Effects of Fiber Content and Particle Size of Forage on the Flow of Microbial Amino Acids from Continuous Culture Fermenters*

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ABSTRACT

Eight dual-flow continuous culture fermenters (1320 mL) were used in two 10-d consecutive periods to study the effects of fiber content and particle size on rumen fermentation, nutrient flow, and the profile and flow of amino acids (AA) from microbial origin. Treatments were arranged in a 2×2 factorial; the main factors were fiber content (high fiber [HF] = 67% alfalfa hay, 33% concentrate; low fiber [LF] = 39% alfalfa hay, 61% concentrate) and forage stem particle size (ground not to pass a 3-mm sieve = large size [LS]; ground to pass a 1-mm sieve = small size [SS]). On the last day of the experiment, liquid- (LAB) and solid- (SAB) associated bacteria were isolated from each fermenter for chemical analysis. Microbial N and AA flows were estimated using LAB or SAB composition data. Total volatile fatty acid concentration was higher for LF (122 mM) than for HF (102 mM). The proportion of acetate was higher for HF (63.5%) than for LF (58.2%). When SS was fed instead of LS, there was a reduction in the acetate proportion (63.4% vs 58.3%, respectively) and in the acetate to propionate ratio (3.13 vs. 2.41, respectively). Bacterial N flow was higher, and dietary N flow was lower, in SS compared with LS when LAB were used for calculations. Efficiency of microbial protein synthesis (EMPS) was affected by particle size, when LAB were used for calculations, and by fiber content, when SAB were used for calculations. The AA profile of LAB differed from SAB in 4 of 16 AA. The fiber content and particle size had small effects on microbial AA profile. However, the bacterial AA flow was higher in 13 of 16 AA, when SAB instead of LAB were used for calculations. Estimates of bacterial N flow, EMPS, AA profile, and flows of microbial origin were dependent on the type of microbial population used for calculations. The

representativeness of the microbial samples is essential for the accurate prediction of the AA flow from microbial origin.

(Key words: fiber, particle size, amino acid, rumen bacteria)

Abbreviation key: EAA = essential AA, EMPS = efficiency of microbial protein synthesis, HF = high fiber, LAB = liquid-associated bacteria, LF = low fiber, LS = large size, NEAA = non-essential AA, SAB = solid-associated bacteria, SS = small size.

INTRODUCTION

Feeding systems for dairy cattle (O'Connor et al., 1993; Rulquin and Vérité, 1993; NRC, 2001) include the prediction of AA requirements and supply to the small intestine. Between 50 and 80% of the AA flowing to the small intestine are of microbial origin (Storm and Ørskov, 1984). There is considerable evidence that the chemical composition (Clark et al., 1992; Martin et al., 1994; Pérez et al., 1998) and the AA profile (Lallès et al., 1992; Martin et al., 1996) of solid- (SAB) and liquid (LAB) associated bacteria differ. Our first objective was to determine the effect of using LAB or SAB on the estimated flow of AA from microbial origin. The hypothesis was that if the AA profiles of SAB and LAB are indeed different, diets that would be favorable to the growth of SAB (high fiber and small particles to increase microbial attachment surface) or LAB (low fiber and large particles to reduce the microbial attachment surface) will affect the partial contribution of each bacterial fraction and, therefore, their estimated contribution to the supply of AA to the small intestine.

A second objective was to study the direct effect of fiber content and particle size on rumen microbial fermentation. Results from *in vivo* studies indicate that as the level of fiber in the diet decreases, ruminal pH, retention time, and fiber digestibility also decrease. However, the reduction in fiber digestibility cannot be attributed uniquely to the change in fiber content of the diet because the effects are confounded. Similarly, while the reduction in particle size has been associated with an increase in fiber digestion caused by greater

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Table 1. Ingredient and chemical composition of the diets.

Ingredient	Diet ¹			
	HF + LS	HF + SS	LF + LS	LF + SS
Alfalfa hay, ² % of DM				
Stem	41.6	41.6	24.1	24.1
Leaf	25.5	25.5	14.8	14.8
Corn grain (ground), % of DM	23.9	23.9	47.2	47.2
Soybean meal, % of DM	7.3	7.3	11.3	11.3
Minerals and vitamins, ³ % of DM	1.7	1.7	2.3	2.3
Chemical composition				
DM, %	92.1	92.4	91.4	91.5
	(% of DM)			
OM	90.6	90.9	92.1	92.5
CP	17.9	17.4	17.5	17.2
NDF	40.1	40.2	28.1	29.6
ADF	24.3	24.7	15.8	16.1
NFC ⁴	31.9	34.0	45.0	44.0

¹HF = High fiber, LS = large particle size, SS = small particle size, and LF = low fiber.

²Chemical composition of stems: 89.9% DM, 6.0% ash, 9.13% CP, and 65.3 NDF. Chemical composition of leaves: 89% DM, 13.0% ash, 24.7% CP, and 14.2% NDF.

³Contained 17,000 ppm of Co, 600 ppm of Cu, 2000 ppm of Fe, 80 ppm of I, 6000 ppm of Mn, 14 ppm of Se, 3000 ppm of Zn, 600 kIU/kg of DM of vitamin A, 120 kIU/kg of DM of vitamin D, and 360 IU/kg of DM of vitamin E.

⁴NFC = Nonfiber carbohydrate (calculated as 100 – (% CP + % ash + % NDF + % ether extract)).

surface for microbial attachment in situ (Nocek and Kohn, 1988), in vivo studies have reported lower fiber digestion, probably associated with an increased passage rate, a reduction in pH caused by lower rumination, or both. The isolation of these main factors independent of other changes in rumen conditions is essential for mathematical modelling of ruminal fermentation. In this context, the use of in vitro continuous culture systems offers the opportunity to change the fiber content or particle size without affecting intake, pH, or dilution rates.

