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Chemical Composition, Ruminal Degradability and *in vitro* Gas Production of Wheat Straw Inoculated by *Pleurotus ostreatus* Mushrooms

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Abstract: Chopped and pressure-pasteurized wheat straw in the form of compost was seeded with mushroom mycelium. Chemical composition, ruminal disappearance and *in vitro* gas production were carried-out on the samples taken on day 0, 21, 42, 63 and 84 after seeding. NDF, ADF and Lignin content of the treated wheat straw reduced significantly with a rate of 0.20, 0.10 and 0.04% per day. Crude protein content increased significantly from 46.6 g kg^{-1} on day 0-50.90 g kg^{-1} on day 84. The a fraction for dry matter was highest ($p<0.05$) for the straw under the mushroom growing conditions (e.g., 0.188 g g^{-1} for the day 0 and 0.342 for the day 84). In contrast, the b value significantly ($p<0.05$) reduced from day 0-84 (0.487 and 0.336 g g^{-1}). The value for crude protein and NDF parameters increased linearly with increasing the growing period. Generally, the ruminal disappearance of different nutrients increased by growing the *Pleurotus ostreatus*, although, the c rate was independent. In case of gas production, mushroom growing led to a significant more gas production during the early hours (less than 24 h) of incubation. The b fraction of *in vitro* gas production as well as *in situ* results reduced significantly from day 0-84. It was concluded that straw seeding with *P. ostreatus* can be more effective for the longer period of growth.

Key words: *Pleurotus ostreatus* mushroom, wheat straw, ruminal degradability, gas production

INTRODUCTION

Mushroom production has been increased in Iran recently and the residual compost can be an environmental danger. Moreover, Iran faces a scarcity in the quantity and quality of consistent year-round supplies of conventional ruminant feeds. Therefore, better utilization of non-conventional feed resources, which do not compete as human food, could be a recommended strategy (Alipour and Rouzbehan, 2007).

Microbiological delignification is required to be a less energy consuming process where the amount of carbohydrate consumption by the organisms needs to be minimum in respect to delignification rate. This method has now become popular for improving the nutritive value of ruminant feed especially lignocellulosic by-products (Mukherjee and Nandi, 2004).

Pleurotus ostreatus is a typical variety of mushrooms from the wild species which is used by the people as a food source. It has been shown that straw in the form of compost can be degraded by enzymes from *P. ostreatus* growing during mushroom production can be more easily digested by ruminants. The aims of this research were to investigate the chemical composition changes and *in situ* ruminal degradability of wheat straw colonized by *P. ostreatus* under mushroom growing conditions.

MATERIALS AND METHODS

Preparation, sampling and chemical analysis: Wheat straw was chopped to 3-4 cm long pieces and soaked for 24 h in cold water pasteurized and inoculated with *P. ostreatus* mycelium at alevel of 2% w w^{-1} of the substrate fresh weight. The inoculated straw was packed into polyethylene bags of 3 kg. Mycelium incubation was done at room temperature of 24°C and a relative air humidity of 85%.

Straw samples were analyzed before mycelium inoculation (0 day) and on day 21, 42, 63 and 84 after inoculation. Samples were dried in a forced-air oven (56°C), ground to pass a 2 mm screen and analyzed for Dry Matter (DM), Organic Matter (OM), Neutral Detergent (NDF) and Acid Detergent (ADF), organic matter, Crude Protein (CP) and Lignin (AOAC, 2000).

***In vitro* gas production measurements:** Cumulative gas production and its parameters were determined as described by Makkar (2005). Rumen fluid was collected

