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The Effect of *Pleurotus* spp. Fungi on Chemical Composition and *in vitro* Digestibility of Rice Straw

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Abstract: This study was carried out to test the potentially of using rice straw substrate for the cultivation of four *Pleurotus* species including *Pleurotus florida*, *Pleurotus djamor*, *Pleurotus sajor-caju* and *Pleurotus ostreatus* and the effect of these species on the chemical composition, cell wall degradation and digestibility of rice straw. Rice straw soaked in water for 24 h and then it was pasteurized at 100°C for 6 h. Rice straw was inoculated with spawns of four *Pleurotus* fungi (*Pleurotus florida*, *Pleurotus djamor*, *Pleurotus sajor-caju* and *Pleurotus ostreatus*) and packed in the plastic bags and incubated in a fermentation chamber at 23-27°C and 75-85% relative humidity. After 60th day, rice straw samples from all groups were taken and analyzed for chemical composition and *in vitro* digestibility. The data obtained were analyzed according to the complete randomized design model consisting of four treatments plus one control and four replicates. The results of this study showed that fungal treatment increased ($p<0.05$) the Crude Protein (CP), silica, Ca and P contents of the rice straw but the hemicellulose, Organic Matter (OM), Acid Detergent Fiber (ADF), Neutral Detergent Fiber (NDF) and Acid Detergent Lignin (ADL) contents decreased. However, the ability of the fungi to degrade these components varied among the species. The ability of *Pleurotus sajor-caju* and *Pleurotus ostreatus* were higher than the other species in decreasing the hemicellulose, NDF, ADF and ADL contents. The highest Biological Efficiency (BE) was produced by *sajor-caju* species with 56.02 and the lowest was belong to *Pleurotus djamor* species with an average 51.17%. All species of fungi incubated on rice straw showed increased ($p<0.05$) the *in vitro* dry mater and organic matter digestibility. Rice straw treated with *sajor-caju* fungus had the highest *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) with 80.10 and 82.18%, respectively. In general this experiment cleared that treatment with *sajor-caju* can improve the quality of rice straw to be useful feed for ruminant nutrition.

Key words: Rice straw, *Pleurotus* fungi, chemical composition, digestibility

INTRODUCTION

Lignocellulose is the major structural component of woody plants and non-woody plants such as grass and represents a major source of renewable organic matter. Lignocellulose consists of lignin, hemicellulose and cellulose. The chemical properties of the components of lignocellulosics make them a substrate of enormous biotechnological value (Malherb and Cloete, 2003). Large amounts of lignocellulosic waste are generated through agricultural practices and they pose an environmental pollution problem. One of these lignocellulosic wastes which is a readily available in many areas of the world is rice straw. Global production of this feedstuff has been calculated at 546×10^6 kg year^{-1} (FAO, 1995). Unfortunately, the low protein, high content of biogenic silica in the wall of the epidermal cell layer and vascular bundle and poor digestibility of rice straw limits its use in the diets of lactating and growing ruminants (Baldrin and Gabriel, 2003).

Numerous methods of physical, chemical and physicochemical have been worldwide researched and developed in order to improving the utilization of straw and other fibrous by products as feed for ruminant or for producing mushroom as a human food with varying degree of success (Hadar *et al*., 1993; Ko *et al*., 2005).

An alternative method for improving the nutritive value of straws could be the application of biotechnology. Processing involves the use of biocatalysts, whole microorganisms or their enzymes or enzymes from other organisms to synthesize or bioconvert the raw materials into new products; recover/purify such as bioproducts and subsequently any needed downstream modifications. Bioprocessing of lignocellulosic materials for higher value

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products normally requires multi-step processes which include: (i) pretreatment (mechanical, chemical or biological), (ii) hydrolysis of the polymers to produce readily metabolizable molecules (e.g., hexose or pentose sugars), (iii) bio-utilization of these molecules to support microbial growth or to produce chemical products (Zadrazil and Kamra, 1996; Aust *et al*., 2003; Baldrain, 2004).

