ENHANCEMENT IN *IN VITRO* DIGESTIBILITY OF WHEAT STRAW OBTAINED FROM DIFFERENT GEOGRAPHICAL REGIONS DURING SOLID STATE FERMENTATION BY WHITE ROT FUNGI

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The study was carried out to find out the differences in the chemical composition of wheat straw obtained from three different regions of India, to compare their susceptibility to fungal degradation, and subsequently to evaluate the correlation between lignin loss and improvement in *in vitro* digestibility. Four *Phlebia* species were used to degrade different wheat straw samples during 30 days of incubation. In wheat straw obtained from central zone of India, most of the fungi were more selective in ligninolysis, with a moderate loss in total organic matter. The best found fungus, *P. brevispora*, enhanced the *in vitro* digestibility from 172 to 287 g/kg in north western, 165 to 275 g/kg in north eastern, and 145 to 259 g/kg in central zone with a respective loss of 163, 129, and 105 g/kg in total organic matter. Other three fungi *P. fascicularia*, *P. floridensis*, and *P. radiata* were also able to enhance the *in vitro* digestibility of all the wheat straw samples up to a significant extent. The study demonstrated that selective ligninolytic behaviour of fungi is influenced by the overall composition of wheat straw as governed by geographic location.

Keywords: In vitro digestibility; Laccase; Ligninolysis; Phlebia species; Wheat straw constituents; White rot fungi

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INTRODUCTION

Availability of green fodder is a worldwide problem. To overcome problems related to the forage crop crisis, different residual straws after cereal crops harvesting are generally fed to animals directly or sometimes supplemented with green forage crops. Wheat (*Triticum aestivum*) is an important cereal crop, and India is its second largest producer in the world (Nagarajan, 2005). Agricultural fields of north western plains zone (NWPZ), north eastern plains zone (NEPZ), and the central zone (CZ) of India are the main contributors of wheat production, while relatively much less agricultural land is used for the cultivation of forage, which generally results in a shortage of forage crops. As compared to forage, wheat straw (WS) is of poor nutritive value and low digestibility to the ruminants due to its higher acid detergent fiber (ADF) content. Lignin is a main constituent of ADF, and is non digestible by ruminants and resistant to most of the microbial enzymatic systems as well. The presence of lignin and its hemicellulose binding matrix increases the unavailability of other energy-containing constituents present in the agricultural residues for the ruminants. Enhancement in the nutritive quality of WS by minimizing the lignin content may help solve this problem.

Biological removal of lignin has the potential to convert the straw into more nutritive and easily digestible feed of good quality (Bisaria 1997; Jalc et al. 1996). Among a variety of microorganisms, selective ligninolytic white rot fungi are the potentially useful organisms (Arora and Gill 2005; Jalc et al. 1998). Most studied white rot fungus *Phanerochaete chrysosporium* is a quite efficient lignin degrader, but it also degrades a huge amount of organic matter, which limits its practical use for animal feed (Jung et al. 1992). It necessitates the search for organisms that can degrade the lignin, but with lower loss of other important constituents such as hemicellulose and cellulose. *Phlebia* species have been reported to degrade lignin more selectively by leaving behind more organic matter available for ruminants (Arora and Sharma 2009). Degradation of lignin and other components are specific to fungal and plant species. Plant constituents may also vary with respect to the climate, season, soil quality, and temperature, etc., which are responsible for the difference in their quality and digestibility (Ford et al. 1979; Ouédraogo-Koné et al. 2008; Whalley et al. 2008). Environmental conditions may be crucial in governing the selectivity of fungal biodegradation of wood components (Tuor et al. 1995). In the presented study, the bioconversion of WS into more nutritive animal feed by four different *Phlebia* species have been evaluated against three different samples of WS, collected from different geographic locations of India.

