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## Nutritive Value Index of Treated Wheat Straw with *Pleurotus* Fungi Fed to Sheep

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**Abstract:** Wheat straw was inoculated by two species of *Pleurotus* fungi (H-77 and H-82) in polyethylene bags. After 17 days, half of the bags were removed from fermentation room and sampled. Fermentation were sized in an other half of the bags after two flashes of mushroom harvested in seven weeks. Chemical analysis and *in vivo* digestibility were conducted on straw before and after fermentation. In a feeding trial, *in vivo* digestibility and voluntary intake determined by sheep, where the dietary treatments were T1) untreated wheat straw, T2) fungal treated wheat straw (F-77) after complete mycelium run before fruit formation, T3) fungal treated wheat straw (F-77) after harvesting of mushroom, T4) fungal treated wheat straw (H-82) before formation of mushroom and T5) fungal treated wheat straw (H-82) after harvesting of mushroom. Fungal treatment significantly ( $p < 0.05$ ) increased the crude protein but decreased the cell wall components of straw. The digestibility and TDN were significantly ( $p < 0.05$ ) higher in T2 and T3. Average daily intake of DM and OM were significantly ( $p < 0.05$ ) higher in T2 but lower in T5 comparing to the initial straw (T1). In comparison to the untreated straw, fungal treatment increased the digestibility of straw as well as the voluntary intake, at the stage of mycelia running. However, the digestibility and intake of mushroom harvested straw were significantly ( $p < 0.05$ ) decreased. In conclusion, treated straw by two species of fungi, improved the nutritive value index, but the nutritive value of mushroom harvested straw was lower than that of the initial straw.

**Key words:** Wheat straw, treatment, *Pleurotus* fungi, nutritive value

### INTRODUCTION

Straw represents a major quantity of biomass from cereal production. However, most parts of its carbohydrates are in the form of structural cell wall bonded with lignin, which reduces its degradability and limits the availability of nutrients from the straw. In order to break down the lignocellulosic bond of straw to increase its nutritive value, various chemical and physical delignification methods have been extensively tested (Leng, 1991; Zahedifar, 1997). Although these methods have advantages but they are costly, technology requirement, low effectiveness and some are not environmental friendly (Leng, 1991; Sharma *et al.*, 1993) that limit the application of these methods, particularly at small farm levels.

Since last decades, biological de-lignification's of straw by solid-state fermentation (SSF) has been considered because of its capacity to remove lignin preferentially (Fazaeli *et al.*, 1999; Moyson and Verachtert, 1991). Attempts had been made to identify species of white-rot fungi for their ability to grow on straws that improved their nutritive value (Yamakawa *et al.*, 1992; Arora *et al.*, 1994; Zadrazil *et al.*, 1996). The bio-conversion of lignocellulosic materials is

circumscribed to the group of white-rot fungi, of which some species of *Pleurotus* are capable of producing upgraded spent-straws as ruminant feed. The fungi are able to colonize on cereal straw and liberate water soluble substrates from the polymers during SSF and thus improve the *in vitro* DM digestibility (Fazaeli *et al.*, 2003; Zadrazil, 1997; Yamakawa *et al.*, 1992). During the SSF of wheat straw by fungi, its organic matter (OM) and detergent fibre content could be reduced and the lignin selectively removed from the lignocellulosic complex (Singh *et al.*, 1990; Kundu, 1994). The crude protein (CP) and ash were also increased in the treated straw (Moyson and Verachtert, 1991). Such changes were dependent on the strain of fungi and the cultural conditions (Tripathi and Yadav, 1992).

Among the edible white-rot fungi, the *Pleurotus* species have been shown to be more efficient (Zadrazil *et al.*, 1996). The potential of some species of the *Pleurotus* fungi such as *P. ostreatus* and *P. eryngii* to reduce indigestible cell wall components and increase the dry matter digestibility (DMD) of straw has been reported (Agosin *et al.*, 1986; Singh *et al.*, 1990). Some strains of *P. ostreatus* increased the *in vitro* digestibility of wheat straw up to 25.5 unit percent while some others decreased the digestibility by 13.8 unit percent (Zadrazil, 1997).