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from two cross-bred cows offered *ad libitum* access to sorghum-Sudan grass hay supplemented with 1.50 kg Lucerne hay (Grings *et al*., 2005). The collected rumen fluid in a pre-warmed and Co_2 filled thermos bottle was homogenized in a blender, strained through nylon material of 40 μm pore size and filtered through glass wool layers. The filtrated liquor was then mixed with carbonate buffer (containing ammonium bicarbonate at a level of 4 g L^{-1}) and sodium bicarbonate, macro mineral solution (5.7 g anhydrous Na_2HPO_4, 6.2 g anhydrous KH_2PO_4 and 0.6 g $MgSO_4 \bullet 7H_2O$ per liter), deionized water and 0.1 mL micro mineral solution (13.2 g $CaCl_2 \bullet 2H_2O$, 10.0 g $MnCl_2 \bullet 4H_2O$, 1 g $CoCl_2 \bullet 6H_2O$ and 8.0 g $FeCl_3 \bullet 6H_2O$ per 100 mL). The solution was then reduced by addition of 41.7 mL reducing agent (40 mL deionized water, 1 mL 1N NaOH and 1 g $Na_2S \bullet 9H_2O$) per liter of solution. Twenty milliliters of this solution were dispensed into the 100 mL glass syringes through tubing at the tip and placed upright in a 39°C water bath. Blank samples (i.e., medium only, no substrate) were placed throughout, the water bath and used to measure any gas production from the medium alone.

Cumulative gas volume were read manually for incubations in N-low and N-rich media from 4 replicates each after 2, 4, 8, 12, 24, 48, 72, 96 and 144 h of incubation. The kinetic parameters in the rumen were also estimated using this exponential model:

$$P = a + b (1-e^{-ct})$$ of Bl¨ummel and Ørskov (1993)

where,

P = The lolume of gas production at time t.
a = The gas production from soluble fraction (mL g^{-1}).
b = Gas production from insoluble fraction (mL g^{-1}).
c = Gas production rate constant (h^{-1}).
a + b = The potential gas production (mL g^{-1}).
t = The incubation time (h).

***In situ* procedure:** For *in situ* measurements, four Holstein steers (24±3 months of age and 450±11 kg of body weight) with permanent ruminal fistula, housed individually in concrete floored pens were used. The steers were fed at 8:00 and 16:00 h with a TMR diet (Table 1). The dried samples (5 g) were weighed into 12×19 cm Polyester bags (with the pore size of 40 μm). Eight bags were prepared for each sample and each incubation time. Ruminal incubation times were 0, 2, 4, 8, 16, 24, 72 and 96 h. All bags were inserted at the same time, just before the morning feeding.

Table1: Ingredients of the total mixed ration (TMR) diet fed to steers (as fed)

Ingredients	Kg/steer/day
Alfalfa hay	2.500
Corn silage	7.000
Barley straw	0.500
Barley grain	1.064
Wheat bran	0.168
Mineral and vitamin premix	0.032
Total	11.264

At the end of each incubation period, bags were rinsed with cold tap water until the rinse water was clear. Zero time disappearance was obtained by washing unincubated bags in a similar way. All washed bags were dried in a forced-air oven (Memmert 854) at 56°C for 48 h. Disappearance of DM and CP for each incubation time was calculated from the proportion remained after incubation. Dried samples were also analyzed for OM and NDF.

Degradation of DM, CP, OM and NDF was calculated using the following typical equation:

$$P = a + b (1-e^{-ct})$$

Statistical analysis: Data were subjected to analysis as a completely randomized design using the General Linear Model (GLM) procedure of SAS. After significant ($p<0.05$) F-test, means Duncan's multiple range test was used to compare treatment means.

RESULTS

Chemical composition: The chemical composition of the straw samples was shown in Table 2. The fraction for dry matter content was lowest ($p<0.05$) for wheat straw on the final (day 84) of mushroom growing (140.2 g kg^{-1}). Crude protein content increased significantly ($p<0.05$) after mushroom growing, from 46.6 g kg^{-1} on day 0-50.90 g kg^{-1} on 84 days. Organic matter content significantly decreased from 91.14-84.47% on the final day of mushroom growing (day 84). The average daily reduction rate of organic matter was 0.8 g kg^{-1}. NDF content of the wheat straw were 719, 690, 669, 612 and 550 g kg^{-1} for the day 0, 21, 42, 63 and 84 after seeding, respectively. A Similar tendency was found for ADF 474, 456, 443, 416 and 387 g kg^{-1} for the respective samples. The differences between these means were significant $p<0.05$. Lignin content of the wheat straw reduced significantly following seeding; the rate was about 0.27% per day. The obtained results showed that a substantial part of cell-wall components including NDF, ADF and partially lignin were degraded by the

