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Chemical Composition and *In Vitro* Digestibility of *Pleurotus ostreatus* Spent Rice Straw[#]

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ABSTRACT

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The nutritive value of rice straw (RS) and *Pleurotus ostreatus* spent rice straw (SRS) was studied by analyzing its proximate composition, fiber fractions, *in vitro* digestibility, amino acids content and scanning electron microscopy (SEM). The possibility of replacing berseem clover (BC; *Trifolium alexandrinum*) with SRS at different levels also was studied. Results showed higher protein content for SRS compared to RS (3.4 to 11.7%) while, DM, OM, NFE, CF, NDF, ADF, ADL, hemicellulose and cellulose were less for SRS than for RS. Highest concentration of amino acids (mg/100 g) was in SRS compared to RS. The SEM showed an extensive damage of SRS when compared to RS. Data also showed that SRS had higher *in vitro* dry matter disappearance (DMD) and *in vitro* organic matter disappearance (OMD) compared to RS. Results of *in vitro* study also, indicated that the levels of 50 and 90% replacement had the highest values of DMD and OMD compared to the other levels. It could be concluded from this study that treatment of RS with *Pleurotus ostreatus* improved the potential feeding value of the resultant substrates (i.e. SRS) as feed resources for ruminants and possibility of replacing BC with SRS at high levels of up to 50 or 90% from diets.

Key words: Biodegradation, *Pleurotus ostreatus*, Rice straw, Spent rice straw.

INTRODUCTION

Lignocellulosic biomass is not only a renewable resource but also it is the richest abundant source of organic components on the earth (Taniguchi *et al.,* 2005). Rice

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cultivation is one of the most important agriculture practices worldwide. However, it produces large amounts of rice straw (RS) by-products. The FAO has estimated RS production at about 723 million tons annually (FAO, 2013). Most of RS is burnt in the field as a way to clear the field and get rid of disease from previous crop. This practice raises the problems of increasing CO_2 emission which leads to increase global warming.

The use of RS and other lignocellulosic materials as animal feed is limited by their low nutritive value and low nitrogen content (Jalc *et al.,* 1996). Various problems associated with the practical utilization of these materials have not yet been solved. One of the key problems hindering the effective utilization of these resources is the low susceptibility of lignocellulose to hydrolysis, mainly attributable to the crystalline structure of the cellulose fibrils surrounded by hemicellulose, and by the presence of the lignin seal which prevents enzymes penetration (Chahal and Chahal, 1999). Intensive researches and developmental studies on the effective utilization of lignocellulosic materials have been done (Taniguchi *et al.,* 2005). Biological delignification of straws by white-rot fungi seems to be a promising way for improving their nutritive value (Fazaeli *et al.,* 2002).

In recent years, the significance of amino acids has been realized, not only in terms of their nutritional availability for anabolic use but also the fact that they are involved in a number of metabolic pathways. It serves in important functions including protein and energy metabolism, gluconeogenesis, lipid-fatty acid metabolism, and in mammary synthesis of milk protein and lactose (El-Kadi *et al.,* 2006; Chalamcherla *et al.,* 2010). Defining and quantifying amino acid requirements will become an important consideration in the next generation of feeding schemes for dairy cattle beyond the current emphasis on identification of limiting amino acids (Chalamcherla *et al.,* 2010).

The objectives of this work were to study the chemical and histological changes occurred as a result of *Pleurotus ostreatus* fungi cultivation on RS and nutritional evaluation of replacing Egyptian berseem clover (BC; *Trifolium alexandrinum*) with the spent rice straw (SRS), the product after *Pleurotus ostreatus* cultivation and harvest.

MATERIALS AND METHODS

Mushroom cultivation process was carried out at a private mushroom farm, *Ploshia* for mushroom production, Cairo, Egypt. The technique was carried out according to the method described by (Oei, 2005).

