

## Dietary Forage and Nonfiber Carbohydrate Contents Influence B-Vitamin Intake, Duodenal Flow, and Apparent Ruminal Synthesis in Lactating Dairy Cows

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### ABSTRACT

The objective of this experiment was to quantify intakes, duodenal flows, and ruminal apparent synthesis (AS) of B-vitamins in lactating dairy cows fed diets varying in forage and nonfiber carbohydrate (NFC) contents. Eight (4 primiparous and 4 multiparous) ruminally and duodenally cannulated Holstein cows were assigned to 4 dietary treatments in a replicated 21-d period, 4 × 4 Latin square design with a 2 × 2 factorial treatment arrangement. Diets, fed as TMR, contained (DM basis) 2 levels of forage (35 and 60%) and 2 levels of NFC (30 and 40%). The forage portion of the diets contained 50% corn silage, 33% alfalfa hay, and 17% grass hay. Soybean hulls and beet pulp (2:1) and corn meal and ground barley (2:1) were included to achieve desired NFC concentrations. No supplemental B-vitamins were fed. B-vitamin AS was calculated as the amount of a specific B-vitamin flowing to the duodenum minus its daily orts-corrected intake. Dry matter and organic matter intakes were higher for cows fed the 35% forage diets and the 40% NFC diets. Increasing dietary forage content decreased ruminal AS of pyridoxine, folic acid, and B<sub>12</sub>. Increasing dietary NFC content increased ruminal AS of nicotinic acid, nicotinamide, niacin, pyridoxal, B<sub>6</sub>, and folic acid but decreased AS of B<sub>12</sub>. Across diets, amounts of B-vitamins synthesized were highest for niacin, followed by riboflavin, B<sub>12</sub>, thiamin, B<sub>6</sub>, and folic acid. Biotin AS values were negative for all diets, suggesting either no ruminal synthesis or that destruction by ruminal microflora was greater than synthesis. B-vitamin intake, duodenal flow, and ruminal synthesis are influenced by dietary forage and NFC contents.

**Key words:** B-vitamin, ruminal synthesis, duodenal flow, lactating cow

### INTRODUCTION

Historical B-vitamin research identified that alteration of dietary forage to concentrate ratios (Conrad and Hibbs, 1954), dietary CP source (Hollis et al., 1954), and corn grain processing (Hayes et al., 1966) in ruminating calves, sheep, and steers, respectively, altered ruminal B-vitamin concentrations. Other studies in sheep (Sutton and Elliot, 1972) and steers (Miller et al., 1986; Zinn et al., 1987) indicated dietary effects on amounts of B-vitamins either consumed, flowing to the duodenum, or ruminally synthesized; amounts flowing to the duodenum generally exceeded B-vitamin intakes. Using lactating dairy cattle, Breves et al. (1981) varied ruminal OM digestion and duodenal microbial N flow through dietary interventions to study duodenal thiamin flow. Daily duodenal thiamin flow was related to daily microbial N flow ( $r^2 = 0.85$ ) and amounts of OM digested in the total tract ( $r^2 = 0.87$ ).

Past B-vitamin research led to the general dogma that dietary supply and ruminal synthesis are sufficient to meet dairy cow requirements (NRC, 2001). Although ruminal B-vitamin synthesis appears to be sufficient to prevent clinical deficiencies in most situations, supplementing dietary thiamin (Shaver and Bal, 2000), biotin (Zimmerly and Weiss, 2001; Majee et al., 2003), niacin (French, 2004), and folic acid (Girard and Matte, 1998) increased lactation performance. However, in other studies, lactation performance was not improved by supplemental folic acid (Girard et al., 2005), niacin (NRC, 2001), or biotin (Rosendo et al., 2004). Possible reasons for lack of consistent responses to B-vitamin supplementation are numerous, but a potentially important factor is variable amounts of ruminally synthesized B-vitamins. Data regarding amounts of B-vitamins flowing to the duodenum or ruminally synthesized

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in lactating dairy cows are limited. The NRC (2001) estimates of ruminal B-vitamin synthesis in lactating cows were extrapolated from steer data of Miller et al. (1986) and Zinn et al. (1987) as lactating dairy cow data were not available. The objective of this experiment was to quantify B-vitamin intakes, duodenal flows, and apparent synthesis in lactating dairy cows fed production diets differing in forage and NFC concentrations and to relate B-vitamin apparent synthesis to nutrient intakes and digestion parameters.

## MATERIALS AND METHODS

### Experimental Design and Treatment Diets

Four primiparous ( $574 \pm 59$  kg of BW) and 4 multiparous ( $651 \pm 67$  kg of BW) lactating Holstein dairy cows were fitted with ruminal cannulas (10.2 cm; Bar Diamond, Inc., Parma, ID) and open gutter-style plastisol duodenal cannulas before the start of the experiment with surgical procedures as described by Putnam et al. (1997). Four cows (2 primiparous and 2 multiparous) were cannulated while nonlactating; the remaining cows were cannulated during mid to late lactation. All experimental procedures were approved by the Institutional Animal Care and Use Committee, University of New Hampshire.

The experimental design was a replicated  $4 \times 4$  Latin square. Treatments were in a  $2 \times 2$  factorial arrangement, and periods were 21 d. The first 15 d were used for diet adaptation. The experiment was conducted from February to May 2003. Cows were assigned to concurrently run squares based on similar DIM [ $204 \pm 31$  and  $33 \pm 11$  DIM (mean  $\pm$  SD)], respectively, for squares 1 and 2); each square contained 2 multiparous and 2 primiparous cows. The 4 treatment diets (Tables 1 and 2) contained 35 or 60% forage and 30 or 40% NFC (DM basis). The forage portion of all diets contained (DM basis) 50% corn silage, 33% alfalfa hay, and 17% grass hay. Soybean hulls and beet pulp (2:1) and corn and barley (2:1) were used to formulate for desired NFC concentrations. Soybean meal, blood meal, and urea were used in formulations to meet RDP and RUP recommendations (NRC, 2001). Amounts of the vitamin-mineral premix and calcium monophosphate were adjusted (Table 1) to achieve desired Ca and P concentrations (Table 3).

### Feeding and Management

Diets were mixed once daily at 1600 h in a drum-type mixer (Data Ranger, American Calan Inc., Northwood, NH). Alfalfa and grass hays were chopped (Teagle Tomahawk model 5050, Teagle Machinery Ltd., Blackwater, Truro, UK) before incorporation into the TMR.

**Table 1.** Ingredient composition of the diets

Ingredient	Diets <sup>1</sup>			
	35–30	35–40	60–30	60–40
Forages				
	— % of DM —			
Corn silage	17.5	17.5	30.0	30.0
Alfalfa hay	11.7	11.7	20.0	20.0
Grass hay	5.8	5.8	10.0	10.0
Concentrates				
Corn (fine ground)	0.0	15.9	0.0	14.8
Barley (ground)	0.0	8.0	0.0	7.4
Soybean hulls	34.0	17.6	18.7	2.7
Beet pulp	17.0	8.8	9.3	1.4
Soybean meal	10.2	9.6	6.7	6.9
Blood meal	0.0	0.6	1.8	2.2
Fat <sup>2</sup>	1.6	1.6	1.6	1.6
Urea	0.2	0.2	0.0	0.0
Smartamine M <sup>3</sup>	0.07	0.06	0.08	0.07
Vitamin-mineral premix <sup>4</sup>	1.5	2.7	1.4	3.0
Calcium monophosphate	0.5	0.2	0.4	0.1

<sup>1</sup>35–30 = 35% forage–30% NFC, 35–40 = 35% forage–40% NFC, 60–30 = 60% forage–30% NFC, and 60–40 = 60% forage–40% NFC, where NFC was calculated by difference:  $100 - [\text{CP} + (\text{NDF} - \text{NDICP}) + \text{fat} + \text{ash}]$ . NDICP = Neutral detergent insoluble CP.

