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Nutritional evaluation of *Erianthus* spp., *Saccharum spontaneum*, and *Saccharum* spp. hybrids as feed

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Abstract

To obtain nutritional information for sugarcane cross-breeding aimed feed utilization, the morphological characteristics, chemical composition, and *in situ* dry matter degradability (DMD) of each of the ten accessions of *Erianthus* spp., *Saccharum spontaneum* and *Saccharum* spp. hybrids at 6 (6M) and 12 months (12M; after regrowth) were evaluated. For *Erianthus* spp. and *S. spontaneum*, the DMD at 48 h after incubation (DMD48h) and the corrected DMD48h (CDMD48h, expressed as DMD48h minus DMD0h) at 6M, exceeded those at 12M. Conversely, for *Saccharum* spp. hybrids, while the CDMD48h at 6M exceeded that at 12M, the DMD48h at 6M was lower than that at 12M. Compared to all other species, *Saccharum* spp. hybrids exhibited the highest DMD48h and CDMD48h. It was considered that a harvest with highest DMD could be obtained during the early growing stages in *Erianthus* spp. and *S. spontaneum*, but during a later growing stage in *Saccharum* spp. hybrids. DMD48h of *Erianthus* spp. correlated negatively with DM, NDFom and ADFom contents at 6 and 12 months after regrowth respectively. The ADL content of *S. spontaneum* correlated negatively with pooled DMD48h ($r = -0.63$; $P < 0.01$) and tended to correlate negatively with pooled CDMD48h ($r = -0.47$; $P < 0.10$). The NDFom and ADFom contents of *Saccharum* spp. hybrids correlated negatively with pooled DMD48h and positively with pooled CDMD48h, whereas these correlations were more affected by advancing maturity rather than the characteristics of accessions. These results suggest that DM and the fiber degradability of the accessions of genus *Saccharum* and *Erianthus* spp. could be estimated from certain chemical components, when selecting highly digestible accessions.

Discipline: Animal industry
Additional key words: cattle, chemical composition, *in situ* dry matter degradability, morphological characteristics, sugarcane

Introduction

Sugarcane varieties (*Saccharum* spp. hybrids) are widely cultivated for sugar production in tropical and subtropical areas. Their byproducts, e.g. sugarcane top, bagasse, and molasses, are also commonly used to feed ruminants in these regions, while sugarcane stalks and whole sugarcane may also be used as feed (Kawashima et al. 2002a, Kawashima et al. 2002b, Kawashima et al. 2003, Martin 1997, Pate et al. 2002, Suzuki et al. 2010, Suzuki et al. 2014). Developing varieties with higher abiotic stress

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tolerance, regrowth ability, or nutritive value will enhance the utility of sugarcane as feed.

Erianthus spp. and *Saccharum spontaneum*, which are extensively adaptable to adverse environments, have been used as materials for inter-specific or inter-genetic crosses with *Saccharum* spp. hybrids (Matsuoka 2006). For sugarcane crosses to be useful as feed, information on the morphological characteristics, yield, and disease resistance of the parents, as well as nutritional characteristics is necessary. Additionally, it may be possible to select based on nutritional characteristics at an early selection stage of crosses, if the nutritional value correlates with the morphological characteristics or chemical composition. Variation in nutritional characteristics among varieties and changes in nutritional characteristics with advancing maturity were reported well in *Saccharum* spp. hybrids (Kung & Stanley 1982, Lopez et al. 2003, Pate et al. 2002, Suzuki et al. 2010). Conversely, such information about *S. spontaneum* and *Erianthus* spp. is limited.

In this study, to obtain nutritional information for sugarcane cross breeding aimed feed utilization, we evaluated the chemical composition and *in situ* dry matter (DM) degradability of accessions of *Erianthus* spp., *S. spontaneum*, and *Saccharum* spp. hybrids at two different regrowth periods, as well as the potential for estimating *in situ* DM degradability using morphological or chemical characteristics.

