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EFFECT OF FUNGAL TREATMENT ON RUMEN DEGRADABILITY AND CHEMICAL COMPOSITION OF *POPULUS NIGRA* SAWDUST AS LIGNOCELLULOSIC BIOMAS

F. Mirzaei Aghjehgheshlagh[1], M. Bayat[1], B. Darabighane[1], M. Sharari, J. Seifdavati[1]and B. Navidshad[1]

[1]Department of Animal Science, University of Mohaghegh Ardabili, Ardabil, Iran 2. Department of Watershed and Range Management, University of Mohaghegh Ardabili, Ardabil, Iran
Corresponding author Email: f_mirzaei@uma.ac.ir

ABSTRACT

The present study was carried out to determine chemical composition and rumen degradability parameters of dry matter (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF) ether extract (EE) and organic matter (OM) in *Populus nigra* sawdust biodegraded by *Phanerochaete chrysosporium* and *Pleurotus ulmarius*. The samples were incubated by the fungal biomass at 30 ˚C with 65 % relative humidity for 7 and 17 days and chemical composition of substrate was then analyzed. Using *in situ* technique enabled us to evaluate degradability of the samples in the rumen of two Moghani rams with rumen fistula. Significant differences were observed in DM, NDF, ADF, EE and ash contents as well as degradability parameters of DM, NDF, ADF and OM, including highly soluble and readily degradable fraction (a), insoluble and slowly degradable fraction (b), rate constant for degradation of the fraction b (c) and effective degradability (ED) in different times of biodegradation. The findings indicated that effects of white rot fungi on *Populus nigra* sawdust significantly decreased DM, NDF, ADF and OM while EE, ash content, DM and NDF degradability parameters were significantly increased.

Keywords: *Phanerochaete chrysosporium*, *Pleurotus ulmarius*, Chemical composition, Degradability*, Populus nigra.*

INTRODUCTION

Lack of adequate feed is a major concern for livestock farmers despite the large amount of various by-products produced in developing countries. Proper consumption of these by-products not only will lead to lower costs but will also help in reducing environmental pollution (Vasta *et al*., 2008). Straws and industrial wood waste are among the most important lignocellulosic compounds (Sindhu *et al*., 2016) which contain larger quantities of sawdust compared to other lignocellulosic wastes. To improve usability of carbohydrates contained in wood, microorganisms of rumen should be provided with greater access to components of cell walls (Mellenberger *et al*., 1970); although trees are not among the favorite nutrients easily accessible by livestock, processing can enhance their nutritional value and provide captive animals with suitable feed (Baertsche *et al*., 1986). Given the high fiber content in mature poplar trees, it cannot be used as feed by any animal, and since wood wastes have relatively low economic value and can lead to loss of organic materials (Bayat *et al*., 2015). However, presence of lignin in wood acts as a limiting factor for animals that consume cell wall compounds (cellulose and hemicelluloses) (Van Kuijk *et al*., 2015; Shahzad *et al*., 2016). Over the past few years, biological degradation of lignin by fungi has been studied as the best available degradation method for complex structures present in highly fibrous materials (Van Kuijk *et al*., 2015; Ravindran and Jaiswal, 2016). On the other hand, the technique is safer and cheaper than other (chemical and physical) methods (Van Kuijk *et al*., 2015). Among these fungi are white rot fungi that belong to *basidiomycetes* and are highly capable of degradation and consuming lignin and cell wall compounds (Elisashvili and Kachlishvili, 2009). White rot fungi have also been shown to selectively degrade lignin (Sharari *et al*., 2013; Van Kuijk *et al*., 2015; Sindhu *et al*., 2016). These fungi (different strains of white rot fungi) have been used to improve quality of roughage and lignocellulosic substances (wheat or rice straws) (Sindhu *et al*., 2016).

