Electrophoretic Analysis of Ruminal Degradability of Corn Proteins

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ABSTRACT
Albumin, globulin, and prolamin fractions were extracted from corn meal in water, .5 M NaCl, and 50% (vol/vol) 1-propanol and examined by SDS-PAGE and densitometric scanning to investigate fractional degradation rates of corn proteins. Several protein fractions were identified for globulins, zein prolamins, and glutelins. Ruminal degradability of individual subfractions was evaluated by suspension of corn and corn gluten meal samples in the rumen of lactating dairy cows from 0 to 72 h. Electrophoretic and densitometric analysis of protein residues revealed that the prolamin fraction zein for corn and corn gluten meal was more resistant to ruminal degradation than albumins, globulins, and glutelins. Relative rates of degradation of zein and the fraction containing albumins, globulins, and glutelins were .060, .026, and .018, .015/lb for corn and corn gluten meal, respectively. Total degradabilities of corn and corn gluten meal, measured by summation of degradability of subfractional components, were 52.2 and 18.6%. Quantitative measurement of ruminally degradable subfractions and estimation of their degradation rates by electrophoretic and densitometric scanning are useful in understanding ruminal degradability of corn proteins.
(Key words: corn proteins, ruminal degradability, protein subfractions, electrophoresis)

INTRODUCTION
Seed proteins traditionally are classified into four fractions based on their solubility in 1) water (albumins), 2) salt solution (globulins), 3) 70% ethanol solution (prolamins), and 4) dilute alkali or acid (glutelins) (8, 21). In cereal grains, prolamins and glutelins represent the bulk of insoluble protein and are likely resistant to degradation in the rumen (18). The content of albumins and globulins in seed proteins, such as soybean and cottonseed, is higher than in cereals. More importantly, albumins and globulins have a higher biological value than prolamins and glutelins (12). Therefore, the relative proportion of each fraction in feedstuffs may greatly influence total protein degradability in the rumen and, thus, the pattern of AA reaching the intestine.

Digestibility of protein fractions in cereals and legumes is related to their AA composition. Albumins and globulins have a higher content of basic and acidic AA than prolamins and glutelins (23, 24). Conversely, prolamins are rich in polar neutral and nonpolar AA. Although relationships between protein fraction content and protein digestibility have been investigated in monogastrics (5), little information is available concerning the relative contribution of each fractional component to dietary protein escaping the rumen (15). Moreover, evidence exists that intestinal digestion of the ruminal bypass protein may be dependent on its fractional composition (22).

We have reported previously (16) on the use of SDS-PAGE to study subfractional degradation of soybean proteins in the rumen. The SDS-PAGE and densitometric analysis were used advantageously to study the sensitivity of β-conglycinin and glycinin soybean polypeptides to ruminal proteolysis. In the same study (16), subfractional degradation rates were estimated to predict the contribution of various subfractions to undegradable soy protein. Corn protein also contributed significantly to protein
requirements of ruminants. Therefore, the objective of this study was to investigate the use of SDS-PAGE and densitometric techniques in prediction of ruminal degradation rates of corn and corn gluten meal subfractions and their potential contribution to dietary protein ultimately reaching the intestine.

MATERIALS AND METHODS

Corn proteins were fractionated in vitro according to their solubility in different solvents as previously reported (10). Corn meal was ground through a 1-mm screen and defatted with 50 ml of petroleum ether using a Soxtec System™ (Tecator Ab, Hoganas, Sweden). Defatted meal (10 g) was added to 100 ml of solvent for protein extraction. Extraction of albumins was in water at 4°C for 15 min and was repeated three times followed by extraction of globulins in 5 M NaCl at 4°C for intervals of 60, 30, and 30 min. Albumin and globulin extracts were centrifuged at 9000 x g for 25 min. Albumins were dialyzed against water at 4°C with continuous mechanical agitation for 48 h. Prolamins were extracted by dissolving protein residues in 50% (vol/vol) i-propanol for 60, 30, and 15 min, followed by centrifugation at 9000 x g for 25 min. All protein fractions and glutelins remaining after extraction in 50% (vol/vol) i-propanol were dialyzed against water at 4°C and freeze-dried for further analysis by SDS-PAGE.

