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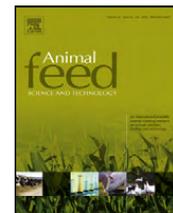
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## Impact of simulated field drying on *in vitro* gas production and voluntary dry matter intake of rice straw

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### ABSTRACT

Due to the recent expansion of the motor fuel ethanol industry, which is largely based upon traditional food and feed crops, supplies of ruminant feeds have become limited in many areas of the world thereby creating a need for alternatives. Rice straw, a substantial biomass source worldwide, could fill this role if its nutritional value was higher. Our aims were to determine changes in the nutritive value of rice straw during long term storage, and short term field drying, as well as the voluntary DM intake of rice straw by heifers before, during and immediately after field drying. In Experiment I, rice straw was macerated and stored for 82 days after baling, but there was no effect on chemical components or 30 h *in vitro* fermentability of neutral detergent fiber (NDF) due to maceration or storage time. In Experiment II, samples of a late maturity tall (M401) and an early maturity short (M202) rice straw variety were collected prior to harvest, during simulated field drying and when fully dried. The M401 had higher acid detergent fiber (473 versus 445 g/kg dry matter (DM);  $P < 0.01$ ), lignin(sa) (38 versus 34 g/kg DM;  $P < 0.01$ ), total Si (55.0 versus 43.0 g/kg DM;  $P < 0.01$ ) and Si in NDF (372 versus 270 g/kg DM of total Si;  $P = 0.01$ ). The M202 produced 14.4, 10.6 and 9.1% more gas than the taller M401 at 4, 24 and 72 h of *in vitro* fermentation, respectively. Fresh plants produced 6.8, 15.6 and 8.9% more gas than plants collected during simulated field drying and as dry plants at 4, 24 and 72 h of *in vitro* fermentation, respectively. In Experiment III, voluntary DM intake of rice plants was measured at the same three stages (*i.e.*, fresh, during simulated field drying, dry), and results were consistent with Experiment II, in that DM intake of fresh plants was higher than plants during both simulated field drying and when dry (5.14 versus 4.13 versus 3.69 kg DM/day;  $P < 0.01$ ). That long term storage of straw after baling did not impact levels of its chemical components or 30 h *in vitro* fermentability of NDF, but gas production of fresh plants was higher than that of plants during simulated field drying and when fully dried, supports the hypothesis that the much higher voluntary DM intake potential of fresh rice plants occurred due to changes during field drying that reduced its fermentability. It seems certain that this depressed DM intake was related to the decrease in fermentability of dry versus fresh plants, although the cause

**Abbreviations:** ADF, acid detergent fiber; ADNDF, sequential analysis with acid detergent followed by neutral detergent; CP, crude protein; D, dry rice straw; DM, dry matter; FD, field drying; dNDF<sub>30</sub>, 30 h *in vitro* digestible NDF; Fz, frozen rice straw; GP, gas production; NDF, neutral detergent fiber; OM, organic matter; Pre, pre-harvest rice plants; SFD, rice straw during simulated field drying; SiADF, Si in ADF; SiADNDF, Si in ADNDF; SiNDF, Si in NDF.

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of the reduced fermentability is not clear. However analyses of Si levels in the detergent fiber fractions provided indications that the location of the Si in the structural carbohydrates may be important in this regard.

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## 1. Introduction

Unstable feed prices, together with increasing plant diversion to biofuel production, has negatively impacted forage availability to the dairy industry in many parts of the world. This has stimulated renewed interest in nutritional studies of plant by-products, particularly cereal straws, as feeds. Rice is the world's second largest cereal crop, first in Asia and third in the USA (FAOSTATS, 2009), but it creates the largest amount of crop residues worldwide (377 million tonnes per year; FAOSTATS, 2009). In California, until 1996, rice crop residues were mostly field incinerated to prevent plant diseases. This strategy is now restricted in California due to air quality concerns and this has created a need for more off-field uses of rice straw.

Rice straw is known to have a low nutritional value and numerous studies have been completed over the years to increase it. Rice straw is unique among feed plants in its high silica (Si) content but, within rice straws, there is a wide range of values (1–100 g/kg dry matter (DM); Epstein, 1999) due mostly to variety and environmental conditions during growth (Khush et al., 1988; Van Soest, 2006). Rice plants need a high Si concentration to enhance strength and rigidity (Ma and Yamaji, 2006), resist disease (Kim et al., 2002; Rodrigues et al., 2005; Ma and Yamaji, 2006), reduce cuticular transpiration and improve water use efficiency (Kim et al., 2002). In the plant, Si is translocated from the soil to the shoot as silicic acid where it is polymerized into silica gel ( $\text{SiO}_2 \cdot n\text{H}_2\text{O}$ ), with more than 0.9 of total Si being in this form, and it is then deposited in the bulliform cells and under the cuticle (Ma and Yamaji, 2006) as the stable  $\text{SiO}_2$  complex. In Si accumulating plants, such as rice, some researchers observed negative effects of higher Si on plant digestibility (Van Soest and Jones, 1968; Bae et al., 1997; Agbagla-Dohnani et al., 2003) and ruminal *in sacco* degradation of rice straw (Agbagla-Dohnani et al., 2003) indicating that Si may, in some way, have a negative effect on plant digestion or degradation. In contrast, Khush et al. (1988) found no effect of Si on *in vitro* organic matter (OM) digestibility.