The present study, therefore, attempts to examine effects of two strains of white rot fungi (*Phanerochaete chrysosporium* and *Pleurotus ulmarius*) on rumen degradability and chemical composition of *Populus nigra* sawdust as a nutritional source for ruminant.

MATERIALS AND METHODS

Substrate preparation: The substrate (poplar sawdust) used in this experiment was prepared using the sawdust from sawmills in Asalem, Gilan, Iran. The sawdust was dried in laboratory and screened using a 0.8 mesh to create a uniform sample.

Solid media culture: *Phanerochaete chrysosporium* was obtained from the Iranian Research Organization for Science and Technology (IROST) and *Pleurotus ulmarius* was obtained from Horticultural Laboratory at the University of Mohaghegh Ardabili, Iran. Each potato

dextrose agar was impregnated with a sterile loop of fungal mycelia to cultivate the fungi. The mixture was kept at 25 °C for 10 days. The petri dishes were kept under sterile conditions to allow the mycelial masses to grow (Sharari *et al.,* 2011).

Liquid media culture and nutritional supplements: The 10-day-old mycelia grown on solid media were used under totally sterile conditions to impregnate liquid media. To achieve maximum growth and multiplication of *Phanerochaete chrysosporium*, a liquid medium was used with these additives per liter: peptone (5 g) as a source of nitrogen, glucose (10 g) as a source of carbon and minerals for fungal growth, including $MgSO_4.7H_2O$ (0.5 g), NH_4Cl (0.1 g), K_2HPO_4 (0.5 g), $FeSO_4.7H_2O$ (0.05 g), $CaCl_2.2H_2O$ (0.1 g), and KH_2PO_4 (2 g). For *Pleurotus ulmarius*, a liquid media was used with the following additives per liter: yeast extract (5 g) as a source of nitrogen, glucose (10 g) as a source of carbon and minerals for fungal growth including $MgSO_4$. $7H_2O$ (0.15 g), KH_2PO_4 (0.025 g), $CaCl_2$ (0.05 g), HPO_4 $(NH_4)_2$ (0.25 g), and $FeCl_3$ (1%; 1.3 ml). Then 50 ml of this liquid was poured into 500-ml Erlenmeyer flasks and put under sterile conditions at 121 °C (15 psi) for 15 minutes to prevent growth of other microorganisms. Each flask was inoculated with one loop of the mycelia before being placed at 30 °C for 10 days in incubator (Sharari *et al.,* 2011).

Fungal inoculation and treatment: Once weighed, dry air substrates were placed in special plastic bags for autoclaving, impregnated with the nutrient liquid and then put at laboratory ambient temperature for 24 hours. The substrates were then put under sterile conditions at 121 °C (15 psi) for 15 minutes. The mycelia were collected from the liquid medium to create a homogenous mixture sprinkled on sterile wood chips. The samples were placed for 17 days in germinator at 30 °C and 65% humidity.

Chemical compositions of the samples were examined on days 0, 7 and 17. DM, EE and ash content of the samples were measured by AOAC (2000) and NDF, ADF measured by Van Soest *et al*. (1991).

***In situ* rumen degradability assay:** The technique was implemented using two Moghani rams (55±2 kg body weight; 2.5 years old) with rumen fistula at the Research Training Station of the University of Mohaghegh Ardabili, Iran. Based on AFRC (1992), animals were fed *ad libitum* a ration consisting of the chopped alfalfa, barley grains, and mineral-vitamin supplement at an amount 10 percent higher than maintenance level. Drinking water was always available. On days 7 and 17, a 3-gram dry treated sample was incubated into the rumen of each sheep at hours 0, 2, 8, 16, 24, 48, 72, and 98 in three replicates (bag size 5 cm × 10 cm, pore size of 50-55 μm) at each time. After incubation, the bags containing samples were removed from the rumen, washed and then dried for 48 hours in an oven at 65 °C (Cottrill and Evans, 1984).

