



Apparent ruminal synthesis of B vitamins in lactating dairy cows fed diets with different forage-to-concentrate ratios

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ABSTRACT

Effects of the forage-to-concentrate ratio on apparent ruminal synthesis of thiamine, riboflavin, niacin, vitamin B₆, folates, and vitamin B₁₂ were evaluated in an experiment using 14 ruminally and duodenally cannulated Holstein cows. The experiment was a crossover design with two 15-d treatment periods and a 14-d preliminary period in which cows were fed a diet intermediate in composition between the treatment diets. Treatments were diets containing low-forage (44.8% forage, 32.8% starch, 24.4% neutral detergent fiber) or high-forage (61.4% forage, 22.5% starch, 30.7% neutral detergent fiber) concentrations. Both diets were formulated with different proportions of the same ingredients. Concentrations of B vitamins were analyzed in feed and duodenal digesta. Apparent ruminal synthesis of each B vitamin was calculated as the duodenal flow minus the intake. The high-forage diet had the highest concentrations of riboflavin, niacin, vitamin B₆, and folates, whereas the low-forage diet had the highest thiamine concentration. Vitamin B₁₂ in the diets was under the level of detection. Consequently, despite a reduction in dry matter intake when the cows were fed the high-forage diet, increasing dietary forage concentration increased or tended to increase intakes of riboflavin, niacin, and vitamin B₆ but reduced thiamine and folate intakes. Increasing dietary forage concentration reduced apparent ruminal degradation of thiamine and apparent ruminal synthesis of riboflavin, niacin, and folates and increased ruminal degradation of vitamin B₆, but had no effect on ruminal synthesis of vitamin B₁₂. As a consequence, increasing the forage-to-concentrate ratio had no effect on the amounts of thiamine, riboflavin, and vitamin B₁₂ reaching the small intestine but decreased the amounts of niacin, vitamin B₆, and folates available for absorption. Apparent ruminal syntheses of riboflavin, niacin, folates, and vitamin B₁₂ were correlated positively with

the amount of starch digested in the rumen and duodenal flow of microbial N, whereas these correlations were negative for thiamine. Apparent ruminal syntheses of thiamine and vitamin B₆ were negatively correlated with their respective intakes, whereas folate intake was positively correlated with its synthesis in the rumen.

Key words: dairy cow, B vitamin, forage-to-concentrate ratio, duodenal flow

INTRODUCTION

The assertion that mature ruminants have no need for an exogenous supply of B vitamins has prevailed for decades. Synthesis by rumen microflora was indeed believed to meet the needs of the animals and to prevent any symptoms of deficiency (McDowell, 2000; NRC, 2001). In the case of dairy cows, the dramatic increase in yields of milk and milk components over the years, and the concomitant increase of nutritional requirements cast doubt over this assertion; as B vitamins act as cofactors for the enzymes involved in the metabolism of carbohydrates, lipids, proteins, and nucleic acids, B-vitamin requirements likely increase with an augmentation of the metabolic activity.

This uncertainty increased interest for research on methods to optimize milk production by ensuring an optimal supply of B vitamins. Various studies on B-vitamin supplementation were then conducted, some showing positive effects of supplementary thiamine (Shaver and Bal, 2000), niacin (Schwab et al., 2005), biotin (Chen et al., 2011; Lean and Rabiee, 2011), and folic acid (Girard and Matte, 1998), whereas others showed no effect (Elliot et al., 1979; Girard et al., 2005).

These contradictory findings led to the necessity of a better understanding of the factors, particularly those related to feeding, involved in ruminal synthesis of B vitamins in dairy cows to define more accurately the need for supplementation. The amounts of B vitamins reaching the small intestine is affected by forage species, alfalfa versus orchardgrass, and within each forage species, by maturity at harvest (Castagnino et al., 2016a,c). Nevertheless, because within each of these

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experiments, the diets have similar proportions of total NDF and NDF from forages, the forage-to-concentrate ratios (**F:C**) differ between treatments. Consequently, effects of forage species or maturity at harvest on B-vitamin duodenal flow (**DF**) are possibly partly confounded with differences in F:C. Effects of F:C ratio on B-vitamin ruminal synthesis had very early attracted the attention of several researchers (Hunt et al., 1941, 1943; Conrad and Hibbs, 1954; Hayes et al., 1966; Sutton and Elliot, 1972; Girard et al., 1994). However most studies were conducted on sheep and steers, and the methodology used (measurement of B-vitamin concentrations in rumen content) provided only a qualitative evaluation of the amounts of vitamins available for the animal. Therefore, the objective of the present experiment was to determine the effects of 2 dietary F:C on B-vitamin supply, apparent ruminal synthesis (**ARS**), and DF in dairy cows comparing diets based on different proportions of the same ingredients.

