Microbial Fermentation of Rice Straw: Nutritive Composition and In Vitro Digestibility of the Fermentation Products

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Rice straw was fermented with Cellulomonas sp. and Alcaligenes faecalis. Microbial cells and undigested residue, as well as chemically treated (NaOH or NH₄OH) and untreated straws, were analyzed for nutrient composition and in vitro digestibility. In a typical fermentation, 75% of the rice straw substrate was digested, and 18.6% of the total substrate weight that disappeared was recovered as microbial protein. The microbial cell fraction was 37% protein and 5% crude fiber; the residue was 12% protein and 45% crude fiber. The microbial protein amino acid profile was similar to alfalfa, except for less cysteine. The microbial cells had more thiamine and less niacin than Torula yeast. In vitro digestibility of the microbial protein was 41.2 to 55%; that of cellulose was 52%.

The major limitations of straw as an animal feed are low digestibility and low protein content. Efforts have been made to increase the feed value of cereal straws by chemical and physical treatments, as well as nutrient supplementation. The digestibility of various crop straws can be increased by treating with NaOH or NH₄OH, but the low protein still requires nitrogen supplementation. Additions of urea, molasses, branched-chain fatty acids, sulfur, and other minerals have met with varying success (2, 4, 5, 6, 12, 17).

Recently efforts have been made to produce high-protein animal feed by microbial fermentation of cellulosic substrates (9, 10). We now report the nutritive composition and in vitro digestibility of the products of microbial fermentation of rice straw.

MATERIALS AND METHODS

Microorganisms, media, and growth. A species of Cellulomonas and Alcaligenes faecalis, isolated by the author, were used. Their cultural characteristics and conditions for growth have been reported elsewhere (9, 10). The growth medium contained (NH₄)₂SO₄ (6.0 g); KH₂PO₄ (1.0 g); K₂HPO₄ (1.0 g); MgSO₄ (0.1 g); CaCl₂ (0.1 g); yeast extract (0.5 g); FeCl₃·6H₂O (16.7 mg); ZnSO₄·7H₂O (0.18 mg); CuSO₄·5H₂O (0.16 mg); CaCl₂ (0.18 mg); ethylenediaminetetraacetic acid (20.1 mg); and 10 to 50 g of cellulosic substrate per liter of distilled water.

Substrate. California rice straw (Oryza sativa Linnaeus), field-dried and chopped to pass through a 7-mm screen, was used as a substrate. Part of the chopped straw was treated with 4% NaOH solution (10:1, water-straw) at 100°C for 15 min, the excess caustic liquid was expressed, and the treated straw was oven-dried (60°C). The other part of the straw was sprayed with aqueous ammonia (5 N NH₄OH) in the amount of 5.2% of the substrate, and kept at ambient temperature for 30 days before being subjected to microbial fermentation (20).

Fermentation. Fermentations were carried out at 35°C in 40-liter Humford fermentors (13). The fermentation mixture was agitated (140 rpm) and aerated (0.5 vol of air/vol of medium per min) with sterilized air. The medium (20 liters) contained 3 to 5% (dry weight) of rice straw. Separately grown active cultures of Cellulomonas sp. and A. faecalis were inoculated. Fermentation continued for 3 to 5 days.

In vitro digestibility. In vitro cellulose digestibility was determined by measuring disappearance of substrate insoluble dry matter upon treatment with cellulase ("Onozuka" SS, Kanezawa-Hosho, Ltd, New York) and protease ("Pronase" B grade, Calbiochem, Los Angeles, Calif.), and was expressed as total solubles after enzymes. In vitro protein digestibility was defined as the decrease in substrate nitrogen after treatment with fungal protease (Streptomyces griseus) and chick pancreas acetone powder (18).

Rumen metabolism. Rumen microbes were obtained from a fistulated sheep that was maintained on alfalfa hay with free access to dried range grass. Susceptibility of substrates to rumen microbial attack was determined by measuring total gas and volatile fatty acid (VFA) production. VFA samples acidified with meta-phosphoric acid were analyzed on an Aerograph 204 gas liquid chromatograph. The rate of ruminal fermentation was measured by anaerobic Warburg procedures and described by Oh et al. (17).

Vitamin, amino acids, and chemical analysis.
All vitamin assays were microbiological: thiamine and niacin by the methods of Gyorgy and Pearson (8), folic acid by the method of Jukes (14), and pyridoxine by the Association of Official Analytical Chemists (1). Amino acids were analyzed by ion-exchange chromatography after 10 mg of protein was hydrolyzed with 10 ml of 6 N HCl under vacuum at 110°C for 24 h. Cystine plus cysteine was determined after pretreatment with performic acid. Lignin was determined according to Van Soest (19). Protein was calculated by multiplying the difference between total nitrogen and NH₃-nitrogen by 6.25. All other chemical analyses, unless otherwise specified, were carried out according to the Association of Official Analytical Chemists methods (1).