Utilization of cereal straw treated with white-rot fungi as animal feed was studied by some workers (Kakkar *et al.*, 1990; Moyson and Verachtert, 1991; Fazaeli *et al.*, 2004). Jalil *et al.* (1998) noted that the *in vitro* dry matter digestibility (IVDMD) of wheat straw was increased from 7 to 10 unit percent when treated with *Pleurotus* fungi for a 30 day fermentation period. Calzada *et al.* (1987) found that during 30 days SSF of wheat straw by *P. ostreatus*, the lignin content decreased significantly and IVDMD increased from 14.3 to 29.5%. Karunanandaa and Varga (1996) reported that treating rice straw with *Cyathus stercoreus* in 30 days of SSF increased the apparent digestion of DM (44 vs. 35.1%) and OM (50.6 vs. 41.5%).

Due to the existence of many species and strains of fungi in nature and their possible different effects on the nutritive value of the substrates, there is an increased research interest on the characteristics of the species and strains including the ability of their growth on the straw and their effects on the nutritive value of the straw. This study was conducted to assess the effect of two species of *Pleurotus* fungi on the digestibility and voluntary intake of wheat straw. Secondly, to compare the nutritive value of fungal treated wheat straw before and after the mushroom was harvested.

## MATERIALS AND METHODS

**Treatment of wheat straw:** Wheat straw was packed in cotton bags (45×90 cm) and soaked in water for 24 h, in a steel water tanks (2×1×0.8 m size) then it was pasteurized in hot water at 80 °C for 60 min. The wheat grain spawns of two *Pleurotus* fungi (coded F-77 and H-82), obtained from the Iranian culture collection center, was used to inoculate the straw. The pasteurized straw was spread in a polyethylene sheet and mixed with the spawn at the rate of 3.5 kg spawn per 100 kg straw (fresh weight basis) in the spawning room. Then the inoculated straw was packed in polyethylene bags (70 cm length and 40 cm diameter and 100 gauge thickness). Each bag that contained approximately 12 kg of straw (fresh weight) was tightened up with nylon thread and transferred to the fermentation room where the temperature of 22±5°C and the relative humidity of 70±5% maintained by means of automatic air condition. During the first week of incubation, when the mycelium run started, all sides of the bags were crushed, to provide a uniform distribution of mycelium for all substrate. After 17 days of incubation, half of the bags were removed from the fermentation room and sun dried and stored for necessary tests. The remaining bags were collected after seven weeks of

fermentation when two flashes of mushroom. Was harvested and the biomasses were sun dried and stored for experiment.

**Feeding trial:** A change over design experiment was conducted in which, five treatments were tested for digestibility and voluntary intake by mature male sheep weighing about 39-42 kg where the treatments

were:

- T1) untreated wheat straw (UWS),
- T2) fungal treated wheat straw (F-77) before formation of mushroom (F-FTWS),
- T3) fungal treated wheat straw (F-77) after harvesting of mushroom (F-SPWS),
- T4) fungal treated wheat straw (H-82) before formation of mushroom (H-FTWS),
- T5) fungal treated wheat straw (H-82) after harvesting of mushroom (H-SPWS).

**Measurements:** Feed intake and digestibility: The animals were fed the dietary treatments *ad libitum* in addition with 100 g of concentrate supplement composed of ground barley, wheat bran, cottonseed meal and mineral supplement. Each treatment was tested for a period of two weeks for adaptation and 10 days of collection period. Daily feed intake and refused were measured and sampled during the collection period. Faeces from individual animals were collected and weighed every morning and sub-sampled. At the end of each collection period, the samples of feeds and refused were dried at 65°C for 48 h and faeces were dried at 65°C until constant weight. The dried samples were ground through 1 mm mash. Aliquots of the samples from each day were pooled and analysed chemically.