Ruminal degradability of corn and corn gluten meal was studied using the in situ dacron bag technique (14) as previously described (16). Briefly, dacron bags were incubated sequentially at 0, 2, 6, 12, 24, 36, 48, and 72 h so that all bags were removed simultaneously from the rumen. Upon removal, bags were washed with water and dried at 50°C in a forced-air oven. Dried residues were ground through a 1-mm screen and analyzed for total protein (N x 6.25) by the Kjeldahl method (2). Rapidly degraded (fraction A), potentially degradable (fraction B), and undegraded (fraction C) protein fractions were estimated using the method of Armentano et al. (1) adapted from that of Ørskov and McDonald (14). Total degradability (fraction D) was estimated using the equation (16)

\[
D = A + \frac{k_d B}{k_d B + k_p B}
\]

where \( k_d B \) = degradation rate of fraction B, \( k_p B \) = rate of passage, assumed to be .05/h.

To compare corn and corn gluten meal protein availabilities with the ruminal microflora immediately after incubation in the rumen, a lag phase (\( t_0 \)) was included in the Ørskov and McDonald model as proposed by McDonald (11). The equation describing the effective protein degradability (\( D' \)) was

\[
D' = A' + \left( \frac{B' k_d B}{k_d B + k_p B} \right) e^{-t_0}
\]

where \( A' \) = protein fraction readily available, \( B' \) = protein fraction degradable at a measurable rate, and \( t_0 \) = lag phase.

Fractional composition of corn protein extracts and of corn and corn gluten meal samples before and after incubation in the rumen was analyzed by SDS-PAGE (9). Protein samples were dissolved in sample buffer containing .625 M Tris·HCl (pH 6.8), 2% SDS, 10% glycerol, .0025% bromophenol blue (6), and 9% 2-mercaptoethanol as previously reported (16). Proteins were separated using a discontinuous SDS-PAGE system consisting of an upper stacking gel of 3.75% acrylamide, .625 M Tris·HCl (pH 6.8), and a linear gradient separating gel consisting of 5 to 15% acrylamide in 1.87 M Tris·HCl (pH 8.8). Polymerization of acrylamide was induced by addition of N,N,N',N'-tetramethylethylenediamine and ammonium persulfate. Molecular mass standard proteins were obtained from Bio-Rad (Bio-Rad Laboratories, Richmond, CA), and purified zein fraction was from United States Biochemical Co. (Cleveland, OH).

Proteins were fixed and stained in a solution of water:methanol:glacial acetic acid (5:5:2, vol/vol/vol) containing .1% Coomassie blue R250 (Bio-Rad Laboratories). Gels were destained in a solution of 30% methanol and 10% acetic acid. Densitometric analysis of proteins stained with Coomassie blue was performed at 580 nm as described previously (16) to estimate degradation rates of individual corn protein subfractions as follows:

\[
\frac{[k_d B B/(k_d B + k_p B)]}{[B_1 k_d B_1/(k_d B_1 + k_p B_1)]} + \frac{[B_2 k_d B_2/(k_d B_2 + k_p B_2)]}{[B_2 k_d B_2/(k_d B_2 + k_p B_2)]}
\]

where \( B_{1,2} \) = percentage of fraction B1 (albumins, globulins, and glutelins) and B2 (zein) at time 0, \( k_d B_{1,2} \) = rate of degradation of frac-
tions B1 and B2, $k_pB$ = rate of passage (assumed, .05/h), $B$ = total B fraction (B1 plus B2), and $k_dB$ = rate of degradation of fraction B.