Fungal treatment as a biological method has been recently considered as a promising method for improving the nutritive value of straw (Zadrazil, 1997; Levin and Forchiassin, 2003). Already (1976) an impressive collection of more than 14000 fungi which were active against cellulose and other insoluble fibers were collected. Despite impressive collection of lignocellulolytic microorganism only a few studies have been conducted to identify species of white-rot fungi for assessing their ability to improve the nutritive value of straw for ruminant nutrition (Esterbabur *et al*., 1991; Yamakava and Okamnto, 1992; Jqgensen *et al*., 2003; Howard *et al*., 2003).

Since there are many species of fungi in nature, there is an interest in characterizing of some species. Therefore, this study was carried out to test the potentially of using rice straw substrate for the growth of four *Pleurotus* species including *Pleurotus florida*, *Pleurotus djamor*, *Pleurotus sajor-caju* and *Pleurotus ostreatus*. In addition to diversify the use of *Pleurotus* species for mushroom production, the ability of basidiomata formation and production on rice straw substrate, biological efficiency and the effect of these species on the chemical composition, cell wall degradation and *in vitro* digestibility of rice straw and to evaluate their effect in upgrading the nutritive value of lignocellulosic materials.

MATERIALS AND METHODS

This experiment was conducted from March to October 2006 at the Animal Science Research Center of Ghaemshahr Branch, Islamic Azad University (Iran). It is located at an altitude of 51.2 m above sea level, latitude 36° 28' N and longitude 52° 53' E. The average annual rainfall is 715 mm.

Mycelial culture and spawn: For establishing mycelial culture of the mushroom for any species a piece of healthy fruit body tissue that species were cut from the stipe, near the point where it joins the cap and inoculated in Petri dish with potato-dextrose agar. The inoculated Petri dishes were incubated at 25°C in the dark for 7 days.

Wheat grains were washed in water and boiled 15 min. The boiled grains were placed on a sieve to drain. The grains were packed into wide-mouth, 1.0 L jars, until each jar was three quarters full and then autoclaved at 121°C and 1.0 atm for 1 h.

The content of each jar was inoculated aseptically with five 1 cm^2 pieces of mycelial agar and its cap closed. The jar contents then was shaken by hand for mixing the mycelia with grains and were incubated in a ventilated incubator at 25°C for 12 days (Sarikaya and Ladish, 1997; Quimio *et al*., 1990).

Preparation of the rice straw and inoculation: Rice straw was chopped into 5 cm long pieces and divided into 10 kg lots. Each lot was packed into polypropylene bags. The end of each bags were tied loosely and were soaked separately in water 24 h, for moisture absorption and then were placed to drain. The bags steamed at 100°C for 6 h in a steel vessel for pasteurization. Sixteen bags, four for each species, were prepared for the experiment.

The spawn was added at 2% (w/w) of the substrate in each bag. The bags containing spawned substrates were placed in a disinfected room with concrete a floor and darkness. Conditions in the spawn-running room were 23-27°C and 75-85% relative humidity. After 18 days, that mycelium has sufficiently colonized on the substrate, fructification condition for providing more light and ventilation were created.

Harvesting of fruit bodies and comparison of biological efficiency: Following the spawn running, when the pinhead formed and the caps were opened, until 60th day in different species all fruit bodies were harvested and kept separately for fresh-weight measurements and biological efficiencies were calculated (Oei, 1991; Miles and Chang, 1997). The Biological Efficiency (BE) is computed as the fresh weight produced divided by the dry weight of the substrate, expressed as a percentage.

Measuring the chemical composition and cell wall degradation: Before spawning and after fruit bodies harvesting, substrate samples from all species were taken and analyzed for organic matter (OM), crude protein (CP), silica, calcium (Ca) and phosphorus (P) according to the procedure outlined by AOAC (1995), cellulose, hemicellulose, acid detergent fiber (ADF), neutral detergent fiber (NDF) and acid detergent lignin (ADL), determined according to the procedure outlined by Goering and Vansoest (1970) and Vansoest *et al*. (1991). *In vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) was estimated by the two-stage technique of Tilley and Terry (1963).

