Forage proportion and particle length affects the supply of amino acids in lactating dairy cows¹

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ABSTRACT

This study was conducted to evaluate the effects of dietary factors that alter ruminal fermentability on intake, duodenal flows and intestinal digestibility of individual amino acids (AA) in lactating dairy cows. The experiment was designed as a 4 × 4 Latin square using 4 ruminally and duodenally cannulated lactating dairy cows. Treatments were arranged in a 2 × 2 factorial design; 2 forage particle lengths (FPL) of alfalfa silage (short and long) were combined with low (35:65) and high (60:40) forage-to-concentrate ratio (F:C; dry matter basis). Four diets were formulated using 2 cuts of alfalfa silage [short (7.9 mm) and long (19.1 mm)], combined with 2 ratios of forage to barley grain concentrate (35:65 and 60:40). Overall, the interactions between dietary F:C and FPL on intake, duodenal flows, and intestinal digestibility of AA were marginal. Intakes of total AA and nonessential AA were not different between low- and high-F:C diets, whereas that of essential AA tended to be less with high-F:C diet as a result of lower intakes of Met, Phe, Arg, and His. The flows of total AA and microbial AA were reduced by 22 and 19%, respectively, with increasing F:C ratio in the diets due to consistently reduced flows of individual AA, whereas AA profiles (% of AA-N) of the duodenal protein were not different. Altering F:C from 35:65 to 60:40 decreased the intestinal digestibility of Ile, Leu, Thr, Val, Ala, Cys, and Ser, and consequently, tended to decrease the digestibility of total AA, essential AA, and nonessential AA. Intakes of total AA, essential AA, and nonessential AA were overall not affected by dietary FPL so FPL did not affect the flows or intestinal digestibility of AA. These results indicate that increasing dietary F:C ratio decreased overall AA supply because flow to the duodenum and intestinal digestibility of AA were decreased. However, increasing FPL

had no effect on AA supply. The measured duodenal flows of AA were consistent with the predictions of the Cornell Net Carbohydrate and Protein System model for the low-forage diet, and were consistent with the National Research Council model for the high-forage diet. Furthermore, the digestibility of individual AA in the intestine varied considerably, regardless of dietary treatment. The results revealed the necessity to consider the both flows and digestibility of individual AA when optimizing ration formulation to meet AA requirements of dairy cows.

Key words: forage-to-concentrate ratio, forage particle length, amino acid, dairy cow

INTRODUCTION

Protein requirements of dairy cows are met with microbial protein that is produced in the rumen and RUP that escapes the rumen and is absorbed in the intestine. Microbial CP supplies an average of 59% (range of 34 to 89%) of the NAN flow to the duodenum (Clark et al., 1992). In high-producing dairy cows, RUP represents a key contributor to metabolizable AA (NRC, 2001). The supply of individual AA that reach, and are digested, in the small intestine ultimately determines whether or not the protein requirement of the lactating dairy cow is met. The current NRC model (NRC, 2001) expresses protein requirements in term of MP, which is the sum of digested rumen microbial, RUP, and endogenous protein. To improve transfer efficiency of dietary protein into milk protein, the models should ideally define the protein requirement in terms of AA rather than MP. However, this approach is limited by the scarcity of data on the AA profiles of the protein entering at the small intestine and on the intestinal digestibility of individual AA. The flow to the duodenum and the intestinal digestibility of individual AA vary with forage source (Vanhatalo et al., 2009) and grain processing (Yang and Beauchemin, 2004). Recently, the intestinal digestibility of individual AA from RUP was shown to vary widely, depending on the feedstuff and specific AA (Boucher et al., 2009a,b; Mjoun et

Received November 29, 2011.

Accepted January 14, 2012.

¹Contribution number 387-11065.

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al., 2010). In contrast, NRC (2001) assumes that the intestinal digestibility of individual AA is equal to the digestibility of RUP. Information concerning the supply of AA derived from the diet and the rumen microbes for a range of feeds is limited, yet this information is required for developing accurate protein evaluation systems.

The variations in intestinal AA profiles that determine quantity of individual AA available in the intestine are mainly caused by differences in the AA composition of dietary proteins and in the ratio between microbial protein and RUP (Rulquin and Vérité, 1993). Composition of the feed consumed and the quantity of microbial protein synthesized have a major influence on passage of AA to the duodenum (Overton et al., 1995). It is well documented that dietary factors that alter ruminal OM fermentability also affect ruminal N metabolism and microbial protein synthesis (Clark et al., 1992; Dewhurst et al., 2000). The duodenal microbial N flows in beef (Beauchemin et al., 2001) and dairy cattle (Yang et al., 2000) were altered by manipulating extent of grain processing. Changing forage-to-concentrate ratio (**F:C**) alters the amount and rate of OM fermented in the rumen and, thus, can vary microbial protein synthesis (Sniffen and Robinson, 1987; Yang and Beauchemin, 2007b). Adequate particle length of forage is necessary to maintain proper rumen function. Reduced forage particle length (**FPL**) often negatively affects ruminal microbial protein synthesis (Oba and Allen, 2000; Beauchemin and Yang, 2005). In addition, interactions among dietary factors, such as between F:C and FPL on microbial protein synthesis, have been reported (Rode and Satter, 1988). Increased forage proportion would affect intake of fermentable OM as well as intake of fiber, which could have a significant effect on nutrient supply to dairy cows.

Our previous results on measuring the flows of AA to the duodenum demonstrated the importance of conducting studies to evaluate multiple dietary factors (grain processing, F:C and FPL) rather than a single dietary factor (Yang and Beauchemin, 2004). The supply of AA is key factor to influence milk production and milk protein content of dairy cows. Limited information is available documenting the influence of ruminally fermentable carbohydrates and the effects of dietary FPL on AA supply.

