Short communication: Effect of fatty acid supplements on apparent ruminal synthesis of B vitamins in lactating dairy cows

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ABSTRACT

The effect of fat supplements (FS) providing different proportions of saturated (SFA) and unsaturated (UFA) fatty acids on supply, apparent ruminal synthesis (ARS), and duodenal flow (DF) of some B vitamins (thiamine, riboflavin, niacin, vitamin B₆, folates, and vitamin B₁₂) were evaluated in an experiment using 8 ruminally and duodenally cannulated lactating Holstein cows. The experiment was a replicated 4 × 4 Latin square design with 21-d treatment periods. The 4 treatments were a control diet without fatty acid supplement and 3 diets with 2.5% additional fatty acids from supplements containing (1) SFA, (2) an intermediate mixture of SFA and UFA, or (3) UFA. All diets were served as a total mixed ration once daily at 115% of the expected intake. B-vitamin concentrations were analyzed in feed and duodenal digesta. Apparent ruminal synthesis of each B vitamin was calculated as the DF minus the intake. B-vitamin concentrations were similar among the 4 treatments; consequently, daily intake of the vitamins followed the same pattern as dry matter intake. Adding FS decreased B-vitamin intakes (except vitamin B₁₂), as did increasing the proportion of UFA. Riboflavin and niacin DF and ARS, expressed as total daily amount or per unit of dry matter intake, were not affected by FS, but increasing the proportion of UFA decreased riboflavin and niacin DF and ARS. Fat supplements decreased DF of vitamin B₆, expressed either as total daily amount or per unit of dry matter intake. No treatment effects were observed on total daily folate DF and ARS. However, when expressed per unit of dry matter intake, folate DF and ARS were greater when

cows were fed FS and they increased linearly with the proportion of UFA in the supplement. Inclusion of fat supplements into the dairy cow diet had a limited effect on the fate of most B vitamins in the rumen although increasing the proportion of UFA in the FS linearly decreased apparent synthesis of riboflavin and niacin in the rumen and the amounts of these vitamins reaching the small intestine.

Key words: dairy cow, B vitamin, fatty acid, duodenal flow

Short Communication

Bechdel et al. (1928) demonstrated that B vitamins are produced by microorganisms present in the rumen. Consequently, B-vitamin supply for dairy cows is the summation of the amounts of vitamins from dietary sources escaping degradation in the rumen and the amounts of vitamins synthesized in the rumen. It is likely that B-vitamin supply is affected by the dietary ingredients themselves as well as their effects on rumen fermentation. However, research aiming to quantify the effect of diet composition on B-vitamin supply for dairy cows is very limited. Fat supplements (FS) are commonly used to increase energy density of lactating dairy cow diets. Products such as prilled hydrogenated fatty acids (FA) and Ca soaps of FA have been developed to limit the negative effects of FA on ruminal microflora and are generally considered as inert in the rumen (Grummer, 1988). However, FA from Ca soaps of FA are partially biohydrogenated in the rumen and this proportion increases with the level of FA unsaturation (Sukhija and Palmquist, 1990; Wu et al., 1991). The effect of these products on the fate of B vitamins in the rumen has never been studied. The present project aimed to evaluate if FS providing different proportions of SFA and UFA could change B-vitamin intake, apparent ruminal synthesis (ARS), and duodenal flow (DF) in dairy cows.

Feed and duodenal digesta samples were collected during a research project conducted at Michigan State

Received April 27, 2017.

Accepted June 18, 2017.

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University following approval by the Institutional Animal Care and Use Committee (Harvatine and Allen, 2006a). Eight multiparous Holstein cows from the Michigan State University Dairy Cattle Teaching and Research Center, equipped with rumen and duodenal cannulas and housed in tie-stalls, were randomly assigned at 77 (SD 8.7) DIM to treatment sequence in a replicated 4×4 Latin square design with 21-d treatment periods. The 4 treatments were a control diet without FS and 3 diets with 2.5% additional FA from supplements containing (1) SFA (**SAT**; prilled, hydrogenated FA, Energy Booster 100, MS Specialty Nutrition, Dundee, IL), (2) an intermediate mixture of SFA and Ca soaps of UFA (**INT**), or (3) UFA (**UNS**; Ca soaps of partially unsaturated FA, Megalac-R, Church and Dwight Company Inc., Princeton, NJ; Harvatine and Allen, 2006a). All rations were offered as a TMR once daily (0900 h) at 115% of the expected intake. The forage-to-concentrate ratio (% of DM) was 37:63. Corn and alfalfa silages were used as forages and their relative contribution to total NDF was 17% (Table 1). Individual feed intake was recorded daily. From d 11 to 14 of each period, samples of all dietary ingredients were collected daily and mixed into a sample per period. Duodenal samples were collected every 9 h from d 12 to 14; then, these 8 samples were pooled per cow per period. All samples were stored at -20°C before processing. Experimental procedures, measurements, and analyses were described in detail by Harvatine and Allen (2006a,b).

