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Cellulase treatment of untreated and steam pre-treated rice straw — effect on in vitro fermentation characteristics

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Abstract

This study was conducted to evaluate the cellulase treatment of the untreated (URS) and steam pre-treated rice straw (STRS) in terms of dry matter loss, sugar composition and in vitro gas production (GP). The STRS was prepared at 15 bar for 5 min with a water-to-straw ratio of 30:70 (w/w). Enzymic hydrolysis of straws was accomplished in a 37°C incubator. Wet samples of the URS and STRS were saccharified with a commercial cellulase (*Penicillium funicalosum*) ranging from 0, 4, 8 to 16 unit per gram straw for 1, 2 and 3 weeks. At lower enzyme loading, dry matter loss from URS was low at 1-week treatment but significantly increased with the prolonged treatment time ($P < 0.01$). With higher enzyme loading and longer incubation time, dry matter loss from URS was high (>150 g kg^{-1}). However, dry matter loss from the STRS was low in all treatments. Water extracts of both the URS and STRS were increased by enzymic hydrolysis, with significantly higher content of water extracts in higher enzyme loading ($P < 0.01$). Enzymic treatment had little effect on the composition of individual and total sugars in either water extracts or insoluble residues of URS, but did increase total soluble sugars of the STRS. Soluble carbohydrates increased significantly with the increase in enzyme dosage ($P < 0.01$), but these were much higher in the STRS than in the URS. While the enzyme treatment had little effects on the GP parameters in the URS, the 24 h GP ($P < 0.05$) and potential GP for the STRS increased with the increment in enzyme loading. Little effect of treatment time was observed in the GP parameters for both URS and STRS. Estimated organic matter digestibility was 46.8–48.7 and 52.0–54.5% for the enzyme treated URS and STRS, respectively. It is inferred that cellulase treatment may further improve the nutritional value of steam pre-treated rice straw, but little could be expected for untreated rice straw only using a pure cellulase.

Keywords: Enzyme treatment; Dry matter loss; Sugar profiles; Gas production; Rice straw

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1. Introduction

In rice-producing countries in the tropics, rice straw (RS) constitutes an important source of roughage for ruminants. The estimated annual amount of RS in Asia was 541 million tonnes, representing 53% of total cereal straw production in Asia and 90% of RS in the world (FAO, 1999). About 200 million tonnes of RS are produced in China annually, but only limited amount of it is used for animal feeding. In some areas of China straws and stovers are burned in the field by farmers, occasionally causing serious pollution problems.

There is widespread interest in the development of an efficient method for hydrolyzing fibrous materials in RS. The possibility of increasing nutritive value of fibrous feedstuffs by enzymic treatment has been studied in recent years (Galbe and Zacchi, 1986; Nakashima et al., 1988; Ryu, 1989; Castro and Ørskov, 1994). Enzymes are environment-friendly, as the same enzymes are naturally occurring in the breakdown process (Ørskov and Smith, 1990). The advantages of biological treatment with either cell-free enzymes or fungi include an increased extent and rate of lignocellulosic digestion, an improved palatability, degradation of potential toxic compounds in the feedstuffs and improved storage characteristics of silage (Milligan et al., 1995). A disadvantage is longer period to complete the treatment compared to physical or chemical treatments, and increased loss of cell wall compounds upon fungal inoculation. High yields are possible by enzymic hydrolysis, but substrate pre-treatment is necessary to give access to the cellulose microstructure. An approach that has shown considerable potential for the cost-effective pre-treatment of lignocellulosics is that of steam explosion (Toussaint et al., 1991; Maekawa, 1992; Castro, 1994).

