Microbial Protein Biosynthesis in the Rumen

E. A. IBRAHIM and J. R. INGALLS
Department of Animal Science, University of Manitoba, Winnipeg, Canada

Abstract
Semipurified and conventional diets with and without diethylstilbestrol (DES) were fed to four rumen fistulated dairy cows in a 4 x 4 Latin square design. Feed was limited to 10 kg daily and fed at intervals of 2 min. Amino acid composition of experimental diets, protozoa, bacteria, and rumen digesta were determined. Experimental diets did not affect (P<.05) amino acid composition of the bacterial protein. Lysine, proline, phenylalanine, and methionine content of protozoa were affected (P<.05) by the experimental diets.

Diaminopimelic acid of bacteria and diaminomethylphosphonic acid of protozoa were used as markers to estimate microbial protein synthesis in the rumen. Rumen microorganisms contributed 54.4 to 91.8% of the total amino acids in rumen digesta with 24.3 to 30.9% in the form of bacterial amino acids. Protozoal amino acids as a percentage of the total rumen amino acids were increased more than twofold with both types of diet when DES was included in the diet.

Total amounts of amino acids passing to lower gut with lignin as a marker were 882 and 937 g daily when the semipurified diets without and with DES were fed and 1,108 and 1,271 g daily when the conventional diet without and with DES was fed.

Introduction
Different methods have been used to assess the microbial contribution to protein in ruminal digesta. Smith (35) and Ellis and Pfander (16) used nucleic acid and McDonald (29) and Ely et al. (18) used differences in solubility of zein and microbial protein in ethanol. Blackburn and Hobson (8) used a method based on solubility differences between casein and microbial protein. McDonald and Hall (30) used organic phosphorus content in casein to estimate the extent of conversion of casein to microbial protein. Some workers (18, 29, 36) determined microbial protein in rumen digesta by differences in lysine content between microbial and dietary proteins. However, these methods cannot be applied in conventional diets because all estimations are based upon purified protein. Some workers (12, 39) have studied microbial protein synthesis in the rumen employing the rate of incorporation of $^{35}$S in microbial cells. Others (17, 41) have estimated bacterial growth with diaminopimelic acid (DAP) as a marker since it has been found in bacterial protein but not in protozoal and dietary protein. Previous studies have indicated that aminomethylphosphonic (AEP) acid is in protozoal protein but not in bacterial or dietary protein (2, 20, 23).

The purpose of the present investigation was to measure microbial protein synthesis in rumen digesta with DAP and AEP as markers and to estimate microbial protein quality as determined by amino acid composition.

Materials and Methods

Rumen digesta. Rumen contents were obtained from four fistulated dairy cows fed semipurified and conventional diets (Table 1) with and without 8 mg/day diethylstilbestrol (DES). The cows were in a 4 x 4 Latin square design. Each experimental period consisted of 28 days. A steady state was established in the tureens of cows through feeding each cow 13 to 15 g (10 kg/day) of diet at intervals of 2 min by a continuous feeding apparatus described by Ibrahim et al. (25). Feed intake was fixed at 10 kg/day for each cow to limit the effect of dry matter intake on composition of rumen digesta.

Rumen fill. The rumen of each cow was manually emptied at the end of each experimental period through the fistulae. Rumen digesta were weighed, mixed thoroughly, sampled, and returned. Samples of rumen digesta were dried at 60°C. Dried samples of rumen digesta were ground and kept for subsequent analysis.

