

## Effect of three strains of *Pleurotus tuber-regium* (Fr.) Sing. on chemical composition and rumen fermentation of wheat straw

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This study was conducted to investigate changes in in vitro dry matter digestibility (IVDMD) and cell wall constituent degradation in wheat straw treated with 3 strains of the fungus *Pleurotus tuber-regium* (PT). The incubation of wheat straw for 30 days at 28°C improved IVDMD from 30.3% (UWS—untreated wheat straw) to 47.1% for strain PT1, to 48.5% for PT4, and was unchanged IVDMD—29.9%—for PT5. The growth of fungi was accompanied by the dry matter loss of wheat straw: 31.5% for PT1, 20.9% for PT4, and 4.8% for PT5. Fungal treatment was characterized by increased crude protein and ash contents (%) in all fungi-treated straws and reduced hemicellulose and lignin content. It is evident that enzymes of all 3 PT strains preferentially degraded hemicellulose and lignin over cellulose. Wheat straw treated with PT1 (TWS-PT1), PT4 (TWS-PT4), and PT5 (TWS-PT5) and barley (80% : 20%) were used as the experimental diets at the fermentation in the artificial rumen. UWS with barley (80% : 20%) served as the control diet. The fermentation of experimental diets was accompanied with increased IVDMD and a very low degree of hemicellulose degradation. Total gas and methane productions were similar in all diets. Moreover, total volatile fatty acid (VFA) production ( $\text{mmol day}^{-1}$ ), mol % of acetate, propionate, butyrate, isobutyrate, and isovalerate were not influenced during the fermentation of experimental diets. From the stoichiometric relations, production, utilization, and recovery of metabolic hydrogen and organic matter fermented were unchanged. Only the recovery of metabolic hydrogen in TWS-PT5 was significantly increased in comparison to control diet. Total microbial production showed the tendency of lower values in experimental diets, and it was accompanied with a significant decrease of ammonia nitrogen ( $\text{mg L}^{-1}$ ). Finally the results showed that the strains of *Pleurotus tuber-regium* can improve the quality of wheat straw, but the loss of dry matter (DM) (mainly hemicellulose) limits the effective utilization of fungi-treated straw in ruminant digestion.

**Key Words**—artificial rumen; detergent fiber composition; fungal treatment; gas production; in vitro dry matter digestibility; VFA production

Agricultural crop residues, especially cereal straw, contribute to a major part of the diet of ruminants in developing countries. Crop residues are lignocellulosic materials rich in energy, low in crude protein, and poor in palatability. 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 by using microorganisms such as white-rot fungi (WRF) have also been reported (Zadrazil, 1984).

Many species of WRF have been screened on a variety of lignocellulosic substrates for their ability to improve the nutritional value of poor quality crop residues for use as a ruminant feedstuff (Fahey et al., 1993; Zadrazil, 1985). In a systematic screening of lignolytic fungi in which preferential degradation of wheat straw lignin was studied, Martinez et al. (1994) conducted that *Pleurotus eryngii* and *Pleurotus ostreatus* are the most promising fungi.

The aim of this study was to screen 3 strains of the tropical fungus *Pleurotus tuber-regium* (Fr.) Sing. by evaluating changes in lignin and polysaccharides of cell walls in wheat straw and to compare fermentation parameters of wheat straw treated by these fungi with

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Table 1. Modification of straw composition by fungi.

Parameter	UWS	Substrates		
		TWS-PT1	TWS-PT4	TWS-PT5
% of DM				
Organic matter	87.70	82.10	79.85	85.36
Ash	6.04	11.30	13.34	9.02
Crude protein	2.14	2.90	3.70	3.45
NDF	79.28	59.28	56.99	67.79
ADF	55.38	49.78	51.58	56.49
Hemicellulose	23.90	9.50	5.41	11.30
Cellulose	47.1	45.13	48.5	50.4
Lignin	8.31	4.65	3.08	6.0
IVDMD	30.28	47.13	48.47	29.86
Fungal degradation (%)				
DM loss	0	31.5±1.0	20.9±1.0	4.8±0.5
NDF loss	—	48.78±1.0	43.14±1.0	18.59±0.5
ADF loss	—	38.42±1.0	26.32±1.0	2.89±0.1
HC loss	—	72.77±2.0	82.09±2.0	54.98±1.0
Cellulose loss	—	34.32±1.0	18.49±1.0	2.11±0.33
Lignin loss	—	61.67±1.0	70.7±1.0	31.26±1.0

UWS, untreated wheat straw; TWS-PT1, 4, 5, treated wheat straw with *Pleurotus tuber-regium* strains. DM, dry matter; IVDMD, in vitro dry matter digestibility; NDF, neutral detergent fiber; ADF, acid detergent fiber; HC, hemicellulose.

untreated wheat straw (UWS) by using the rumen simulation technique (Rusitec).

