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IMPROVING THE NUTRITIVE VALUE OF COMMON REED AND RICE STRAW BY TREATMENT WITH *PLEUROTUS OSTREATUS*

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Abstract

Fungal treatment can used to enhance the nutritive value of agro-industrial by-products. Common reed and rice straw were treated with white strain of *Pleurotus ostreatus* to investigate the effect of fungi on nutrient composition and *in-vitro* digestibility after 21 days. Results revealed that *P. ostreatus* treatment increased (P<0.01) crude protein (CP) and decreased (P<0.01) neutral detergent fiber (NDF) and acid detergent lignin (ADL) contents of common reed and rice straw compared to untreated. Higher increment in CP but higher decrease in ADL content occur with common reed treated with *P. ostreatus.* The *in-vitro* digestibility were increased (P<0.01) of both substrates. However, treated common reed showed higher improvement in *in-vitro* digestibility than of rice straw, it is concluded that *P. ostreatus* improved nutritive value of common reed and could be used in feeding ruminant instead of left unused or controlled by herbicide.

***Key words*:** common reed, rice straw, *Pleurotus ostreatus*, nutrient composition, *in-vitro* digestibility.

Introduction

There are limited areas for cultivation green fodder to use for grazing purpose in Iraq. This leads to depend on natural pastures to feed ruminant animal on agro-industrial by-products such as common reed, barley straw and rice straw (Hassan *et al.*, 1998; 2008). Common reed (*Phragmites communis*) is a wild grass distributed on large areas of marshes, small irrigation canals, and around rice fields in the central and southern of Iraq and it is generally left unused or burnt (Hassan *et al.*, 1998) along with rice straw which is a main agriculture by-product of rice (*Oryza sativa*) cultivation in Najaf Province. Major limitations of using these residues as feed for ruminant are low protein and high cell wall components resulting low digestibility and poor palatability (Van Soest, 2006; Hassan *et al*., 2012). Over the years, researchers have developed methods to overcome these limitations by physical and chemical (alkali) treatment (Hassan *et al.*, 1998; 2008).

Nowadays, there are great attentions towards safety improvement of agricultural by products by using friendly environmental methods instead alkali treatment with the come into view concerns of food safety issues related to animal products such as using exogenous fibrolytic enzymes (Al-Wazeer, 2015). The application of biological treatment is receiving great attention (Hassan *et al.*, 2007). Fungal treatment could be an approach to enhance nutritive value of low quality residue (Hassan *et al.*, 2012). Wheat and barley straw are the most substrates used to grow *Pleurotus Spp.* but the increasing demand of straw and high prices during the shortage of feedstuffs encouraged researchers to searching alternative substrates and local availability (Cohen *et al.*, 2002; Masevhe *et al.*, 2016).Hence, the objective of current study was to evaluate the effectiveness of *Pleurotus ostreatus* on nutrient composition and *in-vitro* digestibility of common reed and rice straw.

Materials and Methods

This study was conducted at Animal Nutrition laboratory belongs to Animal Production Department at Faculty of Agriculture, University of Kufa, Najaf, Iraq.

Fungal treatment

Fungi strain and spawn preparation : The white strain of Oyster mushroom *(Pleurotus ostreatus* Jaq. Fr.) was obtained from College of Agricultural, University of Baghdad. The spawn was grown on sterilized wheat grains (culture) according to the method of Oei (2005).

Substrates preparation, treatment with Fungi Inoculum and Incubation : Two substrate samples of common reed and rice straw were collected from private field in Najaf Province. Both substrates were cut into lengths of 2-4 cm and divided into two parts: first part left without any treatment while the second part of each substrate was soaked in water contain 3.3% calcium carbonate for 24h, and then let stand for 30 min to remove excessive water then sterilized by boiled hot water (100 ºC) for 60 min to destroy any pets and contamination micro-organisms, then cooled to room temperature for 24 h and drained. After cooling, the substrates were packed in polyethylene bags (30cm×50cm). Each bag was inoculated with spawn's grain of *P. ostreatus* (previously prepared spawn) at the rate 3% w/w (Hassan *et al*., 2012). Each bag was tightened up with nylon thread and transferred to the

dark fermentation room where the temperature of 26 ± 3ºC and the relative humidity of 75-80%. After seen the mycelium has colonized on the substrate about 16 days of incubation, large hole were made in the polyethylene bags to provide more light and ventilation. Bags were removed from the fermentation room after 21 days of incubation and the mycelium and substrates were mixed together and oven-dried at 60ºC for 48hand ground with Willey mill and stored in for subsequence analysis.

