STUDIES USING CONTINUOUS CULTURE FERMENTERS AND A THREE-STEP IN SITU/IN VITRO PROCEDURE TO ESTIMATE PROTEIN METABOLISM IN RUMINANTS

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Because in vivo measurement of nutrient digestion in the rumen and small intestine requires ruminally and intestinally cannulated animals that are expensive, labor intensive, and subject to error associated with microbial and passage rate markers, and inherent animal variation, alternative techniques are being used to measure protein metabolism in ruminants. In our laboratory, it is advantageous to use an elaborate continuous culture fermenter system that simulates microbial metabolism in the rumen to evaluate various factors that affect fermentation and nitrogen metabolism. For example, *Penicillium* sp. molds are common contaminants of corn silage and high moisture corn and they produce patulin that can adversely affect fermentation by ruminal microbes. Therefore, fermenters were supplemented with 0, 10, 20 or 40 mg of patulin every 12 h for three consecutive days. Crude protein digestion and bacterial N flows decreased linearly ($P < 0.05$) and conversely, there was a linear increase ($P < 0.05$) in pH and ammonia N with increased patulin. Efficiency of N utilization by ruminal microbes showed a linear decrease ($P < 0.05$) with increasing patulin. Increasing patulin also reduced NDF and ADF fiber digestion at a decreasing rate (linear, $P < 0.01$; quadratic, $P < 0.05$) whereas OM and non-structural carbohydrate digestion decreased linearly ($P < 0.05$). Results from this experiment indicate that patulin can have adverse effects on nitrogen metabolism and digestion by ruminal microbes. Alterations in microbial nutrient digestion and production of microbial end products can impact production and/or health of ruminants. Another technique that is commonly used in our laboratory is the three-step in situ/in vitro procedure to estimate small intestinal protein digestion. Recently, we used this procedure to evaluate consistency in processing procedures. Intestinal protein digestion of three carloads for each of four ruminal protected soybean products [solvent extracted SBM (SE) heat treated (SOLH); SE SBM nonenzymatically browned (SOLNEB); mechanical-extracted (ME) SBM #1 with fresh soy gums (MEC1G); and ME SBM #2 (MEC2)] and three carloads for each of three sources of distillers dried grains with solubles (DDG-A, DDG-B and DDG-C) were evaluated. Means ± s.d. for intestinal CP digestion of SOLH, SOLNEB, MEC1G and MEC2 were 70.8, ± 2.1; 68.2, ± 1.0; 83.0, ± 1.55; and 81.5, ± 2.5%, respectively. Variation in processing of each protected soybean product was not great; however mean intestinal protein digestion was fairly large ranging from 68.2 to 83.0% among the four soybean products. Means ± s.d. for DDG-A, DDG-B and DDG-C were 71.9, ± 3.2; 72.0, ± 0.5; and 80.6, ± 4.2%, respectively. Variation in processing was low for DDG-B (range of 71.7 to 72.6%), but there was a fairly large variation in processing of DDG-A (range of 69.6 to 75.5%), and DDG-C (range of 77.2 to 85.3%). From these types of observations, it appears that the three-step procedure can be a useful method for evaluating quality control of protein within and among processing procedures. Because there is limited data regarding intestinal CP digestion of microbes, we used the pepsin-pancreatin part of the three-step procedure (steps 2 and 3) to estimate intestinal CP digestion of various microbial fractions. Whole ruminal contents were separated into three microbial fractions including solids-associated bacteria (SAB), liquid-associated bacteria (LAB) and protozoa. Crude protein (% of OM) differed ($P < 0.05$) between the bacterial fractions in this study with LAB containing higher CP (70.2) compared with SAB (51.6), however CP of LAB was not different ($P > 0.05$) from protozoa at 60.2. Intestinal CP digestion was numerically greatest for protozoa (96.6%) followed by SAB (92.2%) and LAB (91.6%), however these results were not different ($P > 0.05$). Lysine concentration (g/100 g of total amino acids) among microbial fractions was high for protozoa (11.0), SAB (7.7) and LAB (8.3) fractions compared with most protein supplements fed to ruminants. Results from this study substantiate that microbial protein is not only high in quality but is readily available in the small intestine. Because intestinal CP digestion of microbes was found to be greater than 90%, values used in current protein metabolism models, such as 80% in NRC (2001), should be re-considered.