Rumen cross-inoculation. Rumen cross-inoculations were performed on the first and third day of each period by mixing about 500 ml of rumen contents from each cow. Rumen cross-
Table 1. Composition of experimental diets and rumen digesta.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Semipurified</th>
<th>Conventional</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No DES</td>
<td>DES</td>
</tr>
<tr>
<td>Wheat straw</td>
<td>38.0</td>
<td>38.0</td>
</tr>
<tr>
<td>Glucose</td>
<td>15.7</td>
<td>15.7</td>
</tr>
<tr>
<td>Corn starch</td>
<td>27.0</td>
<td>27.0</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Alphacel§</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Urea</td>
<td>4.6</td>
<td>4.6</td>
</tr>
<tr>
<td>Mineral mixture†</td>
<td>4.5</td>
<td>4.5</td>
</tr>
<tr>
<td>Choline chloride 50%</td>
<td>2.2</td>
<td>2.2</td>
</tr>
<tr>
<td>Molasses</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Diethylstilbestrol</td>
<td>--</td>
<td>+</td>
</tr>
<tr>
<td>Vitamins A, D, E</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alfalfa hay</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Barley</td>
<td>42.0</td>
<td>42.0</td>
</tr>
<tr>
<td>Soybean</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Trace minerals</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Crude protein</td>
<td>13.2</td>
<td>13.2</td>
</tr>
<tr>
<td>Lignin</td>
<td>4.1</td>
<td>4.1</td>
</tr>
<tr>
<td>Rumen digesta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rumen digesta (kg)</td>
<td>35.0</td>
<td>35.3</td>
</tr>
<tr>
<td>Dry matter (%)</td>
<td>10.3</td>
<td>12.4</td>
</tr>
<tr>
<td>Dry matter (kg)</td>
<td>3.6</td>
<td>4.4</td>
</tr>
<tr>
<td>Crude protein (% DM)</td>
<td>17.0B,b</td>
<td>19.1B,a</td>
</tr>
<tr>
<td>Total amino acid, (% DM)</td>
<td>15.2B,b</td>
<td>17.1B,a</td>
</tr>
<tr>
<td>Lignin (% DM)</td>
<td>9.6</td>
<td>9.1</td>
</tr>
</tbody>
</table>

From Ibrahim et al. (24).

A,B Treatment means within a row not sharing a common letter are significantly (P < .01) different.

a,b Treatment means within a row not sharing a common letter are significantly (P < .05) different.

Protozoal and bacterial fraction. Protozoa and bacteria were harvested during the last week of each experimental period. Rumen fluid was collected from whole digesta by squeezing through two layers of cheesecloth into a warmed Dewar flask, and fractionation began within 5 min after collection. Protozoa were harvested by gravimetric technic (23). One liter of strained rumen fluid was diluted one-to-one (v/v) with an acetate-phosphate buffer (NaCl, 2.15 g; KH₂PO₄, .35 g; K₂HPO₄, 1.00 g; NaCl, 5 g; MgSO₄, .12 g made up to 1 liter with distilled water), bubbled with carbon dioxide, and incubated for 1 hr. The protozoal fraction was transferred to 200 ml centrifuge tubes and repeatedly washed with acetate-phosphate buffer, centrifuged at 200 × g, and examined microscopically for contamination with bacteria and food debris.

Protozoal residue was transferred and spread on glass plates and was then dried at 39 C in a forced air drying oven, and dry weight was recorded. The bacterial fraction was obtained by differential centrifugation. One liter of strained rumen was centrifuged at 100 × g for 10 min to remove protozoa and food debris, then the supernatant recentrifuged at 50,000 × g for 20 min in 50 ml centrifuge tubes. The bacterial fraction was resuspended and washed with acetate-phosphate buffer and recentrifuged three times. The bacterial fraction was transferred and spread on glass plates to dry at 39 C in a forced air oven, and dry weight was determined.

Determination of amino acids. Acid hydroly-
sates of diets, protozoa, bacteria, and rumen digesta were prepared according to the procedure described by Ibrahim et al. (23) for amino acid analysis. Methionine and DAP co-chromatographed at 134 min, but it was possible to distinguish between DAP and methionine through conversion of methionine to methionine sulfone (peak-time 50 min) by performic acid treatment. Ground samples of dried diets, protozoa, oxidized residue of bacteria and rumen digesta were hydrolyzed with 3 N HCl under reduced pressure in sealed flasks at 121°C for 15 hr. Hydrolysates were dried under reduced pressure at 40°C and washed with 0.2 N sodium citrate buffer. The residue was transferred with 2.2 ml of citrate buffer, filtered, and adjusted to 50 ml.

The amino acid AEP was eluted with a peak-time of 185 min in an acid-neutral column with sodium citrate buffer, pH 3.28 (.10 N), 4.25 (.20 N) and 6.25 (.40 N) at 0, 85, and 138 min. Quantitative amino acid analyses were determined on .5 ml of the hydrolysates with a Beckman Model 116 amino acid analyzer with norleucine as an internal standard (4).

**Chemical analysis.** Crude protein of the dried samples of experimental diets and ruminal digesta were determined by the macro-

Kjeldahl technic, and proximate composition of experimental diets and ruminal digesta were determined by AOAC (3) methods. Lignin was determined according to the procedure of Van Soest and Wine (37).

**Statistical analysis.** Data were statistically analyzed according to Cochran and Cox (11) procedure, and differences between means were tested by multiple range test (14).