Materials and methods

1. Sample collection

A total of 30 accessions, including ten accessions each of *Erianthus* spp., *S. spontaneum* and *Saccharum* spp. Hybrids respectively, were used in the present study. These accessions were selected from preserved accessions at Khon Kaen Field Crops Research Center (16°20'N, 102°49'E). The selection was performed to maximize variations in morphological characteristics. All selected accessions were harvested simultaneously at 12 months after regrowth (12M) and then 6 months after regrowth (6M). The grass height, diameter, and juice brix value of the 60 harvests (10 accessions × 3 species × 2 regrowth periods) were determined. To determine the dry weight ratio of stems, fresh leaves, and dead leaves, an aliquot of the harvest was separated into those parts, and weighed after drying (55°C, 48 h). Another aliquot of the harvest was chopped, dried in a forced-air oven (55 °C, 48 h), and then ground (SM2000, Retsch, Haan, Germany) for chemical analysis and *in situ* incubation (pore size, 1 and 2 mm, respectively).

2. Chemical analysis

Ground samples of accessions were analyzed for DM (135°C, 2 h), crude ash, and crude protein (CP) content (AOAC 2000). Additionally, the neutral detergent fiber content [assayed without sodium sulphite and without amylase treatment and expressed exclusive of residual ash (NDFom)] (Van Soest et al. 1991), acid detergent fiber content [expressed exclusive of residual ash (ADFom)] (Robertson & Van Soest 1981), and acid detergent lignin content (ADL) (Robertson & Van Soest 1981) were each determined. The contents of cellulose and hemicellulose were obtained as the differences between ADFom and ADL and NDFom and ADFom, respectively. Limitations on the sample amount meant that for three accessions of *S. spontaneum* at six months after regrowth, only crude ash and CP contents were determined.

3. *In situ* incubation

Two American Brahman steers fitted with ruminal cannula, averaging 429 kg, were used for *in situ* incubation. The steers were penned individually and fed a ration of 60% chopped Ruzigrass hay and 40% commercial concentrate as DM basis, in two equal portions at 0900 h and 1600 h daily, and had free access to water and a mineral block. Approximately 5 g of ground samples were placed into Dacron bags (10 × 14 cm; pore size, 55 μm). Replicate bags were suspended in the rumen of two cattle for 3, 6, 12, 24, 48, and 72 h respectively, in reverse order of incubation time and removed simultaneously. The *in situ* trials were conducted in respective species in the following order: *Erianthus* spp., *S. spontaneum*, and *Saccharum* spp. hybrids. After incubation, the bags were rinsed in cold water using a washing machine and the DM content was determined (55°C, 48 h). Four bags of each accession sample without incubation were also washed in cold water, and the DM contents were determined and represented as 0 h incubated bags. The *in situ* trials were performed in accordance with the Guide for the Care and Use of Experimental Animals (Curtis & Nimz 1988).

4. Statistical Analysis

The *in situ* DM degradability (DMD) of each accession (D,%) with incubation time (t, h) was fitted to the following non-linear model (Mertens & Loften 1980):

$$D = a + b(1 - e^{-c(t - LT)}),$$

where a, b, c and LT are the readily degraded fraction (%), slowly degraded fraction (%), degradation rate of fraction b (/h) and lag time (h), respectively. Fitting of the DMD to the non-linear model was performed by the NLIN procedure of SAS (SAS Institute 1999). The corrected DMD (CDMD) was defined as DMD minus DMD at 0 h (Inoue & Yamamoto 1992).

Analyses showing a simple correlation of the *in situ* degradabilities with morphological and chemical characteristics in each species were performed using the REG procedure of SAS (SAS Institute 1999). A two-way analysis of

Table 1. Morphological characteristics, chemical compositions, and *in situ* dry matter degradabilities of *Erianthus* spp., *Saccharum spontaneum*, and *Saccharum* spp. hybrids at 6 (6M) and 12 months (12M) after regrowth