There are several hypotheses relative to possible mechanisms by which Si negatively impacts digestion of plant cell walls of rice straw. Some researchers have suggested that Si acts as a physical barrier (Bae et al., 1997; Kim et al., 2002), and others that it has an inhibitory action on hydrolyzing enzymes in the rumen (Smith and Urquhart, 1975; Agbagla-Dohnani et al., 2003). Although there are studies that suggest a negative correlation to Si and digestibility, it is generally considered that Si, after being deposited in the plant, does not change chemically and will not react with other plant components, such as non-structural carbohydrates and/or structural carbohydrates (Van Soest, 2006). However, it is possible that Si changes chemical form and/or forms linkages to other structures in the plant that increases or decreases its digestibility, thereby resulting in differing digestibility which also may vary according to the phase of the plant (*i.e.*, green or dried as straw) due to the different chemical forms of the Si in plants in these phases.

It is common that forages lose some nutritional value during field drying and conservation. Losses occur due to plant respiration, leaf crushing, leaching by rain and losses during storage due to oxidation and fermentation that all contribute to a decrease in nutritional value (Lee, 1988). These factors adversely affect the most digestible components of the plant, especially the water-soluble carbohydrates and proteins, which increases the fiber content, reduces OM and crude protein (CP) digestibility, and reduces DM intake (Lee, 1988). The decline in chemical composition and nutritive value upon field drying varies in magnitude and depends on factors such as speed of drying, which can be enhanced by maceration (Nader and Robinson, 2008), and weather conditions (Lee, 1988), who also suggested that the proportional change in nutritive value from fresh plants of dry grasses is  $-0.016$  and  $-0.030$  for OM digestibility and voluntary DM intake, respectively. However when Sharif (1984) compared intake and digestibility of fresh rice plants at harvest (*i.e.*, 590 g/kg dry matter (DM)) to dry rice straw (*i.e.*, 920 g/kg DM) in sheep, he found that they consumed and digested 30% more DM when fed fresh *versus* stored straw. However as these rice plants were not from the same lot, a confounding of lot and treatment occurred which may have impacted the findings.

The aims of our experiments were to determine losses of nutritive value in rice straw during long term storage (*i.e.*, 82 days), as well as during short term field drying of fresh plants to create straw. The possible role of Si in this loss of nutritive value relative to its location in the fibre fractions was also assessed. Finally, voluntary DM intake of rice straw by heifers before, during and immediately after field drying were determined.

## 2. Materials and methods

### 2.1. Experiment 1—impacts of long term storage of rice straw on its nutrient value as impacted by maceration

#### 2.1.1. Rice straw treatments

Rice straw baled from two fields, that were part of the study on maceration of rice straw reported by Nader and Robinson (2008), were used in a split-field design that divided each field into control and macerated sections. These 2.4 ha rice fields, near Williams (CA, USA), had been managed similarly during growth and were harvested with a Hardy Harvester (Hardy Co.,

College City, CA, USA) on the same day with no pre-swathing. Immediately after harvest (*i.e.*, within 1 h), the half fields to be macerated were processed with a Model 6600 Macerator (Agland Industries, Arborg, MB, Canada) to a 6.26 mm theoretical length. The control half fields were not processed. Rice straw in all fields was allowed to dry for 72 h and then baled and stacked separately by treatment.

Immediately after baling, four randomly selected bales from each half field (*i.e.*, 16 in total) were transported to the University of California (Davis, CA, USA) and stored under cover in four stacks of four bales each to represent each half field (*i.e.*, two stacks of control and two stacks of macerated bales).

Macerated and control rice straw bales were sampled on day 0 (*i.e.*, immediately after baling, but before transport to UC Davis) and on days, 3, 6, 10, 14, 18, 23, 28, 33, 38, 44, 50, 56, 62, 68, 75 and 82 after baling. Each rice straw sample, representing each of the four stacks of rice straw, consisted of eight cores collected with a 'golf club' hay probe (*i.e.*, Seifert Analytical, Lodi, CA, USA) from each end of each bale. Samples were stored in a freezer at  $-16^{\circ}\text{C}$  until analysis.

### 2.1.2. Analytical methods

Straw samples were ground to pass a 40 mesh screen on an Intermediate Wiley Mill or a 1 mm screen on a model 4 Wiley Mill. Determination of moisture was by gravimetric loss of free water by heating to  $105^{\circ}\text{C}$  in a forced air oven for 2 h. Neutral detergent fiber (NDF) was determined according to Van Soest et al. (1991) without a heat stable amylase and expressed inclusive of residual ash. Lignin(sa) was determined by the sulfuric acid procedure (AOAC, 1997, method 973.18). *In vitro* digestibility of NDF at 30 h of *in vitro* incubation was determined as described by Robinson et al. (1999).

