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# Ground wheat straw as a substitute for portions of oak wood chips used in shiitake (*Lentinula edodes*) substrate formulae

Daniel J. Royse [a,*], Jose E. Sanchez [b]

[a] *Mushroom Research Center, Department of Plant Pathology, 316 Buckhout Laboratory, Pennsylvania State University, University Park, PA 16802, USA*
[b] *El Colegio de la Frontera Sur. Apdo. Postal 36, Tapachula, Chiapas 30700, Mexico*

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**Abstract**

Oak woodchips, used for production of shiitake *Lentinula edodes* (Berk) Pegler, are increasingly difficult to obtain due to dwindling supplies. We investigated the effect of adding ground wheat straw as a substitute for portions of oak woodchips in substrate formulae on mushroom yield and size. We also determined the effect of mushroom cropping on relative feed value (RFV) by chemical analysis of the substrate at spawning (AS) and after cropping (AC). Three formulae containing 0%, 8% and 16% ground wheat straw and 52%, 44% and 36% oak sawdust, respectively, were bulk pasteurized (111 °C for 20 min) in an autoclaving mixer, subjected to spawn run (21 d), browning (28 d) and a production cycle of three breaks (38 d). Mean (4 crops) mushroom yields were 11% higher when 8% wheat straw was used in the medium and 19% higher when 16% wheat straw was substituted for portions of oak sawdust. There were no significant differences in mushroom sizes between any of the treatments. Relative feed values of shiitake substrates AC increased more dramatically as more wheat straw was added to the formulae. Using mature alfalfa (full bloom) as a base value of 100%, RFVs for substrate AS were 98%, 92%, and 92% for 0%, 8% and 16% straw, respectively; RFVs AC were 118%, 120% and 133%, respectively. Substrate AC containing 16% straw had a RFV comparable to corn silage (well-eared). Fat contents of the substrates decreased by 50–62% AC, whereas potassium contents decreased by 40%. Use of ground wheat straw in synthetic medium would not only increase mushroom yield by up to 19%, but may help alleviate periodic shortages of oak sawdust. In addition, growers would avoid the added expense of aging the wheat straw (for 8–12 week) as is typically done for oak sawdust in the industry. This is the first report of RFVs for spent shiitake substrate (SSS) predicting its excellent potential for use as animal feed.


*Keywords:* *Lentinula edodes*; Shiitake; Wheat straw; Oak sawdust; Relative feed value

## 1. Introduction

In the US, production of shiitake (*Lentinula edodes*) increased 18% from 3398 t in 2002–2003 to 4128 t in 2004–2005 (USDA, 2005a). Most shiitake are grown on synthetic substrates composed of oak sawdust (ca. 50%) and nutrient supplements (white millet, rye and wheat bran). Within the last two years, oak sawdust has become difficult to obtain to meet the growing demand for shiitake production. Shiitake growers are facing increased competition for sawdust from uses such as fuel, animal food and bedding, meat smoking, pulping, mulching, soil conditioning, and particleboard production. The occasional shortages of good quality oak sawdust in the past have now become frequent. As a result, growers have begun to seek alternative raw materials for use as production substrates.

Wheat straw is an abundant and relatively inexpensive raw material that successfully has been used to produce shiitake in France (Delpech and Olivier, 1991; Olivier, 1997). Wheat is grown on more land area (250 million hectares) worldwide than any other crop and is third to only corn and rice for total world production (WORC, 2002). Nearly 16% of the world's wheat production is in North America. Wheat straw production in the United States in 2005/06 is expected to reach 78 million metric tons (USDA, 2005b; Engel et al., 2003).

* Corresponding author. Tel.: +1 814 865 3761; fax: +1 814 863 7217.
*E-mail address:* djr4@psu.edu (D.J. Royse).

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Levanon et al. (1993a,b) demonstrated that a mixture of bulk-pasteurized wheat straw and cotton straw could be used to produce shiitake in Israel. However, production of shiitake on bulk-pasteurized wheat straw has not been widely accepted commercially due to problems with infestation of the substrate with *Trichoderma* spp. (Savoie et al., 2000) and low yields (Olivier, 1997; Gaitan-Hernandez and Mata, 2004) compared to sterilized, supplemented sawdust. The addition of peat moss to straw-based pasteurized substrate and use of specifically formulated spawn has improved substrate selectivity and somewhat lessened the severity of *Trichoderma* sp. infestations (Mata et al., 1998), but this production system has not been widely adopted by growers.

