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ORIGINAL RESEARCH

Enhancement the feeding value of rice straw as animal fodder through microbial inoculants and physical treatments

Mona S. Zayed[1]

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Abstract

Purpose Improving the nutritional value of rice straw by microbial inoculants and some physical treatments for animal feeding during dry seasons.

Methods Different microbial inoculants and some physical treatments were used to improve the nutritional value of rice straw. Rice straw was divided into moist straw, soaked straw for 24 h without pasteurization and soaked for 24 h and pasteurized at 100 °C/1 h, and all of them were inoculated with different microbial inoculants.

Results Moistened rice straw inoculated with *Azotobacter chroococcum* and *Saccharomyces cerevisiae* recorded the highest significant reduction in organic matter percent, 74.21%. The highest significant reductions in crude fiber, neutral detergent fiber, and acid detergent fiber percent were recorded in moistened rice straw inoculated with *Azospirillum brasilense* and *Saccharomyces cerevisiae* which gave 27.54, 55.39 and 42.47%, respectively. The highest significant increase in crude protein percent, 13.71%, was recorded in rice straw soaked for 24 h and inoculated with *Azospirillum brasilense* and *Bacillus megaterium*. The combined interaction between microbial treatments and physical pretreatments of rice straw gave a significant decrease in organic matter, crude fiber, neutral detergent fiber and acid detergent fiber %, as well as a significant increase in crude protein % compared to control. Addition of nitrogen fixers to rice straw improved its nitrogen contents.

Conclusions This study showed the possibility of improving the nutritional value of rice straw using microbial inoculants and some physical treatments to produce safe and cheap animal feeds.

Keywords Rice straw · Nitrogen fixers · Cellulose decomposers · Solid-state fermentation · Agricultural waste management

Introduction

Generally, open-field burning of rice straw after harvesting is a conventional method of rice straw disposal in many rice-based countries (Trinh et al. 2017). However, rice straw could be considered an important feeding material during dry seasons when the availability of pasture decreases and other feeds are inadequate. Rice straw is characterized by low digestibility, low protein content, poor palatability, high bulkiness and low mineral content which discourages its use as the sole source of feed for ruminants (Van Soest 2006). Several investigations have been documented on the use of mechanical, physical, chemical and biological treatments as pretreatments for rice straw, to ameliorate its consumption by ruminants (Liu and Ørskov 2000; Selim et al. 2004). The main goal of any pretreatment is to modify or remove any component which acts as a barrier, to improve digestibility, enhance the hydrolytic enzymes and improve the C/N ratio of the straw (Hendriks and Zeeman 2009). When biomass is exposed to pretreatment, several operations, such as the increase in the surface area and porosity, modification of the lignin structure, removal of lignin, partial depolymerization of hemicellulose and reduction of cellulose crystallinity, can also be accomplished (Loow et al. 2015, 2016).

Most of the researches reported that by supplementing rice straw with protein or nitrogenous compounds, the degradability of rice straw, animal intake, milk yield and meat yield can be enhanced when compared with those feeding on untreated rice straw (Wanapat et al. 2009). High costs of chemical nitrogenous materials restrict their use in proper amounts, causing obstruct in animal feeding and production. In addition, the urea is lost through different mechanisms

✉ Mona S. Zayed
monaszayed@agr.asu.edu.eg

[1] Agricultural Microbiology Department, Faculty of Agriculture, Ain Shams University, 68 Hadayek Shobra, P. O. Box 11241, Cairo, Egypt

which cause environmental pollution problems (Choudhury and Kennedy 2005). The utilization of biological nitrogen fixation technology can decrease the use of urea-N and reduce environmental pollution (Choudhury and Kennedy 2004).

Solid-state fermentation is a microbial growth method used to enhance the nutritive value of agricultural by-products to be used as animal feed (Iluyemi et al. 2006). Microorganisms selected for solid-state fermentation should have the capability to produce sufficient quantity of appropriate enzymes that are able to degrade the cellulose and hemicellulose in the substrate. Recent attention has been devoted to the values of *Pleurotus eryngii*, *Bacillus megaterium* and *Bacillus circulans* and other microorganisms as cellulase producers (Xu et al. 2005). *Saccharomyces cerevisiae* has the ability to produce polyamines, which strongly improve the protein content in the by-products (Srinorakutara et al. 2006; Ubalua 2007).

