

Histology of tissues and cell wall of rice straw influenced by treatment with different chemicals and rumen degradation*

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ABSTRACT

Rice straw (RS) was treated with urea, ammonium bicarbonate (NH_4HCO_3) and sodium hydroxide (NaOH), respectively. Treatments with urea, NH_4HCO_3 and NaOH increased the *in sacco* dry matter degradability of RS from 45.3 (untreated) to 52.5, 53.2 and 63.6%, respectively ($P < 0.01$). The untreated and treated RS stem samples were digested in the rumen of a Huzhou sheep for 12, 24, 48 and 72 h and examined for the degradation of tissues and cell walls by using a scanning electron microscope (SEM), and a transmission electron microscope (TEM). Parenchyma was slightly distorted by treatment with urea or NH_4HCO_3 , but severely distorted by NaOH treatment. The chemical treatments had little effect on other tissues of RS stem. All three treatments accelerated the degradation of parenchyma at 12 and 24 h, and NaOH treatment made the parenchyma degraded to most extent. Sclerenchyma cell wall in the untreated RS was slightly digested and still left partial secondary wall and complete middle lamella and primary wall even at 72 h, but NaOH-treated sclerenchyma cell wall began to be degraded at 48 h and just left the middle lamella and occasional primary wall at 72 h. All three treatments made the phloem absent at 48 h, but had little effect on the digestion of epidermis and xylem of vascular tissue. These results indicated that NaOH treatment had the best effects on the modification of structure of rice straw stems and the digestion of tissues and cell wall, and that the positive effects of NH_4HCO_3 treatment basically paralleled those of urea treatment. The improvement in digestion of histological structures was accordant entirely with the increase of *in sacco* degradability of RS after the three treatments. It is suggested that the histological methods by means of microscopical techniques such as SEM and TEM are conducive to a direct insight into the mechanism with which chemical treatments exerted the effects on the improvement of nutritive value of RS.

KEY WORDS: chemical treatment, rice straw, tissue, cell wall, electron microscopy, degradability

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INTRODUCTION

Rice straw (RS) is the largest by-product of the cereal crops in China, annual yield of which totals currently about 188×10^6 tonnes (Guo et al., 2002), and thus presents a great potential as a useful source of feed for ruminants. However, as feed, RS is poor in palatability and digestibility and is low in nitrogen content (Givens and Angela, 1995; Liu, 1995). Utilization of cell wall constituents of crop residues by rumen microbes is hindered by the presence of nonpolysaccharide compounds such as lignin, phenolic acids and silica in some cereal straws (Besle et al., 1994). Physical, chemical and biological treatments have been used for decades in upgrading and utilizing RS efficiently, and treatment with alkali compounds or ammonia is widely applied as the most successful chemical method (Liu et al., 1997; Chaudhry, 1998). Effects of chemical treatments on intake and digestibility of RS have been studied extensively, but the mechanism with which treatments improve the nutritive value of RS keeps unclear. Knowledge about changes in the histology of RS may help understand how different fractions, tissues and cell wall respond to chemical treatments (Xu et al., 1993; Shen et al., 1999).

Structural methods offer effective ways to evaluate digestion of specific cell types and have increased the understanding of the contribution of plant and rumen microbial factors in digestion of forages (Akin, 1989). In the present study, scanning electron microscope (SEM) and transmission electron microscopy (TEM) were used to observe histological changes of stem related to different chemical treatments before and after digestion in the rumen and to compare the effects of different treatments on degradation of RS structure.

MATERIAL AND METHODS

Plant material and its treatment

Rice (*Japonica*) straw, collected from Zhejiang University Experimental Farm (China) immediately after the rice harvest, was chopped to 3-5 cm, and treated with sodium hydroxide (NaOH, 4% of straw DM, w/w) for two days, urea (5% of straw DM, w/w) and ammonium bicarbonate (NH_4HCO_3 , 10% of straw DM, w/w) for 10 days in an airtight thermostated container (35°C), respectively (Liu et al., 1997). Before pretreatment, moisture content of the RS was adjusted to about 40%. Untreated RS was used as control.

Measurement of chemical composition and in sacco degradability of rice straw

Untreated and treated RS were ground to pass a 1mm sieve. The nitrogen content was determined according to AOAC (1990), and neutral detergent fibre (NDF) following the procedures by Van Soest et al. (1991), respectively. The *in sacco* procedure as described by Ørskov et al. (1980), was applied to determine the degradability of untreated and treated RS. About 3 g of samples were weighed into nylon bags (40 µm mesh, 7 cm × 10 cm inner size) and suspended in the rumen of three Huzhou sheep for 24 h. The sheep were fed twice daily on a diet containing 60% hay and 40% concentrate mixture. Upon removal from the rumen, the bags were immersed in cold water, gently washed by hand for 20 min, and then dried at 60°C for 48 h. For estimation of 0 h values, an additional set of bags was incubated in water at 39°C for 1 h, and washed as above.