## Materials and Methods

**Sample preparation.** Fungal cultures—*Pleurotus tuber-regium* (Fr.) Sing. (strains PT1 and PT4) isolated from Africa (Nigeria) and strain PT5 from Asia (New Caledonia) were used. These fungi were obtained from the Culture Collection of Basidiomycetes (CCBAS), Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague. For solid state fermentation (SSF) with 3 strains of basidiomycetes, the samples of moistened wheat straw (water content 80%) were placed in independent plastic bags. After sterilization, the straw was inoculated with homogenized mycelium of WRF. 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  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  (0.15%). Corn steep liquor was served as the nitrogen source. The fungi mycelial mats were collected by filtration, washed with sterile water, homogenized in 100 ml water, and used (5% v/w) for the inoculation of 300 g wheat straw. The wheat straw was sterilized before inoculation by steaming 2 times for 30 min at 100°C. The inoculated substrates were incubated at 28°C for 30 days. After the 30-day incubation period, substrates, UWS, and wheat straw treated with PT1, PT4, and PT5 (TWS-PT1, 4, 5) were dried at 60°C and ground through a 1 mm sieve before analyses.

**Chemical analyses.** The chemical composition of

UWS and fungi-decayed straws are shown in Table 1. 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 digestibility (IVDMD) was determined by the method (Mellenberger et al., 1970) of incubating the samples for 96 h with the rumen fluid taken from sheep (Slovak Merino) fed alfalfa hay. Losses of dry matter (DM) of fungi-decayed straws were estimated by weighing the dried substrate (2 days, 60°C) at the beginning and end of the solid state fermentation (SSF). Losses of components after fungal treatment (Table 1) were calculated as follows:

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

where DML is a percentage of dry matter lost, PF is a percentage of a component in final treated substrate, and PO is a percentage of a component in the original UWS (Tsang et al., 1987).

**In vitro fermentation system.** Fermentation of the substrates—UWS or treated wheat straw 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 by tongs. The solid digesta (80 g wet weight) was 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 830 to 860 ml. Including the first day of the experiment, the vessels were supplied at daily intervals with 12.0 g DM of UWS and 2.85 g DM of barley ( $V_1$ ); 11.96 g DM of TWS-PT1 and 2.85 g DM of barley ( $V_2$ ); 11.93 g DM of TWS-PT4 and 2.85 g DM of barley ( $V_3$ ); 12.08 g DM of TWS-PT5 and 2.85 g DM of barley ( $V_4$ ). The diets were placed in nylon bags, and each bag remained in the vessel for 2 days. To ensure that all diets contained 13% of crude protein (CP), 566.8 mg ( $V_1$ ), 523.4 mg ( $V_2$ ), 482.4 mg ( $V_3$ ), and 501.9 mg ( $V_4$ ) of urea were dissolved in 1 L of artificial saliva. The buffer was infused into the artificial rumen (Rusitec) using a peristaltic pump.

**Measurements.** The experiment in artificial rumen lasted for 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 to 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 ration in nylon bags) were analyzed for DM, ash, and crude protein contents by AOAC methods (AOAC, 1980). The residues were analyzed also for neutral detergent fiber (NDF), and acid detergent fiber (ADF) according to the method of Goering and Van Soest (1970). Hemicellulose was calculated as the difference between NDF and ADF. Cellulose was calculated as the difference between ADF and lignin. Liquid effluents were analyzed for volatile fatty acid (VFA) concentrations by the gas chromatography procedure (Cottyn and Boucque, 1968), using crotonic acid as the internal standard in gas chromatograph Perkin-Elmer 8500. Effluents were analyzed also for total nitrogen content by the Kjeldahl method and for ammonia nitrogen content by the microdiffusion method (Conway, 1962). The volumes of gas produced were measured with a gas meter, and methane was analyzed by gas chromatograph Perkin-Elmer 8500 as reported by Czerkawski and Clapperton (1968). To determine the microbial biomass in the effluent, 30 ml of preserved suspension was centrifuged at  $15,000 \times g$  for 30 min. The residue was washed twice with water and dried to constant weight at  $105^\circ\text{C}$ . The microbial biomass in the residues was determined by an improved technique, using the treatment with saline (pH 2), Tween 80, MeOH, and tertiary butanol (Whitehouse et al., 1994). The other parameters of fermentation—organic matter fermented (OMF) (Van Nevel and Demeyer, 1977)—production, utilization, and re-