The objective of this investigation is to illustrate a novel in vitro study on the effect of using different microbial inoculants either separately or in combination on the quality and nutritional value of rice straw subjected to different physical treatments to be used as animal fodder.

Materials and methods

Sample collection

Samples of rice straw were collected from a unit of Experimental and Agricultural Research Faculty of Agriculture Ain Shams University. Air-dried rice straw was chopped into 3–5 cm, then packed and used.

Experimental design and treatments

The experiment was designed as a 3 × 3 factorial arrangement in complete randomized design (CRD) with six replicates for each treatment. Factor A was the N_2 fixers (with and without N_2 fixers "*Azotobacter chroococcum* and *Azospirillum brasilense*"), factor B was the microbial treatments which were either cellulose decomposers or polyamines producers or control (untreated) and factor C was the effect of different physical treatments (moist rice straw, soaked rice straw for 24 h and soaked for 24 h then pasteurized at 100 °C/1 h.)

Solid-state fermentation of rice straw was carried out in a 500 ml jar. 20 g rice straw was put in each jar. The jars were plugged with cotton wool. Each jar was inoculated with 5 ml from each inoculum containing 10^8 CFU/ml, either from the bacterial inoculant or yeast, and 1 g fresh weight from the fungal inoculant. The jars were incubated at 25–30 °C for 4 weeks. At the end of the experiment, rice straw samples were oven dried at 65 °C until a constant weight and stored in a refrigerator at 4 °C for chemical determination.

Microorganism

Five different microorganisms were used in this study, namely *Azotobacter chroococcum*, *Azospirillum brasilense*, *Bacillus megaterium*, *Bacillus circulans*, *Saccharomyces cerevisiae* and *Pleurotus eryngii.* All of them were obtained from the Unit of biofertilizers, Fac., Agric., Ain Shams University.

The strains were maintained on their appropriate media. *Azotobacter chroococcum* was cultivated in modified Ashby's broth medium (Abd-el-Malek and Ishac 1968) for 7 days/30 °C, *Azospirillum brasilense* was cultivated in Dobereiner's medium (Dobereiner et al. 1976) for 7 days/30 °C, *Bacillus megaterium* and *Bacillus circulans* were cultivated in nutrient broth medium (Jacobs and Gerstein 1960) for 24 h/30 °C, *Saccharomyces cerevisiae* was cultivated in glucose broth medium for 24 h/30 °C and *Pleurotus eryngii* was cultivated in potato dextrose broth medium at 25 °C for 7 days.

Assessment of some metabolic activities of the selected strains

All the strains were examined for the following activities:

Cellulase activity was determined by the dinitrosalicylic acid method (DNS) according to Miller (1959). One cellulase unit is defined as the amount of enzyme that reducing sugar at the rate of 1 μ mol ml^{-1} min^{-1} under assay condition. Nitrogenase activities of the selected strains were determined according to the method described by Mollica et al. (1985). Cytokinins, indole acetic acid and gibberellic acid were determined by using high-performance liquid chromatography (HPLC) according to the method described by Tien et al. (1979).

Chemical analysis

Dry matter, organic matter (OM), crude fiber (CF) and total nitrogen (TN) content in rice straw were determined by the standard methods described by Horwitz (2000). The amount of crude protein (CP) was calculated (Nx6.25). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by the detergent system as described by Van Soest et al. (1991). All the data was recorded on dry matter basis.

Statistical analysis

The obtained data were statistically analyzed according to statistical analysis system (Littell et al. 2002).

Separation between means was carried out using least significant difference (LSD) test. The collected data were also statistically analyzed according the following model: $Y_{ij} = \mu + T_i + e_{ij}$, where y_{ij} is the represents observation, μ is the overall mean, T_i is the effect of treatment (experimental group) and e_{ij} is the experimental error.