The objective of this research was to study the effects of fiber content and particle size on ruminal fermentation and nutrient flow and the profile and flow of AA from microbial origin using LAB or SAB composition data in a dual-flow continuous culture system.

MATERIALS AND METHODS

Diets

Four diets for lactating dairy cows were formulated with 2 levels of fiber content (high fiber [HF] = 67 to 33 alfalfa hay to concentrate ratio; low fiber [LF] = 39 to 61 alfalfa hay to concentrate ratio) and two forage stem particle sizes (large [LS] = ground not to pass a 3-mm sieve; small [SS] = ground to pass a 1-mm sieve; Table 1). Diets were combinations of the 2 main factors (HF + LS, HF + SS, LF + LS, and LF + SS). Diets were designed with the intention of providing an extreme range of diets, where the surface available for the at-

tachment of bacteria would change from small (LF + LS) to large (HF + SS). It was not the intention of this trial to study the effects of fiber content or particle size on rumen dynamic (salivation, rumination, etc.), but to isolate the effect of type of bacteria population on the flow of AA from microbial origin. The fiber source was early bloom alfalfa hay (*Medicago sativa*) for which stems and leaves were separated. The leaves were ground at 0.8 mm. The stems were ground (hammer mill, Prat SA, Sabadell, Spain) and passed through a 3-mm sieve. The sample retained in a 3-mm sieve was divided in 2 equal parts. The first was considered as particle size ≥ 3 mm, and the other was ground at 0.8 mm and totally passed through a 1-mm sieve. This fraction was considered as ≤ 1 mm. This process was conducted to guarantee that the compositions of the 2 particle sizes of alfalfa stems were similar. The alfalfa hay was reconstituted to a standard of 38% leaves and 62% stems (Jarrige et al., 1995).

Continuous Culture System

Eight 1320-mL dual-flow continuous culture fermenters (Hoover et al., 1976) were used in 2 consecutive 10-d experimental periods. Fermenters were inoculated with ruminal fluid strained through 4 layers of cheesecloth taken from 2 ruminally cannulated cows fed a 50:50 alfalfa hay:concentrate diet. Experimental diets were mixed daily, and 95 g of DM were fed in 4 equal portions throughout the day. Temperature was main-

tained at 39°C, and pH was controlled at 6.4 ± 0.05 by infusion of 3 N HCl or 5N NaOH. The system was well buffered at pH 6.4, and the average use of HCl and NaOH was of 12.0 and 12.5 mL, respectively. Anaerobic conditions were maintained by infusion of N₂ at a rate of 40 mL/min. Artificial saliva (Weller and Pilgrim, 1974) was continuously infused into flasks and contained 0.187 g/d of nonprotein N to simulate recycled N. Liquid and solid dilution rates were maintained at 10 and 5%/h, respectively.

Sample Collection and Bacterial Detachment Procedure

Each experimental period consisted of 7 d for adaptation and 3 d for sampling. During sampling days, collection vessels were maintained at 4°C to prevent microbial growth. Solid and liquid effluent content were homogenized for 1 min, and a 500-mL sample was removed via aspiration. Upon completion of each period, effluents from the 3 sampling d were composited and mixed within fermenter. Three subsamples of mixed effluent were taken; 2 of them were acidified with either HCl (4 mL of effluent in 4 mL 0.2 N HCl) or H₃PO₄ (4 mL of effluent in 1 mL of 0.2% HgCl₂, 2% H₃PO₄, and 0.2% 4-methyl valerate) and frozen until analyzed for ammonia N and VFA concentrations, respectively. A 3rd subsample was taken in triplicate for total N analysis. Two 250-mL portions of each composited sample were freeze-dried, ground through a 1-mm screen, and used for DM, ash, NDF, ADF, purine bases, and AA analysis.

Solid-associated bacteria and LAB were obtained the last day of each experimental period. The LAB were obtained by filtration of total fermenter flask contents through 2 layers of cheesecloth. The solid fraction was washed with saline solution (0.85% wt/vol NaCl in 3:1 saline solution:solid fraction) to remove residual bacteria of the liquid fraction (Olubobokun and Craig, 1990), and the filtrate was added to the LAB preparation. The filtrate was centrifuged for 10 min at $1000 \times g$ to remove feed residues. The supernatant fraction was centrifuged twice for 20 min at $20,000 \times g$ to isolate LAB, washed twice (once with 0.85% wt/vol. NaCl solution and another time with distilled water to reduce mineral contamination of the sample), and recentrifuged. The final bacterial pellet was freeze-dried for subsequent analysis. The SAB were obtained from the washed solid fraction by using a combination of several detachment procedures (Whitehouse et al., 1994) selected to obtain the maximum detachment without affecting cell integrity. The fermenter solid residue combined with the feed residue pellet obtained in the first centrifugation of the LAB separation procedure was suspended in a saline

solution with 0.1% methylcellulose (3:1 saline solution:solid fraction) and incubated in a shaking water bath at 37°C for 30 min to remove attached bacteria (Minato and Suto, 1978). After the incubation, samples were refrigerated for 24 h at 4°C, and solid residues were agitated for 1 h with marbles (thirty 2-mm and fifteen 4-mm diameter marbles) to dislodge loosely attached bacteria. Finally, the solid fraction was filtered through cheesecloth and washed 3 times with saline solution. The SAB were obtained by differential centrifugation as in the LAB isolation procedure. The use of chemical (alcohol, formaldehyde, acids) or physical (homogenization) treatments (Whitehouse et al., 1994) was avoided to prevent changes in microbial cell integrity or modification in their AA composition.