Table 2: Changes in chemical composition of wheat straw incubated with *P. ostraontus* mycelium

		DM (%)				
DI	DM (g kg^{-1} as fed)	CP	OM	NDF	ADF	Lignin (sa)
0	940.47^{a}	46.60^{d}	91.14^{a}	719^{a}	474^{a}	98.5^{a}
21	160.33^{b}	46.36^{d}	90.64^{a}	690^{b}	456^{b}	97^{a}
42	158.93^{b}	47.83^{c}	89.42^{b}	669^{c}	443^{b}	87.5^{b}
63	160.00^{b}	49.46^{b}	87.38^{c}	612^{d}	416^{c}	75.5^{c}
84	140.20^{b}	50.90^{a}	84.47^{d}	550^{e}	387^{d}	68.5^{d}

DI: Days after Incubation; Lignin (sa): Lignin determined by solubilization of fiber

Table 3: Cumulative gas produced at different times of wheat straw incubated and parameters of gas production (mean±S.D.)

	Days after incubation						
Variable	0	21	42	63	84	S.E.	p-value
2 h	2.33^{b}	1.33^{b}	3.00^{b}	3.00^{b}	5.33^{a}	0.577	0.0070
4 h	6.33^{b}	3.33^{c}	5.50^{bc}	6.50^{b}	10.00^{a}	0.749	0.0010
8 h	11.50^{b}	3.83^{c}	12.00^{b}	12.75^{b}	16.83^{a}	1.042	<0.0001
12 h	20.66^{b}	12.33^{c}	12.00^{ab}	20.75^{b}	26.00^{a}	1.367	0.0005
24 h	44.66^{a}	32.66^{b}	46.50^{a}	40.00^{a}	45.66^{a}	1.919	0.0033
48 h	53.16^{a}	42.50^{b}	52.50^{a}	47.25^{a}	49.83^{a}	2.017	0.0256
72 h	59.66^{a}	50.00^{b}	60.00^{a}	54.50^{ab}	55.33^{ab}	2.131	0.0516
96 h	64.16^{a}	54.83^{b}	64.50^{a}	58.75^{ab}	59.16^{ab}	2.300	0.0818
144 h	69.33^{ab}	60.33^{c}	70.00^{a}	62.50^{bc}	62.33^{bc}	2.105	0.0344
b (mL)	68.07^{ab}	62.50^{bc}	68.91^{a}	62.00^{bc}	59.49^{c}	1.926	0.0242
c (mL h^{-1})	0.033^{c}	0.022^{d}	0.034^{bc}	0.037^{b}	0.048^{a}	0.0009	<0.0001

Values with different superscripts with in rows indicate significant differences (p<0.05)

mushroom enzymes. However, its chemical composition may be affected by substrate ingredients, mixing ratios of ingredients and cultivation methods (Adamovic´ *et al*., 1998).

***In vitro* gas production:** Cumulative gas produced at different times of wheat straw incubated and parameters of gas production are shown in Table 3. Mushroom growing led to a significant more gas production during the early hours (<24 h) of incubation. The b fraction of *in vitro* gas production as well as *in situ* results reduced significantly from day 0-84. The crates of gas production measurements were significantly increased between the noted days.

***In situ* ruminal disappearance:** *In situ* degradability of different nutrients of wheat straw incubated with *P. ostreatus* are shown in Table 4. According to this equation:

$$P = a + b\,(1-e^{-ct})$$

the fraction for dry matter was highest (p<0.05) for the straw under the mushroom conditions for 84 days (0.188 g g^{-1} for the day 0 and 0.342 for the day 84). In contrast, the b value significantly (p<0.05) reduced from day 0-84 days (0.487 and 0.336 g g^{-1}). The value for crude

Table 4: *In situ* degradability of different nutrients of wheat Straw incubated with P. ostreatus

