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Effect of dried fermentation biomass on microbial fermentation in continuous culture and *in vitro* intestinal digestibility

A.J. Carpenter[1], E. Binversie[2], M. Ruiz-Moreno[3], M.D. Stern*

Department of Animal Science, University of Minnesota, St. Paul, MN 55108-6118, United States

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ABSTRACT

The objective of the current experiment was to determine if fermentation biomass (FB), a dried bacterial by-product derived from lysine production (Ajinomoto Heartland, Inc.) can be used as a protein source in ruminant diets. Eight dual-flow continuous culture fermenters were inoculated with rumen fluid and used during one experimental period consisting of a 7-d adaptation period followed by 3 sampling days. Microbial substrate was provided by one of two isonitrogenous diets, CON or DFB. In CON, soybean meal (SBM) provided 57% of total CP, and in DFB, SBM and FB provided 12 and 45% of total CP, respectively. CON contained 3% molasses, 16% ground corn, 13% grass hay, 48% corn silage, and 20% SBM on a DM basis; DFB contained 3% molasses, 18.4% ground corn, 13% grass hay, 50% corn silage, 8.5% SBM, and 6.7% FB. On sampling days, liquid and solid effluent were collected, combined, and homogenized to be used for chemical analysis and *in vitro* estimation of intestinal digestibility (ID). Treatment did not affect average, maximum, or minimum fermenter pH. There was no effect on apparent or true OM, NDF, or ADF digestibility (%). Total and branched-chain VFA as well as acetate (m*M*) were higher in CON, and isobutyrate concentration (mol/100 mol) tended to increase with CON treatment. Source of N had no effect on total, dietary, or bacterial-N flows. Addition of FB decreased NH_3-N flow from 0.4 to 0.2 ± 0.05 g/d and tended to decrease effluent NH_3-N concentration from 17.1 to 9.7 ± 2.21 mg/100 mL. His and Met flows increased from 0.48 to 0.53 ± 0.012 and 0.18–0.20 ± 0.005 g/d, respectively, when FB partially replaced SBM in the diet, but there were no effects on other AA or total AA flows. There was a trend in percent non-essential AA input (CON = 73.6% *vs.* DFB = 82.2%; SE = 2.83) in effluent; however, there was no effect on percent of essential or total AA input in effluent. Effluent from the DFB treatment was higher in ID than CON (CON = 70.4% *vs.* DFB = 79.6%; SE = 1.64), although there was no difference in estimated amount of protein available for intestinal absorption (g). These results indicate that FB elicited a similar response in N metabolism and AA flows to SBM but had a greater estimated ID and depressed VFA production, and has potential use as a protein source in ruminant diets.

1. Introduction

The human population worldwide is expected to exceed 9 billion by 2050, and with this growth comes a greater demand for

* Corresponding author.
E-mail address: stern002@umn.edu (M.D. Stern).
[1] Present address: Department of Animal Biosciences, University of Guelph, Ridgetown, ON, N0P 2C0, Canada.
[2] Present address: 1150 Bellevue Street, University of Wisconsin Extension Green Bay, WI 54302, United States.
[3] Present address: Institute of Food and Agricultural Sciences University of Florida, Marianna 32446, United States.

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ruminant-derived products, such as meat, milk, and fiber. Producers and industry groups are faced with the challenge of meeting these needs in an environmentally and socially sustainable way with limited land and water resources. Fortunately, ruminants are capable of effectively converting human inedible by-products of other industries into protein and fiber sources that are available for human consumption and use. Utilizing these by-product feeds in this manner can decrease waste and limit the competition for nutrients between livestock and humans, thereby contributing to the overall sustainability of the agriculture industry. For example, Ertl et al. (2016) demonstrated that replacing common cereal grains with wheat bran and sugar beet pulp in a dairy ration improved the human-edible feed conversion efficiency (defined as human-edible output *via* animal products per human-edible input *via* feedstuffs) as well as the net food production index (human-edible output minus human-edible input) of these rations. Furthermore, feeding by-products derived from industrial processes can in some cases contribute to the economic sustainability of livestock producers by enabling them to reduce feed costs without sacrificing nutrients necessary for production.

The relative amount of protein degraded in the rumen (RDP) or passing to the small intestine (rumen undegradable protein, RUP) is important for providing amino acids (AA) for maintenance, growth, and production. Many by-product or co-product feeds have elevated levels of RUP compared to other commonly utilized ruminant protein supplements (Clark et al., 1987), and are thus useful as a cost-conscious method of increasing protein available to the small intestine. However, several methods of increasing the flow of AA to the small intestine by shielding from degradation in the rumen will also make AA unavailable for digestion and absorption in the small intestine. Therefore, it is important when analyzing characteristics of a protein source for ruminants *in vivo* that RDP, RUP, and intestinal digestibility (ID) be determined. The 3-step *in situ/in vitro* enzymatic procedure (Calsamiglia and Stern, 1995) provides a relatively rapid and reliable way to determine ruminal degradation and ID of the protein in a feedstuff.