<sup>2</sup>Megalac, Church and Dwight Co., Inc., Princeton, NJ.

<sup>3</sup>Adisseo, Alpharetta, GA.

<sup>4</sup>Vitamin-mineral mix contained (% DM): 10% Ca, 8% Mg, 2.5% S, 15.2% Na, 0.2% Zn, 0.2% Mn, 418 mg of Cu/kg, 70.4 mg of Co/kg, 23.5 mg of I/kg, 10.5 mg of Se/kg, 65,300 IU of vitamin A/kg, 15,100 IU of vitamin D/kg, and 249 IU of vitamin E/kg.

Diets were fed for ad libitum intake; amounts offered and refused were recorded daily to maintain approximately 5% orts. Approximately 70% of the daily feed allotment was fed following mixing; the remainder was stored in individual 130-L plastic refuse containers and was fed the following morning at 0400 h. Because of differences in diet composition, cows were acclimated to the dietary treatments during the first 2 d (4 feedings) of each period. At each feeding during the acclimation period, approximately equal proportions of the previous and new treatment diets were combined and fed. Cows were housed in individual tie stalls, had free access to water, and were milked at 0400, 1200, and 2000 h.

All dry dietary ingredients were stored individually in bins or bags, and corn silage was stored in a bunker silo. Soybean hulls and shredded beet pulp were delivered as a mix. Grass hay, ground corn, blood meal, soybean meal, fat, urea, Smartamine M (Adisseo USA, Inc., Alpharetta, GA), and vitamins and minerals fed throughout the experiment were each from a single batch delivered before the start of the experiment. The alfalfa hay, ground barley, and soybean hull-beet pulp mix were each from 2 deliveries received at the beginning of the experiment and before the start of period 4. Individual samples of beet pulp and soybean hulls accompanied each delivery. At the beginning of each

**Table 2.** Nutrient composition of feeds

Nutrient <sup>1</sup>	Corn silage	Grass hay	Alfalfa hay	Corn, ground	Barley, ground	Soybean hulls	Beet pulp	Soybean meal	Blood meal
	% of DM								
CP	8.8	9.7	21.3	8.4	11.7	11.8	10.3	53.9	97.0
ADF	30.4	46.6	27.9	5.2	8.3	48.6	31.8	5.9	—
ADICP	0.8	2.1	1.5	1.2	0.5	1.3	4.4	2.0	2.8
NDF	50.1	63.9	37.7	12.2	23.7	67.8	41.7	8.3	—
NDICP	1.9	4.6	3.7	1.8	2.6	4.9	6.7	2.8	3.8
Lignin	4.6	7.7	8.1	1.9	2.6	2.7	4.0	0.5	—
Fat	3.7	1.9	2.0	4.2	1.3	1.5	0.5	2.0	0.2
NSC	25.2	15.1	9.5	67.7	55.9	5.1	17.7	16.7	7.9
NFC <sup>2</sup>	35.1	23.1	31.7	75.5	63.3	18.9	48.7	32.0	—
Starch	21.6	2.1	1.8	63.6	51.2	1.4	1.4	1.5	0.7
Sugars	3.7	13.1	7.7	4.2	4.7	3.8	16.3	15.2	7.2
Ca	0.4	0.6	1.4	0.02	0.08	0.6	1.1	0.4	0.03
P	0.2	0.2	0.3	0.3	0.5	0.1	0.1	0.8	0.2
Mg	0.2	0.2	0.2	0.1	0.1	0.2	0.2	0.3	0.02
K	1.3	1.3	3.0	0.4	0.6	1.3	0.4	2.2	0.5
S	0.1	0.1	0.3	0.1	0.1	0.1	0.2	0.4	0.5
Ash	4.3	6.0	11.2	1.7	2.6	5.0	5.6	6.7	3.5
mg/kg of DM									
Co	0.12	0.33	0.33	0.03	0.08	0.50	0.35	0.17	0.02
Thiamin	0.57	0.89	1.9	2.7	3.9	1.8	0.62	7.1	0.38
Riboflavin	3.5	9.9	17.5	1.2	1.1	2.4	2.0	4.3	0.62
Nicotinic acid	22.5	11.8	26.4	7.0	18.9	34.2	59.8	16.0	10.1
Nicotinamide	1.5	0.34	7.6	0.0	18.0	194.7	1.1	26.1	35.1
Pyridoxamine	0.24	0.25	0.82	1.6	0.73	0.27	0.0	2.0	0.0
Pyridoxal	0.44	0.15	0.61	1.8	0.28	0.48	5.8	0.84	0.0
Pyridoxine	1.9	1.8	4.5	0.21	0.69	1.2	0.61	1.1	0.0
Biotin	7.3	7.6	7.4	6.4	6.2	7.2	5.7	8.1	6.9
Folates	0.48	0.52	1.6	0.21	0.22	0.66	0.16	1.0	0.0
B <sub>12</sub>	0.03	0.02	0.01	0.01	0.01	0.01	0.03	0.01	0.0

<sup>1</sup>ADICP = Acid detergent insoluble CP; NDICP = neutral detergent insoluble CP; NSC = nonstructural carbohydrate.

<sup>2</sup>NFC calculated by difference: 100 - [CP + (NDF - NDICP) + fat + ash].

period appropriate proportions of fat, urea, Smartamine M, calcium monophosphate, and vitamin-mineral mix were blended on location using a rotary mineral mixer. No supplemental B-vitamins were fed.

Chromic oxide and ammonia <sup>15</sup>N ([<sup>15</sup>NH<sub>4</sub>]<sup>2</sup>SO<sub>4</sub>, 10.6% enriched; Isotec, Miamisburg, OH) were used as digesta and microbial N flow markers, respectively. Separate gelatin capsules (0.5 oz., Tropac, Inc., Fairfield, NJ) containing Cr<sub>2</sub>O<sub>3</sub> (7 g) and (<sup>15</sup>NH<sub>3</sub>)<sub>2</sub>SO<sub>4</sub> (3.33 g) were dosed via the ruminal cannula at 0400, 1200, and 2000 h on d 6 to 20 and 13 to 20, respectively. A 5-g priming dose of (<sup>15</sup>NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> was also given on d 13 at 0400 h.

### Data and Sample Collection

Feed intakes were recorded daily; orts were weighed before the 1600-h feeding. Measurements of DMI taken on d 16 to 20 of each period were used in the statistical analysis. To adjust for changes in ingredient DM, samples of dry feeds were collected on d 12 of each period and dried at 55°C for 48 h in a forced-air

oven. Corn silage DM was determined at least twice weekly (depending on weather conditions) by microwave oven to adjust for changes in DM content. Samples of all feeds (except soybean hulls and beet pulp) were collected at the beginning of wk 3 of each period and dried at 55°C for 48 h in a forced-air oven. All feeds were ground to pass a 1-mm Wiley mill screen (Arthur H. Thomas, Philadelphia, PA) and composited by type for nutrient analysis. Ort samples were collected on d 16 to 20 of each period, dried at 55°C for 48 h in a forced-air oven, ground to pass a 1-mm Wiley mill screen, and composited by cow within period for nutrient analysis.

Milk yields were collected from each milking on d 14 to 20. Milk samples were collected from 2 consecutive 1200-, 2000-, and 0400-h milkings on d 15 to 17. Samples from each of the 3 milkings were composited proportionally by yield and preserved with 2-bromo-2-nitropropane-1, 3-diol.

