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Chemical composition, cell wall features and degradability of stem, leaf blade and sheath in untreated and alkali-treated rice straw

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Three dominant morphological fractions (i.e. leaf blade (LB), leaf sheath (LS) and stem) were analysed for chemical composition and ruminal degradability in three rice straw varieties. In one variety treated with alkali, cell wall features were also characterized using Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy. The highest concentrations of cell wall carbohydrates (hemicellulose and cellulose) were observed in LS, whereas the highest concentrations of non-fibre (silica, phenolic compounds and CP) and lignin were recorded for LB. The stem had the lowest silica and hemicellulose contents but intermediate levels of other components. In terms of ruminal degradability, stem ranked higher than LB, which was followed by LS. Hemicellulose was found to be less degradable than either dry matter or cellulose in all the three fractions investigated. FTIR results indicated that the highest levels of hydrogen bonding, esterification and crystallinity within the cell wall components belonged to LS. In the alkaline treatment, these indices decreased to a larger extent for leaf fractions and a greater improvement was achieved in the degradability of LB and LS compared with that of stem. In the 24-h ruminal incubation, the silicified layer of epidermis and the underlying cell walls showed a rigid structure in the control fractions, whereas the treatment with NaOH resulted in crimping of the silicified cuticle layer and the loss of integrity in cell structure. Despite the highest silica and lignin contents observed in LB, LS showed the lowest degradability, which might be due to its high level of hydrogen bonding, crystallinity and esterification within its cell wall components as well as its high hemicellulose content.

Keywords: morphological fractions, silica, FTIR spectra, SEM, alkali treatment

Implications

Rice straw is a huge, inexpensive source of dietary energy for ruminants. The proportions and chemical characteristics of morphological fractions differ markedly and can potentially determine the nutritive value of the whole straw. Few basic investigations are available on the nature of silica, phenolic materials, and structural carbohydrates; hence, little is known about the consequences of the qualitative and quantitative changes in straw fractions. The present study is designed to investigate the chemical characterization of the morphological fractions in both untreated and alkali-treated rice straw.

Introduction

For every kg of rice grain obtained from the field, 1 to 2 kg of straw is produced. The straw is composed mostly of cell walls (>60%), with cellulose, hemicellulose, lignin and residual ash accounting for the main components. Extensive studies have so far been dedicated to investigate the chemical composition and the physical structure of the cell wall of different plants responsible for limiting its digestibility. In rice, silica and lignin are the major barriers to straw utilization by ruminants (Van Soest, 2006; Agbagla-Dohnani *et al.*, 2012). Competition with more photosynthetically efficient C4 plants may have promoted an evolution towards a greater silica accumulation in C3-plants (e.g. rice) cultivated in tropical environments at the expense of lignin, because of the higher cost for the synthesis of an equal amount of lignin (27 ATP; Van Soest, 2006). This extra amount of silica, however, is associated with an increase in ash content and a decrease in the degradability of rice straw. Lignin also plays a vital role in plant growth and health (Pedersen *et al.*, 2005). Results relating silica and lignin content to degradability of morphological fractions are

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equivocal. European rice straw leaves have been found to be more digestible than stems (Agbagla-Dohnani *et al*., 2001), whereas leaves reportedly tend to be less digestible than stems in Asian straws (Vadiveloo, 2000). Silica and lignin contents may be possible contributors to such variation in response (Van Soest, 2006) but no relationship has been found between microbial degradation and silica content in the European varieties (Agbagla-Dohnani *et al*., 2001 and 2003). In addition, the intrinsic properties of cell wall carbohydrates such as crystallinity, polymerization, esterification and substitution of acetyl and arabinose on xylan backbone as well as particle and pore sizes (Chesson, 1981; Taherzadeh and Karimi, 2008) may be involved in substrate degradability. However, these traits have not received adequate attention by researchers. Phenolic acid-ester cross-links in grass cell walls act as nucleation sites for lignin deposition (Ralph *et al*., 1995) and it is believed (Beauchemin *et al*., 2003) that the crystallized region of cellulose is less hydrolyzed than its non-crystallized counterpart because of its low surface area and its inaccessibility for endocellulase.