Steam treatment has been classified as a physical treatment (Walker, 1984), but in fact it is a thermo-mechanical process (Marchessault and St. Pierre, 1980) and a chemical treatment (Muzzy et al., 1983) as it follows the principles of acid-hydrolysis reactions. The advantages of steam treatment arise from several factors: complete hydrolysis of hemicellulose (Grohmann et al., 1985), lignin depolymerization (Hishiyama and Sudo, 1992; Karina et al., 1992) and redistribution within the cell wall (Michalowicz et al., 1991; Toussaint et al., 1991), swelling of cell walls (Morjanoff and Gray, 1987; Wong et al., 1988) and increase in functional specific gravity and water holding capacity (Castro, 1994). These are associated with the improved utilization of cell wall polysaccharides in the steam pre-treated materials by cell-free enzymes (Grohmann et al., 1985; Brownell and Saddler, 1987) and rumen microbes (Castro and Machado, 1990). By applying the steam explosion process to sugarcane bagasse, Kling et al. (1987) demonstrated that about 60% of the hemicellulose fraction in the bagasse was hydrolyzed and further, the susceptibility of cellulose to enzymic hydrolysis increased.

Effects of steam treatment have been dependent on the different conditions such as pressure, time and moisture. In the literature, pressure of 5–40 kg cm^2 for less than 5 min was generally employed in the steam pressure and explosion treatment (Ryu, 1989). With poplar wood chips treated with steam pressure either at 201–225°C with acid for 2 min or at 218–248°C without acid for 3 min, Toussaint et al. (1991) observed an increase in the available surface area and a good correlation with initial rate of enzymic hydrolysis. In

the previous study, we have shown that optimal conditions for steam treatment of rice straw was at a pressure of 15 bar for 5 min and that a water-to-straw of above 3:7 was needed to obtain optimal results (Liu et al., 1999).

Rice straw is a suitable cellulosic substrate for a bioconversion process because of its relatively low lignin content. In the steam-treated RS (STRS), content of water extracts was more than 30% and the hexoses in the insoluble residues accounted for 89% of total sugars (Liu et al., 1999), indicated for dominant existence of cellulose in the STRS. Treatment with enzyme, especially cellulase may be expected to further upgrade the STRS. This study was therefore, conducted to evaluate the effect of cellulase treatment on in vitro fermentation characteristics of the STRS, in comparison with the effect on the untreated straw (URS). For practical application purpose, the straws were incubated in solid state.

2. Materials and methods

2.1. Substrate

Rice straw, *Indica* sp., was obtained from Experimental Farm, Huajiachi Campus, Zhejiang University, and hammer-milled through a sieve size of 2 mm before enzymic treatment. The STRS were prepared at 15 bar for 5 min, and a water-to-straw of 30:70 was used (Liu et al., 1999). Sufficient water (30/70, w/w) was added to the URS to obtain a homogeneous sample.

Cellulase from *Penicillium funicalosum* was obtained from Sigma.

2.2. Enzymic hydrolysis

Ten grams of URS or STRS samples in duplicate were weighed in 5 cm × 10 cm plastic bags. Cellulase was added to the bags with a range of 0, 4, 8 and 16 unit per gram straw. One unit will liberate 1.0 μml of glucose from cellulose in 1 h at pH 5.0 at 37°C within 2 h treatment time. The desired amount of cellulase for each sample was solubilized in 2 ml distilled water and the solution was then poured into the bag and mixed with the contents thoroughly. After air was removed, the bags were sealed. The bags with samples were kept at a 37°C incubator for saccharification and removed at 1, 2 and 3 weeks treatment. Samples were then immediately heated at 100°C for 1 h to stop enzyme activity.

2.3. Evaluation of treatment effects

The effects of enzymic treatment were evaluated in terms of dry matter (DM) loss, sugar composition and in vitro gas production (GP).

All samples were analyzed for DM content and the pH value was recorded for all treated samples. Samples before and after enzymic treatment were weighed and subsamples were oven-dried. DM loss was directly calculated.

Soluble sugars were extracted by soaking a wet sample in distilled water at 40°C, and water extracts and insoluble residues were then freeze-dried. Both fractions were analyzed for individual and total sugars. Details for determination of sugar composition have been described elsewhere (Liu et al., 1999).