Ruminal fluid was sampled at 0600, 1000, and 1400 h on d 19 and at 0330, 0800, and 1200 h on d 20 of each period. Rumen fluid samples (approximately 600

**Table 3.** Nutrient composition of consumed diets

Nutrient	Diet <sup>1</sup>			
	35–30	35–40	60–30	60–40
DM, % as-fed	58.5	57.5	54.3	54.3
NE <sub>L</sub> , Mcal/kg of DM <sup>2</sup>	1.41	1.52	1.40	1.51
	% of DM			
OM	92.0	92.3	91.7	91.4
CP	16.4	16.2	16.6	16.3
ADF	33.8	24.3	31.5	22.2
NDF	47.4	36.9	45.3	34.8
NDICP	4.1	3.2	3.6	2.7
NFC	29.1	39.2	29.8	38.9
NSC	13.1	26.1	14.9	27.3
Starch	5.2	18.9	7.7	20.7
Sugars	8.0	7.2	7.2	6.6
Lignin	3.8	3.6	4.6	4.3
Fat	3.1	3.6	3.5	3.9
Ca	1.1	1.0	1.1	1.0
P	0.35	0.36	0.36	0.36
Mg	0.34	0.39	0.31	0.40
K	1.4	1.2	1.6	1.4
S	0.20	0.22	0.21	0.24
	mg/kg of DM			
Co <sup>3</sup>	1.3	1.9	1.2	2.1
Thiamin	1.7	2.1	1.5	1.9
Riboflavin	4.6	4.3	6.2	5.9
Niacin	94.8	59.7	65.6	29.5
Nicotinic acid	29.1	22.0	25.5	18.3
Nicotinamide	65.7	37.7	40.1	11.2
B <sub>6</sub>	3.2	3.2	3.2	3.2
Pyridoxamine	0.4	0.7	0.4	0.7
Pyridoxal	1.3	1.1	0.9	0.7
Pyridoxine	1.5	1.4	1.9	1.8
Biotin	6.3	6.4	6.8	6.8
Folates	0.6	0.5	0.7	0.6
Vitamin B <sub>12</sub>	0.02	0.01	0.02	0.02

<sup>1</sup>35–30 = 35% forage–30% NFC, 35–40 = 35% forage–40% NFC, 60–30 = 60% forage–30% NFC, and 60–40 = 60% forage–40% NFC, where NFC calculated by difference: 100 – [CP + (NDF – NDICP) + fat + ash]. NDICP = neutral detergent insoluble CP.

<sup>2</sup>Calculated from NRC (2001).

<sup>3</sup>Cobalt content not orts-corrected.

mL total) were collected from the caudal and distal areas of the medial and ventral rumen into a 1.2-L tubulated polypropylene flask using manual vacuum through a 1.3-cm polyvinyl chloride pipe. Following manual mixing, a subsample (approximately 200 mL) was immediately filtered through 4 layers of cheesecloth, and pH was measured. Duplicate 1-mL samples were acidified with 20  $\mu$ L of 50% H<sub>2</sub>SO<sub>4</sub> and frozen at –20°C until prepared and analyzed for VFA. Separate 40-mL samples were transferred to polypropylene centrifuge tubes containing 2.4 mL of 6 N HCl and frozen at –20°C until NH<sub>3</sub> analysis.

Samples of ruminal digesta were collected immediately following ruminal fluid sampling. Ruminal digesta (approximately 2 L per sampling time) were collected from 9 locations within the rumen, including 3 samples each from the dorsal, medial, and ventral ar-

eas. Digesta samples were homogenized in a 3.8-L commercial blender (Waring Products Division, New Hartford, CT) for 1 min on low (16,000 rpm). Digesta were manually squeezed through one layer of 59- $\mu$ m Dacron mesh (Sefar America, Inc., Briar Cliff Manor, NY) using a 33.1-L bucket with squeezing basket (Rubbermaid Home Products, Fairlawn, OH), and 1.5 L of strained rumen fluid was retained in 2-L polyethylene bottles containing 15 mL of 50% H<sub>2</sub>SO<sub>4</sub>. Rumen bacteria were isolated by differential centrifugation as described by Whitehouse et al. (1991). Microbial pellets were lyophilized then ground using a commercial coffee bean grinder (Gloria Jeans, Irvine, CA). Rumen bacteria were isolated on d 9 of each period for background <sup>15</sup>N analysis.

Duodenal digesta samples (500 mL per sampling) were collected every 3 h from d 16 to 19 with sampling advanced 1 h/d such that 24 samples were taken for each cow each period, representing every 1 h of a 24-h period. Removal of the duodenal cannula plug often resulted in an initial surge of duodenal digesta. Digesta from an initial surge was discarded; only digesta from subsequent flows were retained (500 mL per sampling time), composited by cow within period, and frozen at –20°C. Digesta composites were thawed during the first week of the following period and homogenized (3.8-L Waring blender) in their entirety while still partially frozen. Composites were continually poured between containers during subsampling to maintain homogeneity. A 1.2-L subsample was lyophilized and ground to pass through a 40- $\mu$ m screen before nutrient analysis. Approximately 250 mL of digesta was strained (59- $\mu$ m Dacron mesh), and duplicate 50-mL samples were retained and frozen (–20°C) until NH<sub>3</sub> analysis. Duodenal digesta were collected on d 9 of each period for background <sup>15</sup>N analysis.

### Analytical Procedures

Milk composites were analyzed by infrared analysis for fat and true protein by (DairyOne Milk Laboratories, Ithaca, NY) using a Foss MilkoScan 4000 (Foss Electric, Hillerød, Denmark). Feeds and composited orts were analyzed for OM (AOAC, 1990) and CP, ADF, acid detergent insoluble CP, NDF, neutral detergent insoluble CP, lignin, fat, nonstructural carbohydrates, starch, sugars, and minerals (Table 2) using wet chemistry (DairyOne Forage Laboratories, Ithaca, NY) procedures. Starch was analyzed using a YSI 2700 Select Biochemistry Analyzer (YSI, Inc., Yellow Springs, OH), and sugars were as described by Hall et al. (1999). Nonfiber carbohydrate was calculated by difference: 100 – [CP + (NDF – NDICP) + fat + ash] where NDICP = neutral detergent insoluble CP. Feeds were

**Table 4.** Intake, duodenal flow, and ruminal digestibility of dietary nutrients and duodenal flow of N fractions in lactating cows fed diets containing either 35 or 60% forage and 30 or 40% NFC<sup>1</sup>

Item	Diet <sup>2</sup>				SEM	Effect		
	35-30	35-40	60-30	60-40		Forage	NFC	INT <sup>3</sup>
	P							
<b>DM</b>								
Intake, kg/d	21.3	22.2	18.1	19.8	1.3	<0.001	0.06	0.57
Flow, kg/d	15.1	15.5	12.5	14.8	0.8	0.004	0.01	0.08
NM <sup>3</sup> flow, kg/d	11.3	11.5	9.6	10.9	0.6	0.02	0.11	0.18
Truly digested kg/d	9.9	10.7	8.5	8.9	0.9	0.02	0.35	0.78
% intake	46.3	47.8	46.3	43.8	2.3	0.39	0.81	0.40
<b>OM</b>								
Intake, kg/d	19.6	20.5	16.6	18.1	1.2	<0.001	0.07	0.61
Flow, kg/d	12.0	12.6	10.1	12.1	0.8	0.02	0.009	0.14
NM flow, <sup>4</sup> kg/d	8.4	8.7	7.3	8.4	0.6	0.09	0.10	0.32
Truly digested kg/d	11.1	11.7	9.3	9.7	0.8	0.008	0.44	0.88
% intake	56.9	57.1	56.0	52.8	2.2	0.26	0.51	0.45
<b>NDF</b>								
Intake, kg/d	10.1	8.2	8.2	6.9	0.5	<0.001	<0.001	0.24
Flow, kg/d	6.1	5.5	5.0	5.1	0.4	0.001	0.26	0.10
Digested kg/d	4.0	2.6	3.3	1.8	0.3	0.03	<0.001	0.90
% intake	39.4	31.3	39.8	25.6	3.2	0.41	0.003	0.34
<b>Starch</b>								
Intake, kg/d	1.1	4.2	1.4	4.1	0.2	0.38	<0.001	0.15
Flow, kg/d	0.6	1.6	0.4	1.5	0.1	0.13	<0.001	0.96
Digested kg/d	0.5	2.5	1.0	2.6	0.2	0.09	<0.001	0.17
% intake	45.9	59.5	69.2	62.1	4.6	0.001	0.36	0.007
<b>N</b>								
Intake, g/d	558	575	480	520	33.7	0.002	0.13	0.52
Flow, g/d	605	585	505	569	37	0.03	0.36	0.09
NH <sub>3</sub> N flow, g/d	32.4	27.0	30.2	29.2	2.2	0.98	0.03	0.13
NANM N flow, <sup>5</sup> g/d	230	201	197	182	17	0.10	0.16	0.65
Microbial N flow g/d	342	357	279	359	27	0.15	0.03	0.13
g/kg of OMTD <sup>6</sup>	31	31	31	39	2.0	0.07	0.07	0.04
<b>Intake, kg/d</b>								
NFC	6.2	8.7	5.4	7.7	0.5	0.001	<0.001	0.76
Nonstructural carbohydrates	2.8	5.8	2.7	5.4	0.3	0.20	<0.001	0.38
Sugars	1.7	1.6	1.3	1.3	0.1	<0.001	0.04	0.28

