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Original article

Effect of white-rot basidiomycetes-treated wheat straw on rumen fermentation in artificial rumen

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Summary — This study evaluated three white-rot basidiomycetes for their potential to improve the ruminal degradation of wheat straw. *Pleurotus ostreatus* (PO), *Pleurotus ostreatus-mutant* (PO-M) and *Trametes gibbosa* (TG) were incubated on wheat straw for 30 days at 28 °C. Neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein and in vitro dry matter digestibility (IVDMD) were determined. The results demonstrated increasing crude protein and ash contents (%) in the fungi-treated straws. The IVDMD values were also increased. Compared to untreated wheat straw (UWS), the NDF and ADF contents were reduced in fungi-treated straw (TWS). Out of the three fractions – hemicellulose, cellulose and lignin–hemicellulose showed the largest proportionate loss and lignin the smallest in all three fungi-treated straws. TWS with *Pleurotus ostreatus* (TWS-PO), *Pleurotus ostreatus-mutant* (TWS-PO-M) and *Trametes gibbosa* (TWS-TG) together with barley (80:20%) were used as the experimental diets in the artificial rumen. UWS with barley (80:20%) served as the control diet. The results revealed significantly higher IVDMD values, NDF, ADF and cellulose digestibilities (%) with the experimental diets. The production of propionic acid decreased, *n*-butyric, *n*-valeric and isovaleric acids (mmol/day^{-1}) increased and the volatile fatty acid (VFA) production expressed in mol VFAs.kg^{-1} digested dry matter decreased in experimental diets. The total microbial production also decreased at fermentation in the experimental diets.

basidiomycetes / wheat straw / rumen fermentation / artificial rumen

Résumé — Effet du traitement de la paille de blé par des basidiomycètes sur la fermentation en rumen artificiel. *Ce travail a évalué les trois basidiomycètes du point de vue de la possibilité d'améliorer la dégradation de la paille de froment dans le rumen.* Pleurotus ostreatus *(PO),* Pleurotus ostreatus-mutant *(PO-M),* Trametes gibbosa *(TG) ont été incubés sur la paille de froment pendant 30 jours à la température de 28 °C. Les fibres NDF (détergent neutre), les fibres ADF (détergent acide), les protéines brutes et la digestibilité du résidu sec (IVDMD) in vitro ont été déterminées. Les résultats ont démontré l'augmentation des protéines brutes et des cendres (%) dans la paille traitée par les champignons. Les valeurs de l'IVDMD ont également été augmentées. En comparaison avec la paille de froment non traitée (UWS), le contenu de NDF et de ADF a été réduit dans la paille traitée par les champignons (TWS). Des trois composants : hémicellulose, cellulose et lignine, c'était l'hémicellulose qui subissait une perte proportionnellement la plus grande et la lignine la plus petite dans les trois pailles traitées par les cham-*

pignons. TWS avec Pleurotus ostreatus *(TWS-PO), Pleurotus ostreatus-mutant (TWS-PO-M) et* Trametus gibbosa *(TWS-TG) mélangée à l'orge (80:20%) ont été employées pour les régimes expérimentaux dans le rumen artificiel. UWS mélangée à l'orge (80:20%) servait de régime témoin. Les valeurs d'IVDMD, NDF et ADF, ainsi que celles de la digestibilité cellulaire, étaient significativement plus élevées (%) dans les régimes expérimentaux. La production de l'acide propionique diminuait et celle de l'acide* n*-valérique et iso-valérique (mmol/jour^{-1}) augmentait, tandis que la production des acides gras volatils (VFA) exprimée en mol VFA/kg^{-1} du résidu sec diminuait dans les régimes expérimentaux. La production microbienne totale a été diminuée pendant la fermentation des régimes expérimentaux.*

basidiomycètes / paille de froment / fermentation du rumen / rumen artificiel

INTRODUCTION

The use of straw as animal feed is limited by its low nutritional value and its low nitrogen content. Various chemical and physical delignification methods to improve the digestibility of straw have been extensively researched (Sundstol and Owen, 1984). Biological methods of treating straw using microorganisms such as white-rot basidiomycetes have also been reported (Zadrazil, 1984).

The objective of our study was to screen three species of white-rot basidiomycetes – *Pleurotus ostreatus*, *Pleurotus ostreatus-mutant* and *Trametes gibbosa* – by evaluating the changes in the lignin and polysaccharide content of the cell walls and the in vitro ruminal digestibility of wheat straw. It was also the objective of this study to investigate the effect of treating wheat straw with *Pleurotus ostreatus*, *Pleurotus ostreatus-mutant* and *Trametes gibbosa* during in vitro rumen fermentation using the rumen simulation technique (Rusitec) and to compare the fermentation parameters with those of untreated wheat straw.