covery of metabolic hydrogen (Demeyer, 1991) were calculated from the stoichiometry of rumen fermentation.

**Statistical analyses.** Means of results from treatments were compared with one-way analysis of variance (ANOVA). Treatment means were separated by using Duncan's multiple-range test at the 5% level of probability.

## Results and Discussion

After 30 days of solid-state fermentation, all strains of the fungus, *Pleurotus tuber-regium* (Fr.) Sing. demonstrated extensive growth on wheat straw. Hyphae growth and infiltration into the substrate mats were observed.

### *Alteration of chemical composition of fungal-decayed straw*

The chemical composition of fungal-decayed straw is reported in Table 1. Crude protein and ash contents were enhanced by treatment with all 3 strains, probably caused by increasing the fungal biomass in fungi-treated straw (Zadrazil et al., 1995).

The fungal treatment of wheat straw significantly increased IVDMD (Table 1). The increase of IVDMD by about 17 units was observed for TWS-PT1 and by 18 units for TWS-PT4. IVDMD of TWS-PT5 was similar to that of UWS. It is known that some species of fungi increase wheat straw IVDMD, whereas others decrease IVDMD. Fungal treatment of wheat straw with *P. eryngii*, *P. flabellatus*, and *P. ostreatus* increased IVDMD from 15 to 23%, 21%, and from 15 to 23%, respectively, and decreased IVDMD about 1% with *P. ostreatus* (Zadrazil et al., 1996).

### *Losses of DM and cell-wall constituents of fungal-decayed straw*

The value for DM loss ranged from 5 to 31%, the lowest loss of DM was in straw decayed by PT5, and the highest value was for straw decayed by PT1 (Table 1). Losses in DM by fungi during the 30 days of SSF were primarily due to consumption of carbohydrates, which were mostly associated with the cell wall. Therefore DM loss included the losses in hemicellulose, cellulose, and lignin. Hemicellulose and lignin were degraded mainly in the wheat straw decayed by PT1 and PT4. Cellulose was less degraded by these fungi. The strain PT5 degraded half the amount of total hemicellulose, a third the amount of lignin, and only 2% of cellulose in the wheat straw. The different losses of cellulose, hemicellulose, and lignin were probably caused by different enzymatic activities of these fungi.

Table 2. The effect of fungi-treated straw on DM, OM, detergent fiber degradabilities, and gas production in artificial rumen ( $n=6$ ).

Degradation of feed after 48 h (%)	Diets <sup>1</sup>				SEM
	UWS <sup>a</sup>	TWS-PT1 <sup>b</sup>	TWS-PT4 <sup>c</sup>	TWS-PT5 <sup>d</sup>	
DM	29.29 <sup>b</sup>	36.86 <sup>d</sup>	34.59 <sup>a</sup>	33.02 <sup>a</sup>	0.52
Organic matter	33.07 <sup>b</sup>	40.02 <sup>d</sup>	37.95 <sup>a</sup>	34.98 <sup>a</sup>	0.61
NDF	21.80	21.03 <sup>c</sup>	15.69 <sup>a</sup>	19.81 <sup>c</sup>	0.61
ADF	17.61	25.91 <sup>a</sup>	25.10 <sup>a</sup>	23.20 <sup>a</sup>	0.59
Hemicellulose	29.94	2.89 <sup>a,c</sup>	6.89 <sup>a</sup>	7.5 <sup>a,b</sup>	0.89
Cellulose	18.91	27.89 <sup>a</sup>	18.80 <sup>b</sup>	22.29 <sup>b</sup>	0.60
Lignin	12.02	11.40 <sup>c</sup>	34.81 <sup>a,d</sup>	28.31 <sup>a,b</sup>	0.69
Total gas production (L day <sup>-1</sup> )	2.65	2.82 <sup>c</sup>	2.51 <sup>d</sup>	2.81	0.06
Methane production (mmol day <sup>-1</sup> )	6.81	5.38	5.43	5.39	0.02
Mol methane kg <sup>-1</sup> digested DM	1.14	0.98	0.86	1.07	0.02

