A three-step in vitro procedure for estimating intestinal digestion of protein in ruminants

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A Three-Step In Vitro Procedure for Estimating Intestinal Digestion of Protein in Ruminants[1,2]

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ABSTRACT: A three-step in vitro procedure was developed to estimate intestinal digestion of proteins in ruminants. Dacron bags containing feed samples were suspended in the rumen for 16 h. Residue containing 15 mg of N after ruminal exposure was incubated for 1 h in 10 mL of a .1 *N* HCl solution containing 1 g/L of pepsin. After incubation, pH was neutralized with .5 mL of 1 *N* NaOH and 13.5 mL of a pH 7.8 phosphate buffer containing 37.5 mg of pancreatin were added to the solution and incubated at 38°C. After a 24-h incubation, 3 mL of a 100% (wt/vol) trichloroacetic acid solution were added to precipitate undigested proteins. Preincubation of samples in the rumen did not affect ($P > .05$) pepsin-pancreatin digestion of residual CP in soybean meal (SBM), corn gluten meal (CGM), and blood meal (BM) and reduced ($P < .05$) pepsin-pancreatin digestion of residual CP in hydrolyzed feather meal (HFM), fish meal (FM), and meat and bone meal (MBM) (80 vs 70, 88 vs 81, and 82 vs 56%, respectively, for nonruminal vs ruminal preincubation). Pepsin digestion before pancreatin digestion increased ($P < .05$) CP digestion of all proteins tested by a mean of 23 percentage units. The pancreatin digestion step was validated using 34 duodenal samples from which small intestinal CP digestion was determined in vivo. The regression equation of in vivo estimates on pancreatin digestion had an r value of .91 ($P < .001$). Estimates of pepsin-pancreatin CP digestion expressed as percentages of undegraded intake protein (UIP) were SBM = 89.9 ± 2.6, lignosulfonate-treated SBM = 87.3 ± 4.5, CGM = 87.6 ± 2.7, BM = 80.1 ± 16.7, HFM = 69.5 ± 3.9, FM = 85.4 ± 2.6, and MBM = 54.0 ± 6.2. The three-step in vitro procedure provides an alternative to the use of intestinally cannulated animals for estimating intestinal digestion of protein supplements. Because of variation, differences in intestinal digestion of proteins among and within various sources should be considered when determining protein value for ruminants.

Key Words: In Vitro, Protein Digestion, Ruminants

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Introduction

Current recommendations for feeding proteins to cattle are based on the concept of absorbable protein (NRC, 1989). The total amount of protein available for absorption is dependent on the flow of microbial and dietary N to the duodenum and their respective intestinal digestibilities. As the contribution of undegraded intake protein (**UIP**) increases in the diet, its intestinal digestion becomes increasingly important. Variation in in vivo intestinal digestion among protein supplements has been reported (Stern et al., 1985; Waltz et al., 1989). Obtaining estimates of protein digestion in the small intestine is expensive and labor-intensive, and it requires the use of surgically prepared animals. Development of an in vitro technique to estimate intestinal digestion of proteins may provide the means to determine intestinally absorbable dietary protein of individual feeds. This technique should 1) closely simulate physiological conditions of ruminants, including potential effects of ruminal fermentation; 2) be rapid, reliable, and inexpensive; 3) be applicable to a wide variety of protein supplements, and 4) accurately reflect differences in protein digestion. Various in vitro methods that have been developed, including ADIN (Goering et al., 1972), enzymatic procedures (Britton et al.,

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1986), lysine availability tests (Faldet et al., 1991), and an in situ mobile-bag technique (Hvelplund, 1985), do not meet all of these criteria. The objective of this study was to develop a reliable in vitro technique to estimate intestinal digestion of proteins in ruminants.