The GP was measured by the technique of Menke and Steingass (1988) using 100 ml calibrated glass syringes (Model Fortuna, Lonsee-Ettlenscheiβ, Germany). About 200 mg of samples were introduced into the syringe along with 20 ml of buffer–mineral–distilled water mixture (1:1:2, by volume) and 10 ml of rumen liquor that was collected from two rumen fistulated Suffolk × Dorset sheep, fed on a diet containing the following ingredients: hay 50%, barley 30%, molasses 10%, fish meal 9.1%, and others 0.9%. The syringes were placed in a water bath at 39°C, and the GP was recorded after 2, 4, 6, 9, 12, 24, 36, 48, 72 and 96 h incubation. Data were then fitted to the equation $GP = a + b(1 - \exp(-ct))$ (Ørskov, 1985), where a, b and c are constants and GP is the gas production from the substrate at time t.

2.4. Statistical analysis

Analyses of variance (ANOVA) procedure (SAS Institute Inc., 1988) was used to determine the effect of enzyme loading and treatment time on dry matter loss, water extracts, sugar composition and GP parameters. The difference of means was tested using Duncan's new multiple range test.

3. Results

3.1. pH value, water extracts, and dry matter loss during treatment

The DM contents of the URS and STRS before enzymic treatment were 555 and 537 g kg^{-1}, respectively. There were little changes in DM contents of both straws during enzymic treatment.

Table 1 presents the pH value, water extracts, and DM loss in the URS and STRS after enzymic treatment. The pH was 6.65 and 4.15 for the URS and STRS, respectively, indicating that acidic substances were produced during steam treatment. Some mould developed in the URS samples at 3 days treatment but no mould was found in the STRS at any time. When the bags with the URS were opened after enzymic treatment, the samples had a slight smell of ammonia, which may account for high pH of the URS (around 9.0, Table 1).

At lower enzyme loading, DM loss from URS was small at 1-week incubation but increased significantly with the prolonged time ($P < 0.01$) and with the increased enzyme dosage ($P < 0.05$). At higher enzyme loading and longer time of treatment, DM loss from URS was very high (more than 150 g kg^{-1}). However, DM loss from the STRS was low and did not increase with the enzyme loading and treatment time.

Enzymic hydrolysis increased water extracts of both URS and STRS, and higher values were obtained ($P < 0.05$) at higher enzyme loading and longer time of treatment (Table 1).

Table 1
Effect of enzyme dosage and treatment time on pH value, water extracts content and dry matter loss in untreated or steam-treated rice straw[a]

Item	Original straw	Enzyme dosage (unit)[b]				Treatment time (week)			S.E.M.	Significance of		
		0	4	8	16	1	2	3		E	T	E × T
Untreated rice straw												
pH	6.65	8.94	9.09	8.99	8.97	8.94	8.94	8.97	0.07	NS	NS	NS
Water extracts (g kg^{-1})	169	168 Bd	175 Bd	189 Ac	196 Ab	178 c	187 b	182 bc	2.0	**	*	NS
DM loss (g kg^{-1})	–	109 c	114 c	152 b	146 b	66 B	150 A	175 A	9	*	**	*
Steam-treated rice straw												
pH	4.15	4.09	4.01	4.07	4.07	4.08	4.04	4.02	0.03	NS	NS	NS
Water extracts (g kg^{-1})	308	309 Cd	341 Bc	357 Abb	365 Ab	337 c	340 bc	351 b	4.0	**	*	NS
DM loss (g kg^{-1})	–	5	16	35	10	16	13	31	8	NS	NS	NS

[a] A, B, C: means with different letters within enzyme dosage or treatment time differ significantly ($P < 0.01$); b, c, d: means with different letters within enzyme dosage or treatment time differ significantly ($P < 0.05$); NS, not significant.

[b] One unit will liberate 1.0 μmol of glucose from cellulose in 1 h at pH 5.0 at 37°C within 2 h treatment time.