**Results and Discussion**

**Rumen digesta.** Fixing daily feed intake and feeding at intervals of 2 min were assumed to result in a constant rate of fermentation, of salivary inflow, and of digesta leaving the rumen. Rumen volatile fatty acids and NH₃ data (25) suggest a steady state was reached in the rumen. The weight of rumen digesta was somewhat greater for cows fed semipurified diets compared with cows fed conventional diets (Table 1); however, differences were not significant (P<.05). There was no significant difference (P<.05) in percentage of dry matter of rumen digesta of cows fed experimental diets. Total amino acid content of rumen digesta following protein hydrolysis was higher (P<.01) for cows fed conventional diets compared with those fed semipurified diets. Inclusion of DES in the semipurified diet resulted

<table>
<thead>
<tr>
<th>Amino Acids</th>
<th>Semi-purified</th>
<th>Conventional</th>
<th>Rumen digesta</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Des</td>
<td>No DES</td>
<td>Des</td>
</tr>
<tr>
<td>Aspartic Acid</td>
<td>20.0</td>
<td>12.3</td>
<td>11.3</td>
</tr>
<tr>
<td>Threonine</td>
<td>3.6</td>
<td>4.6</td>
<td>4.3</td>
</tr>
<tr>
<td>Serine</td>
<td>4.5</td>
<td>5.0</td>
<td>3.9</td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>20.1</td>
<td>18.3</td>
<td>16.0</td>
</tr>
<tr>
<td>Proline</td>
<td>4.5</td>
<td>8.1</td>
<td>3.8</td>
</tr>
<tr>
<td>Glycine</td>
<td>5.9</td>
<td>5.2</td>
<td>3.7</td>
</tr>
<tr>
<td>Alanine</td>
<td>12.4</td>
<td>5.9</td>
<td>6.5</td>
</tr>
<tr>
<td>Cystine</td>
<td>.1</td>
<td>.1</td>
<td>3.3</td>
</tr>
<tr>
<td>Valine</td>
<td>4.5</td>
<td>5.4</td>
<td>6.1</td>
</tr>
<tr>
<td>Methionine</td>
<td>3.0</td>
<td>1.7</td>
<td>3.9</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>4.5</td>
<td>4.3</td>
<td>4.4</td>
</tr>
<tr>
<td>Leucine</td>
<td>8.0</td>
<td>8.4</td>
<td>6.6</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>2.6</td>
<td>3.4</td>
<td>4.4</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>3.6</td>
<td>5.5</td>
<td>4.2</td>
</tr>
<tr>
<td>Lysine</td>
<td>3.4</td>
<td>5.1</td>
<td>6.3</td>
</tr>
<tr>
<td>Histidine</td>
<td>.9</td>
<td>1.2</td>
<td>8.4 Ab</td>
</tr>
<tr>
<td>Arginine</td>
<td>.6</td>
<td>5.1</td>
<td>2.3</td>
</tr>
<tr>
<td>Diaminopimelic Acid</td>
<td>.49</td>
<td>.34</td>
<td>.36</td>
</tr>
<tr>
<td>Aminoethylphosphonic</td>
<td>.2</td>
<td>.36</td>
<td>.12</td>
</tr>
<tr>
<td>Essential amino acids*</td>
<td>34.7</td>
<td>44.7</td>
<td>50.9</td>
</tr>
<tr>
<td>Total (% of dry matter)</td>
<td>1.0</td>
<td>11.0</td>
<td>15.2</td>
</tr>
</tbody>
</table>

*Threonine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine and arginine.
in a higher total amino acid content of rumen digesta (P < .05) whereas inclusion of DES to the conventional diet had no significant effect on total amino acid content of rumen digesta following protein hydrolysis.

Amino acid compositions (except histidine) of rumen digesta appear to be similar in spite of differences in quantity and quality of amino acids in the experimental diets (Table 2). Duncan et al. (13) observed a similar amino acid pattern for rumen contents of calves fed a purified diet containing urea and those fed natural diets. The two-sulfur-containing amino acids in rumen digesta as a percentage of total amino acids appear higher than in the experimental diets. Also microbial synthesis increased the ratio of lysine and histidine as in cows (6). The percentage of histidine in rumen digesta was influenced (P < .05) by the diet and by DES inclusion in the diet (Table 2). Virtanen (38) also observed more histidine in hydrolyzed rumen contents of cows fed a urea purified diet than of cows fed a natural diet.