MATERIALS AND METHODS

Animals and Treatments

Feed and duodenal digesta samples were collected during a research project conducted at Michigan State University (East Lansing, MI) following approval by the Institutional Animal Care and Use Committee (Voelker Linton and Allen, 2007). Prior to calving, 14 multiparous Holstein cows from the Dairy Cattle Teaching and Research Center were equipped with rumen and duodenal cannulas and housed in tie stalls. Cows were assigned randomly to treatment sequence in a crossover design with a 14-d preliminary period followed by two 15-d treatment periods. At the end of the preliminary period, cows were 178 ± 120 DIM and their DMI was 25 ± 2.7 kg/d (mean \pm SD). The 2 treatments were a low-forage (**LF**) diet and a high-forage (**HF**) diet served as a TMR once daily (1100 h) at 110% of the expected intake. The F:C (% of DM) were 45:55 and 61:39 for LF and HF, respectively. Corn and alfalfa silages were used as forages and their relative contribution to total forage NDF was similar for both diets. The forages provided 82 and 89% of total NDF for LF and HF, respectively. Both diets were formulated using the same ingredients (Table 1). Experimental procedures, measurements, and analyses were described in detail by Voelker Linton and Allen (2007).

Sample and Data Collection

Individual feed intake was recorded daily. From d 11 to 13 of each period, samples of all dietary ingredients and TMR were collected daily and mixed into a sample

per period. Duodenal samples were collected every 9 h during these 3 d and pooled. All samples were stored at -20°C before processing.

Laboratory Analyses

Frozen duodenal samples were chopped finely using a commercial food processor (84142 Food Cutter, Hobart Manufacturing Co., Troy, OH) and a representative subsample was lyophilized. Dietary ingredients and TMR samples were dried in a forced-air oven at 55°C for 72 h. All samples were ground using a Wiley mill (1-mm screen, Arthur H. Thomas, Philadelphia, PA).

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

| Item | Low-forage diet | High-forage diet |
|--|------------------|------------------|
| Ingredient (% of DM) | | |
| Corn silage ² | 29.7 | 40.5 |
| Alfalfa silage ³ | 15.1 | 20.9 |
| Dry ground corn | 33.9 | 16.1 |
| Soybean meal (48% CP) | 11.0 | 9.1 |
| Vitamin-mineral mix ⁴ | 3.2 | 4.3 |
| SoyPlus ⁵ | 5.9 | 9.1 |
| Urea | 0.2 | — |
| Nutrient | | |
| DM (% as fed) | 47.0 | 39.6 |
| OM (% of DM) | 93.0 | 92.1 |
| NDF (% of DM) | 24.4 | 30.7 |
| Forage NDF (% of DM) | 19.9 | 27.3 |
| Indigestible NDF (% of DM) | 13.2 | 15.1 |
| Starch (% of DM) | 32.8 | 22.5 |
| CP (% of DM) | 16.2 | 16.6 |
| Rumen-undegradable CP ⁶ (% of DM) | 7.2 | 7.3 |
| Total Co ⁷ (mg/kg of DM) | 0.95 | 1.00 |
| B-vitamin (mg/kg of DM) | | |
| Thiamine | 2.09 | 1.49 |
| Riboflavin | 25.9 | 32.4 |
| Niacin ⁸ | 38.1 | 47.2 |
| Vitamin B ₆ ⁹ | 5.80 | 6.83 |
| Folates | 0.53 | 0.57 |
| Vitamin B ₁₂ | ND ¹⁰ | ND |

¹Adapted from Voelker Linton and Allen (2007).

²Corn silage contained 46.4% NDF, 16% indigestible NDF, 18.6% starch and 8.1% CP; 30-h in vitro NDF digestibility was 47.5%.

³Alfalfa silage contained 40.6% NDF, 26.4% indigestible NDF, 3.5% starch and 18.3% CP; 30-h in vitro NDF digestibility was 32.6%.

⁴Vitamin-mineral mix contained (DM basis) 10.1% dicalcium phosphate, 4.1% trace-mineral mix, 5.7% sodium bicarbonate, 1.2% magnesium oxide, 124.2 kIU/kg of vitamin A, 40.3 kIU/kg of vitamin D, 671.6 kIU/kg of vitamin E, and 60.1% ground corn as a carrier. Analyzed Co: 5.53 mg/kg of DM.

⁵West Central Soy Cooperative (Ralston, IA). Nutrient composition: 86% DM, 7% ash, 16% NDF, 5% starch and 51% CP.

⁶Estimated by using values from NRC (2001).

⁷Sum of Co present in the mineral premix as Co carbonate and Co naturally present in feed ingredients.

⁸Sum of nicotinic acid and nicotinamide.

⁹Sum of pyridoxamine, pyridoxal, and pyridoxine.

¹⁰ND = not detected.