Materials and Methods

Development of the Technique

Intestinal Digestion. A standard soybean meal (**SBM**) was used in all preliminary tests to determine optimum conditions of the enzymatic assays. A SBM sample (15 mg of N) was incubated in a buffer solution containing digestive enzymes. A pancreatin (Sigma P-7545, Sigma, St. Louis, MO) procedure modified from Akeson and Stahmann (1964) was tested. Dose-response studies were used to determine optimum level of pancreatin (0, 10, 20, 25, 37.5, 50, and 70 mg) for maximizing protein digestion. Comparative studies were conducted to determine optimum buffer type (bicarbonate vs phosphate), buffer molar concentration (.1, .2, .5, or 1.0 molar), pH (7.0, 7.2, 7.4, 7.6, 7.8, or 8.0), incubation time (2, 4, 6, 12, 18, 24, or 30 h), and mixing protocol (vortexing at 0, 2, 3, or 4 times per day). Thymol was added to the solution at 50 ppm to prevent microbial growth. Undigested protein remaining after incubation was precipitated with trichloroacetic acid (**TCA**) and separated by centrifugation. Trichloroacetic acid was added at different concentrations (0, 3, 6, 12, 20, or 40% final concentration [wt/vol]), and centrifugation speeds (5,000, 10,000, or 15,000 $\times g$), and times (5, 10, or 15 min) were tested. In the evaluation of each assay step, conditions were chosen that provided maximal digestion. Optimization of the protein separation step was based on maximizing recovery of precipitable protein.

Freeze-dried duodenal samples were obtained from experiments in which intestinal digestion had been determined in vivo and were used to validate the pancreatin digestion assay. Diets from these studies contained SBM, or SBM heat-processed in the presence of water, xylose, or lignosulfonate (Windschitl and Stern, 1988); SBM, raw soybeans, and soybeans extruded at 132 and 149°C (Stern et al., 1985); a cob-, starch-, and molasses-based diet (Titgemeyer and Merchen, 1990); SBM, blood meal (**BM**), hydrolyzed feather meal (**HFM**), or a mixture of BM and HFM (Waltz et al., 1989); and a basal diet (61% corn silage, 20% wheat straw, and 19% concentrate) with no supplemental protein or the basal diet supplemented with SBM, corn gluten meal (**CGM**), fish meal (**FM**), or BM (Titgemeyer et al., 1989). A total of 34 duodenal samples were collected and processed through the pancreatin digestion assay. In vivo estimates of intestinal CP digestion were regressed on estimates from in vitro pancreatin digestion. Regression analysis weighted for the number of in vivo replications was conducted using the GLM procedures of SAS (1985) with the following model: $\text{In vivo}_{ijkl} = \text{experiment}_i + \text{cow}_j(\text{experiment}_i) + \text{period}_k(\text{experiment}_i) + \text{pancreatin}_l + e_{ijkl}$, where experiment, cow(experiment), and period(experiment) were considered random variables.

Pepsin Digestion. An in vitro pepsin digestion assay adapted from AOAC (1980) was used to determine the effect of HCl-pepsin predigestion on protein digestion by pancreatin. Residues containing 15 mg of N were preincubated with 10 mL of .1 *N* HCl solution containing 1 g/L of pepsin (Sigma P-7012, Sigma) at pH 1.9. Samples were incubated for 0, .5, 1, 2, and 4 h at 38°C. After incubation, pH was neutralized with .5 mL of a 1 *N* NaOH and 13.5 mL of a buffer-pancreatin solution (.5 *M* phosphate solution, pH 7.8, containing 3 g/L of pancreatin [Sigma P-7545, Sigma]) were added. For the zero time, the HCl-pepsin solution was immediately neutralized. Samples were vortexed and incubated at 38°C for 24 h in a shaking water bath. After incubation, 3 mL of a 100% (wt/vol) TCA solution were added, samples centrifuged at 10,000 $\times g$ for 15 min, and TCA-insoluble N measured. Soybean meal, CGM, BM, HFM, FM, and meat and bone meal (**MBM**) were tested to determine the effect of pepsin predigestion on pancreatin digestion of protein.

