Short communication: Intestinal digestibility of amino acids in fluid- and particle-associated rumen bacteria determined using a precision-fed cecectomized rooster bioassay

A. C. Fonseca,* S. M. Fredin,* L. F. Ferraretto,* C. M. Parsons,† P. L. Utterback,† and R. D. Shaver*¹

*Department of Dairy Science, University of Wisconsin, Madison 53706

†Department of Animal Science, University of Illinois, Urbana-Champaign 61801

ABSTRACT

Microbial protein represents the majority of metabolizable protein absorbed by ruminant animals. Enhanced understanding of the AA digestibility of rumen microbes will improve estimates of metabolizable protein. The objective of this experiment was to determine the digestibility of AA in fluid- (FAB) and particle-associated bacteria (PAB) using the precision-fed cecectomized rooster bioassay. Bacteria were isolated from 4 ruminally cannulated lactating Holstein cows by differential centrifugation, including particle suspension in 0.1% Tween-80 for increased removal of PAB from ruminal digesta. Samples of FAB and PAB were fed to 9 cecectomized roosters to determine standardized digestibility of AA. Total AA digestibility was 76.8 and 75.5% for FAB and PAB, respectively, but did not differ. Differences existed in AA digestibilities within bacterial type when compared with the mean essential AA digestibility value. Compared with previous literature estimates of AA digestibility in microbes (mean = 76%; range = 57–87%) and relative to National Research Council estimates of total AA from rumen bacteria (80%), the precision-fed cecectomized rooster assay is an acceptable in vivo model to determine AA digestibility of rumen bacteria.

Key words: digestibility, rumen bacteria

Short Communication

Ruminally synthesized microbial protein typically supplies most of the AA flowing to the small intestine of dairy cows (Clark et al., 1992), and most of the microbes flowing to the small intestine are of bacterial origin (NRC, 2001). The NRC (2001) assumes that microbial protein is 80% digestible, but individual AA digestibilities of microbes, and in particular bacteria, are not considered. Estimates of individual AA digest-

ibilities of rumen bacteria may improve predictions for absorbed metabolizable AA and help to improve N efficiency by dairy cows.

Estimates of AA digestibilities of microbes are difficult and expensive to collect in ruminant animals. Previous work has used duodenal- and ileal-cannulated animals sustained entirely on gastric infusions of VFA and micronutrients infused with large quantities of rumen microbes (Ørskov et al., 1979; Storm et al., 1983). Furthermore, common in vitro techniques to measure postruminal AA digestibility, such as the mobile bag technique (NRC, 2001) or the modified 3-step procedure (Gargallo et al., 2006), are not viable alternatives because microbes, with an average size of 1 to 2 µm, will wash out of the bags. The precision-fed cecectomized rooster bioassay is an alternative in vivo model to measure AA digestibilities and has been used to determine the intestinal digestibility of AA in duodenal digesta (Titgemeyer et al., 1990) and ruminally undegraded feed ingredients high in protein commonly fed to dairy cows (Aldrich et al., 1997; Boucher et al., 2009a,b). The objective of this experiment was to determine and assess the digestibility of AA in fluid- (FAB) and particle-associated bacteria (PAB) collected from lactating dairy cows using the precision-fed cecectomized rooster bioassay.

Four ruminally cannulated multiparous lactating Holstein cows were used for the collection of rumen contents. Research was conducted at the University of Wisconsin-Madison Dairy Cattle Center under a protocol approved by the Institutional Animal Care and Use Committee of the University of Wisconsin-Madison. Rumen contents were collected from the anterior ventral sac near the reticulum using a 250-mL bottle and were immediately placed on ice and transported back to the laboratory for isolation of bacteria by differential centrifugation (Whitehouse et al., 1994). Five hundred-milliliter rumen content samples were squeezed through 1 layer of cheesecloth. Solids retained on the cheesecloth were washed with 400 mL of 0.85% (wt/vol) NaCl solution and squeezed again. Filtrates were pooled and refrigerated at 5°C for less than 1 h until processed for