MATERIALS AND METHODS

Organisms

Fungal cultures of *Trametes gibbosa* and *Pleurotus ostreatus-mutant* were obtained from the Culture Collection of Basidiomycetes (CCBAS) of the Institute of Microbiology, Academy of Sciences of the Czech Republic in Prague. *Pleurotus ostreatus* was obtained from Y Hadar of the Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot, Israel. *Pleurotus ostreatus-mutant* was obtained by ultraviolet mutagenesis by the method described by Homolka et al (1995). The fungi *Pleurotus ostreatus* and *Pleurotus ostreatus-mutant* were used for the comparison of the maternal fungus and mutant. *Pleurotus ostreatus-mutant* in previous experiments showed increased production of the lignolytic enzymes. *Trametes gibbosa* was used for the rapid colonization of the wheat straw and for the production of three main lignolytic enzymes: ligninase, manganese-peroxidase and laccase.

Culture conditions

Seven days before the substrate (wheat straw-whole fiber) inoculation, each fungus was grown at 28 °C in a medium (pH 5.5) containing glucose (1%), corn steep liquor (1.5%) and $MgSO_4.7\ H_2O$ (0.15%). The fungi mycelial mat was separated by filtration, washed with sterile water, homogenized in 100 ml water and used to inoculate 300 g of wheat straw. The wheat straw was sterilized before inoculation by steaming for 1 h at 100 °C. The inoculated substrate was incubated at 28 °C for 30 days.

Chemical analyses

After the 30 day incubation period, the substrates – untreated wheat straw (UWS), wheat straw treated with the fungus *Pleurotus ostreatus* (TWS-PO), *Pleurotus ostreatus-mutant* (TWS-PO-M) and *Trametes gibbosa* (TWS-TG)– were dried at

laboratory temperature (21 °C) before analysis. The samples were analyzed for their detergent fiber content (Goering and Van Soest, 1970). Crude protein and ash were determined by the methods of the Association of Official Analytical Chemists (Horowitz, 1980). In vitro dry matter disappearance (IVDMD) (Mellenberger et al, 1970) was determined by incubating the sample for 96 h with the ruminal fluid taken from sheep (Slovak Merino) fed alfalfa hay.

In vitro fermentation system

Fermentation of the substrates UWS, TWS-PO, TWS-PO-M and TWS-TG with barley (80:20%) was performed in the four fermentation vessels (V_1–V_4) of an artificial rumen, as described by Czerkawski and Breckenridge (1977). The rumen contents to be used as inocula were obtained from three sheep (Slovak Merino) given a diet of 1 200 g hay and barley (80:20%) per animal per day. Liquid samples of the rumen contents were taken through a rumen cannula by suction and samples of the solid digesta were removed with tongs. The solid digesta (80 g wet weight) were placed into nylon bags of 200 μm pore size in each of the fermentation vessels. The vessels were filled to overflowing with strained rumen fluid and artificial saliva (1:1) (McDougall, 1948). The nominal volume of each of the four fermentation vessels was 850 ml and the daily flow of the artificial saliva was 800–830 ml. Including the first day of the experiment, the vessels were supplied at daily intervals with 11.71 g DM of UWS and 2.88 g DM of barley (V_1); 11.61 g DM of TWS-PO and 2.88 g DM of barley (V_2); 11.64 g DM of TWS-PO-M and 2.88 g DM of barley (V_3); 11.67 g DM of TWS-TG and 2.88 g DM of barley (V_4). The diets were placed into nylon bags and each bag remained in the vessel for 2 days. To ensure that all diets contained 13% of crude protein (CP), 403.03 mg (V_1), 309.56 mg (V_2), 353.04 mg (V_3) and 316.30 mg (V_4) of urea were dissolved in 1 L of McDougall's buffer. The buffer was then infused into the Rusitec fermentation system using a peristaltic pump.