Preparation of samples for microscopy

Digestion of plant material. The RS was separated into four parts: node, stem, blade and sheath. Stem materials were used for microscopy. Representative stem samples from untreated and treated materials were selected, cut in length of 5 mm, placed in nylon bags and then subjected to *in sacco* digestion in a rumen fistulated Huzhou sheep for 12, 24, 48 and 72 h, respectively. At the end of digestion, bags were gently washed by hand in cold water, and then the samples were immediately prepared for electron microscopy observation.

Scanning electron microscopy (SEM). Both undigested and digested stems were cut into small pieces (1 mm × 3 mm); then fixed in 0.1 mol/L phosphate buffer (pH 7.2) containing 2% (V/V) para formaldehyde and 2.5% (V/V) glutaraldehyde for 2 h at 4°C; rinsed three times in the same buffer; fixed with 1% osmic acid; rinsed three times in the same buffer; dehydrated in 70, 80, 90, 95 and 100% (V/V) ethanol, respectively; treated with a mixed solution of isoamyl acetate and ethanol (1:1, v/v) and with 100% isoamyl acetate; followed by “critical point dry procedure”; coated with platinum under vacuum (JEE-4X, JEOL Ltd., Japan), and finally observed with SEM (KYKY-1000B, KYKY Technology Development Ltd., China).

Transmission electron microscopy (TEM). Both undigested and digested stems were cut into small pieces (3 mm × 1 mm) and then treated with the same steps as above before being treated with a mixed solution of isoamyl acetate and ethanol. After dehydrating, these pieces were embedded at -20°C in Lowicryl K₄M according to the following procedure: 100% ethanol/resin (1:1, V/V) for 1 h, 100% ethanol/resin (1:2, V/V) for 1 h, pure resin for 1 h and then overnight. Samples were transferred to tubes filled with resin and polymerized under UV light for

72 h at -20°C , then for 24 h at room temperature. The blocks were sectioned on an ultra-microtome (Reichert-Jung ULTRACUT E). Ultra-thin sections were placed on nickel slot grids, and then viewed in TEM (Model JEM-1200EX, JEOL. Ltd., Japan).

RESULTS

Chemical composition and in sacco degradability of rice straw. Table 1 presents the chemical composition and *in sacco* degradability of RS at 24 h. The nitrogen content was increased due to treatments with urea and NH_4HCO_3 , but not influenced by NaOH treatment. Values for NDF were reduced by NaOH and urea treatments, but showed little response to NH_4HCO_3 treatment. *In sacco* degradability of dry matter of RS showed a great difference between the untreated and treated RS. The degradability was the highest for NaOH-treated RS ($P<0.01$), followed by urea- and NH_4HCO_3 -treated ($P<0.01$) and the lowest for untreated straw.

TABLE 1
Chemical composition and *in sacco* degradability of dry matter of rice straw

Indices	Rice straw treated by				SEM
	None	NaOH	Urea	NH_4HCO_3	
Dry matter, g kg^{-1}	950	933	933	938	-
Nitrogen, $\text{g kg}^{-1}\text{DM}$	17	16	28	23	-
Neutral detergent fibre, $\text{g kg}^{-1}\text{DM}$	700	664	683	698	-
<i>In sacco</i> 24 h DM degradability, %	45.3 ^C	63.6 ^A	52.5 ^B	53.2 ^B	0.86

^{A,B,C} means with different letters within the same row differ significantly ($P<0.01$)

Evaluation of stem tissues degradation by SEM. Histological changes of RS stem after different chemical treatments are shown in Figure 1. Intact epidermis, parenchyma, sclerenchyma and vascular bundles were clearly observed in the untreated stem, and vascular bundles included large and small bundles located in parenchyma and sclerenchyma, respectively (Figure 1A). The parenchyma in NaOH-treated stem was so distorted that there were only rudiments left for parenchyma (Figure 1B). Treatments with urea and NH_4HCO_3 resulted in a moderate and slight distortion of parenchyma, respectively (Figures 1C and 1D). However, neither of the treatments in this study had great effects on other tissues of RS stem before rumen digestion.