Table 2. B-vitamin concentrations (mg/kg of DM) of ingredients used in the experimental diets (mean \pm SD; n = 2 samples)

| Ingredient | Corn silage | Alfalfa silage | Corn, dry ground | Soybean meal | SoyPlus ¹ |
|-------------------------------------|------------------|------------------|------------------|------------------|----------------------|
| Thiamine | 0.60 \pm 0.03 | 1.00 \pm 0.51 | 4.09 \pm 0.05 | 2.65 \pm 0.34 | 1.44 \pm 0.18 |
| Riboflavin | 37.53 \pm 0.88 | 55.54 \pm 7.23 | 7.02 \pm 0.06 | 20.98 \pm 0.33 | 27.93 \pm 3.8 |
| Niacin ² | 47.74 \pm 0.14 | 62.24 \pm 4.97 | 13.23 \pm 0.72 | 34.53 \pm 1.67 | 104.80 \pm 43.45 |
| Vitamin B ₆ ³ | 6.57 \pm 0.17 | 13.52 \pm 0.31 | 3.17 \pm 0.25 | 3.75 \pm 0.25 | 5.31 \pm 0.33 |
| Folates | 0.08 \pm 0.01 | 0.57 \pm 0.05 | 0.25 \pm 0.01 | 1.91 \pm 0.13 | 2.18 \pm 0.09 |
| Vitamin B ₁₂ | ND ⁴ | ND | ND | ND | ND |

¹West Central Soy Cooperative (Ralston, IA).

²Sum of nicotinic acid and nicotinamide.

³Sum of pyridoxamine, pyridoxal, and pyridoxine.

⁴Not detected.

Methods used to determine DM, OM, ash, N, NDF, indigestible NDF, and starch in feed and duodenal digesta samples as well as digestive parameters (ruminal digestion of true OM, NDF, and starch, ruminal pH, and VFA profile, and DF of DM and microbial N, and passage rate of indigestible NDF) were described by Voelker Linton and Allen (2007).

The 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). 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) as described by Castagnino et al. (2016c). Folates 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 (⁵⁷Co)/Folate (¹²⁵I), MP Biomedicals, Solon, OH] as described by Castagnino et al. (2016c). Dietary Co concentrations were determined by atomic absorption spectrometry with flame stoichiometric air-acetylene at 240.7 nm based on an adaptation of the method of Bellanger (1988) as described by Castagnino et al. (2016b). Samples were analyzed in duplicate (except for folates, which were analyzed in triplicate), and a coefficient of variation of less than 10% was accepted between replicates.

Calculations and Statistical Analyses

The B-vitamin concentrations in the 2 TMR were the summation of B-vitamin concentration in each ingredient (Table 2) multiplied by the proportion of this ingredient 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 Voelker Linton and Allen (2007). Duodenal flow was calculated as B-vitamin concentrations in the 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 vitamin B₆ daily intake, DF, and ARS 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 SAS mixed model (SAS Institute Inc., Cary, NC) in which periods and treatments were considered as fixed effects and cows as random effect. Differences were declared significant at $P < 0.05$ and as a tendency at $0.05 \leq P \leq 0.10$.

RESULTS AND DISCUSSION

The effects of F:C on daily intakes, DF, and ARS of B vitamins expressed as milligrams per day are presented in Table 3. In Table 4, DF and ARS are expressed as milligrams per kilogram of DMI to allow comparisons independent of differences in DMI, which were 27 and 24 kg/d for LF and HF, respectively (SE = 0.9; $P < 0.0001$). Dry matter DF was also greater (SE = 0.9; $P = 0.002$) for LF (21 kg/d) than for HF (18 kg/d).

Assessing ARS does not allow distinguishing between microbial use, ruminal catabolism, or eventual absorption of B vitamins across the rumen wall. Consequently, the calculation of ARS probably underestimates the actual amounts of individual vitamins synthesized by ruminal microflora. This measurement, nevertheless, provides an estimate of the relative importance of ruminal microflora for vitamin supply.

Thiamine

As observed previously (Schwab et al., 2006; Castagnino et al., 2016a,b,c), corn and soybean meal had higher thiamine concentrations than forages (Table 2). As a result of the low concentrations of thiamine in for-

