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# Yield, Nutrient Characteristics, Ruminal Solubility and Degradability of Spent Mushroom (*Agaricus bisporus*) Substrates for Ruminants

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**ABSTRACT**: This study was conducted to evaluate the yield, nutrient characteristics, ruminal solubility, degradability and disappearance of spent mushroom (*Agaricus bisporus*) substrates for ruminants. The annual yield of spent *Agaricus bisporus* substrates was measured to be about 210,000 tons (M/T) in South Korea. The surface soil-removed spent substrates had nutritional characteristics of high crude ash (375 g/kg) and Ca (32 g/kg), medium protein (134 g/kg CP), and high fiber (384 g/kg NDF on a DM basis). Compared with initial mushroom substrates, spent mushroom substrates had twice higher (p<0.0001) CP content and 22.0% lower (p<0.0001) NDF content on an organic matter basis. Compared with raw rice straw, spent rice straw had much higher (p<0.05) predicted ruminal degradabilities and disappearances of DM and CP and a little lower (p<0.05) predicted degradability and disappearance of NDF. In conclusion, the general feed-nutritional value of spent mushroom (*Agaricus bisporus*) substrates appeared to improve after cultivation of mushrooms. (**Key Words**: Spent Mushroom Substrate, Spent Mushroom Compost, Degradability, Feed, Ruminant)

#### INTRODUCTION

Spent mushroom (*Agaricus bisporus*) substrates are the substrates that remain after harvesting of mushrooms. In Asian countries, the fresh substrates normally containing straw, poultry litter, urea, and limestone are piled up outside for 15-25 days. The substrates are then put on the growing bed, after which they are sterilized, fermented, and inoculated with the seed culture. After the seed culture grows, clay loam soil with medium viscosity is put on them to produce *Agaricus bisporus* mushrooms (fruiting bodies). The methods used for mixing wheat straw, animal litter, and limestone to make mushroom substrates (Bakshi and Langar, 1991; Riahi et al., 1998) are common among Asian countries.

The world-wide production of edible *Agaricus bisporus* was estimated to be about 2 million tons in 1997 (Chang, 1999). Then at least more than 10 million tons of spent *Agaricus bisporus* substrates are annually produced in the world. Although traditionally spent *Agaricus bisporus* 

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substrates (or composts) have been used as a fertilizer for plants, they have been studied also for their use as a feed source for animals.

Langar et al. (1982) reported that spent Agaricus bisporus substrates could be used as sources of minerals for animals, as they are rich in major and trace minerals. However, Bakshi and Langar (1991) reported that spent Agaricus bisporus substrates had limited use as animal feed due to their high crude ash content (380-530 g/kg). White rot mushroom fungi were found to degrade lignocelluloses (Tuomela et al., 2000; Makela et al., 2002), possibly resulting in improved feed-nutritional value of spent mushroom substrates. Mycellial action on the mushroom substrates may alter solubility and degradability of their nutrients in the rumen, but in situ information is not available to describe these characteristics of spent mushroom substrates. An assumption was made that mycellial action would improve feed-nutritional value of spent mushroom substrates by increasing in situ degradability of their nutrients in the rumen of ruminants. This study was conducted to determine nutrient characteristics, ruminal solubility, degradability disappearance of spent mushroom (Agaricus bisporus) substrates for ruminants on the basis of their annual yield measured.

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#### **MATERIALS AND METHODS**

### Field investigation and specimen collection

Field investigation and specimen collection of spent *Agaricus bisporus* substrates were made on 11 farms that intensively grew *Agaricus bisporus* in Seokseong-myoen, Chungnam province in South Korea. The ingredients, input amounts, and cultivation periods of the substrates for each farm were investigated to estimate the yield of spent *Agaricus bisporus* substrates. A total of 21 specimens including raw and spent *Agaricus bisporus* substrates were collected for analysis of the chemical composition. The specimens were stored at -20°C in a freezer (Lassele LOC-520F, Korea) until analysis.

