Net flux of nutrients across splanchnic tissues of lactating dairy cows as influenced by dietary supplements of biotin and vitamin B₁₂¹

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ABSTRACT

Biotin and vitamin B₁₂ are coenzymes in reactions that are essential to propionate metabolism in dairy cows. The objective of the present studies was to determine whether an increased dietary supply of these vitamins would change the net flux of nutrients through the rumen, the portal-drained viscera (PDV), the total splanchnic tissues (TSP), and the liver. Four lactating cows equipped with ultrasonic flow probes around the right ruminal artery and the portal vein and catheters in the right ruminal vein, the portal vein, one hepatic vein, and one mesenteric artery were fed 12 times per day a mixed ration at 95% of ad libitum dry matter intake. Daily supplements of 500 mg of vitamin B₁₂ + 20 mg of biotin or no vitamin supplement (study 1) or 500 mg of vitamin B₁₂ alone or with 20 mg of biotin (study 2) were fed according to a crossover design with two 4-wk periods in each study. On the last day of each period, blood flow was recorded and blood samples were collected every 30 min for 4 h. In study 1, biotin and vitamin B₁₂ given together increased milk production and milk protein yields compared with the control diet. The supplement increased appearance of the 2 vitamins across the PDV and TSP. It also reduced the net portal appearance of ammonia and total volatile fatty acids across the PDV. In study 2, compared with the 2 vitamins together, vitamin B₁₂ alone increased glucose flux across PDV and TSP as well as its arterial concentration and PDV flux of ammonia. With the diet used in the present experiment, the major effects of the vitamin supplements seem to be mediated through changes in ruminal fermentation and gastrointestinal tract metabolism rather than by effects on hepatic metabolism.

Key words: dairy cow, portal-drained viscera, biotin, vitamin B₁₂

INTRODUCTION

The nature of the ruminant digestive system imposes a huge dependence on gluconeogenesis because very little glucose is being absorbed (Reynolds, 2006). In ruminants, the rate of gluconeogenesis increases with feed intake (Hocquette and Bauchart, 1999), and one of the major substrates for gluconeogenesis is propionate (Reynolds, 2006). Seal et al. (1992) observed that, in growing steers, 83% of the absorbed propionate was used for glucose synthesis representing approximately 50% of the whole-body glucose flux.

Metabolic utilization of propionate after its absorption from the gastrointestinal tract is dependent on the transformation of propionate into succinyl-coenzyme A (CoA), requiring the successive actions of biotin- and vitamin B₁₂-dependent enzymes. Propionate is first transformed into propionyl-CoA, which is carboxylated to methylmalonyl-CoA by a biotin-dependent enzyme, propionyl-CoA carboxylase. Methylmalonyl-CoA is then isomerized in succinyl-CoA under the action of the vitamin B₁₂-dependent enzyme, methylmalonyl-CoA mutase. Succinyl-CoA finally enters into the Krebs cycle, from where it can be directed toward gluconeogenesis (Le Grusse and Watier, 1993; McDowell, 2000). Moreover, biotin is also a coenzyme for the pyruvate carboxylase, which catalyzes the carboxylation of pyruvate in oxaloacetate for glucose synthesis (McMahon, 2002).

The effects of dietary supplements of biotin or vitamin B₁₂ on dairy cow metabolism are, however, not clearly defined. On one hand, dietary supplementation with biotin is frequently reported to increase milk production (Girard and Matte, 2006; Ferreira et al., 2007; Enjalbert et al., 2008), but its effect on plasma glucose concentrations is variable (Girard and Matte, 2006; Reynolds et al., 2007). On the other hand, compared with a control diet, a combined supplement of vitamin B₁₂ and folic acid tended to increase whole-body glucose flux and milk lactose yield with a similar quantitative magnitude (Preynat et al., 2009). Moreover, Graulet et al. (2007) observed that, in cows fed supplementary folic acid, a supplement of vitamin B₁₂ increased

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plasma concentrations of glucose while decreasing plasma concentrations of biotin. Nevertheless, dietary supplements of B-vitamins could also possibly affect ruminal microflora altering ruminal fermentation and nutrient availability for the cow, especially at the doses used in the production studies reported above. In vitro, omission of biotin from the culture media decreased VFA production by ruminal bacteria (Milligan et al., 1967). Scott and Dehority (1965) demonstrated that some cellulolytic bacteria had absolute requirements for biotin and vitamin B₁₂ whereas other species require only biotin. In vivo studies, however, failed to detect any effect of dietary supplements of biotin on molar concentrations of VFA in ruminal fluid (Zimmerly and Weiss, 2001; Majee et al., 2003; Ferreira et al., 2007).

The present studies aimed to determine whether an increased dietary supply of biotin and vitamin B₁₂, 2 vitamins essential to propionate utilization and gluconeogenesis could change the net release or uptake of nutrients by the rumen wall, portal-drained viscera (PDV), liver, and total splanchnic tissues (TSP).

MATERIALS AND METHODS

Care of cows followed the recommended code of practice of Agriculture Canada (1990) and the guidelines of the Canadian Council on Animal Care (1993). Surgical procedures were reviewed and accepted by the Institutional Committee on Animal Care of the Dairy and Swine Research and Development Centre (Sherbrooke, Québec, Canada).