A variety of chemical and biological treatments have been employed to improve straw quality (Van Soest, 2006). Van Soest (1981) extracted rice straw using a neutral detergent solution to achieve an increased NDF degradability of organic matter per unit of silica removed. Silica in rice straw in opal form is rendered soluble at pH $>$ 10 (Inglesby *et al*., 2005), and the majority of phenolic acids esterified to cell wall components are also alkali-labile (Sun *et al*., 2002). Obviously, the chemical characteristics of rice straw and its degradability also depend on the plant fraction considered. In this work, the chemical composition and degradability of the morphological fractions in three rice straw varieties grown under identical agronomic conditions were evaluated. Moreover, an alkaline treatment was used in one of the three varieties to evaluate changes in the intrinsic properties and histology of cell wall components.

Material and methods

Rice straw collection and morphological fractions

Three genetically modified varieties of mature rice plants including Zayanderood, Sazandegi (both conventional rice varieties) and Firoozan (a non-native variety) were collected 3 to 4 days before harvesting from Lenjan fields in September 2008 (Isfahan, Iran). The varieties had been cultivated and grown under identical agronomic (sowing, flooding and fertilization) conditions in adjacent rice fields (ca. 3 ha). After cutting the plants about 10 cm above the ground, the leaves were removed from stem internodes and divided into LB (leaf blade) and LS (leaf sheath) to obtain a total of nine aliquots per variety (Figure 1). The relative contribution by weight was determined after oven drying at 105°C for 16 h.

Chemical composition

Samples of each straw fraction were pooled per variety, milled through a 1 mm screen, and stored at −10 °C until they were subjected to chemical analyses, degradability measurements and alkali treatment. Chemical analyses were

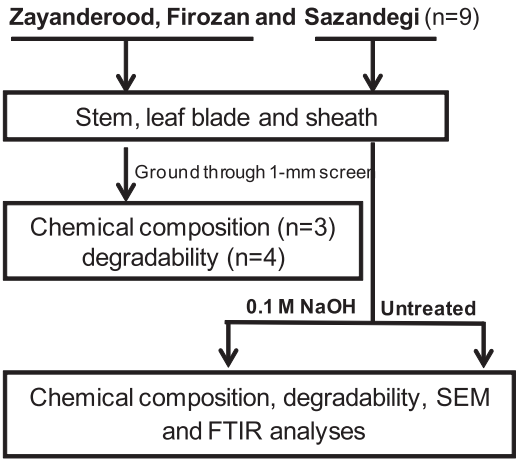

Figure 1 The outline of the characterization and analysis of the rice straw varieties

performed in triplicates. Ash (600°C for 2 h) and CP contents were analysed according to the Association of Official Analytical Chemists (AOAC, 2000). The sequential fibre analyses included residual ash performed according to the method proposed by Van Soest *et al.* (1991). NDF (using a heat-stable α amylase; Sigma A-3306) and ADF were measured using an Ankom$^{200/220}$ fibre analyzer (Ankom Technology Corp., Fairport, NY, USA). The ADL content of the residues was determined using sulphuric acid (72%) in a Daisy incubator (Ankom Technology Corp.) for 3 h at room temperature. Cellulose and hemicellulose contents were estimated as ADF minus ADL and as NDF minus ADF, respectively. To determine the concentrations of the free phenolic compounds (g/kg) in the straw fractions (250 mg), 45 ml of a Na-K buffer (containing KH_2PO_4, 7.92 g; $Na_2HPO_4 \cdot 7H_20$, 10.87 g; EDTA, 10 g; dissolved in 1 l of distilled water) was used in polyethylene tubes (Lau and Van Soest, 1981). The tubes were then capped and placed in an incubator shaker (20 r.p.m.) at 39°C for 24 h. After centrifuging the tubes (10 000 $\times$ **g**, 20 min), 1 ml of the aliquots was diluted to 20 ml with deionized water. The optical density was measured at 280 nm using a UV-Photometer (GeneRay, Biometra, Germany) using tannic acid as standard. For the determination of silica, a sample of straw weighing 250 mg was burned to ash at 600°C for 2 h. After cooling, the sample was transferred into a 50 ml polyethylene tube and digested with 2.5 ml of 35% H_2O_2 and 5 ml of 50% NaOH in an autoclave at 150 kPa for 40 min (Elliott and Snyder, 1991). The mixture was subsequently diluted with deionized water up to 250 ml and silica content in the diluted sample was determined colorimetrically (method 965.07, AOAC, 2000). The optical density was measured at 650 nm (UV 2100-VIS Spectrophotometer Shimadzu, Kyoto, Japan).