### Calculation of the yield of spent *Agaricus bisporus* substrates

The yield of spent *Agaricus bisporus* substrates was calculated using the method (Equation 1) described by Kim et al. (2007), in which the cultivation area of spent mushroom substrates was multiplied by the input amount of raw substrates for the production of *Agaricus bisporus* and the number of cultivation cycles. The results were adjusted for the dry matter of raw and spent *Agaricus bisporus* substrates.

Equation 1:

Yield of substrates

$$= \frac{A}{\% \text{ dry matter of substrates}} \times \text{ cultivation cycles per year}$$

where A = Cultivation area  $(3.3 \text{ m}^2) \times \text{input}$  of raw substrates per  $3.3 \text{m}^2 \times \%$  dry matter of raw substrates.

The nationwide cultivation area of *Agaricus bisporus* was based on the annual yield of mushroom published from 2005 to 2008 by the Korean Ministry of Agriculture, Fishery and Food (KMAFF, 2005, 2006, 2007, and 2008). Six farms that grew *Agaricus bisporus* in Seokseong-myoen, Chungnam province were selected for the on-site estimation of the inputs of raw substrates per 3.3 m<sup>2</sup>. The average input of raw substrates slightly differed between farms, and averaged 243±8.2 kg per 3.3 m<sup>2</sup>. The number of cultivation cycles per year of *Agaricus bisporus* was 3.1, based on the on-farm survey.

### Chemical analysis

The specimens were dried at 65°C for 4 h, and then crushed into a 1-mm particle using the Sample Mill (Cemotec, Tecator, Sweden) grinder. The dry matter (DM, method 925.40), crude protein (N×6.25, CP, method 984.13), and ether extract (EE, method 920.39) were analyzed according to the methods of AOAC (2000).

Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were analyzed according to the method of Van Soest et al. (1991). Neutral detergent fiber was analyzed using both sodium sulfite and alpha amylase. It was expressed as ash-free NDF. Organic matter (OM) was calculated by deducting crude ash from 100; hemicellulose, by deducting ADF from NDF; and nonfibrous carbohydrate (NFC), by deducting NDF%+CP%+ EE%+ash% from 100. The amount of indigestible protein (ADF-CP) was calculated by diluting it in an acid detergent solution and then measuring CP of the filtered substance. True protein (TP) was calculated by measuring the precipitated amount in a 5% trichloroacetic acid solution. Non-protein nitrogen (NPN) was calculated by deducting TP from CP. Cellulose was calculated by deducting ADL from ADF.

For analysis of minerals, a blank solution was prepared by putting 5 g of sample in an ash crucible, ashing it at 600°C for 3 h, then adding 10 ml of a 1:1 HCl solution and leaving it at room temperature for 8 h to reach a final volume of 50 ml according to the mineral analysis method of Standard Analysis Protocol for Feeds (KMAFF, 2010). The analysis was performed using Inductively Coupled Plasma (ICP-OES OPTIMA 5300 DV, PerkinElmer, USA).

#### In situ bag test for ruminal incubation

To increase the accuracy of test results by minimizing the compositional variation of the specimen, the rice straw (a major component of spent substrates) was separated from the remaining soil, broiler litter, urea and lime in the substrates. For a complete separation, the surface soil was manually removed from the substrates. Then the collected specimen was dried at  $60^{\circ}$ C in a drying oven for 48 h, and the rice straw was thoroughly separated from the other remaining materials. The straw was crushed using a Wiley mill (Thomas Scientific, Model 4, New Jersey, USA);  $100~\mu\text{m}-2~\text{mm}$  particles were filtered using an experimental sieve (filter diameter:  $100~\mu\text{m}$  or 2~mm); and these particles were used for the test.