Statistical analysis: Data obtained in the experiment were subjected to analysis of variance (ANOVA) in a completely randomized design model consisting of four treatments plus one control and four replicates using General Linear Model procedure of the SAS program (SAS, 1990). Significant means were compared using the Duncan's multiple range tests.

RESULTS AND DISCUSSION

Chemical composition and cell wall degradation: Chemical composition of untreated and fungal treated rice straws are in Table 1. Fungal treatment significantly increased the CP content of the rice straw. CP content ranged from 5.22 to 6.75 g/100 g dry mater basis for untreated rice straw and rice straws treated with *Pleurotus djamor*, respectively.

Organic Matter (OM) content ranged from 84.67 for untreated rice straw to 64.6 g/100 g dry mater basis for rice straws treated with *Pleurotus ostreatus*. Silica content varied from 12.23 in untreated rice straw to 25.23 g/100 g dry mater basis in rice straw treated with *Pleurotus florida*. Calcium and phosphorus contents in Table 1 show that fungal treatment increases the amounts of these minerals.

Cell wall constitutions of rice straw before and after fungal treatment are shown in Table 1. All fungal treatments reduced ($p<0.05$) the hemicellulose, ADF, NDF and ADL contents of the rice straw, however the ability of the fungi to degrade these components varied among the species. Cellulose content in treated and untreated rice straw showed a similar variation. More effect was observed from the *Pleurotus sajor-caju* and *Pleurotus ostreatus* than the other species in degrading cell wall constitutions.

Silica content determined for untreated rice straw in this study was intermediate to reported values of 5 to 15% (Bae *et al*., 1997). This variation arises because silica deposition varies among rice varieties and with concentration of silica in the soil.

In this study the results of fungal treatment effects on the chemical composition of rice straw is in agreement with other studies (Gutpata *et al*., 1988; Tripathi and Yadav, 1992; Jalk *et al*., 1996, 1998; Magnigo *et al*., 2004). These studies have reported that *Pleurotus ostreatus* fungus decrease cellulose, hemicellulose and lignin content of wheat straw. Also, in this study, *Pleurotus* species have decreased the amount of these constituents in rice straw.

The reasons of these changes may be because white-rot fungi belonging to the basidomycetes produce enzymes such as lignin peroxidase, manganese peroxidase, H_2O_2 producer enzymes, arylalchol oxidase and laccase. These fungi are unable to supply all their carbon and energy requirements from lignin and therefore require substrates such as cellulose or other carbon sources for their growth and delignification therefore they have greatest potential for degradation (Akin *et al*., 1995; Martins, 2002; Ruggeri and Sassi, 2003). As result, they consume cellulose and hemicellulose and thus organic matter decreases and ash content increases. As well, delignification probably increases hemicellulose solubility, but cellulose remains insoluble and its content changes less than hemicellulose.

Comparison of BE, IVDMD and IVOMD: Of the four species of *Pleurotus* mushroom used, the highest mushroom fresh weight were produced by *sajor-caju* species with 56.02 and the lowest was belong to *djamor* species with an average 51.17 g/100 g dry mater basis (Table 2). Fungal treatment has also significantly effect on IVDMD and IVOMD and rice straw treated with *sajor-caju*

Table 1: Chemical composition and cell wall characteristics of untreated and fungal treated rice straw (g/100 g dry mater basis)

Chemical composition	Treatments: Control	*Ostreatus*	*Florida*	*Sajor-caju*	*Djamor*	SE
OM	84.67^{a}	64.60^{b}	64.80^{b}	68.40^{b}	66.20^{b}	4.580
CP	5.22^{c}	5.23^{c}	6.17^{b}	5.92^{b}	6.75^{a}	0.280
Silica	12.23^{d}	24.69^{ab}	25.23^{a}	21.29^{c}	23.26^{b}	0.950
Ca	0.31^{c}	0.70^{a}	0.70^{a}	0.62^{b}	0.67^{ab}	0.040
P	0.07^{c}	0.13^{a}	0.13^{a}	0.11^{b}	0.12^{ab}	0.004
Cellulose	36.12^{a}	36.02^{a}	36.32^{a}	35.18^{b}	36.38^{a}	0.410
Hemicellulose	23.19^{a}	14.15^{c}	17.20^{b}	14.92^{c}	17.76^{b}	0.810
ADL	9.55^{a}	5.14^{b}	5.63^{b}	4.96^{b}	5.84^{b}	0.510
ADF	45.76^{a}	41.23^{b}	42.03^{b}	40.20^{b}	43.30^{b}	2.250
NDF	68.95^{a}	55.38^{b}	59.23^{b}	55.12^{b}	61.06^{b}	3.650