Once growers have completed a cropping cycle, they must dispose of the used or "spent" shiitake substrate (SSS). Disposal of the SSS is an increasingly difficult and costly prospect because of stringent environmental regulations. It is estimated that ca. 6000 t of SSS had to be disposed of in the US last year. Some growers have investigated burning SSS, but other alternatives, such as recycling or possible use as an animal feed, are needed to make better use of this valuable resource.

## 2. Methods

### 2.1. Isolate and spawn

Isolate R26 of *L. edodes* was selected for this study because it is a commercial cultivar. The isolate was maintained on potato-dextrose yeast-extract agar (Jodon and Royse, 1979) and spawn prepared as outlined by Royse and Bahler (1986).

### 2.2. Wheat straw preparation

Baled (11–14 kg) wheat straw (*Triticum aestivum* L.) was collected in Centre County, Pennsylvania and chopped in a two-stage process. First, the baled straw was chopped to a 5–15 cm length with a bale chopper (WIC, Wickham, Que., Canada). Next, the material was collected and processed through a hammer mill (Mighty Mac, MacKissic, Parker Ford, PA) with a 4 mm screen. The chopped, processed straw was bagged and kept in an enclosed building at room temperature until used.

### 2.3. Substrates and preparation

Three substrates containing three ratios of sawdust:wheat straw (52%:0%, 44%:8%, and 36%:16% dry substrate wt.) were prepared from mixtures of chopped wheat straw and mixed hardwood sawdust collected from a commercial sawmill in Centre County, Pennsylvania. Northern red oak (*Quercus rubra* L.) was the predominant species (ca. 90%) in the sawdust although some white oak (*Quercus alba* L.) sawdust was also present. The sawdust was collected in the fall of 2004 and was stored in an enclosed building. The moisture content of the sawdust as determined by weighing, drying, and re-weighing was approximately 30%. The general substrate formulation (all ingredients based on oven-dry substrate wt.) consisted of 52% sawdust/wheat straw, 24% millet (*Panicum miliaceum* L.), 12% wheat bran (*Triticum aestivum* L.), and 12% rye (*Secale cereale* L.).

Mixed substrate ingredients were pasteurized, cooled, inoculated and bagged with a 0.283 $m^3$ paddle mixer following procedures published previously (Royse and Sanchez-Vazquez, 2001, 2003). Dry substrate wt was determined by drying 100 g of the processed substrates in an oven for 48 h at 80 °C.

Spawn run, log browning, and soaking were conducted as outlined by Royse and Sanchez-Vazquez (2001, 2003). Mushrooms were harvested daily from the substrates, when the veil had broken and the gills were fully exposed. The mushrooms were counted and weighed. At the end of the harvest period (38 d; 3 flushes), the accumulated data were used to calculate the biological efficiency (BE; ratio of fresh mushrooms harvested per dry substrate wt and expressed as a percentage). Mushroom size was determined by dividing the total wt of harvested fresh mushrooms by the total number of mushrooms harvested (per replicate).

### 2.4. Experimental design and statistical treatment

The experiments were conducted in a completely randomized design (Steel et al., 1997) with 14 (Crop 1), 16 (Crop 2), 21 (Crop 3) and 22 (Crop 4) replicates per treatment. The SAS program JMP (SAS Institute, 2004) was used to analyze data. Data were examined with a one-way analysis of variance (ANOVA) and the Tukey–Kramer Honestly Significant Difference (HSD) was used to separate treatment means (SAS Institute, 2004).