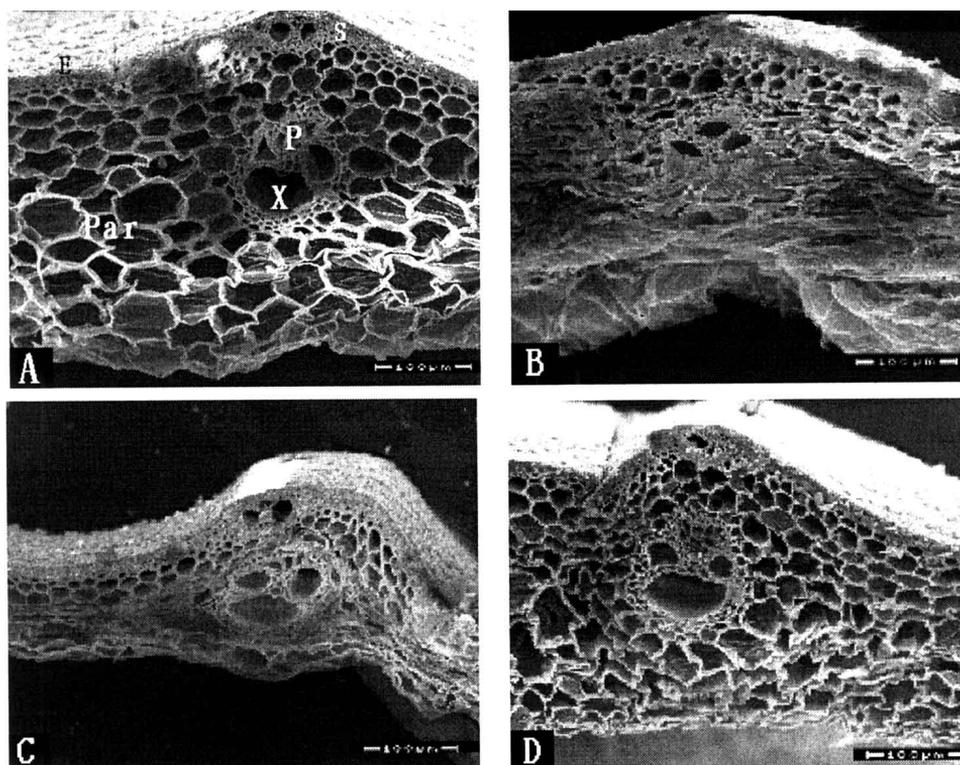


Figure 1. Histological changes of rice straw stem after different treatments. (A) In the untreated stem, epidermis (E), sclerenchyma (S), phloem (P) and xylem (X) of vascular tissue and parenchyma (Par) were intact. (B) NaOH-treated stem, showing that the whole parenchyma was severely collapsed. (C) After treatment with urea, stem parenchyma was moderately distorted. (D) NH_4HCO_3 -treated stem showing slight distortion of parenchyma (SEM \times 200)

At 24 h after *in sacco* degradation, untreated stem still maintained its general structure except for the partial degradation of parenchyma (Figure 2A). NaOH-treated stem showed slight remnants of parenchyma in occasional sites, a great distortion of large vascular bundles and degraded phloem (Figure 2B). In urea- or NH_4HCO_3 -treated stem, most of parenchyma was degraded, only a little of distorted parenchyma near the sclerenchyma was left and large vascular bundles appeared distorted (Figures 2C and 2D). After 48 h *in sacco* degradation, the detectable tissues by SEM were small vascular bundles, epidermis and sclerenchyma, because large vascular bundles had been broken off wholly due to the complete degradation of parenchyma (Figure 3). Untreated stem maintained the integrity of the small vascular bundle (including xylem and phloem), epidermis

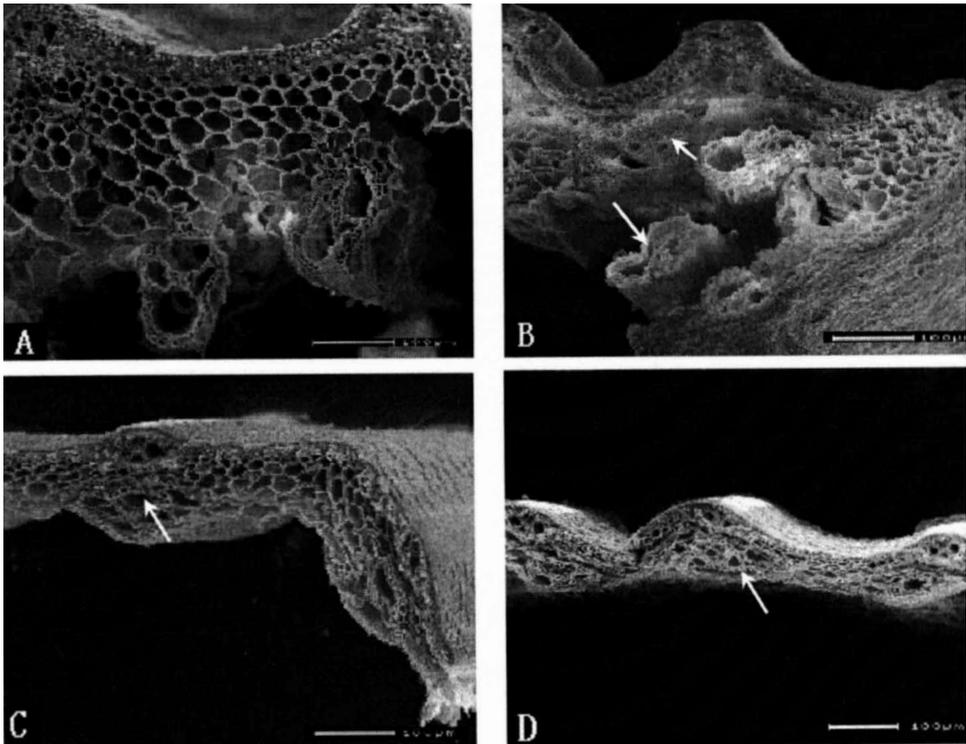


Figure 2. Histological changes of rice straw stem after 24 h *in sacco* degradation. In the untreated stem (A), partial parenchyma was degraded. (B) NaOH-treated stem showing slight remnants of parenchyma, slight degradation of phloem and great distortion of large vascular bundles (arrow). In the urea-treated (C) and NH_4HCO_3 -treated stem (D), only a little of distorted parenchyma nearby sclerenchyma was not degraded and large vascular bundles appeared distorted (arrow) (SEM \times 175)