Cows and Treatments

Eight weeks after calving, 4 lactating cows were surgically equipped with ultrasonic flow probes around the right ruminal artery (8-mm flow probes, R-series, Transonic Systems, Ithaca, NY) and the portal vein (32-mm flow probes, A-series, Transonic Systems) using adaptations of the surgical methods developed for sheep by Rémond et al. (1993; 1998). Indwelling catheters (Tygon tubing, 0.05 mm i.d., 0.09 mm o.d., Norton Performance Plastics, Akron, OH) treated with tridodecylmethylammonium chloride heparin complex [2% (wt/wt), Polysciences Inc., Warrington, PA] were placed in the right ruminal vein, the portal vein, one hepatic vein, and one mesenteric artery. Briefly, a flow probe was placed around the ruminal artery a few centimeters beyond the point of entry of the vessel on the right ruminal wall, and the catheter was placed in the right ruminal vein with its tip equal to the flow probe. The other flow probe was placed around the hepatic portal vein after the junction with the gastrosplenic

vein and before the entry of the portal vein in the liver. A small incision was made in the liver; a first catheter was then inserted in the vein and directed toward the portal vein, its tip placed just at the entry of the portal vein in the liver. A second catheter was then inserted in one hepatic vein and pushed until its tip rested outside the liver. At the time of the surgery, placement of these catheters was verified by echography and blood gas differences from blood drawn from the 2 catheters. The last catheter was placed in one mesentery artery with its entry at the ileal level. At postmortem examination, after exsanguination, the tip of the catheters in the right ruminal vein rested from 0 to 6 cm beyond the flow probe placed around the artery. The tip of the catheters placed in the portal vein was close to the liver, more than 6 cm before the flow probe. The tip of the catheters in a hepatic vein was outside the liver, more than 5 cm before the junction with the vena cava.

Cows were kept in a tie-stall barn under 16 h of light per day (0530–2130 h) and were milked twice daily at a 12-h interval. Cows had free access to water. One kilogram of hay was served at 0700 h and the mixed ration (Table 1) was served in 12 equal meals per day, every 2 h. Vitamin supplements were incorporated in equal amounts into each meal. Before the beginning of each study, voluntary feed intake was measured for each cow for a 1-wk period. Thereafter, during the experiment, the ration was served at 95% of the voluntary feed intake. Refusals, if present, were weighed daily at 1000 h.

This project was divided into 2 studies according to a crossover design with 2 periods of 4 wk each. There was a wash-out period of 7 d between the 2 studies. In study 1, the treatments were 1) no vitamin supplement (**B₈–B₁₂–**) or 2) 20 mg of biotin + 500 mg of vitamin B₁₂ (cyanocobalamin) per day (**B₈+B₁₂+**). In study 2, the treatments were daily supplements of 500 mg of vitamin B₁₂ 1) alone (**B₈–B₁₂+**) or 2) combined with 20 mg of biotin (**B₈+B₁₂+**). Doses were chosen according to Zimmerly and Weiss (2001) and Graulet et al. (2007). Cows were at 118 ± 5.6 and 181 ± 5.6 DIM at the beginning of studies 1 and 2, respectively.

Feed Analysis

At the beginning of the experiment, hay samples were collected in different hay bales harvested in 1 field and pooled. Silage samples were collected twice a week. A subsample was immediately analyzed by near-infrared reflectance spectrometry. Another subsample was frozen at -20°C and pooled by period for further analyses of DM, CP, soluble protein (AOAC, 2000), ADF, NDF, and acid-detergent lignin (Ankom200 fiber analyzer, Ankom Technology Corp., Fairport, NY).

Milk Sampling Procedure and Analyses

Milk production was recorded at each milking during the entire experiment. Milk samples were collected at 2 consecutive milkings at the end of each experimental period. Milk composition (DM, fat, CP, and ash) was determined as described by Girard et al. (2005).

Blood Sampling Procedure and Analyses

On the last day of each 4 wk-period, blood samples ($n = 8$) were taken simultaneously from the 4 catheters every 30 min during 4 consecutive hours starting at the 0800 h meal. Heparinized tubes were used for urea, ammonia, BHBA, glucose, biotin, and VFA. Blood samples were immediately placed on ice and kept chilled until processed. For serum or plasma, blood was centrifuged within 1 h after sampling for 12 min at $1,854 \times g$ and 4°C . Serum for vitamin B₁₂, plasma for biotin and glucose, and whole blood for VFA determinations were kept frozen at -20°C until assayed. For lactate and BHBA determinations, blood was immediately deproteinized and 0.9 mL of distilled water and 100 μL of perchloric acid 6 *N* were added to 1 mL of blood. The tubes were mixed thoroughly and left on ice for at least 2 h. The tubes were then centrifuged for 30 min at $31,000 \times g$. Supernatant was collected and immediately frozen at -20°C until assayed. Blood for ammonia determination was deproteinized as described previously. Then, 1 mL of supernatant was collected and pH was adjusted to 7.0 with KHCO_3 2.0 *M*. It was then thoroughly mixed, let to stand on ice for 15 min, and then centrifuged for 12 min at $1,854 \times g$ and 4°C . Supernatant was collected for immediate analysis.

