Ruminal Fungi Colonisation of Stem Tissue of Untreated and Urea Treated Rice Straw Varieties

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Abstract

The study was conducted to investigate the digestibility untreated and urea treated rice straws varieties from ruminal fungi colonization in the rumen of sheep. The study used straw from 3 varieties of rice namely Illabong, Dongara and Yrm varieties which represented medium and low quality of straw. Each varieties of straw were treated with urea prior incubation in the rumen. Approximately 5mm of 10 cross section of untreated and urea treated straw stem internodes were taken below flag leaves. The materials was placed in nylon bag and incubated in the rumen of fistula sheep for 24, 48 or 72 hours. After withdrawn from the bag bags were washed then samples were removed from bags and observed under scanning electron microscopy (SEM). Observation under SEM revealed that sporangia ruminal fungi had colonised the Dong and Yrl varieties after both 48 and 72 hours incubation. The substrate of Ilb was colonised after 24 and 72 hours incubation. All population tended to be lower after 72 hours incubation than the shorter incubation. Urea treatment decreased the time required for ruminal fungi colonisation. All treated samples were colonised after 24 hours of incubation. From this study can be concluded that digestibility variation due to urea treatment could be explained from ruminal fungi colonisation but not with rice straw varieties.

Keywords: digestibility, rice straw, ruminal fungi, urea treatment, varieties

Introduction

Rice straw is a crop residue that widely available in tropical countries One of the factors that affect the quality of rice straw is varieties. Nutritive quality of rice straw can be evaluated by biological methods and chemical method. Yulistiani *et al.* (2000) reported that different part and varieties has different chemical components content and in vitro digestibility. Dry matter degradability of rice straw varieties in the rumen of sheep was also affected by varieties, botanical fraction and urea treat-

ment (Yulistiani *et al.*, 1998). However, Yulistiani (2010) reported that stem tissue structure between varieties could not be differentiate using scanning electron microscopy observation, but this observation could detect the extent of tissue degradation of untreated and urea treated rice straw varieties after 24 hours incubation.

The primary fibre degrader in the rumen was cellulolytic bacteria (Chen *et al*., 2008). However, the utilization of poor quality, high fibre crop residues by ruminants is enhanced by ruminal fungi (Gordon and Phillips, 1995). Fungi have the ability to colonize lignified cell walls and to weakens fibrous plant tissues in the rumen (Akin and Borneman, 1990) and the ability to degrade the structural components of plant cell walls, due to its ability to produce xylanase and cellulose (Bahramian *et al*., 2011), therefore the ruminal fungi play an important role in the digestibility of fibre in the rumen (Gordon and Phillips, 1995). The objective of this studies were to investigate using scanning electron microscopy the ruminal fungi colonization on untreated and urea treated rice straw varieties incubated in the rumen of sheep.

Materials and Methods

Two fistulated sheep were used in this experiment, the sheep were fed a maintenance ration consisted of 50% oaten chaff and 50% Lucerne chaff supplemented with mineral mixed. The untreated and urea treated stems of the lower part of three varieties of rice straw Dongara (Dong), Ilabong (Ilb) and Yrl obtained from Yanco Agricultural Institute, Yanco, Leeton, N.S.W. were studied. These three varieties was chosen represented for high, medium and low quality from evaluation on their in vitro organic matter digestibility (IVOMD) (Yulistiani *et al*., 2000). The straws were treated with urea at level 40g urea/kg straw DM. Approximately 5 mm of 10 cross sections of untreated and urea treated rice straw stems internodes were taken. The samples were placed in nylon bags and incubated in the rumen for 8, 24 an 48 hours in reverse order. All bags were withdrawn simultaneously and gently washed under running tap water for 30 minutes. Control sample (0 hr incubation) were prepared similarly. For observation under SEM, samples were prepared according to the method of Akin *et al (*1984). Counting of ruminal fungi carried out by observing longitudinal section of samples using Phillips XL 20 scanning electron microscope (SEM), and printed on video print. All video images were captured at the same magnification with a data bar of 500 μm. At least two samples per incubation time and two areas per sample were evaluated. Sporangia that were attached to plant material within the delineated area were counted. The presence of sporangia was verified by comparing un incubated and incubated materials. The ruminal fungi population per cm^2 was calculated by dividing the total numbers of sporangia by the total area and multiplying by 100 data were presented descriptively.

Results and Discussion

The number of fungi colonising of untreated and urea treated straw internode are presented in Table 1. These result showed that, sporangia of ruminal fungi had colonized the untreated Dong and Yrl varieties after samples had been incubated for 48 and 72 hours. Fungi were absent or were present in extremely small numbers, in samples of both varieties after incubation for 24 hours. However, in Ilb variety which had medium quality (Yulistiani *et al*., 2000) sporangia were present after 24 and 72 hours incubation, but not after 48 hours. In 2 of 6 means values of observations fungal population decreased after 72 hours of incubation. Ruminal fungi colonised the thick cell walls sclerenchyma and small vascular bundles of rice straw internodes (Figure 1). Lack of ruminal fungi colonisation of Yrl variety are shown in Figure 2 in contrast to colonisation in Dongara and Illabong varieties.

