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The Effectiveness of Biological Treatment of Wheat Straw by White-Rot Fungi

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ABSTRACT. Out of 13 species of *Basidiomycetes* growing on wheat straw, 9 species enhanced the *in vitro* dry matter digestibility of the substrate. The detergent fiber content (acid and neutral) of the substrate was significantly reduced by most of the fungi tested. Hemicellulose showed the largest proportionate loss, whereas lignin the smallest one.

Utilization of fibrous crop residues by ruminants, even as a source of energy, is limited because the rumen microbial population does not possess lignolytic activity (Zadrazil *et al.* 1995). The use of straw as an animal feed is limited also by its low nutritional value and its low nitrogen content. Biological methods of treating straw were used to identify species of white-rot fungi having the ability to improve the quality of lignocellulosic substrates as ruminant feedstuffs (Zadrazil 1985; Reid 1989). These studies used lignin degradation and improvement of *in vitro* dry matter digestibility (IVDMD) as an index to evaluate the effectiveness of biological treatment. The objective of this study was to determine the effect of fungal colonization of wheat straw by 13 species of white-rot basidiomycetes on *in vitro* dry matter digestibility (IVDMD) and chemical composition of treated substrates.

Fungal cultures — *Dædalea guercina* (L.) PERS. 528, *Hericium clathroides* (PALLAS:FR.) PERS. 662, *Inonotus andersonii* (ELL. et EVER.) ČERNÝ 557, *Inonotus dryophilus* (BERK.) MURR. 703, *Inonotus obliquus* (PERS.:FR.) PILÁT 559, *Lentinus tigrinus* (BULL.:FR.) FR. 826, *Lyophyllum ulmarium* (BULL.:FR.) KÜHN. 408, *Phellinus lævigatus* (P.KARST.) BOURD. et GALZ. 657, *Polyporus brumalis* (PERS.:FR.) FR. 588, *Polyporus ciliatus* FR.:FR 799, *Trametes gibbosa* (PERS.:FR.) FR. 609, *Pleurotus ostreatus* (JACQ.:FR.) KUMM. 476, were obtained from the *Culture Collection of Basidiomycetes (CCBAS), Institute of Microbiology, Academy of Sciences of the Czech Republic*, Prague. *Pleurotus ostreatus* mutant was obtained by ultraviolet mutagenesis (Homolka *et al.* 1995). For solid-state fermentation with basidiomycetes, samples of moistened wheat straw (water content 80 %) were placed into separate plastic bags. After sterilization, the straw was inoculated with homogenized mycelium of white-rot fungi. Seven days before inoculation, each fungus was grown at 28 °C in a medium (pH 5.5) containing glucose (1 %), corn steep liquor (1.5 %) and $MgSO_4 \cdot 7H_2O$ (0.15 %). Corn steep liquor served as the nitrogen source. The fungal mycelial mat was separated by filtration, washed with sterile water, homogenized in 100 mL water and used for inoculating 300 g wheat straw. The wheat straw was sterilized before inoculation by steaming 2 times at 100 °C. The inoculated substrate was incubated at 28 °C for 30 d. After the 30-d incubation period the substrates (untreated wheat straw and treated wheat straw with fungi) were dried at 21 °C and analyzed. The samples were analyzed for neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Goering and Van Soest 1970), nitrogen and ash content. IVDMD was determined in triplicate samples according to Mellenberger *et al.* (1970). The assay was repeated 6 times. Loss of dry matter was estimated by weighing the dried substrate (2 d, 60 °C) at the beginning and end of the solid-state fermentation process. Losses of components after fungal treatment were calculated as follows:

$$100 - [(100 - \mathrm{DML}) \times \mathrm{PF}]/\mathrm{PO}$$

where DML is dry matter lost,
PF per cent final substrate and
PO per cent original substrate (Tsang *et al.* 1987).

After the 30-d incubation period most of the fungi demonstrated extensive growth on wheat straw. Hyphal growth and infiltration into the substrate were observed. *H. clathroides*, *L. tigrinus* and *L. ulmarium* exhibited less extensive growth and infiltration into the substrate. The treatment with 9 species of white-rot fungi significantly reduced (Table I) the content of cell-wall components (NDF,

ADF, hemicellulose) in the wheat straw. Other fungi, *i.e. I. andersonii, I. obliquus, L. tigrinus* and *P. lævigatus* caused only small differences in the contents of cell-wall components in decayed straw. The cellulose content was (*a*) reduced in straw treated with *D. quercina, H. clathroides, P. ostreatus, P. ostreatus* mutant, *P. brumalis, P. ciliatus, T. gibbosa*, (*b*) unchanged with *I. andersonii, I. obliquus, I. dryophilus, L. tigrinus, L. ulmarium*, (*c*) increased with *H. clathroides* and *P. lævigatus* (Table I). Similar effects on the chemical composition of decayed material were described by Karunanandaa *et al.* (1995) and Chen *et al.* (1995). Lignin content was usually reduced after a fungal treatment of wheat straw but it was increased with *L. tigrinus, I. dryophilus* and *P. ciliatus*, possibly because these fungi removed relatively more digestible wall polysaccharides than lignin. Nitrogen and ash content were significantly increased by fungal treatment, possibly as the result of increased fungal biomass (Table I). Biological delignification of straw with white-rot fungi often results in increased IVDMD. Our fungal treatment of wheat straw significantly increased IVDMD (about 6–15 units) in 10 decayed samples (Table I) while it decreased IVDMD (about 4–11 units) in straw samples decayed with *L. ulmarium, P. brumalis* and *L. tigrinus*. The increase of IVDMD in decayed straw was probably related to the decrease of the lignin content. It is known that some species of fungi increase wheat straw IVDMD, whereas others decrease it. About 300 strains of basidiomycetes have been screened for their ability to degrade lignin and cause a change in IVDMD of various lignocellulosic materials (Zadrazil *et al.* 1996).