Measurements

The experiment in Rusitec lasted 13 days. To ensure a steady state within the vessels, an adjustment period for the first 7 days was allowed. Measurements were taken for days 8–13. The general incubation procedure and preparation of the samples for analyses were as described by Czerkawski and Breckenridge (1977). After washing with saliva solution, the residues (undigested samples of the mixed rations in nylon bags) were dried at 105 °C to a constant weight in order to determine the DM digestibility and then analyzed for detergent fiber content. To determine the microbial biomass in the effluent, 30 ml of preserved suspension was centrifuged at 15 000 x *g* for 30 min. The residue was washed twice with water, and was dried to constant weight at 105 °C. The concentrations of volatile fatty acids (VFA) in the effluent were determined by the gas chromatography procedure (Cottyn and Boucque, 1968) using crotonic acid as the internal standard in the Perkin-Elmer 8500 gas chromatograph. The volumes of gas produced were measured with a gas meter and the methane and CO_2 gases were analyzed in a Chrom 4 gas chromatograph as reported by Czerkawski and Clapperton (1968). The concentration of ammonia–nitrogen in the effluent was determined by the microdiffusion method (Conway and O'Malley, 1942). The microbial biomass in the residues were determined by an improved technique using treatment with saline (pH 2.0), Tween 80, MeOH and terciary butanol (Whitehouse et al, 1994).

Statistical analysis

The daily means for all diets were compared using the parametric Student's *t*-test (Snedecor and Cochran, 1971).

RESULTS

In comparison to UWS, *Pleurotus ostreatus*, *Pleurotus ostreatus-mutant* and *Trametes gibbosa* incubated on wheat straw resulted in a significantly increased IVDMD (%) (table I). The fungal treatment increased the crude protein (about 1.0–1.8%) and ash content (3.32–3.76%) in TWS-PO, TWS-PO-M and TWS-TG. The detergent fiber content was reduced in fungi-treated straws. From among the individual fiber components (cellulose, hemicellulose, lignin), the

Table I. Detergent fiber composition, crude protein, ash content and in vitro dry matter digestibility (IVDMD) of wheat straw after 30 days of fungal treatment (% of dry matter)[1,2].

Fungal treatment	*NDF*	*ADF*	*HC (NDF-ADF)*	*Cellulose*	*Lignin (ADL)*	*Crude protein (N x 6.25)*	*Ash*	*IVDMD*
Control	75 ± 0.2	50 ± 0.6	25 ± 0.3	41 ± 0.3	8.9 ± 0.39	4.1 ± 0.16	5.6 ± 0.18	44 ± 0.9
Pleurotus ostreatus	58 ± 0.5[c]	43 ± 0.5[c]	14.9 ± 0.4[c]	36 ± 0.3[c]	7.5 ± 0.34[a]	5.9 ± 0.12[c]	9.1 ± 0.22[c]	51 ± 1.1[c]
Pleurotus ostreatus-mutant	63 ± 0.2[c]	45 ± 0.4[c]	18.4 ± 0.3[c]	39 ± 0.3[c]	6.2 ± 0.28[c]	5.0 ± 0.14[b]	9.0 ± 0.18[c]	54 ± 1.1[c]
Trametes gibbosa	54 ± 0.7[c]	44 ± 0.3[c]	9.4 ± 0.4[c]	38 ± 0.3[c]	6.5 ± 0.26[c]	5.8 ± 0.16[c]	9.4 ± 0.15[c]	57 ± 0.8[c]

[1] Averages of six determinations (NDF, ADF, HC, cellulose, lignin, crude protein and ash) or nine determinations (IVDMD); [2] roughage: wheat straw. The statistical calculations were made between control and fungal treatment of wheat straw; [a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$.

hemicellulose content was reduced to the greatest extent (about 6.67–15.72%) and lignin the least (1.51–2.37%) (table I). The individual substrates UWS, TWS-PO, TWS-PO-M and TWS-TG were used with rolled barley (80:20%) as diets and the parameters of fermentation were compared between experimental diets (V_2, V_3, V_4) and control diet (V_1) in Rusitec. Table II shows that dry matter (DM), organic matter (OM), NDF, ADF and cellulose digestibilities (%) were significantly higher in the experimental diets.

In comparison to the control diet, the total gas production (ml/day^{-1}), as well as CH_4 and CO_2 production (mmol/day^{-1}) were not affected by the fermentation of the experimental diets. Table III shows that total VFA production was unchanged. The total VFA production, however, expressed as VFA production in mol.kg^{-1} digested DM was significantly lower in V_2, V_3, V_4 as compared with V_1. The production of acetic acid and isobutyric acid (mmol/day^{-1}) was not influenced by the fermentation of the individual diets. Differences were found between the experimental diets only. The production of propionic acid (mmol/day^{-1}) was significantly decreased in V_2 and V_4 and unchanged in V_3. The production of *n*-butyric, *n*-valeric and isovaleric acids were significantly increased in V_2–V_4. The decrease of propionic acid production was accompagnied by a significant increase in the acetic–propionic ratio in the experimental diets.