EXPERIMENTAL

Substrate Collection

Wheat straw (WS) samples were collected after the crop harvesting in the month of April from three different geographic locations of India, i.e. north western plains zone (NWPZ) Amritsar (31°38' N 74°52' E) Punjab, north eastern plains zone (NEPZ) Gorakhpur (26°48' N 83°23' E) Uttar Pradesh, and central zone (CZ) Chhindwara (22°4' N 78°58' E) Madhya Pradesh.

Organisms

Four white rot fungi *Phlebia brevispora* (HHB-7030), *Phlebia fascicularia* (FP-70880), *Phlebia floridensis* (HHB- 5325), and *Phlebia radiata* (MJL-1198), were used in the present study. All fungi were procured from T. W. Jeffries (Forest Product Laboratories, Madison, USA). The cultures were maintained by regular subculturing on yeast extract glucose agar (YGA) slants and stored at 4 ºC as well as at -80 ºC in 10 % (v/v) glycerol.

Experimental Setup

WS obtained from different regions was ground (particle size 2 mm ± 0.5), washed, and dried at 90 ºC. Five g of dried sample was taken in 250 ml conical flasks and moistened with 25 ml of malt extract (0.5 %, w/v). The flasks containing substrate were sterilized at 1.6 kg/cm^2 for 15 min and inoculated with three mycelial discs (7-8 mm) per flask, grown on YGA plates for 5-6 days. The inoculated flasks were incubated at 25 ºC. Triplicate sets of flasks for each organism were processed at 10, 20, and 30 days along with an uninoculated control. Enzyme extraction was done as described earlier (Arora et

al. 2002). The contents of each flask were filtered on a tared filter paper and dried at 90 ºC until constant weight. Loss in total organic matter (TOM) was calculated from the difference between the control and inoculated flasks. Dried residue was used for further analytical tests.

Laccase Assay

Laccase (EC 1.10.3.2) activity was measured according to previously described method (Arora et al. 2002). Five ml reaction mixture containing 3.8 ml sodium acetate buffer (13.1 mM, pH 5.0), 1 ml guaicol (2 mM), and 0.2 ml of enzyme extract were incubated for 2 h at 25 ºC, and the absorbance was read at 450 nm and expressed in colorimetric units / ml (CU / ml).

Analytical Methods

A sequential fractionation was carried out with a slightly modified method of Datta (1981), as described earlier (Arora and Sharma 2009), for the estimation of various components of WS. One g of WS was suspended in 100 ml distilled water, kept at 100 °C for 2 h in a water bath, and filtered on a tared crucible. Residue was dried at 90° C to constant weight, and the loss was considered as water soluble part. Dried residue was suspended in 100 ml of 0.5 M H_2SO_4 and kept at 100 °C for 2 h in a water bath; then the contents were filtered, dried, and weighed as described in the first step, and the loss was represented as hemicellulose content. For cellulose and lignin, the dried residue was mixed with 10 ml of 72 % H_2SO_4 and kept at 30 °C for 1 h on a rotary shaker at 200 rpm. The contents were diluted up to 4 % concentration of H_2SO_4 and autoclaved at 1.6 kg/cm^2 for 40 min. The contents were then filtered, dried and weighed according to first step, and the loss was considered as cellulose while residue was considered as lignin.

In Vitro Digestibility

In vitro digestibility of uninoculated and fungal treated WS was estimated as described earlier (Arora and Sharma 2009). Fecal inoculum was prepared by mixing fresh fecal matter of cow in pre warmed (39 ºC) artificial saliva (100 g/l) and filtered through four layered muslin cloth. Five hundred mg WS were taken in a 50 ml centrifuge tube and suspended in 35 ml of fecal inoculum. After flushing with CO_2 gas, these tubes were kept at 39 ºC for 48 h in a water bath. Supernatant was discarded and 35 ml of acidified pepsin was added to the residue. Tubes were again incubated at the same conditions for 48 h and then residue was filtered on a tared filter paper and dried. The weight loss in dry matter during the processing has been expressed as *in vitro* digestibility.

