Ammoniated Rice Straw and Rice Hulls and Rumen Microbial Degradation Investigated by Scanning Electron Microscopy

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Abstract Rice straw (RS), rice hulls (RH) and ammoniated RS and RH, which were treated with 2.5%, 5.0% and 10.0% of ammonia (by wt.) at 45°C for 3 days, were incubated with rumen liquor and artificial saliva for 0, 6, 12, 24 and 48 hours. These specimens were observed by a scanning electron microscope to clarify the structural changes caused by ammonia treatment and bacterial degradation. No remarkable changes in structure were found in the 2.5% ammonia-treated RS except for a slight shrinkage in vascular bundle sheath. Cells of the vascular bundle sheath and phloem were collapsed by 5% ammonia treatment. In the 10% ammonia-treated RS, the outer layer, consisted of epidermis, small bundle sheath and sclerenchyma, was thinned off and most of parenchyma was removed. A less structural change of the original RS was observed during the 6-hr and 12-hr incubation period. Microbial digestion of cell content, especially starch granules, and of cell wall of parenchyma was apparently promoted by the increase in ammonia concentration for treatment and by the incubation time. The decrease in thickness of sclerenchymal cell wall observed in both the ammoniated and incubated RS indicated an abundant supply of digestible materials from this cell. In rice hulls, the structural changes were emphasized at the high ammonia concentration. The thinned sclerenchymal cell wall and the disappearance of parenchyma was observed in the 10% ammonia-treated RH. After 48-hr incubation of the original RH, most of the parenchyma was digested and some of the sclerenchymal cells were collapsed. Microbial degradation of RH was promoted by the increase in ammonia concentration and incubation time. It was observed in rice hulls that lignin and silica were the main factors that impede the structural changes caused by ammonia treatment and microbial degradation.

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The low digestibility of fibrous materials in rice straw and rice hulls is probably related to the extent of lignification of the cell wall constituents and the high content of silicon accumulated in rice plant. These low quality roughages have been treated with ammonia as one of the chemical reagents with the intention of increasing their nutritive values¹⁻⁴. In our previous reports⁴⁻⁷, it was suggested that the improvement of the nutritive values of rice straw and hulls with ammonia treatment is associated with the increased bacterial digestion of fibrous material that results from structural changes of cell wall, in addition to the increase in both the nitrogen content and the bacterial degradation of the cell contents. It is speculated that the increase

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in *in vitro* dry matter disappearance in the ammoniated materials is caused by the enhanced bacterial degradation of fibrous materials brought about by the damage or elimination of undigestive materials such as lignin, silica, cutin and wax.

Numerous studies have been reported on ultrastructural characteristics of plant tissue and rumen bacterial degradation of forage cell walls using the electron microscopes, and AKIN⁸⁾ recently reviewed these problems. However, no investigations using the scanning electron microscope have been conducted yet on the ammoniated roughages and bacterial digestion of these materials. The objectives of this work were to investigate the effect of ammonia treatment on rice straw and hulls and to compare the bacterial digestion of these intact and treated materials by the use of a scanning electron microscope.

Materials and Methods

Rice straw (RS) and rice hulls (RH) were obtained from mid-season rice plant. This plant was seeded in middle-May and harvested in early October, and at the harvest time the leaves and stems demonstrated slightly greenish color. Rice was dehulled late in October after having been dried at 50°C for 12 hours. The internodes of the midportion of the straw cut into about 2 cm and RH were treated with 0, 2.5, 5.0 and 10.0% of ammonia (by wt.) at 45°C for 3 days, which was a modification of the method described by Tohrai *et al.*⁹⁾. These materials (0.5 g) were placed in a flask with rumen liquor (10 ml) and artificial saliva (20 ml) and then continuously bubbled with CO₂ at 39°C for 0, 6, 12, 24 and 48 hours⁵⁾.

