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NUTRITIVE VALUE INDEX OF TREATED WHEAT STRAW WITH *Pleurotus* FUNGI**

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**Plenary invited paper

Abstract Wheat straw was inoculated with spawns of two species of *Pleurotus* fungi (coded: *PF* and *PO*) and incubated in a fermentation room at 22±5ºC and 70±5, relative humidity. After 17 days of spawning, when the substrate was completely covered by fungal mycelia, half of the bags were removed from the fermentation room, sun dried and used for *in vitro* measurements and *in vivo* study as well, using of sheep. For the remainder of the bags, fermentation was allowed for seven weeks during which the fruiting mushroom was harvested two times, and then spent straw was collected and dried under the sun. In a feeding trial, *in vivo* digestibility and voluntary intake of treated straw was compared with initial straw in cattle, when the dietary treatments were:

1) Initial wheat straw (IWS);
2) Fungal (*PF*) treated wheat straw before mushroom formation (FTWS);
3) Fungal (*PF*) spent wheat straw (SPWS) after mushroom was harvested.

Results showed that, the fungal treatments significantly ($P<0.05$) affected the nutrient composition of wheat straw by increasing the CP and ash, but decreasing the OM, NDF, ADF, ADL, cellulose (CL) and hemi cellulose (HCL) contents. The *in vitro* digestibility of DM and OM were significantly ($P<0.05$) the highest for *PF* treated straw but the lowest for the initial straw. The *in vivo* digestibility of most components were significantly ($P<0.05$) increased, in sheep, however treatment *PF* showed the higher amounts of digestibility than that of the *PO* with exception for crude protein CP and HCL. Regarding the feeding trial by cow, results indicated that, the highest amount of DM and OM digestibility were found in FTWS followed by SPWS, but the initial straw had the lowest digestibility ($P<0.05$). Average daily intake of DM, OM, as well as the digestible DM and OM intake were significantly ($P<0.05$) affected by the treatments. In comparison to the IWS, *PO* inoculated straw increased the voluntary intake of digestible DM and OM, at the stage of mycellial running, however, the digestibility and intake

of SPWS were significantly ($P<0.05$) reduced to the level of initial straw. In conclusion, fermentation of wheat straw by *Pleurotus florida* and *P. ostreatus*, improved the nutritive value of straw although the effect of *P.F.* was more than that of the *P.O.* In addition, fungal treatment of straw before mushroom formation, improved the nutritive value index, but no enhancement was found in SPWS comparing with the initial straw.

Key words: wheat straw, treatment, *Pleurotus* spp., nutritive value.

Introduction

Straws represent a major quantity of biomass from cereal production that is a potent source of energy for ruminant nutrition but the availability of this energy is very low. In order to increase the nutritive value of straw, various chemical and physical methods have been extensively studied, but most of them have not been applicable (*Leng*, 1991; *Sharma et al*., 1993; *Zahedifar*, 1997). Since last decades, biological de-lignification of straw by solid-state fermentation (SSF) has been considered because of its capacity to remove lignin preferentially (*Moyson and Verachtert*, 1991; *Fazaeli et al*., 1999). The bio-conversion of straw is circumscribed to the group of white-rot fungi, which are capable to colonize on cereal straw and liberate water soluble substrates from the polymers during SSF and thus improve the digestibility (*Zadrazil*, 1997; *Fazaeli et al*., 2003). Among the edible white-rot fungi, the *Pleurotus* species have been shown to be more efficient (*Zadrazil et al*., 1996; *Jalc et al*., 1997; *Taniguchi et al*., 2005). The potential of *Pleurotus* fungi such as *P. ostreatus* and *P. eryngii* to reduce indigestible cell wall components and increase the dry mater digestibility (DMD) of straw has been reported (*Singh et al*., 1990; *Fazaeli et al*., 2004). The *Pleurotus* fungi have different ability to grow on straw and decompose its structural carbohydrate because of the variation in culture behaviour and culturing conditions (*Jalc et al*., 1996; *Fazaeli et al*., 2002). The level of some cultural and nutritional conditions for this fungus has been reported to govern the selective de-lignification of wheat straw (*Kulkarni and Nagaraj*, 1988; *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). However, fungal treated straw do not always led to a successful growth of fungi and improvement of nutritive values. Due to the existence of many species 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 chemical composition, digestibility and voluntary intake of wheat straw. Secondly, to compare the nutritive value of fungal treated wheat straw before and after harvesting of mushroom.