Sample Analyses

The DM and ash contents in feed, bacteria, and effluents and the ether extract in diets were determined according to the AOAC (1990). The NDF and ADF concentrations in feed and effluents were determined sequentially (using a thermostable amylase; ANKOM Technology, Fairport, NY) and sodium sulfite (Van Soest et al., 1991). Total N in feed, effluents, and bacterial samples were determined by the Kjeldahl method (AOAC, 1990). The ammonia was determined by a colorimetric procedure (Chaney and Marbach, 1962). The VFA concentrations in deproteinized effluent fluid samples were determined by gas chromatography (GLC Hewlett Packard, Palo Alto, CA) using 4-methylvaleric as the internal standard (Jouany, 1982). The purine bases (adenine and guanine) in bacteria and effluent samples were determined by HPLC (Hewlett Packard) using allopurinol as the internal standard (Balcels et al., 1992). Bacteria (1 mg) and effluent samples (5 mg) for AA analysis were hydrolyzed with 200 μ L of 6 N HCl at 110°C for 24 h in sealed, evacuated tubes. Stability of methionine residues was achieved by adding mercaptoethanol as a reducing agent. Derivatization was conducted with 200 nmol per tube of dabsyl chloride at 70°C for 12 min. The AA analysis was performed by reverse-phase HPLC (Beckmann Instruments, Palo Alto, CA) following the procedure of Vendrell and Avilés (1986), and using norleucine as the internal standard. A known mixture of free AA and a standard protein (lisozyme, L-687; Sigma-Aldrich, Madrid, Spain) were processed in duplicate within each run to correct for AA losses during hydrolysis and AA analysis. Recovery rates were used to correct the AA content of bacteria and effluents. The flow of AA from microbial origin was calculated as $\text{g AA/d} = \text{g AA/g DM bact} \times \text{g bact/d}$, where g AA/g DM bact is the AA content expressed in DM

Table 2. Effects of fiber content and particle size of the diets on apparent and true OM, NDF, and ADF digestion in continuous culture.

Item	Fiber content ¹		Particle size		SEM ³	Effect (<i>P</i> <)	
	HF	LF	LS	SS		Fiber	Size
Apparent OM digestibility, %	41.6	44.6	43.7	42.6	3.4	NS	NS
True digestibility ³							
OM _{LAB} , %	44.1	46.2	45.6	44.7	3.4	NS	NS
OM _{SAB} , %	46.6	47.9	47.6	46.9	4.4	NS	NS
Fiber digestibility							
NDF, %	27.9	30.0	31.6	26.3	8.1	NS	NS
ADF, %	26.7	27.6	30.2	24.0	8.1	NS	NS

¹HF = High fiber; LF = low fiber.²LS = Large particle size; SS = small particle size.³Calculated using compositional data of liquid-associated bacteria (LAB) or solid-associated bacteria (SAB).

basis for LAB and SAB, and g bact/d is the amount of bacterial DM synthesized daily.

Statistical Analyses

Data were analyzed as a randomized complete block design with a 2 × 2 factorial arrangement of treatments (Steel and Torrie, 1988). The model used for each dependent variable was

$$Y_{ijkl} = \mu + B_i + F_j + S_k + (F \times S)_{jk} + \varepsilon_{ijkl}$$

where μ is the mean, B is the period as block, F is the fiber content effect, S is the particle size effect, and F × S their interaction.

Statistical analysis was conducted using SAS (SAS Inst. Inc., Cary, NC). Microbial fermentation profile and nutrient flow data were analyzed using PROC GLM. Differences among means were tested by Tukey's multiple comparison test. The AA profile and flow of SAB

and LAB were analyzed as a paired t-test (Steel and Torrie, 1988) using PROC MEANS. Differences were declared at *P* < 0.05.

RESULTS AND DISCUSSION

Chemical Composition of the Diets

Diets were isonitrogenous; NDF and ADF levels were higher, and non-fiber carbohydrates were lower, in the HF diets compared with the LF diets, as expected (Table 1). The alfalfa fractionation and particle size reduction did not affect diet composition. Bowman and Firkins (1993) reported changes in nutrient composition with changes in feed particle size, which were probably due to the uneven distribution of small particles. The careful separation of leaves and stems and the complete grinding of well-defined stem fractions in the present experiment prevented these differences from happening.

Table 3. Effects of fiber content and particle size of the diets on VFA in continuous culture.

Item	Fiber content ¹		Particle size ²		SEM	Effect (<i>P</i> <)	
	HF	LF	LS	SS		Fiber	Size
Total VFA, mM	102.2	121.6	112.1	111.8	8.7	**	NS
Individual VFA, mol/100 mol							
Acetate	63.5	58.3	63.4	58.3	3.7	*	*
Propionate	21.5	25.2	22.2	24.5	4.5	NS	NS
Butyrate	10.9	11.8	9.9	12.9	1.4	NS	**
Iso-butyrate	0.67	0.80	0.79	0.68	0.1	†	†
Valerate	2.45	2.32	2.48	2.30	0.2	NS	†
Iso-valerate	1.05	1.62	1.28	1.39	0.4	*	NS
Branched-chain VFA	1.72	2.42	2.08	2.07	0.5	*	NS
Acetate:propionate	3.05	2.49	3.13	2.41	0.6	†	*

¹HF = High fiber; LF = low fiber.²LS = Large particle size; SS = small particle size.†*P* < 0.10.**P* < 0.05.***P* < 0.01.

Table 4. Effects of fiber content and particle size of the diets on N metabolism in continuous culture.