Vitamin B₁₂ in plasma was determined in duplicate by radioassay with a commercial kit (Quantaphase B₁₂, Bio-Rad Laboratories Ltd., Mississauga, Ontario, Canada) as described by Girard and Matte (1988). Plasma biotin was determined using an ELISA test as described by Santschi et al. (2005a). Plasma glucose, blood BHBA, and lactate were determined using commercial kits (Roche Diagnostics Glucose GmbH, Mannheim, Germany; #310-uv, Sigma Diagnostics, Oakville, Ontario, Canada; and #826-A Sigma Diagnostics, respectively). Blood VFA were determined according to Reynolds et al. (1986). Urea-N and ammonia concentrations were measured in fresh blood on the day of sampling with an automatic analyzer (Technicon Autoanalyzer II, Technicon Instruments Corp., Tarrytown, NY) as described by Huntington (1984) and an enzymatic reaction (glutamate dehydrogenase) as described by Bergmeyer and Beutler (1985). Blood hemoglobin was determined colorimetrically using cyanmethemoglobin as the standard (Manet, 1969). Packed cell volume was

Table 1. Ingredients and nutrient composition of the mixed ration fed to dairy cows¹

Item	Value
Ingredient, % DM basis	
Corn silage ²	20.4
Hay silage ³	30.6
Corn	33.7
Soybean meal 49%	8.6
Micronized soybean	0.8
Distillers grain (wheat)	0.8
Distillers grain (corn)	0.6
Corn gluten meal	1.0
Canola meal	0.8
Calcium carbonate	0.5
Mineral and vitamin premix ⁴	2.2
Nutrient composition	
CP, %	18.1
RDP, % ⁵	12.0
RUP, % ⁵	6.1
ADF, %	19.7
NDF, %	28.9
NE _E , ⁵ Mcal/kg of DM	1.55
MP, g/kg of DM	108

¹The cows also received 1 kg of long hay per day. Composition: 14.1% CP, 6.1% soluble protein, 33.0% ADF, 53.1% NDF, and 3.2% lignin (composite sample).

²Composition: $10.6 \pm 0.4\%$ CP, $5.0 \pm 0.3\%$ soluble protein, $25.9 \pm 1.4\%$ ADF, $44.6 \pm 2.1\%$ NDF, and $2.9 \pm 0.1\%$ lignin ($n = 4$).

³Composition: $19.7 \pm 0.2\%$ CP, $8.3 \pm 0.4\%$ soluble protein, $31.5 \pm 0.6\%$ ADF, $41.5 \pm 0.7\%$ NDF, and $5.4 \pm 0.3\%$ lignin ($n = 4$).

⁴Contained per kg: 67.8 g of Ca, 66.4 g of P, 39 g of Mg, 150.8 g of Na, 107.8 g of Cl, 6.8 g of K, 10 g of S, 2,888 mg of Fe, 2,002 mg of Mn, 1,896 mg of Zn, 462.6 mg of Cu, 57.6 mg of I, 34.2 mg of Co, 24 mg of Se, 398,800 IU of vitamin A, 70,458 IU of vitamin D, and 2,294 IU of vitamin E.

⁵Calculated according to the model of NRC (2001).

determined on fresh samples by microcentrifugation of capillary tubes.

Blood flow in the ruminal artery and the portal vein was recorded continuously from 15 min before the first sampling time to 15 min after the last sample was taken. Hepatic blood flow was calculated as 1.23 times the blood flow recorded in the portal vein; this value was obtained from a compilation of data obtained on lactating dairy cows (Hélène Lapierre, Agriculture and Agri-Food Canada, Sherbrooke, Québec, Canada, personal communication).

Calculations and Statistical Analysis

The net flux of nutrients across the rumen, PDV, and TSP tissues was calculated as described by Girard et al. (2001) except that for each sampling time, the mean blood flow recorded by the flow probe from 15 min before to 15 after blood sample was used for calculations. Net flux of nutrients across the liver was calculated as the differences between TSP and PDV fluxes. Statistical analyses for arterial concentrations and net flux of nutrients across the rumen, PDV, liver, and TSP

Table 2. Effects of dietary supplements of vitamin B₁₂ (500 mg) and biotin (20 mg) on milk production and composition (n = 4; study 1)¹

Item	B ₈ -B ₁₂ -	B ₈ +B ₁₂ +	SEM	P-value
Milk production, kg/d	30.4	31.6	2.8	0.04
DMI, kg/d	23.1	23.1	0.5	0.99
Milk composition, g/kg				
TS	133.3	131.1	5.5	0.26
Fat	43.6	41.2	2.8	0.20
CP	34.8	34.6	2.2	0.61
Ash	7.3	7.4	0.1	0.30
Lactose ²	47.7	48.0	0.7	0.75
Milk component yield, g/d				
TS	4,010	4,091	194	0.30
Fat	1,302	1,278	39	0.59
CP	1,039	1,073	28	0.03
Ash	220	232	19	0.03
Lactose ²	1,449	1,508	119	0.28

¹No vitamin supplement (B₈-B₁₂-) or a daily supplement of 20 mg of biotin + 500 mg of vitamin B₁₂ per day (B₈+B₁₂+).

²Calculated as TS - (fat + CP + ash).

tissues were conducted on average postprandial values. Average DMI and milk production during the last 7 d of each experimental period as well as milk composition were compared. All variables, except for the net fluxes of nutrients across liver and TSP in study 2, were analyzed using the MIXED procedure of SAS (SAS Institute, 2004) according to the following model:

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

where Y_{ijk} is the studied variable, μ is the overall mean, C_i is the cow effect, P_j is the period effect, S_k is the vitamin supplement effect, and ε_{ijk} is the residual error. Period and vitamin supplement effects were analyzed as fixed effects and cow as random effect. In study 2, it was not possible to draw blood from the catheter placed in hepatic veins of 2 cows. Therefore, because there was no period effect ($P \geq 0.1$) on any of the PDV fluxes measured, and in spite of a possible loss of sensitivity of the statistical model, the period effect was removed from the previous model for the analyses of liver and splanchnic fluxes. Fluxes of nutrients across liver and TSP in study 2 were analyzed according to the following model:

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

where Y_{ij} is the studied variable, μ is the overall mean, C_i is the cow effect, S_j is the vitamin supplement effect, and ε_{ij} is the residual error. Vitamin supplement effect was analyzed as a fixed effect and cow as random effect. Results are reported as least squares means and standard errors of the means. The significance level was defined at $P \leq 0.05$ and trends toward significance were considered at $0.05 < P < 0.10$.