Ruminal Incubation. After determining optimal conditions of the pepsin-pancreatin test, six protein supplements (SBM, CGM, BM, HFM, FM, and MBM) were tested to determine the effect of ruminal preincubation on pepsin-pancreatin digestion of feed protein. Approximately 1.5 g of test feed was weighed into a 6-cm × 10-cm Dacron polyester bag and suspended for 16 h in the rumen of a cannulated Holstein cow fed a 60:40 forage:concentrate diet. After 16 h of ruminal incubation, bags were rinsed with tap water until runoff was clear and dried in a 55°C forced-air oven for 48 h. Intestinal digestion of the residual CP was determined using optimal conditions of the pepsin-pancreatin procedure. Results were compared with those obtained when identical samples were processed without ruminal exposure.

Application of the Technique

Two tests were conducted to determine the sensitivity of the technique to heat-damaged proteins and to the presence of anti-trypsin factors. Soybean meal was heated at 165°C for 0, 1.25, 2.5, 3.0, or 3.5 h and tested to determine the sensitivity of the technique to heat-damaged proteins. Whole soybeans were unheated or heated at 141, 149, or 155°C, and tested to determine the sensitivity of the technique to the presence of anti-trypsin factors.

Several samples of protein supplements were used to estimate within- and among-feed variation in intestinal CP digestion using the three-step procedure

described. These samples were obtained from different commercial sources and from various batches, and included SBM (n = 4), lignosulfonate-treated SBM (**LSBM**, n = 5), CGM (n = 4), BM (n = 6), HFM (n = 4), FM (n = 4), and MBM (n = 4). Results were analyzed as a completely randomized design using the GLM procedures of SAS (1985). Statistical differences were declared at $P < .05$ using Tukey's test (Tukey, 1953).

Results

Conditions of the Test

Optimum pH that maximized digestion was 7.6 ± .5 (Figure 1a). However, there were no differences ($P > .05$) in digestion estimates between pH 7.4 and 8.0. This is important because small shifts in pH may occur as protein digestion proceeds. However, pH of the bicarbonate buffer was less stable over time than that of the phosphate buffer, resulting in uncontrolled conditions. A .2 *N* phosphate buffer maintained pH relatively constant at 7.6 and resulted in optimal conditions for digestion (data not shown). The dose-response curve of pancreatin dose vs digestion (Figure 1b) indicated that the addition of 37.5 mg of pancreatin per sample maximized protein digestion. Digestion was also maximized at 24 h of incubation (Figure 1C), and no further benefit was obtained from additional enzyme (up to 75 mg) or incubation time (up to 30 h). Maximal protein precipitation occurred at a final concentration of 12% TCA (data not shown). Vortexing samples up to four times per day did not affect pancreatin digestion. There were no differences among centrifugation speeds or times. However, centrifugation at 5,000 *g* for 5 min tended to yield greater digestion estimates, suggesting suboptimal protein separation.

Results from the pancreatin digestion assay were highly correlated (n = 34, r = .91, $P < .001$) to in vivo estimates of intestinal CP digestion, with an intercept of 15.3 ± 10.7 ($P < .17$) and a slope of .69 ± .12 ($P < .02$). The terms cow(experiment) and period(experiment) were not significant ($P > .05$). The term experiment was significant ($P < .02$), indicating that variation independent of feed (type of animal, location, methodology, etc.) was present.

Pepsin-pancreatin digestion was optimized after 1 h of pepsin predigestion. Pepsin predigestion increased ($P < .05$) estimates of pancreatin digestion of SBM (76.6 vs 95.9%, SEM = 1.7), CGM (76.3 vs 96.3%, SEM = 5), BM (51.8 vs 88.7%, SEM = 1.3), HFM (50.0 vs 78.7%, SEM = 1.6), FM (72.1 vs 85.2%, SEM = 1.1), and MBM (56.7 vs 77.8%, SEM = .6). Digestion of CP remaining after 16 h of ruminal incubation was less ($P < .05$) compared with digestion of samples not incubated in the rumen for MBM, HFM, and FM (82.1 vs 55.6%, SEM = 2.3; 80.1 vs 70.2%, SEM = 2.4; and 87.5 vs 81.1%, SEM = 1.8; for nonruminal vs ruminal preincubation, respectively)