Chemical Analysis

Dry matter (DM), crude protein (CP; nitrogen × 6.25), ash and ADL were determined in accordance with AOAC (2005) procedures. Ash frees NDF, acid detergent fiber (ADF) were determined following the method of Van Soest *et al.*(1991) without α-amylase for NDF. The hemicellulose (HEMI) and cellulose (CELL) contents were calculated by different (NDF-ADF and ADF-ADL, respectively).

***In-vitro* study**

The *in-vitro* of dry matter digestibility (IVDMD) and organic matter digestibility (IVOMD) of common reed and rice straw were determined using Tilly and Terry (1963) with one stage of microbial fermentation (48 h). No Animals were harmed in this study and the source of ruminal liquor obtained from mature Awassi rams after slaughtered, the animals were maintained on diets consisting of alfalfa hay and barley straw (75:25) and 350 g of concentrate diet before slaughtered for one month, immediately after slaughtered, the rumen liquor sample was collected and transferred immediately to the Animal Nutrition laboratory into pre-warmed thermo flask in absence of oxygen, after straining through double layers of muslin cloth. The strained rumen liquor was mixed with buffer previously prepared according to Galyean (1997). The fine ground samples (common reed and rice straw) of 500mg (n=3) were weighed and placed into 100ml plastic centrifuge tubs. Subsequently, the mixture 40 ml strained rumen liquor: 10 ml buffer was added and CO_2 was bubbling slightly. The tube was closed with a rubber cup and incubated in an automatic shaking water bath maintained at 39°C during the process. After 48 h, the tubes were transfer in an ice container to stop further fermentation, then centrifuged at 10,000 rpm for 30 min, and the supernatant was removed. The residue was filtered through a Whatman paper. Blank samples (rumen liquor and buffer only without sample) were incubated as described above and served as a correction factor to the DM and OM residuals. The residual samples were dried in oven at 105ºC for overnight to determine DM residue and then ignition in muffle furnace at 550 ºC for 3h to determine the OM residue after *in-vitro* fermentation.

Statistical analysis

Data were statistically analyzed as completely randomized design with a 2×2 factorial arrangement design by using ANOVA software program of SAS (2004). Least significant differences (LSD) producer was used to separate between treatments means. Data expressed as means ± standard error (n=3).

Results and Discussion

Nutrient composition

The nutrient composition of common reed and rice straw before and after treatment with *P. ostreatus* is illustrated in Table 1. The *P. ostreatus* treatment decreased (P<0.01) DM, OM and increased (P<0.01) CP and ash content of common reed and rice straw compared to the untreated ones. This increase of CP content in treated substrates may be due to the presence of micro-organisms, extra cellular enzymes and residual media ingredients, addition of fungi protein due fermentation, and/or capture excess nitrogen by fermentation of fungi and the proliferation of fungi during degradation (Akinfemi *et al.*, 2010; Khattab *et al.*, 2013). Similar result has been reported when *P. ostreatus* treated common reed (Nakhaei *et al.*, 2017) rice straw (Zadrazil *et al.*, 1996; Akinfemi and Ogunwole, 2012; Khattab *et al.*, 2013; Khan *et al.*, 2015) barley straw (Hassan *et al.*, 2007; 2012). The improvement in CP content of highly fibrous by-product upon fungal treatment is important for feeding ruminant because these substrates are normally low in CP content (Hassan *et al.*, 2007; 2012; Tuyen *et al.*, 2013). In the current study, the reduction in DM and OM content of *P. ostreatus* treated common reed and rice straw may be due to correlated with consumption of substrate carbohydrate in the cell wall and uses as energy source by the fungi, resulting in losses of DM and OM (Tuyen *et al.*, 2012; Khan *et al.*, 2015). The decrease of OM content in this study (5.27 and 5.68%, respectively for common reed and rice straw) was lower than *P. ostreatus* treated rice straw 37.70 % (Jafari *et al.*, 2007) and higher *P. ostreatus* treated common reed 3.7% (Nakhaei *et al.*, 2017). This may be due to the different incubation period, fungi strain and fermentation conditions among studies.