Statistical analysis: Rumen degradation kinetics were calculated by the equation Eq. 1 (Ørskov and McDonald, 1979) in Fitcruve software.

Eq. 1: $P= a+b(1-e^{-ct})$

Where:

a: highly soluble and readily degradable fraction (%), *b:* insoluble and slowly degradable fraction (%), *c:* rate constant for degradation of the fraction b (/h) and *P:* degradability for response variables at time t (%).

In addition, Eq. 2 was used to calculate effective degradability (ED) of DM, NDF, ADF and OM at the rates 0.02, 0.05, and 0.08 per hour.

Eq. 2: $ED=a+bc/(c+k)$

Where:

k: rate constant of passage (/h).

Statistical analysis was performed using the general linear model (GLM) procedure of SAS software (version 9.1, SAS Institute Inc., Cary, NC, USA; 2002) for a completely randomized design with a 2 × 3 factorial arrangement (2 type strains: *Phanerochaete chrysosporium* and *Pleurotus ulmarius*; 3 times treatment: 0, 7 and 17 days). Mean values were compared using Duncan's Multiple Range test at the level of $P<0.05$.

RESULTS AND DISCUSSION

Table 1 presents the results of chemical composition *Populus nigra* sawdust prior and after treatment by *Phanerochaete chrysosporium* and *Pleurotus ulmarius*. Our findings suggest that fungal treatment significantly reduced percentage of DM, NDF, and ADF and this reduction becomes more prominent as treatment time increases, with the largest reduction observed on day 17 along with a significant increase in EE and ash content. The results of this experiment were consistent with Bayat *et al*., (2015) who used *phanerochaete chrysosporium* and *pleurotus ulmarius* to treat *alnus subcordata* sawdust. During the propagation of mycelium over the culture substrate, first the fungi transform easily digestible polysaccharides into low molecular weight carbohydrates. Once these carbohydrates are consumed, the fungi look for new food sources by degrading structural carbohydrates and lignin (Eriksson *et al*., 1990). This leads to reduction in OM content and since lignin has negative effect on digestibility of feed (Van Soest, 1994), nutritional value will be enhanced. Researchers believe that fungal extracellular enzymes cause reduction in cell wall substrate. It has been shown that lignin prevents polysaccharide digestion in the rumen and physically blocks hydrolytic enzymes produced by the rumen

microorganism from affecting tissues with high digestibility (Albores *et al*., 2006) while fungi, with their exoenzymes like laccase, oxidase, aromatic cycles and aliphatic chains, oxidate lignin to produce products with low molecular weight (Alemawor *et al*., 2009; Ravindran and Jaiswal, 2016). Getting best results in enhancing quality of roughage in treatment with white rot fungi depends on fungus strain, culture substrate, environmental conditions and their interactions (Zadrazil and Brunnert, 1981; Van Kuijk *et al*., 2015). Given the numerous evidence suggesting that polysaccharides contained in hemicelluloses are trapped in lignin, removal of lignin will result in increased hydrolysis of hemicelluloses (Van Soest, 1994). Therefore, once easily digestible compounds are finished, fungi probably turn to hemicelluloses to compensate for reduced lignin. Digestibility will lower as this process continues. This is in line with the physiology of white rot fungi (Eriksson *et al*., 1990). Consumption of OMs, particularly cell wall and soluble carbohydrates as substrate, by the fungi leads to reduced OM, thereby relatively increasing ash content (Zadrazil *et al*., 1995).