	Erianthus spp.		*S. spontaneum*		*S.* spp. hybrids		SEM	Significance[†]		
	6M	12M	6M	12M	6M	12M		S	M	S×M
Grass height, cm	180.0	228.8*	128.0	153.3*	155.5	196.7*	11.3	<0.01	<0.01	0.58
	(23.8)[‡]	(23.7)	(18.2)	(19.5)	(11.1)	(17.8)				
Diameter, mm	14.8	14.9	7.3	5.9	23.2	21.6	1.1	<0.01	0.28	0.67
	(29.6)	(24.9)	(56.3)	(13.5)	(17.0)	(11.4)				
Brix, %	8.2	8.0	15.3	14.3	16.9	22.1*	0.7	<0.01	0.03	<0.01
	(24.3)	(23.6)	(17.2)	(25.1)	(10.3)	(5.9)				
Stem ratio, %	61.0	53.5	61.9	60.1	61.8	67.5	2.6	0.02	0.58	0.04
	(16.0)	(13.8)	(12.4)	(14.1)	(15.1)	(6.9)				
DM, %	29.7	46.4*	34.2	50.3	19.9	31.0*	1.5	<0.01	<0.01	<0.01
	(16.2)	(14.6)	(5.4)	(7.3)	(13.3)	(16.3)				
Crude ash, %DM	5.4*	4.2	5.3	4.9	5.4	4.3	0.4	0.47	0.73	0.02
	(8.8)	(17.8)	(30.2)	(28.3)	(13.2)	(27.4)				
CP, %DM	3.6*	2.0	1.9	1.8	4.0	3.4	0.2	<0.01	<0.01	<0.01
	(20.7)	(22.2)	(47.6)	(12.5)	(17.9)	(22.0)				
NDFom, %DM	82.4	82.1	78.7	77.7	74.1*	58.6	1.0	<0.01	<0.01	<0.01
	(2.7)	(2.9)	(3.4)	(3.4)	(2.7)	(9.1)				
ADFom, %DM	55.5	55.1	52.5	53.7	46.2*	38.9	1.3	<0.01	0.04	0.01
	(6.4)	(5.4)	(5.5)	(14.8)	(4.2)	(5.8)				
ADL, %DM	10.3	9.9	8.5	10.8	7.4*	5.9	0.4	<0.01	0.90	<0.01
	(8.5)	(8.7)	(10.2)	(21.4)	(6.7)	(13.3)				
Cellulose, %DM	45.1	45.2	43.9	42.9	38.8*	33.0	1.1	<0.01	0.02	0.03
	(7.0)	(5.8)	(5.1)	(15.8)	(4.6)	(5.8)				
Hemicel, %DM	27.0	27.0	26.3	24.0	27.9*	19.7	1.5	0.10	<0.01	0.02
	(10.7)	(5.5)	(3.7)	(35.7)	(8.1)	(27.9)				
DMD48h, %	35.3*	24.0	38.2*	32.9	48.4	53.7*	1.3	<0.01	<0.01	<0.01
	(9.5)	(17.2)	(10.0)	(12.5)	(11.5)	(7.0)				
CDMD48h, %	20.5*	13.5	19.2	19.3	24.8*	15.4	1.3	0.05	<0.01	<0.01
	(9.6)	(20.7)	(18.2)	(16.7)	(25.6)	(32.5)				

SEM, standard error of mean; DM, dry matter; CP, crude protein; NDFom, ash free neutral detergent fiber; ADFom, ash free acid detergent fiber; ADL, acid detergent lignin; DMD48h, *in situ* DM degradability after 48 h incubation; CDMD48h, DMD48h minus DMD at 0 h after incubation.
† Probability of significance among species (S), between months after regrowth (M), and their interaction (S×M).
‡ coefficient of variance (%)
* Significant difference between months within the same species (P<0.05).

variance with the sugarcane species and month of harvest after regrowth was performed using the GLM procedure. Significance was declared at $P < 0.05$ and a trend was accepted at $0.05 \leq P < 0.10$, unless otherwise stated.

Results and discussion

1. Morphological characteristics, chemical composition, and *in situ* DM degradability

CDMD mainly comprise slowly degraded fractions of CP, ether extracts, NDF, and non-fiber carbohydrates (NFC). The ether extract contents of *Saccharum* spp. hybrids have been reported to be <3% DM (Kung & Stanley 1982, Suzuki et al. 2010). The CP contents of all species in this study were <4%, whereas the NDFom contents were within the range 59 to 82% DM for all species (Table 1). Suzuki et al. (2010) assumed that the bulk of the NFC in sugarcane was sugar and was degraded quickly. It is, therefore, accepted that CDMD in sugarcane mainly reflects fiber degradability.

When fitting DMD of each accession to the non-linear model, no convergence occurred in some accessions. Accordingly, pooled DMD for each sample (6 and 12M per species) were used for model fitting, which meant no statistical comparisons of parameters between months or among species were performed.