The *P. ostreatus* treatment also decreased (P<0.01) NDF, ADF and ADL, but decrease (P<0.05) HEMI and CELL contents of treated common reed and rice straw compared to the untreated. These result could be attributed to the ability of the white rot fungi, including *P. ostreatus*, to produce a wide range of extracellular lignolytic enzymes (laccase, lignin peroxidase and manganese peroxidase) and extracellular hydrolytic enzymes (xylanase and cellulase) that degrade cell wall component of substrates because it was capable to

penetration deep in the cell of substrate (Sánchez, 2009) which means breakdown of lignin bounds structural carbohydrate. However, Akinfemi *et al.* (2010) attributed the reduction in fiber substance might be related with usage of carbohydrates by fungi as a source of energy for mycelia development. In the current study, HEMI content of common reed decreased by 18.97% while in rice straw decreased by 7.67%. This might be due to the degradation of lignin by fungi probably increase the accessibility or solubility of hemicellulose. This results confirmed by previous finding of Jafari *et al*. (2007); Tuyen *et al*. (2013).These observations are in agreement with results reported by Okano and Minemori (2014) they noted that treated common reed with white-rot fungi (*Ceriporiopsis subvermispora*) decrease NDF and Lignin. These results are in harmony with previous results with *P. ostreatus* treated common reed (Gezer *et al.*, 2005; Nakhaei *et al.*, 2017) rice straw (Jafari *et al.*, 2007; Akinfemi and Ogunwole, 2012; Khattab *et al.*, 2013; Khan *et al.*, 2015). In the current study the different between substrates, ADL content of rice straw (P>0.05) did not affected by *P. ostreatus* treatment. CELL content of common reed (P>0.05) not affected by *P. ostreatus* treatment. In addition, the higher increase in CP content (52.17% *VS.* 36.52%) and higher decrease in NDF (10.08% *VS.* 7.08%) and ADL (13.18% *VS.* 8.31%) occurred in common reed than rice straw.

The results obtained in current study regarding changes in fiber fraction of rice straw and common reed after treatment with *P.ostreatus* are in harmony with previous studies (Akinfemi *et al*., 2010; Akinfemi and Ogunwole, 2012; Khan *et al.*, 2015; Tuyen *et al*., 2012; 2013).

Regarding not significant changes in ADL content of rice straw could be related to the considerable amount of silica of rice straw (Van Soest, 2006) or need more time of incubation .This may suggest that longer than 21 days of the treatment with *P. ostreatus* is required for rice straw to degraded lignin and free structural carbohydrates. Khan *et al.* (2015) used different incubation periods (0, 21, 28 and 35 days) when treated rice straw and other straw with *P.ostreatus* and concluded that longer incubation period (35 days) resulted in highest improvement in the nutritive values of rice straw .

Table 1 : Nutrient composition of common reed and rice straw before and after treated with *Pleurotus ostreatus* (%on DM Basis)

Substrate (S)	Common reed		Rice straw		Level of Significance		
Items	Untreated	Treated with *P. ostreatus* (PO)	Untreated	Treated with *P. ostreatus* (PO)	S	PO	S×PO
Dry matter (DM)	93.70±0.15^{a}	92.06±0.02^{b}	91.59±0.51^{b}	88.26±0.53^{c}	**	**	*
Organic matter (OM)	91.39±0.21^{a}	86.57±0.14^{b}	91.03±0.50^{a}	85.86±0.21^{b}	NS	**	NS
Crude protein (CP)	5.52±0.17^{b}	8.40±0.21^{c}	3.76±0.07^{b}	5.13±0.07^{b}	**	**	**
Neutral detergent fiber (NDF)	80.42±0.66^{a}	72.31±0.25^{b}	72.65±0.64^{b}	67.51±0.48^{c}	*	**	NS
Acid detergent fiber (ADF)	57.88±0.94^{a}	54.05±0.34^{b}	52.30±0.15^{b}	48.72±0.50^{c}	**	**	NS
Acid detergent Lignin (ADL)	22.76±0.77^{a}	19.76±0.35^{b}	13.12±0.43^{c}	12.03±0.18^{c}	**	**	*
Cellulose (CELL)	35.12±0.80^{bc}	34.29±0.49^{c}	39.18±0.39^{a}	36.69±0.32^{b}	*	*	*
Hemicellulose (HEMI)	22.54±0.76^{a}	18.26±0.18^{bc}	20.53±0.67^{b}	18.79±0.61^{c}	NS	*	*
Ash	8.60±0.40^{b}	14.14 ±0.94^{a}	8.97±0.20^{b}	13.43±0.94^{a}	NS	**	NS