Table 3. Intake, duodenal flow, and apparent ruminal synthesis (ARS) of B vitamins, expressed as milligrams per day, in response to low-forage and high-forage diets¹

| Item | Treatment | | SEM | P-value |
|-------------------------------------|------------------|-----------------|--------|---------|
| | High-forage diet | Low-forage diet | | |
| Thiamine | | | | |
| Intake | 35.7 | 56.9 | 1.57 | <0.0001 |
| Duodenal flow | 29.1 | 33.4 | 2.32 | 0.21 |
| ARS | -6.6 | -23.5 | 2.35 | 0.0002 |
| Riboflavin | | | | |
| Intake | 778.1 | 703.9 | 5.48 | 0.0004 |
| Duodenal flow | 866.3 | 925.4 | 38.20 | 0.21 |
| ARS | 88.2 | 221.4 | 28.88 | 0.004 |
| Niacin ² | | | | |
| Intake | 1,135.4 | 1,035.3 | 37.84 | 0.001 |
| Duodenal flow | 3,020.2 | 3,866.0 | 296.37 | 0.005 |
| ARS | 1,884.8 | 2,830.8 | 273.86 | 0.002 |
| Vitamin B ₆ ³ | | | | |
| Intake | 163.8 | 157.5 | 5.52 | 0.08 |
| Duodenal flow | 68.3 | 88.3 | 5.21 | 0.003 |
| ARS | -95.5 | -69.2 | 3.61 | 0.0001 |
| Folates | | | | |
| Intake | 13.6 | 14.5 | 0.48 | 0.007 |
| Duodenal flow | 76.6 | 106.1 | 8.09 | 0.001 |
| ARS | 63.0 | 91.6 | 7.83 | 0.001 |
| Vitamin B ₁₂ | | | | |
| Intake ⁴ | 0 | 0 | 0 | — |
| Duodenal flow | 11.9 | 12.5 | 0.55 | 0.26 |
| ARS | 11.9 | 12.5 | 0.55 | 0.26 |

¹Data are LSM.²Sum of nicotinic acid and nicotinamide.³Sum of pyridoxamine, pyridoxal, and pyridoxine.⁴Not detected.**Table 4.** Duodenal flow and apparent ruminal synthesis (ARS) of B vitamins, expressed as milligram per kilogram of DMI, in response to low-forage and high-forage diets¹

| Item | Treatment | | SEM | P-value |
|-------------------------------------|------------------|-----------------|-------|---------|
| | High-forage diet | Low-forage diet | | |
| Thiamine | | | | |
| Duodenal flow | 1.23 | 1.23 | 0.009 | 0.97 |
| ARS | -0.26 | -0.87 | 0.092 | 0.0005 |
| Riboflavin | | | | |
| Duodenal flow | 36.36 | 24.00 | 1.110 | 0.14 |
| ARS | 3.92 | 8.09 | 1.117 | 0.02 |
| Niacin ² | | | | |
| Duodenal flow | 125.52 | 140.15 | 8.509 | 0.05 |
| ARS | 78.32 | 102.07 | 8.495 | 0.004 |
| Vitamin B ₆ ³ | | | | |
| Duodenal flow | 2.83 | 3.22 | 0.138 | 0.05 |
| ARS | -4.00 | -2.58 | 0.138 | <0.0001 |
| Folates | | | | |
| Duodenal flow | 3.18 | 3.87 | 0.262 | 0.008 |
| ARS | 2.61 | 3.34 | 0.262 | 0.006 |
| Vitamin B ₁₂ | | | | |
| Duodenal flow | 0.50 | 0.46 | 0.017 | 0.02 |
| ARS | 0.50 | 0.46 | 0.017 | 0.02 |

¹Data are LSM.²Sum of nicotinic acid and nicotinamide.³Sum of pyridoxamine, pyridoxal, and pyridoxine.

ages, combined with a reduced DMI when feeding HF diet, changes in dietary forage content affected both dietary concentrations (Table 1) and intake of thiamine, which was greater ($P < 0.0001$; Table 3) with LF than HF diets. Similar results were observed by Miller et al. (1986) and Schwab et al. (2006). Reducing dietary forage concentration increased ($P \leq 0.0005$) apparent degradation of the vitamin in the rumen but had no effect on the amount of thiamine reaching the small intestine ($P \geq 0.2$; Tables 3 and 4). As observed in the present experiment, Miller et al. (1986) also reported that degradation of thiamine in the rumen was greater in steers fed a high-grain diet (89% corn-11% alfalfa meal) as compared with a low-grain diet (30% corn-70% alfalfa meal) without an effect on DF. Consequently, as previously observed by Miller et al. (1986) and Castagnino et al. (2016a,b,c), thiamine ARS was negatively correlated with its intake in the present experiment (Table 5). On the contrary, Schwab et al. (2006), in spite of dietary concentrations of thiamine (1.5 to 2.1 mg/kg of DM) similar to those measured in the present experiment, observed an apparent synthesis of the vitamin in the rumen. Moreover, in this latter experiment, the amount of thiamine apparently synthesized in the rumen was

not affected by the F:C, resulting in a greater DF of this vitamin with a 35% forage diet as compared with a 60% forage diet. Nevertheless, it seems that diet composition could affect the fate of thiamine in the rumen. For example, Schwab et al. (2006) reported thiamine ARS for cows fed diets providing NDF and starch at levels of 35 to 47% and 5 to 21% of DM, respectively. However, as observed in the present experiment, Miller et al. (1986) and Castagnino et al. (2016b,c) observed an apparent degradation of thiamine in rumen of animals fed diets with lower NDF contents (8–31% of DM) and greater starch concentrations (23–67% of DM). Comparing 4 diets with different proportions of NDF and starch, although the differences were less marked than in the experiments previously cited, Castagnino et al. (2016a) observed thiamine ARS only when cows were fed the diet with the highest NDF and the lowest starch contents.