The specimen (approximately 10 g) was put into a Dacron bag (R1020, Ankom Technology, NY, USA) (whole size,  $10\times25$  cm; pore size,  $50\pm15$   $\mu m$ ). The ratio of the specimen weight to the Dacron bag size was 20 mg/cm², which was within the range recommended by Nocek (1985). To compare and evaluate *in situ* fraction, degradability and disappearance, two trials were performed using two Holstein beef cattle (an average of 620 kg body weight) with a cannula implanted in the rumen. The observations per treatment were four. The test animals were fed 4 kg of a formulated feed and 4 kg of rye grass straw daily to meet the nutritional requirement for their maintenance (NRC, 2001). The animals had free access to water.

The in situ bag test was performed according to the methods of Ørskov et al. (1980). Two hours after feeding, bags containing test samples were incubated in the ruminal ventral sac for 0, 24, 48 and 72 h. After completion of the incubation, bags were retrieved, washed in running cold water for 24 h until the clear water came out of the bag, and dried at 60°C in a drying oven for 48 h. Then DM, NDF and CP were analyzed. The DM was classified into watersoluble and 50 µm filterable, insoluble degradable, and undegradable fractions (Armentano et al., 1986). It was assumed that the NDF would be completely degraded in the rumen within 72 h (Smith et al., 1971). The portion of NDF that remained after 72 h of incubation was considered the undegradable fraction. The CP was classified into watersoluble and 50 µm filterable, insoluble degradable, and undegradable fractions. The portion of CP that remained after 48 h of incubation was considered the undegradable fraction. Residues at each incubation time minus the undegradable fraction were converted to a percentage, transformed to the natural logarithm, and subjected to linear regression (Armentano et al., 1986). Slope of the regression line was degradation rate of degradable fraction. Computed degradation rates were used to predict ruminal degradability of DM, NDF, and CP in raw and spent rice straws, using the following Equation 2 (Orskov et al., 1983), and assumed passage rates ( $K_pB$ ) of 0.05/h (Miller, 1982):

Equation 2:

Degradability = 
$$A+(K_dB\times B)/(K_dB+K_pB)$$

Where A = soluble and filterable fraction, B = insoluble degradable fraction,  $K_dB =$  degradation rate of degradable fraction,  $K_DB =$  passage rate of degradable fraction.

Disappearances of DM, NDF, and CP were calculated at any given incubation time.

#### Statistical analysis

To compare the average chemical features before and after the *Agaricus bisporus* cultivation, data on chemical and mineral composition were processed by ANOVA, using the GLM procedure of SAS (SAS Institute, 2002). Differences among means were evaluated using Tukey's test (SAS Institute, 2002). *In situ* fractions, predicted degradabilities, and ruminal disappearances of DM, NDF, and CP of raw and spent rice straws were processed using a randomized complete block design. Means were compared using Tukey's test (SAS Institute Inc., 2002). The model used was as follows;

$$Y_{ijk} = \mu + F_i + C_j + e_{ijk}$$

where  $Y_{ijk}$  was the dependent variable;  $\mu$  was the overall mean;  $F_i$  was the fixed effect of feed (i = 1 to 2);  $C_i$  was the



**Figure 1.** Physical appearance of soil layer and spent mushroom (*Agaricus bisporus*) substrate layer.

random and block effect of the cow (j=1 to 2); and  $e_{ijk}$  was error term.

We assumed normality and equal residual variances. Significance was detected at p<0.05 (SAS Institute Inc., 2002).

#### **RESULTS**

### The physical appearance of spent Agaricus bisporus substrates

The physical appearance of spent *Agaricus bisporus* substrates is shown in Figure 1. The initial substrates consisted of 80% rice straw, 14% broiler litter, 2% urea and 4% lime and the upper surface was covered with soil. Since the *Agaricus bisporus* was grown on the soil, it was possible to clearly distinguish the upper soil layer (3.3 cm-thick) and the lower substrates layer (13.1 cm-thick) that consisted of mostly rice straw. The soil was very dry, with a moisture content of 149 g/kg, whereas the substrate layer was not dry at all, with a moisture content of about 600 g/kg. The substrate layer showed a large amount of white mycelium and was easily broken with the hands because it was softened by mycelium during the cultivation.