For *Erianthus* spp., the DMD at each incubation time was lower at 12M than at 6M (Fig. 1). There was no incremental change in brix value (Table 1) and DMD at 0 h (Fig. 1) with an increase in the number of months after regrowth, indicating little sugar accumulation from 6 to 12M. The CDMD at 48 h after incubation (CDMD48h) in the 12M sample was lower than that in the 6M sample (Table 1), indicating a decrease in fiber degradability over time. Accordingly, in *Erianthus* spp., the reduction in DMD with advancing maturity was mainly due to decreasing fiber degradability. This decrease in DMD with advancing maturity is generally observed in tropical grasses (Gomide et al. 1969).

The grass height in *S. spontaneum* at 12M exceeded

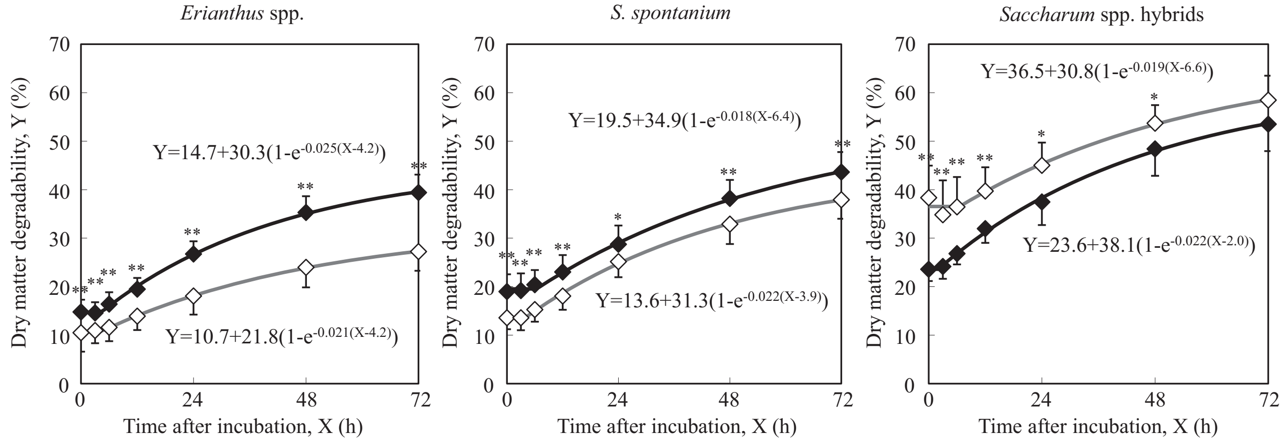

Fig. 1. Means with SD of *in situ* dry matter degradability at 6 (◆) and 12 (◇) months after regrowth and regression curves and equations at 6 (—) and 12 months (—) for *Erianthus* spp., *Saccharum spontaneum*, and *Saccharum* spp. hybrids
*P < 0.05; **P < 0.01.

that at 6M (Table 1), whereas DMD in each incubation time was lower at 12M than that at 6M (Fig. 1). Sultan et al. (Sultan et al. 2007) also found lower *in vitro* DMD of *S. spontaneum* at the mature stage compared to the early bloom stage, and concluded that this reduction was attributable to an increase in fiber content. Other characteristics in Table 1 showed no significant differences between regrowth periods. Since there was little change in the morphological and chemical characteristics between 6 and 12M, *S. spontaneum* might almost mature at 6M regrowth; exhibiting the potential for faster regrowth than the other species.

The brix value and DMD48h in *Saccharum* spp. hybrids at 12M exceeded those at 6M (Table 1). DMD at each incubation time, excluding 72 h, were also higher at 12M than 6M (Fig. 1). Meanwhile, the fiber contents at 12M were lower than those at 6M (Table 1). These results contradicted those in *Erianthus* spp. and *S. spontaneum*. Higher DMD at 0 h and a lower CDMD48h at 12M rather than 6M indicate the accumulation of sugar and decreasing fiber degradability with advancing maturity. Suzuki et al. (2010) also observed the accumulation of NFC, decrease in NDFom content, and decrease in effective NDFom degradability with maturation. Additionally, Kung & Stanley (1982) and Pate et al. (2002) observed an increase in DM degradability and decrease in fiber content with advancing maturity in *Saccharum* spp. hybrids.