and sclerenchyma (Figure 3A). NaOH-treated stem was at the beginning of degradation of sclerenchyma and showed the total loss of phloem, some cracked sclerenchyma cells and intact epidermis (Figure 3B). The phloem was also absent in urea- and NH_4HCO_3 -treated stems, but sclerenchyma and epidermis still kept indigestible (Figures 3C and 3D).

Evaluation of cell wall degradation by TEM. Cross sections of epidermis, parenchyma, sclerenchyma and vascular bundles were evaluated under TEM for cell wall degradation by rumen microorganisms. Untreated epidermal cells showed the thicker outer cell wall region and the thinner inner cell wall that is adjacent to sclerenchyma. Neither of the cell wall layers of untreated and treated epidermis was digestible, even if they were incubated in the rumen for 72 h.

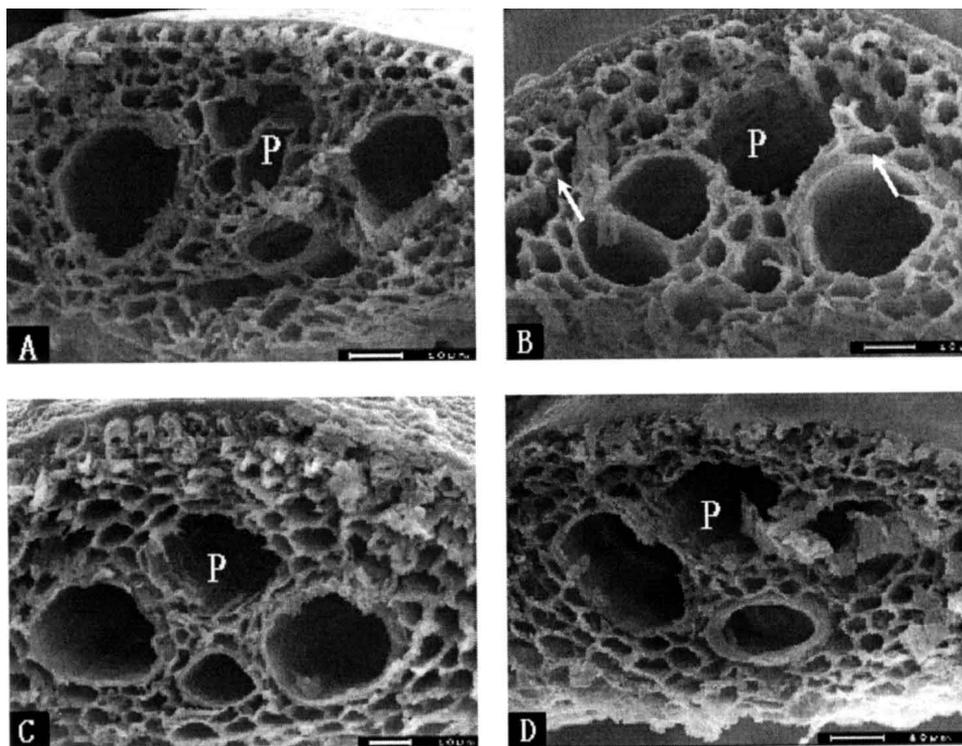


Figure 3. Histological changes of rice straw stem after 48 h rumen degradation. (A) Untreated stem showing the integrity of epidermis, sclerenchyma, xylem and phloem (P). (B) NaOH-treated stem showing total disappearance of phloem (P), a few cracked sclerenchyma cells (arrow) and intact epidermis. (C) Urea-treated and (D) NH_4HCO_3 -treated stem showing the absence of phloem (P) and the integrity of epidermis, sclerenchyma and xylem (SEM \times 1200)

Sclerenchyma of undigested and untreated stem showed the thickness and intactness of cell wall (Figure 4A). After 48 h rumen incubation, the cell wall of NaOH-treated sclerenchyma was colonized by microorganisms, and slight microbial degradation of the secondary wall occurred (Figure 4C), but the degradation was not found in both untreated and other treated sclerenchyma cell walls (Figures 4B and 4D). With further rumen digestion for 72 h, the secondary wall in the untreated sclerenchyma cells was sometimes slightly pitted along its edge (Figure 4E), but there was loss of considerable amounts of the secondary wall in treated sclerenchyma cells. Most of NaOH-treated sclerenchyma cell wall was much thinner than the urea-treated, and the former merely retained the middle lamella and occasional primary wall of cells (Figure 4F), but the