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¹Corresponding author: rdshaver@wisc.edu

isolation of FAB. The solids retained on the cheesecloth were transferred to a 500-mL bottle containing 350 mL of cold solution containing 0.85% (wt/vol) NaCl and 0.1% (vol/vol) Tween-80, mixed thoroughly, and refrigerated at 5°C overnight until processed for isolation of PAB. Filtrates for FAB isolation were centrifuged ($1,000 \times g$ for 5 min at 5°C). Pellets were added to the bottle contents containing PAB, and supernatants were decanted and recentrifuged ($11,330 \times g$ for 30 min at 5°C). These supernatants were discarded and the pellets were resuspended in 100 mL of saline solution and recentrifuged ($11,330 \times g$ for 30 min at 5°C). Supernatants were then discarded and the bottle containing FAB was stored at -20°C until lyophilized. Contents of the PAB bottles were blended for 20 s in a 1-speed Waring blender (Waring Products Division, New Hartford, CT), transferred back to the bottles and stored at 5°C overnight. Contents of PAB bottles were then squeezed through 2 layers of cheesecloth and the filtrates were centrifuged ($1,000 \times g$ for 5 min at 5°C). The pellets were discarded and the supernatant was recentrifuged ($11,330 \times g$ for 30 min at 5°C). Supernatants and final PAB pellets were processed the same as FAB. Bacterial samples were composited by PAB or FAB and ground through a 1-mm Wiley mill screen (Arthur H. Thomas Co., Swedesboro, NJ). All FAB and PAB collections were pooled to yield 1 sample per bacteria type.

Bacterial samples were analyzed for intestinal digestibility of AA using the precision-fed cecectomized rooster bioassay. The experiment was conducted under a protocol approved by the Institutional Animal Care and Use Committee of the University of Illinois (Urbana). The cecectomized rooster assay was conducted as described by Parsons (1985) and Aldrich et al. (1997). Thirteen grams of pooled FAB or PAB, ground through a 1-mm screen, were mixed with 13 g of ground corn, and the total 26 g of sample mixture were crop intubated to cecectomized Single Comb White Leghorn roosters. Four roosters received FAB and 5 roosters received PAB. An additional 4 roosters were crop intubated with 26 g of corn only so that AA digestibilities of the FAB and PAB could be calculated by difference. For crop intubation, the beak was opened and the stem of a funnel was inserted into the crop. Feed was withheld from the roosters for 24 h before and 48 h after intubation of samples, and the birds had ad libitum access to water. Roosters were housed individually in wire mesh cages fitted with excreta collection trays, and total excreta were collected for 48 h and lyophilized. Basal endogenous AA excretion was previously determined by 48-h collection of excreta from fasted birds not used in this experiment according to Parsons (1985). Basal endogenous losses were used to calculate standardized FAB- and PAB-AA digestibilities, where

standardized digestibility is defined as digestibility estimates calculated by subtracting only basal endogenous AA losses from the outflow of AA (Stein et al., 2007).

Intact bacterial samples, corn grain, and rooster excreta from birds fed only corn grain and corn grain plus FAB or PAB were sent to Experimental Station Chemical Laboratories (University of Missouri-Columbia) for analysis of AA content by cation-exchange HPLC coupled with postcolumn ninhydrin derivatization and quantitation (method 982.30; AOAC International, 2012). Samples of the TMR fed to the cows were collected once every 2 wk during the rumen fluid collection phase. Samples were dried in a forced-air oven at 60°C for 48 h to determine DM content, ground the same as the bacterial samples listed above, composited, and sent to Dairyland Laboratories Inc. (Arcadia, WI) for analysis. Samples of TMR, FAB, and PAB were analyzed for DM, OM (method 942.05; AOAC International, 2012), CP (method 990.03; AOAC International, 2012), ether extract (method 2003.05; AOAC International, 2012), and NDF using α -amylase and sodium sulfite (Van Soest et al., 1991). The TMR was analyzed for starch according to Bach Knudsen, (1997; YSI Biochemistry Analyzer; YSI Inc., Yellow Springs, OH). The FAB and PAB were lysed using NaOH for analysis of glycogen according to Hall (2011).

Standardized AA digestibilities for FAB and PAB were calculated as follows: standardized AA digestibility (%) = $\{[AA \text{ intake} - [AA \text{ output} - (\text{undigested AA from corn grain} + \text{basal endogenous AA})]] / AA \text{ intake}\} \times 100$. Data were analyzed as a completely randomized design using PROC MIXED of SAS (2004; SAS Institute Inc., Cary, NC). The model included the fixed effect of the bacteria sample and the random effect of the rooster within bacteria type. To determine differences among individual EAA digestibilities for either FAB or PAB, the mean EAA digestibility was compared against individual EAA estimates using PROC MIXED and included the fixed effect of the AA digestibilities and the random effect of rooster within bacteria type. Means were determined using the least squares means statement. Statistical significance and trends were declared at $P \leq 0.05$ and $P > 0.05$ to $P < 0.10$, respectively.