Spent rice straw preparation

Clean and dried RS was obtained from the field, weighed and soaked overnight for moisture absorption, then let stand for 15 min in order that the excess water can drain off. About 5% calcium carbonate ($CaCO_3$) on DM basis was added to maintain the neutral pH (7.0). The prepared substrates were sterilized by hot water (100°C) for one hour in order to kill competing micro-organisms, and to prevent pests and diseases. After the sterilization, the substrates were put into a clean plastic sheet for draining and

cooling. As soon as the substrates were cooled down to 20-25°C and drained, they were put into the plastic bags (25 cm wide and 40 cm height). Good quality spawns of Oyster mushrooms (*Pleurotus ostreatus*) were obtained from Mushroom Laboratory, Food Industries Department, National Research Centre, Egypt to inoculate the straw in the plastic bags at about 3 to 8% of the weight of the substrate.

The bags were tightly closed and pin holes were made on the bags to ensure that enough oxygen can reach the substrate. The bags were subsequently kept in a spawn running room at 25°C under dark condition until mycelium was formed. After mycelium formation (after about 21 d), large holes were made in the polythene bags to allow the normal development of fruit bodies. The bags were then put in the growing rooms containing shelves at 22°C with a 12 h photoperiod (1500-2000 Lux) and 85-90% relative humidity. Adequate ventilation was provided to prevent increase of CO_2 concentration. The bags were collected after seven weeks when the mushroom fruits were harvested two times and dried under the sun.

In vitro studies

Two laboratory *in vitro* trials were conducted. The first one aimed to compare *in vitro* dry matter disappearance (DMD) and *in vitro* organic matter disappearance (OMD) of both RS and *Pleurotus ostreatus* SRS. The second study aimed to compare different replacement levels of BC with SRS. In the first study, 12 incubation flasks (250 ml volume) were used (6 flasks per each treatment) while, the second study used 72 incubation flasks (6 flasks per each treatment in addition to 6 flasks as blank) to compare OMD and DMD of 11 replacement levels 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100% of BC with SRS. The procedures of the *in vitro* techniques were carried out according to Fondevila and Pérez-Espés (2008). Flasks filled with 140 ml of incubation solution prepared under a CO_2 atmosphere, including a buffer solution, macromineral and micromineral solutions, a reduction solution and rumen inoculum obtained from Baladi bucks fed on good quality alfalfa hay using stomach tube. Flasks were sealed and maintained at 38°C in a shaking water bath (20 oscillations/min) for 48 h.

Chemical analysis

Dried ingredients samples were ground through a Wiley mill (Arthur H. Thomas, Philadelphia, PA, USA) using a 1 mm screen. Samples were analysed for dry matter (DM, method ID 930.15), crude protein (CP, method ID 954.01), ether extract (EE, method ID 920.39), crude fiber (CF, method ID 962.09) and ash (method ID 942.05) according to AOAC (1995). Fiber fractionations were done according to Goering and Van Soest (1970) and Van Soest *et al*. (1991). Organic matter (OM) was calculated.

Determination of amino acids (HCl - hydrolyzed)

Determination of individual amino acids was performed using dried samples of RS and SRS. The procedure was performed according to the method described by (Bailey, 1967) using Eppendorff LC3000 (Germany) amino acid analyser.

Scanning electron microscopy

Scanning electron microscopy (SEM) of RS and SRS was performed according to the method described by Liu *et al*. (2005) using a scanning electron microscope (JSM-6400; JEOL, Tokyo).

Calculations and statistical analysis

Analysis of variance in a completely randomize design using general linear model procedure and Duncan test at 5% level of significance were used to compare the results for the different replacing levels using software SAS/STAT® (SAS, 2001, Version 8.02, SAS Institute Inc., Cary, NC, USA).

RESULTS

The cultivation of *Pleurotus ostreatus* on RS increased its content from CP, EE, ash and silica, while cultivation process decreased RS content of CF, DM, OM, NFE, NDF, ADF, ADL, hemicellulose and cellulose than RS (Table 1).