RESULTS

Study 1

Dietary supplements of biotin and vitamin B₁₂ increased ($P = 0.04$) milk production by 1.2 kg/d over the control group without any effect on DMI ($P = 0.99$; Table 2). There was no effect ($P \geq 0.2$) of supplementary vitamins on milk composition or on milk yields of TS, fat, and lactose, but the vitamin supplement increased ($P = 0.03$) yields of CP by 34 g/d and ash by 12 g/d.

There was no effect of treatments on blood flow in the right ruminal artery (125 ± 4 L/h; minimum = 112 L/h; maximum = 149 L/h; $P = 0.8$) and portal vein ($1,350 \pm 103$ L/h; minimum = 924 L/h; maximum = 1,698 L/h; $P = 0.6$).

The combined supplement of biotin and vitamin B₁₂ increased arterial concentrations of biotin ($P = 0.02$) and its net appearance across PDV ($P = 0.02$) and tended to increase TSP flux of the vitamin ($P = 0.10$; Tables 3 and 4). The vitamin supplement also increased the appearance of vitamin B₁₂ across PDV ($P = 0.04$) and tended ($P \leq 0.10$) to increase TSP flux of the vitamin and its arterial concentration. There was no effect ($P \geq 0.21$) of the vitamin supplement on the flux of the 2 vitamins across the rumen and liver (Table 4).

Supplementary biotin and vitamin B₁₂ given together decreased ($P = 0.006$) the appearance of ammonia across PDV. Arterial concentration of urea-N was lower ($P = 0.04$) in B₈+B₁₂+ cows, but removal of urea-N by the rumen or PDV as well as its liver and TSP fluxes remained unchanged ($P \geq 0.14$; Tables 3 and 4).

The flux of acetate, i-butyrate, and total VFA across PDV decreased ($P = 0.04$) and the flux of i-butyrate across the rumen tended to decrease ($P = 0.10$) in

Table 3. Effects of dietary supplements of vitamin B₁₂ (500 mg) and biotin (20 mg) on arterial concentrations of nutrients (n = 4; study 1)¹

Item	B ₈ -B ₁₂ -	B ₈ +B ₁₂ +	SEM	P-value
Plasma biotin, ng/mL	1.45	1.88	0.07	0.02
Serum vitamin B ₁₂ , pg/mL	222.9	817.5	107.5	0.06
Blood ammonia, mM	0.059	0.061	0.008	0.91
Blood urea-N, mM	9.32	8.81	0.82	0.04
Blood VFA, mM				
Acetate	2.20	2.27	0.07	0.06
Propionate	0.061	0.085	0.005	0.04
I-butyrate	0.011	0.004	0.002	0.06
Butyrate	0.033	0.033	0.003	0.95
Total	2.30	2.39	0.07	0.05
Plasma glucose, mM	3.66	3.67	0.09	0.91
Blood lactate, mM	0.48	0.55	0.01	0.02
Blood BHBA, mM	0.52	0.51	0.05	0.57

¹No vitamin supplement (B₈-B₁₂-) or a daily supplement of 20 mg of biotin + 500 mg of vitamin B₁₂ per day (B₈+B₁₂+).

B₈+B₁₂+ cows, but there was no other treatment effect ($P \geq 0.11$) on VFA flux across the rumen, liver, and TSP (Table 4).

The combined supplement of vitamins decreased the appearance of lactate across the rumen ($P = 0.03$) but increased its arterial concentration ($P = 0.02$; Tables 3 and 4). The net liver flux of BHBA tended to increase ($P = 0.09$) with the supplement, whereas the vitamin supplement had no effect ($P \geq 0.8$) on arterial concentration and net flux of glucose across the studied tissues.

Study 2

Milk production and composition, yields of milk components, and DMI were not different ($P \geq 0.2$) when cows were fed supplementary vitamin B₁₂ alone or combined with biotin (Table 5), except for milk lactose yield, which was higher ($P = 0.003$) when cows were fed vitamin B₁₂ alone.

There was no effect of treatments on blood flow in the right ruminal artery (122 ± 4 L/h; minimum = 112 L/h; maximum = 147 L/h; $P = 0.7$) and portal vein ($1,261 \pm 93$ L/h; minimum = 837 L/h; maximum = 1,565 L/h; $P = 0.8$).

Supplementary biotin and vitamin B₁₂ given together increased ($P = 0.04$) arterial concentration of biotin and tended to increase ($P \leq 0.09$) the net flux of the vitamin across PDV, liver, and TSP tissues (Tables 6 and 7). Arterial concentration of vitamin B₁₂ and its net flux across rumen, PDV, liver, and TSP did not differ ($P \geq 0.12$) between the 2 vitamin supplements.

The appearance of ammonia across PDV as well as its removal by the liver were reduced ($P \leq 0.03$; Table 7) in B₈+B₁₂+ cows compared with B₈-B₁₂+ cows. Urea-N removal by the PDV tissues increased ($P = 0.02$) in B₈+B₁₂+ cows, whereas blood arterial concentration of urea-N decreased ($P = 0.008$; Tables 6 and 7).

