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Cellulase degradation of whole rice straw

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ABSTRACT

The dry matter degradation in a cellulase-buffer solution of the whole straw of six Malaysian rice varieties was estimated before and after treatment for 21 days in a 4% urea or 4% sodium hydroxide (NaOH) solution. Degradation characteristics were estimated from the equation $p = a + b\ (1-e^{-ct})$. Both urea and NaOH treatment significantly increased the rapidly degradable (constant a) and slowly degradable (constant b) fractions. The degradation rate (constant c) was significantly reduced by urea treatment but not by NaOH treatment. The degradation constants were independent of the agronomic and morphological characteristics of the untreated straws.

KEY WORDS: degradation, rice straw, urea treatment, NaOH treatment

INTRODUCTION

The rumen dry matter degradation of feeds has been conventionally carried out using the *in situ* nylon bag (Mehrez and Ørskov, 1977) or the *in vitro* gas production technique (Menke and Steingass, 1988; Blummel and Ørskov, 1993). Both of these are regarded as being unsuitable for routine use due to the requirement for fistulated animals, the tediousness of extracting rumen liquor and the high degree of standardisation expected before comparisons can be made (Bughrara et al., 1992; Lopez et al., 1998). In the case of the nylon bag procedure, variation in bag size, pore size, substrate particle size, variation in animal diet, particle loss and variation in the microbial population inside and outside the bag are factors which contribute to the low reproducibility of the results (Carro et al., 1994; Pike et al., 1996; Madsen et al., 1997). Variation in the quality of fresh inoculum and the non-adaptation of the rumen inoculum to test feeds have been cited as contributing to the low reproducibility of results from the gas production technique (Prasad et al., 1994).

The conventional method of improving the feeding value of rice straw and other cereal crop residues is through physical and chemical treatment. Lignocelluloses from monocotyledonous crops such as cereal straws respond well to chemical treatment because the action is hydrolysable in nature. Sodium hydroxide and urea treatment of Malaysian rice straw varieties improved the chemical composition and *in vitro* dry matter digestibility of the straw (Vadiveloo, 1996) and increased its voluntary intake and digestibility by goats (Vadiveloo, 1986, 1989; Tuen et al., 1991). These results complement other studies on urea-treated rice straw which have recorded improvements in nitrogen content, dry matter degradability and dry matter intake (Mgheni et al., 1993; Shen et al., 1998 a,b).

Enzymatic procedures using cellulase have rarely been employed to study the characteristics of degradation although they have been proposed for the routine estimation of end-point digestion (Bughrara and Sleper, 1986). As a technique for the latter, it is cheap, fast and sufficiently precise (Carro et al., 1994; Magai et al., 1994; Givens et al., 1995) although insensitive to factors such as associative effects and toxins (Prasad et al., 1994).

Studies on the nutritive value of Malaysian rice straw varieties have employed cellulase to estimate end-point digestion (Vadiveloo, 1992, 1995). As the degradation characteristics of Malaysian rice straw varieties have not been documented, the present study was initiated.

MATERIAL AND METHODS

Rice straw

Six straw varieties, MR 84, MR 151, MR 162, MR 164, MR 166 and MR 167 hand-harvested 12 cm above the ground in June 1994 from the Tanjung Karang district of Selangor were field-dried. The relative proportion by weight of leaf blade, leaf sheath and stem (including inflorescence) in approximately 500 g of whole straw was estimated in triplicate for each variety. Whole straw was ground to pass through a 1mm sieve and treated with 4% urea or 4% sodium hydroxide solution in the ratio of 50 g straw to 200 ml of solution. The treated samples were kept in screw-top plastic jars at 27°C for 21 days after which they were oven dried at 60°C to constant weight.