Materials and methods

Wheat straw was packed in nylon bags (45×90 cm) and soaked in water for 24 h, in a steel water tanks (2×1×0.8m size) then it was pasteurized in hot water at 80ºC for one hour. The wheat grain spawn of two *Pleurotus* fungi including *P. florida* (*PF*) and *P. ostreatus* (*PO*), were used to inoculate the straw, at the rate of 3.5 kg spawn per 100 kg straw (fresh weight basis). The inoculated straw was packed in polyethylene bags (70 cm length and 40 cm diameter and 100 gauge thickness). The bags that contained approximately 12 kg of straw (fresh weight) were tightened up with nylon thread and transferred to the fermentation room, where the temperature was adjusted for 22±5ºC and the relative humidity of 70±5% maintained by means of air condition and water sprinkling. During the first week of incubation, when the mycelial running started, all sides of the bags were crashed, to provide an aeration that was necessary for aerobic fermentation. After 17 days of incubation, half of the bags were removed from the fermentation room, allowed to dry under the sun and sampled for *in vitro* digestibility and chemical composition that were determined according to the method of *Tilley and Terry*, (1963) and *AOAC*, (1990) respectively. The *in vivo* digestibility was determined by sheep, using of fecal collection method. The remainder bags were collected after seven weeks of incubation when the mushroom was harvested two times and dried under the sun. In a completely randomised design, three treatments including:

1) Initial wheat straw (IWS),
2) Fungal (*PF*) treated wheat straw before formation of mushroom (FTWS),
3) Fungal (*PF*) treated wheat straw after harvesting of mushroom (SPWS),

were compared for digestibility and intake, using of native mature male cows, weighing about 300 kg. The animals were fed the dietary treatment *ad libitum* in addition with 350 g of concentrate supplement composed of ground barley, wheat bran, cottonseed meal and mineral supplement. Each

experimental period consisted of two weeks for adaptation and one week of measurement. Daily feed intake and refused were measured and sampled during the collection period. Faeces from individual cows were collected and weighed every morning and sub-sampled. The samples of feeds and refused were dried at 65°C for 48 h and faeces were dried at 65°C until constant weight, ground through 1-mm screen and analysed chemically. Data were analysed by the general linear model procedure of SAS (1992) using the following model:

$$Y_{ijk} = \mu + A_j + T_k + E_{ijk}.$$

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

μ = Overall sample mean,

T_k = Treatment k effect,

E_{ijk} = Ordinary least squares residual error.

Results and discussion

Chemical composition. As it is shown in table 1, fungal treatment had significantly ($P<0.05$) increased the CP content from 3.2% in the initial straw to 5.1% and 5.5%. The protein content of the mycelium was reported relatively high (*Ragunathan et al*., 1996), so it was expected that the treated

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

	treatments			SEM
Item	INWS	*PF*	*PO*	n = 24
OM	94.5 [a]	90.2 [b]	90.0 [b]	1.5
ASH	5.9 [b]	9.2 [a]	10 [a]	1.5
CP	3.2 [b]	5.1 [a]	5.5 [a]	0.33
NDF	83.5 [a]	73.7 [b]	75 [b]	2.83
ADF	62.8 [a]	55.4 [b]	56.5 [b]	2.14
ADL	8.2 [a]	7.4 [b]	7.2 [b]	0.14
CL	54.8 [a]	48.3 [c]	50.3 [b]	1.07
HCL	20.7 [a]	18.7 [b]	18.5 [b]	1.36
IVDMD	28.1 [c]	40.3 [a]	37 [b]	2.21
IVOMD	27.5 [c]	40.2 [a]	36.8 [b]	2.07

Means with the different superscripts within each row are significantly ($P<0.05$) different.
SEM = Standard error of mean. CL = Cellulose. HCL = Hemi cellulose.

straw, that contained fungal mycelium to have a higher concentration of CP. An increase of CP content in wheat straw incubated with *Pleurotus* species had also been reported (*Zadrazil et al*., 1996; *Fazaeli et al*., 2003). Both of the fungi 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-rot 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). Fungal treatment also significantly (P<0.05) reduced the concentration of cellulose and hemi-cellulose. Among the treatments, wheat straw treated with *PF* had significantly (p<0.05) 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, 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; *Taniguchi et al*., 2005).

***In vitro* digestibility.** Fermentation of wheat straw by the fungi, significantly (P<0.05) increased the digestibility of DM and OM (Table 1). The Duncan comparison test indicated that wheat straw treated with *PF* had significantly (P<0.05) higher digestibility than the straw treated with *PO*. Lignin binds with hemi-cellulosic components of cell wall, and through covalent linkages and physical binding, prevents the biodegradation of straw carbohydrates by microorganisms (*Eriksson et al*., 1990; *Flachowsky and Klappach*, 1993). Improvement of digestibility in treated straw could be as a result of the solubilisation of the structural polymers by fungi (*Jalc et al*., 1998; *Fazaeli et al*., 1999). Similar results were reported by *Gupta et al*. (1993) and *Fazaeli et al*. (2006). However, the ability of fungi to improve the digestibility of straw could be different. An increasing of DMD of wheat straw fermented with *Pleurotus* fungi has been reported from 15 to 46% (*Zadrazil*, 1997). Beside the culturing conditions, the ability of various strains of white-rot fungi in cell wall degradation and digestibility improvement of straw may be different (*Tripathi and Yadav*, 1992; *Jalc et al*., 1997; *Fazaeli et al*., 2002).