Specimens were prepared for the scanning electron microscopy (SEM) by a modified method of AKIN and AMOS⁹⁾. The intact and incubated materials were cut into cross or longitudinal sections of 2 to 5 mm in length and were degassed in distilled water and then kept in a refrigerator to restore the structure to its original form for a few days. Thereafter sections were fixed in 4% buffered glutaraldehyde at 4°C for 3 days and postfixed in 1.5% buffered osmium tetroxide at 4°C for 4 hours. Those were blotted free of excess moisture and affixed to stubs of the SEM in a vertical position. The stubs were quickly dried at the critical point in dry ice-amyl acetate, coated in a vacuum evaporator with palladium alloy, and then observed in a field emission SEM (Hitachi Ltd., S 450) at about 25 kV. In addition to these samples, the neutral detergent fiber (NDF) and the acid detergent fiber (ADF) of RS and RH prepared according to the procedures of VAN SOEST and WINE¹⁰⁾ and VAN SOEST¹¹⁾, respectively, were also observed by SEM in the same manner to compare the structural changes between these fibrous materials and the bacterial degraded materials.

Results

Rice Straw and Ammoniated Rice Straw (ARS). A tissue construction model of transverse section of the intact rice straw internode is illustrated in Fig. 1, to compare with the extent of degradation in tissues by the treatment with ammonia

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Fig. 1. A tissue construction model of transverse section of the intact rice straw and rice hull.

or detergent solution and the rumen microbial digestion. The micrograph of RS (Fig. 2) shows that all cell walls of tissues are intact and have maintained structural integrity. Numerous globular single grains, which are confirmed as starch granules according to the histochemical method using the light microscope¹²⁾, are observed in most of the parenchyma and no other substrates are found as the cell content there. The cavity of sclerenchymal cell surrounding the small vascular bundle is not observed distinguishably. No remarkable change in structure is observed in the RS treated with 2.5% of ammonia (2.5% ARS) except a slight shrinkage in vascular bundle sheath compared to that in the intact RS. In the 5% ammoniated RS (Fig. 3), cells of the vascular bundle sheath and phloem are collapsed although the other cells are structurally intact. Micrograph of ARS treated with 10% ammonia (Fig. 4) shows a severe destruction, and the outer layer, consists of epidermis, small bundle sheath and sclerenchyma, is thinned off giving it the appearance of being too fragile. Although the cells of the inner vascular bundle are kept in a distorted and swelled form, most of the parenchyma are completely removed. No starch granules are found in these transverse sections, but the longitudinal section of the 10% ARS (Fig. 5) shows that unless cell wall is broken down, thereby giving them their globular or oval shape numerous starch granules fill cells of parenchyma. The neutral detergent fiber of RS (Fig. 6) shows a slight expansion of the whole structure and a decrease in cell wall thickness especially in the sclerenchyma. All tissues, particularly the inner vascular bundle and parenchyma, in acid detergent fiber of RS (Fig. 7) have completely collapsed.

Rumen Microbial Digestion of RS and ARS. A less structural change is observed on the micrographs of RS during the 6 and 12-hr incubation period. After the 24 hr incubation of RS, cell contents, mainly starch granules, were digested and cell walls were partially collapsed although the soft tissues were not removed from the cross section (Fig. 8). After the 48-hr incubation, the parenchyma were completely removed from the section and only the large vascular bundle sheath attached to the outer mechanical layer (Fig. 9). The thickness of the sclerenchymal cell begins to decrease after the 24-hr incubation. The 2.5% ARS is more digestible than RS

during the 48-hr incubation period and the inner layer including the vascular bundle has been completely removed from the outer layer (Fig. 10). The thickness of the cell walls of the sclerenchyma is decreased and shown to be the same as that of the intact parenchymal cell. In the 10% ARS, the large vascular bundle and the parenchyma have easily been collapsed after the 6-hr incubation similar to the micrograph in Fig. 10, and to what has happened to the small vascular bundle after 24 hours. It seems that the digestion is promoted during the shorter period of incubation depending upon the increase in ammonia concentration to treat the RS.