The ammonia–nitrogen production in the effluent was significantly decreased in V_2 and V_4 and unchanged in V_3 as compared with V_1 (table II). Our calculation showed that nitrogen (N) utilization (calculated from nitrogen content of feed + N of urea = input and N of effluent + N of undigested feed = output) was lower in the experimental diets (V_2: 80.8; V_3: 94.9; V_4: 97.2%) as compared with V_1 (99.8%).

The total microbial production (microbial biomass of effluent + microbial biomass of residual [undigested] feed) was significantly decreased in the experimental diets (table III).

Table II. The effect of fungi-treated straw on dry matter, detergent fiber digestibilities and gas production in artificial rumen (mean ± SEM, $n = 6$).

Degradation of feed after 48 h (%)	*Diet* [1]				*Statistics*
	UWS vessel 1	*TWS-PO vessel 2*	*TWS-PO-M vessel 3*	*TWS-TG vessel 4*	
Dry matter	34.65 ± 0.29	41.77 ± 0.81	46.62 ± 1.34	42.84 ± 1.20	V_1-$V_{2,3}$ [c] V_2-V_3 [a] V_1-V_4 [b]
Organic matter	39.32 ± 0.31	46.59 ± 0.91	51.57 ± 1.26	47.98 ± 1.14	V_1-$V_{2,3}$ [c] V_2-V_3 [a] V_1-V_4 [b]
NDF	28.08 ± 0.31	26.24 ± 1.05	37.12 ± 1.61	28.48 ± 1.50	V_1-V_3 [b] V_2-V_3 [c] V_3-V_4 [b]
ADF	14.04 ± 0.37	16.60 ± 1.19	24.36 ± 1.92	33.23 ± 1.41	V_1-V_3, V_2-V_3, V_3-V_4 [b] V_1-V_4, V_2-V_4 [c]
Hemicellulose	50.72 ± 0.25	45.83 ± 0.78	59.98 ± 0.98	15.87 ± 1.79	V_1-V_2 [b] V_2-$V_{3,4}$ [c] V_1-$V_{3,4}$ [c] V_3-V_4 [c]
Cellulose	29.68 ± 0.34	31.07 ± 0.99	44.99 ± 1.39	61.52 ± 0.83	V_1-$V_{3,4}$ [c] V_2-$V_{3,4}$ [c] V_3-V_4 [c]
Total gas production (ml/day^{-1})	2 607.5 ± 59.42	2 815 ± 82.51	2 477.5 ± 136.44	2 779.0 ± 62.85	NS
CO_2 production (mmol/day^{-1})	34.64 ± 2.51	38.01 ± 2.86	37.71 ± 5.08	38.77 ± 2.11	NS
Methane production (mmol/day^{-1})	7.67 ± 0.44	7.70 ± 0.34	8.02 ± 1.09	7.77 ± 0.30	NS
NH_3-N of effluent (mg/L^{-1})	187.55 ± 6.81	147.22 ± 5.78	171.42 ± 10.08	151.25 ± 6.81	V_1-$V_{2,4}$ [b]

[1] All diets contained substrate and barley (80:20%). Substrates were: UWS: untreated wheat straw; TWS-PO: wheat straw treated with *Pleurotus ostreatus*; TWS-PO-M: wheat straw treated with *Pleurotus ostreatus-mutant*; TWS-TG: wheat straw treated with *Trametes gibbosa*; [a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$; NS: not significant.

DISCUSSION

After a 30 day incubation period, *Pleurotus ostreatus*, *Pleurotus ostreatus-mutant* and *Trametes gibbosa* demonstrated extensive growth on wheat straw. Fungal treatment caused the wheat straw to have an increased crude protein and ash content (%). Similar results were presented with white-rot fungi-treated wheat straw (Agosin et al, 1986).

Fungal treatment of wheat straw reduced the detergent fiber content in three white-rotted straws. Of the three straw fractions

– hemicellulose, cellulose and lignin – hemicellulose showed the largest proportionate loss whereas lignin showed the smallest loss during the growth of the three species of basidiomycetes. Streeter et al (1982) found that *Pleurotus ostreatus* decomposed hemicellulose to a greater extent than the other cell wall components. The IVDMD values (%) were higher after 96 h of fermentation in the fungi-treated straws. It is well

Table III. The effect of fungi-treated straw on volatile fatty acid (VFA) production and total microbial production in Rusitec (mean ± SEM, $n = 6$).