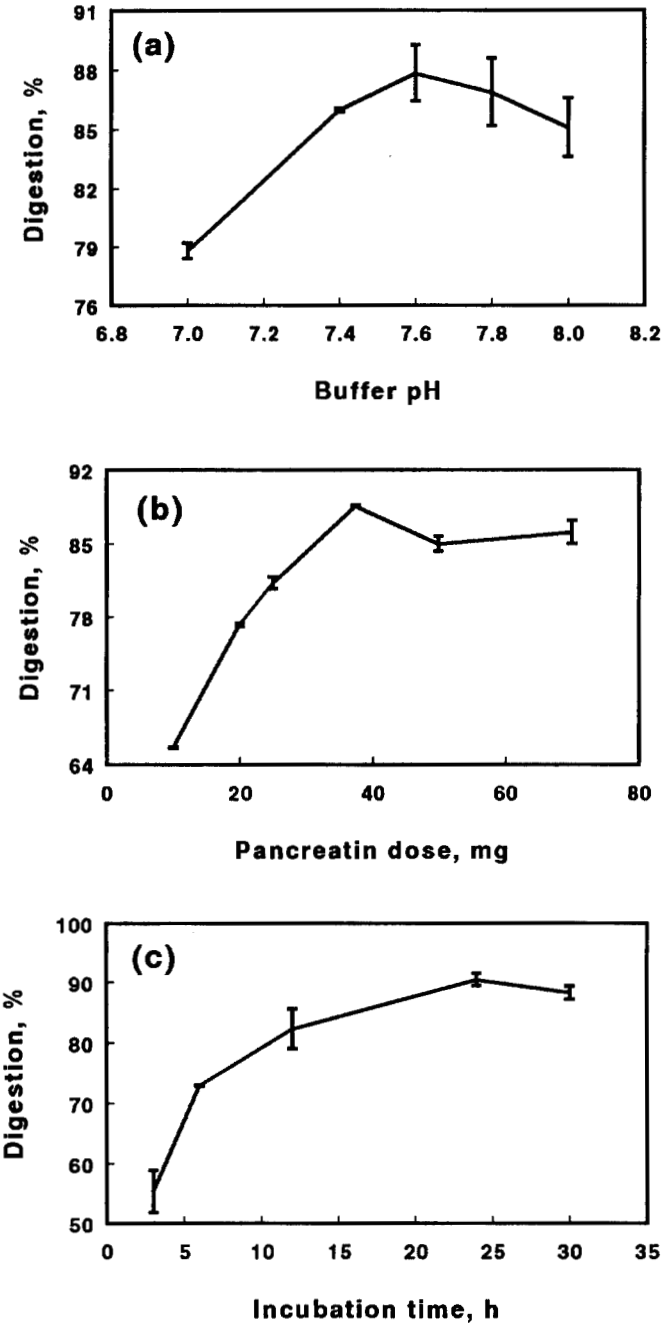

Figure 1. Effect of (a) buffer pH, (b) pancreatin dose, and (c) incubation time on pancreatin digestion of soybean meal protein.

and had no effect ($P > .05$) on SBM, CGM, and BM (91.5 vs 95.7%, SEM = 1.0; 89.7 vs 92.8%, SEM = 2.0; 91.7 vs 89.9%, SEM = 1.8; for nonruminal vs ruminal preincubation, respectively). The technique assumes that microbial contamination of protein supplements is negligible. Erasmus et al. (1994) indicated that microbial contamination of residual matter after ruminal incubation ranged from .9 to 8.6% of total N in protein supplements. Results from the current

study indicate that digestion of the protein leaving the rumen may be different from that of the original feed and justifies the need to use a three step procedure (ruminal, pepsin, and pancreatin) to estimate intestinal digestion of the UIP fraction of feeds.

Protocol for Determining Intestinal Digestion of Proteins in Ruminants

Following is the recommended protocol for determination of intestinal digestion of protein developed from the preliminary tests.