Statistical Analysis

Data were represented as mean values, along with standard deviations. Correlation between different parameters was established by calculating Pearson's correlation coefficient.

RESULTS

Wheat straw obtained from three different geographic regions showed certain variations in their chemical compositions (Table 1).

Table 1. Chemical Composition and Digestibility of Wheat Straw Collected from Different Indian Geographic Locations (g/kg dry weight basis)

	NWPZ	NEPZ	CZ
Water solubles	100 ± 06	070 ± 05	090 ± 05
Hemicellulose	330 ± 12	310 ± 10	350 ± 12
Cellulose	370 ± 13	410 ± 14	320 ± 11
Lignin	205 ± 09	210 ± 09	240 ± 10
Digestibility	172 ± 04	165 ± 04	145 ± 03

Each value represents the mean of three individual samples along with their standard deviation.

Wheat straw obtained from north western plains zone was lowest in lignin content and showed maximum digestibility as compared to straw collected from central zone having the highest lignin content and lowest digestibility, which showed a strong negative correlation (correlation coefficient r = -0.993) (Fig. 1). Solid-state fermentation of wheat straw obtained from different regions was performed with four different white-rot fungi viz. *P. brevispora, P. fascicularia, P. floridensis* and *P. radiata*. All the tested fungi were able to grow on WS under the experimental conditions.

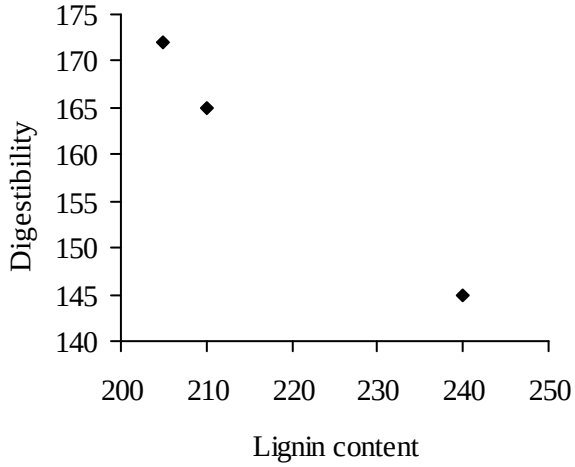

Figure 1. Correlation between *in vitro* digestibility and lignin content (g/kg) (r = -0.993)

Table 2. Changes in Wheat Straw Composition during 30 days of Solid State Fermentation with four White rot fungi. (g/kg dry weight basis)

Wheat straw	Fungal species/ Days of Incubation											
	P. brevispora			*P. fascicularia*			*P. floridensis*			*P. radiata*		
	10	20	30	10	20	30	10	20	30	10	20	30
NWPZ												
TOM loss	028±04	087±04	163±07	058±04	102±05	167±08	044±04	104±06	163±08	065±04	083±05	108±07
Water solubles	150±05	126±05	220±08	124±06	112±05	150±05	138±06	162±06	167±04	124±04	090±05	152±07
Hemicellulose loss	090±04	158±05	291±07	088±04	175±06	294±09	067±06	124±04	171±06	048±05	151±06	192±08
Cellulose loss	072±03	095±04	169±06	096±04	102±05	222±07	046±03	133±05	226±08	056±03	140±06	263±08
Lignin loss	060±05	190±07	306±10	083±04	170±07	231±06	126±05	238±07	275±07	096±03	167±-05	279±06
Digestibility	190±03	224±05	287±04	186±03	233±04	284±06	221±04	253±03	269±05	181±03	216±03	245±05
NEPZ												
TOM loss	061±06	106±05	129±05	025±03	063±05	123±06	059±05	107±07	168±07	073±04	175±08	242±08
Water solubles	078±06	107±06	125±07	070±05	067±05	088±04	081±06	102±04	125±05	098±04	090±05	112±07
Hemicellulose loss	080±05	210±08	276±09	016±02	081±03	212±07	035±03	268±08	353±12	129±06	248±07	310±10
Cellulose loss	043±04	119±05	194±05	022±03	105±03	164±07	130±04	106±03	166±07	084±04	183±07	299±09
Lignin loss	120±03	219±04	322±10	090±04	162±04	209±07	111±05	198±08	361±13	135±07	253±08	332±12
Digestibility	214±03	259±04	275±05	175±04	192±03	248±05	220±06	252±03	278±06	179±04	226±04	285±05
CZ												
TOM loss	033±04	061±04	105±04	015±02	065±04	108±06	024±03	087±05	102±04	038±02	081±04	159±07
Water solubles	082±06	080±05	120±06	085±04	072±04	098±06	077±03	100±05	152±07	097±04	068±03	094±03
Hemicellulose loss	061±04	077±04	118±05	041±02	092±03	189±05	093±04	113±05	128±07	113±05	133±07	150±08
Cellulose loss	025±02	122±04	190±08	009±02	113±06	263±06	015±02	094±05	134±03	038±04	161±06	173±08
Lignin loss	130±06	249±06	305±08	131±05	179±04	221±07	107±06	263±05	303±08	170±05	253±08	289±09
Digestibility	162±03	220±03	259±05	145±03	192±04	231±04	158±05	210±04	250±04	148±03	206±05	232±04

