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Nutritional evaluation of *Pleurotus florida* and *Pleurotus sajor-caju* harvested spent wheat-rice straw as livestock feed

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ABSTRACT

A study was undertaken to assess potential use of *Pleurotus florida* and *Pleurotus sajor- caju* harvested spent wheat-rice straw as livestock feed. Wheat or rice straw [wheat straw ($WS_{100}RS_0$), wheat-rice straw in 50:50 ($WS_{50}RS_{50}$), wheat-rice straw in 33:67 ($WS_{33}RS_{67}$), rice straw (WS_0RS_{100})] were used as substrate for the cultivation of these 2 fungi. The fibrolytic enzyme activity during spawning the substrate was highest in $WS_{50}RS_{50}$ and $WS_{33}RS_{67}$. The higher enzyme activity and mushroom yield in these 2 combinations were also responsible for high total ash and CP; low cell wall constituents in these combinations as compared to WS_{100} or RS_{100}. Irrespective of straw combinations, the *P. florida* as compared to *P.sajor-caju* harvested spent straw had low OM, ADL and cell wall constituents. The net gas production (NGP) and NDF digestibility, irrespective of *Pleurotus* species was highest and similar in spent WS_{100} and RS_{100} and lowest in spent WS_{33} RS_{67} straw combination. Irrespective of straw combinations, the *P. florida* harvested spent straws had highest net gas production, digestibility of NDF and OM as compared to *P. sajor-caju* harvested spent straw combinations. The effective degradability of DM and NDF in spent straws increased linearly with increase in level of rice straw in spent straw mixture. The rumen fill values of spent straws declined with increase in proportion of rice straw, predicting higher voluntary DM intake of spent straw containing higher proportion of rice straw. The values were highest in spent straw mixture containing 100% rice straw. Irrespective of straw proportion in different combinations, the digestion kinetic parameters for DM and NDF revealed that *P. florida* as compared to *P. sajor-caju* spent straw harvested spent straws, had higher degradable fractions (a and b), effective and true degradability and low undegradable fraction and low rumen fill values predicted higher voluntary DM intake. The *P. florida* harvested spent straw was effectively utilized in ruminant system as compared to *P. sajor caju*.

Key words: Fibrolytic enzymes, *In-sacco, In- vitro* degradability, *Pleurotus florida*, *Pleurotus sajor- caju,* Spent wheat-rice straw

In India, three edible mushrooms namely *Agaricus bisporus* – white button mushroom, *Pleurotus* spp. – oyster mushroom and *Volvariella volvaceae* – paddy straw mushroom are currently being cultivated on commercial scale. Out of which, *Agaricus bisporus* contributes 85% of total mushroom production and 15% was contributed by *Pleurotus* spp. and *Volvariella volvaceae*. Cow dung and sand/soil are used for casing during the cultivation of *Agaricus bisporus,* the spent straw is being used as soil conditioner. Reports from this lab revealed that *Agaricus bisporus* harvested spent wheat straw could be used as livestock feed only after washing (Bakshi and Langar 1985, 1991). As there is no need of casing in the cultivation of *Pleurotus* spp. and *V. volvaceae* the spent straw of these species could be used as livestock feed (Bakshi *et al*. 1985, Langar and Bakshi 1986). Till date wheat straw is preferred as substrate for the cultivation of *Pleurotus* spp. Keeping in view the surplus availability of rice straw in northern region of our country, where it is extensively burnt, an attempt was made to see if rice straw could be used exclusively or in combinations with wheat straw as substrate for the cultivation of *Pleurotus* spp. and the spent straw obtained after harvesting the edible mushroom was evaluated as livestock feed.

MATERIALS AND METHODS

Spawn preparation: Spawn was prepared by boiling wheat grains for 30 min and mixed with $CaCO_3$ and gypsum @ 4% and 2% (w/w), respectively. Sterilzed wheat grains were inoculated with either *Pleurotus florida* or *Pleurotus sajor-caju* in glass bottles and incubated at 25°C for 8–10 days. During incubation, the contents were shaken occasionally to

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prevent formation of lumps. The spawn was considered ready to inoculate the substrate when a thick growth of fungal mycelium was observed on the grains.