Except for a tendency ($P = 0.06$) to an increased appearance of butyrate across the rumen with B₈+B₁₂+, net appearance of VFA across rumen and PDV did not differ ($P \geq 0.11$) between the vitamin supplements (Table 7). The combined supplement of vitamins increased ($P = 0.03$) removal of i-butyrate by the liver and decreased ($P = 0.03$) TSP flux of propionate (Table 7).

Arterial concentrations of glucose was lower ($P = 0.02$) in B₈+B₁₂+ than B₈-B₁₂+ cows. There was a net appearance of glucose across PDV tissues with B₈-B₁₂+ but a net removal with B₈+B₁₂+ ($P = 0.02$); TSP flux of glucose was also lower ($P = 0.02$) when the cows were fed the 2 vitamins together. Net appearance of BHBA from the liver and its arterial concentration tended to increase ($P \leq 0.10$) with the combined supplement of biotin and vitamin B₁₂ compared with vitamin B₁₂ given alone (Tables 6 and 7). There was no treatment effect ($P \geq 0.3$) on arterial concentration and net flux of lactate across the studied tissues.

DISCUSSION

Production Data

In study 1, the combined supplement of biotin and vitamin B₁₂ increased milk production in cows compared with cows fed no vitamin supplement, but it had no effect in study 2 when compared with cows fed vitamin B₁₂ alone, suggesting that the addition of vitamin B₁₂ was responsible for this increment. Nevertheless, comparisons between the 2 present studies could be misleading because cows produced more than 30 kg/d in study 1 whereas, as lactation progressed, milk production was lower in study 2. Supplementary biotin is generally reported to increase milk production in cows producing more than 30 kg/d, but not in low-producing cows (Cooke and Brumby, 1982; Bonomi et al., 1996; Midla et al., 1998; Fitzgerald et al., 2000; Zimmerly

Table 4. Effects of dietary supplements of vitamin B₁₂ (500 mg) and biotin (20 mg) on net flux of nutrients across the right rumen wall, portal-drained viscera, splanchnic tissues, and liver (study 1)¹

Item, mmol/h unless noted	B ₈ -B ₁₂ -	B ₈ +B ₁₂ +	SEM	P-value
Flux across right rumen wall (n = 4)				
Plasma biotin, µg/h	-9.6	-0.3	2.5	0.21
Serum vitamin B ₁₂ , µg/h	-12.7	3.2	11.5	0.46
Blood ammonia	137.5	108.2	9.6	0.26
Blood urea-N	-56.9	-61.6	10.6	0.79
Blood VFA				
Acetate	630.4	542.3	27.7	0.22
Propionate	208.6	182.7	12.4	0.35
I-butyrate	5.9	4.8	0.5	0.10
Butyrate	35.5	29.3	2.6	0.18
Total	883.4	759.1	40.2	0.24
Plasma glucose	-2.3	-1.5	4.3	0.91
Blood lactate	26.9	25.0	1.6	0.03
Blood BHBA	53.2	46.0	3.7	0.36
Flux across portal-drained viscera (n = 4)				
Plasma biotin, µg/h	-67.2	135.6	32.3	0.02
Serum vitamin B ₁₂ , µg/h	24.4	146.2	27.8	0.04
Blood ammonia	677.9	568.8	84.4	0.006
Blood urea-N	-583.8	-686.3	197.1	0.73
Blood VFA				
Acetate	2,726.4	2,407.7	164.1	0.0006
Propionate	1,031.1	974.1	120.5	0.38
I-butyrate	34.4	26.9	5.2	0.04
Butyrate	213.4	187.1	28.6	0.11
Total	4,005.3	3,695.8	238.1	0.01
Plasma glucose	-17.1	-29.9	56.0	0.87
Blood lactate	122.5	101.0	14.4	0.40
Blood BHBA	162.7	136.1	19.2	0.16
Net liver flux (n = 4)				
Plasma biotin, µg/h	119.8	113.3	57.9	0.95
Serum vitamin B ₁₂ , µg/h	-34.5	-40.9	23.4	0.64
Blood ammonia	-782.7	-592.9	78.1	0.31
Blood urea-N	972.5	870.5	199.3	0.76
Blood VFA				
Acetate	193.7	362.6	141.3	0.53
Propionate	-1,089.8	-939.4	134.0	0.55
I-butyrate	-38.3	-31.2	5.7	0.35
Butyrate	-181.3	-168.0	29.1	0.15
Total	-1,150.8	-776.0	254.7	0.46
Plasma glucose	805.2	827.3	50.9	0.79
Blood lactate	-77.9	-41.7	34.5	0.17
Blood BHBA	194.3	239.7	31.1	0.09
Flux across splanchnic tissues (n = 4)				
Plasma biotin, µg/h	16.5	225.8	28.3	0.10
Serum vitamin B ₁₂ , µg/h	-14.8	126.7	52.3	0.10
Blood ammonia	-31.9	-23.5	16.8	0.77
Blood urea-N	352.9	169.4	64.2	0.14
Blood VFA				
Acetate	3,302.6	2,784.7	290.8	0.40
Propionate	59.8	35.0	14.7	0.37
I-butyrate	-1.7	-3.2	3.0	0.77
Butyrate	31.0	20.1	6.6	0.43
Total	3,390.5	2,836.6	301.6	0.39
Plasma glucose	804.7	789.4	119.2	0.87
Blood lactate	53.3	58.5	34.9	0.84
Blood BHBA	365.6	377.2	42.0	0.21