Table I. Chemical composition (%) and *in vitro* dry matter digestibility (IVDMD, %) of wheat straw after fungal treatment[a]

Variable	NDF	ADF	HC	Cellulose	Lignin	Nitrogen	Ash	IVDMD
UWS[b]	71.7	56.1	15.5	46.2	8.8	0.59	6.0	41.4
D. quercina	58.6***	47.4***	11.2***	41.9***	5.5***	0.75***	8.2**	59.2*
H. clathroides	68.4***	54.5*	13.9*	49.4**	5.9***	0.75***	6.5*	56.3*
I. andersonii	70.1*	55.0	15.1	46.4	8.6	0.78***	6.5**	51.4*
I. dryophilus	71.3	57.6*	13.7*	46.5	11.1***	0.61	6.6**	55.9*
I. obliquus	70.4	54.9	15.4	47.4	8.1**	0.67***	7.7***	52.0*
L. tigrinus	70.2	56.2	12.6**	44.1	12.1***	0.85***	7.9	33.1**
P. lævigatus	71.2	56.0	15.2	48.7**	7.4***	0.64*	6.8**	50.2*
SEM	0.4	0.3	0.3	0.6	0.2	0.1	0.4	0.6
UWS[b]	75.0	50.0	25.0	41.2	8.9	0.65	5.6	44.0
L. ulmarium	71.2***	49.9	21.2***	42.0	8.0	0.72*	9.4***	36.0***
P. ostreatus	58.0***	43.1***	14.9***	36.1***	7.5*	0.94***	9.1***	51.0***
P. ostreatus[c]	63.1***	45.0***	18.4***	39.1***	6.2***	0.81**	9.0***	54.0***
P. brumalis	64.1***	45.2***	19.4***	38.0***	6.3***	0.88***	9.0***	40.1*
P. ciliatus	57.0***	46.2***	11.2***	34.2***	11.1**	1.01***	7.6**	50.1***
T. gibbosa	54.1***	44.1***	9.4***	38.1***	6.5***	0.93***	9.4***	57.0***
SEM	0.4	0.4	0.3	0.3	0.3	0.2	0.2	0.6

[a]NDF – neutral detergent fiber, ADF – acid detergent fiber, HC – hemicellulose, IVDMD – *in vitro* dry matter digestibility, SEM – standard error of the mean.
[b]Untreated wheat straw. [c]Mutant. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

Losses of dry matter and cell wall constituents (NDF, ADF, hemicellulose, cellulose, lignin) were determined only in straw, inoculated with *D. quercina, H. clathroides, P. lævigatus, I. andersonii, I. obliquus* and *I. dryophilus*. The degradation rates of dry matter were different and are presented in Table II. The losses of wheat straw dry matter caused by fungi during the 30-d solid-state incubation include losses of cellulose, hemicellulose and lignin (Table II). More than half of the amount of total hemicellulose was degraded by *D. quercina*. The other fungi degraded 25–30 % of hemicellulose. A pattern similar to hemicellulose degradation was found with wheat straw cellulose, where the degradation by *D. quercina* reached 49 %. *P. lævigatus, I. andersonii, I. obliquus* and *I. dryophilus* degraded about 20 % of the cellulose, while *H. clathroides* degraded cellulose to a small extent. The extensive degradation of lignin by fungi (except for *I. dryophilus*) indicated that the chemical structure of wheat straw is broken down by these fungi. Out of the three fractions (hemicellulose, cellulose and lignin) hemicellulose and lignin showed the largest proportionate loss after incubation with *D. quercina, H. clathroides, P. lævigatus* and *I. obliquus*. The other two fungi caused the largest proportionate loss in cellulose and hemicellulose contents.

Table II. Loss (%) of structural components of wheat straw after treatment with six white-rot fungi

Fungus	Dry matter	NDF	ADF	Hemicellulose	Cellulose	Lignin
D. quercina	43 ± 2	54 ± 2	52 ± 2	59 ± 2	49 ± 2	65 ± 2
H. clathroides	12 ± 1	16 ± 1	14 ± 1	27 ± 1	6 ± 1	41 ± 1
P. lævigatus	24 ± 1	24 ± 1	24 ± 1	26 ± 1	20 ± 1	36 ± 1
I. andersonii	24 ± 2	26 ± 1	26 ± 1	32 ± 1	24 ± 1	19 ± 1
I. obliquus	24 ± 1	26 ± 1	26 ± 1	30 ± 1	22 ± 1	31 ± 1
I. dryophilus	22 ± 1	22 ± 1	20 ± 1	31 ± 1	21 ± 1	2 ± 1

Fungal treatment is a promising means to convert low-quality wheat straw into a higher quality ruminant feed. The treatment of wheat straw with 9 strains of basidiomycetes effectively enhanced the digestibility of wheat straw, caused varying dry matter loss and increased nitrogen content. The other 3 species (*L. tigrinus*, *L. ulmarium*, *P. brumalis*) decreased IVDMD of wheat straw and *D. quercina* caused a high dry matter loss (43 %) of the straw. These fungi showed a lower effectiveness in the treatment.

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