### 2.1.3. Statistical analysis

Data were analyzed statistically using PROC MIXED of SAS (2002). The model included maceration and time (*i.e.*, 0–82 days) as fixed effects, with 'time' as a repeated measure. Significant differences were accepted if  $P \leq 0.05$  and trends to significance were accepted if  $0.05 < P \leq 0.10$ .

## 2.2. Experiment II—impacts of simulated field drying of rice straw on its nutrient value as impacted by plant height, stage of growth and simulated field drying

### 2.2.1. Rice straw treatments

Two medium grain semi-dwarf rice varieties, a late maturity variety with an average height of 98 cm (var. M401) and an early maturity shorter variety of 92.7 cm (var. M202) were hand-harvested approximately 10 cm above the ground (*i.e.*, just above the water line) on several occasions prior to harvest, and at harvest, from two fields each on two commercial rice farms between Williams and Maxwell (CA, USA). The farm locations were separated by approximately 2 km and were on the same soil type with the same weather conditions and management.

On days  $-14$ ,  $-10$ ,  $-6$  and  $-2$  relative to harvest,  $0.3 \text{ m}^2$  replicates of standing rice plants were sampled *in situ* between 9:30 and 11:00 h with 8 m spacing along a 16 m linear transect in two fields per variety. Sampling started when superficial water had disappeared from the rice leaves in order to avoid erroneous DM values. A  $0.3 \text{ m}^2$  area was measured using a metal square, and plants were cut with scissors and placed on a portable table. Sub-samples were created at each sampling that were 4 and 10 randomly selected plants. Rice, hulls and senesced flag leaves were removed from the plants during sampling and all sub-samples were placed in plastic sealable bags, which were placed in a chest with freezer packs. During the 1 h travel time from the field to UC Davis, the eight samples of four plants each were chopped to approximately 2–3 mm lengths with a commercial paper cutter (EPI 26315, Elmer's Products, Inc., Columbus, OH, USA) and placed in 100 ml plastic containers with sealable tops. Immediately upon arrival at the laboratory, these chopped samples were weighed into glass syringes with pistons lubricated with Vaseline and incubated with buffered rumen fluid (described in Section 2.2.2), as well as being weighed for determination of DM at  $105^{\circ}\text{C}$  for 24 h in a forced air oven. Thus, the time between harvest and incubation was 2.0–3.5 h. The reserve sample with 10 rice straw plants was stored in a freezer at  $-16^{\circ}\text{C}$ .

At harvest, a  $1 \text{ m}^2$  sample was sampled *in situ* with 8 m spacing along the same 16 m linear transect in two fields (*i.e.*, 8 samples). All material was transported to UC Davis and stored under cover in simulated windrows in a naturally ventilated area for subsequent sampling. The rice straws were turned daily by hand for 6 days in order to facilitate drying. On days 1, 2, 3, 4, 6, 8, 12, 19 and 33 relative to harvest, rice plants were sampled from each simulated windrow between 8:00 and 10:00 h. Sub-samples were created as before at each sampling with 4 and 10 randomly selected plants. Rice, hulls and flag leaves were removed from plants during sampling, and all sub-samples were placed in plastic sealable bags. During this time, the 8 samples of 4 plants each were chopped to approximately 2–3 mm lengths with the same EPI 26315 commercial paper cutter and placed in 100 ml plastic containers with sealable tops. These chopped samples were weighed into glass syringes with pistons lubricated with Vaseline and incubated with buffered rumen fluid, as described above, as well as being weighed for DM determination at  $105^{\circ}\text{C}$  for 24 h in a forced air oven. The remaining sample was stored in a freezer at  $-16^{\circ}\text{C}$  for later fiber and Si analyses. Thus, the time between sampling and incubation was 1–3 h. The reserve sample with 10 rice straw plants was stored in a freezer at  $-16^{\circ}\text{C}$ .

### 2.2.2. *In vitro* gas production

Gas production from chopped straw was determined according to Menke and Steingass (1988). Fresh material was weighed in duplicate to allow an approximate 200 mg DM sample, which was introduced into 100 ml calibrated glass syringes