***In vivo* digestibility.** Total tract digestibility of nutrients and cell wall components as well as the gross energy (GE) significantly (P<0.05) affected by the treatments, when fed to sheep (Table 2). Where the straw was fermented with *Pleurotus* fungi, the digestibility of the most components

were increased however treatment *PF* showed the higher amounts of digestibility than that of the *PO* with exception for crude protein (CP) and hemi-celluloses (HCL). These results are supported with the findings of *in vitro* digestibility of this study but are not in accordance with those reported by *Yamakawa et al.* (1992) who studied the digestibility of fungal treated rice straw in sheep.

Table 2. Effect of treatments on the *in vivo* digestibility of the nutrients.

Items (%)	Treatment			SEM (n= 20)
	UTS	PF	PO	
DM	30.4[c]	40.1[a]	35.2[b]	1.39
OM	33.4[c]	45.1[a]	37.8[b]	0.33
CP	34[c]	43.1[a]	40.8[a]	0.33
NDF	30.3[c]	39.1[a]	33.1[b]	0.83
ADF	27.8[c]	41.8[a]	33.7[b]	1.04
CL	32.2[b]	61.0[a]	44.4[b]	1.07
HCL	31.3[b]	37.1[a]	39.3[a]	1.36
GE	31.6[c]	42.0[a]	35.3[b]	2.21

Means with the different superscripts within row are significantly (P<0.05) different.
SEM = Standard error of mean. CL = Cellulose. HCL = Hemi cellulose

Regarding the feeding trial on cows, it was found that DM and OM digestibility were significantly (P<0.05) higher in FTWS and SPWS than the IWS (Table 3). The digestibility of DM and OM were 34.8 and 35.0%, respectively, in the initial straw, whereas there were 45.2 and 44.8% in FTWS; 41.0 and 41.5% in SPWS respectively. These results are supported by the findings of the previous results of this study and other reports (*Kundu*, 1994; *Zadrazil et al*., 1996). 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 wheat straw cultivated with *P. ostreatus*. *Fazaeli et al*. (2006) found that *in vivo* digestibility of treated straw with *P. florida* was increased when fed to sheep, however the spent straw remained from mushroom harvesting had lower digestibility and nutritive value index. *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 TDN. *Marwaha et al*. (1990) reported that the *in vivo* DM digestibility of wheat straw, fermented with fungi *P. sajor-caju*,

was decreased when fed to Jersey calves. It appears that the changes in the nutritive value of straw may be related to the type of fungi and cultural conditions (*Tripathi and Yadav*, 1992).

Table 3. Effect of treatments on the digestibility and nutrients intake.

Item	treatments			SEM (N=12)
	IWS	FTWS	SPWS	
Digestibility				
DMD (%)	34.8 [b]	45.2 [a]	41.0 [ab]	2.9
OMD (%)	35.0 [b]	44.8 [a]	41.5 [ab]	2.7
Intake				
DMI (g/d)	4460 [b]	5440 [a]	4280 [b]	290
OMI(g/d)	4140 [b]	4900 [a]	3800 [b]	260
DMI (% of body weight)	1.49 [b]	1.81 [a]	1.43 [ab]	0.15
OMI (% of body weight)	1.38 [b]	1.63 [a]	1.27 [ab]	0.13
DMI (g/kgBW$^{0.75}$)	63 [b]	77 [a]	59 [b]	2.8
OMI (g/kgBW$^{0.75}$)	58 [b]	68 [a]	53 [b]	2.6
dDMI (g/d)	1550 [b]	2400 [a]	1800 [b]	165
dOMI (g/d)	1440 [b]	2200[a]	1620 [b]	136
dDMI (g/kgBW$^{0.75}$)	21.5 [b]	33.3 [a]	24.4 [b]	3.6
dOMI (g/kgBW$^{0.75}$)	20.0 [b]	30.5 [a]	22.5[b]	2.9
Index				
NVI (based on the dDMI)	100 [b]	155 [a]	113 [b]	18.8
NVI (based on the dOMI)	100 [b]	153 [a]	112 [b]	15.9

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

IWS = Initial wheat straw.
FTWS = Fungal treated wheat straw (before mushroom formation).
SPWS = Spent wheat straw (treated straw after harvesting of mushroom).
SEM = Standard error of mean.
NVI = Nutritive Value Index = Relative Intake (RI) x digestibility,
RI = Amount of intake from treated straw/amount of intake from un treated straw.