¹No vitamin supplement (B₈-B₁₂-) or a daily supplement of 20 mg of biotin + 500 mg of vitamin B₁₂ per day (B₈+B₁₂+).

and Weiss, 2001; Bergsten et al., 2003; Majee et al., 2003; Rosendo et al., 2004; Ferreira and Weiss, 2007; Ferreira et al., 2007; Reynolds et al., 2007; Enjalbert

et al., 2008). The effect of supplementary biotin on milk composition is generally limited, but increases in milk yields of protein had been reported (Zimmerly and

Table 5. Effects of dietary supplements of vitamin B₁₂ (500 mg) with or without biotin (20 mg) on milk production and composition (n = 4; study 2)¹

Item	B ₈ -B ₁₂ +	B ₈ +B ₁₂ +	SEM	P-value
Milk production, kg/d	28.0	27.1	2.7	0.28
DMI, kg/d	22.5	22.5	0.5	0.81
Milk composition, g/kg				
TS	132.5	133.5	5.4	0.57
Fat	43.2	44.4	2.9	0.46
CP	35.8	34.9	2.3	0.22
Ash	7.3	7.4	0.1	0.37
Lactose ²	46.2	46.8	0.6	0.62
Milk component yield, g/d				
TS	3,664	3,579	184	0.26
Fat	1,185	1,185	51	0.99
CP	986	930	46	0.25
Ash	203	199	20	0.57
Lactose ²	1,290	1,265	57	0.003

¹Daily supplements of 500 mg of vitamin B₁₂ (B₈-B₁₂+) or 20 mg of biotin + 500 mg of vitamin B₁₂ (B₈+B₁₂+

²Calculated as TS - (fat + CP + ash).

Weiss, 2001; Majee et al., 2003; Ferreira et al., 2007). Blends of B-vitamins including vitamin B₁₂, at doses 500 and 1,000 times lower than the amount used in the present studies given in addition to biotin supplements, had no effect on milk production or composition compared with biotin given alone (Majee et al., 2003).

Blood Flow

Rémond et al. (1993) reported that in sheep, blood flows in the right and left ruminal arteries are generally equal, although in some animals, blood flows in the right ruminal artery could be greater. In dairy cows, however, variations between the areas irrigated by the right and left ruminal arteries among animals seem to be larger than in sheep. In the present experiment, blood flow in the right ruminal artery varied between 7.4 and 13.0 L/h for the 4 cows. This variability among

animals is confirmed by a later study recording blood flow in the right and left ruminal arteries of 5 lactating dairy cows. Averaged blood flow recorded for the left ruminal artery represented 90 ± 10% of the flow in the right ruminal artery, but this ratio varied from 68 to 124% among the 5 studied cows (our unpublished data). In the present experiment, there was no correlation between blood flows in the right ruminal artery and the portal vein ($r = 0.03$; $P = 0.91$).

Rémond et al. (1998) demonstrated that in sheep, portal blood flow measured using A-series flow probes, like those used in the present studies, gave similar values as blood flow measured with an indicator dilution method. One should keep in mind that, in the present experiment, blood flow in the portal vein was measured and liver and TSP blood flows were calculated as described previously, whereas in experiments reported by Reynolds (1995), using indicator dilution methods, PDV

Table 6. Effects of dietary supplements of vitamin B₁₂ (500 mg) with or without biotin (20 mg) on arterial concentrations (n = 4; study 2)¹

Item	B ₈ -B ₁₂ +	B ₈ +B ₁₂ +	SEM	P-value
Plasma biotin, ng/mL	1.58	1.68	0.07	0.04
Serum vitamin B ₁₂ , pg/mL	605.1	758.5	147.0	0.12
Blood ammonia, mM	0.075	0.073	0.005	0.69
Blood urea-N, mM	10.46	9.34	1.06	0.008
Blood VFA, mM				
Acetate,	2.05	2.02	0.09	0.79
Propionate	0.082	0.066	0.016	0.52
I-butyrate	0.015	0.011	0.001	0.04
Butyrate	0.025	0.027	0.002	0.46
Total	2.17	2.12	0.11	0.72
Plasma glucose, mM	3.74	3.61	0.10	0.02
Blood lactate, mM	0.55	0.55	0.02	0.99
Blood BHBA, mM	0.44	0.50	0.08	0.07

¹Daily supplements of 500 mg of vitamin B₁₂ (B₈-B₁₂+) or 20 mg of biotin + 500 mg of vitamin B₁₂ (B₈+B₁₂+

Table 7. Effects of dietary supplements of vitamin B₁₂ (500 mg) with or without biotin (20 mg) on net flux of nutrients across the right rumen wall, portal-drained viscera, splanchnic tissues, and liver (study 2)¹