Table 2 shows the results for degradability of DM in *Populus nigra* sawdust at different incubation times by using *in situ*. As seen in the table 2, treatment of the sawdust has improved degradability of DM. Maximum degradability over experiment time was observed for *Phanerochaete chrysosporium* on day 17 while minimum degradability was found for untreated sawdust. Researchers believe that for degrading lignin, fungi require another source of energy, usually carbohydrates (Zadrazil *et al*., 1995). Based on this hypothesis, all major compounds within cell wall (cellulose, hemicelluloses and lignin) will be degrading but the important point to note is the ratio of these compounds and how they are consumed since the goal of this biological process is to enhance nutritional value of roughage with poor nutritional quality. Strong chemical bonds between lignin and polysaccharides in plants prevent digestive enzymes from reaching plant fibers (Ravindran and Jaiswal, 2016), thereby reducing digestibility. Sawdust treatment with fungi enhances microorganism access to structural carbohydrates due to degradation of lignin and breaking chemical bonds between structural carbohydrates and lignin as a result of enzymes produced by fungi (Van Kuijk *et al*., 2015). Cellulose decomposition by the rumen microorganisms is in inverse relation with cellulose crystallization and factors can prevent that are also able to facilitate cellulose decomposition by *T.viride* and *Aspergillusniger* (Fogarty and Kelly, 2012).

Table 3 shows how fungi and treatment affect degradability parameters of *Populus nigra* sawdust. In general, sawdust treatment led to increased degradation in fast degradable fraction (*a*), slowly degradable fraction (*b*), rate constant for degradation of the fraction *b* (*c*), and PD (potential degradability) and ED at different rates. The largest fast and slow degradable parts were observed on day 17 for *Phanerochaete chrysosporium* while the smallest parts were found in untreated sawdust. Treated sawdust had fewer cell wall components and smaller amount of lignin compared to the control group, with the smallest amount of cell wall components found on day 17. Hence, soluble parts are the smallest in untreated sawdust which has greater cell walls. Lignin, NDF, and ADF were reduced while intracellular substances were increased in the untreated substrate. Researchers reported inverse relation between the lignin content and the amount of fibrous materials with digestible DM and this demonstrates that treatment improves digestibility (Hoffman *et al*., 1993). Sawdust treatment enhanced ED at different nutrition levels because of the reduction of the proportion of structural carbohydrates, in particular lignin. The difference in the findings is attributable to different lignin structure in substrates, impacts of fungal enzymes and fungal strains.

Our findings for effects of fungi and treatment on degradability parameters of NDF and ADF in *Populus nigra* sawdust are reported in Tables 4 and 5. As seen in the tables, further treatment improved fast degradation part, slow degradation part, potential degradability and effective degradability. The differences in these parameters can be ascribed to different fungal strains, treatment system and types of fungal enzymes. The small amount of dissolved DM in the untreated substrates is probably the result of physical structure of these substrates. As seen in the table 1, the untreated sawdust had large NDF and ADF. Researchers suggested that digestibility of DM depends on solubility of digested fiber. In other words, greater solubility and smaller amount of fibrous content mean greater digestibility (Nocek and Grant, 1987). Comparison of ADF and NDF effective degradability at the rates 0.02, 0.05, and 0.08 per hour indicated significant differences, as the presence of lignin, cellulose and raw fibers in the untreated substrate reduced degradability of cell wall.

Table 6 presents the results for effects of fungi and treatment on degradability parameters of OM in *Populus nigra* sawdust. As seen in the table 6, further treatment improved fast degradable part, potential degradability, and effective degradability. No particular trend was observed for constant rate of degradability per hour. Apart from this constant rate, treatment had significant impact on other degradability parameters of OM ($P<0.01$). In fact, sawdust treatment has decreased the amount of structural carbohydrates and increased dissolved carbohydrates which required shorter time for degradation since microorganisms facilitate faster degradation and, in the absence of cell wall components, degradability is enhanced and OM becomes more degradable because of biological degradation of substrate (Albores *et al*., 2006).

Table 1. Chemical composition (%) of *Populus nigra* sawdust prior (0 day) and after (7 day and 17 day) treatment by *Phanerochaete chrysosporium* and *Pleurotus ulmarius* (n=3).