<sup>1</sup> All diets contained substrate and barley (80% : 20%). Substrate were UWS, untreated wheat straw; TWS-PT1, 4, 5, treated wheat straw with *Pleurotus tuber-regium* strains.

Values in a row with different superscript letters (a, b, c, d) differ at  $p<0.05$ .

Table 3. The effect of fungi-treated straw on VFA production, pH, and ammonia-nitrogen production in artificial rumen ( $n=6$ ).

	Diets <sup>1</sup>				SEM
	UWS <sup>a</sup>	TWS-PT1 <sup>b</sup>	TWS-PT4 <sup>c</sup>	TWS-PT5 <sup>d</sup>	
Total VFA production (mmol day <sup>-1</sup> )	29.55	29.34 <sup>d</sup>	27.77	26.56 <sup>a</sup>	0.59
Mol VFA kg <sup>-1</sup> digested DM	6.81	5.38	5.43	5.39	0.09
Mol% acetic acid	56.07	55.47	52.21	54.05	0.49
Mol% propionic acid	24.87	27.43	27.47	24.57	0.46
Mol% butyric acid	11.31 <sup>b,d</sup>	9.91 <sup>c</sup>	11.11	12.16 <sup>b</sup>	0.24
Mol% isobutyric acid	0.99	0.69	0.83	0.80	0.03
Mol% <i>n</i> -valeric acid	4.50 <sup>c,d</sup>	5.02 <sup>c</sup>	6.43	6.04 <sup>b</sup>	0.13
Mol% isovaleric acid	0.81	0.43	0.65	0.52	0.06
NH <sub>3</sub> -N in effluent (mg L <sup>-1</sup> )	252.07 <sup>b,c,d</sup>	212.73	214.77	212.73	7.12
pH	7.03 <sup>b,c,d</sup>	6.94	6.97	6.96	0.01

<sup>1</sup> All diets contained substrate and barley (80% : 20%). Substrate were UWS, untreated wheat straw; TWS-PT1, 4, 5, treated wheat straw with *Pleurotus tuber-regium* strains.

Values in a row with different superscript letters (a, b, c, d) differ at  $p<0.05$ .

#### Digestibilities of DM, organic matter, and fiber constituents of diets

The DM and organic matter digestibilities of experimental diets were significantly higher in comparison to control diet during fermentation in semicontinuous fermenter. The apparent digestibility of DM and organic matter for the TWS-PT1, TWS-PT4, and TWS-PT5 diets was increased by 25.8 and 21%; 18.1 and 14.7%; 12.7 and 5.7%, respectively, in comparison with the UWS diet (Table 2). Digestibilities for fiber and fiber constituents are reported as a percentage of initial quantities fed to the fermenter, as well as actual quantities digested. It is evident that NDF digestibility was slightly reduced (significantly in the TWS-PT4 diet), and ADF digestibility was significantly increased during the fermentation of experimental diets. This different effect of detergent fiber constituents was

caused by very low digestibility of hemicellulose. Hemicellulose digestibility was decreased about 90, 77, and 75% in TWS-PT1, TWS-PT4, and TWS-PT5 diets, respectively, in comparison with the control diet. Cellulose and lignin digestibilities were increased during fermentation of the experimental diets.

#### Fermentation characteristics

The digestion of all diets was carried out at pH 6.94–7.03 (Table 3). The fermentation of experimental diets was characterized by a significant drop in pH. These results suggest that the pH may be influenced by lignin degradation products in fungi-treated straw (Agosin and Odier, 1985). The significant decrease in the pool of NH<sub>3</sub>-N effluent in the experimental diets can be explained by a decreased degradation of feed nitrogen in the diets containing fungi-treated straw.

Table 4. The effect of fungi-treated straw on stoichiometric parameters of rumen fermentation and microbial matter production in artificial rumen ( $n=6$ ).