### 2.5. Chemical analysis substrate at spawning AS and after cropping AC

Samples (150 g wet wt.) of substrate AS were collected at time of bagging while samples of substrate AC were collected from the centers of three shiitake logs (50 g each) and pooled into one sample for each treatment for analysis. Samples were oven-dried at 80 °C for 48 h then ground to 1 mm. Crude protein, acid detergent fiber (ADF), neutral detergent fiber (NDF), mineral (calcium, phosphorus, potassium and magnesium), fat and ash contents were determined by Agri Analysis (2006), a commercial analysis laboratory. RFVs were calculated for each substrate as outlined by Jeranyama and Garcia (2004).

## 3. Results

Mixtures of chopped wheat straw/oak sawdust/supplements flowed through the mixer similar to standard mixtures of oak sawdust/supplements only. Water added to wheat straw/oak sawdust/supplement mix was readily absorbed and no substantial differences in heat treatment and cool-down of the mixtures were observed.

### 3.1. Yields

As the percentage of wheat straw increased from 0% to 16%, yields tended to increase (Table 1). While yield increases were generally observed with increasing levels of wheat straw, not all increases were statistically significant, most often only when comparing 0–16% wheat straw. In Crop 1 for example, yields increased an average of 6% for each 8% increase in the amount of wheat straw used in the substrate (up to 16%) but these differences were not statistically different. In Crop 2, yields increased an average of 8.5% for each additional increment of wheat straw and significant differences in yield were found between 0% and 16% straw addition. In Crop 3, yield increases averaged 13% for each additional 8% increase in chopped straw addition with each increment in straw addition showing a significant increase in mushroom yield. In Crop 4, yields increased an average of 9.5% for each 8% increase in wheat straw with a significant difference between 0% and 16% straw addition. Overall means (4 crops) of yields increased an average of 9.5% for each 8% increase in the amount of wheat straw added. There was no significant difference in mushroom size between any of the treatments for each of the four crops (data not shown).

### 3.2. Chemical analysis of substrates

Crude protein increased in all shiitake substrates by the end of cropping (Table 2). Conversely, both ADF and NDF contents decreased, thereby increasing RFV. The magnitudes of RFV increases were greater as the proportion of wheat straw increased. By the end of cropping, RFV (133%) for shiitake substrate containing 16% wheat straw was similar to corn silage and nearly as high as alfalfa hay harvested at early bloom (138%; Jeranyama and Garcia, 2004). In contrast, the RFV (118%) for substrate without wheat straw (AC) was similar to sorghum silage (114%) but still higher than alfalfa hay cut at full bloom (100%; Jeranyama and Garcia, 2004).

Mineral, fat and ash contents and total digestible nutrients (TDN) for substrates AS and AC are shown in Table 3. Potassium and fat levels decreased substantially (40–60%) while calcium, phosphorus and magnesium levels increased (dry wt. basis). Total digestible nutrients increased in all substrates during cropping by 0.6–1.4%.

Table 1
Yield (g/log), percentage biological efficiency (BE) and means for four crops of *Lentinula edodes* grown on combinations of wheat straw (WS), oak sawdust (OS) and nutrient supplements

| Substrate mixture (%) | | | Crop 1 | | | Crop 2 | | | Crop 3 | | | Crop 4 | | | Mean (Crops 1–4) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WS | OS | S[a] | Yield (g)[c] | Change (%) | BE[b] (%) | Yield (g)[c] | Change (%) | BE (%) | Yield (g)[c] | Change (%) | BE (%) | Yield (g)[c] | Change (%) | BE (%) | Yield (g) | Change (%) | BE (%) |
| 0 | 52 | 48 | 915 a | – | 88.8 | 735 a | – | 71.4 | 767 a | – | 74.5 | 889 a | – | 86.7 | 827 | – | 80.4 |
| 8 | 44 | 48 | 982 a | +7 | 95.3 | 852 ab | +14 | 82.7 | 902 b | +15 | 87.6 | 966 ab | +8 | 94.2 | 926 | +11 | 90.0 |
| 16 | 36 | 48 | 1036 a | +12 | 100.6 | 883 b | +17 | 86.2 | 1041 c | +26 | 101.1 | 1102 b | +19 | 107.5 | 1016 | +19 | 98.9 |

[a] Supplements included white millet (24%), rye (12%) and wheat bran (12%).
[b] Biological efficiency calculated as the ratio of fresh mushroom harvested/dry substrate wt., expressed as a percentage.
[c] Means followed by the same letter in the same column are not significantly different at the $P = 0.05$ level according to Tukey–Kramer HSD.