Dry matter (DM) and fibre degradability

To measure DM, NDF and ADF degradability, straw samples (500 mg) were weighed into Ankom F57 filter bags (25 μm pore size; ANKOM Technology Corp.). Thirty six bags thus filled were then put into two larger loose mesh cloth sacks

(50 × 50 cm; 2 mm pore size) and incubated in the rumen of two fistulated non-lactating Holstein cows for 48 h (four replicates including two replicates/straw fraction per cow). The cows were individually housed in 4 × 4 m covered pens where they received a dry cow total mixed ration consisting of 38% maize silage, 25% lucerne hay, 15% barley straw and 22% concentrate (barley grain, canola meal, wheat bran and a mineral/vitamin mix). After incubation, the bags were removed from the rumen, hand-washed with cold water until the rinsing water was clear, and dried at 60°C for 48 h in a forced-air oven.

Alkaline treatment of morphological fractions

The morphological fractions of the Sazandegi variety were treated with sodium hydroxide to be used for the chemical, Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM) analyses. The F57 bags (500 mg of the straw sample) were soaked in 0.1 M NaOH (50 ml/bag) in a Daisy incubator jar and incubated at 39°C for 24 h. Ruminal degradability (48 h) of the samples was measured using the method described above. To determine solubilization of silica and phenolic compounds by alkali treatment, the treatment described above was conducted. Five hundred milligrams of the untreated sample (the control) was mixed and stirred with 45 ml of 0.1 M NaOH into a 50 ml polyethylene tube in a shaker incubator at 39°C for 24 h. Some of the samples were subsequently filtered through a filter paper (Whatman No. 41) for silica determination, whereas others were centrifuged (10 000 × **g**, 20 min) for phenolic compound analysis. The IR absorbance band of the cell wall constituents, that is, cellulose, hemicelluloses and lignin in the morphological fractions (LB, LS and stem) was measured using a FTIR system (Bruker Tensor 27, Bruker Optics Inc., Billerica, MA, USA) coupled with a universal attenuated total reflectance accessory. Cell contents were removed by extracting the straw samples using a neutral detergent solution (Van Soest *et al.*, 1991). The samples were then finely ground and pressed uniformly against the diamond surface using a spring-loaded anvil, and the mid-IR spectra were recorded from a resolution of 4000 to 600 cm^{-1} at 2 cm^{-1}.

Morphological fractions (untreated and NaOH-treated) were incubated in the rumen of fistulated cows for 24 h. After incubation, the bags were removed, rinsed and stored in distilled water in a refrigerator for a few days. The straw samples were then fixed with 3% glutaraldehyde in 0.2 M cacodylate buffer (pH 7.4) for 24 h at room temperature (Agbagla-Dohnani *et al.*, 2001). Afterwards, the samples were dehydrated for 5 min in 50%, 10 min in 75% and twice for 15 min in 95% ethanol (v/v) to be later affixed and sputter-coated with gold (BAL-TEC SCD 005) and, finally, subjected to SEM (PHILIPS XL30, Philips, Eindhoven, The Netherlands) observation at 10 kV.

Statistical analysis

Data from the morphological fraction proportions were analysed using the GLM procedure of SAS (SAS Institute, 2001) according to the following model:

$$Y_{ij} = \mu + V_i + e_{ij}$$

where μ is the overall mean; V_i is the ith variety effect; e_{ij} is the random residual effects with mean 0 and the variance homogeneity, σ^2. Data obtained on the chemical composition and degradability of the morphological fractions of each variety were analysed using the MIXED procedure as a split-plot design with variety and morphological fraction as the main plot and the subplot, respectively. The following model was used:

$$Y_{ijk} = \mu + V_i + \delta_{ik} + F_j + (V \times F)_{ij} + e_{ijk}$$

where μ is the overall mean; V_i is the effect of level i of factor V, variety; F_j is the effect of level j of factor F, morphological fraction; $(V \times F)_{ij}$ is the effect of the ijth interaction of $V \times F$; δ_{ik} is the main plot error (the main plots within factor V) with mean 0 and variance $\sigma^2\delta$; e_{ijk} is the split-plot error with mean 0 and the variance σ^2. Data from the alkaline treatment were analysed based on a 3 × 2 factorial arrangement using the GLM procedure according to the following model:

$$Y_{ij} = \mu + F_i + T_j + (F \times T)_{ij} + e_{ij}$$

where μ, F, T, $F \times T$ and e are overall mean, morphological fraction, treatment, interaction between F and T and residual effect, respectively.