Rice Hulls and Ammoniated Rice Hulls (ARS). As shown in a tissue construction model of transverse section of the intact rice hulls (Fig. 1), the outer mechanical tissues consist of the row of hemispherical tubercular processes including sclerenchyma and are covered with heavy silicon layer. Inside the sclerenchymal tissue, the vascular bundle is surrounded with parenchyma and sclerenchyma. The remarkable changes, such as the deformation of silica layer, the thinness of sclerenchymal cell wall and the disappearance of parenchyma are observed in 10% ARH as shown in Fig. 11. The structural changes caused by ammonia treatment for RH are emphasized with the increase in ammonia concentration. The micrograph of RH-NDF (Fig. 12) indicates that none of the sclerenchyma are removed from the outer layer and some of the parenchyma, from the inner layer. In the acid detergent fiber of rice hulls (Fig. 13), the sclerenchyma which are covered with silica layer are removed and cell wall of the other cells are thinned off.

Rumen Microbial Digestion of RH and ARH. The rumen microbial digestion for rice hulls is lower than that in RS, and parenchyma is the chief digestible portion after the 12-hr incubation. After the 48-hr incubation of RH, some of the sclerenchymal cells in the process are collapsed, their cell walls are remarkably thinned off, and the tissues of the inner layer have been sloughed off from the section (Fig. 14). Microbial digestion of RH is increased by the ammonia treatment and promoted with the increase in ammonia concentration similar to that observed in the ARS. The

Explanation of Figures

Fig. 2. Intact rice straw. It has maintained structural integrity. Starch granules are found in the parenchyma.

Fig. 3. The 5% ammoniated RS. The cells of vascular bundle sheath and phloem are collapsed.

Fig. 4. The 10% ammoniated RS. It shows a severe destruction and most of parenchyma are completely removed.

Fig. 5. The longitudinal section of the 10% ammoniated RS. It shows numerous starch granules in parenchymal cells.

Fig. 6. NDF of RS. It shows a slightly expanded structure and decrease of cell wall thickness.

Fig. 7. ADF of RS. All tissues have completely collapsed.

Fig. 8. Cross section of intact RS incubated with rumen microbes for 24 hours. Cell contents are digested and cell walls are partially collapsed.

Fig. 9. Intact RH incubated for 48 hours. Parenchyma are completely removed and large vascular bundle sheath has attached to the outer mechanical layer.

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micrograph of the 10% ARH after the 12-hr incubation (Fig. 16) shows that microbial digestion takes place not only on the cross-section but also on the surface, particularly the lane between the rows of processes which are covered with silica layer (Fig. 15). Digestion of all the tissues including sclerenchyma is increased after the 48-hr incubation of 10% ARH (Fig. 17). Parenchyma and vascular bundle are swollen and cell wall constituents are digested.

Discussion

It has been observed by electron microscope¹³⁾ that the plastid of various organs and tissues of rice plant contains numerous starch grains and the process of accumulation and distribution of starch in each organ differs with its development of growth¹⁴⁾. In some tropical grasses, most starches are found in the chloroplasts of parenchyma bundle sheath cell¹⁵⁾. It is evident from the micrographs of the cross section of RS (Fig. 2) and the longitudinal section of 10% ARS (Fig. 5) that starch granules are exactly intact in parenchyma provided that the cell wall is not broken out and that ammonia treatment has less effect on them, even though the ammonia concentration is extremely high (10%). In spite of the comparatively low effects of ammonia on cell content in parenchyma, it has been known that NDF content in RS is decreased by the ammonia treatment^{4,5)}. It is assumed, therefore, that the decrease in NDF content is accompanied by a decrease in the thickness of sclerenchymal cell wall as observed in micrographs of ARS (Fig. 4) and NDF (Fig. 6).

It has been reported that starch¹⁶⁾ and other contents¹⁷⁾ in cell were completely degraded by rumen bacteria and protozoa. A similar observation is shown on the exposed starch granules in Fig. 8. Although the bacterial digestion of parenchymal cell wall which occurs prior to that of starch is comparatively low, it appears that the potential nutrients including starch in parenchymal cell are finally utilized by rumen microbes. The existence of starch in the rice straw which still keeps the slight green color may be profitable from the view point of ruminant nutrition.

Explanation of Figures

Fig. 10. The 2.5% ARS incubated with rumen microbes for 48 hours. The inner layer is completely removed from the outer layer and the thickness of cell walls of sclerenchyma are remarkably decreased.