Each value represents mean of six individual samples along with their standard deviation; TOM - total organic matter

Solid State Fermentation of Wheat Straw Collected from North Western Plains Zone (NWPZ)

Maximum TOM loss of 167 g/kg was caused by *P. fascicularia* during 30 days of incubation, which was closely followed by *P. brevispora* and *P. floridensis*, while *P. radiata* caused minimum loss of 108 g/kg (Table 2). *P. radiata* was highly efficient to cause maximum loss in TOM during the first 10 days, in comparison to other fungi, which were more effective after 10 days. Maximum water-soluble components (220 g/kg) were liberated during 30 days from WS inoculated with *P. brevispora*, which was followed by *P. floridensis* (167 and 162 g/kg in 30 and 20 days, respectively). *P. fascicularia* liberated 150 g/kg water soluble during 30 days, which was insignificant with *P. radiata* (152 g/kg, 30 days) and *P. brevispora* (150 g/kg, 10 days). Minimum water soluble components were liberated by different fungi on the 20th day, except for *P. floridensis*. Maximum hemicellulose was degraded by *P. fascicularia* (294 g/kg), closely followed by *P. brevispora* (291 g/kg), whereas *P. floridensis* degraded only 171 g/kg during 30 days of incubation. Comparatively, *P. radiata* was less selective initially, and it degraded only 48 g/kg hemicellulose, which increased up to 192 g/kg during further 20 days. Cellulose degradation was maximum in *P. radiata* (263 g/kg), followed by *P. floridensis* and *P. fascicularia*I, with a respective insignificant (P>0.05) loss of 226 and 222 g/kg, whereas *P. brevispora* degraded least cellulose (169 g/kg) during 30 days of incubation. All fungi, except *P. fascicularia* degraded more lignin than other components. Lignin degradation was more selective in *P. brevispora* (306 g/kg), followed by *P. radiata* (279 g/kg) and *P. floridensis* (275 g/kg). During first 20 days of incubation *P. floridensis* attacked lignin selectively and caused a loss of 238 g/kg, which was insignificant (P>0.05) in comparison to the loss caused by *P. fascicularia* (231 g/kg) during 30 days. Maximum enhancement in *in vitro* digestibility was obtained in the WS inoculated with *P. brevispora* (287 g/kg) and *P. fascicularia* (284 g/kg), followed by *P. floridensis* 269 g/kg, while in *P. radiata* it was minimum (245 g/kg). Laccase production was maximum in *P. floridensis* on 10th day, followed by *P. brevispora*, while in *P. fascicularia* it was least on 10th day and increased gradually, whereas *P. radiata* showed lower laccase activity (Fig. 2 a).