* $P < 0.05$; ** $P < 0.01$.

Table 2
Composition of individual and total sugars as anhydrous sugars in water extracts and insoluble residues from rice straw treated with enzyme at different dosage and treatment time (%)

Enzyme dosage (unit)[a]	Time (week)	Sugar composition[b]									
		Rhe + Fuc	Ara	Xyl	Man	Gal	Glu	UA	Total	C_5	C_6
Water extracts											
0	1	0.13	0.55	0.91	0.51	1.18	1.25	0.56	5.09	29	71
	2	0.39	0.88	1.36	0.42	1.32	1.26	0.52	5.76	39	61
	3	0	0.90	1.42	0.41	1.40	1.13	0.68	5.94	26	74
4	1	0	0.64	1.13	0.60	1.21	1.38	1.35	6.31	28	72
	2	0.49	0.77	1.25	0.64	1.40	1.43	0.69	6.18	33	67
	3	0.39	1.06	1.58	0.62	1.55	1.63	1.15	7.82	34	66
8	1	0.48	0.64	0.94	0.62	1.10	1.37	0.38	5.05	31	69
	2	0	0.73	1.15	0.66	1.43	1.49	0.75	6.21	30	70
	3	0.44	0.90	1.37	0.63	1.46	1.26	0.67	6.29	36	64
16	1	0.20	1.31	0.98	0.97	1.20	2.37	0.96	7.79	29	71
	2	0	0.65	0.99	0.69	1.24	1.39	0.69	5.65	29	71
	3	0	0.88	1.33	0.68	1.51	1.29	0.83	6.52	34	66
Insoluble residues											
0	1	0.19	2.88	17.78	0.34	1.21	31.62	1.98	55.93	37	63
	2	0.19	3.20	18.63	0.25	1.14	34.71	1.99	60.03	36	64
	3	0.18	3.08	18.00	0.29	1.16	32.45	1.96	57.00	37	63
4	1	0.19	2.93	17.95	0.29	1.25	31.78	2.50	56.80	37	63
	2	0.13	3.11	18.06	0.32	1.20	32.94	1.76	57.43	37	63
	3	0.22	3.12	17.53	0.46	1.11	31.01	2.08	55.45	37	63
8	1	0.20	2.90	18.14	0.24	1.22	31.08	2.24	55.92	38	62
	2	0.21	3.06	18.62	0.26	1.22	31.98	1.67	56.93	38	62
	3	0.12	3.00	18.10	0.43	1.16	31.68	2.01	56.41	37	63
16	1	0.26	2.75	17.02	0.31	1.16	28.69	2.03	52.12	38	62
	2	0.19	2.88	18.21	0.29	1.16	30.74	1.96	55.33	38	62
	3	0.26	3.11	18.15	0.44	1.24	30.01	1.87	54.98	39	62

[a] One unit will liberate 1.0 μmol of glucose from cellulose in 1 h at pH 5.0 at 37°C within 2 h treatment time.
[b] Rha + Fuc, rhamnose + fucose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glu, glucose; UA, uronic acids; total, total monosaccharides; C_5, total pentoses; C_6, total hexoses.

3.2. Sugar composition

Compositions of individual and total sugars in the URS and STRS are presented in Tables 2 and 3. Enzymic treatment had little effect on sugar compositions in either water extracts or insoluble residues of the URS (Table 2). The small change in total soluble sugars and proportion of hexose in water extracts suggested that little saccharification occurred during enzymic treatment of the URS. However, total soluble sugars in the STRS did increase with the increased enzyme loading, mainly due to an increase in glucose content (Table 3). The proportion of total hexoses in the STRS increased with the increasing dosage of enzyme and treatment time. Treatment time had little influence on individual composition of sugar in both water extracts and insoluble residues.