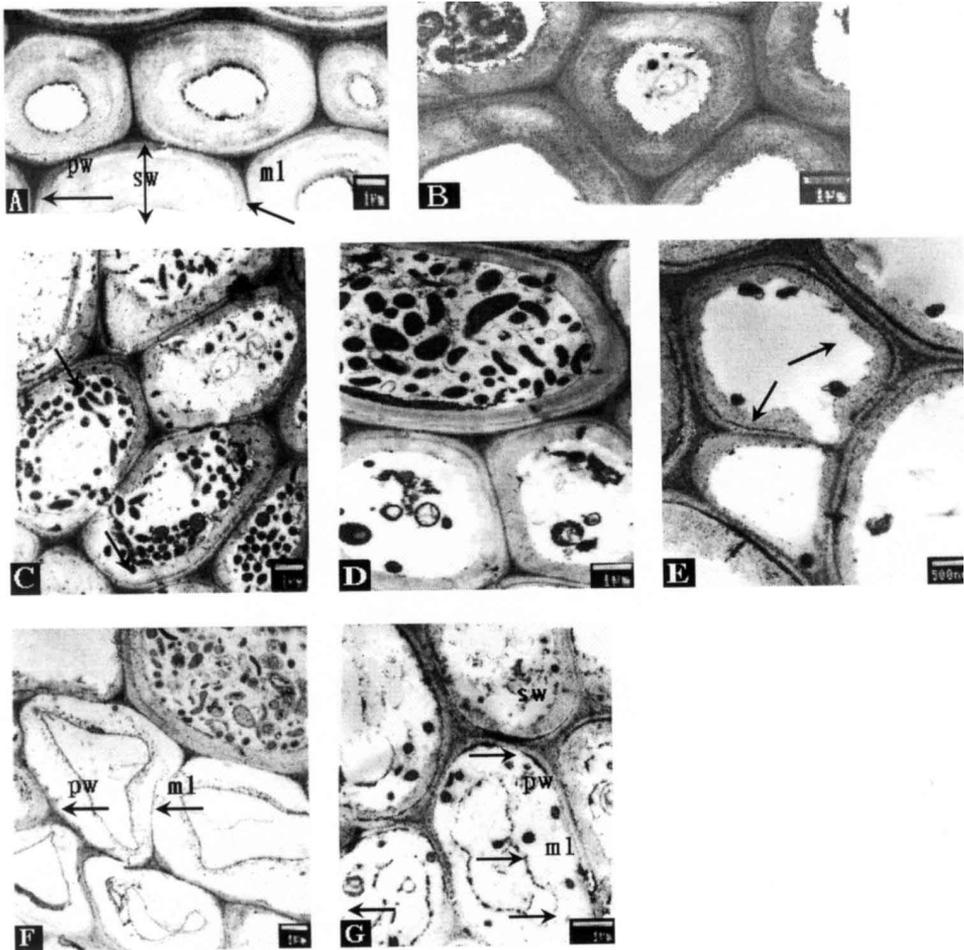


Figure 4. Sclerenchyma cells in rice straw stem. (A) Untreated cells before rumen digestion, showing the thickness of the whole cell wall and the intactness of middle lamella (ml), primary wall (pw) and secondary wall (sw) (TEM×5000). (B) After 48 h rumen digestion, few or no microorganisms appeared in the lumens and the intact cell wall of untreated stem (TEM×5000). (C) In NaOH-treated cells after 48 h rumen digestion, substantial microorganisms were visual in the lumens and the secondary wall began to be degraded (arrow) (TEM×5000). (D) Urea-treated cells after 48 h rumen digestion, showing no degradation of cell wall and an uneven distribution of microorganisms (TEM×5000). After 72 h rumen digestion, the secondary wall of the untreated cells was sometimes lightly pitted along its edge (arrow) (E) (TEM×12000); there merely left the middle lamella (ml) and occasional primary wall (pw) of sclerenchyma cells in the NaOH-treated stem (F) (TEM×4000); and there still left the secondary wall (sw) in partial cells, in addition to the middle lamella (ml) and primary wall (pw) of sclerenchyma cells in the urea-treated straw (G) (TEM×7500)