Substrate preparation: Wheat or rice straw as such or in combination [wheat straw ($WS_{100}RS_0$), wheat-rice straw in 50:50 ($WS_{50}RS_{50}$), wheat-rice straw in 33:67 ($WS_{33}RS_{67}$), rice straw (WS_0RS_{100})] were used as substrate for the cultivation of *P. florida* or *P. sajor-caju* species. Chopped wheat and rice straw (1.5–2.0 cm) in 500g (DM basis) batches in above combinations were moistened to 70% with water. Each batch of respective straw combination was inoculated with the spawn of either *P. florida* or *P.sajor-caju* species @ 5% on dry matter basis and packed into polyethylene bags having breathing slits (50–60; 5 mm slits/bag). The bags were incubated at 27°C till the appearance of fruiting bodies. Activities of various extra cellular fiber degrading enzymes in different compost mixtures were studied to ascertain growth behavior of 2 fungal species. Samples of colonized substrate were taken at different crop stages i.e. spawn run, pinning, first flush, second flush and spent straw following spawning.

In-vitro *gas production studies:* The nutritional value of WS-RS and spent WS-RS in different combinations of *P. florida* and *P. sajor-caju* species were evaluated by *in vitro* gas production technique (Menke *et al*. 1979, Menke and Steingass 1988). About 375 mg of the substrate was incubated at 39°C for 24h in triplicate in 100 ml calibrated glass syringes containing substrate and a buffered rumen fluid (Menke *et al*. 1979). Blank and sample of standard hay were run in triplicate with each set. Rumen contents were collected from 3 rumen fistulated buffalo calves, maintained on 2.0 kg conventional concentrate mixture (maize 25, mustard cake 10, de-oiled mustard cake 20, rice bran 15, de-oiled rice bran 27, mineral mixture 2 and common salt 1 part each) supplemented with 4.0 kg green fodder and 6.0 kg rice straw.

In-sacco *studies:* The digestion kinetic parameter for DM and NDF were assessed by *in sacco* technique (Mehrez and Ørskov 1977). Three male buffalo calves fitted with permanent rumen fistulae were selected and maintained on 2.0 kg concentrate mixture supplemented with 4.0 kg green fodder and 6.0 kg rice straw. The nylon bags (pore size of 50±10 μm) of 8 cm×17 cm stitched with monofilament polyester thread were used. Sample (3g) was placed in each nylon bag. The bags were incubated in the rumen for 3, 6, 9, 12, 24, 36, 48, 60 and 72h in triplicate. After the stipulated period, bags were taken out, washed under tap water until rinsing water from the bag became colourless and dried in a forced air oven maintained at 55°C for 48 h. The bags containing sample without incubation in the rumen were washed in the running tapwater to assess the loss at 0h. The residue was analyzed for DM and NDF. The rapidly soluble fraction (a), insoluble but potentially degradable fraction (b), degradation rate (c) and effective degradability (ED) were calculated according to McDonald (1981). The rumen fill was calculated according to Van Eys (1982). The DM intake was predicted by using the equation of Orskov *et al*. (1988).

Chemical and statistical analysis: About 50 g of fresh composite sample of colonized straw for *Pleurotus* spp. were immersed in 100 ml sterilized distilled water, agitated for 30 min, filtered through nylon gauze and the filtrate was centrifuged at 12 000 rpm for 15 min. The supernatant was used fresh for estimation of carboxymethyl cellulase (Mandels *et al*. 1976), cellobiase (Toyama and Ogawa 1977), filter paper activity (Mandels and Sternberg 1976) and xylanase activity and hemicellulases (Bucht and Erikson 1968). After cropping, the spent straw samples of different straw combinations were analysed for total ash and CP (AOAC 1995), cellulose (Crampton and Maynard 1938) and other cell wall constituents (Robertson and Van Soest 1981). The data were analyzed by 4 × 2 factorial design (Snedecor and Cochran 1968) by using SPSS (1996) version 12.0 and the means were tested for the significant difference by using Tukey's b.