### Yield of the spent Agaricus bisporus substrates

In South Korea, *Agaricus bisporus* was mostly produced in Chungnam and Gyeongbuk provinces. The annual yields of spent *Agaricus bisporus* substrates according to province in South Korea are presented from 2004 until 2008 in Table 1. The yield of spent *Agaricus bisporus* substrates was measured to be about 660,000 tons (M/T) in 2004 and has been decreasing thereafter. Since 2006, the yearly yield of spent *Agaricus bisporus* substrates has been on the level of 200,000 tons (M/T). In 2008, the yield of spent *Agaricus bisporus* substrates was 210,000 tons (M/T).

The average amount of substrates input for mushroom production per area unit was 242.9 kg per 3.3 m<sup>2</sup>. Based on the yearly 3.1 cultivation cycle of mushrooms, the amount of spent *Agaricus bisporus* substrates generated was 1,118

<b>Table 1.</b> Calculated annual regional yields of spent mushroom (Agaricus bisporus) substrates according to province in Sout
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Province	Year							
	2004	2005	2006	2007	2008			
	M/T							
Gyeonggi	31,528	36,404	16,776	3,355	10,066			
Gangwon	1,689	463	-	-	-			
Chungbuk	872	-	-	-	-			
Chungnam	348,531	317,922	154,341	137,565	137,565			
Jeonbuk	10,155	9,978	6,710	3,355	3,355			
Jeonnam	13,443	6,307	6,710	3,355	3,355			
Gyeongbuk	233,642	168,058	50,329	36,908	33,552			
Gyeongnam	671	-	-	-	6,710			
Jeju	-	-	-	-	-			
Metropolises	17,380	50,487	16,776	16,776	13,421			
Total	657,911	589,620	251,643	201,314	208,025			

kg per  $3.3 \text{ m}^2$ , 4.6 times higher than that of the inputs and 23.4 times higher than that of the yield of mushrooms.

### Chemical composition of spent *Agaricus bisporus* substrates

Chemical compositions of substrates before and after cultivation of *Agaricus bisporus* are presented in Table 2. After cultivation of *Agaricus bisporus*, crude ash contents of spent substrates increased up to 548 g/kg due to the introduction of soil. Crude ash content (DM basis) of soil-removed spent *Agaricus bisporus* substrates was 375 g/kg, much higher than the 169 g/kg in the pre-cultivation stage (p<0.05).

When the data were analyzed on the OM basis, CP and ADL contents of substrates increased (p<0.05), NDF, ADF, hemicellulose and cellulose contents decreased (p<0.05), and EE and NFC contents were not different (p>0.05) after cultivation of *Agaricus bisporus*. The decrease in NDF and ADF contents was considered attributable to the fiber degradation by the *Agaricus bisporus* mycelium and the 2-fold increase in CP content due to the mycelium proliferation. The appreciable decrease (p<0.05) in TP/CP and ADF-CP (indigestible protein)/CP percentage proves the active breakdown of protein by mycelium. For a reference, the chemical composition on the DM basis of soil-removed spent substrates was 134 g/kg CP, 0.4 g/kg EE,

Table 2. Chemical compositions (g/kg) of the initial and spent mushroom (Agaricus bisporus) substrates with and without surface soil

