could be due to an enhancement of propionate usage for gluconeogenesis. However, in the current study, in spite of the effects of the vitamin supplements on the hepatic abundance of *MUT* and holomutase activity, the glucose WB Ra and the glucose Ra from propionate, either as absolute flux or proportion, were not affected by vitamin B₁₂ supplementation ($P \geq 0.39$; Table 5). The proportion of glucose Ra from propionate averaged $59.7 \pm 8.5\%$ and is in accordance with the contribution of propionate to glucose synthesis levels reported in the literature (50 to 58%) for cows in early lactation (Amaral et al., 1990; Danfær et al., 1995; Larsen and Kristensen, 2013).

Whole-body glucose Ra represents the sum of glucose synthesis via gluconeogenesis, glucose absorption from the hepatic portal-drained viscera, and glycogenolysis (Lemosquet et al., 2009b; Galindo et al., 2011). Whole-body glucose Ra for cows receiving the folic acid supplement tended to be lower by 229 g/d ($P = 0.10$) compared with cows that did not, which is in line with the lower lactose synthesis (-250 g/d) observed in these cows. In contrary, WB glucose Ra in the study of Preynat et al. (2009a) tended to increase by 160 g/d with the combined supplement of folic acid and vitamin B₁₂ at wk 12 of lactation and was associated with a similar increase in milk lactose secretion ($+165$ g/d). In the current study, DMI was not different among treatment groups; it is then unlikely that the decrease of WB glucose Ra observed with supplementary folic acid was due to change in portal glucose absorption, and neither was the WB Ra of propionate affected by treatments.

In the current study, the proportion of WB glucose Ra to milk lactose tended to decrease with the folic acid supplement by 5.0% ($P = 0.09$; Table 5). Mammary gland is the primary user of glucose, mammary glucose uptake varying between 69 to 85% of WB glucose Ra, whereas milk lactose yield to mammary glucose uptake ratio varied from 57 to 79% (Lemosquet et al., 2009b; Galindo et al., 2011). In the current experiment, either glucose utilization in tissues other than mammary gland or glucose utilization in the mammary gland for other purposes than lactose synthesis could have been favored with the use of the folic acid supplement. The former would agree better with results observed in those cows during the first 7 wk of lactation when the folic acid supplement had no effect on DMI and milk TS yields, but reduced BCS losses, decreased plasma concentration of NEFA, and increased plasma concentrations of glucose and insulin (Duplessis et al., 2017). Results from Graulet et al. (2007), Preynat et al. (2009b), and Duplessis et al. (2014) also indicated that the vitamin supplements altered the energy partitioning.

The major difference between our study and those of Graulet et al. (2007) and Preynat et al. (2009b) seems to be the vitamin status of the studied animals. Concentrations of folates and vitamin B₁₂ in plasma, milk, and liver, and plasma concentrations of Cys, Hcy, and Met (related to the methylation pathway) of the control cows (B₉-B₁₂- cows) at wk 9 of lactation were compared with concentrations at wk 8 postpartum from these 2 previous studies (Graulet et al., 2007; Preynat et al., 2009b, 2010). Plasma, milk and liver concentrations of folates of control cows were higher on average by 18, 33, and 119%, respectively, in the present experiment than in the 2 other experiments as

well as plasma concentration of vitamin B₁₂ by 72 and 78%. These comparisons indicate that, even without vitamin supplementation, control cows in the current study already had less potential to be deficient in folate and vitamin B₁₂ at wk 9 than in the 2 compared studies. In opposition to liver folate concentration, hepatic vitamin B₁₂ concentration of control cows was lower on average by 13% in the current trial compared with the 2 other studies. As folates and vitamin B₁₂ are stored in the liver (Le Grusse and Watier, 1993), it shows that, in opposition to folates, less vitamin B₁₂ was stored in the liver of the control cows in the present trial than in the 2 other studies although the plasma concentration of vitamin B₁₂ was higher.

Regarding AA related to the methylation pathway, plasma concentrations of Cys and Met in control cows were higher on average by 16 and 28%, respectively, and plasma concentration of Hcy was lower on average by 14% in this experiment than in the 2 other studies. In the Met cycle, Hcy could either be remethylated to Met or be irreversibly catabolized to Cys through the transsulfuration pathway (Bertolo and McBreairty, 2013). When Met is in excess, conversion of Met to Hcy (transmethylation) and transsulfuration are upregulated as Met is toxic at relatively low concentrations (Bertolo and McBreairty, 2013). As plasma Met and Cys concentrations were higher in control cows than in the 2 other trials, it could be hypothesized that the transsulfuration pathway was already upregulated to eliminate extra Met.

CONCLUSIONS

Plasma concentrations of folic acid, vitamin B₁₂, Met, and Cys of control cows in the present experiment were higher than previously reported and suggest that the supply of folic acid and vitamin B₁₂ from ruminal synthesis was sufficient to optimize transmethylation pathway and performance of dairy cows under conditions of the current study. Moreover, the absence of treatment effect on plasma concentrations of methylmalonic acid as well as on the proportion of glucose synthesized from propionate, averaging 60%, also supports the fact that vitamin B₁₂ supply was sufficient. However, milk and lactose yields tended to decrease with the folic acid supplement as did the WB glucose Ra. Consequently, as previously observed in other studies, supplementary folic acid and vitamin B₁₂ may alter energy partitioning in cows but, in the present experiment, it seems to have been at the expense of milk lactose secretion. These results highlight the necessity to develop a tool to predict supplies of folic acid and vitamin B₁₂ from ruminal synthesis according to diet management, to predict

when supplementation with these vitamins would be appropriate.

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