The objective of this study was to evaluate the effects of dietary F:C, FPL, and their interaction on intakes, duodenal flows, and intestinal digestibility of individual AA. The effects on ruminal pH and fermentation, digestibility, and milk production were also measured but were reported separately (Yang and Beauchemin, 2007a,b).

MATERIALS AND METHODS

Cows and Diets

A complete description of the experimental methodology was presented previously (Yang and Beauchemin, 2007b), so only the relevant details are given herein. Four ruminally and duodenally cannulated lactating cows were used in an experiment designed as a 4 × 4 Latin square with a 2 × 2 factorial arrangement of treatments; 2 FPL of alfalfa silage (short and long) were combined with low (35:65) and high (60:40) F:C (DM basis). The diets were formulated using the NRC model (NRC, 2001) to supply sufficient energy and protein for a 650 kg cow to produce 35 kg/d of milk containing 3.5% fat and 3.2% protein (Table 1).

Cows were housed in individual tie-stalls and offered a TMR 3 times daily at 0600, 1500, and 1800 h for ad libitum intake. The cows, averaging 628 ± 55 kg of BW and 65 ± 20 DIM, were cared for according to the Canadian Council on Animal Care (Ottawa, ON, Canada) guidelines.

Second-cut alfalfa was harvested at the early bloom stage of maturity and ensiled in large silo bags (200-t capacity) for 2 mo before being used. A forage harvester (model 6910; John Deere, West Bend, WI), equipped with a 37-tooth sprocket and 8 knives, was used to obtain silage chopped at a theoretical chop length of 7.9 and 19.1 mm for short and long cut silage, respectively. Chemical compositions (DM basis) of alfalfa silage (short vs. long) were DM, 54.9 vs. 51.1%; NDF, 44.7 vs. 46.2%, and CP, 21.1 vs. 21.3%, respectively.

Each period consisted of 14 d of adaptation to diets and 7 d of experimental measurements. Feed offered andorts were measured and recorded daily during the last 7-d of the period to calculate feed intake. Feed samples including alfalfa silage and TMR were collected once weekly, and orts were collected daily and composited weekly for particle length and DM determination. Samples were then composited by period, dried in an oven at 55°C for 48 h, and then ground through a 1-mm diameter screen (standard model 4; Arthur Thomas Co., Philadelphia, PA) for chemical analysis.

Duodenal Flow and Apparent Digestion

Duodenal flow and apparent digestion of protein and AA were determined using YbCl₃ (Rhône-Poulenc Inc., Shelton, CT) as a digestive marker. The marker was mixed into the concentrate portion of the diets to supply approximately 1.5 g of Yb/d per cow. Ammonia ¹⁵N ([¹⁵NH₄]₂SO₄, 10.6% atom % ¹⁵N; Isotec Inc., Miamisburg, OH) was used as a ruminal microbial marker.

Table 1. Ingredients and chemical composition of the experimental diets (DM basis)

Composition	Forage ¹ :concentrate (F:C)			
	35:65		60:40	
	Short	Long	Short	Long
Ingredient, %				
Alfalfa silage, short cut	35.7	—	59.5	—
Alfalfa silage, long cut	—	35.7	—	59.5
Barley grain, steam rolled ²	56.1	56.1	31.1	31.1
Corn gluten meal	3.33	3.33	3.33	3.33
Canola meal (Alberta Gold) ³	1.43	1.43	1.19	1.19
Soypass ⁴	1.43	1.43	1.19	1.19
Beet molasses	0.48	0.48	0.95	0.95
Calcium carbonate	0.24	0.24	0.24	0.24
Dicalcium phosphorus	0.24	0.24	0.24	0.24
Monosodium phosphate	0.05	0.05	0.05	0.05
Vitamin-mineral mix ⁵	0.71	0.71	0.71	0.71
Canola oil	0.19	0.19	0.48	0.48
Rumen inert fat ⁶	—	—	0.95	0.95
Binding agent (Aka) ⁷	0.11	0.11	0.12	0.12
Chemical composition				
DM, %	72.5	71.1	66.0	62.0
OM, % of DM	93.4	93.1	91.2	90.9
CP, % of DM	19.9	20.5	21.6	21.8
NDF, % of DM	30.3	30.6	34.5	36.4
NDF from forage, % of DM	16.0	16.5	26.6	27.5
ADF, % of DM	20.4	21.6	26.9	27.4
Starch, % of DM	30.6	30.6	17.8	17.8
NE _L , ⁸ Mcal/kg	1.65	1.65	1.65	1.65

¹Alfalfa forage was harvested at a theoretical chop length of 19.1 or 7.9 mm for long- and short-cut silages, respectively.

²Chemical composition of barley grain (DM basis) was 97.7% for OM; 20.8% for NDF; 7.2% for ADF; and 12.4% for CP.

³A registered trademark for heat-processed canola meal product (Canbra Foods, Lethbridge, Alberta, Canada).

⁴A registered trademark for heat-processed soybean meal product (Borregaard LignoTech, Rothschild, WI).

⁵Contained 58.8% NaCl, 16.0% Dynamate (Pitman Moore Inc., Mundelein, IL; 18% K, 11% Mg, 22% S, and 1,000 mg of Fe/kg), 2% ZnSO₄·H₂O, 2.4% MnSO₄·4H₂O, 0.01% CoSO₄·6H₂O, 0.009% Na₂SeO₃, 0.012% ethylenediamine dihydroiodide, 0.8% CuSO₄·5H₂O, 2,000,000 IU of vitamin A/kg, 200,000 IU of vitamin D/kg, and 2,000 IU of vitamin E/kg.

⁶Enertia (ADM Animal Health and Nutrition, Quincy, IL).

⁷Bear River Zeolite of Canada Corp. (Lethbridge, Alberta, Canada).

⁸Estimated from NRC (2001).