Results

Morphological fractions, chemical composition and degradability

Similar overall average proportions were obtained for LB (31%), LS (31%) and stem (38%) among the varieties investigated (Table 1). However, they exhibited differences in their chemical compositions (fibre and non-fibre contents). The highest values of non-fibre (CP, ash, silica and phenolic compounds) as well as ADL contents were detected for LB, whereas LS recorded the highest levels of NDF, ADF, hemicelluloses and cellulose ($P < 0.01$). Stems showed intermediate values for all parameters except for their hemicellulose and silica. LB was found to have three times more soluble phenolic compounds than LS and stem fractions. A significant interaction was found to exist between variety and morphological fraction with respect to NDF, ADF, CP, ash and phenolic contents ($P < 0.01$). Although LS and stem exhibited virtually identical ash quantities, the silica content of stems was extremely lower than that of LS ($P < 0.01$). The contributions of silica to ash content were recorded as 54%, 44% and 21% for LB, LS and stems, respectively. Average values of DM degradability, hemicelluloses and cellulose in rice straw varieties were 47%, 40% and 50%, respectively (Table 1). DM degradability was substantially higher in stems than it was in leaf fractions ($P < 0.01$). The lowest values for DM and fibre degradability were observed in LS. However, the interaction of variety by fraction was significant in the case of fibre component degradability ($P < 0.05$).

Table 1 *Morphological fractions, fibre and non-fibre compositions and degradability of three rice straw varieties*

	Firozan			Sazandegi			Zayanderood				*P*-value		
Item	Blade	Sheath	Stem	Blade	Sheath	Stem	Blade	Sheath	Stem	s.e.m.	V	F	V × F
Morphological fraction (%)													
Proportion	31.3	31.4	37.4	30.8	31.4	37.7	30.9	31.0	38.0	–[+]	ns	–	–
Chemical composition (g/kg dry matter)													
NDF	693	790	707	669	785	732	621	744	676	3.9	***	***	***
ADF	395	475	453	388	470	474	351	444	434	3.0	***	***	***
ADL	67	29	38	66	27	30	53	26	34	2.9	ns	***	ns
Hemicellulose	298	315	254	280	315	258	270	300	242	4.3	***	***	ns
Cellulose	328	446	415	322	443	443	299	417	399	3.6	***	***	***
Hemi/cellulose	0.91	0.71	0.61	0.87	0.71	0.58	0.90	0.72	0.61	<0.01	ns	***	ns
Crude protein	43	39	48	46	32	41	77	53	52	2.2	**	***	***
Ash	167	157	132	204	177	136	165	129	134	0.5	*	***	***
Silica	93	65	26	112	77	30	86	62	29	2.9	ns	***	ns
Phenolic compounds	16	6	7	17	7	7	22	8	9	0.1	**	***	***
48 h *in sacco* degradability (%)													
Dry matter	44.4	37.3	50.7	44.7	39.5	50.2	46.2	44.7	57.6	1.03	**	***	ns
NDF	44.7	36.4	41.7	44.3	40.7	43.6	43.0	41.7	49.1	1.11	*	***	ns
ADF	46.7	37.0	44.6	45.1	40.9	47.2	45.0	43.9	52.8	1.08	**	***	**
Hemicellulose	42.1	35.5	36.4	43.0	40.5	36.9	40.3	38.4	42.5	1.43	ns	*	*
Cellulose	50.8	40.2	48.8	56.3	43.8	50.8	53.1	47.2	57.0	1.28	*	***	*

s.e.m. = standard error of mean; V = variety; F = fraction; ns = not significant.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
[+]s.e.m. values of the varieties were 0.11, 2.04 and 2.10 for leaf blade, leaf sheath and stem, respectively.

Table 2 *Fibre and non-fibre compositions and degradability (48 h) of Sazandegi after treatment with 0.1 M NaOH*

	Fraction				*P*-value		
Item	Blade	Sheath	Stem	s.e.m.	F	T	F × T
Chemical composition (g/kg in dry matter)							
NDF	498	623	610	4.3	***	***	***
ADF	375	475	476	1.9	***	ns	*
ADL	43	15	21	1.1	***	***	*
Hemicellulose	123	148	134	4.1	***	***	***
Cellulose	332	460	455	2.1	***	ns	ns
Silica	51	12	6	0.7	***	***	***
Phenolic Compounds	61	50	53	0.2	***	***	**
Degradability 48 h (%)							
Dry matter	80.9	78.1	79.9	1.76	***	***	**
Hemicellulose	85.1	81.3	78.9	5.40	**	***	ns
Cellulose	85.9	73.7	76.7	3.52	***	***	ns

s.e.m. = standard error of mean; F = fraction; T = alkali treatment; ns = not significant.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
Data of untreated straw were presented in Table 1.