Solid State Fermentation of Wheat Straw Collected from North Eastern Plains Zone (NEPZ)

During 30 days of incubation, maximum loss in TOM was caused by *P. radiata* (242 g/kg), followed by *P. floridensis* (168 g/kg), *P. brevispora* (129 g/kg), and *P. fascicularia* (123 g/kg) (Table 2). Maximum water-soluble contents (125 g/kg) were liberated by *P. brevispora* and *P. floridensis*, followed by *P. radiata* (112 g/kg) and *P. fascicularia* (88 g/kg) during 30 days of incubation. Maximum hemicellulose was degraded by *P. floridensis* (353 g/kg), followed by *P. radiata* (310 g/kg), *P. brevispora* (276 g/kg), and *P. fascicularia* (212 g/kg) during 30 days. Cellulose was best degraded by *P. radiata* (299 g/kg), which was followed by *P. brevispora* (194 g/kg), while *P. floridensis* and *P. fascicularia* degraded 166 and 164 g/kg cellulose, respectively. Maximum lignin was degraded by *P. floridensis* (361 g/kg), followed by *P. radiata* (332 g/kg), *P. brevispora* (322 g/kg) and *P. fascicularia* (209 g/kg). Maximum *in vitro* digestibility of WS was enhanced by *P. radiata*, *P. floridensis* and *P. brevispora* ranging

from 285 to 275 g/kg, followed by *P. fascicularia* (248 g/kg). Maximum laccase was produced by *P. floridensis* on 10th day of incubation, similarly followed by *P. radiata* and *P. brevispora* with subsequent decline while it increased gradually up to 30 days in case of *P. fascicularia* (Fig. 2 b).