RESULTS AND DISCUSSION

The highest yield of *P. florida* was observed in $WS_{33}RS_{67}$ followed by that in $WS_{50}RS_{50}$ combinations (Table 1), while the lowest yield was observed when wheat straw was exclusively used as substrate. *P. sajor-caju* did not fruit on any of the straw combinations, while on exclusive wheat or rice straw mushrooms appeared but the yield was much lower than that of *P. florida*.

As *P.sajor-caju* did not fruit on any of the combinations tested, therefore, the cellulase and hemicellulase activity was determined at different crop stages in different WS:RS mixtures during growth of *P. florida* only. Carboxy methyl cellulase (CMCase) and cellobiase activity increased rapidly from spawn run to pinning stage, showing a maxima at pinning approximately after 30 d of spawning, followed by progressive decline up to final harvest in all the combinations. The CMCase and cellobiase activity was highest in $WS_{50}RS_{50}$ almost comparable with $WS_{33}RS_{67}$ but considerably higher than WS_{100} and RS_{100} (Table 2). The cellobiase activity was lower than CMCase activity. In contrast to the CMCase and cellobiase activity, which was maximum near pinning and first flush, filter paper activity (FPase) was high even at initial stages of spawn run (15 d after spawning). At this stage FPase activity was comparable in all straw combinations except

Table 1. Yield (g/kg dry substrate) of *Pleurotus* spp. on different WS-RS combinations

Parameters	*Pleurotus florida*	*Pleurotus sajor-caju*
$WS_{100}RS_0$	420	292
$WS_{50}RS_{50}$	710	
$WS_{33}RS_{67}$	738	
WS_0RS_{100}	475	125

WS, Wheat straw; RS, rice straw.

Table 2. Enzyme activity (μmol/min/g compost) of *Pleurotus florida* on different WS-RS combinations

Parameters		$WS_{100}RS_0$	$WS_{50}RS_{50}$	$WS_{33}RS_{67}$	WS_0RS_{100}
CMCase	Pinning	0.418	0.594	0.583	0.431
	Spent straw	0.032	0.218	0.060	0.232
Cellobiase	Ist Flush	0.260	0.368	0.387	0.222
	Spent straw	0.282	0.132	0.148	0.201
FPase	Spawn run	0.332	0.331	0.362	0.272
	Spent straw	0.020	0.040	0.002	0.040
Xylanase	Spawn run	0.798	0.950	1.052	0.848
	Spent straw	0.242	0.040	0.120	0.020

WS, Wheat straw; RS, rice straw.

considerably low activity in RS_{100}. In all the combinations the FPase activity showed a progressive decline after completion of spawn run. Among different cellulases, CMCase activity was highest while that of FPase activity was lowest. The xyalnase activity of $WS_{50}RS_{50}$ and $WS_{33}RS_{67}$ combinations was highest at initial spawn run stages with a progressive decline thereafter. Higher xylanase activity by both the fungi over the cellulase activity probably explained their selective preference for hemicelluloses degradation. The activity of most of the fibrolytic enzymes was lowest in spent straws.

The chemical composition revealed that WS and RS as compared to spent WS-RS had low total ash, CP and ADL content; high OM and cell wall constituents (Table 3). The higher fibrolytic enzyme activity and mushroom yield in $WS_{50}RS_{50}$ and $WS_{33}RS_{67}$ combinations was responsible for high ($P<0.01$) total ash and CP; low ($P<0.01$) cell wall constituents in these straw combinations as compared to WS_{100} or RS_{100} (Table 3). Irrespective of straw combinations, the *Pleurotus florida* as compared to *Pleurotus sajor caju* harvested spent straw had low ($P<0.01$) OM, ADL and cell wall constituents.