Fig. 11. Cross section of the 10% ammoniated rice hull. It shows the deformation of silica layer, the thinness of sclerenchymal cell wall and the disappearance of parenchyma.

Fig. 12. NDF of rice hulls. Some of parenchyma are removed from the inner layer.

Fig. 13. ADF of RH. The sclerenchymal cells are removed and cell wall of the other cells are thinned off.

Fig. 14. Cross section of RH incubated with rumen microbes for 48 hours. Some parenchymal cells are collapsed, cell wall of these cells are thinned off and tissues of the inner layer are sloughed off.

Fig. 15. Surface of the intact RH. It is covered with silica layer.

Fig. 16. Surface of the 10% ammoniated RH incubated with rumen microbes for 12 hours. Microbial digestion takes place on the lane between the rows of processes.

Fig. 17. Cross section of 10% ARH incubated with rumen microbes for 48 hours. Sclerenchyma, parenchyma and vascular bundle are swollen and cell wall constituents are digested.

Microbial digestion of the parenchymal cell wall of RS was apparently promoted by the increase in ammonia concentration and by the incubation time. It has been stated in our previous report⁹⁾ that the higher dry matter digestibility observed in ARS as compared with that in RS is mainly derived from a significantly high DMD of NDF fraction in ARS. It is assumed, therefore, that the decrease in the thickness of the sclerenchyma's cell wall, caused by the increase in the ammonia concentration for treatment and by the prolonged incubation time, indicates the decrease in cell wall constituents of sclerenchyma. It further suggests that there is an abundant supply of the digestible material from sclerenchymal cell. KAWAMURA *et al.*¹⁸⁾ have reported, in the histochemical study on RS and their rumen microbial digestion, that the cell wall of parenchyma, which was not lignified, was readily digested and that the epidermis and mechanical tissues such as vascular bundle and sclerenchyma, containing silica and lignin, stopped the invasion of microbes into the inner tissues. In the study on delignified cell walls with SEM¹⁹⁾, it has been reported that while lignin does impede the digestion, the cell wall polysaccharides show marked differences in their rate and extent of digestion when lignin is not a barrier. Observations in this report on rice straw were in good agreement with the results of the other reports described above.

Silica is another main factor that impedes the digestibility of fibrous materials with lignin. In rice plant, silica is localized in the epidermis, vascular bundle plus bundle sheath, and the sclerenchyma linking them. Also it may play some important roles in regulating the rate of transpiration and the increased resistance of rice plant to some diseases and insect pests²⁰⁻²²⁾. It has been known that particles of formed silica gel fill up intraspaces of cellulose micelle of the epidermal cell and make up a silica cellulose membrane²¹⁾. Silica in rice hulls is mainly deposited in the space between the cuticle and the epidermal cells constituting a cuticle-silica double layer²⁰⁾. Silica content of hulls is particularly higher than that of any other portions and often reaches about 20% of dry matter^{20,22)}. It is considered that the quantities of lignin and silica in these materials affect the rate of microbial degradation. It seems that the higher contents of these undigestible materials in RH, as compared with those in RS, may give some unfavorable effects on the microbial digestion of RH.

The fact that the rate and extent of microbial digestion in both RS and RH are promoted by the ammonia treatment and further increased by the higher concentration of ammonia, is apparently shown in the micrographs and confirmed with IVDMD values previously reported⁵⁾. It is clearly shown, therefore, that the damages and elimination of the rigid structure of non-nutritive materials such as lignin and silica are an important role of ammonia treatment and that bacteria readily invade into the cells or tissues through the spaces provided by the treatment.