Item, mmol/h unless noted	B ₈ -B ₁₂ +	B ₈ +B ₁₂ +	SEM	P-value
Flux across right rumen wall (n = 3)				
Plasma biotin, µg/h	-8.9	1.8	4.0	0.13
Serum vitamin B ₁₂ , µg/h	5.7	4.0	2.9	0.75
Blood ammonia	112.3	102.5	10.9	0.65
Blood urea-N	-58.4	-24.5	28.4	0.50
Blood VFA				
Acetate	534.7	507.1	5.3	0.17
Propionate	202.7	166.3	31.3	0.53
I-butyrate	4.5	4.6	0.5	0.76
Butyrate	28.6	33.8	2.3	0.06
Total	770.5	711.8	34.6	0.45
Plasma glucose	-1.3	-0.8	1.5	0.87
Blood lactate	27.1	22.0	2.6	0.26
Blood BHBA	43.5	46.2	0.9	0.20
Flux across portal-drained viscera (n = 4)				
Plasma biotin, µg/h	-22.0	289.4	62.1	0.07
Serum vitamin B ₁₂ , µg/h	42.1	32.1	19.6	0.75
Blood ammonia	611.1	533.3	77.7	0.03
Blood urea-N	-314.3	-530.5	158.4	0.02
Blood VFA				
Acetate	1,949.5	1,997.9	171.3	0.55
Propionate	868.9	793.4	103.6	0.31
I-butyrate	23.2	23.9	3.2	0.72
Butyrate	139.0	154.5	15.9	0.11
Total	2,980.7	2,969.6	273.5	0.61
Plasma glucose	39.9	-50.6	54.2	0.02
Blood lactate	122.9	115.1	18.6	0.68
Blood BHBA	127.9	121.9	16.2	0.71
Net liver flux (n = 2)				
Plasma biotin, µg/h	145.2	222.0	46.1	0.09
Serum vitamin B ₁₂ , µg/h	-13.0	-33.3	42.9	0.75
Blood ammonia	-821.2	-588.8	36.8	0.006
Blood urea-N	1,259.5	1,695.9	231.9	0.41
Blood VFA				
Acetate	555.7	383.6	177.0	0.30
Propionate	-940.4	-790.2	148.7	0.40
I-butyrate	-19.8	-26.5	4.1	0.03
Butyrate	-130.2	-155.8	18.1	0.28
Total	-534.8	-588.9	310.8	0.82
Plasma glucose	905.1	927.2	49.5	0.61
Blood lactate	-9.8	-138.3	74.1	0.44
Blood BHBA	224.5	290.4	45.1	0.10
Flux across splanchnic tissues (n = 2)				
Plasma biotin, µg/h	116.9	446.3	26.3	0.07
Serum vitamin B ₁₂ , µg/h	64.0	22.9	44.3	0.35
Blood ammonia	-22.7	3.2	15.0	0.38
Blood urea-N	706.9	848.8	125.6	0.57
Blood VFA				
Acetate	2,565.8	2,648.2	187.8	0.77
Propionate	50.0	30.2	8.0	0.03
I-butyrate	1.3	-2.1	0.6	0.17
Butyrate	12.6	20.4	4.1	0.20
Total	2,629.6	2,696.7	197.2	0.81
Plasma glucose	1,013.0	764.8	9.7	0.02
Blood lactate	111.8	27.0	77.0	0.48
Blood BHBA	374.5	424.4	26.0	0.34

¹Daily supplements of 500 mg of vitamin B₁₂ (B₈-B₁₂+) or 20 mg of biotin + 500 mg of vitamin B₁₂ (B₈+B₁₂).

and TSP blood flows were measured and liver blood flow was calculated. In the present experiment, liver blood flow represents only 61 to 64% of the expected

liver blood flow calculated according to the equation by Reynolds (1995). However, calculated according to the equations described by Reynolds (1995) and

notwithstanding the treatment effects, liver removal of propionate in the 2 studies ranged between 70 and 94% of the calculated values and liver release of glucose ranged between 87 and 103% of these values. Therefore, although lower than the expected values but based on liver fluxes of glucose and propionate, liver blood flows measured in the present studies seem reasonable.

Vitamins

There was no appearance of vitamin B₁₂ through the rumen wall, even in cows fed the vitamin supplements. A positive flux of vitamin B₁₂ across the rumen wall of cows has already been observed after a single ruminal bolus of the vitamin but at a dose (7.8 g; Girard et al., 2001) greater than that used in the present experiment (500 mg/d, or 42 mg/meal). In study 1, the net PDV release of vitamin B₁₂ per day was 2.6 mg greater in B₈+B₁₂+ cows than in B₈-B₁₂- cows, representing 0.5% of the dose ingested. This proportion is of the same magnitude as that previously observed by Girard et al. (2001), who reported that 0.3% of a dietary supplement of 500 mg of cyanocobalamin given in a single dose was released from the PDV during the following 24 h. Ingestion of supplementary vitamin B₁₂ increased the TSP flux of the vitamin by 3.2 mg/d as well as its arterial concentration, but without any significant effect on its uptake by the liver compared with unsupplemented cows.

In the 2 studies, the flux of biotin across the rumen wall was not different from zero. However, there was a net removal of biotin by the gastrointestinal walls in cows not fed supplementary biotin, whereas in B₈+B₁₂+ cows, there was a net appearance of 4.9 to 7.5 mg/d of the vitamin through PDV. These amounts represented 25 to 38% of the biotin supplement served daily. Santschi et al. (2005b) reported that, in cows fed a daily supplement of 20 mg of biotin, 37% of the amount reaching the duodenal cannula disappeared in the small intestine. As observed for vitamin B₁₂, TSP flux and arterial concentration of biotin were increased in cows fed the vitamin supplement. The flux of biotin in the liver was similar in cows fed no vitamin supplement or the 2 vitamins together, but it tended to be decreased when the cows were fed vitamin B₁₂ alone, possibly indicating an increased need of hepatic tissues for biotin.