Thiamine ARS (mg/d) was correlated positively with ruminal pH and negatively with the amount of OM ruminally digested and microbial flow of N (Table 5; Figure 1), contrary to the findings of Schwab et al. (2006). Breves et al. (1981) also reported positive correlations between thiamine DF and microbial flow of N and the

Table 5. Pearson correlation coefficients between apparent ruminal synthesis and dietary and digestive parameters

| Item | B-vitamin apparent ruminal synthesis (mg/d) | | | | | |
|---------------------------------------|---|------------|---------------------|-------------------------------------|----------|-------------------------|
| | Thiamine | Riboflavin | Niacin ¹ | Vitamin B ₆ ² | Folates | Vitamin B ₁₂ |
| Intake (kg/d) | | | | | | |
| DM | -0.55** | NS | 0.63*** | NS | 0.57** | 0.60*** |
| OM | -0.57** | NS | 0.64*** | NS | 0.58** | 0.60*** |
| NDF | NS | NS | NS | -0.59*** | NS | 0.39* |
| Starch | -0.76*** | 0.50** | 0.63*** | 0.53** | 0.61*** | 0.42* |
| N | -0.50** | NS | 0.62*** | NS | 0.59*** | 0.61*** |
| Intake of vitamin ³ (mg/d) | -0.74*** | NS | NS | -0.41* | 0.48** | — ⁴ |
| Ruminally digested (kg/d) | | | | | | |
| True OM | -0.34† | NS | 0.49** | -0.33† | 0.35† | 0.39* |
| NDF | 0.44* | -0.53** | -0.35† | -0.68*** | NS | NS |
| Starch | -0.66*** | 0.49* | 0.80*** | NS | 0.68*** | 0.43* |
| Ruminal measure | | | | | | |
| VFA (mM) | | | | | | |
| Total | NS | 0.37† | 0.45* | NS | 0.60*** | 0.49** |
| Acetate | NS | NS | NS | NS | NS | NS |
| Propionate | -0.48** | 0.43* | 0.71*** | 0.46* | 0.83*** | 0.48** |
| Butyrate | NS | NS | NS | 0.35† | 0.41* | 0.34† |
| pH | 0.38* | NS | -0.45* | -0.37† | -0.60*** | -0.65*** |
| Flow to duodenum | | | | | | |
| DM (kg/d) | -0.53** | 0.44* | 0.54** | NS | 0.56** | 0.54** |
| Microbial N (g/d) | -0.42* | 0.50** | 0.51** | NS | 0.54** | 0.51** |
| Passage rate (%/h) | | | | | | |
| Indigestible NDF | NS | NS | 0.39* | NS | NS | 0.52** |

¹Sum of nicotinic acid and nicotinamide.

²Sum of pyridoxamine, pyridoxal, and pyridoxine.

³Intake of the corresponding B vitamin.

⁴Not calculated.