The present experiment (Table 2) indicates that the rumen microorganisms in the cows synthesized considerable quantities of essential and nonessential amino acid from nonprotein nitrogen, agreeing with results by Loosli et al. (28) and Duncan et al. (13). The total of essential amino acids of rumen digesta, expressed as percentage of total amino acid, from cows fed the semipurified diet was similar to that for cows fed the conventional diet, and values in percent are similar to those observed by Schelling et al. (34) on rumen content of lambs fed a purified diet.

The amino acid content of protozoa as a percentage of dry weight is high as compared with bacteria (Table 3). These results agree with those by Johnson et al. (26). The concentration of lysine and glutamic acid tended to be higher in the protozoa than in bacteria whereas the histidine, threonine, serine, cystine, and methionine content of bacteria tended to be higher than that of protozoa. Similar results have been reported in sheep by many investigators (5, 32, 33).

Table 3. Amino acid composition of protozoal and bacterial fractions from dairy cows fed the experimental diets.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Protozoa</th>
<th>Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Semipurified</td>
<td>Conventional</td>
</tr>
<tr>
<td></td>
<td>No DES</td>
<td>DES</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>10.9</td>
<td>11.7</td>
</tr>
<tr>
<td>Threonine</td>
<td>4.5</td>
<td>4.6</td>
</tr>
<tr>
<td>Serine</td>
<td>3.5</td>
<td>3.9</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>18.0</td>
<td>18.1</td>
</tr>
<tr>
<td>Proline</td>
<td>2.9b</td>
<td>3.3a,b</td>
</tr>
<tr>
<td>Glycine</td>
<td>5.0</td>
<td>4.9</td>
</tr>
<tr>
<td>Alanine</td>
<td>6.4</td>
<td>7.1</td>
</tr>
<tr>
<td>Cystine</td>
<td>3.0</td>
<td>2.1</td>
</tr>
<tr>
<td>Valine</td>
<td>5.4</td>
<td>5.7</td>
</tr>
<tr>
<td>Methionine</td>
<td>2.0a,b</td>
<td>2.5a</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>5.4</td>
<td>5.3</td>
</tr>
<tr>
<td>Leucine</td>
<td>6.6</td>
<td>6.6</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>4.1</td>
<td>4.5</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>4.5b</td>
<td>4.3b</td>
</tr>
<tr>
<td>Lysine</td>
<td>6.4b</td>
<td>7.5a,b</td>
</tr>
<tr>
<td>Histidine</td>
<td>1.3</td>
<td>1.6</td>
</tr>
<tr>
<td>Arginine</td>
<td>3.3</td>
<td>3.7</td>
</tr>
<tr>
<td>Diaminoethylphosphonic</td>
<td>.62</td>
<td>.54</td>
</tr>
<tr>
<td>Diaminopimelic</td>
<td>. .</td>
<td>. .</td>
</tr>
</tbody>
</table>

Essential amino acids\(^1\) 43.5 46.2 45.8 46.9 50.7 51.9 52.4 55.9
Total (% of dry matter) 33.6 37.7 40.4 38.9 23.7 23.4 27.6 19.1

\(^1\) Threonine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine and arginine.

\(a\) and \(b\) Protozoa treatment means within a row not sharing a common letter are significantly (P < .05) different.

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In the present experiments, neither DES nor diet influenced amino acid composition of rumen bacteria. These results support the previous conclusion that there is little difference for rumen bacterial protein composition even under widely differing dietary regimes (1, 5, 33). A constant amino acid composition was observed with 22 strains representing nine species of rumen bacteria grown in pure culture and in a nonselective medium (33). Nitrogen content of bacteria may be varied, but the range of amino acid composition is small (33).

The experimental diets did not affect amino acid composition of the protozoal fraction except for lysine, proline, phenylalanine, and methionine content (P<.05) (Table 3). The methionine content of protozoa from cows fed the DES semipurified diet was higher (P<.01) than that of protozoa from cows receiving the conventional diet. Lysine, phenylalanine, and proline were significantly higher for protozoa from rumen contents of cows fed DES conventional diets than those from cows fed the semipurified diet without DES. Differences in amino acid composition would be expected if protozoa have varying amino acid composition as the diet and DES influenced population and number of ciliate protozoa (24). However, some workers (5, 32) reported no effect of diet on protozoal amino acid content. The low net protein utilization of bacterial protein (60%) compared with protozoal protein (73%) obtained by McNaught et al. (31) suggests that proportion changes in bacterial and protozoal protein fractions will change the quantity and quality of protein available to the host.