The ingredient and chemical composition of the TMR offered to the cannulated cows is listed in Table 1. The TMR contained 88.5% forage and may have reduced population diversity of FAB-specific microbes and increased the biomass of PAB relative to FAB. However, the majority of bacterial biomass in a typical lactating dairy cow diet is associated with the particle fraction. Martin and Michalet-Doreau (1995) found that 74% of total bacteria was PAB using ^{15}N labeling and Mullins et al. (2013) determined that 92% of total bacterial

Table 1. Ingredient and chemical composition (% of DM, unless otherwise noted) of the TMR offered to the cows

Item	Amount
Alfalfa silage	44.5
Corn silage	26.8
Alfalfa hay	10.7
Straw	6.5
Concentrate mix ¹	11.5
Nutrient composition	
DM, % as fed	47.4
OM	92.1
CP	14.7
NDF	46.1
Starch	10.6
Ether extract	3.4
NFC ²	28.6
TDN	82.1
NE _L , Mcal/kg	1.4

¹Concentrate mix = 37.6% corn; 28.1% dried distillers grain plus solubles; 18.0% soybean meal; 9.0% corn gluten feed; 2.0% calcium carbonate; 1.8% soy hulls; 1.1% sodium bicarbonate; 0.7% salt; 0.4% vitamins A, D, and E; 0.4% monocalcium phosphate; 0.3% Dynamate (Mosaic Co., Plymouth, MN); 0.3% magnesium oxide; 0.2% trace mineral salt; and 0.2% biotin.

²Nonfiber carbohydrates = 100 – (NDF + CP + ether extract + ash).

DNA was associated with the particle fraction using real-time quantitative PCR.

The chemical and AA composition of FAB and PAB are listed in Table 2. The OM (% of DM) was 83.2 and 87.4% for FAB and PAB, respectively. In published literature, FAB comprised 62 to 85% OM and PAB comprised 74 to 92% OM, whereas bacteria comprised 6 to 9% N (% of DM; Hvelplund and Hesselholt, 1987; Rodríguez et al., 2000; Yang et al., 2001; Vicente et al., 2004), and total lipids ranged from 5 to 13% for FAB and 11 to 28% for PAB (Bauchart et al., 1990; Rodríguez et al., 2000; Vlaeminck et al., 2006). In the present experiment, the N and OM fractions are similar to other published studies, whereas the lipid fraction is reduced. Vlaeminck et al. (2006) determined that total lipids (% of DM) increased in FAB and PAB with decreasing dietary forage:concentrate levels. The high-forage diet fed in the present experiment may have resulted in the lower concentration of total lipids in rumen bacteria. The EAA composition of FAB and PAB is similar to values reported by Storm and Ørskov (1983) and Clark et al. (1992). The AA composition of rumen bacteria can vary substantially (Clark et al., 1992); however, it is unknown if changes to the AA or nutrient composition of rumen microbes can affect AA digestibility of the microbes. In addition, differences among studies in AA composition of rumen microbes may represent analytical issues related to AA quantification or bacterial isolation and not true differences among samples.

The AA digestibilities of FAB and PAB are listed in Table 3. The digestibility of total AA for FAB and

Table 2. Chemical and AA composition of fluid- (FAB) and particle-associated bacteria (PAB)

Item ¹	FAB	PAB
Nutrient composition, % of DM		
OM	83.2	87.4
N	8.5	8.1
TAA	39.9	37.2
Glycogen	3.5	4.8
Ether extract	2.4	8.8
AA composition, % of TAA		
Ala	7.4	6.9
Arg	4.8	4.9
Asp	11.9	11.8
Cys	1.2	1.2
Glu	12.5	12.2
Gly	5.8	5.7
His	1.9	1.9
Ile	5.8	6.1
Leu	7.8	8.1
Lys	8.3	8.4
Met	2.4	2.3
Ser	3.9	3.6
Phe	5.1	5.4
Pro	3.8	3.9
Thr	5.3	5.1
Trp	1.0	1.1
Tyr	4.8	5.0
Val	6.4	6.4
BCAA	20.0	20.6
EAA	48.8	49.7
NEAA	51.2	50.3

¹TAA = total AA; BCAA = branched-chain AA.