Marker solution was continuously infused into the rumen of the cows via the ruminal cannulas using an automatic pump (model 60 RPM/7524–10, Masterflex L/S microprocessor pump drive; Cole-Parmer Instrument Co., Vernon Hills, IL) during the last 11 d of the period. Daily amounts infused were 140 mg of ¹⁵N dissolved in 800 mL of water for each cow. Ruminal samples were collected once daily for 4 d to prepare a mixed bacterial pellet using a differential centrifugation technique (Yang and Beauchemin, 2007b). Bacterial pellets were accumulated by period, freeze dried, ground using a mortar and pestle, and then further ground using a ball mill (Mixer Mill MM2000; Retsch GmbH, Haan, Germany) to a fine powder for determination of N content and ¹⁵N enrichment.

Duodenal samples were collected 4 times daily every 6 h, moving ahead 2 h each day for the last 4 d of infusion. This schedule provided 12 representative samples of duodenal contents taken at 2-h intervals. Each duodenal sample was split into 3 fractions that were pooled by cow within period and retained for ammonia analysis, DM determination after oven-drying at 55°C, or for chemical analysis after freeze drying. Fecal samples (approximately 200 g of wet weight) were collected for each cow from the rectum twice daily at various times during the last 6 d of the period. Fecal samples were immediately subsampled (about 50 g), composited across sampling times for each cow and each period, dried at 55°C, ground to pass a 1-mm sieve (standard model 4; Arthur Thomas Co.), and stored for analysis.

Chemical Analyses

Feed DM was determined by oven drying at 55°C for 48 h. Analytical DM content of the samples was determined by drying at 135°C for 2 h (AOAC, 1990). The OM content was calculated as the difference between DM and ash contents, with ash determined by combustion at 550°C overnight. The NDF and ADF contents were determined using the methods described by Van Soest et al. (1991) with amylase and sodium sulfite used in the NDF procedure. Starch was determined by enzymatic hydrolysis of α -linked glucose polymers, as described by Rode et al. (1999). Content of N in the samples was determined by flash combustion (model 1500; Carlo Erba Instruments, Milan, Italy). Amino acids in feed and digestive samples were analyzed as the phenylisothiocyanate derivatives by HPLC (WM02; Waters Corp., Milford, MA) according to the procedure of Hagen et al. (1989), with minor modifications in the oxidation step. A sample containing 10 mg of protein was oxidized with 5 mL of performic acid. One milliliter of concentrated HCl and 3 drops of 1-octanol were added to allow samples to sit at 20°C before evaporation the next day. For derivatization, 40 μ L of a derivatized MeOH-water-triethylamine (2:2:1) solution was added into the derivatization tubes. The contents were dried by adding the phenylisothiocyanate mixture at 20°C and atmospheric pressure.

Calculations and Statistical Analyses

Flows of CP and AA to the duodenum or the excretion of CP and AA in feces were calculated by multiplying DM flow by the respective concentration of CP or AA in duodenal or fecal DM. The calculations of the flows of DM to the duodenum and DM excreted in feces were described previously (Yang and Beauchemin, 2007b). Flows of microbial AA to the duodenum were estimated based on the microbial CP flow and AA composition of bacterial samples isolated from ruminal contents.

Data for intakes and duodenal flows of protein and AA were analyzed using the mixed model procedure of SAS (SAS Institute Inc., Cary, NC) for a 4 $\times$ 4 Latin square design. The model included treatments (F:C and FPL) and the interaction between F:C and FPL as a fixed effect and period and cow as random effects. The estimation method was restrictive maximum likelihood (REML) and the degrees of freedom method was Kenward-Rogers. Effects of the factors were declared significant at $P < 0.05$ and trends were discussed at $P < 0.10$, unless otherwise noted.

RESULTS AND DISCUSSION

Intake of AA

No interactions were observed between F:C and FPL on intakes of total AA, essential AA (EAA) and nonessential AA (NEAA), in contrast to the tendency ($P < 0.10$) for an interaction between F:C and FPL for CP intake (Table 2). Intake of CP was lower ($P < 0.05$) with high- than with low-F:C diets, whereas the intakes of total AA and NEAA were not different, but the intake of EAA tended ($P < 0.09$) to be less with the high-F:C diet. The greater variation for total AA (SE = 209) than for CP (SE = 109) was the reason that no interaction was observed between F:C and FPL, and no effect of F:C occurred. The trend toward less intake of EAA with high F:C primarily resulted from less intake of Met ($P < 0.01$) and Phe ($P < 0.02$) as well as a tendency for less intake of Arg ($P < 0.07$) and His ($P < 0.08$) for cows fed the high-F:C diet. The decrease in intakes of Arg, His, Met, and Phe probably resulted from the substitution of alfalfa silage for barley grain, canola meal, and soybean meal, which had marginally higher concentration (% of CP) of Arg, His, Met, and Phe than alfalfa silage (NRC, 2001). Intakes of total AA, EAA, and NEAA were not affected by dietary FPL, except for the intakes of Gly ($P < 0.03$) and Ser ($P < 0.02$), which were greater for long than for short FPL. The lack of difference in intakes of total AA, EAA, and NEAA due to FPL was in agreement with a previous report (Yang and Beauchemin, 2004). This effect was expected because the dietary formulation was the same between short and long FPL, and the intake of CP was not affected by FPL. Nevertheless, AA intake is a less meaningful measurement of the real supply of AA to dairy cattle.