Table 3
Composition of individual and total sugars as anhydrous sugars in water extracts and insoluble residues from rice straw treated with steam pressure followed by enzyme at different conditions (%)

Enzyme dosage (unit)[a]	Time (week)	Sugar composition[b]									
		Rhe + Fuc	Ara	Xyl	Man	Gal	Glu	UA	Total	C_5	C_6
Water extracts											
0	1	0.10	1.07	9.98	0.57	1.58	5.77	0.43	19.40	57	43
	2	0.09	1.05	9.88	0.22	1.54	5.93	0.48	19.19	57	43
	3	0	1.02	9.82	0.50	1.54	5.83	0.40	19.11	57	43
4	1	0.20	1.06	10.81	0.35	1.40	8.34	0.55	22.51	53	47
	2	0	0.97	9.75	0.56	1.41	9.49	0.36	22.54	48	52
	3	0	0.97	8.76	0.37	1.38	10.59	0.42	22.49	43	57
8	1	0	1.01	10.35	0.69	1.44	10.41	0.67	24.57	46	54
	2	0	1.05	10.13	0.37	1.56	10.85	0.48	24.44	46	54
	3	0.15	0.98	8.69	0.69	1.35	11.81	0.42	23.91	40	60
16	1	0.11	1.03	10.47	0.37	1.41	12.45	0.72	26.53	43	57
	2	0	1.03	10.08	0.57	1.52	13.29	0.46	26.95	41	59
	3	0.14	0.92	8.00	0.61	1.31	12.69	0.43	23.96	37	63
Insoluble residues											
0	1	0.14	0.38	5.60	0.23	0.30	37.66	0.54	44.85	13	87
	2	0.12	0.34	5.21	0.31	0.23	38.07	0	44.28	13	87
	3	0.12	0.32	5.15	0.16	0.23	37.43	0.32	43.61	13	87
4	1	0.16	0.30	4.29	0.40	0.30	36.12	0	41.57	11	89
	2	0.12	0.33	3.84	0.21	0.22	35.55	0	40.07	10	90
	3	0.12	0.28	3.97	0.19	0.22	36.67	0	41.33	10	90
8	1	0.12	0.34	4.19	0.18	0.20	35.50	0	40.53	11	89
	2	0.14	0.33	4.30	0.20	0.22	37.57	0	42.76	11	89
	3	0.13	0.31	4.00	0.17	0.23	36.20	0.22	41.26	10	90
16	1	0.12	0.34	4.55	0.18	0.26	36.47	0	41.92	12	88
	2	0.11	0.31	4.17	0.21	0.24	35.78	0	40.82	11	89
	3	0.11	0.29	3.82	0.21	0.25	33.97	0	38.65	11	89

[a] One unit will liberate 1.0 μmol of glucose from cellulose in 1 h at pH 5.0 at 37°C within 2 h treatment time.
[b] Rha + Fuc, rhamnose + fucose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glu, glucose; UA, uronic acids; total, total monosaccharides; C_5, total pentoses; C_6, total hexoses.

3.3. In vitro gas production

Total soluble and insoluble carbohydrates and in vitro gas production are shown in Table 4 for both the URS and STRS. Total soluble carbohydrates increased significantly with the increase in enzyme dosage ($P < 0.05$), but these were much higher in the STRS than in the URS. Little differences were observed between incubation times in total soluble and insoluble carbohydrates for URS and STRS.

The GP parameters reflected the sugar contents for all samples. The URS was lower in the 24 h GP, rate of GP and potential GP than did the STRS. While all GP parameters for the URS showed little change with the enzyme treatment, for the STRS the 24 h GP ($P < 0.05$) and potential GP increased with the increment in enzyme loading. Little effect of treatment time on the GP parameters was observed in both URS and STRS.