Item	Fiber content ¹		Particle size ²		SEM	Effect (<i>P</i> <)	
	HF	LF	LS	SS		Fiber	Size
Ammonia N, mg/100 mL	17.8	18.6	18.6	17.8	3.5	NS	NS
Total N flow, g/d	3.02	3.01	3.00	3.03	0.1	NS	NS
Ammonia N flow, g/d	0.56	0.58	0.58	0.56	0.1	NS	NS
Non-ammonia N flow, g/d	2.46	2.43	2.42	2.47	0.1	NS	NS
Purine bases:N, μ mol/g N							
LAB ³	20.1	24.5	24.5	23.2	4.2	†	†
SAB ³	24.3	18.8	20.1	19.9	5.1	†	NS
Dietary N flow, g/d							
LAB	1.32	1.42	1.46	1.29	0.1	NS	*
SAB	1.17	1.41	1.35	1.23	0.3	†	NS
Bacterial N flow, g/d							
LAB	1.13	1.00	0.96	1.18	0.2	NS	*
SAB	1.29	1.02	1.08	1.24	0.3	NS	NS
Protein degradation, %							
LAB	51.9	46.9	46.7	52.1	7.2	NS	NS
SAB	57.5	47.5	50.7	54.3	10.6	†	NS
EMPS, ⁴ g/kg OMTD							
LAB	30.9	27.5	27.0	31.3	3.8	NS	*
SAB	33.3	26.8	28.8	31.3	3.8	**	NS

¹HF = High fiber; LF = low fiber.²LS = Large particle size; SS = small particle size.³LAB = Liquid-associated bacteria; SAB = solid-associated bacteria.⁴EMPS = Efficiency of microbial protein synthesis (g N/kg OM truly digested [TOMD]).†*P* < 0.10.**P* < 0.05.***P* < 0.01.

Because there were no interactions between fiber content and particle size (*P* > 0.10), main effects are presented and discussed, unless otherwise indicated.

Digestibility Coefficients

Fiber content did not affect apparent or true OM and fiber digestion, although it was numerically higher in LF (Table 2) than in HF. In contrast, fiber digestion decreased in vivo as the content of fiber decreased in the diet (Bourquin et al., 1994). Under in vivo conditions, low fiber diets resulted in a decrease in ruminal pH, and the cause of the decrease in fiber digestion was confounded between fiber level and pH (Hoover, 1986). In the present trial, the lack of effect of treatments on fiber digestion when pH was controlled suggests that the reduction in fiber digestion observed in vivo was due to changes in pH rather than a direct effect of fiber content.

Particle size did not affect the apparent or true digestion of OM or fiber (Table 2). In vivo, the reduction in particle size has often been related to a decrease in ruminal degradation of DM, probably because of a lower retention time of feed particles in the rumen or a decrease in pH (Grant et al., 1990). In contrast, in vitro studies have shown that a reduction in particle size results in an increase in ruminal degradation of DM

and fiber (Fahey et al., 1993), which was attributed to an increase in the surface area available for microbial attack (Bowman and Firkins, 1993) and the prevention of the changes in pH and dilution rates observed in vivo. Under the conditions of the present experiment, it is likely that the control of pH and dilution rates resulted in no differences in OM and fiber digestion. These results suggest that most changes observed in vivo on OM and fiber digestion caused by the content of fiber and particle size are mediated through changes in pH or dilution rates. Therefore, mechanistic models may predict accurately these effects by proper simulation of pH and dilution rate effects on nutrient digestion.

Effects on Ruminal Fermentation

VFA concentration. Total VFA concentration was higher (*P* < 0.05) in LF than in HF (Table 3) and is consistent with the non-significant trend for higher OM digestion in LF (Table 2). In vivo (Bourquin et al., 1994) and in vitro (Russell, 1998) studies reported that low fiber diets resulted in higher VFA concentration because of the greater rate of fermentation of starch compared with fiber. Molar proportion of acetate was higher, and that of branched-chain VFA was lower, in HF than in LF diets (*P* < 0.05) and was consistent with

Table 5. Chemical composition of solid- (SAB) and liquid-associated bacteria (LAB) in continuous culture.

Variable	Bacteria		SEM ¹	P <
	LAB	SAB		
Bacterial N, %	9.37	8.71	0.18	**
PB, ² μ mol/g DM	209.7	188.3	7.7	**
PB:N, μ mol/g N	22.28	21.55	0.87	NS
Bacterial N flow, ³ g/d	1.07	1.16	0.06	NS
EMPS ⁴	29.18	30.03	0.17	NS
AA profile, g AA/100 g AA				
Asp	9.95	10.57	0.20	**
Glu	8.26	8.40	0.11	NS
Ser	5.84	5.86	0.09	NS
Thr	6.17	6.10	0.08	NS
Gly	10.05	10.19	0.31	NS
Ala	11.34	11.52	0.07	*
Arg	3.51	3.62	0.04	**
Pro	4.11	4.18	0.07	NS
Val	5.63	5.53	0.07	NS
Met	1.25	1.22	0.04	NS
Ile	4.81	4.65	0.10	NS
Leu	6.26	6.17	0.06	NS
Phe	3.50	3.49	0.03	NS
Lys	12.06	11.34	0.18	***
His	2.53	2.61	0.05	†
Tyr	4.41	4.53	0.06	†
EAA ⁵	45.73	44.75	0.33	**
NEAA ⁶	54.27	55.25	0.33	**

¹Standard error of the mean of differences between SAB and LAB.

²PB = Purine bases (μ mol [Adenine + Guanine]/g DM).

³Total N flow of microbial origin.

⁴EMPS = Efficiency of microbial protein synthesis.

⁵EAA = Essential AA (Thr, Arg, Val, Met, Ile, Leu, Phe, Lys, and His).

⁶NEAA = Nonessential AA (Ala, Asp, Glu, Gly, Pro, Ser, and Tyr).

† $P < 0.10$.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

previous reports (Bourquin et al., 1994), as was the trend for lower acetate:propionate in the LF diets ($P < 0.10$; Siciliano-Jones and Murphy, 1989; Russell, 1998).

Total VFA concentration was not affected by particle size. However, the proportion of acetate and acetate:propionate was higher in LS, and the butyrate concentration was lower in LS, compared with SS ($P < 0.05$; Table 3). Although the increase in the surface area for attachment of bacteria in SS did not increase fiber digestion and acetate concentration as expected (Bowman and Firkins, 1993), the higher butyrate concentration may justify, at least in part, the reduction in acetate concentration.