	Diets <sup>1</sup>				SEM
	UWS <sup>a</sup>	TWS-PT1 <sup>b</sup>	TWS-PT4 <sup>c</sup>	TWS-PT5 <sup>d</sup>	
Organic matter fermented (g day <sup>-1</sup> )	2.80	2.75	2.67	2.62	0.45
Production of H <sub>2</sub> (mmol day <sup>-1</sup> )	56.95	55.45	52.89	52.52	1.01
Utilization of H <sub>2</sub> (mmol day <sup>-1</sup> )	42.47	45.20	40.81	43.48	1.65
Recovery of H <sub>2</sub> (%)	74.33 <sup>d</sup>	81.44	77.19	84.28	2.32
Total microbial matter (g day <sup>-1</sup> )	0.51	0.48	0.45	0.42	0.02

<sup>1</sup> All diets contained substrate and barley (80%:20%). Substrate were UWS, untreated wheat straw; TWS-PT1, 4, 5, treated wheat straw with *Pleurotus tuber-regium* strains.

Values in a row with different superscript letters (a, b, c, d) differ at  $p<0.05$ .

This was confirmed by the lower utilization rate for nitrogen (N) found in the experimental diets. The N utilization (%) was calculated from N content of feed+N of urea=input and N of effluent+N of undigested feed=output. The N utilization was calculated as a ratio output/input (mg day<sup>-1</sup>) $\times$ 100. The values were lower in the experimental diets ( $V_2$  93.8,  $V_3$  93.4,  $V_4$  82.7%), in comparison with control  $V_1$  (97.6%).

In studies of rumen fermentation, the production of gases as methane and output of VFA are used as indices of substrate breakdown in the rumen (Asiegbu et al., 1995). Gas production as the result of fermentation of carbohydrates was not influenced in the fermentation of experimental diets. Also, methane production (mmol day<sup>-1</sup>) and methane production expressed as mol kg<sup>-1</sup> digested DM of diet were not changed (Table 2). Total VFA production (mmol day<sup>-1</sup>) and VFA production expressed as mol kg<sup>-1</sup> digested DM were characterized by similar values of all fermented diets (Table 3). The proportions of individual VFAs on total VFA production were expressed as mol%. Molar proportions of acetate, propionate, and isoacids—isovalerate and isovalerate were unchanged during the fermentation of experimental diets and were comparable to the control diet. The differences were in mol% *n*-valerate and butyrate only (Table 3). It is possible to calculate the fermentation products from the stoichiometry of rumen fermentation (Table 4). The parameters of fermentation—OMF (g day<sup>-1</sup>), production, utilization, and recovery of metabolic hydrogen were not altered during fermentation of experimental diets. Only a higher recovery of H<sub>2</sub> was observed during fermentation of TWS-PT5 diet (Table 4). The microbial matter production was similar in all diets, and slightly lower values were observed in experimental diets.

## Conclusions

The strains of the fungus *Pleurotus tuber-regium*

(Fr.) Sing.—PT1, PT4, and PT5 preferentially degraded hemicellulose and lignin over cellulose during the colonization of wheat straw. PT1 and PT4 significantly increased IVDMD. The growth of fungi was accompanied by the DM loss—31.5% for PT1, 20.9% for PT4, and 4.8% for PT5, respectively. The fermentation characteristics during in vitro experiment in Rusitec showed that the treatment of wheat straws with the strains PT1, PT4, and PT5 did not improve the nutritional values that were comparable to untreated wheat straws.