Nutrient intake. As it is shown in Table 3, daily consumption of DM and OM based on the kg or g/kg BW$^{0.75}$ were significantly (P<0.05) different among the treatments. The FTWS significantly (P<0.05) increased the DM and OM intake but when the animals received SPWS the intake was reduced. It may be due to the longer fermentation period (49 vs. 17 days), which led greater depletion of the carbohydrate source of the straw by fungi during the fruiting body formation. *Calzada et al*. (1987) reported that dry mater intake was similar for wheat straw remained from harvesting mushroom of *P. sajor-caju* and normal straw when fed to lamb. Similar results was found by

Dhanda et al. (1996) when fed SPWS from *P. sajor-caju* to buffalo. *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 g/kg of $BW^{0.75}$, in treated straw, by sheep. The digestible DM and OM intake (DDMI and DOMI) were significantly ($p<0.05$) affected by the treatments. In comparison to the IUWS or SPWS a significantly ($P<0.05$) higher amount of DDMI and DOMI was obtained when the animals fed FTWS. The nutritive value index was significantly ($P<0.05$) higher in FTWS but it was similar in IWS and SPWS.

Conclusion

Treatment of wheat straw by *Pleurotus* fungi resulted in a reduction of cell wall components and increasing of CP, *in vitro* and *in vivo* digestibility. In addition, the voluntary intake of treated straw before mushroom growing, increased by cow but it was reduced when the fermentation process prolonged for mushroom production and harvesting.

INDEKS HRANLJIVE VREDNOSTI PŠENIČNE SLAME TRETIRANE GLJIVAMA IZ RODA *Pleurotus*

H. Fazaeli

Rezime

Pšenična slama je inokulisana sa dve vrste jestivih gljiva iz roda *Pleurotus* (šifra: *PF* i *PO*) i inkubirana u sobi za fermentaciju na 22 ±5ºC i 70 ±5, relativne vlažnosti. Nakon 17 dana, kada je supstrat bio pokriven micelijom gljive, polovina vreća je uklonjeno iz sobe za fermentaciju, osušeno na suncu i korišćeno za *in vitro* merenja i *in vivo* ispitivanja, u ishrani ovaca. Za preostale vreće, fermentacija je nastavljena 7 nedelja, tokom kog perioda su pečurke brane dva puta, a slama sakupljena i osušena na suncu. U hranidbenom ogledu, *in vivo* svarljivost i konzumacija tretirane slame su poređeni sa početnom slamom i to kod goveda, kada su inicijalni hranidbeni tretmani bili: 1) početna pšenična slama (IWS); 2) (*PF*) slama

tretirana glivama pre formiranja pečurki (FTWS); 3) (*PF*) slama tretirana gljivama (SPWS) nakon branja pečurki. Rezultati su pokazali da je tretman gljivama signifikantno (P<0.05) uticao na sastav nutrijenata u pšeničnoj slami i to povećanje SP i pepela, ali smanjenje sadržaja OM, NDF, ADF, ADL, sadržaja celuloze (CL) i hemi celuloze (HCL). Vrednosti i*n vitro* svarljivosti suve i organske materije su bile signifikantno (P<0.05) najviše kod tretirane slame *PF* a najniže kod početne slame. Svarljivost *in vivo* većine komponenti je bila signifikantno povećana (P<0.05), kod ovaca, međutim, tretman *PF* je pokazao veću svarljivost nego kod *PO* sa izuzetkom SP i HCL. U vezi sa hranidbenim ogledom kod krava, rezultati ukazuju da su najveće vrednosti svarljivosti SM i OM utvrđene kod FTWS, a zatim SPWS, ali početna slama je imala najnižu svarljivost (P<0.05). Prosečan dnevni unos SM, OM, kao i unos svarljive SM i OM su bili pod signifikantnim (P<0.05) uticajem tretmana. U poređenju sa IWS, *PO* inokulisana slama je uticala na povećanje unosa svarljive SM i OM, u stadijumu micelije gljive, međutim, svarljivost i unos SPWS su bili signifikantno (P<0.05) smanjeni na nivo ovih parametara u početnoj slami. Kao zaključak, fermentacija pšenične slame korišćenjem *Pleurotus florida* i *P. ostreatus*, je poboljšala nutritivnu vrednost slame iako je uticaj *P.F*. bio veći nego uticaj *P.O.* Takođe, tretman gljivama slame pre formiranja pečurki je povećao indeks nutritivne vrednosti, ali nije utvrđeno povećanje u SPWS u poređenju sa početnom slamom.

Ključne reči: pšenična slama, tretman, *Pleurotus* spp., nutritivna vrednost.

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