**Chemical analyses and *in vitro* digestibility:** The Organic Matter (OM) was measured by ashing the samples at 500°C for 4 h. Crude Protein (CP) was analysed by Kejltek Auto 1030 analyser (N×6.25) and Crude Fibre (CF) was determined according to the method represented in AOAC (1990). Cell wall component including Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF) were determined by using the methods of Van Soest *et al.* (1991). Acid Detergent Lignin (ADL) was measured according to the AOAC (1990). The *in vitro* digestibility was determined using by the method of Tilley and Terry (1963).

**Statistical analysis:** Data obtained were analysed for parametric statistics, including analyses of variance by

GLM procedure of SAS software (1992) using the following model and tested for significance, using Duncan multiple range test.

$$Y_{ijk} = \mu + P_j + T_k + C_1 + E_{ijk}$$

$Y_{ijk}$  = Responses of animal  $i$  in treatment  $k$  of period  $j$ ,

$\mu$  = Overall sample mean,

$T_k$  = Treatment  $k$  effect,

$E_{ijk}$  = Ordinary least squares residual error.

## RESULTS AND DISCUSSION

**Chemical composition:** Table 1 shows the chemical composition of treated and untreated wheat straw. Fungal treatment had significantly ( $p < 0.05$ ) decreased the OM but increased the CP content of the straw. The CP content was 3.2% in the initial straw, but it was 5.1% (F-77) and 5.5% (H-82) for the fungal treated straw. These results are in agreement with some reports (Singh *et al.*, 1990; Fazaeli *et al.*, 2004). During the fermentation period, the substrate is decomposed and fungal biomass accumulated in which a considerable part of organic matter may be mineralised to  $CO_2$ . An increase of CP content in wheat straw incubated with *Pleurotus* species had also been reported (Ardon *et al.*, 1996; Zadrazil *et al.*, 1996). The protein content of the mycelium was reported relatively high (Ragunathan *et al.*, 1996), so it was expected that the treated straw, that contained fungal mycelium to have a higher concentration of CP.

Both of the treatments significantly ( $p < 0.05$ ) reduced NDF, ADF and ADL contents of the straw and no differences was observed among the ability of the fungi to degrade these components. This was due to the natural habitats of the white-rote fungi that largely depend on organic carbon (for their energy requirement) including carbon in the form of structural material such as lignocellulosic (Jennings and Lysek, 1996). The losses of NDF and ADF from the straw suggested that these fungi could solubilise and utilise the cell wall as carbon source and thus changed the ratio of soluble to un-soluble carbohydrates in the straw (Taniguch *et al.*, 2005). The decrease in NDF and ADF contents of the treated straw has been supported by other reports (Singh *et al.*, 1990; Okanon *et al.*, 2005). However, the potential of NDF degradation among these species of fungi could be different (Jalc *et al.*, 1996; Zadrazil *et al.*, 1996). Decrease of ADL in wheat straw had been reported when it was treated with *P. pulmonarius* and *P. sajor-caju*, (Moysen and Verachtert, 1991). It could be as a result of lignin degradation enzymes, produced by the *Pleurotus* fungi during the fermentation period (Singh *et al.*, 1990; Boyle *et al.*, 1992).

Table 1: Chemical composition and *in vitro* digestibility of the treatments (% of DM basis).