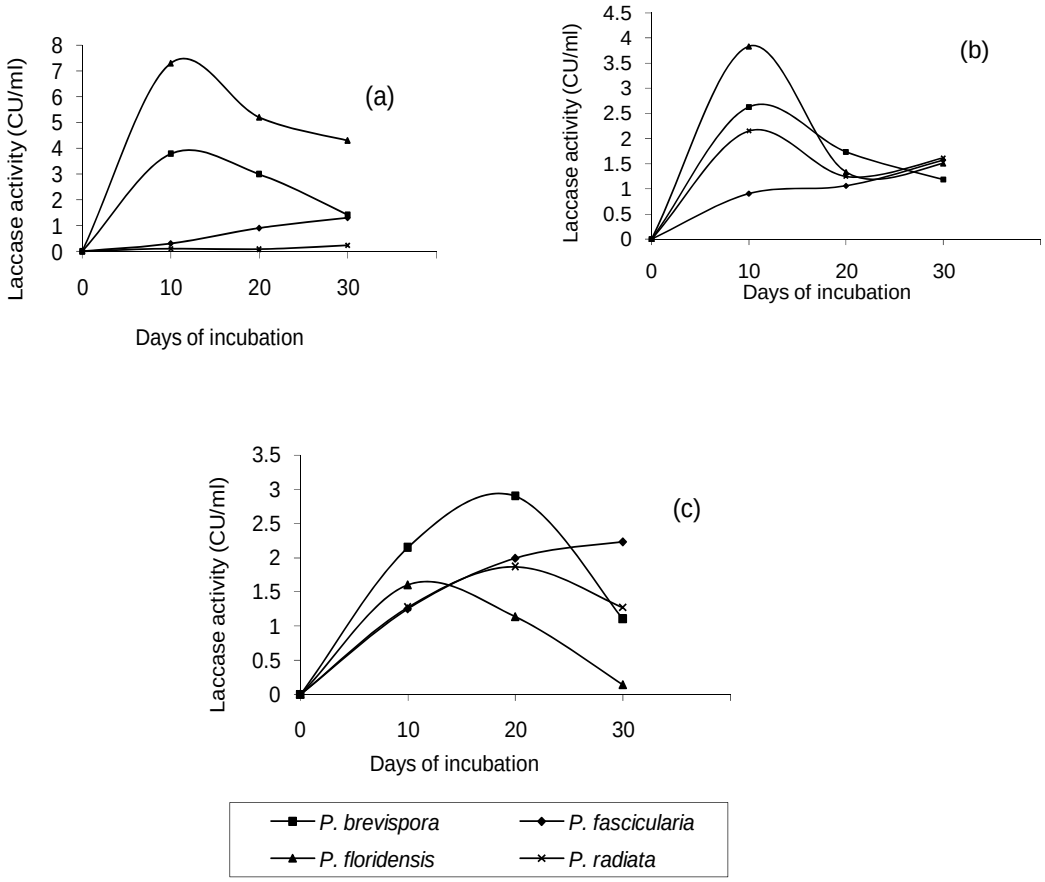

Figure 2. Laccase production by different white rot fungi spp. during solid state fermentation of wheat straw obtained from (a) North Western Plains Zone, (b) North Eastern Plains Zone, and (c) Central Zone

Solid State Fermentation of Wheat Straw Collected from Central Zone (CZ)

Maximum TOM loss was caused by *P. radiata* (159 g/kg), while the other three fungi spp. caused an insignificant (P>0.05) loss of 102 – 108 g/kg during 30 days of incubation (Table 2). Maximum water soluble components were liberated by *P. floridensis* (152 g/kg), followed by *P. brevispora* (120 g/kg) and *P. fascicularia* (98 g/kg) during 30 days, whereas *P. radiata* liberated only 97 g/kg of water solubles during the first 10 days. A maximum amount of hemicellulose (189 g/kg) was degraded by *P. fascicularia*, followed by *P. radiata* (150 g/kg), *P. floridensis* (128 g/kg), and *P. brevispora* (118 g/kg). Cellulose degradation was also maximum in WS inoculated with *P. fascicularia* (263 g/kg), followed by *P. brevispora* (190 g/kg), *P. radiata* (173 g/kg), and *P. floridensis* (134 g/kg) during 30 days. A maximum amount of 305 and 303 g/kg

lignin was degraded by *P. brevispora* and *P. floridensis*, which was followed by *P. radiata* (289 g/kg) and *P. fascicularia* (221 g/kg). Similarly, *in vitro* digestibility was maximum in WS inoculated with *P. brevispora* (259 g/kg) and *P. floridensis* (250 g/kg). *P. radiata* and *P. fascicularia* enhanced the digestibility up to 232 and 231 g/kg, respectively. *P. brevispora* gave maximum laccase production during 20 days followed by *P. radiata*. On the other hand, *P. floridensis* produced maximum laccase on 10th day while in *P. fascicularia* the activity increased gradually up to 30 days (Fig. 2 c).

DISCUSSION

The composition of plant biomass governs their susceptibility to microbial degradation. Holocellulose consists of a large amount of digestible fibers, while lignin is an indigestible biopolymer, which also binds to holocellulose and makes it inaccessible for the ruminants. The ratio of these plant cell wall constituents may vary depending upon the various environmental factors (Cherney et al. 2003; Whalley et al. 2008).

Lignin is the most abundant heterogenous natural biopolymer after cellulose, and it is comprised of three main phenolic propanoid units. The ratio of these monomer alcohols vary in lignin of different origin and affect the degradability of plant cell wall (Grabber 2005). It has been proposed that highly branched lignin is more inhibitory to cell wall degradability (Jung and Deetz 1993). Thus, lignin composition may govern susceptibility of agro residues to fungal attack.

In the present study, WS was collected from climatologically three different regions NWPZ, semi arid; NEPZ, humid subtropical; and CZ, tropical wet and dry. The climate difference may affect the crop production as well as the constitutional quality. Variation in the WS constituents may also be due to the variations in several other factors, e. g. invariably wheat is sown at the same time in the month of November, during which there is a significant variation in the temperature of these regions. CZ is warm (35 ºC - 15 ºC) as compared to NEPZ (20 ºC - 0 ºC), which may be a reason for its high lignin content and lower digestibility. A similar correlation between temperature, lignin content, leaf strength, and *in vitro* digestibility was reported earlier during the study on grass (*Phalaris aquatic*) leaves (Henry et al. 2000).