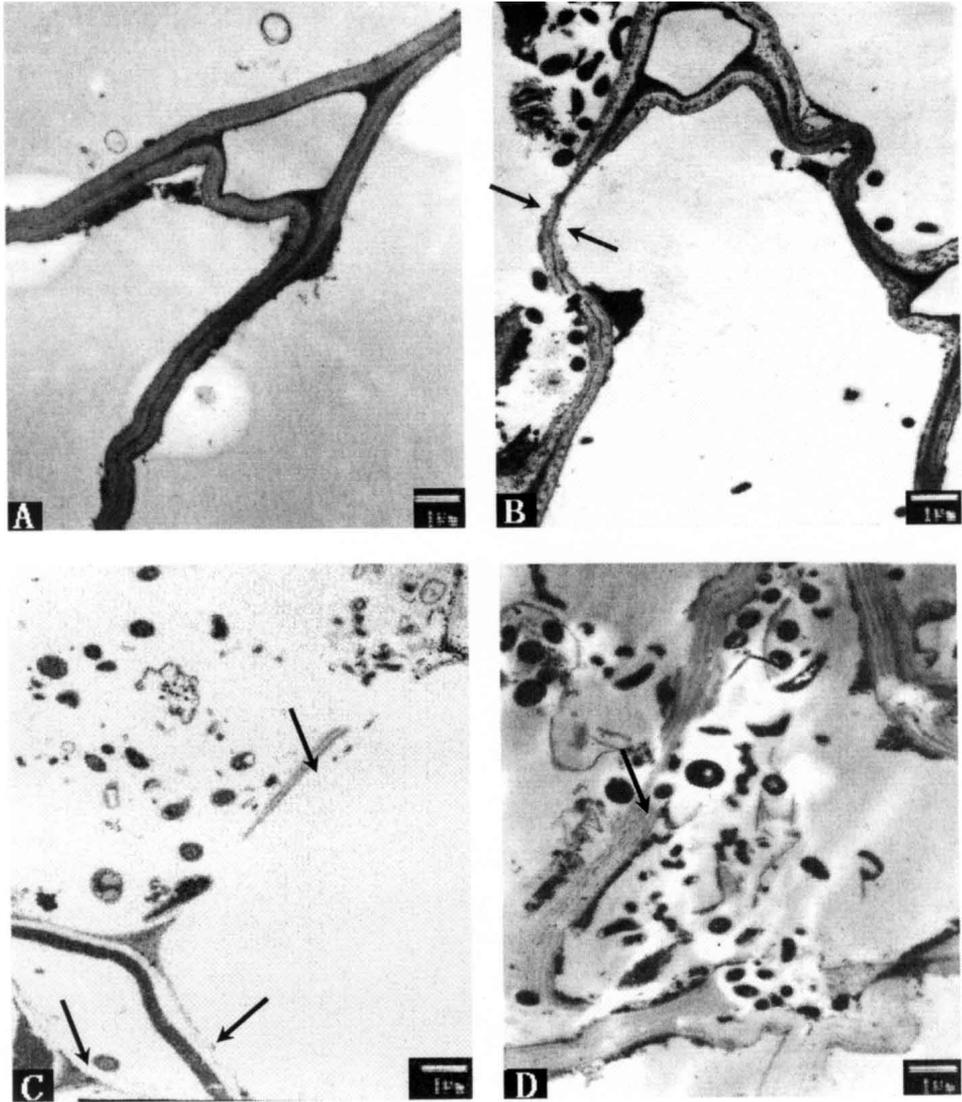


Figure 5. Ground parenchyma cell wall in rice straw stem. (A) Untreated cell wall before rumen digestion. (B) Untreated cell wall after 12 h rumen digestion, showing some rumen microorganisms near the cell wall and little evidence of removal in the cell wall except for in several occasional sites (arrow). (C) NaOH-treated cell wall after 12 h rumen digestion, showing that most components of parenchyma cell wall were removed leaving only in the middle lamella (arrow). (D) Urea-treated ground parenchyma cell wall after 12 h rumen digestion, showing the discontinuous degradation of cell wall (arrow) and the middle lamella in some areas (TEM×5000)