<sup>1</sup>Calculated by difference: 100 - [CP + (NDF - NDICP) + fat + ash]. NDICP = neutral detergent insoluble CP.

<sup>2</sup>35-30 = 35% forage-30% NFC, 35-40 = 35% forage-40% NFC, 60-30 = 60% forage-30% NFC, and 60-40 = 60% forage-40% NFC (DM basis), where NFC = non-fiber carbohydrates calculated by difference: 100 - [CP + (NDF - NDICP) + fat + ash]. NDICP = neutral detergent insoluble CP.

<sup>3</sup>INT = Interaction of dietary forage and NFC content.

<sup>4</sup>NM = Nonmicrobial.

<sup>5</sup>NANM = Nonammonia, nonmicrobial N.

<sup>6</sup>OMTD = OM Truly digested ruminally, corrected for duodenal microbial OM flow.

analyzed for Co at the University of Wisconsin-Madison Soil and Plant Analysis Laboratory by inductively coupled plasma mass spectrometry using a VG PlasmaQuad PQ2 Turbo Plus (Thermo Electron Corp., Waltham, MA).

Ruminal fluid was analyzed for VFA by GLC (Varian 2100, Sunnyvale, CA), and pH was measured using a portable pH meter (Orion model 230A, pH triode

electrode, Orion Research, Inc., Boston, MA). Ammonia analysis was performed using a gas-ammonium electrode (Orion model 407A meter with 95-12 electrode; Orion Research, Inc.).

Duodenal digesta were analyzed for OM, CP, NDF, and starch (DairyOne). Duodenal Cr content was analyzed by inductively coupled plasma atomic emission spectrophotometry as described by Williams et al.

**Table 5.** Ruminal pH, VFA, and NH<sub>3</sub> N concentrations in lactating cows fed diets containing 35 or 60% forage and 30 or 40% NFC<sup>1</sup>

Item	Diet <sup>2</sup>				SEM	Effect		
	35–30	35–40	60–30	60–40		Forage	NFC	INT <sup>3</sup>
pH <sup>a,b,c</sup>	6.0	6.0	6.3	6.4	0.05	<0.001	0.16	0.89
0 <sup>4</sup>	6.1	6.3	6.5	6.2	0.08			
2	6.0	6.1	6.1	6.3	0.07			
4	6.0	5.9	6.3	6.2	0.07			
6	5.9	5.9	6.2	6.2	0.07			
8	5.9	6.0	6.4	6.4	0.07			
10	6.0	6.0	6.4	6.4	0.07			
Total VFA, mM <sup>a,b</sup>	111.9	104.9	100.3	102.1	5.0	0.02	0.38	0.14
0 <sup>4</sup>	109.6	98.0	91.8	85.1	6.5			
2	108.7	100.0	109.4	111.2	6.5			
4	117.7	106.4	105.9	118.8	6.5			
6	118.6	108.8	103.4	108.8	6.5			
8	114.5	113.2	101.1	97.1	6.5			
10	106.9	103.0	90.1	91.8	6.5			
NH <sub>3</sub> N, mg/dL <sup>a,b</sup>	12.2	11.4	10.5	11.6	0.6	0.09	0.70	0.04
	mol/100 mol							
Acetate <sup>a</sup> (A)	63.9	61.3	66.7	64.1	0.7	<0.001	<0.001	0.99
Propionate <sup>a,b</sup> (P)	20.6	22.9	18.7	19.9	0.4	<0.001	<0.001	0.10
Butyrate <sup>a</sup>	11.7	11.9	10.7	11.3	0.4	0.02	0.24	0.57
Others <sup>5</sup>	3.8	3.9	3.8	4.7	0.2	0.05	0.02	0.07
A:P <sup>a,b</sup>	3.1:1	2.7:1	3.6:1	3.3:1	0.1	<0.001	<0.001	0.61

<sup>1</sup>Calculated by difference: 100 – [CP + (NDF – NDICP) + fat + ash]. NDICP = neutral detergent insoluble CP.

<sup>2</sup>35–30 = 35% forage–30% NFC, 35–40 = 35% forage–40% NFC, 60–30 = 60% forage–30% NFC, and 60–40 = 60% forage–40% NFC (DM basis).

<sup>3</sup>INT = Interaction of dietary forage and NFC content.

<sup>4</sup>Hour relative to 0400-h feeding.

<sup>5</sup>Others = isobutyrate, isovalerate, valerate.

<sup>a</sup>Repeated measures effect: h ( $P < 0.01$ ).

<sup>b</sup>Repeated measures effect: forage × h ( $P < 0.05$ ).

<sup>c</sup>Repeated measures effect: NFC × h ( $P < 0.05$ ).

(1962) using a Varian Vista AX-CCD (Varian Instruments, Mulgrave, Australia). Duodenal samples were analyzed in triplicate for DM (100°C, forced-air oven to constant weight). Ammonia analysis was performed as described for ruminal fluid. Duodenal digesta and bacterial samples to be used for <sup>15</sup>N analysis were placed in 8- × 5-mm tin capsules (EMAL-Tec USA, Mason, OH), treated with 50 µL of K<sub>2</sub>CO<sub>3</sub> (10 g/L), and placed in a 60°C oven overnight to evaporate residual ammonia. Samples were analyzed for total N and <sup>15</sup>N at the Stable Isotope Facility (University of California, Davis) by isotope ratio mass spectrophotometry using a Europa Scientific Integra (PDZ Europa, Cheshire, England).

Assays for feeds, orts, and duodenal digesta B-vitamin concentrations were developed by the laboratory of C. L. Girard and conducted as described by Santschi et al. (2005a). Thiamin, riboflavin, niacin (nicotinic acid and nicotinamide), and B<sub>6</sub> (pyridoxamine, pyridoxal, and pyridoxine) were analyzed by HPLC. Biotin was analyzed by enzyme-linked immunosorbent

assay. Pancreatic extract (pancreas acetone powder, bovine, Sigma, St. Louis, MO) was added to each sample to release bound biotin for analysis; samples were corrected for pancreatic extract biotin concentrations. Foliates and true B<sub>12</sub> were analyzed by radioassay as described by Girard et al. (1994) and Santschi et al. (2005a).

### Calculations and Statistical Analyses

Apparent synthesis (AS) of B-vitamins was calculated as the daily amount of a specific B-vitamin flowing to the duodenum minus its daily orts-corrected intake. This calculation does not reflect net B-vitamin synthesis, as it ignores ruminal degradation, microbial use, or potential absorption across the rumen wall. Niacin AS and flow were calculated as the sum of nicotinic acid (NA) and nicotinamide (NAM). Vitamin B<sub>6</sub> AS and flow were calculated as the sum of pyridoxamine (PAM), pyridoxal (PAL), and pyridoxine (PYR).