B-vitamin concentrations in feed and duodenal digesta were analyzed at the Sherbrooke Research and Development Centre (Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada) as described by Castagnino et al. (2016b). Thiamine, riboflavin, niacin, and vitamin B₆ were quantified by HPLC (Varian ProStar, Lake Forest, CA) equipped with a solvent delivery system (model 210), an autosampler (model 410), and a fluorescence detection system (model 363). Foliates were determined with a microbiological microtiter plate test (VitaFast Folic Acid, R-Biopharm Inc., Marshall, MI) and vitamin B₁₂ was analyzed by radio-assay [SimulTRAC-S Vitamin B₁₂ (^{57}Co)/Folate (^{125}I), MP Biomedicals, Solon, OH]. Dietary Co concentrations were determined by atomic absorption spectrometry with flame stoichiometric air-acetylene at 240.7 nm as described by Castagnino et al. (2016a). Samples were analyzed in duplicate (except for folates for which samples were analyzed in triplicate), and a coefficient of variation of less than 10% was accepted between replicates.

B-vitamin concentrations in the 4 experimental TMR were the summation of B-vitamin concentration in each ingredient multiplied by the proportion of this ingredi-

ent in the TMR, on a DM basis. Daily intakes of each vitamin were calculated as the concentration of each vitamin in the TMR multiplied by DMI. Calculations of DM DF were described by Harvatine and Allen (2006a). B-vitamin DF was calculated as B-vitamin concentrations in duodenal sample multiplied by the daily DM flowing through the duodenum. Apparent ruminal synthesis was calculated as DF minus the daily intake of each vitamin. Total niacin daily intake, DF, and ARS were calculated as the sum of nicotinic acid and nicotinamide for these variables. Total daily intake, DF, and ARS of vitamin B₆ were calculated as the sum of pyridoxamine, pyridoxal, and pyridoxine for these variables.

Daily intake, DF, and ARS for each vitamin, expressed in milligrams per day or milligrams per kilogram of DMI, were analyzed using the MIXED procedure of SAS (version 9.4, SAS Institute Inc., 2012) in which period and treatment were considered as fixed effects and cow as a random effect. A priori contrasts, (1) effect of addition of FS (control vs. SAT, INT, and UNS), (2) linear effect of substituting UFA for SFA (SAT vs. UNS), and (3) quadratic effect of substituting UFA for SFA (INT vs. SAT and UNS), were used. Differences were declared significant at $P < 0.05$ and as a tendency at $0.05 \leq P \leq 0.10$.

Daily DMI was 26.5, 25.4, 24.5, and 23.0 (SE 0.93) kg for control, SAT, INT, and UNS, respectively. Feeding FS decreased DMI in lactating dairy cows and DMI decreased linearly when increasing the proportion of UFA in FS (Harvatine and Allen, 2006a). Dry matter DF followed a similar trend (FS effect, $P = 0.03$; linear effect, $P < 0.001$), with 18.9, 19.4, 17.5, and 15.7 (SE 0.92) kg of DM/d for control, SAT, INT, and UNS, respectively. B-vitamin concentrations were similar among the 4 experimental diets (Table 1). Consequently, daily intake of the studied vitamins, except for vitamin B₁₂, followed the same pattern as DMI. Fat supplements decreased ($P \leq 0.001$) B-vitamin intakes, which also decreased ($P \leq 0.03$) linearly when the proportion of UFA increased (Table 2).

Fat supplements decreased (FS effect, $P = 0.001$) the amount of vitamin B₆ reaching the duodenum daily compared with control, and daily DF of vitamin B₆ tended to decrease (linear effect, $P = 0.07$) when increasing the proportion of UFA in the supplement (Table 2). However, when expressed per unit of DMI, FS decreased (FS effect, $P = 0.04$) DF of vitamin B₆ and tended to increase (FS effect, $P = 0.08$) degradation of the vitamin in the rumen (Table 2). Treatments did not affect ($P \geq 0.20$) the daily amounts of folates reaching the duodenum or their apparent synthesis in the rumen. However, when expressed per unit of DMI, FS increased folate DF and ARS (FS effect, P

Table 1. Ingredients, nutrient composition, and B-vitamin concentrations of the experimental diets¹