Fermentation biomass (FB) is a by-product of commercial Lys production. *Escherichia coli* is utilized in Lys fermentation because its metabolism is well-understood and its DNA can be easily manipulated to make production more efficient (Wittmann and Becker, 2007). Once the fermentation process has been completed, Lys must be removed from bacterial cells by ultrafiltration or centrifugation, leaving FB (Anastassiadas, 2007). With a growing demand worldwide—particularly in China, Europe, North America, and Latin America—Lys production is expected to continue increasing (Grand View Research, 2015), which would in turn result in increased production of FB. To date, limited research has been published investigating the effects of FB in livestock diets. Subalo et al. (2013) found that ileal crude protein (CP) and AA digestibility of FB was similar to or greater than that of fish meal, and did not differ from soybean meal (SBM) in digestibility of CP and essential AA (EAA) in weanling pigs with the exception of Arg, Met, and Phe, which were lower in FB. When Broderick et al. (2000) supplemented dietary protein with by-products of AA fermentation to dairy cattle, they concluded that they were not as effective as SBM, although just as effective as supplemental non-protein N (NPN) in the form of urea. More research is required to investigate what influence this by-product might have on ruminal protein metabolism and small intestinal digestion in ruminants, and the experiments described here were designed to collect preliminary data regarding the potential of FB as a ruminant feedstuff. The objective of this study was to determine if FB could be utilized as a protein source in ruminant diets by simulating rumen conditions *in vitro* with continuous culture fermentation and estimating ID *in vitro*.

2. Materials and methods

2.1. Experimental diets

Chemical and AA composition of experimental diets are reported in Table 1 and Fig. 1, respectively, and chemical composition of FB and SBM are provided in Table 2. Two diets that were formulated to be isoenergetic and isonitrogenous provided substrate for microbial metabolism in this experiment. The control diet (CON) had no FB added while the experimental diet (DFB) included FB. High-protein SBM was utilized as the main protein source in CON, accounting for approximately 57% of the CP in the diet. Molasses, ground corn, corn silage, and alfalfa hay contributed the remaining 43% of the CP. Fermentation biomass accounted for approximately 45% of CP in the DFB diet with SBM accounting for 12% of CP. Feedstuffs were dried and ground to 2 mm particle diameter with a Wiley Mill (Thomas Scientific, Philadelphia, PA). Diets were mixed for 20 min using a Hobart mixer (Hobart Corporation, Troy, OH) and pelleted with a California pellet mill (California Pellet Mill, San Francisco, CA).

2.2. Continuous culture fermenter operation

Two blocks of 4 dual-flow continuous culture fermenters (1034 ± 40 mL) previously described by Hannah et al. (1986) were inoculated with pooled ruminal fluid strained through 4 layers of cheesecloth. In brief, continuous culture fermenters provide a model for *in vivo* rumen fermentation with continued addition of substrate and removal of inoculum throughout the experimental period, as opposed to batch cultures which are contained. “Dual-flow” refers to the fact that solids and liquids are removed from the fermenter at different rates from each other. Treatments were randomly allocated within 2 blocks of 4 fermenters ($n = 2$ fermenters per treatment per block). Ruminal fluid was obtained from a cannulated Holstein cow fed a 60:40 forage to concentrate (DM basis) total mixed ration. Fermenters were provided with 75 g of dietary DM/d during the 10-d experimental period. Feed was given simultaneously to all fermenters with an automated feeding device (Hannah et al., 1986) that was operated using an automatic timer (DT 17, Intermatic, Spring Grove, IL), which fed 8 equal portions of the total DM every 3 h, with a 1.5-h feeding phase and a 1.5-h rest phase. Liquid dilution rate was set to 10%/h by continuous infusion of artificial saliva buffer (pH = 8.25), and solid dilution rate was set to 5.5%/h by filtrate removal. Individual fermenter pH was recorded every 10 min by an electronic data acquisition system (Daisy Lab®) and maintained between 5.8 and 6.8 by automated addition of either 5 N NaOH or 3 N HCl. Fermenter temperatures were maintained at 39 °C by an electrical heater, and anaerobic conditions were maintained by constant infusion of N_2 at the rate of