Items	Treatments			SEM (n = 24)
	T1 control	T2 F-77	T3 H-82	
OM	94.5 <sup>a</sup>	90.2 <sup>b</sup>	90.0 <sup>b</sup>	1.5
ASH	5.9 <sup>b</sup>	9.2 <sup>a</sup>	10.0 <sup>a</sup>	1.5
CP	3.2 <sup>b</sup>	5.1 <sup>a</sup>	5.5 <sup>a</sup>	0.33
NDF	83.5 <sup>a</sup>	73.7 <sup>b</sup>	75.0 <sup>b</sup>	0.83
ADF	62.8 <sup>a</sup>	55.4 <sup>b</sup>	56.5 <sup>b</sup>	1.04
ADL	8.2 <sup>a</sup>	7.4 <sup>b</sup>	7.2 <sup>b</sup>	0.040
CL	54.8 <sup>a</sup>	48.3 <sup>c</sup>	50.3 <sup>b</sup>	1.07
HCL	20.7 <sup>a</sup>	18.7 <sup>b</sup>	18.5 <sup>b</sup>	1.36
IVDMD	28.1 <sup>c</sup>	40.3 <sup>a</sup>	37.0 <sup>b</sup>	2.21 (n = 20)
IVOMD	27.5 <sup>c</sup>	40.2 <sup>a</sup>	36.8 <sup>b</sup>	2.07 (n = 20)

Means with the different superscripts within row are significantly ( $p < 0.05$ ) different, SEM = Standard Error of Mean, CL = Cellulose, HCL = Hemi cellulose, IVDMD = *In vitro* dry mater digestibility, IVOMD = *In vitro* organic mater digestibility

Table 2: Effect of treatments on the *in vivo* digestibilities of the nutrients

Items (%)	Treatments			SEM (n = 24)
	T1 control	T2 F-77	T3 H-82	
DM	30.4 <sup>c</sup>	40.1 <sup>a</sup>	35.2 <sup>b</sup>	1.39
OM	33.4 <sup>c</sup>	45.1 <sup>a</sup>	37.8 <sup>b</sup>	0.33
CP	34.0 <sup>c</sup>	43.1 <sup>a</sup>	40.8 <sup>b</sup>	0.33
CF	30.4 <sup>c</sup>	40.8 <sup>a</sup>	35.7 <sup>b</sup>	0.36
NDF	30.3 <sup>c</sup>	39.1 <sup>a</sup>	33.1 <sup>b</sup>	0.83
ADF	27.8 <sup>c</sup>	41.8 <sup>a</sup>	33.7 <sup>b</sup>	1.04
CL	32.2 <sup>b</sup>	61.0 <sup>a</sup>	44.4 <sup>b</sup>	1.07
HCL	31.3 <sup>b</sup>	37.1 <sup>a</sup>	39.3 <sup>a</sup>	1.36
GE	31.6 <sup>c</sup>	42.0 <sup>a</sup>	35.3 <sup>b</sup>	2.21 (n = 20)

Means with the different superscripts within row are significantly ( $p < 0.05$ ) different, SEM = Standard Error of Mean, CL = Cellulose, HCL = Hemi cellulose

Fungal treatment also significantly ( $p < 0.05$ ) reduced the concentration of cellulose and hemi-cellulose. Among the treatments, wheat straw treated with F-77 had the lowest cellulose content while the hemi-cellulose content was similar for both of the treatments. The fungi, which their life depends on lignocellulosic materials, mostly release and utilise the hemi-cellulose and cellulose as carbohydrate sources. They are able to produce laccase, cellulase, xylanase and glucosidase enzymes to degrade lignocellulosic compounds and utilise the releasing sugars (Azizi *et al.*, 1990; Zadrazil *et al.*, 1996).

***In vitro* digestibility:** As it is shown in Table 1, solid state fermentation of wheat straw by the fungi, significantly ( $p < 0.05$ ) increased the digestibility of DM from 28.1 to 37 and 40.3 as well as the OM, from 28.1 to 36.8 and 40.2% , respectively, but the effects of the treatments on IVDMD and IVOMD of the straw were significantly ( $p < 0.05$ ) different. The Duncan comparison test indicated that wheat straw treated with F-77 had significantly ( $p < 0.05$ ) higher digestibility than the straw treated with H-82.