NS, $P \geq 0.10$; † $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

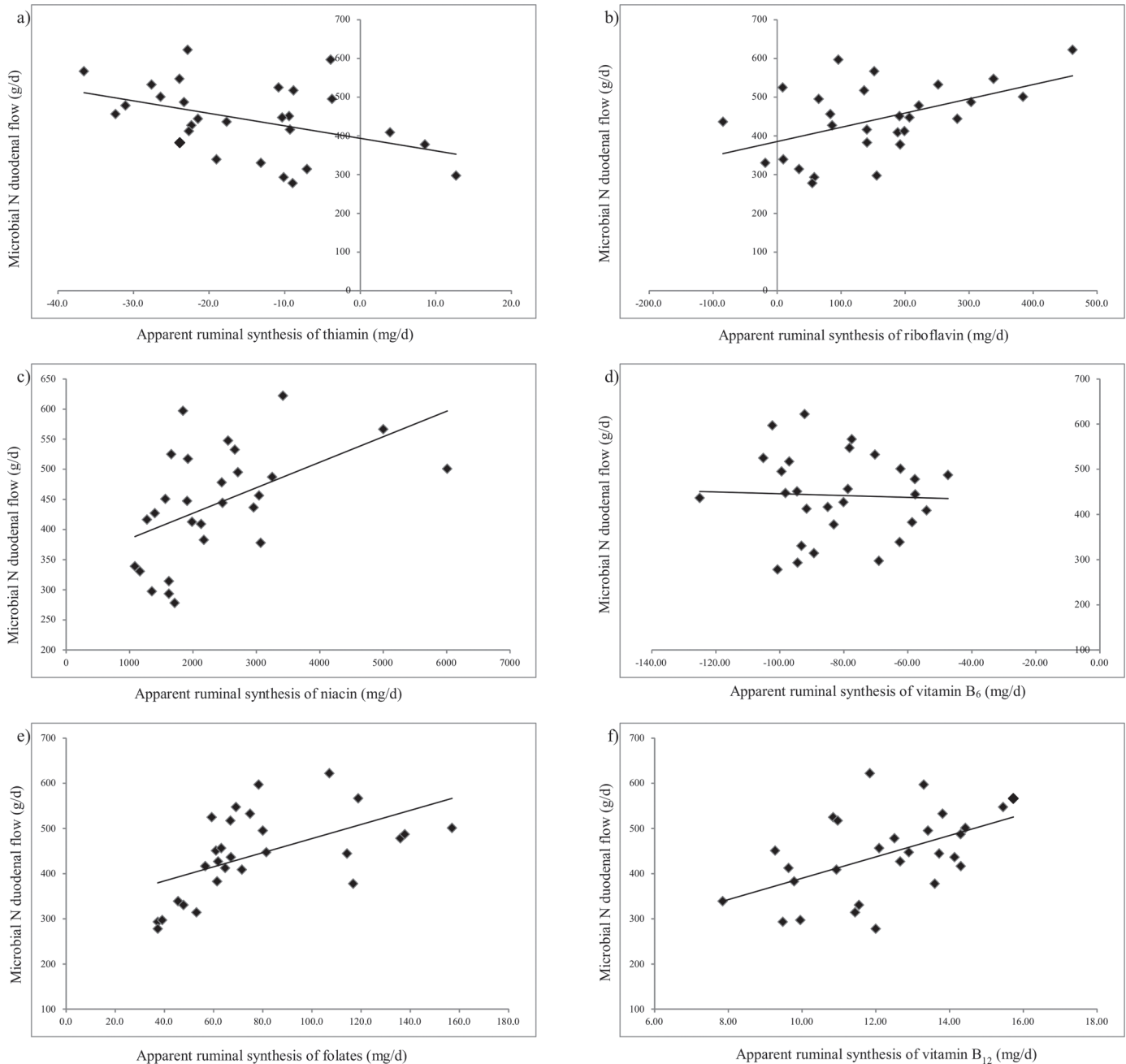


Figure 1. Regression curves between microbial N duodenal flow (g/d) and apparent ruminal synthesis (mg/d) of (a) thiamine, $r = -0.42^*$; (b) riboflavin, $r = 0.50^{**}$; (c) niacin, $r = 0.51^{**}$; (d) vitamin B₆, $r = -0.04$; (e) folates, $r = 0.54^{**}$; and (f) vitamin B₁₂, $r = 0.51^{**}$ ($^*P < 0.05$; $^{**}P < 0.01$).

proportion of OM digested in the total digestive tract. In the present experiment, starch intake, ruminally digested starch, as well as ruminal concentration of propionate were negatively correlated with thiamine ARS. Using ingredients similar to those used in the present experiment, Castagnino et al. (2016b,c) also observed a negative correlation between the vitamin ARS and the amounts of OM and ruminally digested starch.

Riboflavin

The forages were the main sources of riboflavin (Table 2), confirming observations from previous reports (Schwab et al., 2006; Beaudet et al., 2016; Castagnino et al., 2016a,b,c). Indeed, in the present experiment, 76 and 83% of dietary riboflavin were provided by forages in LF and HF diets, respectively. Due to the

high riboflavin concentrations in forages, the HF diet increased ($P < 0.0001$) riboflavin intake (Table 3) despite a reduced DMI as compared with the LF diet. Similar results were reported by Miller et al. (1986) and Schwab et al. (2006). Furthermore, as observed by these 2 groups of authors, the F:C had no effect on riboflavin DF. Hunt et al. (1941), Conrad and Hibbs (1954), and Hollis et al. (1954) observed that increasing the proportion of concentrate in diets increased ruminal concentration of riboflavin in steers, whereas Hayes et al. (1966) found no effect. More recently, Santschi et al. (2005) reported higher riboflavin concentration in solid-associated bacteria when cows were fed a high-concentrate diet, whereas it had no effect on riboflavin ARS according to Schwab et al. (2006). In the current experiment, however, increasing the proportion of dietary concentrate increased ($P \leq 0.02$; Tables 3 and 4) riboflavin ARS.