Item	Treatment ²			
	Control	SAT	INT	UNS
Ingredient, % of DM				
Corn silage ³	24.6	24.7	24.7	24.6
Alfalfa silage ⁴	12.6	12.6	12.6	12.6
Ground corn	28.7	28.8	28.8	28.7
Whole cottonseed	13.5	13.5	13.5	13.5
Protein mix ⁵	10.5	10.6	10.5	10.5
Mineral-vitamin mix ⁶	4.3	4.3	4.3	4.3
Control mix ⁷	5.7	0.5	0.2	—
SAT mix ⁷	—	5.0	2.5	—
UNS mix ⁷	—	—	2.9	5.7
Nutrient				
DM, % as fed	55.6	55.7	55.7	55.7
OM, % of DM	92.6	92.9	93.1	93.1
Total fatty acids, % of DM	5.5	8.3	8.1	7.8
UFA, % of DM	3.6	3.9	4.4	4.9
Starch, % of DM	30.8	30.3	30.5	30.7
NDF, % of DM	29.1	27.3	27.5	27.7
Indigestible NDF, % of DM	11.2	9.7	10.0	9.9
Forage NDF, % of DM	16.9	17.0	16.9	17.0
CP, % of DM	16.2	16.1	16.1	16.1
Rumen-undegraded CP, ⁸ % of DM	5.1	4.8	5.1	4.9
Gross energy, Mcal/kg	4.55	4.72	4.72	4.71
Total cobalt, mg/kg of DM	1.34	1.33	1.13	1.17
B-vitamin concentration, mg/kg of DM				
Thiamine	2.11	2.05	2.06	2.07
Riboflavin	24.59	24.46	24.45	24.47
Niacin ⁹	28.79	28.60	28.35	28.13
Vitamin B ₆ ¹⁰	4.06	4.02	4.04	4.07
Folates	0.37	0.34	0.35	0.35
Vitamin B ₁₂	ND ¹¹	ND	ND	ND

¹Adapted from Harvatine and Allen (2006a).²SAT = SFA from prilled, hydrogenated fat (Energy Booster 100, MS Specialty Nutrition, Dundee, IL); INT = mix of SAT and UNS; and UNS = Ca soaps of partially unsaturated fatty acid (Megalac-R, Church and Dwight Company Inc., Princeton, NJ).³Corn silage contained 34.7% DM (as fed) and 43.4% NDF, 8.4% CP, 10.7% indigestible NDF, 24.1% starch, and 4.8% ash on a DM basis.⁴Alfalfa silage contained 36.3% DM (as fed) and 48.1% NDF, 16.2% CP, 25.7% indigestible NDF, 2.6% starch, and 9.7 ash on a DM basis.⁵Protein mix contained 74.1% soybean meal, 20.1% corn gluten meal, and 5.8% blood meal.⁶Vitamin-mineral mix contained (DM basis) 12.7% sodium bicarbonate, 11.5% limestone, 5.5% salt, 2.2% trace mineral premix, 2.0% urea, 2.0% dicalcium phosphate, 0.6% vitamin D, 0.48% vitamin A, 0.12% vitamin E, and 62.9% dry ground corn as a carrier. Analyzed Co: 5.83 mg/kg of DM.⁷Control mix contained 50.5% rice hulls, 16.7% limestone, and 32.7% ground corn; SAT mix contained 50.5% prilled, hydrogenated fatty acids, 16.7% limestone, and 32.7% ground corn; and UNS mix contained 57.5% Ca soaps of fatty acid, 10.9% rice hulls, and 31.6% ground corn.⁸Estimated by using values from NRC (2001).⁹Sum of nicotinic acid and nicotinamide.¹⁰Sum of pyridoxamine, pyridoxal, and pyridoxine.¹¹ND = not detected.

≤ 0.003) compared with control and both folate DF and ARS increased linearly (linear effect, $P \leq 0.02$) with the proportion of UFA in the supplement. Under the present experimental conditions, neither DMI nor folate intake had an effect on the total amount of this vitamin available for absorption by the cow. Thiamine daily DF decreased linearly (linear effect, $P = 0.04$) when increasing the proportion of UFA, but this effect was no longer significant when reported per unit

of DMI ($P = 0.81$). As no treatment effect ($P \geq 0.16$) was observed on thiamine ARS, the difference in DF was probably due to the greater vitamin intake (linear effect, $P = 0.01$). Riboflavin and niacin DF and ARS, expressed as milligrams per day or milligrams per kilogram of DMI, were not affected by FS ($P \geq 0.25$; Table 2). However, increasing the proportion of UFA in the FS decreased ($P \leq 0.04$) daily riboflavin and niacin DF and ARS. This effect was still present when reported

Table 2. Duodenal flow (DF) and apparent ruminal synthesis (ARS) of B vitamins, expressed as milligrams per day or milligrams per kilogram of DMI, in response to dietary fatty acid supplements¹