All intake, duodenal flow, and production-related data were reduced to cow-period means ( $n = 32$ ) prior to statistical analysis. Data were analyzed as a replicated Latin square using the mixed procedure of SAS (2002). The model included period, square, forage, NFC, and forage  $\times$  NFC interaction as fixed effects; cow within square was a random effect. Ruminal pH ( $n = 184$ ), VFA ( $n = 192$ ), and  $\text{NH}_3$  N ( $n = 192$ ) measurements were analyzed with time as repeated measures using the first-order autoregressive covariance structure, which provided the best fit according to Sawa's Bayesian information criterion. The model included period, square, forage, NFC, hour, interactions for forage  $\times$  NFC, forage  $\times$  hour, NFC  $\times$  hour, and NFC  $\times$  forage  $\times$  hour as fixed effects; cow within square and forage  $\times$  NFC  $\times$  period  $\times$  cow within square were random effects. Degrees of freedom were calculated using the Kenward-Roger option. Because of meter malfunction, pH measurements at 0330 h during period 4 were not made and, thus, were included in the statistical analysis as missing observations. Square  $\times$  treatment interactions were originally evaluated but were removed from statistical models because they were not significant. Pearson correlation coefficients were determined between cow-period B-vitamin AS observations and some parameters. Ruminal pH, VFA, and  $\text{NH}_3$  N measures were averaged across time before correlation analysis. Main effects of forage, NFC, the forage  $\times$  NFC interaction, and correlations were considered significant at  $P < 0.05$ , and tendencies were considered at  $P < 0.10$ .

## RESULTS

Diet ingredient composition, feedstuff nutrient composition, andorts-adjusted diet nutrient composition are presented in Tables 1, 2, and 3, respectively. Dietary NFC contents were similar to formulation targets. Dietary Co concentrations averaged 1.6 mg/kg of DM and were reflective of dietary proportions of the vitamin-mineral premix. Because Co concentrations were approximately 15-fold higher than NRC (2001) guidelines of 0.11 mg/kg of DM, it is unlikely that variation among diets would influence  $\text{B}_{12}$  synthesis.

Milk yields (data not shown in tables) were 32.7, 35.1, 29.3, and 32.1 kg/d (SEM = 1.9) for cows fed the 35% forage–30% NFC, 35% forage–40% NFC, 60% forage–30% NFC, and 60% forage–30% NFC diets, respectively. Average milk yields were 3.2 and 2.6 kg/d higher ( $P < 0.05$ ) for cows fed the 35% forage–40% NFC diets, respectively. Milk fat and true protein contents were 3.58 and 2.89, 3.33 and 2.88, 3.80 and 2.84, and 3.62 and 2.83% for the 35% forage–30% NFC, 35% forage–40% NFC, 60% forage–30% NFC, and 60% for-

age–30% NFC diets, respectively (SEM = 0.13 and 0.05, respectively). Increasing dietary forage increased ( $P = 0.003$ ) milk fat and decreased ( $P = 0.04$ ) milk true protein contents. Increasing dietary NFC decreased ( $P = 0.008$ ) milk fat content. Milk fat yield and milk true protein content were higher ( $P = 0.02$ ) and lower ( $P = 0.01$ ), respectively, for cows in the earlier lactation square.

Daily intakes; ruminal digestibility; duodenal flow of DM, OM, NDF, and starch; daily intakes of NFC, nonstructural carbohydrates (NSC), and sugars; and duodenal N fractions are presented in Table 4. Increasing dietary forage from 35 to 60% decreased DM, OM, NDF, NFC, N, and sugar intakes as well as amounts of DM, OM, and NDF ruminally digested. Increasing dietary NFC from 30 to 40% increased NFC, NSC, and starch intakes and amount of starch ruminally digested; NDF and sugar intakes and amounts of NDF ruminally digested were decreased. Duodenal  $\text{NH}_3$  N and microbial N flow were lower and higher, respectively, for cows fed 40% NFC diets. Increasing NFC had no effect on microbial N flow expressed as grams per kilogram of OM truly digested in the rumen (OMTD) for cows fed 35% forage diets, although it was increased for cows fed 60% forage diets (forage  $\times$  NFC;  $P = 0.04$ ). Microbial N efficiency was higher ( $P = 0.01$ ) for cows in the earlier lactation square. Organic matter digestion and N fraction flow values in this experiment are within the ranges presented by Clark et al. (1992).

Effects of dietary forage and NFC content on ruminal pH, VFA, and  $\text{NH}_3$  N concentrations are presented in Table 5. Increasing dietary forage increased ruminal pH and ruminal acetate molar proportions and decreased total VFA concentrations and ruminal propionate and butyrate molar proportions. Increasing dietary NFC decreased ruminal acetate and increased ruminal propionate molar proportions. Increasing both dietary forage and NFC increased the sum of ruminal isobutyrate, isovalerate, and valerate molar proportions. Increasing NFC decreased ruminal  $\text{NH}_3$  N for cows fed 35% forage diets and increased ruminal  $\text{NH}_3$  N for cows fed 60% forage diets (forage  $\times$  NFC;  $P = 0.04$ ).

Effects of dietary forage and NFC content on B-vitamin intake, flow, and AS are presented in Table 6. Daily B-vitamin AS was expressed as milligrams per kilogram of OM intake (OMI) per day to account for differences in feed intake and as milligrams per kilogram of OMTD for a measure of AS efficiency (Table 7). Differences observed in individual B-vitamin intakes coincided with differences in DMI and dietary B-vitamin concentrations. Except for PYR and biotin, duodenal flows of B-vitamins were higher than intake, indi-

**Table 6.** B-vitamin intakes,<sup>1</sup> duodenal flows, and apparent synthesis (AS) in lactating cows fed diets containing 35 or 60% forage and 30 or 40% NFC<sup>2</sup>

Item	Diet <sup>3</sup>				SEM	Effect		
	35–30	35–40	60–30	60–40		Forage	NFC	INT <sup>4</sup>
	mg/d					<i>P</i>		
Thiamin								
Intake	36.3	46.3	27.0	37.0	2.4	<0.001	<0.001	0.99
Duodenal flow	96.9	94.2	70.7	86.9	6.9	0.005	0.21	0.09
AS	60.6	47.9	43.8	50.0	5.3	0.13	0.38	0.06
Riboflavin								
Intake	98.2	96.1	111.4	117.2	7.1	<0.001	0.65	0.33
Duodenal flow	344.0	350.0	317.1	362.9	24.9	0.70	0.17	0.28
AS	245.9	253.9	205.7	245.6	19.6	0.16	0.16	0.34
NA <sup>5</sup>								
Intake	620	489	462	363	31	<0.001	<0.001	0.38
Duodenal flow	1209	1504	1016	1134	143	0.06	0.15	0.53
AS	589	1015	555	771	133	0.31	0.03	0.44
NAM <sup>5</sup>								
Intake	1399	838	727	221	56	<0.001	<0.001	0.46
Duodenal flow	1256	1370	892	837	106	<0.001	0.77	0.40
AS	-143*	532	165*	615	99	0.06	<0.001	0.27
Niacin								
Intake	2019	1327	1187	584	85	<0.001	<0.001	0.42
Duodenal flow	2464	2874	1908	1970	221	0.003	0.27	0.42
AS	446	1547	720	1386	196	0.78	<0.001	0.28
PAL <sup>5</sup>								
Intake	27.4	23.6	16.6	13.4	1.3	<0.001	<0.001	0.73
Duodenal flow	30.8	36.7	25.6	27.3	2.3	0.001	0.03	0.82
AS	3.4*	13.0	6.0	13.9	2.3	0.42	<0.001	0.69
PAM <sup>5</sup>								
Intake	8.6	14.9	7.7	13.5	0.8	0.03	<0.001	0.62
Duodenal flow	50.1	55.9	44.7	52.7	3.9	0.12	0.02	0.69
AS	41.5	41.0	37.0	39.2	3.3	0.21	0.72	0.59
PYR <sup>5</sup>								
Intake	30.9	30.3	35.1	36.0	2.3	0.002	0.91	0.60
Duodenal flow	9.1	6.1	5.9	9.3	1.5	0.98	0.88	0.05
AS	-21.8	-24.2	-29.2	-26.7	2.7	0.03	0.98	0.27
B <sub>6</sub>								
Intake	66.9	68.9	59.4	62.9	4.2	0.01	0.29	0.77
Duodenal flow	89.9	98.7	73.2	89.3	5.9	0.01	0.02	0.44
AS	23.0	29.8	13.8	26.5	4.7	0.15	0.03	0.49
Biotin								
Intake	134.4	141.1	122.3	133.8	8.8	0.09	0.11	0.66
Duodenal flow	119.5	130.4	106.8	131.2	8.7	0.37	0.01	0.31
AS	-14.9	-10.7*	-15.5	-2.6*	6.3	0.56	0.19	0.50
Folates								
Intake	13.7	12.2	12.4	12.6	0.9	0.88	0.73	0.52
Duodenal flow	28.9	32.4	25.4	29.0	2.4	0.04	0.04	0.98
AS	16.3	20.2	13.0	16.4	2.0	0.04	0.04	0.86
B <sub>12</sub>								
Intake	0.4	0.3	0.3	0.3	0.02	0.18	0.14	0.35
Duodenal flow	102.6	79.0	79.7	60.4	5.9	<0.001	<0.001	0.53
AS	102.2	78.6	78.4	60.1	5.9	<0.001	<0.001	0.54