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References

- 1) WHITE, T.W., J. Anim. Sci., **25**: 25-28. 1966.
- 2) GUGGOLZ, J., G.M. McDONALD, H.G. WALKER, Jr., A.H. BROWN, W.N. GARRETT and G.O. KOHLER, Proc. West Sec. Amer. Soc. Anim. Sci., **22**: 71-76. 1971.
- 3) WAISS, A.C. Jr., J. GUGGOLZ, G.O. KOHLER, H.G. WALKER, Jr., and W.N. GARRETT, J. Anim. Sci., **35**: 109-112. 1972.
- 4) ITOH, H., Y. TERASHIMA, N. TOHRAI and Y. MATSUI, Jpn. J. Zootech. Sci., **46**: 87-93. 1975.
- 5) TOHRAI, N., Y. TERASHIMA and H. ITOH, Jpn. J. Zootech. Sci., **49**: 69-74. 1978.
- 6) ITOH, H., Y. TERASHIMA and N. TOHRAI, Jpn. J. Zootech. Sci., **50**: 54-61. 1979.
- 7) TOHRAI, N., Y. TERASHIMA and H. ITOH, Jpn. J. Zootech. Sci., **50**: 189-194. 1979.
- 8) D.E. AKIN, J. Anim. Sci., **48**: 701-710. 1979.
- 9) D.E. AKIN and H.E. AMOS, Appl. Microbiol., **29**: 692-701. 1975.
- 10) VAN SOEST, P.J. and R.H. WHINE, Assoc. Off. Analytical Chem. J., **50**: 50-55. 1967.
- 11) VAN SOEST, P.J., Assoc. Off. Analytical Chem. J., **46**: 829-835. 1963.
- 12) CHANG, W.W.L. and C.P. LEBLOND, Am. J. Anat., **131**: 73-100. 1971.
- 13) SATO, K. and Y. EHARA, Proc. Crop. Sci. Soc. of Japan, **43**: 111-122. 1974.
- 14) TOGARI, Y. and K. SATO, Proc. Crop. Sci. Soc. of Japan, **22**: 98-99. 1954.
- 15) AKIN, D.E. and D. BURDICK, Crop. Sci., **17**: 529-533. 1977.
- 16) DEKKER, R.F.H., G.N. RICHARDS and M.J. PLAYNE, Carbohydr. Res., **22**: 173-185. 1972.
- 17) VAN SOEST, P. J., J. Anim. Sci., **26**: 119-128. 1967.
- 18) KAWAMURA, O., T. SENSU, M. HORIGUCHI and T. MATSUMOTO, Tohoku J. Agric. Res., **24**: 183-191. 1973.
- 19) BARTON, F.E., II and D.E. AKIN, J. Agric. Food Chem., **25**: 1299-1303. 1977.
- 20) YOSHIDA, S., Y. OHNISHI and K. KITAGAKI, Soil Sci. Plant Nutr. (Tokyo), **8**: 1-5. 1962.
- 21) YOSHIDA, S., Y. OHNISHI and K. KITAGAKI, Soil Sci. Plant Nutr. (Tokyo), **8**: 30-35. 1962.
- 22) YOSHIDA, S., Y. OHNISHI and K. KITAGAKI, Soil Sci. Plant Nutr. (Tokyo), **8**: 36-41. 1962.

走査電子顕微鏡によるアンモニア処理稲わらおよびもみ殻 ならびにルーメン微生物による消化過程の観察

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稲わらともみ殻およびそのアンモニア処理（2.5，5.0 および 10.0% NH_3 ，重量比）した材料をルーメン液と人工唾液の混合液（1：2）で 0，6，12，24 および 48 時間培養した。これらの試料における組織構造の変化を走査電子顕微鏡で観察した。2.5% のアンモニア処理では，稲わらの組織構造に著しい変化は見られなかった。維管束鞘および師管部の細胞は 5% アンモニア処理によって崩壊した。10% 処理によって外層の機械組織は薄くなり，柔組織の大部分は消失した。無処理稲わらの組織構造は 12 時間までの培養でほとんど変化しなかった。柔組織細胞内のデンプン粒およびその細胞壁の微生物消化は処理アンモニア濃度および培養時間の増加によって

促進された。厚膜細胞の厚みはアンモニア処理ならびに培養によって減少し，この細胞から可消化成分が供給されることを示した。もみ殻は 10% アンモニア処理によって厚膜細胞はもろくなり柔組織の一部は消失した。無処理もみ殻は 48 時間培養によって柔組織が消化され，厚膜細胞の一部が崩壊した。もみ殻の微生物消化もアンモニア濃度および培養時間の増加によって促進された。もみ殻ではリグニンおよびシリカの存在がアンモニア処理ならびに微生物消化による構造の変化を妨げているものと見られた。

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