(Model Fortuna, Lonsee-Ettlenscheiß, Germany). A reference alfalfa hay was used as the internal standard, with a 200 mg sample weighed to duplicate syringes. A mixture containing 472.5 ml distilled water, 236.25 ml buffer solution, 236.25 ml main element solution, 0.1185 ml trace element solution and 1.20 ml resazurin solution per liter was prepared in an Erlenmeyer flask. After warming to 39 °C, and continuous flushing with CO<sub>2</sub>, a reduction solution consisting of 45.0 ml distilled water, 1.875 ml 1-n-NaOH, and 296.25 mg Na<sub>2</sub>S·9H<sub>2</sub>O was added. Rumen liquor was collected from a Holstein cow fitted with a large diameter flexible rumen cannula (Bar Diamond Inc., Parma, ID, USA) fed twice daily a diet of oat hay, alfalfa hay, almond hulls and mineral premix. The ratio of rumen liquor to buffer was 1:2. Thirty milliliters of rumen liquor/buffer mixture was added to each syringe which were placed in a water bath at 39 °C and gas production was recorded after 1, 2, 3, 4, 5, 6, 12, 18, 19, 20, 22, 24, 26, 28, 50 and 72 h of incubation. Syringes were shaken gently before each measurement. Total gas values were corrected for blank incubations and the alfalfa hay standard.

### 2.2.3. Analytical methods

The DM was determined by drying at 105 °C for 24 h followed by equilibration in desiccators, with particle size for DM determination being 0.5–1.0 mm. Total N was determined with an N gas analyzer utilizing induction furnace and thermal conductivity (LECO FP-528) by AOAC method 990.03 (1997). Neutral detergent fiber (aNDF) analyses included a heat stable amylase (Van Soest et al., 1991). Acid detergent fiber (ADF) was determined according to AOAC method 973.18 (1997). The aNDF and ADF values include residual ash in order to create residues for Si analysis. Lignin(sa) was determined by the sulfuric acid procedure (AOAC, 1997, method 973.18). ADNDF was determined by sequential analyses (in order to create a residue for Si assay) in which ADF was extracted with ND, the ADF being determined according to AOAC method 973.18 (1997) and NDF determined without a heat stable amylase (Van Soest et al., 1991).

Silica was determined by nitric acid/hydrogen peroxide/hydrofluoric acid digestion by ICP analyses (Feng et al., 1999). Stored rice plants were analyzed for NDF, ADF and ADNDF. Frozen (Fz) samples of varieties M202 and M401 from stage 'Pre' and dry plants from stage 'D' were weighed to 500 mg of estimated DM for ADF and NDF analyses, and to approximately 800 mg of DM to ADNDF analyses. Samples from the frozen and the dry plants, and the residues obtained from their fiber analyses, were analyzed for Si.

### 2.2.4. Calculations and statistical analysis

The rate and extent of gas production were calculated using the model:

$$Y = b(1 - e^{-k \times t})$$

where  $Y$  is gas volume (ml) at time  $t$ ,  $b$  is potential gas production (ml) and  $k$  is rate (/h) at which gas is produced.

Data were analyzed statistically using the PROC MIXED option of SAS (2002) with a model that included fixed effects of variety (*i.e.*, M202, M401) and stage (*i.e.*, growing (Pre), during simulated dry down (SFD) or dry (D)), and all 2-way interactions. Stage 'Pre' are the samples from the 4 days before harvest (*i.e.*, days -14, -10, -6 and -2 relative to harvest), stage 'SFD' is from the 4 days after harvest (*i.e.*, days 1, 2, 3 and 4 relative to harvest) and stage 'D' are the last five samples (*i.e.*, days 6, 8, 12, 19 and 33 relative to harvest). Statistical differences were considered significant if  $P < 0.05$  and tendencies to significance were accepted if  $0.05 < P < 0.1$ . Data in tables are least square means with the standard errors of means.

## 2.3. Experiment III—impacts of simulated field drying of rice straw on its chemical composition and voluntary DM intake

### 2.3.1. Experiment outline

Eight Jersey/local breed crossbred milking purpose heifers of uniform body weight (*i.e.*,  $152 \pm 10.9$  kg predicted from a girth tape) were acquired from local farmers in a rice growing village of India (Belagere village, Channagiri Taluk, Davanagere district, Karnataka State) and housed in a closed cattle barn in a single row. Heifers were provided similar management, and the diet was 0.75 kg of a concentrate heifer/day and *ad libitum* access to Sona Masoori rice straw (var. DPT-5204) in three physical forms that were fed consecutively (relative to their growth in the field or subsequent drying) in an individual feed bunk with fresh water available.

The physical forms were fresh rice plants harvested daily from 11 days pre-harvest through the day of harvest (*i.e.*, stage Pre), followed by post-harvest rice plants wilting from the day of harvest through day 5 post-harvest (*i.e.*, stage field drying, FD) and by post-harvest rice straw completely dried and ready for stacking (*i.e.*, stage dry, D).