RESULTS AND DISCUSSION

When a mixed culture of Cellulomonas sp. and A. faecalis was grown on rice straw, substrate utilized depended on the initial substrate level, inoculum size, fermentation time, and substrate pretreatment. Figure 1 shows material balance of a typical fermentation of NaOH-treated straw. After 3 days, 75% of the initial substrate had been digested, 150 g of undigested fermentation residue remained, and 178 g of microbial cell precipitate had been produced. The residue fraction (mainly undigested straw plus some microbial cells) was 12% protein and the microbial cell fraction (mainly microbial cells, a small amount of fine fibers, and precipitated minerals) was 37% protein. Thus, 84 g of protein (18 g in the residue and 66 g in the cells) was obtained by digesting 450 g of the initial 600 g of rice straw, a net protein yield of 18.6%.

Table 1 lists the chemical composition of the rice straw substrates and products of their microbial fermentation. The untreated rice straw contained 0.67% nitrogen, 29.8% crude fiber, and 15.8% silica that accounted for most of the 18.6% ash. NaOH treatment removed about half of the silica, probably by solubilization of the amorphous silica. It also removed the reducing sugars. The ammonia treatment increased both total and NH₃-nitrogen, possibly by formation of ammonium salts and amides of cellulose. Ammonia treatment, however, removed silica less than NaOH.

The undigested fermentation residue was about 12% protein and 45% crude fiber. This level of protein was equivalent to that obtained
by urea (2.5%) supplementation of straw (6). The increased crude fiber in the residue may have been from preferential microbial utilization of readily digestible carbohydrates, leaving cellulose and lignin. Lignin in the residue increased from 4.3% to 9.2%. Thus, 53% of the initial lignin was recovered in the residue, and the rest was solubilized in the effluent. The digestibility lowering effect of lignin may be caused by its chemical complex formed with cellulose. Therefore, the lignin in the residue, which may be partially dissociated from the plant cell walls by mechanical and microbiological action during the fermentation, should not be as detrimental as the native lignin in the straw.

Ca, P, and S in the undigested residue were 2, 4, and 7 times, respectively, those in the untreated rice straw. These minerals are especially low in rice straw, and their increase could be an additional benefit from fermentation.

The microbial cell fraction was 38% crude protein, 5.7% crude fiber, 25% ash, and 0.9% crude fat. The high ash may be due to silica and salt accumulation during neutralization of alkali-treated substrates. Membrane filtration only reduced ash to 23.4% and silica to 15%. As a comparison, the same microbial species grown on Trypticase soy broth (BBL) contained 11.9% ash and 1% silica. Although silica is nutritionally inert, its abrasiveness may damage the digestive tract of animals. More than 50% of the silica in the filtrate of fermentation mixture could be separated from cells by allowing the mixture to stand for a few hours at room temperature.

Table 2 compares the amino acid composition of microbial and alfalfa protein. The essential amino acid profiles of Cellulomonas sp. and A. faecalis were similar, except for more methio-

| Component | Rice straw (untreated) | Rice straw (NaOH treated) | Rice straw (NH₂OH treated) | Undigested residue* | Microbial cells* |
|-----------|------------------------|---------------------------|----------------------------|---------------------|------------------|
| Total N   | 0.67                   | 0.52                      | 1.59                       | 2.30                | 6.65             |
| NH₄-N     | 0.03                   | 0.03                      | 0.43                       | 0.24                | 0.51             |
| Ether extract | 1.24              | 0.81                      | 1.03                       | 1.48                | 0.93             |
| Crude fiber | 29.8                | 32.5                      | 34.5                       | 45.0                | 5.71             |
| Lignin    | 3.72                   | 4.34                      | 4.96                       | 9.24                | 1.63             |
| Reducing sugar | 0.55              | 0.23                      | 0.15                       | 1.63                |                  |
| Ash       | 18.58                  | 21.84                     | 15.95                      | 20.69               | 25.09            |
| Silica    | 15.8                   | 9.9                       | 13.4                       | 16.9                | 19.9             |
| Ca        | 0.12                   | 0.19                      | 0.19                       | 0.21                | 1.37             |
| P         | 0.10                   | 0.10                      | 0.07                       | 0.41                | 0.98             |
| S         | 0.14                   | 0.08                      | 0.08                       | 1.02                | 0.43             |

*From fermentation of NaOH-treated straw.

| Amino acid | Cellulomonas* | Alcaligenes* | Mixed culture* | Alfalfa* |
|------------|---------------|--------------|----------------|---------|
| Lysine     | 8.00          | 9.92         | 6.62           | 6.70    |
| Histidine  | 2.96          | 2.53         | 2.13           | 2.53    |
| Arginine   | 6.18          | 4.85         | 6.82           | 5.54    |
| Aspartic acid | 8.30      | 9.15         | 10.67          | 12.54   |
| Threonine  | 4.73          | 4.46         | 5.28           | 5.12    |
| Serine     | 4.11          | 3.44         | 3.75           | 5.25    |
| Glutamic acid | 18.49      | 17.08        | 14.47          | 11.30   |
| Proline    | 7.51          | 4.46         | 4.71           | 5.10    |
| Glycine    | 4.17          | 5.16         | 6.51           | 5.73    |
| Alanine    | 8.12          | 8.94         | 11.07          | 6.33    |
| Valine     | 6.79          | 7.01         | 7.15           | 6.70    |
| Methionine | 1.69          | 2.70         | 1.45           | 1.96    |
| Isoleucine | 4.12          | 5.40         | 4.11           | 5.54    |
| Leucine    | 8.66          | 7.64         | 8.70           | 8.43    |
| Tyrosine   | 2.41          | 3.06         | 2.98           | 3.72    |
| Phenylalanine | 3.69      | 4.11         | 3.76           | 5.75    |
| Cystine/cysteine | 0.41 | 0.47         | 0.54           | 1.40    |

