The Effects of Effective Microorganisms (EM) on the Nutritive Values of Fungal-Treated Rice Straw

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Abstract

A study to improve the nutritive values of rice straw by means of biological treatment was conducted. The rice straw was subjected to three treatments: plain rice straw (T1), rice straw treated with *Aspergillus niger* for 10 d (T2) and fungal-treated rice straw inoculated with EM (T3). All samples were subjected to proximate analysis to determine the dry matter (DM), organic matter (OM), crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF), acid detergent lignin (ADL), cellulose and hemicellulose content of rice straw after eight days of fermentation. The effect of fermentation days of T3 on the nutritive values and dry matter degradability (DMD) of the three treatment groups were also carried out on nine fistulated goats using nylon bag technique and data were recorded at six different fermentation times. No significant ($p>0.05$) difference was observed on the chemical composition of rice straw treated with *A. niger* except the CP content increased significantly ($p<0.01$) when compared with untreated rice straw (T1). However, when the fungal-treated rice straw was inoculated with EM, significant ($p<0.01$) difference was observed in CP, OM, NDF, ADF and cellulose content of the rice straw compared with T1. It was suggested that the optimum duration of fermentation by the EM on the fungal-treated rice straw was 6 d of fermentation as hemicellulose content was recorded higher than other days of fermentation, while NDF, ADF, ADL, and cellulose content were relatively lower than previous days. DMD of rice straw was improved when fungal-treated rice straw was inoculated with EM.

Keywords: Effective microorganisms, *Aspergillus niger*, rice straw.

Introduction

Rice straw is a main agricultural byproduct which farmers usually stored for use as ruminant feed in tropical area. The conventional feed resources like pastures are no longer available due to competition of land usage. In 2011, Malaysia produced approximately 2.665 million tons of rice and assuming the ratio of grain to straw is 1:0.9 with 50% collectable straw, a total of about 1,332,500 tons of straw would be made available - enough to feed more than 500,000 ruminants for a year with daily intake of 6 kg straw per day (MOA, 2011). Rice straws contain low nitrogen, vitamins and minerals, which hinder the availability of cellulose to be degraded by rumen microbes and eventually limit the necessary nutrient uptake for a satisfactory performance of animals, especially ruminants. The cellulose and hemi-cellulose content of rice straw range between 25 - 45% and 20 - 30%, respectively, while the lignin content is between 10-15% (Sharma *et al.*, 2001; Vlasenko *et al.*, 1997).

However, wide variation in the chemical composition and digestibility among different rice straw varieties had been reported by many researchers (Doyle *et al*., 1986; Vadiveloo, 1992; Abou-El-Enin, 1999). Despite the limitation due to low nitrogen and high concentration of fibrous materials, which limit the degradation process, many studies have been conducted with efforts dedicated towards attaining most of the potential nutritive value of this abundant agricultural byproduct.

Most of the treatments done on rice straw involved physical, chemical and biological or combination of the three treatments. Physical treatment mainly focuses on reducing the size of rice straw through chopping or grinding and breaking the cellulolytic bond of rice straw through steaming, flaking or boiling (Wanapat, 1999). Chemical treatment involves the usage of highly acidic or alkaline chemical such as NaOH, KOH, or $Ca(OH)_2$ to penetrate the lignified structure of rice straw cell walls and break the bonds that bind the cellulose, hemicelluloses and lignin which eventually increase the availability of those energy source of carbohydrate to be used by the rumen microbes (Chaudhry, 1998). Biological treatment is a much favorable option and is believed to be more environmental friendly and safer than the use of chemicals. Microorganisms like bacteria (Chaji *et al.,* 2010), fungi (Jahromi *et al*., 2010), enzymes (Euna *et al.,* 2006) and other beneficial microorganisms that are able to degrade lignocellulosic component of rice straw are used to improve the availability of nutrients for the usage of rumen microorganisms.

The use of fungi or their derivative enzymes in improving the nutritive values of straw has drawn much attention (Jalc, 2002). *Aspergillus niger*, a fungi that has the capability in degrading lignin component of the straw and at the same time, showing a promising result as white-rot fungi is attracting much interest. Apart from the usage of the fungi, the application of effective microorganisms, popularly known as EM, in improving the quality of animal feed has also received much attention in many regions of the world. It was developed by Professor Teruo Higa, University of the Ryukyus, Okinawa, Japan who used EM as inoculants to increase the microbial diversity of soils and plants (Higa, 1991). He found out that the inoculation of EM cultures to the soil/plant ecosystem can improve soil quality, soil health, and the growth, yield and quality of crops (Higa, 1991).