In several studies lignin loss enhanced the *in vitro* digestibility, and selective lignin degradation minimized TOM loss (Cohen et al. 2002). Selective ligninolysis goes well with choice of substrate. TOM loss was minimum in WS of CZ, as lignin was dominantly degraded over holocellulose, while in other WS samples hemicellulose and cellulose were also degraded to a reasonable extent. This may be either because of the higher content of lignin bound to other digestible fibers more efficiently, and that is why initially fungi had to attack lignin (Table 2) instead of those fibers, or higher lignin concentration might be inducing the lignin modifying enzymatic system of fungi, resulting in relatively selective ligninolysis. Degradation of lignin not only depends upon laccase, but also on variety of other enzymes too, such as manganese peroxidase, and lignin peroxidase (Arora et al. 2002; Arora and Gill 2005).

P. brevispora was the best organism and degraded more than 300 g/kg lignin in all the WS samples, along with good laccase production, which may be a reason for its

better ligninolytic ability. As a result of higher ligninolysis, the organism was also able to enhance the digestibility up to a significant level. There was a very strong positive correlation between enhancement in *in vitro* digestibility and lignin degradation (NWPZ r = 0.914, NEPZ r = 0.863 and CZ r = 0.919, Fig. 3). *P. brevispora* enhanced the *in vitro* digestibility from 172 to 287 g/kg in NWPZ, 165 to 275 g/kg in NEPZ, and 145 to 259 g/kg in CZ, as the net enhancement of 115 g/kg, 110 g/kg, and 114 g/kg respectively. In all these regions net enhancement in *in vitro* digestibility was approximately 11%, lignin loss 30 to 32%, while loss in TOM ranged from 10 to 16%. In the CZ, TOM loss was minimum, and in NWPZ it was maximum, with similar enhancement in digestibility. More selective lignin loss in the CZ minimized the TOM loss, thus making more holocellulose available for the ruminants.

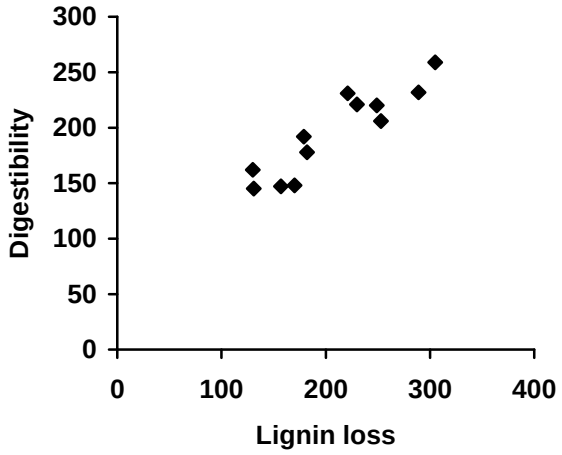

Figure 3 Correlation between *in vitro* digestibility and lignin loss (g/kg) using data obtained from CZ (r = 0.919)

The lignin degradation pattern of other fungi was more specifically governed by WS samples. *P. floridensis* degraded highest amount of lignin (361 g/kg) in WS of NEPZ, along with the higher laccase production, but the ligninolysis was substrate-specific. Though the loss in TOM was almost similar (163 g/kg), lignin degradation was much lower (275 g/kg) in NWPZ. Nevertheless, selective ligninolysis was maintained in all the WS samples. The regional effect was more pronounced in *P. radiata* during WS degradation, it showed a better laccase activity by using WS of NEPZ, and CZ resulted in higher lignin loss and enhanced maximum *in vitro* digestibility, while in NWPZ it was lower. Net enhancement in *in vitro* digestibility by this fungus ranged from 73 to 120 g/kg while for the remaining fungi it ranged from 83 to 115 g/kg. Similarly loss in TOM ranged from 108 to 242 g/kg while none of the fungi caused more than 170 g/kg loss in TOM of WS irrespective of the region.