Lignin binds with hemi-cellulosic components of cell wall and through covalent linkages and physical binding, prevents accessibility and biodegradation of straw

carbohydrates by cellulolytic and hemi-cellulytic micro organisms (Karunanandaa and Varga, 1996). Improvement the digestibility of treated straw could be as a result of solubilization of structural polymers by fungi (Boyle *et al.*, 1992), which made it more accessible to the rumen micro organisms. Similar results were reported by Gupta and Langara (1991). However, the ability of fungi to improve the digestibility of straw could be different. Increase of DMD of wheat straw fermented with *Pleurotus* fungi has been reported from 15 to 46% (Zadrazil *et al.*, 1996). Beside the culturing conditions, the ability of various strains of white-rot fungi in cell wall degradation and digestibility improvement of wheat straw may be different (Tripathi and Yadav, 1992; Jalc *et al.*, 1997).

**Feeding trial:**

**In vivo digestibility:** Total tract digestibility were significantly ( $p < 0.05$ ) affected by fungal treatment (Table 2). The digestibility of DM and OM were 30.4 and 33.4%, respectively, in the initial straw, where as there were 41.5 and 43.5; in T2; 35.2 and 37.8 for T3, respectively. The digestibility of CP, CF, NDF, ADF, CL and HCL were also significantly affected by the treatments. When the straw was fermented with *Pleurotus* fungi, the digestibilities of the most components were increased however T2 showed the higher amounts of digestibility than that of the T3. The digestibility of Gross Energy (GE) was significantly ( $p < 0.05$ ) highest for the T2 and the lowest for the T1. These results are supported by the findings of the *in vitro* digestibility of this study and other reports (Zadrazil *et al.*, 1996; Zadrazil, 1997). There are few reports in which digestibility of fungal treated straw were evaluated *in vivo*. However, these results are in agreement with those of Marwaha *et al.* (1990), who noted that treatment of wheat straw by *P. sajor-caju* led to an increase ( $p < 0.05$ ) in the digestibility of DM, CP, CF and ADF in Jersey calves. Yoshida *et al.* (1993) found an increase (by 11%) in the DM digestibility of straw cultivated with *P. ostreatus*. In contrast, Walli *et al.* (1991) fed fungal (*Cuprinus fimetarius*) treated straw to Holstein Friesian bulls and noted that no enhancement was found in DMI, DMD and the TDN. Marwaha *et al.* (1990) reported that the *in vivo* DM digestibility of wheat straw was decreased after fermented with fungi *P. sajor-caju*. It appears the changes in the nutritive value of straw may be related to the type of fungi and cultural conditions.

**Nutrient intake:** Results obtained from intake are presented in Table 3. Daily consumption of DM and OM ( $\text{g day}^{-1}$  or  $\text{g kg}^{-1} \text{BW}^{0.75}$ ) significantly ( $p < 0.05$ ) increased when the sheep received wheat straw treated

Table 3: Effect of treatments on the nutrients intake by the experimental animals

Items	Treatments					SEM (n=12)
	T1 control	T2 F-77 <sup>1</sup>	T3 H-82 <sup>1</sup>	T4 F-77 <sup>2</sup>	T5 H-82 <sup>2</sup>	
DMI ( $\text{g day}^{-1}$ )	575 <sup>b</sup>	646 <sup>a</sup>	606 <sup>ab</sup>	522 <sup>bc</sup>	483 <sup>c</sup>	0.57
OMI ( $\text{g day}^{-1}$ )	546 <sup>b</sup>	614 <sup>a</sup>	566 <sup>ab</sup>	462 <sup>c</sup>	436 <sup>c</sup>	0.51
DMI ( $\text{g kg}^{-1} \text{BW}^{0.75}$ )	35 <sup>b</sup>	39 <sup>a</sup>	37 <sup>a</sup>	32 <sup>b</sup>	29 <sup>c</sup>	8.38
OMI ( $\text{g kg}^{-1} \text{BW}^{0.75}$ )	33 <sup>b</sup>	37 <sup>a</sup>	34 <sup>ab</sup>	28 <sup>bc</sup>	25 <sup>c</sup>	7.54
dDMI ( $\text{g day}^{-1}$ )	184 <sup>b</sup>	258 <sup>a</sup>	212 <sup>b</sup>	183 <sup>b</sup>	130 <sup>c</sup>	0.21
dOMI ( $\text{g day}^{-1}$ )	176 <sup>b</sup>	252 <sup>a</sup>	215 <sup>b</sup>	157 <sup>c</sup>	135 <sup>c</sup>	0.21
dDMI ( $\text{g kg}^{-1} \text{BW}^{0.75}$ )	11.2 <sup>b</sup>	15.7 <sup>a</sup>	12.9 <sup>ab</sup>	11 <sup>b</sup>	7.9 <sup>c</sup>	1.38
dOMI ( $\text{g kg}^{-1} \text{BW}^{0.75}$ )	11.3 <sup>b</sup>	15.3 <sup>a</sup>	13 <sup>ab</sup>	9.5 <sup>c</sup>	8.2 <sup>c</sup>	1.54
NVI	100 <sup>b</sup>	135 <sup>a</sup>	116 <sup>ab</sup>	84 <sup>bc</sup>	73 <sup>c</sup>	10.45