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## References

- Agosin, E. and Odier, E. (1985) Solid-state fermentation, lignin degradation and resulting digestibility of wheat straw fermented by selected white-rot fungi. *Appl. Microbiol. Biotechnol.*, **21**, 397–403.
- AOAC (1980) Official Methods of Analysis, ed. by Horowitz, W., 13th ed., Association of Official Analytical Chemists, Washington D.C.
- Asiegbu, O. F., Morrison, I. A., Paterson, A., and Smith, J. E. (1995) Effects of cell-wall phenolics and fungal metabolites on methane and acetate production under in vitro rumen conditions. *J. Gen. Appl. Microbiol.*, **41**, 475–485.
- Conway, E. J. (1962) Microdiffusion Analysis and Volumetric Error, 5th ed., Crosby Lockwood, London, UK, 322 pp.
- Cottyn, B. G. and Boucque, C. V. (1968) Rapid method for the gas chromatographic determination of volatile fatty acids in rumen fluid. *J. Agric. Food Chem.*, **16**, 105–107.
- Czerkawski, J. W. and Breckenridge, G. (1977) Design and development of a long-term rumen simulation technique (Rusitec). *Br. J. Nutr.*, **38**, 371–384.
- Czerkawski, J. W. and Clapperton, J. L. (1968) Analysis of gas produced metabolism of microorganisms. *Lab. Pract.*, **17**, 994–996.
- Demeyer, D. I. (1991) Quantitative aspects of microbial metabolism in the rumen and hindgut. In *Rumen Microbial Metabolism and Ruminant Digestion*, ed. by Jouany, J. P., INRA Editions, Paris, pp. 217–237.
- Fahey, G. C., Jr., Bourquin, L. D., Titgemeyer, E. C., and Atwell, D. G. (1993) Postharvest treatment of fibrous feedstuffs to improve their nutritive value. In *Forage Cell Wall Structure and*

- Digestibility, ed. by Jung, H. G., Buxton, D. R., Hatfield, R. D., and Ralph, J., Proc. Symp., Madison, WI, pp. 715–766.
- Goering, H. K. and Van Soest, P. J. (1970) Forage fiber analyses. Agriculture Handbook No. 379, USDA, Washington D.C., pp. 1–20.
- Martinez, A. T., Camarero, S., Guillen, F., Gutierrez, A., Munoz, C., Varela, E., Martinez, M. J., Barrasa, J. M., Ruel, K., and Pelayo, J. M. (1994) Progress in biopulping of non-woody materials—chemical, enzymatic and ultrastructural aspects of wheat straw delignification with lignolytic fungi from the genus *Pleurotus*. *FEMS Microbiol. Rev.*, **13**, 265–274.
- McDougall, E. J. (1948) Studies on rumen saliva. 1. The composition and output of sheep's saliva. *Biochem. J.*, **43**, 99–109.
- Mellenberger, R. W., Satter, L. D., Millett, M. A., and Baker, A. J. (1970) An in vitro technique for estimating digestibility of treated and untreated wood. *J. Anim. Sci.*, **30**, 1005–1011.
- Sundstol, F. and Owen, E. (1984) Straw and Other Fibrous By-Products as Feed, Elsevier Sci. Publ., Amsterdam, 604 pp.
- Tsang, L. J., Reid, I. D., and Coxworth, E. C. (1987) Delignification of wheat straw by *Pleurotus* spp. under mushroom-growing conditions. *Appl. Environ. Microbiol.*, **53**, 1304–1306.
- Van Nevel, C. J. and Demeyer, D. I. (1977) Determination of ruminal growth in vitro from  $^{32}\text{P}$  labelled phosphate incorporation. *Br. J. Nutr.*, **38**, 101–114.
- Whitehouse, N. L., Olson, V. M., Schwab, C. G., Chesbro, W. R., Cunningham, K. D., and Lykos, T. (1994) Improved techniques for dissociating particle-associated mixed ruminal microorganisms from ruminal digesta solids. *J. Anim. Sci.*, **72**, 1335–1343.
- Zadrazil, F. (1984) Microbial conversion of ligno-cellulose into feed. In *Straw and Other Fibrous By-Products as Feed*, ed. by Sundstol, F. and Owen, E., Elsevier Sci. Publ., Amsterdam, pp. 276–292.
- Zadrazil, F. (1985) Screening of fungi for lignin decomposition and conversion of straw into feed. *Angew. Bot.*, **58**, 433–452.
- Zadrazil, F., Kamra, D. N., Isikhuemhen, O. S., Schuchardt, F., and Flachowsky, G. (1996) Bioconversion of lignocellulose into ruminant feed with white-rot fungi—review of work done at FAL, Braunschweig. *J. Appl. Anim. Res.*, **10**, 105–124.
- Zadrazil, F., Punyia, A. K., and Singh, K. (1995) Biological upgrading of feed and feed components. In *Biotechnology in Animal Feeds and Animal Feeding*, ed. by Wallace, R. J. and Chesson, A., Weinheim, New York, Basel, Cambridge, Tokyo, pp. 55–70.