A reduction in acetate:propionate is often observed in in vivo experiments as a result of forage processing (Fahey et al., 1993). This has been implicated as the reason for the reduction in milk fat percentage often observed when lactating dairy cattle consume processed forages (Shaver et al., 1986). However, these observations in vivo are likely indirect effects of changes in pH.

Grant et al. (1990) reported a reduction in acetate:propionate when cows were fed small particle forage, but the effect was attributed to the reduction of pH and the resulting increase in propionate. In the present experiment, the decrease in acetate:propionate in SS was due to a decrease in acetate. It should be noted that the decrease in acetate proportion in SS was parallel to an increase in butyrate concentration, suggesting that there was an increase in the conversion of acetate to butyrate in the SS diets.

N metabolism. Fiber content and particle size did not affect the ammonia N concentration or the flow of total, ammonia and nonammonia N ($P > 0.05$; Table 4). Dietary and microbial N flow, protein degradation, and the efficiency of microbial protein synthesis (EMPS) were estimated using the chemical composition of LAB and SAB independently. Results are dependent of purine bases:N in LAB and SAB, which tended to be affected by fiber content and particle size, when LAB were

Table 6. Effect of fiber content and particle size of diets on amino acid flow (g/d) in continuous culture.

AA	Fiber content ¹		Particle size ²		SEM	Effect (<i>P</i> <)	
	HF	LF	LS	SS		Fiber	Size
Asp	1.94	2.07	2.03	1.97	0.20	*	NS
Glu	2.20	2.49	2.38	2.31	0.23	*	NS
Ser	0.87	0.92	0.90	0.88	0.08	NS	NS
Thr	0.92	0.98	0.96	0.94	0.09	NS	NS
Gly	0.94	0.97	0.96	0.95	0.09	NS	NS
Ala	1.32	1.46	1.41	1.38	0.16	NS	NS
Arg	0.92	0.99	0.92	0.99	0.16	NS	NS
Pro	0.70	0.75	0.74	0.72	0.08	NS	NS
Val	1.04	1.13	1.09	1.08	0.11	NS	NS
Met	0.27	0.28	0.29	0.26	0.04	NS	NS
Ile	0.89	0.96	0.93	0.93	0.10	NS	NS
Leu	1.41	1.55	1.49	1.47	0.14	†	NS
Phe	0.84	0.88	0.88	0.84	0.09	NS	NS
Lys	0.92	0.95	0.94	0.92	0.10	NS	NS
His	0.29	0.29	0.29	0.29	0.03	NS	NS
Tyr	0.66	0.75	0.72	0.68	0.06	*	NS
EAA ³	7.48	8.02	7.80	7.71	0.73	NS	NS
NEAA ⁴	8.63	9.40	9.14	8.89	0.85	†	NS
Total	16.11	17.42	16.94	16.60	1.57	NS	NS

¹HF = High fiber; LF = low fiber.²LS = Large particle size; SS = small particle size.³EAA = Essential AA (Thr, Arg, Val, Met, Ile, Leu, Phe, Lys, and His).⁴NEAA = Nonessential AA (Ala, Asp, Glu, Gly, Pro, Ser, and Tyr).†*P* < 0.10.**P* < 0.05.****P* < 0.0001.

used for calculation, and by fiber content, when SAB were used (Table 4).

The fiber content did not affect flow (g/d) of microbial N and tended (*P* < 0.10) to reduce dietary N flow and increase dietary CP degradation in HF compared with LF when SAB were used in calculations. The EMPS was higher (*P* < 0.05) in HF than in LF when SAB were used for calculation. Although the numerical trends were similar between data obtained using LAB or SAB, differences were only significant when SAB data were used in calculations. Many authors (Hvelplund and Madsen, 1985; Rode et al., 1985) reported that high forage diets fed in vivo result in higher EMPS. In contrast, results of Bourquin et al. (1994) indicated lower EMPS in vivo when a 90% vs. 60% forage diet was fed. Meng et al. (1999) observed in continuous culture that diets with a high concentration of structural carbohydrates resulted in higher EMPS than diets with a high non-structural carbohydrate concentration, which agrees with the results obtained in the present trial.

The reduction in particle size resulted in lower dietary N flow and higher microbial N flow when LAB composition data were used in calculations (*P* < 0.05), although these differences did not result in significant differences in dietary CP degradation. The EMPS was lower in LS than in SS, although it was only significant when LAB composition data were used in calculations

(*P* < 0.05; Table 4). Results from in vivo trials have shown no consistent effects of forage particle size on the EMPS. Beever et al. (1981) observed no effects of forage particle size on the EMPS. In contrast, Rode et al. (1985) found that grinding and pelleting alfalfa hay resulted in a 15% increase in EMPS in lactating dairy cows, but the higher EMPS was attributed to a faster solid dilution rate. However, in the present trial, solid dilution rate was maintained constant. Reduced particle size also results in increased surface area for microbial attachment and digestion (Bowman and Firkins, 1993) and may enhance energy availability and microbial growth, which may explain the higher EMPS observed in SS than in LS, although differences were only significant when LAB composition was used in calculations.

Chemical composition of LAB and SAB indicates differences in N and purine concentration; both were higher in LAB compared with SAB. However, N:purine was not different between microbial populations (Table 5). The higher mean N and purine concentrations in LAB vs. SAB in the present trial have already been reported by others (Olubobokun et al., 1988; Pérez et al., 1998). The higher purine concentration in LAB might reflect a higher rate of protein synthesis and, most probably, a higher growth rate than in SAB (Czerkawski, 1986; Legay-Carmier and Bauchard, 1989).

Table 7. Effect of fiber content and particle size of the diets on AA profile (g AA/100 g AA) of liquid-associated bacteria (LAB) and solid-associated bacteria (SAB) in continuous culture.