Overall, the DMD48h in *Saccharum* spp. hybrids at 12M was the highest among all species and regrowth periods and mainly attributable to sugar accumulation. Each species in the present study showed lower CP content and higher NDFom content (Table 1), compared to forage in the standard feed composition tables in Thailand (The Working Committee of Thai Feeding Standard for Ruminant 2010). There are also many observations concerning the low CP content in *Saccharum* spp. hybrids (Kung & Stanley 1982, Lopez et al. 2003, Pate et al. 2002, Suzuki et al. 2010, Suzuki et al. 2014). For cattle fed on the genus *Saccharum* or *Erianthus* spp., it is essential to supply a CP source as an alternative to the forage generally used.

2. Correlation of *in situ* DM degradability with morphological and chemical characteristics

For *Erianthus* spp., DMD48h pooled at 6 and 12 months after regrowth correlated negatively with grass height and dead leaf ratio, whereas the pooled DMD48h correlated positively with the fresh leaf ratio, as well as CP and crude ash contents (Table 2). These correlations are considered attributable to maturation, i.e. increasing of height and reduction of crude ash and CP contents (Table 1), because no significant correlations emerged between DMD48h and these characteristics at 6 and 12 months respectively. Meanwhile, DMD48h correlated negatively or tended to do so with DM, NDFom and ADFom contents at both 6 and 12 months (Table 2). These correlations indicate that the extent of maturation differs among accessions, even if the regrowth period of accessions is similar, because a significant positive correlation between DM and NDFom content emerged at both growing periods (r = +0.68 at 6 months, r = +0.68 at 12 months), and maturation of tropical grasses increases its DM and fiber contents (Gomide et al. 1969). Accordingly, DM, NDFom and ADFom contents would be available to indicate DM degradability for *Erianthus* spp., while these relationships include the effect of advancing maturity, which differs among accessions. The pooled CDMD48h negatively correlated or tended to correlate negatively with height, dead leaf ratio and DM content, and positively correlated with fresh leaf ratio, CP and crude ash contents (Table 3). These significant correlations or correlation trends with pooled CDMD48h reflect decreasing of CDMD48h with advancing maturity.

Table 2. Correlation coefficients between *in situ* dry matter degradability after 48 h incubation (DMD48h) and morphological and chemical characteristics within accessions of *Erianthus* spp., *Saccharum spontaneum* and *Saccharum* spp. hybrids. at 6 and 12 months after regrowth

	Height cm	Dia mm	Stem %	FL %	DL %	Brix %	DM %	CP %DM	CA %DM	NDFom %DM	ADFom %DM	ADL %DM
DMD48h, %												
Erianthus spp.												
6 months	0.24	0.23	-0.41	0.35	0.21	0.15	-0.78**	0.60+	0.47	-0.58+	-0.81**	-0.72*
12 months	-0.38	-0.37	0.10	0.28	0.20	-0.07	-0.84**	0.69	0.09	-0.82**	-0.85**	-0.40
pooled†	-0.46*	-0.06	0.27	0.46*	-0.71**	0.07	-0.94**	0.87**	0.66**	-0.32	-0.39+	-0.06
S. Spontaneum												
6 months	-0.88**	0.44	0.36	-0.38	-0.22	0.51	0.05	-0.23	-0.03	-0.72+	-0.84*	-0.66
12 months	0.42	0.08	0.36	0.10	-0.45	0.43	-0.57+	-0.41	-0.28	-0.04	-0.42	-0.48
pooled	-0.20	0.11	0.31	0.21	-0.47+	0.46+	-0.43+	-0.20	-0.28	-0.14	-0.44+	-0.63**
S. spp. hybrids												
6 months	-0.58+	-0.19	-0.28	0.18	0.27	-0.52	-0.32	0.51	-0.19	0.40	-0.40	-0.54
12 months	0.54	-0.37	0.22	-0.61+	0.22	-0.20	0.03	-0.36	-0.28	-0.42	-0.61+	-0.26
pooled	0.35	-0.33	0.07	-0.14	-0.01	0.27	0.37	-0.07	-0.21	-0.49*	-0.64**	-0.59**

Dia., diameter; Stem, stem ratio; FL, fresh leaf ratio; DL, dead leaf ratio; Brix, juice brix value; DM, dry matter; CP, crude protein; CA, crude ash; NDFom, ash free neutral detergent fiber; ADFom, ash free acid detergent fiber; ADL, acid detergent lignin.
† Pooled DMD48h at 6 and 12 months after regrowth.
+ P<0.10; *P<0.05; **P<0.01.