Harvesting and feeding of fresh rice plants started on day -11 prior to harvest until day 0 (*i.e.*, Pre), when the plants had reached physiological harvest maturity. Rice plants were harvested manually, with grain completely removed by hand, followed by driving a tractor over the plants five times. The resulting rice plants were fed fresh in small portions over the entire day and residues were measured the following day before feeding new rice plants. Samples of rice plants/straw offered, and refusals, were collected daily and stored in plastic bags at 4 °C until transported to the laboratory. On the day of harvest the rest of the field was harvested by combine harvesters, with about 0.45 ha of the rice crop manually harvested with grains threshed by hand and the straw dried in the sun. As it was drying, a portion of the wilting straw was removed daily to feed to the heifers from day 1 to 5 relative to harvest (*i.e.*, FD) and then dry rice plants from day 6 to 10 (*i.e.*, D). Samples of the straw offered and refused were collected daily and stored in plastic bags as previously described. Samples collected during this study were transported to the laboratory, cut into 10 cm lengths and dried at 60 °C for DM determination. A sub-sample

**Table 1**Effects of rice maceration and time of storage on NDF, dNDF<sub>30</sub> and lignin(sa) of rice straw [Experiment I].

	Treatment (Trt)			P		
	Control	Macerated	SEM	Time	Trt	Time × Trt
Dry matter, g/kg	957	957	1.8	0.07	0.76	1.00
NDF, g/kg DM	680	691	2.2	0.42	<0.01	0.20
dNDF <sub>30</sub> <sup>a</sup> , g/kg NDF	407	406	5.2	0.52	0.63	0.28
Lignin(sa), g/kg DM	65.5	66.6	0.91	<0.05	0.23	0.34

DM, dry matter; NDF, neutral detergent fibre.

<sup>a</sup> 30 h *in vitro* digestion of NDF.

of the dried materials was ground through a 1 mm screen, pooled by stage (*i.e.*, Pre, FD, D) and stored at room temperature (*i.e.*, 30 °C) for later chemical analysis.

### 2.3.2. Analytical methods

Standard methods as described in AOAC (1990) were used for straw chemical analysis being: determination of DM (930.15), ash (924.05), CP (984.13) and ADF (973.18). The NDF was determined according to Van Soest et al. (1991) without amylase or sodium sulfite, and NDF and ADF are expressed inclusive of residual ash. Lignin(sa) was determined according to Robertson and Van Soest (1981). Silica was determined by nitric acid/hydrogen peroxide/hydrofluoric acid digestion by ICP analyses (Feng et al., 1999).

### 2.3.3. Statistical analysis

Data were analyzed using the PROC MIXED option of SAS (2002) with a model that included fixed effects of stage (*i.e.*, Pre, SFD and D) with repeated measures. Stage 'Pre' is the samples from the 11 days before harvest (*i.e.* days –11, –10, –9, –8, –7, –6, –5, –4, –3, –2, –1 relative to harvest), stage 'FD' are the 5 days after harvest (*i.e.*, days 1, 2, 3, 4, 5 relative to harvest) and stage 'D' are the last 5 days (*i.e.* days 6, 7, 8, 9, 10 relative to harvest). Statistical differences were considered significant if  $P < 0.05$  and tendencies to significance were accepted if  $P < 0.10$ . Data in tables are least square means.

## 3. Results

### 3.1. Experiment I—impacts of long term storage of rice straw on its nutrient value as impacted by maceration

Time of storage from baling through 82 days did not affect the NDF level of straw, or its dNDF<sub>30</sub>, but the lignin(sa) content declined ( $P < 0.05$ ) slightly with time (Table 1). Maceration increased the NDF content of straw (680–691 g/kg;  $P < 0.01$ ), without affecting dNDF<sub>30</sub>. There were no maceration by time interactions.

### 3.2. Experiment II—impacts of simulated field drying of rice straw on its nutrient value as impacted by plant height, stage of growth and simulated field drying

The taller later maturity variety (*i.e.*, M401) had higher ADF (473 versus 445 g/kg DM;  $P < 0.01$ ) and lignin(sa) (38 versus 34 g/kg DM;  $P < 0.01$ ; Table 2).

A small decrease ( $P < 0.05$ ) in CP content occurred between the Pre stage and after the plants were dry (*i.e.*, 43 versus 39 g/kg of DM). There was a variety by stage interaction for ADF ( $P = 0.03$ ), with a larger difference between M401 and M202 at the Pre stage.

Gas production at 4, 24 and 72 h of *in vitro* incubation was affected by variety and stage but there were no variety by stage interactions (Table 3). The shorter early maturity variety (*i.e.*, M202) produced 14.4, 10.6 and 9.1% more gas than the tall late maturity variety (*i.e.*, M401) at 4, 24 and 72 h, respectively; which is consistent with Khush et al. (1988) who concluded,

**Table 2**

Chemical components (g/kg DM) of rice varieties M202 and M401 pre-harvest (Pre), during simulated field drying (SFD) and as dry straw (D) [Experiment II].