AA		Fiber content ¹		Particle size ²		SEM	Effect (<i>P</i> <)	
		HF	LF	LS	SS		Fiber	Size
Asp	LAB	10.4	9.5	9.8	10.1	0.5	**	NS
	SAB	10.4	10.7	10.5	10.6	0.4	NS	NS
Glu	LAB	8.4	8.1	8.3	8.2	0.2	**	NS
	SAB	8.3	8.5	8.6	8.2	0.3	NS	†
Ser	LAB	5.8	5.9	5.8	5.9	0.3	NS	NS
	SAB	5.8	5.9	5.8	5.9	0.2	NS	NS
Thr	LAB	6.1	6.3	6.2	6.1	0.2	NS	NS
	SAB	6.1	6.1	6.0	6.2	0.2	NS	*
Gly	LAB	10.2	9.9	10.1	10.0	0.2	***	NS
	SAB	10.2	10.1	10.2	10.2	0.2	NS	NS
Ala	LAB	11.4	11.3	11.4	11.2	0.2	NS	NS
	SAB	11.5	11.5	11.7	11.4	0.3	NS	†
Arg	LAB	3.6	3.4	3.5	3.5	0.1	†	NS
	SAB	3.7	3.6	3.6	3.6	0.1	†	NS
Pro	LAB	4.2	4.1	4.2	4.0	0.3	NS	NS
	SAB	4.2	4.1	4.2	4.1	0.1	†	NS
Val	LAB	5.6	5.6	5.6	5.7	0.4	NS	NS
	SAB	5.5	5.5	5.5	5.5	0.1	NS	NS
Met	LAB	1.2	1.3	1.3	1.2	0.1	NS	NS
	SAB	1.2	1.2	1.3	1.2	0.2	NS	NS
Ile	LAB	4.7	4.9	4.9	4.7	0.2	*	NS
	SAB	4.8	4.5	4.5	4.7	0.2	*	†
Leu	LAB	6.2	6.3	6.4	6.2	0.2	NS	†
	SAB	6.2	6.1	6.2	6.1	0.1	NS	NS
Phe	LAB	3.5	3.5	3.5	3.5	0.1	NS	NS
	SAB	3.5	3.5	3.5	3.5	0.1	NS	NS
Lys	LAB	11.7	12.4	12.0	12.1	0.6	*	NS
	SAB	11.3	11.3	11.1	11.5	0.4	NS	*
His	LAB	2.6	2.5	2.6	2.5	0.2	NS	NS
	SAB	2.7	2.5	2.6	2.6	0.2	NS	NS
Tyr	LAB	4.4	4.4	4.4	4.4	0.3	NS	NS
	SAB	4.5	4.6	4.6	4.5	0.2	NS	NS
EAA ³	LAB	45.2	46.3	45.9	45.5	0.1	†	NS
	SAB	45.0	44.5	44.4	45.1	0.8	NS	NS
NEAA ⁴	LAB	54.8	53.7	54.1	54.5	1.1	†	NS
	SAB	55.0	55.5	55.6	54.9	0.8	NS	NS

¹HF = High fiber; LF = low fiber.²LS = Large particle size; SS = small particle size.³EAA = Essential AA (Thr, Arg, Val, Met, Ile, Leu, Phe, Lys, and His).⁴NEAA = Nonessential AA (Ala, Asp, Glu, Gly, Pro, Ser, and Tyr).†*P* < 0.10.**P* < 0.05.***P* < 0.01.

Changes in the purine and N concentrations in LAB and SAB may affect the magnitude and the significance of the differences in estimates of N metabolism in fermenters. Although numerical differences between estimates of N metabolism had the same direction, the magnitude of these differences was higher in SAB than in LAB. The standard error of most measurements in SAB was larger than that in LAB and was probably due to either incomplete or altered recovery of the microbial population through the bacteria detachment procedures. The differences observed in microbial composition between LAB and SAB suggest that many of the estimates of N metabolism changed more because of

the microbial population considered than because of the main factors studied. Therefore, the identification of significant effects depended on the microbial population selected for calculations. These observations indicate the importance of obtaining representative samples of the microbial population including the LAB and SAB.

Effects on the AA Profile and Flow

Total flow of AA in effluents. Fiber content and particle size did not affect the flow (g/d) of essential (EAA), non-essential (NEAA), nor most individual AA (Table 6). Clark et al. (1992) indicated that, because of

Table 8. Differences in amino acid flow (g/d) of microbial origin between solid-associated bacteria (SAB) and liquid-associated bacteria (LAB) in continuous culture.

AA flow, g/d	Bacteria		SEM ¹	Effect (<i>P</i> <)
	LAB	SAB		
Asp	0.62	0.77	0.05	***
Glu	0.73	0.86	0.05	*
Ser	0.32	0.37	0.02	**
Thr	0.39	0.45	0.02	*
Gly	0.34	0.39	0.02	*
Ala	0.46	0.55	0.03	**
Arg	0.33	0.40	0.02	**
Pro	0.20	0.24	0.01	*
Val	0.46	0.52	0.03	*
Met	0.13	0.15	0.01	†
Ile	0.42	0.47	0.02	*
Leu	0.49	0.57	0.03	*
Phe	0.30	0.35	0.02	*
Lys	0.54	0.59	0.04	NS
His	0.10	0.12	0.01	***
Tyr	0.27	0.32	0.02	**
EAA ³	3.15	3.62	0.19	*
NEAA ⁴	2.94	3.50	0.19	**
Total	6.10	7.12	0.38	*

¹Standard error of the mean of differences between SAB and LAB.²EAA = Essential AA (Thr, Arg, Val, Met, Ile, Leu, Phe, Lys, and His).³NEAA = Nonessential AA (Ala, Asp, Glu, Gly, Pro, Ser, and Tyr).†*P* < 0.10.**P* < 0.05.***P* < 0.01.****P* < 0.001.

the large contribution of microbial protein to the total AA flow, it was difficult to modify the AA profile in the digesta unless large amounts of supplemental ruminal undegradable protein sources were fed. In the present study, between 39.7 and 52.4% of the protein leaving the fermenters was of microbial origin, and diets did not contain supplemental undegradable protein sources. The flow of Asp, Glu, and Tyr was higher (*P* < 0.05) in the LF diets, resulting in a trend for higher NEAA flow in LF diets compared with HF diets (*P* < 0.10). The flow of Leu tended to increase (*P* < 0.10) in LF diets compared with HF diets and was attributed to the higher Leu content of corn (Rulquin and Vérité, 1993).