Several studies have demonstrated the effects of fungal inoculation on rice straw in improving its nutritive content (Arora *et al.,* 1994; Jahromi *et al.,* 2010; Zadrazil *et al.,* 1997). Fungal treatment could be an approach to convert low quality wheat straw into a higher quality ruminant feed. However, to our knowledge, there is no available information on the effect of EM inoculation on the fungal treated rice straw to see if it could further improve the straw nutritive values. Thus, the current study was carried out to evaluate the chemical composition and dry matter digestibility (DMD) of rice straw subjected to fungal and EM treatments and to determine the optimum day of EM fermentation on the fungal-treated rice straw.

Materials and Methods

Fungal spore preparation

The fungus used in this study was obtained from the Institute of Tropical Agriculture, Universiti Putra Malaysia. Preparation of the fungal spore was according to the method described by

Jahromi *et al.* (2001). Briefly, *Aspergillus niger* was cultured in potato dextrose agar. Spore suspension of the fungal strain was prepared by washing 5-day old culture slants with sterilized saline solution (0.9% NaCl) and vigorously shaken for 1 min. The spores were manually counted using a haematocytometer and the numbers of spore were adjusted to contain approximately 10^7 spores/ml.

Pretreatment of rice straw

Rice straw was obtained from local rice fields in Selangor, Malaysia. The straw was oven dried and ground to obtain uniform size (mesh size 6) before stored in plastic bags at 4℃ to be used as a substrate in this study. Approximately 30g of rice straw was weighed and put inside a 500 ml Erlenmeyer flask. Then, 60ml distilled water was added to give about 70% of moisture content. No additional mineral was added to the substrate. The flask was plugged with cotton and autoclaved at 121℃ for 15 min. Later the flask was inoculated with 10% (v/w) inoculums, containing 10^7 spores per ml prepared earlier for the fermentation process to take place.

Experimental procedure

The rice straw were subjected to three treatments: plain rice straw (T1), rice straw treated with *Aspergillus niger* (T2) and fungal-treated rice straw inoculated with EM (T3). Each of the treatments was prepared in triplicates and incubated at 30℃ for 10 consecutive days after which the straw from all treatments were autoclaved at 121℃ for 15 min to stop any fungal activities, especially in T2 and T3 groups. The T3 flasks were further inoculated with EM and incubated for another eight days at 37℃. A small fraction of rice straw from T3 fermented from day 2, 4, 6 and 8 were subjected to nutrient analysis. This was to determine the optimum time required for the EM to further improve the nutritional value of rice straw treated with the fungi.

Chemical analysis

Dry matter (DM) content of each sample was determined by drying to constant weight at 103℃ for 12 h followed by equilibration in a dessicator. Ash was determined after incineration for 4 h at 550℃ (AOAC, 1990) while crude protein (CP) was determined by micro-Kjeldahl technique (AOAC, 1990). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined by the detergent system (Van Soest *et al.,* 1991) and acid detergent lignin (ADL) was determined by the method described by AOAC (1990). Hemicellulose content was estimated as the difference between NDF and ADF while cellulose content was estimated as the difference between ADF and ADL.

In sacco degradability

Solubility and degradability *in sacco* of DM of each rice straw treatment was determined according to the protocol described by Orskov *et al*. (1980). Approximately 1g of sample was weighed and inserted into a clean nylon bag. The bags were inserted into nine fistulated goats' rumen for a specific incubation time (0, 6, 12, 18, 24, and 36 h). The goats went through an adaptation period for 10 d and fed at maintenance level allowing them to consume only rice straw and concentrate in the DM ratio of 60:40. The nylon bag was taken out from the goat's rumen and washed thoroughly, before drying inside the oven at 60℃ until constant weight was achieved. Later when the sample residue was dried

thoroughly, it was removed from the nylon bag and put into the crucible. The standard procedure for dry matter was carried out. The difference between initial sample weight and final sample weight was calculated.

Statistical analysis

The data were analyzed using the ANOVA procedure using SAS package version 9.02 (SAS Institute Inc. USA). Treatment means were compared by using Duncan's multiple range test.