Tabel 1. Total number of fungal sporangi/cm^2 on rice straw stems before and after treatment with urea (mean at least two observation)

Varieties	Treatments	Incubation time (hours)		
		24	48	72
Dong	untreated	0	0.13	0.36
	urea treated	2.1	1.93	0.63
Ilb	untreated	0.71	0	0.91
	urea treated	0.22	0.06	0.03
Yrl	untreated	0	0.23	0.20
	urea treated	0.5	0.17	0.21

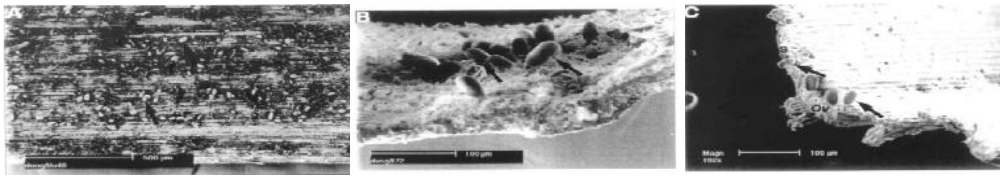

Figure 1. Scanning electron microscopy of ruminal fungi (arrow) colonization on the internode of rice straw. (A. After incubation for 24 hours (magnification 70x); B. After incubation for 72 hours (magnification 234 x); C. Ruminal fungi colonized lignified tissue of the small vascular bundles/ov and schlerenchyma/s) (magnification 162x).

From previous study it was reported that from various variety of straw evaluated showed that the lower part of the straw from Dong had the highest IVOMD and DM degradability (Yulistiani *et al*. 2000). The number of ruminal fungi colonising

untreated Dong and Ilb samples was also higher than that for Yrl, which has a lower IVOMD than either of these varieties. In addition, the fungal population numerically was higher in the treated, than the untreated, straw. This results suggest that there is a tendency for ruminal fungi to colonize the more digestible straw in higher numbers. The results on ruminal fungi population in this study could not be statistically analysed because there was no sample replication for sheep. Even though the sheep were fed on a similar diet it appeared that there were differences in diet preferences for different part of the diet. The differences in ruminal population could therefore be due to differences between sheep or to substrate.

The ruminal fungi population in the untreated dong variety extremely small after the straw had been incubated in the rumen for 24 hours, while that of urea treated Ilb was higher after the same incubation time (Table 1), eventhough the IVOMD and DM degradability of Dong were higher than those of Ilb (Yulistiani *et al*., 2000; 1998). This indicates that tissue degradation may have occurred in the presence or absence of ruminal fungi. This might indicates that the fungal population was not as active in fibre digestion as bacteria in mixed rumen microbial population, and this results agrees with the conclusion of Chen *et al*. (2008).

Ruminal fungi preferentially colonized lignified tissues of sclerenchyma and the small vascular bundles (Figure 1). Similar results has been observed by Grenet and Barry (1988). In spite of this, these walls did not degrade significantly, as previously reported by Yulistiani (2010). On the other hand, Rezaeian *et al.* (2005) reported in *in vitro* pure culture the higher fungal biomass of sodium hydroxide treated barley straw was higher and followed by the higher degradation of cellulose than that of untreated straw. Therefore, in mixed rumen microbes, the development of large numbers of sporangia on fibre may not indicate that ruminal fungi have a substantial role as a forage digester.

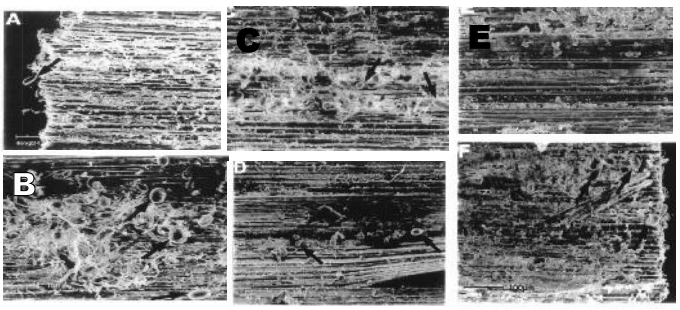

Figure 2. Scanning electron microscopy of a longitudinal section of internodes of rice straw varieties after incubation in the rumenfor 24 hours, with presence (arrow) or absence of ruminal fungi. (A. Untreated Dongara variety; B. Urea treated Dongara variety; C. Untreated Illabong variety; D. Urea treated Illabong variety; E. Untreated Yrl variety; F. Urea treated Yrl variety)

Urea treatment reduced the time required for fungi to colonise stem tissue. All area treated samples had been colonized by ruminal fungi after 24 hours of incubation. However, only with the Dong variety was the number of fungi in the treated straw higher at all incubation times compared to untreated straw (Tabel 1; Figure 2). On the other hand the IVOMD and dry matter loss (at all incubation time) of all rice straw varieties was increased after urea treatment (Yulistiani *et al*., 2000; 1998). These results indicate that digestibility increased even in the absence of ruminal fungi colonization.

Conclusion

Observation using scanning electron microscopy shows that ruminal fungi colonisation could not explain the variation of rice rice straw varieties and the effect of urea treatment.

Acknowledgment

I would like express my appreciation to Dr. Laurie Lewin from Yanco Agricultural Institute, Leeton New Soth Wales, Australia for providing rice straw to be used in this experiment. And thank also to Dr. John D. Brooker, from University of Adelaide for his advice in this experiment.

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