Chemical composition and degradability

Treated and untreated straw varieties were analysed for total ash and crude protein (AOAC, 1984), neutral detergent fibre and ash insoluble in neutral detergent solution (Van Soest et al., 1991). *In vitro* dry matter digestibility (IVD) was estimated by the cellulase-neutral detergent solution procedure of Bughrara and Sleper (1986) using the Onozuka 3S enzyme (Yakult Biochemicals). For the estimation of cellulase dry matter degradability, 0.5 g samples of whole straw were incubated in a cellulase-buffer solution (25 g cellulase in 1000 ml of 0.05 M sodium acetate/glacial acetic acid, pH 4.6) for 3, 6, 12, 24, 48 and 72 h at 38°C. After incubation, the residues were completely recovered by filtering in a Tecator Fibertec System, washed with water, oven-dried at 100°C and weighed. Degradation characteristics were described by the exponential equation $p = a + b\ (1-e^{-ct})$ where p is degradation at time t (hours) and a, b and c are constants (%) representing the rapidly degradable fraction, slowly degradable fraction and degradation rate, respectively (Ørskov and McDonald, 1979).

Statistical analyses

Straw varieties were compared for differences in their proportions of leaf blade, leaf sheath and stem by Kruskal-Wallis non-parametric analysis of variance. Within treatments, differences between varieties in chemical composition, IVD and cellulase dry matter degradation were compared by a one-way analysis of variance. Due to the absence of replication within varieties, differences in degradation characteristics between varieties could not be compared by an analysis of variance. Instead, the method of principal components was used.

Principal components is one of several multivariate statistical procedures wherein several criterion variables can be used simultaneously to evaluate mean differences. In principal components analysis, linear combinations of the criterion variables are computed, the number of linear combinations computed being equal to the number of criterion variables. The first combination or first principal component accounts for the largest variance. The criterion variables in each component can be summarised in a principal component score, which on the basis of its sign and magnitude, can be used to rank the means. The degradation constants (a, b and c) were used as criterion variables and varieties were compared for their scores on the first principal component.

The mean effect of treatment on chemical composition, IVD, cellulase dry matter degradation and degradation characteristics were compared by a one-way analysis of variance with six replicates (varieties) per treatment. For each treatment, simple correlations between the degradation constants and the morphology, agronomy and chemical composition of the varieties were also calculated.

All statistical analyses were run on a SAS Version 6 statistical package (Statistical Analysis Systems Institute Inc., 1987).

RESULTS

The agronomic and morphological characteristics of the six varieties are shown in Table 1. Varieties differed significantly (P<0.05) in the percentage of leaf blade and leaf sheath only.

TABLE 1

Agronomic and morphological characteristics of the straw varieties

Variable	Variety					
	MR162	MR166	MR151	MR84	MR167	MR164
Culm height, cm	69	79	84	81	79	75
Maturity, days	116	117	138	124	121	117
Yield, kg/ha	4262	4340	5063	4687	4065	3875
Leaf blade, %	31.4	28.0	34.2	31.6	29.6	31.5
Leaf sheath, %	41.1	41.2	37.6	38.8	42.0	41.0
Stem, %	26.2	29.7	27.6	28.9	28.2	26.3

Varietal differences in chemical composition and IVD before and after treatment were significant (P<0.001) but small in magnitude and hence are not reported. Varietal differences in cellulase dry matter degradation show that differences were generally more pronounced following chemical treatment (Table 2).

The degradation characteristics of each variety are shown in Table 3. Principal component analysis of the degradation constants showed that the first principal component accounted for 73, 60 and 70%, respectively, of the variance in the untreated, urea and NaOH data. Principal component scores were ranked in sign and magnitude, the highest negative score being ranked first. A large response to urea or NaOH treatment would be reflected in a change in the sign and magnitude of the component score. Compared to untreated straw, urea or NaOH treatment did not consistently alter the ranking of the straw varieties – MR 84 which was lowest ranked before treatment was second ranked after urea treatment but remained lowest ranked after NaOH treatment; MR 164 retained its ranking after urea treatment but was highest ranked after NaOH treatment.

The mean effects of urea and NaOH treatment are shown in Table 4. Both treatments increased IVD and ash content but reduced NDF content. Urea treatment also increased CP content. Dry matter degradation increased significantly for all incubation times (P<0.001). Urea and NaOH treatments significantly increased the rapidly degradable (constant a), slowly degradable (constant b) and potentially degradable (a + b) fractions (P<0.001). The rate of dry matter degradation (constant c) was significantly reduced after urea treatment (P<0.05) but not after NaOH treatment (P>0.05).