Means with the different superscripts within row are differ ($p<0.05$)

Table 2: Comparison of biological efficiency in different species and *in vitro* digestibility of untreated and fungal treated rice straw

Biological characteristics	Treatments: Control	*Ostreatus*	*Florida*	*Sajor-caju*	*Djamor*	SE
BE	---	54.38^{ab}	53.78^{b}	56.02^{a}	51.17^{c}	1.09
IVDMD	67.11^{c}	78.73^{a}	75.25^{b}	80.10^{a}	69.68^{c}	1.55
IVOMD	56.16^{d}	79.13^{b}	76.29^{bc}	82.18^{a}	74.91^{c}	1.29

Biological Efficiency (BE) = (mushroom fresh weight/substrate dry weight)×100; IVDMD = *In vitro* dry mater digestibility, IVOMD = *In vitro* organic mater digestibility, (g/100 g dry mater basis); Means with the different superscripts within row are differ ($p<0.05$)

fungus had the highest IVDMD and IVOMD with 80.10 and 82.18%, respectively.

There was a negative relationship between biological efficiency and cell wall contents of rice straw after mushroom harvesting. Cell wall contents were lower in species that had higher biological efficiency. In these species, IVDMD and IVOMD were higher than species with low biological efficiency and high cell wall content.

It has been suggested that lignin is linked to both hemicellulose and cellulose forming a physical seal around the latter two compounds that is impenetrable barrier preventing penetration of solutions and enzymes (Arora *et al*., 2002; Howard *et al*., 2003). Therefore, delignification results in changes in cell wall structure beyond the simple removal of lignin and cell content is readily available for rumen microorganisms. This could cause to increase rice straw digestibility.

Present results about *in vitro* digestibility is in agreement with many other finding (Zadrazil, 1997; Ko *et al*., 2005) but some researchers reported that fungal delignification causes low digestibility values (Hadar *et al*., 1993; Jalk *et al*., 1996). This difference in reported results can be because of many factors such as silica content of rice straw and fungus growth stage influence cell wall degradation and their digestibility.

Silica has shown to exert an anti-microbial effect on the rumen bacteria, inhibit cellulose and cellulolytic microbes and thus reduce digestion of plant cell wall. But it must be attended that the digestibility of lignocellulosic materials contains of silica improved by solubilization of silica (Bae *et al*., 1997). Therefore in this study high digestibility in fungal treated rice straw, in spite of higher silica content can be related to solubilization of silica.

White-rot fungi at the primary growth phase that termely named primary metabolism degrade high digestible polysaccharides into low weight molecules and consume lignin and hemicellulose relatively more than cellulose. On the contrary, at the secondary growth phase or termely secondary metabolism, these fungi intend to degrade cellulose more than hemicellulose and lignin (Zadrazil and Kamra, 1996). Thus, as time passes after the mushroom inoculation, cell wall constituent and their digestibility decrease. Therefore, in our study high digestibility value for fungal treated rice straw can be related to little time passed after the mushroom inoculation that include all of the primary phase and apart of secondary phase.

CONCLUSION

This study suggests that rice straw is suitable substrate for growing of all the *Pleurotus* species tested and rice straw fermentation by fungi can improve its nutritive value for ruminant. Although all of the fungi species show high ability in improving the nutritive value of rice straw and *sajor-caju* species seems to be more potent for mushroom production and upgrading of rice straw, but it must be attended that saprophytic strength and mycelium extension of any species relates to many factors such as substrate type, processing method, cultivation conditions etc. Therefore selection an appropriate species is a little difficult and more works should be done.

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