Effects on the AA profile of microbial origin. The AA profile of LAB and SAB (Table 5) was within the ranges reported by Clark et al. (1992), except for Ser, Gly, Ala, and Lys, which were higher in both bacterial groups, and Asp, Arg, Glu, and Phe, which were lower in both bacterial groups. Between-population comparisons indicated that the concentration of Asp, Ala, and Arg was higher (*P* < 0.05), that of His and Tyr tended to be higher (*P* < 0.10), and that of Lys was lower (*P* < 0.05) in SAB compared with LAB. However, the numerical differences between microbial populations were

smaller, and the number of AA that differed were fewer, than data reported by Martin et al. (1996), who reported a larger difference in 7 of 11 EAA or semi-essential AA depending on the time of sampling (2 vs. 23 h postfeeding) and in 6 of 11 AA depending on the type of diet (hay vs. hay and barley).

Fiber content and particle size had a small effect on the profile of essential AA from LAB and SAB (Table 7). The interaction of fiber content × particle size was only significant for the proportion of Thr and Lys in LAB. The proportion (g AA/100 g AA) was 5.97, 6.18, 6.41, and 6.11, for Thr, and 11.31, 12.07, 12.72, and 12.12 for Lys in HF + LS, HF + SS, LF + LS, LF + SS, respectively. Results of other AA are presented by main effects.

The AA profiles of LAB and SAB were different in HF and LF (*P* < 0.05) in 5 of 16 AA studied. However, the numerical differences were small and dependent on the bacterial population considered (Table 7). In LAB, the profiles (g AA/100 g AA) of Asp, Glu, and Gly were higher, and those of Ile and Lys were lower, in HF than in LF. In SAB, the proportion (g AA/100 g AA) of Ile was lower (*P* < 0.05) in LF than in HF. Overall, the proportion (g AA/100 g AA) of EAA was 2.43% lower (*P* < 0.10) in HF than in LF when LAB was used for calculations. In contrast, the concentration (g AA/100 g AA) of EAA was not affected by the content of fiber when SAB was used for calculations. However, these differences in AA profile were smaller than those reported by Hussein et al. (1995), who reported a higher concentration of AA in a pool of ruminal bacteria (LAB and SAB) in a high forage diet (70% corn silage) compared with a low forage diet (30% corn silage).

Fiber content and particle size had a small effect on the AA profile of LAB and SAB. Lallés et al. (1992) and Martin et al. (1996) indicated that the AA composition of ruminal bacteria was a characteristic of each bacterial group and, therefore, difficult to change by dietary manipulation. Others (Ørskov, 1988; Putnam et al., 1997) reached the same conclusion when comparing the AA profile of LAB and a mixture of LAB and SAB obtained from animals fed different diets. In contrast, data summarized from literature reports (Storm and Ørskov, 1984; Clark et al., 1992) and some in vivo trials (Hussein et al., 1995) suggested that diet might affect the AA profile of ruminal bacteria. However, Clark et al. (1992) suggested that the differences observed in the AA profile of ruminal bacteria across different experiments might be related to differences in the methodologies used for microbial isolation, the microbial marker used, or the method of AA analysis.

Effects on the AA flow of microbial origin. The estimated flow of microbial AA was higher (*P* < 0.05) for 14 of 16 AA measured when SAB were used instead

Table 9. Effect of fiber content and particle size of diets on the estimated flow of AA (g/d) of liquid-associated bacteria (LAB) and solid-associated bacteria (SAB) in continuous culture.

AA		Fiber content ¹		Particle size ²		SEM	Effect (<i>P</i> <)	
		HF	LF	LS	SS		F	S
Asp	LAB	0.75	0.51	0.54	0.71	0.22	†	NS
	SAB	0.93	0.62	0.69	0.86	0.34	NS	NS
Glu	LAB	0.86	0.62	0.66	0.82	0.15	†	NS
	SAB	1.04	0.70	0.78	0.96	0.37	†	NS
Ser	LAB	0.37	0.28	0.28	0.37	0.10	NS	NS
	SAB	0.44	0.31	0.32	0.43	0.14	†	NS
Thr	LAB	0.45	0.34	0.35	0.44	0.13	NS	NS
	SAB	0.54	0.36	0.39	0.51	0.18	†	NS
Gly	LAB	0.39	0.29	0.29	0.39	0.12	NS	NS
	SAB	0.48	0.31	0.35	0.44	0.16	†	NS
Ala	LAB	0.54	0.40	0.42	0.52	0.15	†	NS
	SAB	0.67	0.44	0.50	0.62	0.23	†	NS
Arg	LAB	0.40	0.29	0.30	0.38	0.11	†	NS
	SAB	0.50	0.32	0.35	0.46	0.16	*	NS
Pro	LAB	0.24	0.17	0.18	0.22	0.07	†	NS
	SAB	0.29	0.19	0.21	0.27	0.09	†	NS
Val	LAB	0.53	0.39	0.40	0.52	0.16	NS	NS
	SAB	0.64	0.42	0.46	0.59	0.21	†	NS
Met	LAB	0.15	0.12	0.12	0.14	0.04	NS	NS
	SAB	0.18	0.12	0.14	0.16	0.06	†	NS
Ile	LAB	0.47	0.37	0.37	0.47	0.13	NS	NS
	SAB	0.58	0.40	0.41	0.54	0.18	*	NS
Leu	LAB	0.57	0.42	0.44	0.55	0.16	NS	NS
	SAB	0.69	0.45	0.50	0.64	0.22	†	NS
Phe	LAB	0.35	0.26	0.27	0.33	0.09	NS	NS
	SAB	0.43	0.28	0.31	0.39	0.14	†	NS
Lys	LAB	0.61	0.48	0.48	0.62	0.16	NS	NS
	SAB	0.73	0.48	0.52	0.69	0.23	†	NS
His	LAB	0.12	0.08	0.09	0.11	0.03	†	NS
	SAB	0.15	0.09	0.10	0.14	0.05	*	NS
Tyr	LAB	0.31	0.23	0.24	0.30	0.10	NS	NS
	SAB	0.39	0.26	0.29	0.36	0.14	†	NS
EAA ³	LAB	3.65	2.74	2.82	3.57	1.00	NS	NS
	SAB	4.43	2.89	3.18	4.13	1.43	†	NS
NEAA ⁴	LAB	3.46	2.49	2.62	3.33	1.00	†	NS
	SAB	4.25	2.84	3.14	3.94	1.47	†	NS
Total	LAB	7.11	5.24	5.44	6.90	2.00	†	NS
	SAB	8.68	5.73	6.33	8.07	2.90	†	NS