Weigh approximately 1.5 g of feed, ground through a 2-mm screen, into 6-cm × 10-cm Dacron polyester bags and suspend them in the rumen for 16 h. Depending on N content and CP degradability of the feed tested, four to eight bags may be required to provide at least 60 mg of residual N per feed. After the incubation period, rinse bags with tap water until runoff is clear and dry them in a 55°C forced-air oven for 48 h. Pool samples from bags and determine N content. Weigh samples to contain 15 mg of residual N into a 50-mL centrifugation tube. Add 10 mL of a pH 1.9, .1 *N* HCl solution containing 1 g/L of pepsin (Sigma P-7012, Sigma), vortex, and incubate for 1 h in a 38°C shaker water bath. After incubation, add .5 mL of a 1 *N* NaOH solution and 13.5 mL of a pancreatin solution (.5 *M* KH_2PO_4 buffer standardized at pH 7.8 containing 50 ppm of thymol and 3 g/L of pancreatin [Sigma P-7545, Sigma]). Vortex and incubate samples at 38°C for 24 h in a shaker water bath. Vortex samples approximately every 8 h. After incubation, immediately add 3 mL of a 100% (wt/vol) solution of TCA to the tubes to stop enzymatic action and precipitate undigested proteins. Vortex all tubes and allow them to stand for 15 min. Centrifuge samples at 10,000 × *g* for 15 min and analyze the supernatant for soluble N by the Kjeldahl method (AOAC, 1980). Pepsin-pancreatin digestion of protein is calculated as TCA-soluble N divided by amount of sample N (Dacron bag residue) used in the assay.

Application of the Technique

The above protocol was tested on soybean proteins to determine the sensitivity of the technique to heat damage and to the presence of anti-trypsin factors. Heating soybean meal at 165°C for more than 2.5 h resulted in a decrease in intestinal CP digestion (Figure 2a). In contrast, mild heating of whole soybeans resulted in an increase in protein digestion (Figure 2b), probably due to the inactivation of anti-trypsin factors present in raw soybeans. These results indicate that the technique is sensitive to effects of heat damage and to the presence of anti-trypsin factors.

Estimates of pepsin-pancreatin digestion (percentage of UIP) of protein supplements (Figure 3) using the three-step procedure were SBM = 89.8 ± 2.6, LSBM = 87.3 ± 4.5, CGM = 87.6 ± 2.7, BM = 80.1 ± 16.7, HFM = 69.5 ± 3.9, FM = 85.4 ± 2.6, and MBM = 54.0 ± 6.2. Estimates of pepsin-pancreatin digestion of CP were highest ($P < .05$) for SBM, LSBM, FM, CGM, and BM, intermediate for HFM, and lowest ($P < .05$) for MBM.