Nitrogen Metabolism

Reynolds et al. (2007) observed that supplementary biotin given alone, at the dose used in the present studies, increased net PDV release of ammonia, whereas Bonomi et al. (1996) observed no effect of biotin

supplements on blood concentrations of urea-N. However, in the present experiment, the combined supplement of biotin and vitamin B₁₂ decreased the release of ammonia across PDV and its uptake by the liver as well as the arterial concentrations of urea-N. Ammonia absorbed from the PDV is mostly from microbial fermentation of dietary protein, although some of it is derived from urea transferred to the lumen of the gastrointestinal tract via blood and saliva or tissue metabolism (Huntington, 1989). Because feed intake did not differ between treatments within each study, differences in release of ammonia across PDV, in spite of an increased removal of urea-N by gastrointestinal walls of B₈+B₁₂+ cows in study 2, demonstrated that the combined vitamin supplement altered nitrogen metabolism in rumen compared with cows fed no biotin supplements. This decreased PDV flux of ammonia could be caused by either an increased utilization for microbial protein synthesis or, on the contrary, a reduced ruminal degradation of dietary proteins.

Energy Metabolism

Bonomi et al. (1996) observed a decrease in blood concentration of acetate in cows fed supplementary biotin alone. However, most studies reported no marked effect of supplementary biotin given alone on molar concentrations of VFA in ruminal fluid (Zimmerly and Weiss, 2001; Majee et al., 2003; Ferreira et al., 2007) or on PDV, TSP, and liver flux of VFA (Reynolds et al., 2007). In the present experiment, the combined supplement of biotin and vitamin B₁₂ decreased the release of most individual and total VFA, except propionate, across PDV compared with B₈-B₁₂- cows (study 1), but had no such effect compared with B₈-B₁₂+ cows (study 2). This observation, in combination with observations on nitrogen metabolism described previously, further supports the hypothesis that a dietary supplement of the 2 vitamins altered ruminal fermentation, although for VFA flux specifically, the effect was more likely caused by vitamin B₁₂ supplements. Moreover, the release of VFA across PDV was 20% lower in study 2 than in study 1 despite a DMI difference of less than 3%; this further reduction of the VFA flux is possibly caused by a carryover effect of the vitamin supplementation given in study 1 on ruminal microflora.

Some studies observed an increase in plasma glucose with biotin supplements given alone (Bonomi et al., 1996; Rosendo et al., 2004), but most studies observed no effect (Zimmerly and Weiss, 2001; Majee et al., 2003; Reynolds et al., 2007). Graulet et al. (2007) observed that a dietary supplement of vitamin B₁₂ increased plasma glucose in cows also fed supplementary folic acid but had no effect in cows fed vitamin B₁₂ supplement

alone. In study 1, the combined supplement of biotin and vitamin B₁₂ had no effect on glucose metabolism although it tended to increase the release of BHBA by the liver; the latter could be caused by an augmentation of the demand for energy to sustain the increased milk production observed with this treatment. In study 2, however, compared with the 2 vitamins given together, vitamin B₁₂ alone seems to improve energy metabolism. It increased glucose release from PDV and TSP and tended to decrease liver release of BHBA. Arterial concentrations of glucose and BHBA followed the same trends. The increased PDV and TSP fluxes and arterial concentrations of glucose in B₈-B₁₂+ cows probably explained the minor augmentation of the milk yield of lactose observed in these cows.

Early works indicated that, in sheep, a large proportion of propionate is metabolized in rumen wall whereas, in cattle, this proportion is only 5 to 15% (Elliot, 1979). However, using radioisotopes, Seal and Parker (1994) demonstrated that propionate is extensively metabolized in the rumen wall of steers where it enters the gluconeogenic pathway, drastically reducing glucose utilization by gastrointestinal tissues and leaving more glucose available for the other tissues. Utilization of propionate requires the transformation of propionate into succinyl-CoA through 4 reactions; 2 of these reactions use biotin and vitamin B₁₂ as coenzymes. Supplementary biotin given to dairy cows increased the activity of pyruvate carboxylase, the enzyme essential for the carboxylation of pyruvate in oxaloacetate in liver, but had no effect on propionyl-CoA activity; because of the importance of propionate metabolism in lactating dairy cows, propionyl-CoA has a higher priority for biotin than pyruvate carboxylase (Ferreira and Weiss, 2007). However, there is no obvious explanation for the fact that in the present experiment, supplementary vitamin B₁₂ given alone decreased removal of glucose from the gastrointestinal walls more efficiently than when given with biotin.

CONCLUSIONS

A dietary supplement of biotin and vitamin B₁₂, at the doses used in the present experiment, seems to affect ruminal fermentation, decreasing the amounts of ammonia and VFA reaching the portal blood. Nevertheless, in study 1, milk production and milk protein yield were increased with this supplement, possibly because with the diet used in the present studies, AA composition of the dietary protein not degraded in rumen completed the AA profile of microbial protein to match more closely dairy cow requirements for milk protein synthesis. However, the increased release of BHBA from the liver and arterial concentrations seem to indicate that

increasing milk yield while decreasing PDV flux of VFA put some strain on energy metabolism. In study 2, even if DMI was similar for the 2 treatments, supplementary vitamin B₁₂ given alone increased glucose appearance from PDV and TSP compared with the 2 vitamins given together. This increase probably explained the minor milk lactose yield increment observed in cows fed only vitamin B₁₂ supplement. With the diet used in the present experiment, a dietary supplementation in biotin and vitamin B₁₂ changed the net PDV flux of nutrients, probably through alterations of the rumen fermentations, whereas vitamin B₁₂ supplement alone mostly modified glucose metabolism within the gastrointestinal walls. However, the vitamin supplements had limited effects on hepatic metabolism. Therefore, under the conditions of the present experiment, responses of lactational performance to vitamin supplementations were likely caused by changes in nutrient supply from the gastrointestinal tract. However, with the design used, effects on metabolism of extrahepatic tissues cannot be ruled out.

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