Flows of AA to the Duodenum

The interactions between F:C and FPL on flows of CP and total and individual AA to the duodenum were, overall, not significant (Table 3). The flows of total ($P < 0.01$), microbial ($P < 0.04$), and feed CP ($P < 0.03$) were less with the high-F:C than with the low-F:C diet. Increasing F:C from 35:65 to 60:40 decreased the intake of CP and the microbial protein production in the rumen without altering ruminal degradability of CP (Yang and Beauchemin, 2007b). Similarly, the flows of total AA, EAA, NEAA, and individual AA to the duodenum were lower ($P < 0.01$) with the high-F:C than with the low-F:C diet. The present finding contrasts somewhat to the study by Clark et al. (1992) who reviewed several digestion trials in which diets contained a wide range

Table 2. Effects of forage-to-concentrate ratio (F:C) and forage particle length (FPL) on AA intake

Item	Forage ¹ :concentrate (F:C)				SE	<i>P</i> -value		
	35:65		60:40			F:C	FPL	F:C × FPL
	Short	Long	Short	Long				
Intake of DM, ² kg/d	20.3	20.9	18.6	18.0	0.7	0.01	NS ³	NS
Intake of CP, g/d	4,057	4,288	4,025	3,963	109	0.05	NS	0.10
AA, g/d								
Arg	134	146	133	122	9.4	0.07	NS	NS
His	63	72	64	53	6.3	0.08	NS	NS
Ile	108	118	103	112	9.2	NS	NS	NS
Leu	251	314	268	273	23.8	NS	NS	NS
Lys	110	130	113	111	13.1	NS	NS	NS
Met	54	53	38	26	6.8	0.01	NS	NS
Phe	154	207	138	139	15.1	0.02	NS	0.10
Thr	85	89	83	93	6.7	NS	NS	NS
Trp	12	18	17	15	2.2	NS	NS	0.06
Val	150	160	140	158	9.9	NS	NS	NS
Ala	153	175	160	176	11.4	NS	0.09	NS
Asp	193	219	228	225	25.8	NS	NS	NS
Cys	35	38	42	47	2.8	0.02	NS	NS
Glu	664	712	715	694	73.9	NS	NS	NS
Gly	105	123	105	121	6.4	NS	0.03	NS
Pro	272	306	241	238	19.8	0.03	NS	NS
Ser	106	118	113	143	7.4	0.04	0.02	NS
Tyr	89	105	81	69	9.7	0.05	NS	NS
Total AA	2,958	3,134	2,809	2,846	201	NS	NS	NS
Essential AA	1,212	1,306	1,097	1,101	85.3	0.09	NS	NS
Nonessential EAA	1,746	1,827	1,712	1,746	128.6	NS	NS	NS

¹Alfalfa forage was harvested at a theoretical chop length of 19.1 and 7.9 mm for long- and short-cut silages, respectively.

²Data were previously reported (Yang and Beauchemin, 2007b).

³NS = nonsignificant at $P > 0.10$.

of forage (from 24 to 81%), but failed to establish an optimal F:C that maximized ruminal fermentation and passage of NAN and AA to the duodenum. The authors suggested that simply altering F:C does not optimize ruminal fermentation and passage of AA to the duodenum because the composition of both forages and concentrates contribute to the optimization. Further, the effects of F:C may be related more to the amount and rate of OM fermentation in the rumen than to the specific F:C in the diet. This suggestion is supported by several studies. Moorby et al. (2006) reported a linear decrease of microbial N flow, along with a linear decrease of ruminal fermented OM, with increasing F:C from 35:65 to 50:50, 65:35, and 80:20. The decrease in microbial AA was consistent with a 1.5 kg/d decrease in ruminal fermented OM when feeding a high-F:C rather than a low-F:C diet. This decrease was the result of substituting 25% silage for 25% barley grain (Yang and Beauchemin, 2007b). However, altering the F:C did not change the passage of AA to the duodenum in another study (Yang and Beauchemin, 2004) because the high- and low-forage diets had equal fermentable OM and that may explain why no difference existed due to forage level (Yang et al., 2001a). The type of forages and

experimental design were different between the present and the previous study (Yang and Beauchemin, 2004) in which alfalfa silage, alfalfa hay, and barley silage were fed, and 3 dietary factors (grain particle size, F:C, and FPL) were investigated, so possible confounding among the factors would be expected. Vanhatalo et al. (2009) reported higher omasal canal AA flows for red clover- than for grass silage-based diets. The results suggest that ruminal fermentability of feeds rather than F:C was the primary factor determining the flows of AA.

Decreased flows of AA to the duodenum with the high-F:C diet resulted from both lower microbial AA ($P < 0.05$) and dietary AA contents. The relative contribution changed with increasing F:C; the contribution to total AA flows by microbial AA increased from 55 to 57%, but the contribution by dietary AA decreased from 45 to 43%. Overton et al. (1995) showed that microbial protein production has a major influence on passage of AA to the duodenum. In another study (Yang and Beauchemin, 2004), the effects of F:C on microbial AA contribution to duodenal flow of AA depended upon grain particle size; for coarsely rolled barley, increasing F:C from 35:65 to 55:45 increased the proportion of

Table 3. Effects of forage-to-concentrate ratio (F:C) and forage particle length (FPL) on duodenal flows of AA

Item ¹	Forage ² :concentrate (F:C)				SE	P-value		
	35:65		60:40			F:C	FPL	F:C × FPL
	Short	Long	Short	Long				
AA, g/d								
Arg	155	151	128	125	12.5	0.01	NS ³	NS
His	70	72	62	53	4.9	0.01	NS	NS
Ile	182	184	154	139	15.4	0.01	NS	NS
Leu	292	308	244	216	21.0	0.01	NS	NS
Lys	193	194	151	128	17.4	0.01	NS	NS
Met	48	52	40	36	2.6	0.01	NS	0.04
Phe	163	169	142	120	11.0	0.01	NS	NS
Thr	144	146	117	108	12.9	0.01	NS	NS
Trp	6	8	8	9	2.2	NS	NS	NS
Val	221	219	180	169	18.0	0.01	NS	NS
Ala	222	238	179	168	19.4	0.01	NS	NS
Asp	303	308	249	234	30.5	0.01	NS	NS
Cys	45	51	40	41	4.0	0.01	0.08	NS
Glu	522	585	406	413	58.7	0.01	NS	NS
Gly	190	207	172	153	14.0	0.01	NS	0.06
Pro	264	240	209	181	19.5	0.01	0.02	NS
Ser	179	172	137	133	15.0	0.01	NS	NS
Tyr	122	130	107	93	7.5	0.01	NS	0.06
Total AA	3,321	3,433	2,725	2,518	266.8	0.01	NS	NS
EAA	1,475	1,502	1,226	1,102	109.1	0.01	NS	NS
NEAA	1,846	1,930	1,499	1,416	159.8	0.01	NS	NS
Bacterial, g/d								
Total AA	1,778	1,944	1,578	1,426	233.0	0.05	NS	NS
EAA	867	943	757	692	108.7	0.04	NS	NS
NEAA	911	1,001	821	734	126.0	0.07	NS	NS
Flows of CP, g/d								
Total	4,359	4,758	3,900	3,808	387	0.01	NS	NS
Feed and endogenous	2,015	2,089	1,858	1,779	146	0.03	NS	NS
Microbial	2,233	2,535	1,938	1,926	283	0.04	NS	NS