	Days after incubation				
Parameters	0	21	42	63	84
DM					
a	0.188^{e}	0.195^{d}	0.224^{c}	0.289^{b}	0.342^{a}
b	0.487^{a}	0.495^{b}	0.473^{a}	0.392^{b}	0.336^{c}
c	0.019^{b}	0.021^{b}	0.021^{b}	0.024^{ab}	0.029^{a}
CP					
a	0.101^{e}	0.117^{d}	0.153^{c}	0.229^{b}	0.276^{a}
b	0.408^{a}	0.379^{a}	0.375^{a}	0.315^{b}	0.267^{b}
c	0.020^{a}	0.022^{a}	0.020^{a}	0.022^{a}	0.030^{a}
OM					
a	0.149^{e}	0.168^{d}	0.191^{c}	0.256^{b}	0.310^{a}
b	0.496^{a}	0.504^{a}	0.482^{a}	0.418^{b}	0.375^{b}
c	0.019^{a}	0.022^{a}	0.023^{a}	0.024^{a}	0.026^{a}
NDF					
a	0.032^{c}	0.028^{c}	0.056^{b}	0.064^{b}	0.086^{a}
b	0.583^{a}	0.612^{a}	0.603^{a}	0.580^{a}	0.573^{a}
c	0.021^{a}	0.022^{a}	0.021^{a}	0.023^{a}	0.021^{a}

Values with different superscripts with in rows indicate significant differences (p<0.05)

protein and NDF parameters increased linearly with increasing the growing period. The rapidly degradable CP and NDF fractions (g g^{-1}) increased linearly and significantly with increasing the growing period, but the slowly degradable for both fractions (g g^{-1}) decreased significantly (p<0.05) from day 0-84. The rapidly degradable fractions of OM and NDF of the colonized wheat straw for day 0 (0.149 and 0.032, respectively) were lower than that for day 84 (0.310 and 0.086, respectively). In contrast, the b value of OM and NDF (g g^{-1}) significantly (p<0.05) reduced with increasing the growing period of samples from day 0-84, but this constant was not affected for CP and NDF components. Although, the c rates were significantly different between the samples from day 0-84, but this constant was not affected for CP and NDF.

DISCUSSION

Ligno-cellulose complex of the fibrous by-products such as wheat straw can be degraded hy the Enzyme complexes including cellulase, cellobiase, hemicellulase and ligninase in certain mushroom strains from the *Pleurotus* among others fungal genus. Some researchers have noted that straw (compost) degraded by these enzymes during mushroom production can be more easily digested by ruminants. This straw contained more free sugars, more protein, less cellulose and lignin, with an increased content of ash compared with beginning material (Williams *et al*., 2001).

In wheat straw treated with mushrooms, solubiliation of hemicelluloses from the cell wall may also lead to

increased proportion of ADF and the NDF components of the treated straw. Mushrooms are able to degrade between 25 and 60% of the dry weight of plant tissues although their efficiency varies according to the species, strain and the plant type (Broudiscou *et al*., 2003).

The obtained results in this study showed that a substantial part of cell-wall components including NDF, ADF and partially lignin were degraded by the mushroom enzymes. However, chemical composition and biological quality of proteins from fungal fruit bodies are affected by different strains, substrate composition and method of preparation, growing techniques, age and the stage of development of fruit bodies (Valencia del Toro *et al*., 2006).

The presented results suggest that generally rumen degradability of different nutrients can be increased by growing the *P. ostreatus*, although the c rate was independent. The results also showed that nutritive values of cell-wall components of wheat straw colonized by *P. ostreatus* not only depend on increased its digestibility due to higher delignification but also on the availability of polysaccharide fractions remaining as energy source for the ruminal microorganisms. Furthermore, the increased CP content of wheat straw from mushroom residues can enhance the growth of lignocellulotic microbes and performing the higher feeding value.

CONCLUSION

It was concluded the wheat straw as a suitable mushroom compost can be used as a feed for ruminants with high feeding value in comparison with the untreated straw. This recommendation is important in a country of Iran that more than half of its feed resources are in the form of fibrous roughages.

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