Means with the different superscripts within a row are significantly ( $p < 0.05$ ) different.

F-77<sup>1</sup> = Fungal treated straw *Pleurotus* F-77 before mushroom yield

F-82<sup>1</sup> = Fungal treated straw *Pleurotus* F-82 before mushroom yield

F-77<sup>2</sup> = Fungal treated straw *Pleurotus* F-77 after harvesting mushroom

F-82<sup>2</sup> = Fungal treated straw *Pleurotus* F-82 after harvesting mushroom

SEM = Standard error of means

dDMI = Digestible dry mater intake

dOMI = Digestible organic mater intake

NVI = Nutritive value index = Relative intake x digestibility

Relative intake = amount of intake from treated straw/ amount of intake from initial straw

by *Pleurotus* (F-77<sup>1</sup>) at mycelium running stage. The digestible DM and OM intake were also significantly ( $p < 0.05$ ) increased for the above treatment. Improvement of intake could be due to the physical (softness of the straw structure) and chemical (cell wall degradation) changes of wheat straw through the solid state fermentation process by fungi. In addition, fungal treatment increased the DM and OM digestibility of the straw, which increased the voluntary intake. Yamakawa *et al.* (1992) reported an increase of DM intake of *P. ostreatus* treated rice straw from 12-13 (in normal straw) to about  $20 \text{ g kg}^{-1}$  of  $\text{BW}^{0.75}$  (in treated straw) by sheep. The voluntary intake of DM, OM, as well as the digestible DM and OM were reduced when the residual straw after harvesting of mushroom fed to the animals. In general digestible OM intake and of nutritive value index (NVI) were the highest for T2 and the lowest for T4 and T5. It may be due to the longer fermentation period (7 vs. 2.5 weeks), which led greater depletion of the carbohydrate source of the straw by fungi during the fruiting body formation (Pant *et al.*, 2006). Calzada *et al.* (1987) fed either fungal treated straw after harvesting the edible mushroom of *P. sajor-caju* or normal straw to lamb and found that both groups showed similar DMI. In buffalo, Dhanda *et al.* (1996) noted that fermented paddy straw obtained after *P. sajor-caju* (PAU-3) was harvested, had no effect on the nutrient utilization and nitrogen balance when compared with untreated straw. However, when the fermentation period of the straw was reduced to two weeks, the DMI was significantly ( $p < 0.05$ ) increased. Therefore, it showed that

the duration of treatment was equally important as the species of fungi to improve the nutritive value of straw.

### CONCLUSION

Treatment of wheat straw by *Pleurotus* fungi resulted in a reduction of cell wall and its components and increasing of CP, *in vitro* and *in vivo* digestibility. In addition, the voluntary intake of treated straw before mushroom growing, increased by sheep but it was reduced after mushroom harvesting. From two *Pleurotus* fungi which were used in this experiment, F-77 showed the better ability for degradation of straw with the highest nutritive value index although the nutritive value of the residual straw from mushroom collection of other species of fungi was lower than the initial straw.

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