<sup>1</sup>Orts-corrected.<sup>2</sup>Calculated by difference: 100 - [CP + (NDF - NDICP) + fat + ash]. NDICP = neutral detergent insoluble CP.<sup>3</sup>35–30 = 35% forage–30% NFC, 35–40 = 35% forage–40% NFC, 60–30 = 60% forage–30% NFC, and 60–40 = 60% forage–40% NFC (DM basis).<sup>4</sup>INT = Interaction of dietary forage and NFC content.<sup>5</sup>NA = Nicotinic acid, NAM = nicotinamide, PAL = pyridoxal, PAM = pyridoxamine, and PYR = pyridoxine.\*Value not different from zero ( $P > 0.05$ ).

cating ruminal production. Increasing NFC tended (forage × NFC;  $P = 0.06$ ) to decrease daily thiamin AS (expressed as mg or mg/kg of OMTD) for cows fed 35%

forage diets; the opposite effect occurred for cows fed 60% forage diets. Daily niacin AS (mg, mg/kg of OMI, or mg/kg of OMTD) was higher for cows fed 40% NFC

**Table 7.** Daily B-vitamin apparent synthesis (AS) [expressed as mg/kg of OM intake (OMI) and mg/kg of OM truly digested ruminally (OMTD)] in lactating cows fed diets containing either 35 or 60% forage and 30 or 40% NFC<sup>1</sup>

Item	Diet <sup>2</sup>				SEM	Effect		
	35–30	35–40	60–30	60–40		F	NFC	INT <sup>3</sup>
						<i>P</i>		
Thiamin AS								
mg/kg of OMI	3.2	2.3	2.6	2.7	0.3	0.67	0.12	0.08
mg/kg of OMTD	5.7	4.1	4.7	5.2	0.5	0.95	0.32	0.06
Riboflavin AS								
mg/kg of OMI	12.6	12.4	12.5	13.6	0.8	0.45	0.58	0.39
mg/kg of OMTD	22.8	21.9	22.7	27.0	2.2	0.26	0.45	0.24
Niacin AS								
mg/kg of OMI	25.2	73.2	43.9	78.7	10.2	0.25	<0.001	0.53
mg/kg of OMTD	47.2	130.1	81.5	162.2	22.2	0.15	0.002	0.96
B <sub>6</sub> AS								
mg/kg of OMI	1.2	1.5	0.8	1.5	0.3	0.42	0.05	0.33
mg/kg of OMTD	2.3	2.5	1.5	2.9	0.5	0.66	0.09	0.25
Folates AS								
mg/kg of OMI	0.8	1.0	0.8	0.9	0.1	0.51	0.16	0.93
mg/kg of OMTD	1.5	1.7	1.4	1.8	0.2	0.96	0.19	0.64
B <sub>12</sub> AS								
mg/kg of OMI	5.3	3.8	4.8	3.3	0.2	0.02	<0.001	0.89
mg/kg of OMTD	9.4	6.7	8.6	6.4	0.6	0.33	<0.001	0.70

<sup>1</sup>Calculated by difference: 100 – [CP + (NDF – NDICP) + fat + ash]. NDICP = neutral detergent insoluble CP.

<sup>2</sup>35–30 = 35% forage–30% NFC, 35–40 = 35% forage–40% NFC, 60–30 = 60% forage–30% NFC, and 60–40 = 60% forage–40% NFC (DM basis).

<sup>3</sup>INT = Interaction of dietary forage and NFC content.

diets, as were amounts of NA and NAM. Duodenal flow of PAL and PYR were higher ( $P < 0.05$ ) for cows in the later lactation square. Daily vitamin B<sub>6</sub> AS (mg or mg/kg of OMTD) was higher for cows fed 40% NFC diets. This increase in B<sub>6</sub> AS (mg/d) was due to an increase in PAL AS as there was no effect of dietary NFC content on PAM or PYR AS. Ruminal PYR disappearance (negative AS) was greater for the 60% forage diets. Vitamin B<sub>6</sub> and PAL AS were higher ( $P < 0.05$ ) for cows in the later lactation square. Folate AS (mg/d) was lower and higher for cows fed 60% forage and 40% NFC diets, respectively; daily B<sub>12</sub> AS (mg, mg/kg of OMI, mg/kg of OMTD) was lower for cows fed 40% NFC diets. Similarly, increasing dietary forage decreased daily B<sub>12</sub> AS (mg and mg/kg of OMI).

Pearson correlation coefficients between B-vitamin AS and nutrient intakes, amounts of nutrients ruminally digested, and duodenal flow of microbial N are presented in Table 8.

## DISCUSSION

Dietary forage and NFC effects on AS could be due to changes in populations or functions of ruminal microbial species, their interrelationships, and subsequent effects on microbial B-vitamin metabolism. Wolin et al. (1997) suggested that B-vitamin “cross-

feeding” occurs between ruminal bacteria; B-vitamins that are produced by one species may be required by another. To our knowledge, no research has been conducted investigating the relationship between changes in ruminal microbial populations and B-vitamin synthesis and (or) degradation. Although ruminal degradation of supplemental B-vitamins clearly occurs (Zinn et al., 1987; Santschi et al., 2005a), information is lacking with regard to how degraded B-vitamins are used by rumen microbes.

Studies (Hollis et al., 1954; Hayes et al., 1966; Girard et al., 1994) have reported that altering dietary ingredients affects ruminal B-vitamin concentrations. Ruminal concentrations, however, may not represent total microbial B-vitamin synthesis because rumen fill and digesta passage rates—particularly where large differences in dietary forage content exist—may be influenced by diet composition. Estimating B-vitamin AS as the difference between amounts flowing to the duodenum and dietary intake ignores microbial destruction, possible ruminal absorption, and the potential contribution of feed-derived B-vitamins available for intestinal absorption. To our knowledge, no data measuring ruminal destruction or bioavailability of feed-derived B-vitamins are available.