Chemical composition		DM	NDF	ADF	EE	Ash	OM
Fungi 1	0 day	93.41^{a}	92.58^{a}	68.92^{a}	3.88^{b}	0.27^{d}	99.74^{a}
	7 day	88.94^{b}	89.16^{c}	65.55^{c}	4.91^{ab}	0.43^{bc}	99.56^{bc}
	17 day	82.99^{d}	87.40^{e}	64.95^{d}	5.32^{a}	0.56^{a}	99.43^{d}
Fungi 2	0 day	93.41^{a}	92.58^{a}	68.92^{a}	3.88^{b}	0.27^{d}	99.74^{a}
	7 day	85.45^{b}	90.15^{b}	66.91^{b}	5.78^{a}	0.38^{c}	99.62^{b}
	17 day	85.55^{c}	88.33^{d}	65.83^{c}	6.03^{a}	0.48^{b}	99.51^{c}
SEM		0.22	0.08	0.15	0.35	0.02	0.02
Significantly	Fungi	**	**	**	*	**	**
	Treatment time	**	**	**	n.s	**	**
	Treatment time × Fungi	**	n.s	n.s	n.s	n.s	n.s

Means in a column with different superscripts differ significantly (P < 0.05).
DM, dry matter; NDF, neutral detergent fiber; ADF, acid detergent fiber; EE, ether extract; OM, organic matter.
Fungi 1, *Phanerochaete chrysosporium;* Fungi 2, *Pleurotus ulmarius.*
n.s, not significant; *, P<0.05; **, P<0.01
SEM, standard error of the mean

Table 2. Degradation of dry matter in *Populus nigra* sawdust at different incubation times *(%).*

Incubation times in rumen (hour)		0	2	8	16	24	48	72	98
Fungi 1	0 day	7.19^{e}	7.94^{e}	8.52^{d}	10.62^{d}	11.58^{e}	12.57^{e}	13.25^{e}	13.50^{e}
	7 day	9.72^{c}	10.35^{c}	11.56^{b}	13.57^{c}	14.27^{c}	15.62^{c}	16.27^{c}	16.46^{c}
	17 day	11.20^{a}	11.63^{a}	13.58^{a}	15.95^{a}	18.65^{a}	20.22^{a}	20.84^{a}	21.04^{a}
Fungi 2	0 day	7.19^{e}	7.94^{e}	8.52^{d}	10.62^{d}	11.58^{e}	12.57^{e}	13.25^{e}	13.50^{e}
	7 day	8.58^{d}	8.70^{d}	10.18^{c}	11.78^{d}	12.81^{d}	13.58^{d}	14.53^{d}	14.74^{d}
	17 day	10.54^{b}	10.98^{b}	11.82^{b}	14.90^{b}	16.56^{b}	17.69^{b}	18.89^{b}	19.06^{b}
SEM		0.13	0.02	0.12	0.13	0.07	0.06	0.09	0.02
Significantly	Fungi	**	**	**	**	**	**	**	**
	Treatment time	**	**	**	**	**	**	**	**
	Treatment time × Fungi	n.s	**	n.s	*	**	**	n.s	**

Means in a column with different superscripts differ significantly (P < 0.05).
Fungi 1, *Phanerochaete chrysosporium;* Fungi 2, *Pleurotus ulmarius.*
n.s, not significant; *, P<0.05; **, P<0.01
SEM, standard error of the mean

Table 3. Degradability of dry matter in *Populus nigra* sawdust.