Results and Discussion

Chemical composition

The proximate parameters of OM, CP, NDF, ADF, ADL, cellulose, hemicelluloses and ash were analyzed to determine the nutritive values of untreated rice straw and fungal-treated rice straw with and without EM (Table 1). There were significant differences ($p<0.05$) recorded among the treated rice straw groups (T2 and T3) and untreated rice straw group (T1) in DM, OM, CP, NDF, ADF, and cellulose content. The reduction of NDF and ADF contents from the rice straw suggested that *A. niger* could solubilise and utilise the cell wall as carbon source and thus changed the ratio of insoluble to soluble carbohydrates in the straw. The decrease in NDF and ADF contents of the fungal-treated straw was in agreement with Fazaeli *et al.* (2004) and Singh *et al.* (1990). The reduction was due to the nature of the fungi that largely obtained their energy requirement from an organic carbon present in the form of structural material such as lignocelluloses (Jennings and Lysek, 1996). The losses of NDF and ADF contents from the rice straw suggested that the ability of *A. niger* to solubilise and utilize the cell walls as carbon sources and thus changed the ratio of insoluble to soluble carbohydrates in the straw. This soluble carbohydrate was further utilized by microorganisms present in the EM. This was possibly the explanantion for the inoculation of EM on the fungal-treated rice straw had significantly reduced ($p<0.05$) the NDF and ADF content from 79.54 to 74.02% for NDF and 63.69 to 59.74% for AD, respectively. The present result also showed that inoculation of EM on the fungal-treated rice straw had beneficial effect of reducing hemicellulose and cellulose content of rice straw. This was due to the reduction of lignocellulosic contents of the straw after the fungal fermentation process had taken place. It was assumed that the activities of fibrolytic enzymes of *A. niger* such as cellulase, β-glucosidase and xylanase had reduced the lignocellulose compound of the rice straw as reported by several studies previously (Pothiraj *et al.*, 2006; Madamwar *et al.*, 1989). Further reduction of lignocellulose compounds of fungal treated rice straw proved that EM did carry out the degradation process to the next level. This further reduction increased the nutrients availability, but not affecting the CP content. It is speculated that the microorganisms present in the EM has utilized some of the N content produced from *A. niger* cell walls as a protein source.

Table 1: Effect of biological treatments on chemical composition of rice straw (% DM)

Composition (%)	Treatments			Significant level
	T1	T2	T3	
Dry matter	88.24±0.05	34.42±1.86	45.30±0.78	**
Organic matter	83.84±0.57[a]	57.41±4.45[c]	68.90±3.08[b]	**
Crude protein	5.00±0.04[c]	6.10±0.09[a]	5.96±0.07[b]	**
Natural detergent fibre	82.83±1.77[a]	79.54±3.68[a]	74.02±1.04[b]	**
Acid detergent fibre	66.45±0.66[a]	63.69±0.47[b]	59.74±0.12[c]	**
Acid detergent lignin	4.31±0.19[ab]	4.09±0.45[a]	4.01±0.02[b]	NS
Cellulose	59.60±0.50[b]	62.14±0.61[a]	55.73±0.19[c]	**
Hemicellulose	15.85±3.71[a]	16.37±1.66[a]	14.28±1.08[a]	NS
Ash	14.26±0.51[a]	14.62±1.16[a]	14.10±1.55[a]	NS

T1: untreated, T2: fungal-treated, T3: fungal+EM-treated, NS: Not significantly different (p>0.05), **significantly different at 1% level (p<0.01)
[abc] Means with different letters within a row differed significantly at p < 0.05.

Effect of fermentation days on the nutritive values

The nutritive values of fungal-treated rice straw inoculated with EM were analyzed to determine the optimum days of fermentation (Table 2). There were significant differences (p<0.01) among different fermentation day treatments on the nutritive parameters observed in this study except in ADL composition. The NDF, ADF and ADL composition were lower in day 6 of fermentation than in the other days. Reduction of lignocellulose compounds on day 6 of fermentation showed that the digestibility of the rice straw had increased. Such improvements could be a result of the changes in non-structural carbohydrates to structural carbohydrates. As fermentation day increased, the lignocellulose compounds decreased, thus proving that fungal-treated rice straw inoculated with EM produced enzymes that further degraded the lignocellulose compounds of rice straw.

Table 2. Effects of fermentation day of fungal-treated rice straw inoculated with EM on rice straw nutritive values.