¹EAA = essential AA; NEAA = nonessential AA.²Alfalfa forage was harvested at a theoretical chop length of 7.9 and 19.1 mm for short- and long-cut silages, respectively.³NS = nonsignificant at $P > 0.10$.

microbial AA in the flows of total AA from 51 to 69% and for finely rolled barley, increased F:C increased proportions from 52 to 57%. Those results revealed the complexity of interactions among dietary factors in terms of microbial activity in the rumen of dairy cows.

Flows of CP, total AA, EAA, NEAA, and individual AA to the duodenum were not affected by FPL, except for a reduction ($P < 0.02$) of Pro with long FPL (Table 3). The result is consistent with the lack of difference in the amount of ruminally fermented OM between short and long FPL (Yang and Beauchemin, 2007b). Several studies also reported no differences in bacterial flow or total duodenal flows of EAA, NEAA, or total AA due to FPL for steers (Firkins et al., 1986) and dairy cows (Yang and Beauchemin, 2004). The flows of Met ($P < 0.04$), Gly ($P < 0.06$), and Tyr ($P < 0.06$) depended upon an interaction between F:C and FPL; flows were higher in low-F:C than in high-F:C diets with short FPL, but the opposite occurred for the long FPL. The

results indicated that the effect of dietary FPL on the flows of most AA to the duodenum is minimal.

AA Profiles of Duodenal Protein

Interactions between F:C and FPL on AA profiles were, overall, not significant except for Pro and Ser, which were reduced ($P < 0.02$) with long FPL in low F:C, but not with high F:C (Table 4). In general, the profiles of individual EAA were not different either between low and high F:C or between short and long FPL. Only a few NEAA profiles were affected by dietary treatment. The differences in AA profiles of the duodenal protein primarily depended on the variation of AA profiles of ruminal microbial protein, RUP, and the ratio of microbial protein to RUP. The AA profiles of ruminal bacteria are not typically affected by F:C and FPL (Yang et al., 2001b). Therefore, the present results agree with the conclusion by Rulquin and Vé-

rité (1993) that variations in intestinal AA profiles are mainly caused by differences in the AA composition of the dietary proteins and in the ratio of microbial protein to RUP. The lack of overall differences in the AA profiles of duodenal protein suggests that the variation in AA profiles of RUP was minimal because the ratio of microbial protein to RUP was relatively constant across the treatments. The present result is consistent with our previous study (Yang and Beauchemin, 2004), which found that the F:C and FPL affected only a few NEAA profiles. However, Mjoun et al. (2010) showed a substantial variation in in situ ruminal degradation of individual AA among and within feedstuff, indicating a potential important influence of RUP on the AA profiles in the duodenal protein. In addition, the slightly decreased Ala with increasing F:C was not expected because ruminal bacterial protein have higher Ala (Lud-den and Cecava, 1995; Yang et al., 2001b) compared with dietary AA profiles. The discrepancy could be due to the differences in feeds and animals used between the studies. Nevertheless, the present results indicate that dietary factors such as F:C and FPL have minimal effects of biological significance on duodenal AA flows.

Digestibility of AA in the Intestine

No interaction was observed between F:C and FPL on the intestinal digestibility (% of duodenal flow) of total and individual AA, except for Ser for which the digestibility tended ($P < 0.08$) to be lower with long compared with short FPL in the low-F:C diet, yet no effect of FPL was found in the high-F:C diet (Table 5). Feeding a high- versus low-F:C diet decreased ($P < 0.05$) the digestibility of Val and Ser, and tended ($P < 0.10$) to decrease the digestibility of Ile, Leu, Thr, Ala, Cys, EAA, NEAA, and total AA. Ben-Ghedalia and Solomon (1988) reported a consistent decrease in the intestinal digestibility of individual AA with increasing proportion of wheat straw (from 0, 30, 70, to 100%) in place of barley grain in the diets of sheep. In that study, the decrease in AA digestibility was associated with increased fiber-bound N at the ileum, which indicated that the amount of fiber-bound N was greater in the ileum than in the diet. These findings suggest that increasing F:C in the diets enhances particle-associated bacteria production but its digestion in the small intestine can be partly prevented by the protection of

Table 4. Effects of forage-to-concentrate ratio (F:C) and forage particle length (FPL) on AA profiles of duodenal protein