* Cultures of Cellulomonas sp. and A. faecalis were grown on Trypticase soy broth.
* Mixed culture of Cellulomonas sp. and A. faecalis grown on rice straw.
* Data from Livingston et al. (15) was converted to g/100 g protein base.

| Vitamin | Microbial cells* | Torula yeast* |
|---------|------------------|--------------|
| Folic acid | 20.0             | 21.5         |
| Thiamine  | 36.0             | 5.3          |
| Niacin    | 222.0            | 417.3        |
| Pyridoxin | 35.4             | 33.4         |

* Mixed culture of Cellulomonas sp. and A. faecalis grown on rice straw.
* Bressani (3).
nine in the latter. Organisms grown on Trypti-
case soy broth contained a higher essential
amino acid content than those grown on the
straw-mineral solution. The amino acid profile
of the mixed culture grown on rice straw was
similar to that of alfalfa, except for less cyste-
ine. Eighty percent of total nitrogen in mi-
crobial cells was recovered as protein nitrogen;
the rest was the nucleic acids and other nitroge-
nous compounds.

Table 3 compares the vitamins in microbial
cells grown on straw with Torula yeast (Candida
utilis). The microbial cells contained more thi-
amine and less niacin than yeast. Folic acid and
pyridoxin in the microbial cells were equivalent
to Torula yeast. Thus, microbial cells from
straw fermentation may provide vitamins as well
as protein.

The in vitro digestibility of protein in the
fermentation products ranged from 41% to 55%
(Table 4). This relatively low protein digestibil-
ity may be attributed to microbial cell walls,
since Yang (21) has shown that sonication of
microbial cells improved their protein utiliza-
tion by animals. Crude fiber digestibility (total
solubles after enzymes) was 30% for raw rice
straw, 73% for NaOH-treated straw, and 57% for
NH₄OH-treated straw. Some of the digestible
matter in straw was apparently used by micro-
organisms for protein production. However,
enough digestible matter (52% total solubles
after enzymes) was left in the fermentation
residue to warrant its potential use as a rumin-
ant feed.

Total in vitro gas production for the fer-
mentation residue was lower than that for untreated
straw and alkali-treated straw (Table 5). VFA
production, however, paralleled in vitro di-
gestibility; the fermentation residue produced
more VFA than untreated rice straw but less than
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straw and alkali-treated straw (Table 5). VFA
production, however, paralleled in vitro di-
gestibility; the fermentation residue produced
more VFA than untreated rice straw but less than
alkali-treated straw. None of the straw or

products from straw fermentation produced as
much gas or VFA as alfalfa.

Thus, the laboratory data indicate that the
microbial cell fraction, with its high protein
content, may be used as a protein source for the
nonruminants, and the fermentation residue for
the ruminants.

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the rumen metabolism study; A. C. Weiss, who supplied
NH₄-treated straw; and the members of Field Crops and
Chemical Physics Laboratories of WRRL, ARS, who per-
formed the chemical analyses.

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| Table 4. In vitro digestibility of the fermentation products |
|-----------------------------------------------|
| Product                | Protein Content (%) | Digestibility (%) | Crude fiber Content (%) | Digestibility (%) |
|------------------------|---------------------|-------------------|-------------------------|-------------------|
| Rice straw             | 4.2                 | 29.8              | 30.3 ± 0.50             |
| NaOH-treated straw     | 3.1                 | 32.5              | 73.2 ± 0.27             |
| NH₄OH-treated straw    | 7.2                 | 34.5              | 57.4 ± 0.41             |
| Fermentation residue   | 12.8                | 55.5 ± 0.69       | 51.7 ± 0.44             |
| Microbial cells        | 38.2                | 41.6 ± 0.25       | 5.7                     |

*Percentage of dry matter.
*Means and standard deviation.
*From fermentation of NaOH-treated straw.

| Table 5. Ruminal metabolism of the products |
|-----------------------------------------------|
| Product                     | Gas production (mmol/8 h/10 g of rumen content) | Net VFA production (mg/100 ml) |
|-----------------------------|-----------------------------------------------|-------------------------------|
| Rice straw                  | 0.799                                         | 207                           |
| NaOH-treated straw          | 0.977                                         | 378                           |
| NH₄OH-treated straw         | 0.885                                         | 345                           |
| Fermentation residue        | 0.579                                         | 312                           |
| Control (alfalfa)           | 1.307                                         | 409                           |

*From fermentation of NaOH-treated straw.

Thus, the laboratory data indicate that the
microbial cell fraction, with its high protein
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