Results and discussion

Assessment of some metabolic activities of selected strains

Considerable variations were recorded among the tested strains regarding their capabilities for cellulase, nitrogenase activities, as well as cytokinins, indole acetic acid (IAA) and gibberellic acid production (Table 1). Data show that *Azotobacter chroococcum, Azospirillum brasilense* and *Bacillus circulans* were able to fix atmospheric nitrogen and the highest nitrogenase activity was obtained with *Azotobacter chroococcum*, 133.9 µmol C_2H_4 ml^{-1} h^{-1}. These results are in agreement with that reported by (Zayed 2012). The strains of *Bacillus megaterium*, *Bacillus circulans* and *Pleurotus eryngii* were able to produce cellulase enzyme and the highest cellulase activity was obtained with *Bacillus megaterium*, which gave 3521.73 µmol min^{-1}. All the strains were able to produce cytokinins, IAA and gibberellins, except *Saccharomyces cerevisiae* which produced cytokinins and gibberellins only. *Saccharomyces cerevisiae* recorded the highest production capability of cytokinins, 3.52 µg ml^{-1}, *Azospirillum brasilense* recorded the highest production capability of IAA, 4.7 µg ml^{-1}, and the highest gibberellic acid was recorded with *Pleurotus eryngii,* 39.1 µg ml^{-1}. Different researches have reported the ability of microorganisms to produce cytokinins, IAA and gibberellins with different quantities depending on the species of microorganism as well as the growth conditions (Manaf and Zayed 2015; Zayed 2012).

Nutritional value of rice straw as affected by combined interactions between microbial inoculants and physical treatments

The combined interaction between microbial treatments and physical pretreatments of rice straw (Table 2) shows significant decrease in organic matter (OM) % in all treatments when compared with control. Moist rice straw inoculated with *Azotobacter chroococcum* as a nitrogen fixer and *Saccharomyces cerevisiae* as a single cell protein recorded the highest significant reduction in organic matter content of 74.21%.

The crude fiber (CF) % recorded significant reduction in all treatments when compared with the control. The highest significant reduction in CF% was recorded with moist rice straw inoculated with *Azospirillum brasilense* and *Saccharomyces cerevisiae* of 27.54%.

The crude protein (CP) % recorded significant increment in all treatments when compared with the control. The interaction between nitrogen fixers and microbial treatments either as cellulose decomposers or single cell protein gave significant differences between all treatments in CP%. The highest significant increase,13.71%, in CP% was recorded in rice straw soaked for 24 h and inoculated with *Azospirillum brasilense* as a nitrogen fixer and *Bacillus megaterium* as a cellulose decomposer.

Neutral detergent fiber (NDF) % and acid detergent fiber (ADF) % (Table 3) show that combined interaction between microbial treatments and some physical pretreatments of rice straw gave significant reduction in NDF and ADF% in all treatments when compared with the control. Moist rice straw inoculated with *Azospirillum brasilense* as a nitrogen fixer and *Saccharomyces cerevisiae* as a single cell protein recorded the highest significant reduction in NDF and ADF% and gave 55.39 and 42.46%, respectively. Valdez et al. (2008) recorded great decrease in OM in rice straw, wheat straw and barley straw treated with some microbial inoculants compared to untreated materials. The decrease of CF value and the increase of CP value agree with those reported by Akinfemi et al. (2010). These results were elucidated by different researchers from diverse points of view,

Table 1 Some metabolic activities of selected strains

Metabolic activities of micro-organisms	Nitrogenase activity (µ mol C_2H_2 ml^{-1} h^{-1})	Cellulase activity (µmol min^{-1})	Plant hormones		
			Cyto. (µg ml^{-1})	IAA (µg ml^{-1})	Gb. (µg ml^{-1})
Azotobacter chroococcum	133.90	–	2.41	1.20	1.73
Azospirillum brasilense	0.61	–	2.88	4.70	12.50
Bacillus megaterium	–	3521.73	1.29	1.20	2.10
Bacillus circulans	0.11	1215.79	1.31	0.90	2.40
Saccharomyces cerevisiae	–	–	3.52	–	9.50
Pleurotus eryngii	–	1170.92	2.05	0.69	39.10