Ruminal degradability parameters		a	b	c	PD	ED(0.02)	ED(0.05)	ED(0.08)
Fungi 1	0 day	7.16^{d}	6.37^{d}	0.045^{d}	13.55^{e}	11.55^{e}	10.15^{e}	9.40^{e}
	7 day	9.68^{b}	6.78^{c}	0.048^{bc}	16.45^{c}	14.50^{c}	13.00^{c}	12.20^{c}
	17 day	10.31^{a}	10.86^{a}	0.051^{a}	21.15^{a}	18.10^{a}	15.85^{a}	14.60^{a}
Fungi 2	0 day	7.16^{d}	6.37^{d}	0.045^{d}	13.55^{e}	11.55^{e}	10.15^{e}	9.40^{e}
	7 day	8.11^{c}	6.52^{d}	0.049^{a}	14.65^{d}	12.75^{d}	11.40^{d}	10.60^{d}
	17 day	9.76^{b}	9.35^{b}	0.047^{cd}	19.10^{b}	16.30^{b}	14.30^{b}	13.30^{b}
SEM		0.04	0.06	0.0006	0.05	0.03	0.03	0.02
Significantly	Fungi	**	**	**	**	**	**	**
	Treatment time	**	**	n.s	**	**	**	**
	Treatment time × Fungi	**	**	**	*	n.s	n.s	**

Means in a column with different superscripts differ significantly (P < 0.05).
a, highly soluble and readily degradable fraction (%); b, insoluble and slowly degradable fraction (%); c, rate constant for degradation of the fraction b (/h); PD, potential degradability (%); ED, effective degradability (%).
Fungi 1, *Phanerochaete chrysosporium;* Fungi 2, *Pleurotus ulmarius*
n.s, not significant; *, P<0.05; **, P<0.01
SEM, standard error of the mean

Table 4. Degradability of NDF in *Populus nigra* sawdust.

Ruminal degradability parameters			a	b	c	PD	ED(0.02)	ED(0.05)	ED(0.08)
Fungi 1		0 day	2.40^{d}	4.12^{d}	0.093^{a}	6.50^{a}	5.80^{e}	5.15^{e}	4.70^{e}
		7 day	4.26^{c}	5.54^{c}	0.067^{c}	9.75^{c}	8.60^{c}	7.60^{c}	7.10^{d}
		17 day	7.17^{a}	8.59^{a}	0.094^{a}	15.75^{a}	14.35^{a}	12.90^{a}	12.05^{a}
Fungi 2		0 day	2.40^{d}	4.12^{d}	0.093^{a}	6.50^{a}	5.80^{e}	5.15^{e}	4.70^{e}
		7 day	5.61^{b}	4.40^{d}	0.012^{c}	10.00^{c}	7.30^{d}	6.50^{d}	6.20^{d}
		17 day	6.19^{b}	6.69^{b}	0.069^{b}	12.90^{b}	11.40^{b}	10.15^{b}	9.45^{b}
SEM			0.19	0.23	0.003	0.09	0.02	0.03	0.03
Significantly	Fungi		**	**	**	**	**	**	**
	Treatment time		**	**	**	**	**	**	**
	Treatment time × Fungi		**	n.s	**	**	**	**	**

Means in a column with different superscripts differ significantly (P < 0.05).
a, highly soluble and readily degradable fraction (%); b, insoluble and slowly degradable fraction (%); c, rate constant for degradation of the fraction b (/h); PD, potential degradability (%); ED, effective degradability (%).
Fungi 1, *Phanerochaete chrysosporium;* Fungi 2, *Pleurotus ulmarius.*
n.s, not significant; *, P<0.05; **, P<0.01
SEM, standard error of the mean

Table 5. Degradability of ADF in *Populus nigra* sawdust.

Ruminal degradability parameters			a	b	c	PD	ED(0.02)	ED(0.05)	ED(0.08)
Fungi 1		0 day	1.44^{e}	3.31^{d}	0.019^{d}	4.75^{e}	3.05^{e}	2.35^{d}	2.10^{d}
		7 day	4.96^{c}	4.01^{c}	0.019^{d}	8.95^{c}	6.85^{c}	6.05^{c}	5.70^{c}
		17 day	6.88^{a}	7.79^{a}	0.054^{a}	14.70^{a}	12.55^{a}	10.90^{a}	10.05^{a}
Fungi 2		0 day	1.44^{e}	3.31^{d}	0.019^{d}	4.75^{e}	3.05^{e}	2.35^{d}	2.10^{d}
		7 day	4.28^{d}	3.21^{d}	0.028^{c}	7.50^{d}	6.15^{d}	5.45^{c}	5.15^{c}
		17 day	5.75^{b}	6.31^{b}	0.042^{b}	12.05^{b}	10.00^{b}	8.65^{b}	8.00^{b}
SEM			0.07	0.19	0.001	0.02	0.05	0.05	0.03
Significantly	Fungi		**	**	**	**	**	**	**
	Treatment time		**	**	**	**	**	**	**
	Treatment time × Fungi		*	n.s	**	*	**	**	**