Although limited, data do suggest that there is no appreciable ruminal absorption of B-vitamins in fed ruminants. Rérat et al. (1958a) observed little ruminal

**Table 8.** Pearson correlation coefficients between B-vitamin apparent synthesis (AS) and dietary and digestive parameters

Item	B-vitamin AS					
	Thiamin	Riboflavin	Niacin	B <sub>6</sub>	Folates	B <sub>12</sub>
Intake, kg/d						
DM	0.51***	0.72****	0.23	0.12	0.63****	0.61****
OM	0.51***	0.71****	0.23	0.12	0.63****	0.61****
NDF	0.50***	0.47***	-0.23	-0.07	0.31*	0.88****
NFC	0.32*	0.65****	0.55****	0.26	0.67****	0.16
NSC	0.16	0.51***	0.65****	0.29	0.57****	-0.15
Starch	0.06	0.41**	0.67****	0.28	0.48***	-0.31*
Sugars	0.59****	0.62****	-0.01	0.06	0.53***	0.82****
Dietary content, % of DM						
NDF	0.14	-0.19	-0.61****	-0.26	-0.28	0.57****
NFC <sup>1</sup>	-0.10	0.23	0.62****	0.30*	0.34*	-0.49***
NSC <sup>2</sup>	-0.13	0.20	0.62****	0.27	0.29*	-0.55****
Starch	-0.14	0.18	0.61****	0.26	0.27	-0.57****
Sugars	0.28	-0.04	-0.50***	-0.11	-0.05	0.69****
Ruminally digested, kg/d						
DM <sup>3</sup>	0.37**	0.51***	0.10	0.11	0.52***	0.52***
OM <sup>3</sup>	0.40**	0.49***	0.04	0.14	0.50***	0.54****
NDF	0.19	0.13	-0.32*	-0.15	0.05	0.67****
Starch	0.13	0.46***	0.62****	0.29*	0.54****	-0.21
Microbial N flow, g/d	0.65****	0.79****	0.38**	0.14	0.77****	0.44***
Ruminal measures						
VFA						
Total, mM	0.51***	0.46***	0.13	0.23	0.43***	0.54****
Acetate, mol/100 mol	-0.41**	-0.42**	-0.29*	-0.35**	-0.49***	-0.18
Propionate, mol/100 mol	0.20	0.32*	0.33*	0.40**	0.57****	0.18
Butyrate, mol/100 mol	0.39**	0.31*	0.11	0.16	0.21	0.24
pH	-0.42**	-0.39**	0.04	-0.21	-0.39**	-0.67****
NH <sub>3</sub> N, mg/dL	0.35**	0.23	-0.18	-0.01	0.15	0.17

<sup>1</sup>NFC calculated by difference: 100 - (CP + (NDF - NDICP) + fat + ash). NDICP = Neutral detergent insoluble CP.

<sup>2</sup>NSC = Nonstructural carbohydrates.

<sup>3</sup>Digestibility corrected for duodenal flow of microbial DM and OM.

\* $P < 0.10$ , \*\* $P < 0.05$ , \*\*\* $P < 0.01$ , and \*\*\*\* $P < 0.001$ .

B-vitamin absorption in fed sheep but did observe absorption when B-vitamins were infused into an evacuated rumen (Rérat et al., 1958b). During a 1-h period, approximately 31% of a 3.2-g NAM dose was absorbed from evacuated rumens, yet NA was not absorbed (Erickson et al., 1991). Harmeyer and Kollenkirchen (1989) suggested that NAM is instantly converted to NA in the rumen. Girard et al. (2001) calculated that only 0.015 and  $4 \times 10^{-6}$ % of 2.6- and 7.8-g doses of folic acid and B<sub>12</sub>, respectively, were ruminally absorbed within 1 h of dosing. Hoeller et al. (1977) showed in vitro that the rumen wall is largely thiamin-impermeable. Santschi et al. (2005b) indicated that B-vitamin concentrations were 10- to 5,000-fold higher in bacteria than in particle-free supernatant. Those results are in accordance with Rérat et al. (1959), who reported that in the rumen, B-vitamins are primarily sequestered within bacteria, which would reduce the possibility of ruminal absorption. Because ruminal B-vitamin absorption appears negligible, calculations of AS should provide a reasonable estimate of B-vitamin

supply to the animal by the ruminal microbial population.

Zinn et al. (1987) reported linear increases in duodenal flow of thiamin, niacin, vitamin B<sub>6</sub>, and B<sub>12</sub> when intakes of grain-fed steers increased incrementally from 1.2 to 2.2% of BW. When AS was estimated from Zinn et al. (1987), amounts of B-vitamins ruminally synthesized also appeared to increase with increasing feed intake.

When averaged across diets, thiamin AS was 50.6 mg/d, which is similar to the values of 51.7 mg/d reported by Breves et al. (1981) and greater than the 26.1 mg/d reported by Santschi et al. (2005a) in lactating dairy cows. Similarly, Steinberg and Kaufmann (1977) reported that thiamin AS ranged from 23 to 50 mg/d with a mean of 32 mg/d. Using steers, Miller et al. (1986) reported a net thiamin loss, which they hypothesized was due to destruction by thiaminase enzymes. Daily thiamin AS was 4.0 mg/kg of OMI calculated from Breves et al. (1981) vs. an average of 2.7 mg/kg of OMI in our study. Using raw cow-period data

provided by Breves et al. (1981), thiamin AS was correlated ( $r = 0.76$ ,  $P < 0.001$ ) with duodenal microbial N flow, which was similar to our observed correlation between these parameters.

Increasing dietary forage increased riboflavin intake in our study and that of Miller et al. (1986). However, riboflavin flow and AS were not different between high- and low-forage fed steers (Miller et al., 1986). Santschi et al. (2005a) reported that riboflavin AS averaged  $267 \pm 17$  mg/d, similar to our across-diet average of 238 mg/d. Calculated daily riboflavin AS ranged from 0.4 (Zinn et al., 1987) to 9.8 mg/kg of OMI (Miller et al., 1986), which are lower than our across-diet average of 12.8 mg/kg of OMI. Positive correlations for thiamin and riboflavin AS with nutrient intake (but not nutrient content), ruminal DM and OM digestibility, and duodenal microbial N flow, and the correlations with ruminal VFA parameters suggest that AS of these vitamins is increased as dietary intake, ruminal digestibility, and microbial N production increase.

Niacin AS was 4- to 62-fold greater than the other B-vitamins, excluding biotin. Considerably lower AS values for NAM than NA may support the hypothesis of Harmeyer and Kollenkirchen (1989) that NAM is hydrolyzed to NA. Similarly, a portion of NAM could be converted to NA during analysis with extraction procedures involving acids (Ndaw et al., 2002) such as those used in the present experiment. In our study, increasing dietary NFC content increased niacin AS by 884 mg/d, or about 2.5-fold, in contrast to Miller et al. (1986) where no difference ( $P > 0.20$ ) was observed between high and low-forage diets. In our study, niacin AS was positively correlated with NFC, NSC, and starch intakes, as well as dietary starch content and amounts ruminally digested. In contrast, niacin AS was negatively correlated with amounts of NDF digested ruminally, dietary NDF content, and ruminal molar percentage of acetate but positively correlated with ruminal molar percentage of propionate. Santschi et al. (2005a) reported that niacin AS was  $2213 \pm 224$  mg/d in lactating cows fed diets containing 58% forage, 28% NDF, and 47% NFC with average DMI of 19.8 kg/d. The niacin AS value reported by Santschi et al. (2005a) is approximately 830 mg/d higher than that for our 60% forage–40% NFC diet, which may be partially due to dietary differences between the studies.