TABLE 2

Effect of time of incubation on cellulase dry matter degradation, %

Treatment	Variety	Time of incubation, h					
		3	6	12	24	48	72
Untreated	MR162	20.5	24.4	26.8	30.3	32.4	33.9
	MR166	19.6	23.6	26.4	30.3	30.6	30.3
	MR151	19.4	22.8	26.8	30.7	31.2	34.5
	MR84	19.0	21.7	26.8	29.7	30.2	29.4
	MR167	20.1	23.9	27.5	30.5	34.9	31.8
	MR164	20.0	22.9	28.0	30.6	30.9	32.0
	SE diff +	0.41ns	0.63ns	0.51ns	0.44ns	0.64**	0.47***
Urea	MR162	26.3	32.1	35.0	41.5	44.7	46.3
	MR166	25.4	31.7	35.4	41.6	44.8	46.0
	MR151	24.3	30.3	33.7	39.4	42.8	44.0
	MR84	26.7	32.5	36.5	41.6	45.5	46.5
	MR167	25.0	30.3	35.0	40.1	43.0	45.0
	MR164	24.5	30.8	34.6	40.7	43.4	46.3
	SE diff +	0.74ns	0.42**	0.47**	0.91ns	0.77ns	0.46***
NaOH	MR162	42.7	50.1	57.6	61.9	65.7	66.9
	MR166	43.9	50.6	59.7	63.3	67.4	69.4
	MR151	43.9	50.7	58.7	62.5	65.5	66.6
	MR84	42.7	50.9	59.0	63.2	67.3	67.8
	MR167	41.4	48.7	57.1	59.7	65.1	68.2
	MR164	40.2	50.8	55.8	60.1	65.8	69.5
	SE diff +	0.62**	0.85ns	0.35***	0.41***	0.26***	0.74*

*** P<0.001, ** P<0.01, * P<0.05, ns P>0.05

+ SE of the difference between two means

Significant correlations (P<0.05) were obtained between the following pairs of variables:

Untreated : potentially degradable fraction and insoluble ash content, r = 0.82
Urea-treated : degradation rate and stem percentage, r = 0.89
rapidly degradable fraction and IVD, r = 0.85
NaOH-treated : degradation rate and grain yield, r = 0.91
degradation rate and IVD, r = 0.84.

DISCUSSION

The morphological composition of the present six varieties was similar to the varieties reported by Vadiveloo (1992) but varied from the varieties reported by Vadiveloo (1995) being higher in leaf content.

TABLE 3

Degradation constants and statistics on the first principal component

Treatment	Variety	Constants					Statistics	
		a, %	b, %	c, /hr	a + b	RSD+	score	rank
Untreated	MR162	18.3	15.2	0.0691	33.5	0.77	-1.68	1
	MR166	14.9	15.7	0.1234	30.6	0.51	0.22	4
	MR151	16.0	17.2	0.0801	33.2	1.04	-0.22	3
	MR84	13.1	16.9	0.1319	30.0	0.80	1.24	6
	MR167	16.3	16.9	0.0906	33.2	1.25	-0.23	2
	MR164	14.6	16.9	0.1214	31.5	0.76	0.69	5
Urea	MR162	22.9	23.1	0.0670	46.0	1.24	-1.57	1
	MR166	21.2	24.5	0.0769	45.7	1.00	1.16	6
	MR151	20.7	23.1	0.0723	43.8	0.94	0.02	3
	MR84	22.9	23.3	0.0731	46.2	0.88	-0.74	2
	MR167	20.9	23.4	0.0756	44.3	0.88	0.49	4
	MR164	20.8	24.6	0.0703	45.4	1.28	0.63	5
NaOH	MR162	35.1	31.0	0.1032	66.1	1.14	0.02	4
	MR166	36.0	32.1	0.1002	68.1	1.35	-0.11	3
	MR151	35.3	30.5	0.1148	65.8	0.94	0.04	5
	MR84	33.5	33.6	0.1142	67.1	1.11	1.83	6
	MR167	35.6	30.9	0.0839	66.5	1.97	-0.70	2
	MR164	36.3	31.4	0.0748	67.7	2.51	-1.07	1

+ residual standard deviation

The absolute values for constant a and constant b for untreated straw (Tables 3 and 4) were lower than similar values obtained by the nylon bag or gas production techniques (Walli et al., 1988; Nakashima and Ørskov, 1990; Shen et al., 1998b) probably because cellulase dry matter degradation only mimics part of the complex process taking place in the rumen (Lopez et al., 1998). The increase in potential dry matter degradability of rice straw after urea or NaOH treatment was in accordance with the findings of Nakashima and Ørskov (1990) and Walli et al. (1988) with ammonia-treated rice straw. In the case of urea-treated straw, this may be attributed to an increased extraction of biogenic silica (Shen et al., 1998a). With wheat straw, the improvement in dry matter degradability after ammonia treatment is attributed to an increased solubilisation of lignin (Kondo et al., 1992).