Table 1: Chemical composition and *in vitro* digestibility of rice straw and spent rice straw (% of DM basis)

	Rice straw	Spent rice straw
Chemical composition		
DM	93.1	83.1
OM	83.9	70. 9
CP	3.4	11.7
CF	35.6	24.0
EE	1.6	2.7
Ash	16.1	29.1
Silica	11.7	18.5
NDF	63.5	39.6
ADF	36.2	30.2
ADL	9.4	4.3
Hemicellulose	27.24	9.42
Cellulose	26.87	25.91
In vitro digestibility (%)		
DMD (±SE)	50.8^{b}±0.5	60.4^{a}±2.5
OMD (±SE)	54.4±2.5	60.6±5.5

Each value is a mean of 6 samples

NDF, Neutral detergent fibers; ADF, Acid detergent fibers; ADL, acid detergent lignin

^{ab}Means with different superscripts in the same column are significant ($P<0.05$)

Total amino acids concentration (mg/100 g) was increased in SRS (8151) than in RS (5951) due to the cultivation of *Pleurotus ostreatus*. Only three individual amino acids were lowered in SRS; leucine, tyrosine and phenylalanine, while the methionine and lysine concentrations were higher in SRS than in RS (Table 2).

Table 2. Amino acids content (mg/100 g) of rice straw and spent rice straw

Amino acid	Rice straw	Spent rice straw
Essential amino acid		
Threonine	202	387
Methionine	42	54
Phenylalanine	496	208
Histidine	220	567
Lysine	190	440
Non- essential amino acids		
Serine	254	576
Glutamic acid	1133	1382
Alanine	524	890
Tyrosine	526	99
Arginine	217	569
Aspartic	521	858
Branched chain amino acids		
Leucine	691	601
Isoleucine	196	221
Glycine	130	341
Valine	250	312
Proline	359	646
Total	5951	8151

The surface of the SRS tissues (Fig. 1B and 1D) seemed fragile, while the papillae, wart-like structures and micro-hairs were nearly disappeared and the cuticle wax silica layer was partly dissolved to clearly show the short-cells (Fig 1A and 1C). Moreover, an extensive damages of RS tissues by the fungus *Pleurotus ostreatus* on the surface of SRS when compared with RS.

Table 3. *In vitro* dry matter disappearance (DMD) and *in vitro* organic matter disappearance (OMD) at different levels of replacement of berseem clover with spent rice straw

Level of replacement (%)	Disappearance (%)	
	DMD	OMD
0	$56.2^{b}\pm1.2$	$66.7^{ab}\pm1.3$
10	$56.2^{b}\pm2.0$	$63.9^{b}\pm1.6$
20	$57.2^{ab}\pm2.2$	$65.7^{ab}\pm1.6$
30	$57.4^{b}\pm2.9$	$65.4^{ab}\pm1.5$
40	$58.1^{ab}\pm1.5$	$64.3^{ab}\pm1.3$
50	$60.7^{ab}\pm1.8$	$69.\ 6^{a}\pm1.5$
60	$59.4^{ab}\pm1.6$	$66.4^{ab}\pm1.4$
70	$59.9^{ab}\pm2.5$	$65.6^{ab}\pm2.0$
80	$60.3^{b}\pm3.3$	$67.7^{ab}\pm2.8$
90	$62.3^{a}\pm3.2$	$70.9^{a}\pm2.7$
100	$56.5^{b}\pm1.9$	$59.9^{c}\pm1.1$

Each value is a mean of 6 samples

[abc]Means with different superscripts in the same column are significant ($P<0.05$).