PAB were 76.8 and 75.5%, respectively. These values are similar to the AA digestibility of microorganisms (80%) reported in NRC (2001). A trend existed for a difference between FAB and PAB for Met digestibility ($P = 0.08$). Otherwise, no difference existed in digestibility between FAB and PAB. The AA digestibility results in the present study are greater than the estimates of apparent AA digestibility of rumen microorganisms determined in sheep (mean = 71%; Armstrong and Hutton, 1975), and goats (mean = 57%; Lee and Tasaki, 1977); however, apparent digestibilities will be lower than true digestibilities. Storm et al. (1983) determined the true digestibility of AA in sheep and found greater digestibility (82%) compared with the present study. However, Storm et al. (1983) did not find any difference between AA in their digestibilities, except for diaminopimelic acid (**DAPA**), an AA component of bacterial cell walls. Furthermore, Tas et al. (1981) determined that true absorption of microbial AA was 87% in sheep, using multiple regression analysis, but this estimate did not include Cys, Trp, Pro, or DAPA, which are AA that typically have lower digestibilities. Hvelplund and Hesselholt (1987) found that true digestibility of AA in microbial protein varied between 80 to 91% in sheep. Intestinal digestibilities among individual AA was not significantly different; however,

Table 3. Digestibility of amino acids in fluid- (FAB) and particle-associated bacteria (PAB) using the precision-fed cecectomized rooster bioassay

Item ¹	FAB	PAB	SEM	P-value
Ala	72.8*	70.8	2.79	0.62
Arg	82.4	80.1*	2.01	0.44
Asp	78.2	76.4	2.24	0.56
Cys	71.1	61.9	4.83	0.20
Glu	74.7	71.8	2.96	0.49
His	83.1*	75.2	3.60	0.14
Ile	79.2	76.3	1.78	0.28
Leu	78.2	74.7	2.39	0.31
Lys	79.6	78.4	2.28	0.73
Met	82.2*	76.5	2.07	0.08
Phe	78.3	74.1*	2.13	0.19
Pro	74.3	73.6	2.64	0.85
Ser	71.2	65.3	4.22	0.34
Thr	75.7	72.8	3.47	0.56
Trp	76.2*	76.2	2.68	0.98
Tyr	76.5	75.1	2.33	0.69
Val	72.4*	70.9*	3.12	0.73
BCAA	76.6	75.9	2.20	0.81
EAA	78.7	75.5	2.36	0.35
NEAA	74.1	72.7	2.73	0.71
TAA	76.8	75.5	2.41	0.70

¹BCAA = branched-chained AA; TAA = total AA.

*Least squares EAA means within the same column differed ($P < 0.05$) from the mean EAA.

DAPA (40%), Phe (69%), and Cys (74%), exhibited lower digestibilities than the other AA.

Differences existed among AA when compared with the mean EAA value within bacteria type (Table 3). Arginine, His, and Met yielded greater digestibilities than the mean EAA in FAB, whereas Trp and Val digestibilities were lower. Arginine and Lys were greater than the mean EAA in PAB, whereas Phe and Val were lower. Storm et al. (1983) found the digestibility of Arg (89%) to be greater and His (68%) to be lower than the mean total AA digestibility in microbial protein, whereas Hvelplund and Hesselholt (1987) found greater digestibilities for Arg (91%) and His (89%), and a lower digestibility for Phe (69%) in microbial protein. Digestibility of Met has also been found to be greater compared with total AA in some (Armstrong and Hutton, 1975; Lee and Tasaki, 1977), but not all studies (Storm et al., 1983; Hvelplund and Hesselholt, 1987).

In conclusion, based on mean total AA and EAA digestibilities in bacteria relative to NRC (2001) and previous research, the precision-fed cecectomized rooster bioassay appears to be a viable in vivo model for the determination of AA digestibilities in rumen bacteria. No difference existed in AA digestibilities between FAB and PAB, but differences among AA digestibilities within bacterial type exist. Future research using the cecectomized rooster bioassay may allow for measurements of AA digestibilities of other rumen microorganisms such as protozoa.

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