Means in a column with different superscripts differ significantly (P < 0.05).
a, highly soluble and readily degradable fraction (%); b, insoluble and slowly degradable fraction (%); c, rate constant for degradation of the fraction b (/h); PD, potential degradability (%); ED, effective degradability (%).
Fungi 1, *Phanerochaete chrysosporium;* Fungi 2, *Pleurotus ulmarius.*
n.s, not significant; *, P<0.05; **, P<0.01
SEM, standard error of the mean

Table 6. Degradability of organic matter in *Populus nigra* sawdust.

Ruminal degradability parameters			a	b	C	PD	ED(0.02)	ED(0.05)	ED(0.08)
Fungi 1		0 day	4.97^{d}	8.73^{c}	0.071^{a}	13.70^{e}	11.85^{e}	10.30^{e}	9.40^{e}
		7 day	9.48^{b}	8.26^{d}	0.048^{d}	17.70^{c}	15.25^{c}	13.50^{c}	12.55^{c}
		17 day	10.03^{a}	12.33^{a}	0.055^{bc}	22.35^{a}	19.10^{a}	16.55^{a}	15.15^{a}
Fungi 2		0 day	4.97^{d}	8.73^{c}	0.071^{a}	13.70^{e}	11.85^{e}	10.30^{e}	9.40^{e}
		7 day	7.00^{c}	8.46^{d}	0.058^{b}	15.45^{d}	13.35^{d}	11.70^{d}	10.80^{d}
		17 day	9.47^{b}	10.57^{b}	0.052^{cd}	20.05^{b}	17.10^{b}	14.90^{b}	13.70^{b}
SEM			0.14	0.07	0.001	0.1	0.06	0.02	0.03
Significantly	Fungi		**	**	**	**	**	**	**
	Treatment time		**	**	n.s	**	**	**	**
	Treatment time × Fungi		**	**	**	n.s	n.s	*	**

Means in a column with different superscripts differ significantly (P < 0.05).
a, highly soluble and readily degradable fraction (%); b, insoluble and slowly degradable fraction (%); c, rate constant for degradation of the fraction b (/h); PD, potential degradability (%); ED, effective degradability (%).
Fungi 1, *Phanerochaete chrysosporium;* Fungi 2, *Pleurotus ulmarius.*
n.s, not significant; *, P<0.05; **, P<0.01
SEM, standard error of the mean

Conclusion: Insufficient feed source for livestock is a major obstacle in producing livestock products and researchers believe that the limitations on human and animal food will get more severe over the coming years, a concern which drew attentions toward lignocellulosic compounds. Our findings, in general, supported positive effects of fungal treatment of *Populus nigra* sawdust. Therefore, given the limitations on exploiting pastures and high costs of fodder on one hand and considerable amount of wood waste as an industrial by-product on the other, biological treatment of this waste may be helpful in providing an inexpensive source of feed for ruminants. In addition, given the nutrition value of this waste, the processing will not only provide an inexpensive valuable feed source to cut feeding costs of livestock. It will also considerably reduce environmental pollution by converting a major source of contamination to a food usable in animal feed. Advantages of fungal treatment depend on several factors: fungal strain, substrate, culture conditions and time of incubation. It is recommended that future experiments be conducted on other fungal strain, different time of incubation and culture conditions.

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