Table 4
Soluble and insoluble carbohydrates and in vitro gas production in the untreated or steam pre-treated rice straw after treatment with enzyme at different dosage and treatment time[a]

Item	Enzyme dosage (unit)[b]				Treatment time (week)			S.E.M.	Significance of		
	0	4	8	16	1	2	3		E	T	E × T
Untreated rice straw											
Carbohydrate (g kg^{-1} DM)											
Soluble	9 d	12 c	11 c	13 b	11	11	12	1	*	NS	*
Insoluble	479 Ab	467 Abc	458 ABc	435 Bd	454	467	442	6	**	NS	NS
In vitro gas production (GP)											
GP at 24 h (ml)	20.2	19.8	20.1	19.0	18.3	20.5	20.5	0.7	NS	NS	NS
Rate of GP (% h^{-1})	3.47	3.15	3.08	3.23	3.12	3.43	3.15	0.20	NS	NS	NS
Potential GP (ml)	34.8	33.6	35.3	34.6	35.6	34.2	34.0	0.7	NS	NS	NS
Steam pre-treated rice straw											
Carbohydrate (g kg^{-1} DM)											
Soluble	59 Ce	77 Bd	87 ABc	94 Ab	79	80	78	2	**	NS	NS
Insoluble	306 A	272 B	267 B	257 B	280	278	268	4	**	NS	NS
In vitro gas production (GP)											
GP at 24 h (ml)	24.5 e	25.8 d	26.6 c	27.5 b	26.1	26.1	26.0	0.4	**	NS	NS
Rate of GP (% h^{-1})	4.25	4.63	4.32	4.45	4.59	4.30	4.35	0.09	NS	NS	NS
Potential GP (ml)	37.1	37.1	37.8	39.1	35.3	38.4	39.7	0.8	NS	NS	NS

[a] A, B, C: means with different letters within enzyme dosage or treatment time differ significantly ($P < 0.01$); b, c, d, e: means with different letters within enzyme dosage or treatment time differ significantly ($P < 0.05$); NS, not significant.

[b] One unit will liberate 1.0 μmol of glucose from cellulose in 1 h at pH 5.0 at 37°C within 2 h treatment time.

* $P < 0.05$; ** $P < 0.01$.

4. Discussion

In the present study enzymic treatment exerted small effect on the URS, with the increased pH and DM loss, slightly increased water extracts and soluble carbohydrates and little change in the GP parameters. The results were not consistent with those of Nakashima et al. (1988), who investigated the effects of polysacchridase and moisture content (500–700 g kg^{-1}) on quality and degradation characteristics of ensiled rice straw. They obtained a lowered pH, improved fermentation characteristics, and increased rumen degradation in the cellulase treated rice straw. Moisture contents before enzymic treatment were 445 and 463 g kg^{-1} for the URS and STRS, which were above the critical level for enzyme action reported by Castro and Ørskov (1994). However, higher contents of moisture were used in the experiment of Nakashima et al. (1988). Rice straw is bulky material, and high moisture may therefore, advantageous for the enzymic treatment of rice straw.

Mould was observed in the URS but not in the STRS. This is partly due to the difference in pH between the URS and STRS, as the mould growth might be inhibited in the acidic condition attributed to the low pH in the STRS. Pre-treatment of rice straw with steam improved the effects of the enzymic treatment. Compared with the enzyme-treated URS, the treated STRS had a much lower DM loss during the treatment and higher content of water extracts (Table 1), higher soluble sugars (Tables 2 and 3), and hence higher parameters of in vitro GP (Table 4).

The contents of water extracts and soluble carbohydrates are the important measures of treatment effects. Steam treatment increased the water extracts of RS to 308 g kg^{-1} from 169 g kg^{-1} in the original RS (Table 1), and enzyme hydrolysis further increased the water extracts of the STRS to 365 g kg^{-1} at enzymic dosage of 16 unit, while little effect was observed in the URS. Nakashima and Ørskov (1990) increased the water extracts from rice straw by ammonia or cellulase treatment However, the increase, by 4% with ammonia and 23% with cellulase from 207 g kg^{-1} in the original straw, was less than that by steam pre-treatment in the present study (Table 1). Through pre-treatment of barley straw with sodium hydroxide, Nakashima and Ørskov (1989) could only increase the water extracts from 168 to 193 g kg^{-1}. It is indicated that steam pre-treatment was more effective in increasing the lignocellulosic microstructure of rice straw.