¹HF = High fiber; LF = low fiber.²LS = Large particle size; SS = small particle size.³EAA = Essential AA (Thr, Arg, Val, Met, Ile, Leu, Phe, Lys, and His).⁴NEAA = Nonessential AA (Ala, Asp, Glu, Gly, Pro, Ser, and Tyr).†*P* < 0.10.**P* < 0.05.

of LAB (Table 8). The average difference was 15% in EAA and 19% in NEAA. Klusmeyer et al. (1991) also reported differences in the calculated amount of EAA from bacteria passing to the duodenum when using different bacterial population isolates (mixed, LAB, or SAB) because of differences in the concentration of some AA in bacterial OM.

The proportion of Met and Lys of microbial origin flowing from the fermenters changed depending on the bacterial population used for calculations (4.13 and 4.69% Met and 17.4 and 16.3% Lys, when using LAB and SAB, respectively) and were close to the optimal level (5 and 15%, for Met and Lys, respectively) recom-

mended for optimal milk protein productions (Rulquin and Vérité, 1993; NRC, 2001) in SAB vs. LAB.

The flow of each individual AA of microbial origin depends on the measurements of AA concentration and the estimated flow of microbial DM. Therefore, results are largely dependent on the representativeness of the microbial sample obtained and the accuracy of the analysis of microbial marker and AA profile. The impact of these errors on the estimation of the supply of AA of microbial origin to the small intestine is important. Because the differences in the estimated AA flow of microbial origin (g/d) resulting from the use of LAB or SAB were larger than the actual differences in their

AA profiles, the estimation of the microbial DM contribution to the total protein flow was more determinant to the differences than the actual differences in the AA composition of the 2 microbial populations. These data reinforce the recommendations of Martin et al. (1996), who indicated the need to improve the accuracy of measuring the total and partial contribution of each microbial fraction to the total flow of N to the small intestine. Results indicate that the estimation of the microbial protein synthesis in the rumen and factors affecting it and the relative contribution of each microbial population (LAB and SAB) to the total flow of microbial protein are essential to improving the precision of the estimation of the flow of AA of microbial origin to the small intestine.

The effects of fiber content and particle size on the estimated flow of AA of microbial origin using LAB and SAB are presented in Table 9. There were no effects of particle size on bacterial AA flow. The fiber content $\times$ particle size interaction was significant for the estimated flow of Lys ($P < 0.05$) when LAB was used for the calculation and was lower in HF + LS (0.45 g/d) and LF + SS (0.45 g/d) vs. HF + SS (0.78 g/d) and LF + LS (0.51 g/d).

The estimated flow (g/d) of Arg, Ile, and His of bacterial origin was lower in LF than in HF when SAB instead of LAB were used for calculations ($P < 0.05$; Table 9). Total flow of AA (g/d) of microbial origin was lower ($P < 0.05$) in LF than in HF, but differences in individual AA were dependent on the microbial population used for calculations. Particle size did not affect the estimated flow of total or individual AA of microbial origin.

The flow of AA of microbial origin was calculated by multiplying bacterial flow (g/d) by the AA profile (%). Because differences in the AA profile (%) between LAB and SAB were small, and differences in the estimated flow (g/d) of AA of microbial origin between LAB and SAB (dependent on purine bases:N; Table 4) were large, it can be concluded that differences in the estimation of microbial protein flow had a higher impact on the estimation of the flow of AA of microbial origin than the AA profile. Therefore, it appears that the accurate measurement of the purine content of bacteria is more important than the measurement of the AA profile in determining the relative contribution of bacterial AA to the total flow of AA from fermenters. In addition, the accurate estimation of the flow of AA of microbial origin requires the accurate estimation of the contribution of LAB and SAB to the total N flow to the small intestine.

CONCLUSIONS

Fiber content and particle size had no effect on OM and fiber digestion in continuous culture of rumen fluid.

High fiber diets had lower total VFA and higher acetate concentrations compared with LF diets. The LS diet had a higher proportion of acetate and acetate:propionate and a lower proportion of butyrate compared with the SS diets. These differences were independent of intake, dilution rates, and pH. The effects of fiber content and particle size on microbial and dietary N flow, protein degradation, and EMPS were dependent on the microbial population used for calculations. The AA profile of microbial groups was relatively independent of diet. However, accurate estimation of microbial N flow and the partial contribution of each microbial population may affect the estimated flow of AA of microbial origin.

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