Microbial amino acid synthesis was estimated in rumen digesta with diaminoethylphosphonic and diaminopimelic acids as markers for protozoa and bacteria. The experimental diets affected (P>.05) neither the diaminoethylphosphonic acid content of protozoa nor the diaminopimelic content of bacteria (Table 3). However, values for individual animals and periods for AEP and DAP were used for estimating microbial amino acid fractions. Protozoal amino acids appeared to make up a larger fraction of the total amino acids than bacterial amino acids when DES was included in experimental diets. However, the quantitative relationship between protozoa and bacteria is one of the questions in rumen microbiology. The number of bacteria in control faunated animals is less when compared with those in nonfaunated animals (10, 15).

Microbial amino acid fractions were greater (P<.05) in rumen digesta of cows fed the semipurified diet compared with those fed conventional diet when DES was not included in either diet. The difference was not significant (P>.05) when DES was included; however, the same trend was noted. The inclusion of DES in experimental diets increased (P<.01) the protozoal amino acid fraction in rumen digesta from 31.5 to 67.5% for cows receiving the semipurified diet and from 24.2 to 56.7% for cows receiving the conventional diet. There was no effect (P>.05) of inclusion of DES in experimental diets on the bacterial amino acid fraction, but the trend was for a decreased proportion from bacteria with DES diets. The extent of microbial amino acid contribution to total amino acid in the rumen digesta ranged from 54.4 to 91.8%. These results are similar to those by different technics (29, 30, 35). Smith (35) estimated that about 70% of total nonammonia nitrogen was microbial in calf ruminal digesta. Microbial nitrogen represented 41.1 to 51.6% of total ingesta nitrogen in sheep, based on microbial polynucleotide nitrogen (16). Ely et al. (18) reported that 26

### Table 4. Fractions of amino acids in the rumen digesta of dairy cows fed the experimental diets.

<table>
<thead>
<tr>
<th>Amino acids:</th>
<th>Semipurified</th>
<th>Conventional</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No DES</td>
<td>DES</td>
</tr>
<tr>
<td></td>
<td>1 2</td>
<td>1 2</td>
</tr>
<tr>
<td>Rumen digesta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protozoal</td>
<td>15.2Bb</td>
<td>100.0</td>
</tr>
<tr>
<td>Bacterial</td>
<td>4.8</td>
<td>31.5B</td>
</tr>
<tr>
<td>Microbial</td>
<td>4.7</td>
<td>30.9</td>
</tr>
<tr>
<td>Unspecified</td>
<td>9.5</td>
<td>62.4Ba</td>
</tr>
</tbody>
</table>

| Amino acids content as a percentage of dry matter of rumen digesta. |
| Amino acids as a percentage of total amino acids of rumen digesta. |

3 Degraded and residual dietary protein; microbial and tissue debris; peptides, free amino acid, etc.

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Table 5. Estimated amino acid passing into the lower gut daily.

<table>
<thead>
<tr>
<th>Amino acids:</th>
<th>Semipurified</th>
<th>Conventional</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No DES</td>
<td>DES</td>
</tr>
<tr>
<td>Protozoal (g)</td>
<td>278</td>
<td>628</td>
</tr>
<tr>
<td>Bacterial (g)</td>
<td>273</td>
<td>232</td>
</tr>
<tr>
<td>Microbial (g)</td>
<td>551</td>
<td>860</td>
</tr>
<tr>
<td>Unspecified (g)</td>
<td>331</td>
<td>77</td>
</tr>
<tr>
<td>Total (g)</td>
<td>882</td>
<td>937</td>
</tr>
<tr>
<td>N/diet a</td>
<td>67</td>
<td>71</td>
</tr>
<tr>
<td>Total/dietary b</td>
<td>882</td>
<td>937</td>
</tr>
</tbody>
</table>

*Total amino acid nitrogen passing to the lower gastrointestinal tract as a percentage of dietary nitrogen (dietary crude protein was 13.2 and 13.4% for semipurified and conventional diets).

Protein synthesized by rumen organisms appears to be a main source of amino acids for the host. The conclusion is true when either nonprotein nitrogen or protein nitrogen make up a large proportion of dietary nitrogen. Therefore, the host protein synthesis depends to some degree upon the magnitude of microbial protein synthesis in the rumen.

Acknowledgment

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References

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