Row means with different subscripts differ significantly at (P<0.05); NS: not significant; *: P<0.05; **P<0.01

***In- vitro* digestibility**

As it is shown in Table (2), the *P. ostreatus* treatment increased (P<0.01) IVDMD, IVOMD and ME of treated common reed and rice straw compared to untreated. The reason of improved *in-vitro* digestibility of both substrates by *P. ostreatus* could be attributing to reduction in cell wall components and an increase in crude protein content of substrate after fermentation. This finding is consist with finding (Mukherjee and Nandi, 2004; Akinfemi *et al*., 2010; Tuyen *et al.*, 2013) when treated rice straw and corn stover with *P. ostreatus.* It has been suggested that lignin is linked to both cellulose and hemicellulose forming physical barrier around them, preventing the accessibility of hydrolytic enzymes of the rumen micro-organisms (Karunanandaa *et al.*, 1995), ruminal micro-organisms do not have ability to emit any lignolytic enzymes (Zadrazil *et al.*, 1996). However, *Pleurotus spp.* releases lignolytic enzyme that degraded lignin (Sánchez, 2009). Therefore, delignification results in changes in cell wall structure beyond the removal lignin and release cellulose and hemicellulose which made them more accessible to the rumen microorganisms (Jafari *et al*., 2007; Khattab *et al*., 2013). Similarly, Hassan *et al.*

(2007; 2012) found significant increased IVDMD and IVOMD of barley straw after incubation with *P. ostreatus* for 21 days. Improved *in-vitro* organic matter digestibility and metabolizable energy estimated via gas production technique of rice straw treated with *P. ostreatus* was also observed by Jafari *et al.* (2007), Akinfemi and Ogunwole (2012), Khattab *et al.* (2013) and Khan *et al.* (2015). Nakhaei *et al*. (2017) reported improve IVOMD, ME and *in situ* degradability of common reed treated with *P. ostreatus*. Okano and Minemori (2014) who found improved IVDMD and *in-vitro* NDF digestibility of common reed treated with white rot fungus. The improvement in IVDMD and IVOMD of common reed were (11.69 and 15.88%, respectively) higher than improvement in rice straw (7.64 and 9.46%, respectively) in the current study. This result could be attributed to the significant decrease in lignin content of common reed by *P. ostreatus* (Table 1) the higher than reported by others. The results obtained in the current study for common reed and rice straw treated with *P. ostreatus* are contrasted from the previously mentioned, which may be clarified by differences in incubation time and/or morphological nutrient composition of these substrates (Shrivastava *et al*., 2011) this need further investigation.

Table 2 : *In-vitro* digestibility (%)and estimated ME (MJ/kg.DM) of common reed and rice straw before and after treated with *Pleurotus ostreatus*

Substrate (S)	Common reed		Rice straw		Level of Significance		
Items	Untreated	Treated with *P.ostreatus* (PO)	Untreated	Treated with *P.ostreatus* (PO)	S	PO	S×PO
IVDMD%	27.83±0.17^{d}	31.08±0.36^{c}	40.72±0.10^{b}	43.83±0.38^{a}	**	**	*
IVOMD%	29.34±0.31^{d}	34.00±0.43^{c}	41.87±0.14^{b}	45.83±0.60^{a}	**	**	*
ME(MJ/kg.DM)	4.40±0.05^{d}	5.10±0.06^{c}	6.28±0.21^{b}	6.88±0.09^{a}	**	**	*

Row means with different subscripts differ significantly at (P<0.05); *: P<0.05; **P<0.01
IVDMD: *in-vitro* dry matter digestibility, IVOMD: *in-vitro* organic matter digestibility,
ME: Metabolizable energy estimated according to equation ME (MJ/kg.DM) =IVOMD × 0.15 (MAFF, 1975)

Conclusions

From the results of the current study it can be concluded that *Pleurotus ostreatus* has a marked increase the crude protein content and *in-vitro* digestibility of common reed and rice straw. Fungi treatment with *P. ostreatus* of common reed would considerably be of beneficial effect if included in the diet of ruminant animal and reduce using of herbicides in weed control.

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