Table 2 Nutritional values of rice straw (OM, CF, and CP%), affected by combined interactions between different microbial inoculants and some physical treatments

Straw treatments	Moist straw			Straw soaked for 24 h			Straw soaked for 24 h and pasteurized at 100 °C/1 h		
Parameters measured	OM%	CF%	CP%	OM%	CF%	CP%	OM%	CF%	CP%
Control	82.44	40.42	5.66	82.50	39.95	5.42	82.39	40.63	4.62
Microbial treatments									
Without nitrogen fixers									
Bacillus megaterium	76.87	34.88	12.02	76.61	30.69	12.26	79.64	38.87	7.06
Bacillus circulans	80.41	31.46	8.57	76.61	38.83	11.44	79.64	34.49	8.65
Saccharomyces cerevisiae	80.41	36.70	9.47	81.56	36.47	9.07	79.64	36.62	9.08
Pleurotus eryngii	76.53	32.59	8.06	80.39	31.87	9.04	81.58	36.90	8.35
Azotobacter chroococcum									
Bacillus megaterium	79.18	32.85	11.81	79.04	34.03	11.03	80.50	34.85	9.15
Bacillus circulans	76.41	29.69	13.03	82.60	36.97	8.65	81.98	33.28	6.50
Saccharomyces cerevisiae	74.21	29.17	10.75	80.41	32.63	9.49	80.84	34.98	10.74
Pleurotus eryngii	76.07	28.69	11.34	80.41	32.44	9.12	80.23	36.94	8.86
Azospirillum brasilense									
Bacillus megaterium	75.67	32.48	12.39	80.41	32.47	13.71	82.55	38.28	7.15
Bacillus circulans	80.41	35.94	8.78	79.46	36.70	10.68	80.53	36.64	10.26
Saccharomyces cerevisiae	80.41	27.54	9.60	79.96	30.38	10.57	79.10	37.57	8.79
Pleurotus eryngii	75.09	30.76	11.40	78.88	33.63	11.22	82.60	36.26	8.78
SE*	0.2975	0.718	0.329	0.2975	0.718	0.329	0.2975	0.718	0.329
LSD	0.5357	1.168	0.4837	0.5357	1.168	0.4837	0.5357	1.168	0.4837

*Significant error

Table 3 Nutritional values of rice straw (ADF and NDF%), affected by combined interactions between different microbial inoculants and some physical treatments

Straw treatments	Moist straw		Straw soaked for 24 h		Straw soaked for 24 h and pasteurized at 100 °C/1 h	
Parameters measured	NDF%	ADF%	ADF%	NDF%	NDF%	ADF%
Control	68.69	49.97	68.20	49.70	68.91	50.10
Microbial treatments						
Without nitrogen fixers						
Bacillus megaterium	62.98	46.75	58.65	44.31	67.09	49.07
Bacillus circulans	59.44	44.75	67.05	49.05	62.57	46.52
Saccharomyces cerevisiae cerevisiae	64.85	47.81	64.61	47.67	64.77	47.76
Pleurotus eryngii	60.61	45.41	59.86	44.99	65.06	47.93
Azotobacter chroococcum						
Bacillus megaterium	60.88	45.56	62.10	46.26	62.94	46.73
Bacillus circulans	57.62	43.72	65.13	47.96	61.32	45.81
Saccharomyces cerevisiae	57.07	43.41	60.65	45.44	63.08	46.80
Pleurotus eryngii	56.58	43.14	60.45	45.32	65.09	47.95
Azospirillum brasilense						
Bacillus megaterium	60.50	45.35	60.48	45.34	66.48	48.73
Bacillus circulans	64.06	47.36	64.85	47.81	64.79	47.77
Saccharomyces cerevisiae	55.39	42.46	58.32	44.12	65.75	48.32
Pleurotus eryngii	58.72	44.34	61.68	46.02	64.39	47.55
SE*	0.7414	0.418	0.7414	0.418	0.7414	0.418
LSD	1.2052	0.6805	1.2052	0.6805	1.2052	0.6805