Alkaline treatment of morphological fractions

The Sazandegi variety was subjected to alkali treatment because of its higher silica content. Chemical composition and degradability of treated morphological fractions are summarized in Table 2 and the same data for the untreated fractions are presented in Table 1. Treatment of straw fractions with 0.1 M NaOH reduced ($P < 0.01$) NDF, ADL, hemicelluloses and silica, but it did not change ADF or cellulose ($P > 0.05$) and increased both the release of phenolic compounds as well as DM and fibre degradability ($P < 0.01$). An interaction effect was established between treatment and morphological fractions for chemical composition and DM degradability ($P < 0.05$). Compared with leaf fractions, stems were found to be less affected by alkali treatment with respect to their NDF, ADL and hemicellulose contents or DM degradability but more affected in terms of their silica content. The increments observed in ruminal degradability levels were 98%, 81% and 59% in LS, LB and stem, respectively.

Supplementary Figure S1 shows the FTIR spectra and Table 3 presents the positions and assignments of IR absorbance bands

Table 3 *Position of FTIR absorbance bands of morphological fractions, their assignment and the ratios of absorbance bands of treated to untreated morphological fractions*

No.*	Wavenumber	Functional group**	Treated/untreated		
			Blade	Sheath	Stem
1	3334	–OH stretching of hydrogen bonds in cellulose	0.84	0.79	1.36
2	2922	C–H stretching in cellulose	0.80	0.74	1.20
3	1728	C=O stretching of acetyl or carboxylic acid in hemicellulose and lignin	0.56	0.49	0.77
4	1641	C=C stretching of the aromatic ring in lignin	0.79	0.70	1.06
5	1548	C=C in lignin	0.72	0.67	0.95
6	1423	Aromatic skeletal vibrations combined with C-H_2 deformation in lignin and cellulose	0.78	0.68	1.11
7	1367	Aliphatic C–H vibrations in cellulose and hemicellulose	0.77	0.69	1.12
8	1317	C–H_2 wagging in cellulose	0.83	0.74	1.21
9	1242	Syringyl ring breathing and C–O stretching out of lignin and xylan	0.65	0.53	0.92
10	1159	C–O–C vibrations at β-glucosidic linkages in cellulose and hemicellulose	0.77	0.62	1.12
11	1033	Overlapping peaks from carbohydrate, lignin and silicon dioxide functional groups	0.79	0.61	1.20
12	896	C–O–C vibrations at β-glucosidic linkages in hemicellulose and cellulose	0.94	0.78	1.31

*Peak number as indicated in Supplementary Figure S1.
**Inglesby *et al.* (2005), Kumar *et al.* (2009), Kobayashi *et al.* (2009) and Nieves *et al.* (2011).

and the absorbance band ratios of treated/untreated morphological fractions (treatment results). An intense band at 3336 cm^{-1} due to both inter- and intramolecular hydrogen bond stretching within the cell wall components was the highest for LS but the lowest for stem. A similar intensity for the band at 1423 cm^{-1} assigned to crystalline cellulose I (the form abundantly occurring in nature) was observed for the morphological fractions. With the alkali treatment, these bands reduced for the leaf fractions but an inverse effect was observed in the stem fraction. Degrees of esterification (ester-linked acetyl, feruloyl and p-coumaroyl groups) as indicated by the absorbance band at 1728 cm^{-1} were similar in leaf fractions but higher than those of stems. Exposure to the alkaline condition caused a decline in the corresponding band in all morphological fractions, whereas the difference was greatest for LS (Table 3).

SEM images of untreated and NaOH-treated LBs are shown in Supplementary Figure S2. A similar change was observed in the case of LS and stem (data not shown). The silicified surface layer in the untreated straw remained completely intact even after 24 h rumen incubation (Supplementary Figure S2a), whereas it was crimped but still observable in the NaOH-treated one (Supplementary Figure S2c). In the untreated LB, the underlying tissues were not released from the surface layer (Supplementary Figure S2b), with the cells having a rectangular structure and completely adherent to one another. In the NaOH-treated straw, no systematic ultrastructure or attachments were observed in the cells lying under the epidermis because of the disappearance of cemented material between cells (Supplementary Figure S2d).