Data regarding total protein and subfractional degradability of corn and corn gluten meal samples in the rumen were analyzed as described previously (16). Briefly, in situ incubations in the rumen were repeated twice, and protein degradability was analyzed using a model containing cow, replicate, time, cow × time, and cow × replicate. A simple within- and between-comparison method was used to analyze total degradability of fraction B, estimated by summation of subfractional degradabilities, versus total fraction B.

RESULTS AND DISCUSSION

Electrophoretic analysis of corn protein fractions extracted by differential solubility is illustrated in Figure 1. Several albumin and globulin polypeptides over a large range of molecular mass were separated by SDS-PAGE. Although some protein bands were present in both protein extracts, the globulins appeared to be composed mainly of subunits with a molecular mass ranging from 25 to 50 kDa (23). Corn prolams were easily identified after SDS-PAGE and consisted of two major subunits of 22 and 24 kDa. Evidence of the bifractional pattern for zein when separated by SDS-PAGE in a denatured state has been reported previously (7). Densitometric analysis of corn prolams extracted in 50% (vol/vol) 1-propanol confirmed that the in vitro extraction procedure used in this study yielded prolamin fractions similar to purified standard zein (Figure 2). This finding was not surprising because zein is the main component of corn prolams (24).

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Figure 1. The SDS-PAGE linear gradient (5 to 15%) slab gel analysis of corn proteins. Lane 1, albumins (water); lane 2, globulins (5 M NaCl); lane 3, corn prolams [50% (vol/vol) 1-propanol]; lane 4, standard zein; lane 5, SDS-sample buffer glutelins; lane 6, molecular mass markers, phosphorylase b, 97.4 kDa; BSA, 66.2 kDa; ovalbumin, 42.7 kDa; carbonic anhydrase, 31.0 kDa; trypsin inhibitor, 21.5 kDa; and lysozyme, 14.4 kDa.
TABLE 1. Protein degradability of corn subfractions B.

<table>
<thead>
<tr>
<th>Protein source</th>
<th>Fraction B&lt;sup&gt;1&lt;/sup&gt;</th>
<th>k&lt;sub&gt;d&lt;/sub&gt;B&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>84.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Corn gluten meal</td>
<td>55.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>Means in the same column with no common superscripts differ (P < .05).

<sup>1</sup>Values for subfractions B are based on percentage of contribution to total protein degradability.

<sup>2</sup>Degradation rates.

Because corn glutenins consist of several polypeptides linked through disulfide bonds, the use of reducing agents in the electrophoretic sample buffer generates several additional protein components that can be characterized by SDS-PAGE (10). In this study, three main polypeptides of 31, 50, and 55 kDa were identified by SDS-PAGE analysis of glutenins residues in presence of the reducing agent mercaptoethanol. A similar profile of corn glutenins in 1% SDS sample buffer has been reported previously (24).

The sensitivity of corn protein subfractions to ruminal proteolytic breakdown was studied by suspension of corn and corn gluten meal samples using the in situ bag technique. Fractions A, B, and C for corn meal and corn gluten meal were 11.4, 83.9, 4.7 and 5.2, 55.5, and 39.3% of total protein (Figure 3). Degradation rates of fraction B estimated by linear regression were .047 and .016/h for corn and corn gluten meal, respectively (Table 1). Protein degradabilities of corn and corn gluten meal were 52.2 and 18.6%, respectively, in agreement with previous values (12, 19). Nevertheless, others (20) have suggested that corn gluten meal forms a viscous mass inside the bag, and attack by microorganisms was severely delayed (4). In the present study, a lag phase of 2.27 h was estimated for corn gluten compared with .24 h for corn meal, suggesting that the in situ bag technique may not effec-

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Figure 2. Densitometric analysis of corn prolamin. a) standard zein; b) corn prolamins extracted in 50% (vol/vol) 1-propanol. Protein separation was performed on a 5 to 15% linear gradient SDS-PAGE.