Item	Forage ¹ :concentrate (F:C)				SE	<i>P</i> -value		
	35:65		60:40			F:C	FPL	F:C × FPL
	Short	Long	Short	Long				
AA, % of AA-N								
Arg	4.66	4.40	4.70	4.99	0.07	NS ²	NS	NS
His	2.11	2.14	2.36	2.09	0.12	NS	NS	NS
Ile	5.51	5.37	5.67	5.52	0.14	NS	NS	NS
Leu	8.80	9.03	9.06	8.63	0.26	NS	NS	NS
Lys	5.80	5.65	5.55	5.13	0.26	NS	NS	NS
Met	1.44	1.53	1.52	1.43	0.09	NS	NS	NS
Phe	4.93	4.97	5.32	4.76	0.25	NS	NS	NS
Thr	4.35	4.24	4.29	4.31	0.13	NS	NS	NS
Trp	0.04	0.05	0.08	0.08	0.02	0.08	NS	NS
Val	6.70	6.38	6.60	6.70	0.16	NS	NS	NS
Ala	6.67	6.95	6.56	6.69	0.11	0.01	0.01	NS
Asp	9.13	8.95	9.06	9.27	0.37	NS	NS	NS
Cys	1.35	1.48	1.47	1.65	0.08	NS	NS	NS
Glu	15.73	16.93	14.63	16.45	0.96	NS	NS	NS
Gly	5.72	6.06	6.43	6.13	0.25	0.07	NS	NS
Pro	7.99	7.00	7.70	7.19	0.18	NS	0.01	0.02
Ser	5.40	5.04	4.97	5.27	0.14	NS	NS	0.02
Tyr	3.67	3.83	4.03	3.72	0.18	NS	NS	NS
CP, % of DM	29.0	31.5	27.2	27.8	1.0	0.01	0.02	0.08
AA-N, % N	80.2	77.7	76.9	74.0	4.8	NS	NS	NS
EAA-N, ³ % AA-N	44.3	43.8	45.1	43.6	0.7	NS	0.10	NS

¹Alfalfa forage was harvested at a theoretical chop length of 7.9 and 19.1 mm for short- and long-cut silages, respectively.

²NS = nonsignificant at $P > 0.10$.

³EAA = essential AA.

Table 5. Effects of forage-to-concentrate ratio (F:C) and forage particle length (FPL) on AA digestibility in the intestine

Item ¹	Forage ² :concentrate (F:C)				SE	<i>P</i> -value		
	35:65		60:40			F:C	FPL	F:C × FPL
	Short	Long	Short	Long				
AA, % of duodenal flow								
Arg	77.0	74.7	74.2	73.9	2.5	NS ³	NS	NS
His	63.4	67.0	63.3	57.4	4.5	NS	NS	NS
Ile	68.8	68.3	65.7	62.8	2.9	0.09	NS	NS
Leu	71.3	71.7	68.7	65.8	2.8	0.07	NS	NS
Lys	71.1	65.8	66.8	63.8	4.9	NS	NS	NS
Met	68.1	73.1	67.7	66.5	4.1	NS	NS	NS
Phe	65.5	67.6	65.2	58.8	3.4	NS	NS	NS
Thr	68.2	64.3	62.0	60.6	3.7	0.10	NS	NS
Trp	40.4	50.1	61.2	56.4	12.7	0.02	NS	NS
Val	69.6	67.3	65.2	64.2	3.0	0.05	NS	NS
Ala	62.1	59.0	55.9	55.9	4.7	0.08	NS	NS
Asp	63.8	59.3	57.5	58.0	4.5	NS	NS	NS
Cys	65.0	64.1	60.7	59.6	3.8	0.10	NS	NS
Glu	77.4	73.4	70.5	72.6	5.0	NS	NS	NS
Gly	56.0	60.1	56.2	51.1	4.7	NS	NS	NS
Pro	68.9	65.8	66.2	62.5	3.0	NS	NS	NS
Ser	73.4	66.6	62.8	65.7	3.9	0.05	NS	0.08
Tyr	64.2	62.8	57.1	53.4	6.7	NS	NS	NS
Total AA	68.9	67.2	64.6	63.4	3.5	0.08	NS	NS
EAA	69.9	68.9	66.9	64.2	3.0	0.09	NS	NS
NEAA	68.1	65.8	62.7	62.6	3.9	0.08	NS	NS
Intestinal AA digested, g/d								
Total AA	2,337	2,363	1,808	1,615	255.9	0.01	NS	NS
EAA	1,041	1,045	829	707	99.9	0.01	NS	NS
NEAA	1,296	1,319	980	908	158.6	0.01	NS	NS

¹EAA = essential AA; NEAA = nonessential AA.²Alfalfa forage was harvested at a theoretical chop length of 7.9 and 19.1 mm for short- and long-cut silages, respectively.³NS = nonsignificant at *P* > 0.10.

particles. Therefore, the trend of decreased intestinal digestibility of total AA, EAA, and NEAA with increased F:C may have resulted from a change in the relative proportion of microbial AA to dietary AA. The intestinal digestibility of microbial AA is typically lower than that of dietary AA (Hvelplund and Madsen, 1985; Mjoun et al., 2010). The decrease in the digestibility of Thr, Cys, and Ser with increasing F:C is consistent with previous findings (Yang and Beauchemin, 2004), but a greater number of individual AA were affected by F:C in the present study.

The intestinal digestibility (% of duodenal flow) of total AA, EAA, NEAA, and individual AA were not affected by FPL (Table 5). The results are consistent with the digestibility of CP, which was not different between short and long FPL (Yang and Beauchemin, 2007b). The flows of NDF, CP, and the ratio of microbial N to dietary N were not affected by FPL, which may have lead to the similar digestibility of AA in the intestine for the 2 FPL. Digestibility of AA can be affected by the passage of NDF through fiber-bound N

and the relative proportion of microbial N and dietary N in the intestinal flow.

The digestibility (% of duodenal flows) of the total AA (averaged 66%) was higher than that of CP in the intestine (averaged 61%; Yang and Beauchemin, 2007b). Santos et al. (1984) reported 4 percentage unit higher intestinal digestibility of AA than that of total N. The greater intestinal digestibility of AA compared with that of CP is consistent with the report by Hvelplund and Madsen (1985) that the digestibility of either bacterial CP or feed CP is lower than that of the respective AA. The difference in the intestinal digestibility of CP and AA is in contrast to the previous conclusion that intestinal N digestibility is a good predictor of AA digestibility (Weisbjerg et al., 1996; Yang and Beauchemin, 2004). Further, the intestinal digestibility of EAA was about 2 to 7% higher than that of NEAA, which was consistent with studies using dairy cattle (Santos et al., 1984; Yang and Beauchemin, 2004) or a rooster after rumen incubation of samples in dairy cows (Boucher et al., 2009b). These findings

indicate that preferential absorption of EAA occurred in the small intestine. However, this phenomenon is not accounted for by *in vitro* (Mjoun et al., 2010) or in mobile bag (Wang et al., 1999; Harstad and Prestløkken, 2001) studies because these techniques usually measure single feeds, no microbial CP production is measured, and no absorption occurs (*in vitro*).