Table 3. Correlation coefficients between corrected *in situ* dry matter degradability after 48h incubation (CDMD48h) and morphological and chemical characteristics within accessions of *Erianthus* spp., *Saccharum spontaneum* and *Saccharum* spp. hybrids. at 6 and 12 months after regrowth

	Height cm	Dia mm	Stem %	FL %	DL %	Brix %	DM %	CP %DM	CA %DM	NDFom %DM	ADFom %DM	ADL %DM
CDMD48h, %												
Erianthus spp.												
6 months	0.36	-0.25	-0.43	0.35	0.25	0.15	-0.28	0.27	0.21	-0.22	-0.28	-0.58+
12 months	-0.25	-0.71*	-0.48	0.14	0.44	0.10	-0.59+	0.59+	0.89**	0.08	-0.12	-0.30
pooled†	-0.41+	-0.28	0.13	0.54*	-0.66**	0.12	-0.85**	0.80**	0.85**	0.04	-0.05	0.01
S. Spontaneum												
6 months	-0.82*	0.25	0.23	-0.27	-0.13	-0.08	0.18	-0.04	-0.08	-0.21	-0.40	-0.35
12 months	0.50	0.20	0.50	0.02	-0.59+	0.03	-0.56+	-0.35	-0.37	0.00	-0.35	-0.62+
pooled	-0.03	0.21	0.38	-0.05	-0.40	0.01	-0.30	-0.11	-0.28	-0.07	-0.33	-0.47+
S. spp. hybrids												
6 months	-0.49	-0.08	-0.22	0.19	0.19	-0.61+	-0.42	0.67*	-0.24	0.70*	-0.13	-0.50
12 months	-0.75*	-0.52	-0.84**	0.11	0.61+	-0.15	-0.38	0.31	0.05	0.45	0.21	-0.37
pooled	-0.75**	0.00	-0.52*	0.15	0.51*	-0.74**	-0.70**	0.59**	-0.06	0.74**	0.59**	0.32

Dia., diameter; Stem, stem ratio; FL, fresh leaf ratio; DL, dead leaf ratio; Brix, juice brix value; DM, dry matter; CP, crude protein; CA, crude ash; NDFom, ash free neutral detergent fiber; ADFom, ash free acid detergent fiber; ADL, acid detergent lignin.
† Pooled CDMD48h at 6 and 12 months after regrowth.
+ P<0.10; *P<0.05; **P<0.01.

In *S. spontaneum*, relationships between degradabilities and morphological or chemical characteristics were weak compared to the other species. The pooled DMD48h negatively correlated with ADL content (Table 2), and also pooled CDMD48h tended to correlate negatively with ADL content (Table 3). Meanwhile, there was no correlation between DMD48h and ADL content in each month. A trend showing negative correlation emerged between CDMD48h and ADL content at 12 but not 6 months. The lack of significant differences in chemical components between 6 and 12 month periods (Table 1) means negative correlations of pooled DMD48h and CDMD48h with ADL content were not attributable to the regrowth period. Accordingly, these negative correlations might indicate that the accession with lower ADL content involves higher fiber degradability, and consequently higher DM degradability.

In *Saccharum* spp. hybrids, pooled DMD48h correlated negatively with NDFom, ADFom, and ADL contents, whereas no correlations emerged between the DMD48h and those contents in each month, excepting ADFom content at 12 months (Table 2). The pooled CDMD48h correlated positively with NDFom and ADFom contents, whereas no correlations of CDMD48h with those contents of each month emerged, except for NDFom content at 6 months (Table 3). Additionally, the chemical components and degradabilities at 6 and 12 months differed significantly (Table 1). Accordingly, the correlations of pooled degradabilities with fiber components are considered far more affected by advancing maturity rather than the characteristics of accessions.

Conclusion

It is indicated that DM and fiber degradabilities in *Erianthus* spp. and *S. spontaneum* decrease with advancing maturity. Conversely, in *Saccharum* spp. hybrids, only fiber degradability decreased with maturation, but DM degradability increased. Estimations of DM and fiber degradabilities are partially possible using several chemical components as an indicator. In *Erianthus* spp., DMD48h could be estimated from DM, NDFom or ADFom contents. In *S. spontaneum*, it is suggested that the ADL content would be used as an indicator of DMD48h and CDMD48h. In *Saccharum* spp. hybrids, NDFom and ADFom contents correlate with DMD48h and CDMD48h, but these relationships were more affected by advancing maturity rather than the characteristics of accessions.

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