Riboflavin ARS was correlated positively with starch intake, ruminally digested starch, ruminal concentration of propionate, and microbial flow of N (Table 5; Figure 1) in accordance with the findings of Schwab et al. (2006). Contrary to the current experiment, however, Schwab et al. (2006) did not observe a negative correlation with the amount of NDF ruminally digested. Finally, no correlation was found between the amount of riboflavin ingested and its ARS.

Niacin

Niacin concentration in SoyPlus (West Central Soy Cooperative, Ralston, IA) was greater in period 1 than in period 2 (135 vs. 74 mg/kg), respectively. Nevertheless, in both periods, SoyPlus followed by alfalfa and corn silages had the highest concentrations of niacin among the feedstuffs used in the current experiment (Table 2). These 3 ingredients represented 70.5% of the HF diet as compared with 50.7% of the LF diet. Such variation in experimental TMR composition may explained why increasing dietary forage enhanced ($P = 0.001$) daily intake of niacin from 1,035 to 1,135 mg/d (Table 3), even if DMI was lower for cows fed the HF diet. Daily intake of niacin reported in the literature (Schwab et al., 2006; Niehoff et al., 2013; Beaudet et al., 2016; Castagnino et al., 2016a,b,c) varies greatly, ranging from 325 to 4,434 mg/d, and reflects differences in dietary concentrations of this vitamin, from 22 to 170 mg/kg of DM. As stated by Niehoff et al. (2013), niacin concentrations in forages are highly variable.

Duodenal flow and ARS of niacin (mg/d) were increased ($P < 0.005$) respectively by 28 and 50% with LF as compared with HF. When expressed as milligrams per kilogram of DMI, to take into account the greater DMI when cows were fed the LF diet, DF and ARS

were still increased by 12 and 30%, respectively, when feeding the LF diet. Niehoff et al. (2013) also observed an increase in niacin DF and ARS when increasing the proportion of concentrate in the diet from 33 to 50%, but increasing this proportion to 66% had no further effect. Schwab et al. (2006) reported similar findings regarding niacin DF, which decreased by 38% when the dietary forage increased from 35 to 60%, although the proportion of forage had no effect on niacin ARS. These results are consistent with experiments (Hollis et al., 1954; Hayes et al., 1966) showing an augmentation of the ruminal concentration of niacin in steers and sheep when the level of concentrate in the diet increased, although Santschi et al. (2005) observed no effect of a change in dietary forage concentration on niacin concentrations in ruminal bacteria and fluid of dairy cows.

Duodenal flow of microbial N, ruminal concentration of propionate, and amount of ruminally digested starch were correlated positively with niacin ARS, supporting previous findings from Schwab et al. (2006). Mean ruminal pH was correlated negatively and amount of ruminally digested OM was positively correlated with niacin ARS, in contrast to the aforementioned authors who reported no correlation. Finally, niacin ARS was not correlated with the intake of the vitamin (Table 5; Figure 1).

Vitamin B₆

Dietary vitamin B₆ concentration was 17% higher in HF than in LF (Table 1) due the high concentrations of this vitamin in alfalfa silage (Table 2), but HF only tended to increase ($P = 0.08$) vitamin B₆ intake (Table 3) because the decrease in DMI with HF partially counterbalanced for the higher dietary vitamin concentration. However, LF reduced ($P < 0.0001$) vitamin B₆ ruminal degradation compared with HF, which increased ($P \leq 0.05$) vitamin B₆ DF (Tables 3 and 4) as observed by Schwab et al. (2006).

However, Schwab et al. (2006) observed positive vitamin B₆ ARS without an effect of the F:C. Apparent ruminal degradation of vitamin B₆ has already been observed with diets using ingredients similar to the present experiment (Castagnino et al., 2016a,b,c).

Contrary to what was observed for thiamine, riboflavin, and niacin, but as observed by Schwab et al. (2006), no correlation was found between vitamin B₆ ARS and microbial flow of N (Table 5; Figure 1). Mean ruminal pH, as well as the amounts of ruminally digested OM and NDF, were correlated negatively with vitamin B₆ ARS, whereas no correlation among these variables were reported by Schwab et al. (2006). Finally, vitamin B₆ intake was negatively correlated with its ARS.

Folates

It is noteworthy that folate concentration was very low in corn silage, whereas the highest concentrations were measured in soybean meal and SoyPlus (Table 2). These last 2 ingredients provided 63 and 66% of folates in LF and HF diets, respectively. Consequently, daily intake of folates was greater ($P = 0.007$) with the LF diet due to the greater DMI. As observed previously by Schwab et al. (2006), reducing the proportion of forage in the current experiment increased ($P \leq 0.008$) folate DF and ARS (Tables 3 and 4).