As observed in our study, Zinn et al. (1987) reported positive B<sub>6</sub> AS, whereas Santschi et al. (2005a) reported negative AS ( $-14 \pm 7$  mg/d). In our experiment and that of Santschi et al. (2005a), PYR AS values were negative, yet PAM and PAL AS were positive. Pyridoxine intakes were greater than PAL, which in turn were greater than PAM intakes, yet AS values of these vitamers were reversed. This suggests that

there is ruminal metabolism of PYR or microbial conversion of PYR to another B<sub>6</sub> vitamer, conceivably PAM. This hypothesis is further supported by the observation that PYR represented >60% of total vitamin B<sub>6</sub> in diets, but accounted for  $\leq 5\%$  in ruminal bacterial fractions (Santschi et al., 2005b). When averaged across diets, daily B<sub>6</sub> AS was 1.3 mg/kg of OMI and lower than the 4.6 mg/kg of OMI calculated from Zinn et al. (1987). Increased B<sub>6</sub> AS with increasing dietary NFC content combined with positive correlations with kilograms of starch digested, dietary NFC content, and positive and negative correlations with ruminal molar percentages of propionate and acetate, respectively, suggests that B<sub>6</sub> synthesis may be closely linked to NFC digestion.

In our study and that of Santschi et al. (2005a), biotin AS values were negative and averaged  $-10.9$  and  $-1.4$  mg/d, respectively. Miller et al. (1986) reported that ruminal biotin synthesis was  $< 1$  mg/d in steers fed either high-corn grain (88% dietary DM) or high-alfalfa meal (70% dietary DM) diets. Calculated biotin AS was positive in the study of Zinn et al. (1987). In vitro data (Abel et al., 2001) indicate that ruminal microflora synthesize more (or degrade less) biotin as hay replaces barley grain. Utilizing healthy hay- and grain-fed fistulated sheep, Duncan et al. (1999) isolated *Pseudomonas aeruginosa* from ruminal digesta, a bacterial genus that has been shown to degrade biotin (Brady et al., 1965). Positive biotin AS values observed by Miller et al. (1986) and Zinn et al. (1987) could have occurred as the microbiological assays used by these researchers might not have completely accounted for biocytin (biotin bound to lysine) in feed and duodenal samples. The link between lysine and biotin is cleaved only by biotinidase (McMahon, 2002), an enzyme present in the pancreas, intestinal brush-border cells, and some bacterial species. If the aforementioned researchers did not include exogenous enzyme in their assays, diet-derived biotin might have been underestimated. Without treatment with pancreatic extract, biocytin was not detected with the assay used in the present experiment, but the rate of conversion of biocytin to biotin following the enzymatic treatment was 92%. When duodenal and various feed samples were analyzed with and without pancreatic extract, we (unpublished observations) found that approximately 93% of total biotin was present as biocytin. However, it is possible that the assay used in the present experiment also detected inactive metabolites such as biotin sulfoxide or bisnorbiotin, overestimating total biotin concentrations in feed and duodenal digesta.

Daily folate AS averaged 16.5 mg and was similar to the 21 mg reported by Santschi et al. (2005a). However,

folate AS calculated from Zinn et al. (1987) was negative. This discrepancy could be partially due to absorption of folates prior to the duodenal cannula (Santschi et al., 2005a), leading to underestimation of folate AS. However, the extent of absorption from the proximal duodenum has not been precisely quantified. Girard et al. (1994) observed numerically lower ruminal folate concentrations in steers fed high-forage diets (70% timothy hay, DM basis) than those fed low-forage (30% timothy hay, DM basis) diets, although folic acid intakes were higher for the high-forage diets. However, ruminal concentrations may not reflect the amount of folates escaping from the rumen. Although increasing dietary forage and NFC content increased duodenal flow and AS of folates, these effects were not significant when AS was reported per kilogram of OMI. Positive correlations with microbial N flow and intake and ruminal digestion of all measured nutrients except NDF and correlations with ruminal VFA parameters suggest that folates AS is increased when overall nutrient intake and ruminal digestibility are improved.

Daily B<sub>12</sub> AS averaged 79.8 mg, which is similar to the 73-mg value of Santschi et al. (2005a). Decreased AS of B<sub>12</sub> with increased dietary corn grain content was observed in sheep (Sutton and Elliot, 1972). In the present experiment, increasing both dietary forage and NFC—whether expressed as milligrams per day or milligrams per kilogram of OMI per day—reduced B<sub>12</sub> AS. Vitamin B<sub>12</sub> AS was highest for the 35% forage diets, on which cows consumed the greatest quantity of sugars. *Selenomonas* bacteria have been shown to synthesize appreciable amounts of B<sub>12</sub> (Dryden et al., 1962; Anderson et al., 2001) and use sugars as fermentative substrates (Stewart and Bryant, 1988). Though purely supposal, the nutrient content of the 35% forage diets might have fostered proliferation of these bacteria. Vitamin B<sub>12</sub> AS was positively correlated with measurements of sugars and NDF and was negatively correlated with dietary NFC, NSC, and starch content.

The NRC (2001) provides estimates of apparent ruminal B-vitamin synthesis in lactating dairy cows based on steer data of Miller et al. (1986) and Zinn et al. (1987). To investigate the validity of this approach, we adapted those data to AS measurements from our experiment by expressing the NRC (2001) estimates on the basis of milligrams per kilogram of DMI per day (NRC assumed DMI of 22.9 kg/d) and multiplying by our across-diet average DMI measurements. These adjusted B-vitamin AS means are compared with our measured mean, minimum, and maximum AS values in Table 9. Relative to our measured AS values, the NRC (2001) closely estimated riboflavin AS; overestimated thiamin, B<sub>6</sub>, and biotin AS; and underestimated folic acid AS. The NRC (2001) estimates of niacin and

**Table 9.** Comparison of NRC (2001) adapted and measured B-vitamin apparent synthesis (AS)

	B-vitamin AS			
	NRC <sup>1</sup>	Measured		
	Mean	Mean	Minimum	Maximum
Thiamin	127	51	44	61
Riboflavin	232	238	206	254
Niacin	1603	1025	446	1547
B <sub>6</sub>	85	23	14	30
Biotin	12	-11	-16	-3
Folates	6.2	16	13	20
B <sub>12</sub>	62	80	60	102

<sup>1</sup>NRC (2001) ruminal synthesis (mg/kg of DMI per d) adjusted to average DMI measured in the current study.

B<sub>12</sub> AS were similar to measured maximum and minimum values, respectively. With regard to ranking B-vitamins by amounts synthesized, the 2 approaches are similar; niacin and riboflavin syntheses were highest followed by thiamin, B<sub>6</sub>, and B<sub>12</sub>. The lowest values were for folic acid and biotin. Because of the variation in relationships between individual B-vitamin AS and nutrient intakes, nutrient digestibility and microbial N flow, and forage and NFC effects on AS, future prediction equations estimating B-vitamin AS will likely need to be multifactorial and B-vitamin specific.

## CONCLUSIONS

When averaged across diets, AS of individual B-vitamins as a percentage of B-vitamin intake was thiamin, 142; riboflavin, 228; total niacin, 120; total B<sub>6</sub>, 39; folic acid, 137; and B<sub>12</sub>, 24,276, clearly exemplifying the importance of ruminal synthesis for most B-vitamins. Increasing dietary forage and NFC contents influenced B-vitamin intakes, duodenal flow, and AS. When expressed as milligrams per day, B-vitamin duodenal flow and AS were influenced more frequently by dietary NFC content than by dietary forage content. When adjusted for OMI, only B<sub>12</sub> AS was influenced by dietary forage content, whereas niacin, B<sub>6</sub>, and B<sub>12</sub> were influenced by NFC content. Thiamin, riboflavin, and folic acid AS appear to be increased when overall dietary intake and digestibility and microbial N production increase; B<sub>6</sub> and niacin AS are enhanced with increased dietary NFC content. Negative AS values for biotin suggest minimal ruminal synthesis and/or appreciable ruminal degradation. Vitamin B<sub>12</sub> AS was highest for 35% forage–30% NFC diets and was increased with increasing dietary sugars. Additional research investigating dietary effects on duodenal B-vitamin flows and ruminal AS, coupled with lactating cow requirement and intestinal B-vitamin absorption

research, will allow for determination of dietary B-vitamin supplementation strategies.

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