	Diet [1]				*Statistics*
	UWS vessel 1	*TWS-PO vessel 2*	*TWS-PO-M vessel 3*	*TWS-TG vessel 4*	
Total VFA production (mmol/day^{-1})	37.74 ± 0.82	35.90 ± 0.74	40.16 ± 1.27	34.95 ± 0.69	V_1-V_4, V_2-V_3 [a] V_3-V_4 [b]
Acetate (mmol/day^{-1})	21.33 ± 0.56	20.20 ± 0.37	22.99 ± 0.68	20.14 ± 0.48	V_2-V_3, V_3-V_4 [b]
Propionate (mmol/day^{-1})	12.85 ± 0.20	10.63 ± 0.23	11.96 ± 0.40	9.96 ± 0.23	V_1-$V_{2,4}$ [c] V_2-V_3 [a] V_3-V_4 [b]
Butyrate (mmol/day^{-1})	2.18 ± 0.09	3.02 ± 0.12	3.41 ± 0.15	3.07 ± 0.09	V_1-$V_{2,3,4}$ [c]
Isobutyrate (mmol/day^{-1})	0.34 ± 0.03	0.47 ± 0.07	0.37 ± 0.05	0.36 ± 0.02	NS
n-valerate (mmol/day^{-1})	0.55 ± 0.02	0.85 ± 0.04	0.65 ± 0.03	0.68 ± 0.03	V_1-V_2 [c] V_1-V_3 [a] V_1-V_4 [b] V_2-$V_{3,4}$ [b]
Isovalerate (mmol/day^{-1})	0.49 ± 0.02	0.78 ± 0.04	0.77 ± 0.03	0.73 ± 0.03	V_1-$V_{2,3,4}$ [c]
Acetate–propionate ratio	1.66 ± 0.03	1.90 ± 0.02	1.92 ± 0.02	2.02 ± 0.04	V_1-$V_{2,3,4}$ [c] V_2-V_4 [a]
mol VFA.kg^{-1} digested DM	7.46 ± 0.16	5.93 ± 0.10	5.94 ± 0.19	5.62 ± 0.13	V_1-V_2, V_3, V_4 [c]
Total microbial matter production (g/day^{-1})	1.47 ± 0.07	1.41 ± 0.09	1.43 ± 0.02	1.42 ± 0.01	V_1-V_2 [c] V_1-V_3 [a] V_1-V_4 [b]

[1] All diets contained substrate and barley (80:20%). Substrates were: UWS: untreated wheat straw; TWS-PO: wheat straw treated with *Pleurotus ostreatus*; TWS-PO-M: wheat straw treated with *Pleurotus ostreatus-mutant*; TWS-TG: wheat straw treated with *Trametes gibbosa*; [a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$; NS: not significant.

known that some species of fungi increase wheat straw IVDMD, whereas others decrease it (Zadrazil, 1985).

Fermentation of the fungi-treated straw TWS-PO, TWS-PO-M and TWS-TG with barley (80:20%) was accompanied by a significant increase in DM, OM, NDF, ADF and cellulose digestibilities as compared to the control diet. The hemicellulose digestibility was significantly increased in the TWS-PO-M diet and decreased in TWS-PO and TWS-TG (table II). This was probably caused by the considerable loss of the hemicellulose after the fungal treatment.

Total gas production, CO_2 and methane production did not seem to be influenced in this experiment. The fermentation of the experimental diets caused considerable changes in the VFA production. These changes were probably caused by differences in the detergent fiber composition of the fungi-treated straw as compared to the untreated wheat straw.

Similar results were presented in an experiment with *Lentinus tigrinus* - and *Polyporus ciliatus* - treated wheat straw (Jalč et al, 1994). Decreases in the total VFA production and in individual VFAs (acetic, propionic, butyric acids) were observed in the experiment with *Coprinus fimetarius* - treated rice straw (Demeyer et al, 1988).

The observed decrease in the pool of NH_3-N effluent in the experimental diets can be explained by a decreased degradation of feed nitrogen in the diets containing fungi-treated straw. This was confirmed by the lower utilization rate for nitrogen found in the experimental diets. The production of microbial matter was decreased in the experimental diets and reflects the changes in VFA production (mol.VFA.kg^{-1} digested DM, acetate–propionate ratio) at the fermentation of all three fungi-treated straw.

Finally, the results showed that wheat straw was upgraded to a more digestible material with *Pleurotus ostreatus*, *Pleurotus ostreatus-mutant* and *Trametes gibbosa*. The fungal treatment increased the crude protein and ash contents and reduced the detergent fiber content in the fungi-treated straws. The rumen fermentation of fungi-treated straw was characterized by an increase of IVDMD and IVOMD values, NDF, ADF and cellulose digestibilities in artificial rumen. However, total VFA production expressed in mol.kg^{-1} digested DM and total microbial production (g/day^{-1}) in effluent and undigested feed were decreased. We can speculate that low molecular weight phenolic compounds forming from lignin breakdown could serve as the inhibitors of microorganisms found in rumen.

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