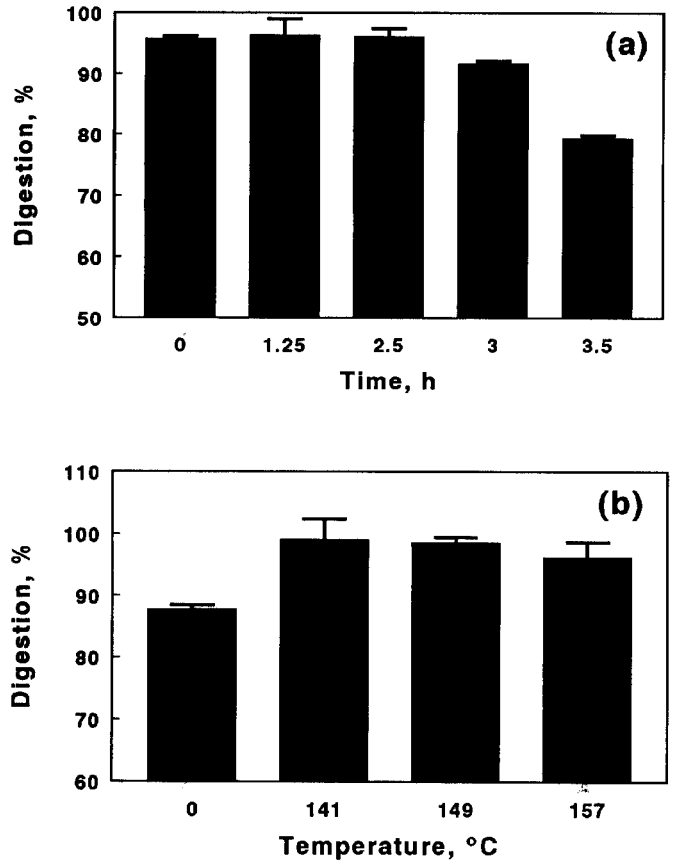

Figure 2. Effect of (a) heating time on protein digestion of the undegraded intake protein (UIP) fraction of soybean meal, and (b) heating temperature on protein digestion of the UIP fraction of soybeans.

Discussion

Feeding systems currently in use recognize that intestinal digestion of proteins varies among feed sources (Jarrige and Alderman, 1987). Because of the difficulties in determining intestinal digestion of protein, current formulation systems use corrected fecal N (Jarrige, 1989), ADIN (ARC, 1984; Sniffen et al., 1992), or assume constant values (NRC, 1989). However, efforts are currently underway to incorporate more accurate estimates into these feeding systems (Jarrige and Alderman, 1987). Acid detergent insoluble N has been the most commonly used indicator of unavailable protein in the small intestine. However, ADIN as an estimate of protein digestion in the small intestine was developed for forages (Goering et al., 1972) and the reliability of this measurement in non-forage feeds has been strongly criticized (Britton

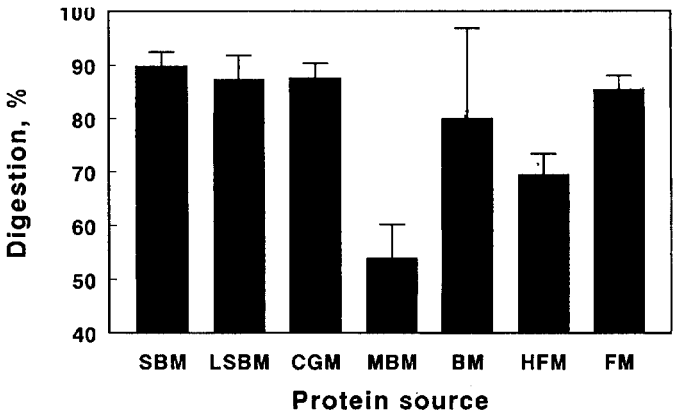

Figure 3. Estimates of intestinal digestion of soybean meal (SBM), lignosulfonate-treated SBM (LSBM), corn gluten meal (CGM), blood meal (BM), hydrolyzed feather meal (HFM), fish meal (FM), and meat and bone meal (MBM) protein using the three-step in vitro procedure.

et al., 1986; Klopfenstein and Britton, 1987; Weiss et al., 1989). Britton et al. (1986) compared apparent total tract digestion of N with ADIN content of feeds and found a poor relationship ($n = 19$; $r = -.39$), suggesting that ADIN was not a good indicator of protein digestion in the small intestine. Merchen (1990) suggested that heating would affect N availability differently depending on the protein supplement, and that the relationship between ADIN and intestinal availability of N may be non-linear over a wide range of feedstuffs.

The mobile-bag technique (Hvelplund, 1985) uses a more physiological approach. Although results from fecal collection of bags showed reasonable correlation with rat growth rates ($r = .92$, Rooke, 1985) and in vivo intestinal CP digestion ($r = .81$, Hvelplund, 1985), an interaction between type of feed and site of collection (ileal vs fecal) has been reported (Hvelplund, 1985), and may invalidate these results. Hvelplund (1985) calculated that the amount of protein within the nylon bag that was digested in the large intestine was 50 and 27% of that leaving the ileum for SBM and rapeseed meal, respectively. In addition, bag pore size, animal, diet, large intestinal fermentation, and bacterial contamination may also contribute to variation (Hvelplund, 1985; Rooke, 1985; Voigt et al., 1985).