*Significant error

as it could be attributed to utilization of rice straw's carbohydrates (including the CF) by the microbial inoculants as carbon source to produce energy for their growth (El-Ashry et al. 2001). Also, this may be due to the presence of *Azotobacter* sp. and *Azospirillum* sp., which are nitrogen fixers as they supply the other microbial inoculants used in the treatments with their allowances from nitrogen that consequently increase their degradation activity and decrease the organic matter through fermentation processes (El-Bordeny et al. 2015). The presence of some other nitrogen fixers in the un-pasteurized straw may lead to an increase in the nitrogen content of the treated straws (Rao and Naik 1990) as well as secretion of extracellular enzymes by the microorganisms inoculated into the treatment during their breakdown of rice straw which are protein substances (Kadiri 1999). All the previous factors decrease the C/N ratio of rice straw, which leads to an improvement in the ability of microbial inoculants as well as the dominant microorganisms in the rice straw to degrade the roughages in the rice straw. Biological treatment of rice straws resulted in reducing the NDF and ADF contents. These findings are in agreement with Mahrous and Abou Ammou (2005). The presence of high concentrations from ADF and NDF in rice straw is responsible for its poor nutritive value and lower digestibility as animal feed, since high concentrations from NDF and ADF could be responsible for low digestibility in animals (Falls et al. 2017; Sath et al. 2012). Reducing the concentration of ADF and NDF in rice straw converted it into more nutritive and easily digestible animal feed (Sharma and Arora 2011). The decrease in CF and CF fractions (NDF, ADF) may be caused due to cellulase enzymes secreted by microbial inoculants (Akinfemi et al. 2010).

Effect of nitrogen fixers on the nutritional quality of rice straw

It is obvious from data recorded in Table 4 that, in general, no significance difference was recorded between all the treatments in the OM, while rice straw inoculated with *Azotobacter chroococcum* recorded the highest significant reduction in CF, NDF and ADF which gave 33.04, 61.08, and 45.68%, respectively. However, in accordance with CP, the highest significant increment, 10.28%, was recorded with rice straw inoculated with Azospirillum *brasilense*. No significant difference was recorded between rice straw either inoculated with *Azotobacter chroococcum* or *Azospirillum brasilense* in CF, CP, NDF and ADF. Rice straw is rich in C and poor in N, which limits its degradation process. This high C/N ratio could be decreased by increasing the basal N content of rice straw by adding a nitrogen source which may have originated from non-symbiotic nitrogen-fixing bacteria. Among the nitrogen fixers, *Azotobacter* sp. and *Azospirillum* sp. were selected as they play a key role in harnessing the atmospheric nitrogen through fixation process and converting it into ammonium ion (El-Fattah et al. 2013; Zayed 2012). Also, Ahlawat and Rai (1997) reported that inoculation of mushroom seed spawn substrates with nitrogen fixers increased the growth of *P. eous* and *Agaricus bisporus.*

Effect of different microbial inoculants on the nutritional quality of rice straw

It is noticeable from the data recorded in Table 5 that all microbial inoculants gave significant records in all parameters documented when compared with raw rice straw (uninoculated). Rice straw treated with different microbial inoculants recorded significant reduction in OM, CF, NDF and ADF when compared with raw rice straw.

The best significant reduction in OM, 78.94%, was recorded in rice straw treated with *Bacillus megaterium,* while the best significant reduction in CF, NDF and ADF was recorded in rice straw treated with *Pleurotus eryngii* and gave 33.34, 61.38 and 45.85%, respectively. No significant difference was recorded between all other treatments in these parameters. Crude protein recorded significant increase in all microbial treatments compared to raw rice straw. The highest significant increment in crude protein, 10.73%, was recorded with rice straw inoculated by *Bacillus megaterium,* compared to all treatments. Mixed cultures of cellulolytic bacteria or fungi can improve the degradation of rice straw fibers (Silanikove et al. 1988). Yeasts are a group of unicellular fungi which are used as a source of high nutritional value