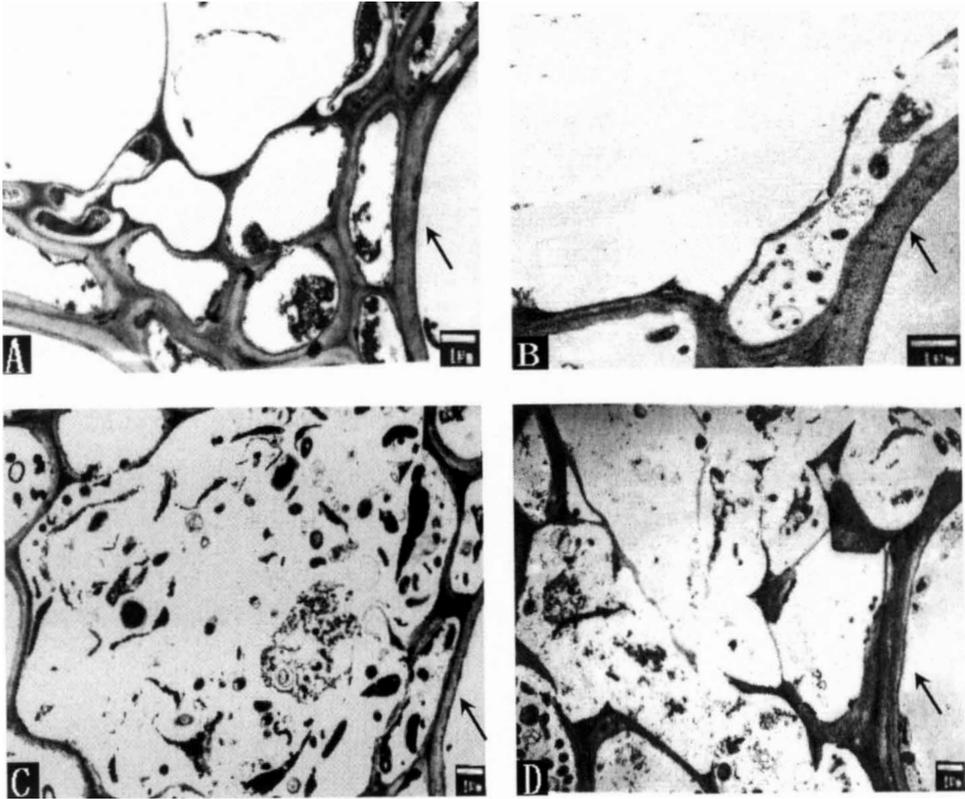


Figure 6. Phloem and xylem of small vascular tissues in the rice straw stem after 48 h rumen digestion. Arrow shows the undegraded xylem vessel wall. (A) Untreated phloem showing the structural integrity (TEM \times 5000). (B) NaOH-treated phloem, showing that phloem was completely cleared (TEM \times 5000). In NH_4HCO_3 -treated (C) and urea-treated phloem (D), there were numerous cell wall residues left (TEM \times 4000)

latter still left some secondary wall in partial sclerenchyma cells in addition to the middle lamella and primary wall (Figure 4G). The changes in NH_4HCO_3 -treated sclerenchyma cell wall were similar to those in the urea-treated (not presented).

Figure 5A showed the thin wall of parenchyma cells in untreated stem before digestion. After 12 h rumen incubation, the untreated ground parenchyma cells showed some microorganisms occurring near the cell wall but little evidence of removal of cell wall except for in several occasional sites (Figure 5B). In contrast, microorganisms degraded most of NaOH-treated ground parenchyma cell wall leaving a residue of the middle lamella (Figure 5C). The parenchyma cell wall

treated with urea was degraded discontinuously and left the middle lamella in some areas (Figure 5D), which was similar to the degradation of NH_4HCO_3 -treated stem. After prolonged rumen incubation for 24 and 48 h, all parenchyma tissues in both untreated and treated stems were extensively degraded up to complete removal (not presented), which confirmed the results obtained with SEM.

Untreated phloem cells of small vascular bundles kept intact structurally even after 48 h rumen digestion (Figure 6A) and presented the total loss after 72 h incubation (not presented). The chemical treatments were effective in improving the degradation rate of phloem. After 24 h rumen incubation, slight degradation occurred in NaOH-treated phloem cells (not presented). Rumen microorganisms completely cleared phloem cells in NaOH-treated stem (Figure 6B) and removed a most portion of phloem cells in urea- and NH_4HCO_3 -treated stems with numerous cell wall residues at 48 h incubation (Figures 6C and 6D).

DISCUSSION

Plant tissues may be classified into two groups based on their thickness of the cell wall, and tissues with thick lignified cell wall (sclerenchyma, vascular tissue and outer wall of the epidermis) were generally lower in digestibility compared to thin cell wall type (phloem, inner wall of the epidermis and parenchyma) (Wilson, 1991). Lignified tissues are further divided into syringyl (sclerenchyma) and coniferaldehyde types (xylem); the former may be attacked more easily by microorganisms than the latter (Akin et al., 1987). The present study has demonstrated that parenchyma in RS stem is first degraded and epidermis and xylem are the least digestible tissues.

Gross examination of chemically treated and untreated stems which had undergone *in sacco* degradation for 72 h, revealed that all epidermis was still intact, with little evidence of structural destruction. Besides the thick cell wall of epidermis, it may still be related to abundant silica deposited in epidermis polymerized with cuticle waxes in RS stem (Doyle et al., 1986). Some data indicated that this cuticle wax silica layer seems to be an important barrier to rumen microbial entering epidermis cell (Wilson, 1990; Nakashima et al., 1991). However, because chemical treatments have little effect on the localization of silica in straw stem, rumen microorganisms would not be expected to penetrate this barrier (Harbers et al., 1982; Zhang, 1995), which is confirmed by our observations.

Because of the different extents of lignification between the secondary and primary wall of sclerenchyma cells, the secondary wall can be relatively easily degraded and the middle lamella and primary wall are resistant to bacterial degradation (Cone and Engels, 1990; Akin et al., 1992; Engels and Schuurmans,

1992), as shown in Figure 4. Morrison (1988) and Mulder et al. (1992) reported that alkali treatment damaged the lignin-polysaccharide bond and remove partial polysaccharides and phenolic compounds (lignin) in the secondary cell wall. In the present study, we observed that NaOH treatment greatly facilitated the digestion of sclerenchyma (Figure 4F), and urea and NH_4HCO_3 treatments had a slight influence on the degradation (Figure 4G), indicating that three treatments have varying effects on the degradation of sclerenchyma due to the different intensity of alkalescency.