Table 2
Crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), and relative forage values (RFV) of shiitake substrate containing three levels of wheat straw at spawning (AS) and after cropping (AC) and for some forage types

| Wheat straw content of substrate (%) | Sample time | CP (%) | ADF (%) | NDF (%) | RFV (%) |
|---|---|---|---|---|---|
| 0 | AS | 7.1 | 39 | 55 | 98 |
| 0 | AC | 8.2 | 37 | 48 | 118 |
| 8 | AS | 7.7 | 39 | 59 | 92 |
| 8 | AC | 8.2 | 38 | 46 | 120 |
| 16 | AS | 7.6 | 38 | 60 | 92 |
| 16 | AC | 8.5 | 35 | 43 | 133 |
| *Forage type*[a] | | | | | |
| Alfalfa | Early bloom | 18 | 33 | 43 | 138 |
| Alfalfa | Full bloom | 16 | 41 | 53 | 100 |
| Bromegrass | Late vegetation | 10 | 35 | 63 | 91 |
| Bromegrass | Late bloom | 7 | 49 | 81 | 58 |
| Corn silage | Well eared | 10 | 28 | 48 | 133 |
| Sorghum silage | Not specified | 8 | 32 | 52 | 114 |

[a] *Source*: Jeranyama and Garcia (2004).

Table 3
Mineral, fat and ash contents and total digestible nutrients (TDN) of shiitake substrates containing various levels of wheat straw (0%, 8%, 16%) at spawning (AS) and after cropping (AC)

| % Analysis[a] | Wheat straw in substrate (%) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | | 8 | | 16 | |
| | AS | AC | AS | AC | AS | AC |
| Calcium | 0.09 | 0.18 | 0.10 | 0.18 | 0.11 | 0.19 |
| Phosphorus | 0.31 | 0.33 | 0.33 | 0.38 | 0.36 | 0.39 |
| Potassium | 0.33 | 0.13 | 0.39 | 0.25 | 0.43 | 0.27 |
| Magnesium | 0.11 | 0.24 | 0.13 | 0.21 | 0.13 | 0.23 |
| Fat | 1.6 | 0.6 | 1.3 | 0.5 | 1.0 | 0.5 |
| Ash | 2.1 | 3.0 | 2.5 | 3.2 | 2.6 | 3.5 |
| TDN | 62.5 | 63.9 | 62.7 | 63.3 | 63.3 | 64.6 |

[a] Agri Analysis (2006).

## 4. Discussion

The use of wheat straw as a substrate ingredient for the production of shiitake is not new. In the early 1990s, several researchers (Olivier and Delpech, 1990; Delpech and Olivier, 1991; Levanon et al., 1993a,b) reported the successful production of shiitake on bulk, pasteurized wheat straw. Olivier and Delpech (1990) and Delpech and Olivier (1991) were the first to develop cultivation techniques based on substrates containing wheat straw enriched with chicken feather meal. This technique required the addition of 75 μg/g benomyl or carbendazim to the substrate to control *Trichoderma* spp., a relatively high spawn rate (7% w/w) and strict control of spawn run temperatures to avoid mold development in the substrate (Delpech and Olivier, 1991). Later, European growers who adopted this production methodology stopped adding fungicide to the substrate and relied more on hygienic measures applied at spawning (Olivier, 1997). Mushroom yield improvements also were made through the addition of 15% sawdust to the wheat straw substrate. Further improvements occurred through the development of improved spawn formulations and with the use of *Sphagnum* peat added to the substrate to suppress green mold (Mata et al., 1998). Overall BEs (25–56%, Gaitan-Hernandez and Mata, 2004) on pasteurized wheat straw, however, are about 50% of those obtained from sterilized substrates with oak sawdust as the basal ingredient (62–122%, Royse and Sanchez-Vazquez, 2001, 2003). However, *Trichoderma* spp. infestation of the substrate remained a serious problem for growers producing shiitake on straw (Savoie et al., 2000).