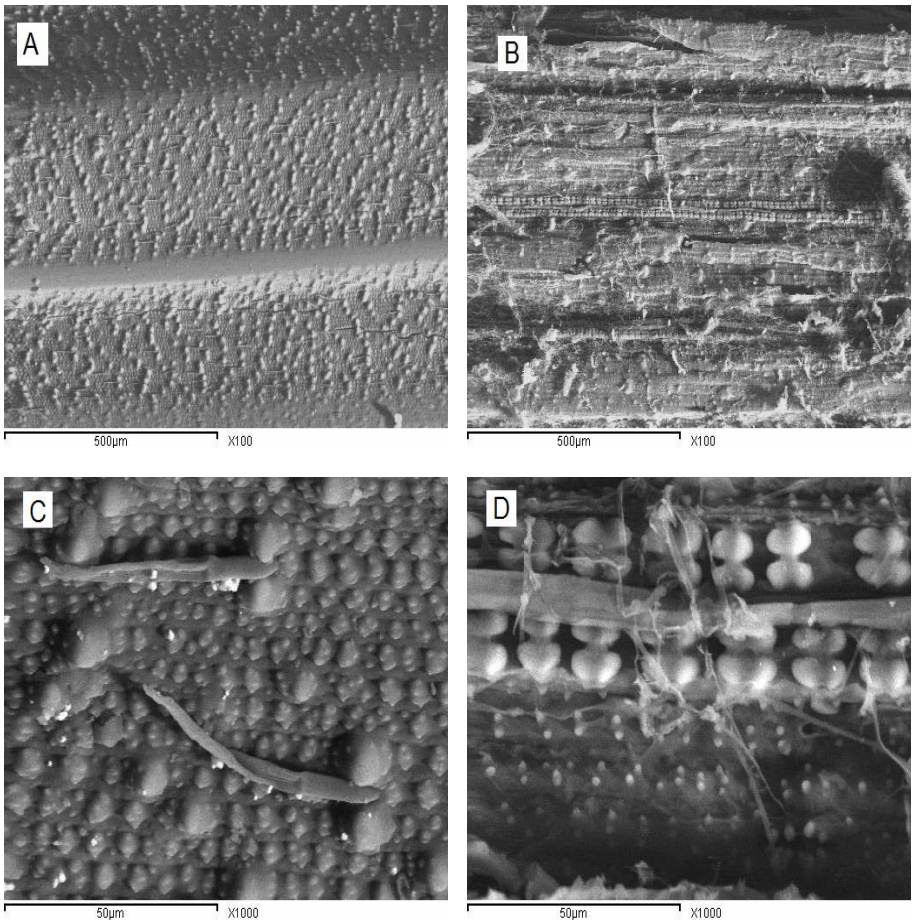

Fig. 1: Scanning electron microscope of rice straw and *Pleurotus ostreatus* spent rice straw- a) Rice straw (*100X*); b) *Pleurotus ostreatus* SRS (*100X*); c) Rice straw (*1000X*); d) *Pleurotus ostreatus* SRS (*1000X*)

Results of the first experiment showed that *Pleurotus ostreatus* SRS had higher ($P < 0.05$) DMD and OMD (Table 1) compared to RS.

Results of the second *in vitro* experiment indicated that the levels of 50 and 90% replacement increased values of DMD and OMD compared with the other levels. The lowest values of DMD, OMD were observed with 10, and 100% replacement levels compared with the other levels. On the contrary, the addition of SRS almost lowered OMD compared with 0% replacement level (Table 3).

DISCUSSION

Chemical composition and cell wall constituents

Fungus obtains their requirements from decaying the OM, in particular, the lingo cellulolytic constituents. This finding could explain the changes resulted from *Pleurotus* cultivation on RS. *Pleurotus ostreatus* belongings to the basidomycetes which produce enzymes such as lignin peroxidase, manganese peroxidase, H_2O_2 producer enzymes, arylachol 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 (Ruggeri and Sassi, 2003). They consume of cellulose and hemicellulose with the growth of *Pleurotus ostreatus* on RS and thus decreased OM and ash contents in SRS. Delignification of RS probably increases hemicellulose solubility, but cellulose remains insoluble and its contents changes less than hemicellulose (Jafari *et al.,* 2007). The higher CP content in SRS compared with RS may be due to one of the following reasons: the presence of micro-organisms, extracellular enzymes and residual media ingredients (*i.e.* mycelium) in SRS (Ball and Jackson, 1995; Siddhant and Singh, 2009); the capture of access N by aerobic fermentation by fungus (Akinfemi, 2010); the proliferation of fungi during degradation (Akinfemi and Ogunwole, 2012).