No direct correlation could be established between the contents of water soluble and digestibility, as observed in earlier studies (Rolz et al. 1986). In most of the cases,

except for *P. floridensis*, the water-soluble components were at a minimum level on the 20th day, which increased during further incubation period, while the digestibility was minimum on the 10th day and increased steadily during 30 days of incubation. The correlation between water solubles and *in vitro* digestibility was not strong (r = 0.574). However, digestibility depends upon the availability of other polysaccharides as well as the structure of polymers (Rolz et al. 1986) that are modified by the fungal enzymatic treatment.

Correlation between hemicellulose loss and *in vitro* digestibility was found to be positive in the WS of NWPZ (r = 0.869), NEPZ (r = 0.860), and CZ (r = 0.625), respectively. The same findings regarding the relation between hemicellulose loss and *in vitro* digestibility were reported earlier in WS with two different white rot fungi (Agosin et al. 1985). During the degradation of wood and other lignocellulosic substrates hemicellulose-lignin matrix is primarily attacked by white rot fungi, as reported earlier (Martinz et al. 2005). All the fungi degraded relatively higher amounts of lignin and hemicellulose during the degradation of WS obtained from NEPZ. In the WS collected from the CZ, lignin and hemicellulose were degraded initially, but during the later period of incubation, cellulose degradation was more dominant than hemicellulose. In the WS obtained from NWPZ, *P. brevispora* degraded lignin and hemicellulose efficiently, while *P. fascicularia* degraded more hemicellulose than lignin. *P. floridensis* was again selective in lignin and hemicellulose degradation initially, but during the later period cellulose was more effectively degraded than hemicellulose and followed by *P. radiata*.

While looking at the ruminant digestibility of feed, it is important that the strategy chosen does not cause much loss in TOM available to the animal. In the present study, high holocellulose loss was found to be closely related with TOM loss; e.g. in the WS obtained from NEPZ, *P. radiata* degraded 24% TOM, which was the maximum loss caused by any fungus during the study. This was a result of non-selective degradation, which degraded hemicellulose, 31%; cellulose, 30% and lignin, 33%. Enhancement in the *in vitro* digestibility of WS by *P. radiata*, *P. brevispora*, and *P. floridensis* was statistically insignificant and ranged from 285 to 275 g/kg whereas *P. brevispora* caused approximately half loss in TOM (12%), and *P. floridensis* also degraded a lesser amount of TOM (16%).

In all the treatments TOM loss increased along with hemicellulose and cellulose degradation. In *P. brevispora* and *P. fascicularia* TOM loss was highest in NWPZ, followed by NEPZ and then CZ, while not much difference was observed in lignin degradation, but holocellulose has followed the pattern of TOM loss. Similar remarks regarding selective lignin loss and TOM loss were made earlier during the degradation study of oat straw and alfalfa stems with *Phanerochaete chrysosporium* (Jung et al. 1992).

The present study corroborates the earlier observations, where it has been demonstrated that the improvement in nutritional quality and digestibility of feed material is not only dependent up on the fungal strain used but also on the type of substrate employed for the purpose (Okano et al. 2006; Chalamcherla et al. 2009).

CONCLUSIONS

1. Biochemical constituents of wheat straw varied with respect to their climatologically different geographical locations. These biochemical differences may not only be attributed to climate, but also to strain variation.
2. The profile of fungal degradation of wheat straw and laccase production varied depending upon wheat straw constituents.
3. *P. brevispora* was the best organism to provide a practically promising approach in selective lignin degradation and enhancement of *in vitro* digestibility of wheat straw, irrespective of region.
4. The study thus provide an opportunity to exploit the situation further where more natural feeding conditions, i.e. moistening agent, particle size, and supplements, can be simulated in the studies.

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