Our work shows that it is possible to replace up to 16% of the oak sawdust with chopped wheat straw and obtain as good or better yields. On oak sawdust without wheat straw, BEs ranged from 71% to 89% while on oak sawdust/wheat straw (36/16%) BEs ranged from 86% to 108%. These BEs are considerably higher than those reported by Philippoussis et al. (2003). They were able to obtain 25% BE on a mixture of 60% oak sawdust/15% wheat straw/25% nutrients. When they increased the amount of wheat straw to 75% (no oak sawdust), BE increased to 54%. One of the major differences between our experiments and theirs was the amount of nutrient supplements added to the mix. Philippoussis et al. (2003) used only 25% nutrient supplements while we used nearly twice as much (48%). Other differences in their study compared to ours included soaking and leaching of the substrates, non-aging of the sawdust, and browning inside the bag.

Thus far, growers have not considered using SSS as animal feed. This may be due to lack of knowledge of the potential value of the material. Work with another white rot mushroom, *Pleurotus ostreatus* (oyster mushroom) revealed that wheat straw-based, spent oyster substrate (SOS) would not be an adequate sole source of ruminant feed due to its relatively low digestibility (Adamovic et al., 1998; Royse et al., 1991). The RFV for SOS (Royse et al., 1991) was 55% and 66% when the substrate was non-supplemented or supplemented with 12% SpawnMate IISE® at spawning, respectively. Based on RFV, our work predicts that the SSS from wheat straw-amended substrate would be as good as corn silage (RFV = 133%) and better than alfalfa (RFV = 100%, harvested at full bloom) for ruminant feed. The RFV index estimates the digestible dry matter (DDM) of the substrate from ADF, and calculates the DM intake potential (as a percent of body weight, BW) from NDF. RFV has been used for years to compare the quality of legume and legume/grass hays and silages (Jeranyama and Garcia, 2004). Both ADF and NDF were reduced during mushroom cropping with NDF values showing greater decreases. At the end of cropping, substrates containing more wheat straw had greater decreases of both ADF and NDF. In fact, ADF and NDF were reduced from 38% to 35% and 60% to 43%, respectively, in substrates containing 16% wheat straw. These values are similar to alfalfa hay at early bloom (the best quality alfalfa). In substrates containing no wheat straw, ADF and NDF were reduced from 39% to 37% and 55% to 48%, respectively, and indicate that *L. edodes* was much more efficient in consuming fiber in wheat straw compared to oak sawdust.

It should be noted that differences in the digestibility of the fiber fraction may result in a difference in animal performance when forages with a similar RFV index are fed (Jeranyama and Garcia, 2004). In addition, our data are observational so the variance associated with the samples is unknown. Therefore, additional work is needed to determine how much variation exists between samples and how suitable SSS is for animal feed. Considering the fact that CP is relatively low for SSS (ca. 8.5%) compared to alfalfa hay (16%), some protein supplementation with concentrates may be necessary to improve feed value of the SSS. In addition, fat content of SSS only is about 0.5% whereas alfalfa hay and corn silage is ca. 2% and 1%, respectively (Belyea and Steevens, 2006). Therefore, a concentrate containing both high protein and high fat content (such as whole soybean or whole cottonseed) may suffice to increase relative quality of SSS. In addition, it would be desirable to know what percentage of the SSS could be used in a ration that ruminants would consume. Adamovic et al. (1998) discovered that rations containing more than 17% SOS was rejected by cattle (probably due to high NDF content). Additional work is needed to determine how much SSS could be used in a ration and still have acceptable daily weight gain for animals. In addition, calcium and potassium contents of a ration composed of SSS may have to be adjusted because both calcium and potassium levels are only about 13% of those found in alfalfa (UCTFC, 1969).

The ability to substitute a portion of the sawdust with wheat straw in a shiitake formula without sacrificing yield gives growers the flexibility of using alternative materials should they experience acute shortages of oak sawdust in the future. Further work on the physical characteristics (particle size, water holding capacity, nutritional status) of wheat straw and how altering those characteristics may impact mushroom yield and quality could lead to further increases in production efficiency.

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