Significant positive linear relationships have been observed between the GP parameters and soluble carbohydrate content (Liu et al., 1999). Soluble carbohydrate was very low in all the URS and did not increase so much by enzymic hydrolysis (Table 4). However, it increased significantly with the increasing dosage of enzyme. The GP was reflective of the soluble carbohydrate content, and the GP at 24 h of incubation increased significantly with the increasing dosage of enzyme ($P < 0.05$).

Treatment time had little influences on the pH (Table 1), sugar compositions in water extracts and insoluble residues (Tables 2 and 3), and the GP parameters for both straws (Table 4), though the water extracts of both URS and STRS were significantly but slightly increased with the prolonged time of treatment ($P < 0.05$). Nakashima and Ørskov (1989) found that the differences in the rumen degradation of barley straw were small between cellulase-treating periods of 10–40 days. With the steam-treated wheat straw incubated with enzymes from *Trochoderma reesei* at liquid buffer, Ternrud et al. (1989) observed that a third of the total amount of sugar obtained after 24 h has been released

during the first 30 min, and that 90% of the polysaccharides has been hydrolyzed after 24 h. A long period of treatment seemed to be disadvantageous, as the DM loss from the URS significantly increased with time (Table 1).

The organic matter digestibility (OMD) may be estimated by the equation of Menke and Steingass (1988): $\mathrm{OMD}(\%) = 31.55 + 0.8343\ \mathrm{GP}_{24}$, where GP_{24} (ml) is the GP at 24 h incubation. The estimated OMD was 46.8–48.7 and 52.0–54.5% for the enzyme-treated URS and STRS, respectively. The digestibility of the treated-STRS in the present study was comparable to the hay of medium quality (FAO, 1998), but slightly inferior to wheat straw treated with steam followed by single cellulose hydrolysis (Castro and Ørskov, 1994).

Since the hemicellulose fraction of rice straw was mostly hydrolyzed after steam treatment (Liu et al., 1999), the improvement in nutritional value by cellulase treatment in the present study was less than what we expected. Furan derivatives (furfural and hydroxymethyl furfural) and phenolic compounds can be formed during steam treatments, and these may be toxic to the rumen microbes (Forsberg et al., 1986; Kyuma et al., 1991) or have an inhibitory activity on cell-free enzymes (Mes-Hartree and Saddler, 1983, Sharma et al., 1985). Contrary to the these authors, Zahedifar (1996) observed that the phenolic compounds and furan derivatives were not toxic to rumen microbes. Castro (1994) also observed that rumen microbes were tolerant to and could quickly metabolize both furfural and hydroxymethyfurfural, and that inhibitory compounds present in steam-treated samples are phenolic-type compounds but these negative effects were observed only at harsh treatment conditions. These compounds were not measured in the present study, but the effects of enzymic hydrolysis should not be severely inhibited by these possible harmful products due to the relative low content of lignin in RS and to the mild condition under which the STRS was prepared (at 15 bar for 5 min).

Many workers have used the enzyme mixtures consisting of different ratios of enzyme preparations to examine the effect of enzymatic hydrolysis (Galbe and Zacchi, 1986; Nakashima et al., 1988; Castro, 1994). Further work is needed to study the effects of different enzyme mixtures.

5. Conclusion

The cellulase treatment hydrolyzed the cellulose fraction of steam-treated rice straw into monosaccharides and improved the nutritional value of steam pre-treated rice straw, while using a pure cellulase little effect could be achieved for rice straw. The extent to which the nutritional value of the steam-treated rice straw was improved increased with an increment in enzyme loading, but with little effect of treatment time. Further study is needed to examine the effect of enzyme mixtures consisting of different enzyme preparations to optimize the hydrolysis of cellulose in the steam-treated rice straw.

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