Digestibilities of individual AA in the intestine were variable among AA and ranged from 40% for Trp to 77% for Arg and Glu. The high digestibilities for Arg and Glu are in agreement with Weisbjerg et al. (1996) and Wang et al. (2011). Considerable variation in the digestibility of individual AA was also reported in other *in vivo* results (Ben-Ghedalia and Solomon, 1988; Yang and Beauchemin, 2004; Boucher et al., 2009b). However, the variation in the digestibility of individual RUP AA for single feeds was small *in vitro* (Mjoun et al., 2010) or in mobile bags (Wang et al., 1999; Harstad and Prestløkken, 2001), whereas it was great when the residues were fed in roosters. These results demonstrated the differences in the intestinal digestibility of individual AA between *in vitro* (or in mobile bags) and *in vivo*.

Supply of AA

A slight difference in AA supply in the intestine compared with duodenal AA flows would be expected due to digestibility of individual AA. For instance, the ratio of EAA to NEAA increased from 0.79 to 0.83 (average of 4 treatments) due to higher digestibility of EAA than of NEAA. Duodenal flow of Phe was 8.6 g/d greater than that of Arg, whereas it was 8.9 g/d less

after adjusted for the digestibility in the intestine. The result suggests that the both duodenal flow and the digestibility of AA need to be considered to improve the accuracy of predicting AA supply to dairy cows.

The NRC (2001) suggested that maximal milk protein production is achieved by formulating diets at concentrations of 7.2 and 2.4% of MP for Lys and Met, respectively. These concentrations correspond to a Lys-to-Met ratio of 3:1. The ratio of Lys to Met was greater than 3:1 in the current study (3.8) and in the model predictions [NRC, 2001, 3.2; Cornell Net Carbohydrate and Protein System (CNCPS) version 6.1 (<http://www.cncps.cornell.edu/downloads.html>), 3.1], except for CPM-Dairy (Cornell-Penn-Miner System, version 2.023; University of Pennsylvania, Kennett Square; Cornell University, Ithaca, NY; and William H. Miner Agricultural Research Institute, Chazy, NY), which was 2.7 (Table 6). However, the proportion (% of EAA flow) of Lys (13.0 and 11.9, respectively, for low- and high-F:C diets), and that of Met (range of 3.2 and 3.4) were lower than recommended by these models. The models recommend Lys and Met at 16.2 and 5.5%, respectively, for NRC (2001) and 16.8 and 5.3%, respectively, for CPM-Dairy (version 2.023). The models (NRC, 2001; CPM-Dairy, version 2.023; and CNCPS, version 6.1) predicted a 20 to 27% deficiency of Lys and a 40% deficiency of Met.

The duodenal flow of EAA measured in the present study was comparable to that estimated by the CNCPS and higher than that estimated by NRC (2001) and CPM-Dairy (version 2.023) for the low-F:C diet (Table 6). However, the measured flow of EAA was close to the estimation by NRC (2001), but different from that

Table 6. Measured versus predicted duodenal flows of essential AA (EAA) using various models¹ for dairy cows fed low- or high-forage diets

Duodenal flow, g/d	Diet							
	Low forage (35%)				High forage (60%)			
	Measured	NRC	CPM-Dairy	CNCPS	Measured	NRC	CPM-Dairy	CNCPS
Arg	153	128	146	190	127	129	123	184
His	71	57	64	83	57	57	54	80
Ile	183	136	126	170	147	138	108	168
Leu	300	263	212	279	230	265	181	276
Lys	194	175	151	194	139	177	130	190
Met	50	54	57	64	38	54	48	58
Phe	166	143	135	182	131	145	114	177
Thr	145	136	113	148	113	138	97	145
Trp	7	—	36	49	9	—	31	48
Val	220	154	144	193	174	156	123	191
Total EAA	1,488	1,247	1,184	1,551	1,164	1,260	1,009	1,516

¹NRC = NRC (2001); CPM-Dairy = Cornell-Penn-Miner System, version 2.023 (University of Pennsylvania, Kennett Square; Cornell University, Ithaca, NY; and William H. Miner Agricultural Research Institute, Chazy, NY); CNCPS = Cornell Net Carbohydrate and Protein System, version 6.1 (<http://www.cncps.cornell.edu/downloads.html>).

estimated by the other 2 models. The flows of EAA estimated by CPM-Dairy decreased with increasing F:C, which is consistent with the measurements; however, the flows estimated by NRC (2001) and the CNCPS were similar between the low- and high-F:C diets. These results indicate that a difference in estimates of AA supply exists not only between in vivo measurements and prediction models, but also among the models.

Milk Production and Composition

The effects of F:C and FPL on milk yield and milk composition from the 4 dairy cows used in this study (Table 7) were similar to those for all 12 cows used in the full experiment reported by Yang and Beauchemin (2007b). The actual milk yield of the 4 cows (averaged 27.3 kg/d) was lower than the average of all 12 cows (32.2 kg/d; Yang and Beauchemin, 2007b) because of smaller frame size. Increasing the F:C ratio of the diet decreased the production of actual milk ($P < 0.02$), 4% FCM ($P < 0.03$), and SCM ($P < 0.01$), as well as the yield and content of milk protein ($P < 0.01$), but increased ($P < 0.03$) milk fat content. However, no interactions existed between F:C and FPL and no effects of FPL on milk yield and milk composition were observed. Further details of the effects of F:C and FPL on milk production and composition, as well as on DMI, ruminal pH and fermentation, and nutrient digestibil-

ity were discussed previously (Yang and Beauchemin, 2007b). Therefore, only the relationship between the duodenal flows of AA and milk protein production is discussed herein.