Figure 3. Fractional protein degradability of corn and corn gluten meal (CGM) by dacron bag suspension technique. Protein fractions A (rapidly degraded), B (potentially degradable), and C (undegraded) were estimated. Bars represent the least squares means (±SEM) of six samples assayed in duplicate.
Figure 4. Densitometric analysis of corn (A) and corn gluten meal (B) proteins. Following incubation in the rumen, residual proteins were analyzed by electrophoretic separation on a 5 to 15% linear gradient SDS-PAGE and densitometric scanning. Hours are specific times of in situ incubation in the rumen; B2, zein fractions (22.0 and 24.0 kDa). Peaks represent relative contribution of individual protein fractions at each period.
tively measure ruminal protein degradability of corn gluten meal. Furthermore, adjustment for the lag phase did not alter total degradability for this protein source (data not shown).

Preliminary electrophoretic analysis of corn protein fractions based on their differential solubility and migration pattern in SDS-PAGE provided an in vitro system for monitoring protein degradation in the rumen. Zein, the B2 prolamin fraction, was relatively resistant (Figure 4) to ruminal proteolysis for corn and corn gluten meal. Conversely, fraction B1 components (albumins, globulins, and glutelins) contributed very little to the protein residues following incubation in the rumen. Densitometric analysis, after incubation in the rumen, was used to measure quantitatively the fractions B1 and B2 and to estimate their degradation rates (Table 1). Corn zein (B2) was degraded quite slowly (.026/h) compared with fraction B1 (.06/h) and represented 16.2 and 68.7%, respectively, of total protein. Corn gluten meal subfractions B1 and B2 were degraded at lower rates, .018 and .015/h, respectively, and constituted 30.0 and 25.5% of total protein. Therefore, a smaller percentage of total protein in corn gluten meal was available to the ruminal microflora than with corn meal. Analysis of densitometric scans revealed that, by 48 h, zein represented the bulk of the residual protein for both protein sources (Figure 4). Previous in vitro studies (3) showed that zein was degraded minimally compared with cottonseed meal and casein when zein was incubated with ruminal fluid. The hydrophobic nature of zein may be responsible for its low utilization by microorganisms (24).

The marked variability in ruminal rates of degradation may be associated with different AA composition (12) and tertiary structure of different protein sources (13). Corn prolamins are rich in nonpolar AA and have a rodlike molecular structure compared with globular water-soluble proteins (7). Furthermore, presence of three-dimensional disulfide linkages in corn glutelins, which increases the complexity of the tertiary structure, may limit the action of microbial proteases and, thus, reduce ruminal degradation of zein.

Previous attempts to identify subfractional B components of corn proteins (21) showed that corn prolamins were relatively resistant to ruminal degradation compared with glutelins. However, knowledge of their rate of degradation in vivo is necessary to predict quantitatively and qualitatively the contribution of protein bypass to the intestinal tract (12). This study provides further evidence that SDS-PAGE and densitometric analysis of protein subfractions can be used to predict degradational rates of dietary protein in the rumen. This methodology will combine with digestibility studies of the ruminally undegradable fractions and qualitative and quantitative identification of the individual AA contributing to these fractions to strengthen our ability to predict the contribution of ruminally undegradable protein to the AA profile delivered to the small intestine.

CONCLUSIONS

In vitro fractionation of corn proteins associated with SDS-PAGE analysis prior to and after incubation in the rumen of corn and corn gluten meal samples demonstrated that the alcohol-soluble corn prolamins were relatively resistant to ruminal degradation. This study suggests that increased hydrophobicity of proteins may be associated with reduced degradability in the rumen. Densitometric scanning of protein bands after electrophoretic separation allows determination of the relative rates of fractional digestion of corn proteins. Because a balanced supply of AA is important to satisfy microbial and animal needs, knowledge of the relative degradation rates of protein subfractions may provide a more accurate estimate of the AA profile reaching the small intestine.

REFERENCES

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