Preliminary in vitro enzymatic tests were conducted using a purified multi-enzyme (chymotrypsin, trypsin, protease, and peptidase) procedure developed by Hsu et al. (1977). Estimates of intestinal CP digestion were lower for the multi-enzyme procedure than for pancreatin digestion (58 vs 75%, $P < .05$, for multi-enzyme vs pancreatin, respectively). This difference was likely due to interference of nonprotein components (such as starch or fats) on protein digestion. Amylase, lipase, and ribonuclease enzymes present in the pancreatin, but not in the purified multi-enzyme preparation, were likely responsible for the increased protein digestion with the pancreatin assay. Therefore, all further tests were conducted only with the pancreatic extract.

Because the proteolytic activity in the intestine of cows is high, the extent of protein digestion is not limited by retention time (van Bruchen et al., 1985; Voigt et al., 1985). Therefore, the objective of the preliminary tests in the development of the pancreatin assay was to optimize digestion conditions to maximize enzymatic activity. Validation of the pancreatin assay used duodenal samples of animals fed diets containing a wide variety of protein supplements and resulted in a high correlation value ($n = 34$, $r = .91$, $P < .001$). The fact that the slope ($.69 \pm .12$) was different ($P < .05$) from 1 may be due to the unquantified contribution of endogenous N in vivo or to intrinsic problems of the in vivo technique (markers, animal variation, or cannula location), and not necessarily to the pancreatin assay. The correlation provides strong support for the adequacy of the pancreatin assay as a means to determine intestinal digestion of proteins. Results from the current study agree with those of Akeson and Stahmann (1964), who reported an r value of .98 when estimates from a pepsin-pancreatin assay were regressed on in vivo estimates of protein quality in rats. In contrast, Britton et al. (1986) reported a low correlation between total tract and pepsin-pancreatin CP digestion in ruminants. However, pepsin-pancreatin digestion should be correlated to intestinal and not to total tract digestion.

Pepsin predigestion increased ($P < .05$) estimates of postruminal availability of protein in all supplements tested, suggesting that the abomasum plays an important role in protein digestion. Cherian et al. (1988) reported an increase in intestinal digestion of SBM, MBM, and canola meal protein when samples were predigested with pepsin. In contrast, other researchers (Hvelplund, 1985; van Bruchen et al., 1985; Voight et al., 1985) suggested that the role of HCl-pepsin on protein digestion was negligible.

Because feed reaching the abomasum is modified by ruminal fermentation, it was reasonable to test the effect of ruminal preincubation on pepsin-pancreatin digestion of feed protein. A 16-h preincubation in the rumen was selected as an estimate of mean retention time of feeds in the rumen. In addition, preliminary tests indicated no differences in pepsin-pancreatin digestion of proteins when samples were preincubated in the rumen for 12 to 18 h (data not shown).

The effect of ruminal preincubation was small for SBM, CGM, and BM, and indicates that UIP protein in these feeds is readily digestible in the small intestine. However, pepsin-pancreatin digestion of CP was reduced ($P < .05$) by 9.9, 6.4, and 26.5 percentage

units for HFM, FM, and MBM, respectively, following ruminal preincubation. It is hypothesized that digestible protein in these sources is mostly degraded in the rumen, and only a small fraction of the UIP is available for digestion postruminally. A reduction in estimates of intestinal digestion or biological value of MBM and FM protein after being incubated in the rumen has been previously reported (Rooke, 1985; de Boer et al., 1987). No similar reports have been found in relation to HFM.