	Variety (V)		SEM	Stage (S) <sup>a</sup>			SEM	P		
	M202	M401		Pre	SFD	D		V	S	V × S
CP	42	39	1.4	43a	39b	39b	1.3	NS	0.04	NS
aNDF	606	612	4.5	613	609	605	4.0	NS	NS	NS
ADF	445	473	2.2	456	460	460	2.8	<0.01	NS	0.03
Lignin(sa)	34	38	0.6	36	37	35	0.7	<0.01	NS	NS

Different letters (a and b) within rows and stage differ ( $P < 0.05$ ). ADF, acid detergent fibre; CP, crude protein; DM, dry matter; aNDF, neutral detergent fibre.<sup>a</sup> Pre, fresh plants sampled before harvest; simulated field drying (SFD), rice plants sampled during the simulated field drying period immediately after harvesting (*i.e.*, 0–4 days); Dry (D), rice plants sampled during the storage of rice straw (*i.e.*, 5–19 days after harvest).

**Table 3**

Gas production (ml/g DM) *in vitro* at 4, 24 and 72 h after incubation of fresh material from two rice varieties, M202 and M401, sampled pre-harvest, during simulated field drying and as dry straw [Experiment II].

	Variety (V)		SEM	Stage (S) <sup>a</sup>			SEM	P		
	M202	M401		Pre	SFD	D		V	S	V × S
Gas production, ml/g DM										
4 h	27	23	0.7	27b	24a	25a	0.6	<0.01	0.02	NS
24 h	127	113	2.1	132b	115a	112a	2.0	<0.01	<0.01	NS
72 h	188	171	3.1	190b	176a	173a	2.9	<0.01	<0.01	NS
Gas production constants										
b, ml/g DM	200	183	3.3	198b	191a	187a	3.0	0.01	0.02	NS
k, /h	0.041	0.037	0.0019	0.042	0.037	0.037	0.0022	NS	NS	NS

Different letters (a and b) within rows and stage differ ( $P < 0.05$ ). DM, dry matter.

<sup>a</sup> Pre, fresh plants sampled before harvest; simulated field drying (SFD), rice plants sampled during the simulated field drying period immediately after harvesting (*i.e.*, 0–4 days); Dry (D), rice plants sampled during the storage of rice straw (*i.e.*, 5–19 days after harvest).

based upon a review, that semi-dwarf varieties had similar or higher *in vitro* OM digestion than tall varieties. The extent of gas production (*i.e.*, 'b') was also higher for the M202 (8.4% more than M401), but rate of gas production was not influenced.

Pre plants produced 6.8, 15.6 and 8.9% more gas than SFD and D plants at 4, 24 and 72 h of *in vitro*, respectively. The extent of gas production also declined from pre to SFD and D by 5.7%, but rate of gas production was not affected by stage.

The M401 variety had more Si (55.0 versus 43.0 g/kg DM;  $P < 0.01$ ) and more Si in aNDF (372 versus 270 g/kg DM of total Si;  $P = 0.01$ ). The D rice straw had higher SiADF (808 versus 746 g/kg of total Si;  $P < 0.04$ ) than frozen plants. Si in ADNDF of D plants was the same for both stages.

### 3.3. Experiment III—impacts of simulated field drying of rice straw on its chemical composition and voluntary DM intake

The CP level of Pre plants was higher than SFD plants (34.3 versus 25.3 g/kg DM;  $P < 0.05$ , respectively). The NDF level of Pre plants was lower than the D plants (787 versus 807 g/kg DM;  $P < 0.05$ ).

Voluntary DM intake of Pre plants by the heifers was higher than that of FD plants which was also higher than of D plants (5.14 versus 4.13 versus 3.69 kg/day;  $P < 0.05$ ).

## 4. Discussion

### 4.1. Impacts of long term storage of rice straw on its nutrient value as impacted by maceration

Chemical changes resulting in losses and/or changes of nutrients inevitably arise during field drying due to action of plant and microbial enzymes, chemical oxidation, leaching and mechanical damage, with the magnitude of these changes being dependent to a large extent upon the speed of drying (Lee, 1988; McDonald et al., 2002), which can be increased in rice straw by maceration at harvest (Nader and Robinson, 2008). During field drying there can be considerable losses of soluble sugars as a result of respiration and aerobic fermentation, thereby leading to increased concentrations of other constituents in the plant, particularly fiber components. Thus, faster field drying tends to conserve sugars and, since cellular contents are essentially fully digestible in ruminants (Van Soest, 1994), it could also contribute to minimizing the reduction of OM digestibility due to field drying (Lee, 1988). However it would seem unlikely that these processes would result in further loss of nutrient value of rice straw after it is fully dry, which seemed to be suggested by the large decrease in voluntary DM intake of rice straw stored for extended periods (Sharif, 1984).

The lack of effect of maceration on *in vitro* NDF<sub>30</sub> digestibility contrasts to results of Ware et al. (2005), who observed that maceration increased digestibility of rice straw, and Plascencia et al. (2007) also observed that maceration increased ruminal digestion of rice straw OM and NDF, as well as increased total tract digestion of OM. In these studies, conducted *in vivo*, positive effects on fiber digestibility were related to the maceration that broke the structural organization of the cell wall to provide more open surfaces for rumen bacterial and/or enzyme access that promoted a faster, and to a higher extent, digestion of structural components of the plant. In our study, this effect did not occur, probably due to the modest degree of maceration (Nader and Robinson, 2008) since, in our study with the same rice variety using a higher level of maceration, digestibility of aNDF<sub>30</sub> increased by 11.9% (383 versus 435 g/kg aNDF;  $P = 0.06$ ).