Vitamin	Treatment, ² mg/d				P-value ³				Treatment, ² mg/kg of DMI				P-value ³			
	Control	SAT	INT	UNS	SE	FS	L	Q	Control	SAT	INT	UNS	SE	FS	L	Q
Thiamine																
Intake	56.0	52.2	49.5	47.6	1.80	<0.001	0.01	0.77								
DF	74.8	74.6	69.0	61.7	6.07	0.17	0.04	0.87	3.94	3.83	3.88	3.89	0.176	0.66	0.81	0.92
ARS	18.9	22.6	19.3	13.8	5.23	0.95	0.16	0.83	0.74	0.88	0.78	0.56	0.205	0.99	0.20	0.78
Riboflavin																
Intake	654	627	589	563	20.4	<0.001	0.002	0.69								
DF	714	815	683	560	45.0	0.45	<0.001	0.92	27.0	31.8	28.2	24.3	1.48	0.45	0.001	0.93
ARS	60.4	187.5	90.6	-3.1	38.40	0.40	0.001	0.97	2.29	7.29	3.70	-0.20	1.475	0.37	0.001	0.92
Niacin⁴																
Intake	763	727	695	646	24.9	0.0001	0.0004	0.61								
DF	1,852	2,506	1,922	1,673	238.4	0.50	0.03	0.58	70.4	95.9	78.2	70.9	8.9	0.26	0.07	0.64
ARS	1,089	1,769	1,230	1,019	227.5	0.35	0.04	0.58	41.6	67.3	49.9	42.8	8.9	0.25	0.07	0.64
Vitamin B₆⁵																
Intake	107.4	103.2	97.7	93.9	3.36	0.001	0.008	0.74								
DF	29.5	25.3	21.8	20.7	2.24	0.001	0.07	0.55	1.10	1.00	0.91	0.90	0.083	0.04	0.31	0.60
ARS	-78.0	-78.1	-75.4	-73.1	3.34	0.43	0.23	0.97	-2.96	-3.01	-3.14	-3.17	0.088	0.08	0.17	0.62
Folates																
Intake	9.7	8.7	8.3	8.1	0.32	<0.001	0.03	0.60								
DF	41.1	41.0	43.1	42.8	3.02	0.57	0.53	0.62	1.51	1.60	1.78	1.83	0.077	0.003	0.01	0.40
ARS	31.2	32.2	34.8	34.7	2.75	0.20	0.36	0.58	1.15	1.26	1.43	1.48	0.078	0.002	0.02	0.40
Vitamin B₁₂																
Intake ⁶	0	0	0	0	0	—	—	—								
DF	5.9	5.3	6.0	5.1	0.61	0.33	0.83	0.13	0.22	0.20	0.24	0.22	0.021	0.92	0.48	0.12
ARS	5.9	5.3	6.0	5.1	0.61	0.33	0.83	0.13	0.22	0.20	0.24	0.22	0.021	0.92	0.48	0.12

¹Least squares means.²SAT = SFA from prilled, hydrogenated fat (Energy Booster 100, MS Specialty Nutrition, Dundee, IL); INT = mix of SAT and UNS; and UNS = Ca soaps of partially unsaturated fatty acid (Megalac-R, Church and Dwight Company Inc., Princeton, NJ).³FS = effect of fatty acid supplement; L = linear effect of substituting UFA for SFA; and Q = quadratic effect of substituting UFA for SFA.⁴Sum of nicotinic acid and nicotinamide.⁵Sum of pyridoxamine, pyridoxal, and pyridoxine.⁶Not detected.

per unit of DMI ($P \leq 0.07$; Table 2), suggesting that differences in ruminal conditions also affected the fate of these vitamins in the rumen. Neither DF nor ARS of vitamin B₁₂, expressed as milligrams per day or milligrams per kilogram of DMI, were affected by FS or the proportion of UFA in the FS ($P \geq 0.12$; Table 2). Harvatine and Allen (2006a) observed a linear increase in ruminal digestibility of NDF when increasing the proportion of UFA in the supplements due to a slower passage rate of potentially digestible NDF, but a more rapid passage rate of indigestible NDF. Castagnino et al. (2016a) reported that riboflavin and niacin ARS are positively correlated with NDF digested in rumen and negatively correlated with indigestible NDF passage rate. Moreover, these authors observed that conditions promoting riboflavin and niacin ARS had the opposite effect on folate ARS.

Although the effects of the FA supplements on ruminal digestibility of NDF and its passage rate were attributed to physiological effects from greater DF of UFA (Harvatine and Allen, 2006b), these changes likely modified microbial activity in the rumen, which, in turn, altered the amounts of some B vitamins, especially riboflavin and niacin, available for the dairy cow.

ACKNOWLEDGMENTS

The authors thank Chrystiane Plante and Valérie Beaudet (Sherbrooke Research and Development Centre, Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada) for their technical support. Vitamin analyses were financially supported by the Programme de recherche en partenariat pour l'innovation en production et transformation laitière Novalait, Agriculture et

Agroalimentaire Canada, Fonds de recherche du Québec Nature et Technologies, Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec, Québec, QC, Canada.

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