The rate of dry matter degradation (constant c) was significantly reduced after urea treatment ($P<0.05$), but not significantly altered following NaOH treatment ($P>0.05$; Table 4). These effects may be explained by the different response of the leaf and stem fractions to urea and NaOH treatment observed by

Vadiveloo (1999). Urea treatment resulted in a significant reduction in the degradation rate of the stem fraction but did not result in a significant change in the degradation rates of the leaf blade and leaf sheath fractions. With NaOH treatment however, degradation rate was significantly reduced in the stem and leaf sheath fractions but was significantly increased in the leaf blade fraction.

Chemical treatment did not consistently affect the ranking of straw varieties based on their degradation characteristics (Table 3). With temperate cereal straws, the potential degradability after ammonia or urea treatment is greater for straw varieties of initially lower degradability (Tuah et al., 1986; Goto et al., 1991; Colucci et al., 1992). However the response to NaOH treatment of wheat, barley and oat straw varieties was independent of initial digestibility (Moss et al., 1990).

Insoluble ash (mainly silica) content and potential dry matter degradability in the untreated straw was significantly correlated ($r = 0.82$; $P<0.05$) but since the range of the two variables was narrow (standard deviation = 0.84 and 1.49, respectively) the significance may be, for now at least, empirical only. It is interesting to note however that Nakashima and Ørskov (1990) also reported a significant correlation ($r=0.91$; $P<0.01$) between silica and potential degradabi-

TABLE 4

Treatment effects on chemical composition (% in DM), cellulase dry matter degradation (%) and degradation constants

Parameter	Variable	Untreated	Urea	NaOH	SE diff [+]
Chemical composition	Total ash	17.8	19.5	22.6	0.27***
	NDF	63.5	62.3	54.6	0.49***
	Insoluble ash	5.3	9.1	10.0	0.40***
	IVD	54.0	62.0	86.4	0.41***
	CP	3.3	5.0	2.8	0.16***
Time of incubation, h	3	19.7	25.3	42.4	0.45***
	6	23.2	31.3	50.3	0.41***
	12	27.0	35.0	58.0	0.42***
	24	30.3	40.8	61.8	0.45***
	48	31.7	44.0	66.1	0.54***
	72	32.0	45.7	68.1	0.59***
Degradation constants	a, %	15.6	21.6	35.2	0.76***
	b, %	16.3	23.7	31.5	0.52***
	c, hr^{-1}	0.1019	0.0725	0.0990	0.01032*
	a+b, %	32.0	45.2	66.9	0.67***
	RSD [++]	1.18	1.27	1.75	

[+] SE of the difference between two means

[++] residual standard deviation

lity in rice straw. The degradation constants of the untreated straw varieties were not significantly correlated to their agronomic or morphological characteristics suggesting that both sets of characteristics may be jointly selected in rice breeding programmes (Vadiveloo, 1995).

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STRESZCZENIE

Określenie rozkładu całej słomy ryżowej przy zastosowaniu celulazy

Oznaczono rozkład suchej masy całej słomy sześciu odmian ryżu malajskiego, stosując roztwór celulazy przed lub po traktowaniu słomy 4% roztworem mocznika lub 4% roztworem wodorotlenku sodu (NaOH).

Dla scharakteryzowania przebiegu rozkładu zastosowano równanie $p = a + b (1-e^{-ct})$. Tak traktowanie słomy mocznikiem jak i NaOH istotnie zwiększyło szybko- jak i wolno rozkładalne frakcje. Tempo rozkładu było istotnie obniżone w słomie traktowanej mocznikiem, lecz nie NaOH. Stałe rozkładu nie zależały od agronomicznych i morfologicznych właściwości nietraktowanej słomy.