J. Dairy Sci. 97:3855–3859
http://dx.doi.org/10.3168/jds.2013-7880

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 digestibilities 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

Received December 27, 2013.
Accepted March 6, 2014.
[1]Corresponding 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 × *g* for 5 min at 5°C). Pellets were added to the bottle contents containing PAB, and supernatants were decanted and recentrifuged (11,330 × *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 × *g* for 30 min at 5°C). Supernatants were then discarded and the bottle containing FAB were 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 × *g* for 5 min at 5°C). The pellets were discarded and the supernatant was recentrifuged (11,330 × *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 intake − [AA output − (undigested AA from corn grain + basal endogenous AA)]}/AA intake) × 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