As reported by Schwab et al. (2006), intakes of DM, OM, and starch, amounts of OM and starch digested in the rumen, concentrations of total VFA and propionate in the rumen, and DF of microbial N were positively correlated with folate ARS, whereas the correlation with the ruminal pH was negative (Table 5; Figure 1). Finally, folate ARS was positively correlated with the amount of this vitamin ingested.

Vitamin B₁₂

No vitamin B₁₂ was detected in the studied diets in accordance with the very low concentrations reported, for example, by Schwab et al. (2006) and Castagnino et al. (2016c). As a consequence, vitamin B₁₂ DF and ARS were identical. When reported on a daily basis (Table 3), no effect ($P = 0.26$) was found of the F:C on vitamin B₁₂ ARS. However, contrary to what was observed with all the other studied B vitamins, except thiamine, increasing dietary forage increased ($P = 0.02$) vitamin B₁₂ ARS when expressed on a DMI basis (Table 4). Similarly, Sutton and Elliot (1972) observed a 36% decline in ruminal production of the active form of vitamin B₁₂ in sheep when the F:C dropped from 70:30 to 40:60, whereas Santschi et al. (2005) reported a decrease of vitamin B₁₂ concentrations in solid-associated bacteria with a LF diet. In contrast with these findings, Kon and Porter (1953) and Hayes et al. (1966) observed a reduction of vitamin B₁₂ concentration in ruminal fluid of steers when the proportions of dietary forages were increased. Schwab et al. (2006) also reported higher daily vitamin B₁₂ ARS with a 35% forage diet as compared with a 60% forage diet. According to the latter, LF diets provided more dietary sugars, nutrients positively correlated with vitamin B₁₂ ARS. Moreover, Stewart and Bryant (1988) reported that *Selenomonas* bacteria, which synthesized large quantities of vitamin B₁₂, used sugars as fermentative substrates.

Positive correlations were observed between vitamin B₁₂ ARS and (1) the amounts of DM, OM, and NDF ingested, (2) the amount of OM digested in rumen, and (3) DF of microbial N, and a negative correlation was

observed with ruminal pH (Table 5; Figure 1). Similar relationships have also been reported by Schwab et al. (2006). However, in the present experiment, a positive correlation was found between the amount of starch digested in rumen but no correlation with ruminal digestion of NDF as opposed to Schwab et al. (2006).

In summary, in the present experiment, increasing dietary forage content from 45 to 61% increased intakes of riboflavin, niacin, and vitamin B₆ by 11, 10, and 4%, respectively, whereas thiamine and folate intakes were reduced by 59 and 7%, respectively. Meanwhile, the HF diet increased the degradation of vitamin B₆ and reduced the apparent synthesis of riboflavin, niacin, and folates. Increasing forage supply had no effect on vitamin B₁₂ ARS but reduced apparent degradation of thiamine. Consequently, increasing the F:C had no effect on the amounts of thiamine, riboflavin, and vitamin B₁₂ reaching the small intestine but decreased niacin, vitamin B₆, and folate DF. By comparison, Schwab et al. (2006) observed that increasing the proportion of forage in the diet from 35 to 60% reduced thiamine, niacin, and vitamin B₆ intakes, increased riboflavin intake and had no effect on folate intake. Meanwhile, increasing the F:C only decreased folate and vitamin B₁₂ ARS, consequently reducing the amounts of all studied B vitamins, except riboflavin, reaching the small intestine (Schwab et al., 2006). As emphasized by differences in vitamin intake between both studies, it is noteworthy that chemical composition of the studied diets differs between these 2 experiments. Schwab et al. (2006) compared 2 low-forage (35% forage) and 2 high-forage (60% forage) diets differing in their proportions of NFC, 30 versus 40%. Within each concentration of NFC, low and high forage diets have similar NDF and starch values: 47.4 and 5.2 versus 45.3 and 7.7 for diets with 30% NFC and 36.9 and 18.9 versus 34.8 and 20.7 for diets with 40% NFC. In the present study, however, both experimental diets had lower NDF (24.4 vs. 30.7% for the low and high forage diets, respectively) and higher starch (32.8 and 22.5%) contents than the experimental diets studied by Schwab et al. (2006). Therefore, based on this comparison as well as on those described in the previous sections, the F:C did not seem to be a good predictor of the amounts of B vitamins available for absorption in dairy cow. Indeed, diets with the same F:C can be completely different in terms of nutrient composition, whereas diets formulated with different F:C can have relatively similar nutrient composition. Consequently, and as suggested when comparing correlations between vitamin ARS and nutrient or digestive parameters, for most B vitamins reported by Schwab et al. (2006) and the present experiment, dietary ingredients and nutrient composition are probably more likely to help predict the fate of these vitamins in the rumen

than simply considering the F:C. However, due to the limited information available on the effects of changing the F:C on the fate of B vitamins in the rumen, this hypothesis needs to be confirmed by complementary studies.

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