Discussion

Morphology, physicochemical characteristics and degradability
Compared with other varieties, greater proportions of stems or lower leaf/stem ratios (1.63) were observed for the varieties used in the present study (Vadiveloo, 2000; Agbagla-Dohnani *et al.*, 2001); however, differences in variety did not cause any differences in leaf to stem ratio. A higher ratio of stem to whole plant is preferred, particularly in varieties with high silica content (Asian and United States). In these varieties, the epidermis of leaves is more silicified, resulting in a lower degradability of the leaf fractions as compared with the stems. Unlike the silica content or its contribution to ash that were much higher in the current varieties, the ash contents were similar to the values reported elsewhere (Vadiveloo and Fadel, 2009). The differences may be related to the differences between the methods used for measuring silica as acid detergent insoluble ash had been used in most previous studies for the determination of silica. Degradability levels of morphological fractions decreased in the following order: stem >LB>LS. This finding is consistent with that reported by Agbagla-Dohnani *et al.* (2003). The difference between the degradability levels of stem and LB might be related to the higher silica and lignin contents in LB, which might not explain the lower degradability of LS compared with that of LB. The higher cell wall and, hence, the lower cell content of LS *v.* LB might be the reason for its reduced degradability. Hemicellulose/cellulose ratio decreased from stem to LB, but LS had the greatest hemicelluloses content. Ruminal microorganisms in this study seemed to exhibit a lower capability for degrading hemicellulose as compared with cellulose or whole DM, with the difference being particularly larger in the case of stems. This might be somewhat unexpected as cellulose has an insoluble and crystalline structure but hemicellulose has a random, amorphous and branched one with little resistance to hydrolysis (Taherzadeh and Karimi, 2008). However, hemicellulose is directly bound to lignin (Van Soest, 2006) and, perhaps, to silica, which might reduce its potential for hydrolysis. The lower degradability in LS *v.* LB and LB *v.* stem in rice straw fractions could be attributed to the hemicellulose content together with

such inherent factors as crystallinity, esterification and hydrogen bonding as evidenced by the FTIR analysis, which showed the highest and lowest values for LS and stem, respectively.

Alkaline treatment

The stem's NDF content was less affected by the NaOH treatment compared with that of leaf fractions. This is probably a reflection of the lower hemicellulose/cellulose ratio of the stem as cellulose (and also ADF) was not reduced by the alkaline treatment. In the case of hemicellulose, it has been shown that ester and some glycoside (1−3) bonds in the branching region are hydrolyzed by alkaline treatments (Van Soest, 1994). In contrast, the release of silica occurred to a greater extent in LS (84%) and stem (81%) than in LB (54%) by applying 0.1 M NaOH. This may confirm a two-phase silica extraction in rice straw (Van Soest and Lovelace, 1969), and further indicate the higher solubility of silica in LS and stem fractions (or possibly a difference in the location of silica, for example, less silica in the cell wall and cuticle layer but more in the cell content). Additionally, FTIR and chemical analyses revealed that LS contained more linkages in the cellulose, hemicelluloses or lignin structure, which might be susceptible to alkaline treatment (e.g. hydrogen bonds, crystallization, acetylation, esterified phenolic acids). As a result, degradability was improved to a larger extent in LS than in LB or stem. Although cellulose and ADF contents were unaffected by the alkaline treatment, their degradability was significantly increased. This may be due to the reduction of certain factors limiting hydrolysis such as silica, lignin and hemicellulose or due to changes in the internal structure such as crystallinity or hydrogen bonds. A breakdown in the structure and a reduction in crystallinity have been observed in response to alkaline treatment (Nieves *et al*., 2011). This might be responsible for the improved attachment of microbial cellulosome (Fontes and Gilbert, 2010), which results in a better digestion after alkaline treatment. SEM analysis indicated that the silicified layer was entirely intact and that the cell wall had a distinct structure in the untreated straw samples. This layer, however, was crimped but not completely dissolved, while the cell configuration under the surface layer was extensively destroyed by silica solubilization and de-esterification as a result of the NaOH treatment. As a structural element, silica complements lignin and strengthens and rigidifies cell walls (Van Soest, 1994). The negative effect of silica may be due to the physical barrier formed by the external layer or an inhibitory action on hydrolyzing enzymes (Herbers *et al*., 1981). Moreover, it may be claimed that the rigid silicified epidermal layer in rice straw is probably a barrier against the external environmental or pathogenic factors in the rice plant but that it has no limiting effect on ruminal degradation (LB *v.* LS). Agbagla-Dohnani *et al.* (2003) reported that epidermal silica did not hinder degradation from the interval cavity of rice straw.

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Supplementary material

For supplementary material referred to in this article, please visit http://dx.doi.org/10.1017/S1751731113000256.

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