Table 4 Effect of nitrogen fixers on the nutritional quality of rice straw

Treatments parameters measured	Without nitrogen fixers	*Azotobacter chroococcum*	*Azospirillum brasilense*	SE*	LSD
Organic matter (OM)%	79.16	79.32	79.59	0.38	1.05
Crude fiber (CF)%	35.03	33.04	34.05	0.52	1.45
Crude protein (CP)%	9.42	10.04	10.28	0.28	0.79
Cell wall contents					
NDF%	63.13	61.08	62.12	0.53	1.50
ADF%	46.83	45.68	46.26	0.30	0.85

*Significant error

Table 5 Effect of different microbial inoculants on the nutritional quality of rice straw

Treatments parameters measured	Untreated straw**	*Bacillus megaterium*	*Bacillus circulans*	*Sacch. cerevisiae*	*Pleurotus eryngii*	SE*	LSD
Organic matter (OM)%	82.44	78.94	79.78	79.61	79.09	0.42	2.66
Crude fiber (CF)%	40.33	34.38	34.89	33.56	33.34	0.59	5.44
Crude protein (CP)%	5.23	10.73	9.62	9.73	9.58	0.32	1.01
Cell wall contents							
NDF%	68.60	62.45	62.98	61.61	61.38	0.61	5.61
ADF%	49.92	46.45	46.75	45.98	45.85	0.34	3.17

*Significant error

**Raw straw

proteins and vitamins and high biomass production, making them a preferable additive to livestock's feeds (Shridhar 2012). Also, it is safe and resistant to antibiotics, as well as has the ability to produce polyamines, which are compounds that strongly affect cell growth and differentiation (Costalos et al. 2003; McFarland 2007). Also, Valdez et al. (2008) found that the growth of *Pleurotus pulmonarius* on wheat straw increased the organic matter content and improved the nutritional quality of agricultural by-products to be used as a ruminant feeding.

Effect of moistening, soaking and pasteurization of rice straw on the feeding value of the final product

Clear significant differences between the physical treatments in all the tested parameters were recorded. Moist straw recorded the best significant reduction in the parameters OM, CF, NDF and ADF of 78.01, 32.55, 60.57 and 45.39%, respectively, as well as the highest significant increase in protein content of 10.22% when compared to other treatments (Table 6). During solid-state fermentation of rice straw, the microbial inoculants encounter the presence of some inhibiting compounds in rice straw including low molecular weight organic acids, and phenolic and inorganic compounds. These compounds are released and formed during pretreatment (pasteurization) and/or hydrolysis of the residues (Dashtban et al. 2009). It should be noticed that the significant use of soaking methods is to remove and/or dilute the concentration of the inhibiting compounds that exist in rice straw. Although pasteurization treatments did not give the highest significant data when compared with other treatments, it recorded non-contaminated products which ensure clean and healthy product for animal feed when compared with other treatments.

Conclusions

This investigation elucidates the possibility of improving the nutritional value of rice straw using different microbial inoculants and some physical treatments. The combined inoculation of different microbial inoculant either as nitrogen fixers or cellulose decomposers increased the crude protein percentage and decreased OM, CF, ADF and NDF in rice straw without using chemical compounds to produce safe, non-contaminated and cheap animal feeds. Different microbial inoculants with different enzymatic activities recorded different nutritional values for treated rice straw. Therefore, the type of microbial inoculant that should be used to improve the nutritional value of the roughage could be determined on the basis of the nutritional content of the roughage used

Table 6 Effect of some physical treatments on the nutritional quality of rice straw

Treatments parameters measured	Moist straw	Soaked straw for 24 h	Soaked for 24 h and pasteurized at 100 °C/1 h	SE*	LSD
Organic matter (OM)%	78.01	79.91	80.86	0.09	0.26
Crude fiber (CF)%	32.55	34.39	36.64	0.20	0.56
Crude protein (CP)%	10.22	10.13	8.31	0.08	0.23
Cell wall contents					
NDF%	60.57	62.46	64.79	0.21	0.58
ADF%	45.39	46.46	47.77	0.12	0.32

*Significant error

as animal feed. Using pasteurization and soaking for treating rice straw prevented its contamination and improved fiber digestion parameters.

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