The reduction in production of milk, milk protein, and milk protein content were likely due to the deficit in energy supply as a result of decreased DMI with increased F:C. Lower DMI with high-forage diets is thought to be due to the rumen-filling effect of forage. Less starch was digested in the rumen and in the intestine, which would have provided less propionate for glucose synthesis and less glucose for absorption in the intestine with high-F:C diets (Yang and Beauchemin, 2007b). The relationship between dietary energy supply and milk protein concentration is well documented (Sutton, 1989); an energy deficit can depress protein percentage by 0.1 to 0.4 percentage units. However, the supply of protein and AA did not limit milk protein production in the present study because milk protein yields were higher (0.9 kg/d) in other reports (Overton et al., 1995; Brito et al., 2007; Broderick et al., 2007) than the present value (0.78 kg/d) when the same amount of total AA flow to the duodenum was compared (i.e., 2,500 g of total AA/d). That level corresponds to the lowest flow of total AA observed in the present study. The truly digested OM in the rumen was higher in the studies of Overton et al. (1995; 9 kg/d) and Brito et al. (2007; 14 kg/d) than in the present study (8 kg/d; Yang and Beauchemin, 2007b).

Table 7. Effects of forage-to-concentrate ratio (F:C) and forage particle length (FPL) on milk production and composition for lactating dairy cows

Item	Forage ¹ :concentrate (F:C)				SE	P-value		
	35:65		60:40			F:C	FPL	F:C × FPL
	Short	Long	Short	Long				
Milk yield, kg/d								
Actual	28.5	29.0	25.6	26.1	2.2	0.02	NS ²	NS
4% FCM	25.1	25.2	23.8	24.0	1.9	0.03	NS	NS
SCM	24.9	25.2	23.1	23.4	1.9	0.01	NS	NS
Milk fat								
%	3.24	3.14	3.53	3.48	0.17	0.03	NS	NS
kg/d	0.91	0.91	0.90	0.91	0.08	NS	NS	NS
Milk protein								
%	3.17	3.19	2.99	2.97	0.09	0.01	NS	NS
kg/d	0.89	0.92	0.77	0.78	0.06	0.01	NS	NS
Milk lactose								
%	4.61	4.65	4.63	4.61	0.12	NS	NS	NS
kg/d	1.32	1.35	1.18	1.20	0.11	0.02	NS	NS
Milk efficiency								
Actual/DMI	1.40	1.39	1.39	1.44	0.08	NS	NS	NS
FCM/DMI	1.23	1.21	1.28	1.33	0.08	0.01	NS	NS
SCC × 10 ⁵	1.03	1.02	1.14	1.33	0.77	NS	NS	NS

¹Alfalfa forage was harvested at a theoretical chop length of 7.9 and 19.1 mm for short- and long-cut silages, respectively.

²NS = nonsignificant at $P > 0.10$.

Mackle et al. (2000) suggested that the mechanism by which increased dietary energy level influences milk protein is related to increased microbial N synthesis in the rumen, possibly because microbial protein has an AA profile more closely resembling that of milk protein (Santos et al., 1998). In the present study, although the flow of microbial N (g/d or % of N intake) decreased with high-F:C diets, the differences in AA profiles of duodenal protein among treatments were minimal. In addition, the significant interaction between F:C and FPL on the duodenal flow of Met did not alter the milk protein content or milk protein yield in a same manner. In fact, the proportions of Met in the EAA were not different among treatments even though they were lower than recommended by the models as discussed above. In the present study, the protein supply seemed to be largely sufficient to meet the requirement of milk protein production due to high CP content in the diets and high flows of AA. Thus differences in the AA profiles of the duodenal protein did not affect milk protein production.

CONCLUSIONS

Increasing the F:C in the diet reduced the flows of AA to the duodenum as a result of reduced flows of both microbial and feed AA. The intestinal digestibility of AA, EAA, and NEAA tended to decrease with increased rate of forage inclusion in the diets. Consequently, the amount of AA digested in the intestine was significantly decreased by feeding high-F:C compared with low-F:C diets. However, altering FPL had minimal influence on the flows of AA to the duodenum and on the intestinal digestibility of AA. Minimal interaction occurred between F:C and FPL for the flows and digestibility of AA. It was concluded that increasing dietary F:C decreased overall AA supply because of a reduction in the flow to the duodenum and decreased intestinal digestibility of AA; however, increasing FPL had no effects on AA supply. Duodenal flows of AA were more accurately predicted by the CNCPS model for the low-forage diet and by the NRC (2001) model for the high-forage diet. The digestibility of EAA in the intestine was slightly but consistently greater than that of NEAA. Furthermore, the digestibility of individual AA varied considerably, regardless of dietary treatment. The results demonstrate the necessity of considering the both flows and digestibility of individual AA for optimizing diet formulation to meet AA requirements of dairy cows. The flows of AA to the duodenum and the AA profiles had minimal effects on the milk protein production even when energy supply was in deficit because the protein supply was sufficient.

ACKNOWLEDGMENTS

This experiment was financially supported by the Dairy of Farmers of Canada (Ottawa, ON) and Agriculture and Agri-Food Canada's (Ottawa, ON, Canada) Matching Investment Initiative. The authors thank K. Andrews, B. Farr, A. Furtado, D. Vedres, and R. Wuerfel (of Research Centre, Agriculture and Agri-Food Canada, Lethbridge, AB, Canada) for their assistance in performing sampling and laboratory analyses, and as well as the staff of the Lethbridge Research Centre (Lethbridge, AB, Canada) dairy unit for care of the cows.

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