Such results were obtained by Jafari *et al.* (2007), Akinfemi (2010) and Akinfemi and Ogunwole (2012) when *Pleurotus ostreatus* cultivated on RS causing an increase of CP, ash content and decrease in the content of hemicelluloses, OM, CF, ADF, NDF and ADL.

Amino acid content

Comparing amino acids content before and after cultivation of *Pleurotus ostreatus* on RS showed an increase in the content of total amino acids which associated with the presence of fungus mycelium and mushroom bodies by-products. The improvement in the total amino acids content of the RS was the result of an increased in the quantity of different amino acids. However, the increase in the quantity was not uniform for all the amino acids.

Histological changes of RS tissues

Epidermis is an important protective tissue in plants due to its wax and silica layers, and it may decrease water loss from evaporation and defend against bacterial and fungal pathogens. However, it becomes an obstacle to degradation by rumen micro-organisms (Wang *et al.,* 2007). The morphological changes induced by *Pleurotus ostreatus* were examined by SEM to obtain insight into the structural modification in the RS. An extensive damage was observed in RS tissues treated with *Pleurotus ostreatus* (i.e., SRS) when compared with untreated RS, suggesting the efficacy of *Pleurotus ostreatus* to produce lignocellulolytic enzymes and degrade of lignocellulose in RS.

Photos of SEM emphasized that the cultivation of *Pleurotus ostreatus* resulted in an increase in susceptibility of RS to enzymatic hydrolysis due to partial degradation of the lignin which is responsible for preventing penetration of cellulase in the RS. Based on histological changes of stem epidermis, it may be suggested that cultivation of *Pleurotus ostreatus* affect epidermis of straw stem differently, and result in different degradability of epidermis.

In vitro digestibility

Increased DMD in SRS may be due to the decreased CF content and different fiber fractions due to enzymes action, and also due to the increased CP content of SRS (Jafari *et al.,* 2007). Likewise, delignification results in changes in cell wall structure beyond the simple removal of lignin and cell constituents are readily available for rumen micro-organisms (Mirzaei *et al.*, 2007) and this could lead to increase RS digestibility. However, some researchers reported that fungal delignification causes low digestibility values (Jalc *et al.,* 1996). This difference in reported results may be possibly related to many factors such as silica content of RS 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. However, this effect depends on solubilization of silica (Bae *et al.,* 1997). However, increased digestibility in SRS, in spite of higher silica content, might be related to solubilization of silica.

The presences of highly lignified tissues in RS makes a physical barrier and prevents the accessibility of highly digestible tissues to the action of hydrolytic enzyme for rumen micro-organisms (Karunanandaa *et al.,* 1995) and increased digestibility associated with the degradation of structural carbohydrates (Mukherjee and Nandi, 2004). Results reported here are in agreement with many other findings (Ko *et al.,* 2005; Jafari *et al.,* 2007).

Replacing BC with SRS

Increased DMD and OMD with both 50 and 90% replacement levels compared with the other levels may be related with the enhancement of structure occurred with SRS compared with RS. This was related with the delignification and hydrolysis occurred with the extracellular enzymes secreted from the fungus. The addition of SRS decreased OMD compared with 0% replacement level which may be due to the high content of ash in SRS. The high content of ash shown to exert an anti-microbial effect on the rumen microflora which inhibits digestion. The complete replacement of BC with SRS may increase the level of phenolic compounds which resulted from lignin decomposition which exert negative effects on media microflora.

CONCLUSION

Results suggest that *Pleurotus ostreatus* is a suitable fungus for improving the nutritive value of RS as a ruminant feed not only by improving the chemical composition but also enhancing the digestibility. *Pleurotus ostreatus* has a good potential as feed stuff for ruminant animals and could be used in combination with other feedstuffs. However, further work may be required involving its validation under *in vivo* conditions.

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