The lack of an impact of time of storage post baling on the nutritive value of our rice straw shows that once it was dried, it did not undergo degradation of nutrient value. This suggests that the findings of Sharif (1984) relative to reduced nutritive value and voluntary DM intake of straws stored for an extended period of time was not caused by changes after the straw was fully dried.

**Table 4**

Silica in the ADF, NDF and ADNDF fractions of frozen (Fz) and dry rice straws (D) from two varieties [Experiment II].

	Variety (V)		SEM	Stage (S) <sup>a</sup>		SEM	P		
	M202	M401		Fz	D		V	S	V × S
Total Si (g/kg DM)	43.0	55.0	1.50	49.8	48.2	1.49	<0.01	0.45	0.43
SiADF <sup>b</sup>	797	757	17.7	746	808	17.6	0.15	0.04	0.33
SiNDF <sup>b</sup>	270	372	22.1	305	337	21.9	0.01	0.33	0.42
SiADNDF <sup>b</sup>	187	260	34.4	192	255	34.0	0.18	0.24	0.25

SiADF, Si in acid detergent fibre; SiNDF, Si in neutral detergent fibre; SiADNDF, Si in neutral detergent extracted acid detergent fibre.

<sup>a</sup> Fz, frozen plants sampled before harvest; D, rice plants sampled as dry rice straw.<sup>b</sup> Values as g/kg of total Si.

#### 4.2. Impacts of simulated field drying of rice straw on its nutrient value as impacted by plant height and stage of growth and drying

Chemical analysis confirmed the expected high NDF and lignin(sa) levels, as well as the low N content, of these rice straws, and values were in the range reported by Bainton et al. (1991), Vadiveloo (1995) and Agbagla-Dohnani et al. (2001). The shorter M202 variety and the longer M401 variety did not differ in CP, which is not consistent with Bainton et al. (1991) and Wang et al. (2006), who reported an effect of rice variety height on the CP level of straw when comparing short and tall varieties, although they compared different varieties than those used in our study. That ADF was higher for M401 (473 versus 445 g/kg DM;  $P < 0.001$ ) was probably due to M401 being taller, thereby containing longer stems and leaf sheaths, as Wang et al. (2006) reported higher ADF values for leaf sheaths versus stems at various stages of maturity (Tables 4 and 5).

That rice plants before harvest had higher CP values than rice straw could be due to physical and/or chemical losses during field drying, as loss of leaves during drying (*i.e.*, the part of the rice plant with higher CP (Vadiveloo, 2000; Wang et al., 2006)), dissipation of non-protein N formed by respiration (Carpintero et al., 1979), formation of chemical linkages between proteins and structural polysaccharides (McDonald et al., 2002; Hindrichsen et al., 2006), or to reactions with fermentable carbohydrates (McDonald et al., 2002). The lack of differences among our 'stages' in ADF levels could be because the proportion of ADF increases in the plant during maturation and, consequently, the increase in ADF may have occurred before stage Pre (Wang et al., 2006).

Despite the similarity in the chemical composition among rice plants collected prior to harvest, during field drying and as dry straw, there were substantial differences in gas production at 4, 24 and 72 h of *in vitro* fermentation. Gas produced by fermentation arises largely from the carbohydrate fraction of the forage and gas produced up to 4 h of incubation can be considered to mainly reflect fermentation of non-structural carbohydrate, while gas produced between 4 and 24 h can be considered to estimate the amount of structural carbohydrate that will be digested in cattle fed at production levels. The higher gas production at 4 h for Pre rice plants probably reflects its higher level of non-structural carbohydrate, which are almost certainly rapidly fermented. However, since the fiber content of rice straw was not affected by stage, differences in gas production at 24 h suggests that it underwent changes during field drying which were not identified by traditional chemical assays, but which reduced its fiber fermentability. For example, the lignin(sa) content did not differ among stages, suggesting that this was not what caused the sharp decrease in gas produced due to drying. However it seems clear that field drying has a negative impact on fermentability of rice straw, and it may be that reactions occurring in the fiber matrix that create chemical, perhaps related to Si, or physical alterations (although Nader and Robinson (2008) failed to show a positive impact of maceration) were responsible.

Total Si levels in our rice straw are within the ranges reported by Agbagla-Dohnani et al. (2001), but lower than reported by Abou-El-Enin et al. (1999). This could be due to genetic variation among rice varieties and/or differences in Si concentration in soil or because senesced flag leaves were removed at sampling in our study. The taller variety (*i.e.*, M401) had higher Si

**Table 5**

Chemical composition (g/kg DM) and voluntary dry matter (DM) intake of rice straw of three physical forms of rice straw in relation to simulated field drying [Experiment III].