Estimates of postruminal digestion of SBM, LSBM, CGM, and FM were between 85 and 90%. Values obtained using the mobile-bag technique agree with results from the three-step procedure for SBM, CGM, FM, and MBM (de Boer, 1987) but were greater than true digestion estimated in vivo (Titgemeyer et al., 1989). However, it should be noted that, in contrast with data from experiments using animals fitted with duodenal and ileal cannulas, the three-step procedure estimates abomasal (pepsin) and intestinal (pancreatin) digestion. Because protein may be digested and absorbed from the abomasum (Webb et al., 1992), less digestible dietary protein reaches the small intestine. Therefore, values obtained with the three-step procedure are expected to be somewhat higher than those estimated using cows fitted with duodenal and ileal cannulas.

The most important findings of the application of this procedure to feed samples were the low intestinal digestion of MBM and HFM protein and the large variation observed among BM samples. The consistently low intestinal protein digestion of HFM agrees with in vivo observations by Waltz et al. (1989). Apparent digestion of nonammonia N from the small intestine of cows fed HFM (62.0%) was lower than that of cows fed diets containing SBM (73.7%), BM (78.9%), or a combination of BM and HFM (79.6%). A similar pattern was observed for the absorption of total, essential, and nonessential AA. Palmquist et al. (1993) also reported that intestinal protein digestion of HFM was lower than that of BM (68.3 vs 98.9%, respectively) when measured using the mobile-bag technique. These results are in agreement with results of others (Goedeken et al., 1990; Harris et al., 1992), who reported a reduction in total tract digestion of CP in diets containing HFM. Blasi et al. (1991) suggested that processing time had small effects on the quality of HFM, and that the low performance frequently reported was due to the intrinsic characteristics of the protein. The presence of disulfide bridges may be responsible for the low intestinal digestion of HFM. In contrast, no detrimental effects of HFM were reported in growing calves (Goedeken et al., 1990; Blasi et al., 1991).

Analysis of various MBM samples also resulted in low estimates of pepsin-pancreatin digestion and is consistent with estimates obtained using the mobile bag technique (Rooke, 1985; de Boer et al., 1987; Erasmus et al., 1994). Lower weight gains and higher fecal N were reported in cattle fed diets containing meat meal than in those fed diets containing BM, CGM, or SBM (Stock et al., 1981). The source of the raw material (meat, tendons, or bones) is the major factor affecting MBM quality (Atkinson and Carpenter, 1970; Skurray and Herbert, 1974; Stock et al., 1981). Tendons and bones are rich in collagen and ossein, respectively. These proteins contain peptide bonds that are not sensitive to trypsin hydrolysis, preventing the unfolding of the protein and decreasing accessibility of other enzymes. When tendons and bones are added to the MBM, its intestinal digestibility is decreased (Atkinson and Carpenter, 1970). An increase in ash and hydroxyproline content, an amino acid characteristic of collagen, has been strongly correlated with the nutritive value of MBM (Atkinson and Carpenter, 1970; Rooke, 1985).

Pepsin-pancreatin digestion of BM protein averaged 80.1%, but individual samples ranged from 50 to 98%. Similar variation has also been reported in estimates obtained using the mobile-bag technique, ranging from 99% (Palmquist et al., 1993) to 57% (Erasmus et al., 1994). Much of this variation can be attributed to processing methods. Waible et al. (1977) reported that batch-drying greatly reduced intestinal digestion of the BM protein, and decreased lysine and methionine availability, compared with ring-dried BM. These results agree with Harvey and Spears (1989), who reported that cattle fed batch-dried BM grew slower and were less efficient than those fed ring-dried BM, possibly due to lower intestinal availability of the protein.

Implications

The three-step procedure closely simulated physiological conditions in the animal. The technique was sensitive to heat damage of soybean protein and to the presence of anti-trypsin factors in soybeans. Compared with in vivo determination of intestinal digestion of CP, the three-step procedure provided evidence of reliability, resulted in a substantial reduction in cost and labor, and could be routinely used for screening intestinal digestion of proteins in ruminants. The use of estimates of intestinal digestion in combination with estimates of protein degradation in the rumen may provide estimated values of intestinally absorbable dietary protein derived from individual ingredients. These data would be very useful for quality control of processed proteins and for determining an overall value of protein supplements for ruminants.

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