Generally, parenchyma can be completely degraded by rumen microorganisms with the lasting digestion (Akin et al., 1992; Engels and Schuurmans, 1992). In this study, all parenchyma was completely degraded after 48 h digestion (Figure 3), as indicated by Akin and Burdick (1981) and Zhang (1995). However, different treatments increased the degradation rate of parenchyma to different extents (Figures 2 and 5). Rumen microbial digestion of RS stem tissues first began from the thin-wall parenchyma of stem due to the absence of cuticle wax layer and epidermis on it (Shen et al., 1999). Treatments with urea, NH_4HCO_3 and NaOH distorted parenchyma in different degrees (Figure 1) and made the parenchyma cell wall of stem easily ruptured, which may help rumen microbes to invade parenchyma cells easily.

There are different rates and extents of degradation between large and small vascular bundles because of their differences in location as revealed by SEM. Treatments had little effect on vascular bundles (Figure 1), in contrast with the observation by Shen et al. (1999) who reported that urea treatment cracked the wall of vascular tubes. Although the phloem tissue is, in general, readily digestible (Harbers et al., 1982), the result observed under TEM in this study illuminated the phloem of untreated stem still retained structural integrity even at 48 h incubation (Figure 6A), and chemical treatments seemed to help rumen bacteria to degrade the phloem of large and small vascular bundles at dissimilar digestion times (Figures 2B, 3 and 6). However, chemical treatments did not enhance the breakdown of xylem (Figures 3 and 6), which is consistent in the result of Harbers et al. (1982).

CONCLUSIONS

Of all three treatments in this study, NaOH treatment had the best effects on the digestion of parenchyma, sclerenchyma and phloem of vascular tissue, and the positive effects of NH_4HCO_3 treatment on these tissues basically paralleled to those of urea treatment. The improvement in digestion of histological structures was accordant entirely with the increase of *in sacco* degradability of RS after

the three treatments. These results indicated that the histological methods by means of microscopical techniques such as SEM and TEM helped a direct insight into the mechanism with which chemical treatments exerted the effects on the improvement of nutritive value of RS.

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STRESZCZENIE

Histologiczne badania tkanek i błon komórkowych słomy ryżowej traktowanej różnymi związkami chemicznymi oraz jej rozkład w żwaczu

Słomę ryżową (SR) traktowano mocznikiem, dwuwęglanem amonu (NH_4HCO_3) i tlenkiem sodu (NaOH), w następstwie czego wzrósł ($P < 0,01$) rozkład s.m. oznaczany *in sacco*, z 45,3 SR nietraktowanej do 52,5; 53,2 i 63,6%, odpowiednio. Próby SR nietraktowanej i traktowanej były trawione w żwaczu owiec Huzhou przez 12, 24, 48 i 72 godz. celem oznaczenia rozkładu tkanek i błon komórkowych, przy zastosowaniu skaningowego (SEM) i transmisyjnego (TEM) mikroskopu elektronowego. Parenchyma była nieco uszkodzona na skutek traktowania mocznikiem lub NH_4HCO_3 , a znacznie po traktowaniu NaOH. Wpływ chemicznego traktowania na inne tkanki był mały. Wszystkie zastosowane związki przyspieszały rozkład parenchymy w 12 i 24 godz., NaOH - w większym stopniu. Sklerenchyma błon komórkowych RS nietraktowanej była trawiona w nieznacznym stopniu; po 72 godzinach pozostała niestrawiona część ścianki wtórnej oraz cała lamela środkowa i pierwotna ścianek. Natomiast sklerenchyma ściany komórek traktowana NaOH zaczynała być trawiona po 48 godzinach, a środkowa lamela i częściowo ścianka pierwotna po 72 godzinach. Wszystkie trzy sposoby traktowania słomy powodowały strawienie łyka po 48 godzinach. W 48 godzinie nie stwierdzono obecności łyka, a traktowanie w małym stopniu wpłynęło na trawienie naskórka i ksylemy tkanki naczyniowej.

Uzyskane wyniki wskazują, że przy traktowaniu RS NaOH otrzymuje się najlepsze wyniki dotyczące zmian struktury ździebeł słomy ryżowej oraz trawienia tkanek i błon komórkowych, i że podobne wyniki uzyskuje się przy stosowaniu NH_4HCO_3 . Poprawa trawienia histologicznych struktur jest zgodna ze zwiększeniem rozkładu *in sacco* RS po traktowaniu różnymi związkami chemicznymi. Można zasugerować, że metody histologiczne przy użyciu technik mikroskopowych, takich jak SEM i TEM, umożliwiają bezpośredni wgląd w mechanizmy chemicznego traktowania wywierającego wpływ na polepszenie wartości pokarmowej słomy ryżowej.