	Stage <sup>a</sup>			SEM
	Pre	FD	D	
Organic matter	849	852	849	3.6
Crude protein	34a	25b	31ab	2.2
Neutral detergent fibre	787c	800cd	807d	6.6
Acid detergent fibre	532	465	551	38.2
Lignin(sa)	71	71	66	4.0
Silica	92	101	103	9.0
DM intake, kg DM/day	5.14a	4.13b	3.69c	0.090

Different letters (a–d) within rows and stage differ ( $P < 0.05$ ).<sup>a</sup> 'Pre', 'FD' and 'D': rice straw fed before harvest, during field drying and as dry rice straw.

levels, and this was probably due to it having a higher leaf to stem ratio, since studies on rice straw fractions have shown that leaves are higher in Si than stems (Antongiovanni and Sargentini, 1991; Shen et al., 1998), and that leaves of tall varieties are higher in Si than shorter varieties (Antongiovanni and Sargentini, 1991).

Silica levels in rice plants are usually higher than in other plants, which has led many to suggest that Si is responsible for the low digestibility of rice straw but, as discussed in Section 1, the evidence for this view is weak. However Wang et al. (2006) suggested that the negative impacts of Si on digestibility of rice plants may be due to its location in the fiber matrix rather than in its total level in the plant. In our rice plants, approximately 780 g/kg DM of the Si was located in the ADF versus 320 g/kg DM located in NDF, which is consistent with expectations of the detergent fiber system (Van Soest, 1994). Stage D had more Si in the ADF than stage fresh frozen (808 versus 746 g/kg DM, respectively), for which there is no obvious explanation, although direct comparison of these values to gas production values of fresh rice plants is not possible as the fresh samples were frozen prior to AD and ND analysis to isolate residues for Si analysis, and the impact of freezing is unknown. Nevertheless, a possible reason for this difference could be related to the chemical form of Si in the rice plant, as M401 had more Si in NDF (372 versus 272 g/kg DM for M202 and M401, respectively) and this may be the reason for the lower gas production of M401. Overall, these results may support suggestions of Wang et al. (2006) that the location of the Si in the plant, perhaps related to its chemical form and reactivity with organic components, could be the cause of changes in its fermentability.

#### 4.3. Impacts of simulated field drying of rice straw on its chemical composition and voluntary DM intake

Similar to results of Experiment II, there were no differences in the chemical composition of rice plants relative to stage, but a reduction in voluntary DM intake from Pre to D straw of 1.45 kg/day occurred. This 28% decrease, very similar to the 30% decrease of Sharif (1984), confirms these previous observations and suggests that the Sharif (1984) reduction in DM intake were the result of changes in field drying, rather than during long term storage.

These DM intake results are also supported by gas production data from Experiment II where the 20% reduction in 24 h gas production, which is considered to estimate the amount of carbohydrate that will be digested in cattle, is quantitatively similar to the 28% reduction in DM intake. According to Allen (1996), voluntary DM intake of low digestibility feeds is limited by physical distention in the gastrointestinal tract, which diminishes as digestibility increases.

## 5. Conclusions

There was no preservation of non-structural carbohydrate contents in rice straw due to maceration, as was desired, and dNDF<sub>30</sub> was not affected. In addition, storage of straw from baling for 82 days did not impact levels of its chemical components or dNDF<sub>30</sub>.

The tall late maturity rice variety had higher levels of ADF, lignin(sa), total Si and Si in NDF than did the short early maturity rice straw, and this was associated with lower gas production at 4, 24 and 72 h of *in vitro* fermentation. Gas production of fresh plants was higher than in the plants during simulated field drying and when fully dried. However this occurred in the absence of differences in chemical composition, except the amount of Si in ADF (which was higher for dried versus fresh frozen plants), leading to the hypothesis that the lower gas production of plants during field drying and when fully dried, versus fresh plants, is not identifiable through common chemical analyses of fiber (*i.e.*, NDF, ADF, lignin(sa)).

The much higher voluntary DM intake potential of fresh straw at harvest, as demonstrated by Sharif (1984), was confirmed in our study with heifers, and it seems clear that this depression in DM intake potential of dried rice straw occurred during field drying. It appears certain that the cause of this depressed DM intake was related to the decrease in fermentability of dry versus fresh plants, although the cause of the reduced fermentability is not clear. However analyses of the Si levels in detergent fiber fractions provided indications that the location of the Si in the plant fiber matrix may be important.

Results clearly demonstrate the substantial differences in the nutritional value of fresh versus dry rice plants, and it is obvious that field drying converts forage with a moderate nutritional value into a low quality forage with limited feeding options. Studies are needed to better understand what occurs in the rice plant during the field drying process to create this decrease in nutritional value, and it is essential to create strategies to maintain its nutritional value, rather than use treatments to improve the nutritional value of rice straw after field drying. More research is needed on Si location and form in the rice plant, especially as it relates to its fermentability and digestibility.

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