Table 3. Chemical composition (%) of spent WS-RS irrespective of fungal species and effect of fungal species irrespective of straw combination

Parameters	Straw		Straw combinations				PSE	Fungal species		PSE
	Wheat	Rice	$WS_{100}RS_0$	$WS_{50}RS_{50}$	$WS_{33}RS_{67}$	WS_0RS_{100}		*Pleurotus florida*	*Pleurotus sajorcaju*	
Ash**	8.50	15.25	13.12^a	24.25^c	25.25^c	20.75^b	0.43	23.12^B	18.56^A	0.30
OM**	92.5	84.75	86.88^c	75.75^a	74.75^a	79.25^b	0.43	76.88^A	81.44^B	0.30
CP**	3.50	4.92	3.91^a	6.45^b	7.14^c	7.15^c	0.14	5.66^A	6.66^B	0.10
NDF**	82.0	76.0	71.50^b	63.50^a	63.0^a	62.0^a	0.74	58.50^A	71.50^B	0.52
ADF**	55.5	52.0	51.25^b	45.0^a	46.0^a	44.50^a	0.44	43.0^A	50.38^B	0.32
ADL**	7.50	5.50	7.11^a	7.75	8.50	8.25	0.32	5.88^A	9.93^B	0.23
Cellulose**	37.0	34.0	33.0^d	20.0^a	21.50^b	24.5^c	0.36	22.0^A	27.5^B	0.25
Hemicellulose*/**	26.5	24.0	20.25^b	18.50^{ab}	17.0^a	17.50^{ab}	0.65	15.50^A	21.12^B	0.46

WS, Wheat straw; RS, rice straw; Values with different superscripts$^{a,\ b}$ for different straw combinations and superscripts$^{A,\ B}$ for fungal species with in a row differ significantly *$P<0.05$; **$P<0.01$; PSE, pooled SE.

Table 4. Effect of combination of straws irrespective of fungal strain and effect of fungal strain irrespective of straw combination on *in-vitro* digestibility of spent straw

Parameters	Straw combinations				Pooled SE	Fungal species		Pooled SE
	$WS_{100}RS_0$	$WS_{50}RS_{50}$	$WS_{33}RS_{67}$	WS_0RS_{100}		*Pleurotus florida*	*Pleurotus sajorcaju*	
NGP	61.74^c	47.46^b	41.77^a	61.24^c	0.14	72.58^B	33.53^A	0.10
NDFD	17.96^c	17.57^b	16.86^a	21.58^d	0.10	22.34^B	14.61^A	0.07
TOMD	44.76^a	47.22^c	48.28^d	45.95^b	0.04	57.36^B	35.75^A	0.02

WS, Wheat straw; RS, rice straw; NGP, net gas production (ml/gDM/24h); NDFD, neutral detergent fibre digestibility;
TOMD, true organic matter digestibility; Values with different superscripts$^{a,\ b}$ for different straw combinations and superscripts$^{A,\ B}$ for fungal species with in a row differ significantly **$P<0.01$; PSE, pooled SE.

Table 5. Effect of combination of straws irrespective of fungal strain and effect of fungal strain irrespective of straw combination on digestion kinetic parameters for DM and NDF of spent straw

Parameters	Straw combinations				Pooled SE	Fungal species		Pooled SE
	$WS_{100}RS_{0}$	$WS_{50}RS_{50}$	$WS_{33}RS_{67}$	$WS_{0}RS_{100}$		*Pleurotus florida*	*Pleurotus sajor-caju*	
			Digestion kinetic parameters for DM, %					
a**	18.35^{a}	33.30^{d}	31.30^{c}	27.42_{b}	0.13	36.81^{B}	18.38^{A}	0.09
b**	32.80^{c}	22.40^{a}	28.21^{b}	39.85^{d}	0.12	33.35^{B}	28.28^{A}	0.08
c**, h^{-1}	0.038^{a}	0.036^{a}	0.047^{b}	0.046^{b}	0.002	0.044^{B*}	0.039^{A*}	0.002
UDF**	48.85^{d}	44.35^{c}	40.50^{b}	32.70^{a}	0.18	29.85^{A}	53.35^{B}	0.13
ED**	38.15^{a}	46.25^{b}	49.71^{c}	53.25^{d}	0.14	57.83^{B}	35.85^{A}	0.10
TD**	60.35^{b}	59.05^{a}	63.60^{c}	64.45^{d}	0.10	62.88^{B}	60.85^{A}	0.07
PED**	58.80^{b}	57.10^{a}	62.00^{c}	62.85^{d}	0.09	61.28^{B}	59.10^{A}	0.06
AED**	31.65^{a}	32.90^{b}	40.00^{c}	44.00^{d}	0.10	45.28^{B}	29.20^{A}	0.07
RF**	27.85^{d}	27.40^{c}	24.60^{b}	22.90^{a}	0.09	22.58	28.80	0.07
			Digestion kinetic parameters for NDF, %					
a**	7.34^{a}	22.10^{d}	21.25^{a}	15.72^{b}	0.17	19.85^{B}	13.36^{A}	0.12
b**	40.15^{c}	26.35^{a}	29.95^{b}	44.30^{d}	0.69	41.32^{B}	29.05^{A}	0.06
c*, h^{-1}	0.042^{c}	0.036^{a}	0.037^{ab}	0.041^{c}	0.001	0.041^{B}	0.037^{A}	0.001
UDF**	52.55^{b}	51.55^{c}	48.85^{b}	40.00^{a}	0.08	38.88^{A}	57.60^{B}	0.06
ED**	32.65^{a}	37.50^{b}	39.40^{c}	43.25^{d}	0.10	45.62^{B}	30.78^{A}	0.07
TD**	62.40^{c}	58.85^{a}	59.60^{b}	62.40^{c}	0.09	62.22^{B}	59.45^{A}	0.06
PED**	60.85^{c}	57.35^{a}	58.25^{b}	60.85^{c}	0.08	60.68	57.98	0.05
AED**	26.85^{a}	29.65^{b}	31.70^{c}	38.45^{d}	0.08	38.90^{B}	24.12^{A}	0.06
RF**	29.70^{d}	28.85^{c}	27.85^{b}	25.25^{a}	0.07	25.08^{A}	30.75^{B}	0.05