Composition (%)	Days				Significant level
	2	4	6	8	
NDF	74.08±0.47[b]	73.52±1.82[b]	73.05±0.61[b]	73.90±0.18[b]	**
ADF	66.72±0.30[ab]	66.02±0.50[b]	58.67±0.35[d]	59.74±0.23[c]	**
ADL	4.13±0.35[ab]	4.27±0.09[a]	3.82±0.07[b]	4.01±0.02[ab]	NS
Cellulose	62.60±0.50[ab]	61.75±0.53[b]	54.85±0.37[d]	55.73±0.22[c]	**
Hemicellulose	7.36±0.59[c]	7.50±2.31[c]	14.39±0.38[a]	14.16±0.34[a]	**
Crude protein	5.69±0.04[c]	5.81±0.03[b]	5.81±0.03[b]	5.95±0.05[a]	**

NS: Not significantly different (P>0.05), **significantly different at 1% level (P<0.01)
[abcd]Means with different letter within a row differed significantly

Inoculation of EM on the fungal-treated rice straw had significantly ($p<0.01$) increased the CP content of straw, but among the treatments, CP was recorded higher in day 8 of fermentation (5.95%) compared to day 6 (5.81%), day 4 (5.81%) and day 2 (5.69%). Apart from the changes in the nutritive values of the rice straw, the fermentation carried out by EM on fungal-treated rice straw can be assumed as successful when these physical aspects were observed; 1) a marked change of color from yellowish to brown or dark brown of the rice straw, 2) a sweet and strong smell, without any trace of bad fermentation smell, and 3) absence of mould (Chenost, 1995). Decrease in lignocellulosic compounds gives the indication that improving nutrients availability can successfully be achieved through application of EM. Increase in CP contents from day to day is possibly due to the multiplication of EMs, as sufficient nutrients and favorable environment presents in the substrate enhanced the growth and numbers of these beneficial microorganisms. Based on the result, sample from day 8 of fermentation was chosen for further study using *in sacco* approach to determine the DM degradability in goats.

In sacco degradability

Time of incubation and its relation with DM degradability of the rice straw had demonstrated the EM inoculation on fungal-treated rice straw had the highest means compared to other treatment groups. The DM degradability of fungal-treated rice straw inoculated with EM (T3) had increased one-fold starting from 12 h onwards (Table 3) than the other two treatments. The plain rice straw (T1) had the lowest DM degradability among the incubation times. The DM degradability of T3 was recorded highest at 36 h of incubation. This may be explained by the NDF content in these treatments that was lower than other two treatment groups (Table 1). Fermentation of the rice straw with fungi decreased the cell wall components and increased the soluble fraction of the carbohydrates in the straw which was further utilized by the EM. The DM degradability of fungal-treated rice straw (T2) was found to be not much different with the data recorded for T1.

Table 3. . Effects of fermentation day of fungal-treated rice straw inoculated with EM on value of DMD over incubation time (hours)

Time (h)	Treatments			Significant level
	T1	T2	T3	
6	8.09 ± 0.06^{a}	8.46 ± 0.24^{a}	12.03 ± 0.21^{b}	**
12	8.31 ± 0.31^{a}	9.30 ± 0.03^{b}	17.43 ± 0.19^{c}	**
18	9.01 ± 0.15^{a}	9.58 ± 0.11^{b}	18.20 ± 0.20^{c}	**
24	9.09 ± 0.20^{a}	10.25 ± 0.26^{b}	19.35 ± 0.04^{c}	**
30	10.09 ± 0.23^{a}	11.07 ± 0.12^{b}	20.36 ± 0.20^{c}	**
36	10.14 ± 0.29^{a}	11.30 ± 0.09^{a}	20.25 ± 0.29^{b}	**

** significantly different at 1% level ($p<0.01$)
[abc]Means with different letter within a row differed significantly at $p < 0.01$.

The result is similar to the finding of Maqbool *et al*. (1997) that reported the application of rice straw with EM which contained mixtures of few genera of microorganisms had further degraded the lignocellulosic contents, thus improving the rumen DM degradability which would affect the weight gain of animal. By further degrading the lignocellulosic contents of rice straw, more nutrients are made available for ruminal microflora, which in turn will sustain the longevity of the microbes. More importantly, harmful microorganisms such as toxin-producing fungi that contaminate the rice straw could be suppressed by application of EM on the feedstuff, proving its ability to restrain the growth of the harmful toxin-producing fungi (Maqbool *et al*., 1997).

Conclusions

The present sudy shows a promising result in improving the nutritive value of rice straw that were treated with biological treatments: fungal treated and with EM. Reduction in lignocellulosic contents as shown by decreased value of NDF, ADF and ADL of treated rice straw may increase the nutrients availability to animals. Biological treatments give us a safer yet similar quality to the widely used chemical treatments in treating poor quality roughages to be fed to the ruminants.

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