WS, Wheat straw; RS, rice straw; a, rapidly soluble fraction; b, insoluble but potentially degradable fraction; C, degradation rate; ED, effective degradability; UDF, undegradable fraction; TD, true degradability; PED, potential extent of digestion; AED, apparent extent of digestion;RF, rumen fill; Values with different superscripts$^{a,\ b}$ for different straw combinations and superscripts$^{A,\ B}$ for fungal species with in a row differ significantly $^{*}P<0.05$, $^{**}P<0.01$; PSE, pooled SE.

In-vitro *studies:* The net gas production (NGP) in spent straws irrespective of the *Pleurotus* species was highest ($P<0.01$) and similar in spent WS_{100} and RS_{100} and lowest ($P<0.05$) in spent WS_{33} RS_{67} straw combination (Table 4). The NDF digestibility was highest ($P<0.01$) in WS_{0} RS_{100} spent straw combination followed by WS_{100} RS_{0} and lowest in WS_{33} RS_{67} spent straw combination. However, the true OM digestibility was highest in WS_{33} RS_{67} spent straw combination. Irrespective of straw combinations, the *P. florida* harvested spent straws had highest ($P<0.01$) net gas production, digestibility of NDF and OM as compared to *P. sajor-caju* harvested spent straw combinations.

In-sacco *studies:* The digestion kinetic parameters for DM and NDF revealed that irrespective of *Pleurotus* species, the degradable fractions (a and b) in spent straws increased ($P<0.01$) linearly, while the undegradable fraction decreased ($P<0.01$) with the increase in level of rice straw in spent straw mixture (Table 5). The effective degradability of DM and NDF in spent straws increased linearly ($P<0.01$) with the increase in level of rice straw in the spent straw mixture. The rumen fill values of spent straws declined ($P<0.01$) with the increase in proportion of rice straw, predicting higher voluntary DM intake of spent straw containing higher proportion of rice straw. The values were highest ($P<0.01$) in spent straw mixture containing 100% rice straw. The studies indicated that spent rice straw could be effectively utilized for livestock feeding.

Irrespective of the straw proportion in different combinations, the digestion kinetic parameters for DM and NDF revealed that *P. florida* as compared to *P.sajor-caju* harvested spent straws had higher degradable fractions (a and b), effective and true degradability and low ($P<0.01$) undegradable fraction (Table 5). The low rumen fill values for DM and NDF in *P. florida* as compared to *P.sajor-caju* harvested spent straws predicted higher voluntary DM intake in the former than latter. Overall it was indicated that the *P. florida* as compared to *P. sajor-caju* harvested spent straw was effectively utilized in ruminant system.

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