Item	I:4:-1 MC2	After harvesting			
	Initial $MS^2$ (n = 5)	Soil-removed SMS <sup>3</sup> $(n = 5)$	Whole SMS (n = 11)	SEM	p value
Dry matter	223°	428 <sup>b</sup>	544 <sup>a</sup>	18.5	< 0.0001
Dry matter basis					
Crude ash	169 <sup>c</sup>	375 <sup>b</sup>	548 <sup>a</sup>	28.2	< 0.0001
Organic matter basis					
Crude protein	109 <sup>c</sup>	216 <sup>b</sup>	$224^{a}$	12.1	< 0.0001
True protein/CP	928 <sup>a</sup>	675 <sup>c</sup>	781 <sup>b</sup>	27.8	< 0.0001
Non-protein/CP	72°	325 <sup>a</sup>	$219^{b}$	27.8	< 0.0001
ADF-CP/CP	520 <sup>a</sup>	393 <sup>b</sup>	511 <sup>a</sup>	33.5	0.0249
Ether extract	6	6	6	0.7	0.7391
Neutral detergent fiber	792 <sup>a</sup>	618 <sup>b</sup>	595 <sup>b</sup>	22.6	< 0.0001
Acid detergent fiber	633 <sup>a</sup>	528 <sup>b</sup>	531 <sup>b</sup>	17.3	0.0004
Hemicellulose	158 <sup>a</sup>	$90^{b}$	64 <sup>b</sup>	14.3	0.0003
Cellulose	423 <sup>a</sup>	100 <sup>b</sup>	109 <sup>b</sup>	15.6	< 0.0001
Acid detergent lignin	211 <sup>b</sup>	$428^{a}$	422 <sup>a</sup>	15.3	< 0.0001
Non-fibrous carbohydrate	93	161	175	25.6	0.0608

<sup>&</sup>lt;sup>1</sup> Dry matter basis. <sup>2</sup> Mushroom substrates. <sup>3</sup> Spent mushroom substrates.

<sup>&</sup>lt;sup>a,b,c</sup> Means with different letters within the same row are significantly different (p<0.05).

**Table 3.** Mineral composition (dry matter basis) of spent mushroom (*Agaricus bisporus*) substrates with and without surface soil after harvesting of the fruit body<sup>1</sup>

Item	Soil-removed SMS <sup>2</sup>	Soil	Whole SMS	SEM	p value
Major minerals (g/kg)					
Ca	$32.2^{a}$	6.2 <sup>b</sup>	$22.0^{a}$	3.92	< 0.0001
P	$6.7^{a}$	$0.4^{b}$	$4.9^{a}$	1.54	0.0044
Mg	3.9	3.7	4.0	1.04	0.9505
Na	2.4	0.6	1.7	0.82	0.1223
K	21.9 <sup>a</sup>	3.6 <sup>b</sup>	$14.1^{ab}$	5.84	0.0271
Trace minerals (mg/kg)					
Fe	3,114 <sup>b</sup>	16,430 <sup>a</sup>	9,103 <sup>ab</sup>	3,345.5	0.0063
Mn	1,014	537	976	2398.9	0.1268
Zn	71.5	51.6	93.2	25.67	0.3065
Cu	6.4	4.5	15.3	5.40	0.1462

<sup>&</sup>lt;sup>1</sup> Means of five observations. <sup>2</sup> Spent mushroom substrates from which the upper soil layer was removed.

384 g/kg NDF, 329 g/kg ADF, and 104 g/kg NFC. To evaluate the potential use as a mineral source of spent *Agaricus bisporus* substrates, their mineral contents were analyzed and presented in Table 3. The Ca content of soil-removed spent substrates was 32 g/kg, five times higher than for the covering soil (p<0.05).

For trace minerals, Mn, Zn and Cu contents in spent substrates were not affected (p>0.05) by the soil removal. However, Fe content abundant in the soil was affected (p<0.05) by the soil removal.

# In situ fractionation and degradability of spent rice straw nutrients

For the accuracy of the in situ test, spent rice straw, a

major component of spent *Agaricus bisporus* substrates, was thoroughly separated from other ingredients and the test results were compared with those of raw rice straw. *In situ* DM, NDF and CP fractions, and degradabilities of spent rice straw are presented in Table 4. The water-soluble and filterable fraction of spent rice straw DM was 2.7 times higher than that of raw rice straw DM (p<0.05). The insoluble, degradable fraction and the undegradable fraction of spent rice straw DM were 48.5% and 13.6% lower than those of raw rice straw DM, respectively (p<0.05).

The degradable fraction of spent rice straw NDF was 20.2% lower than that of raw rice straw NDF (p<0.05). As a result, the degradable fraction of rice straw NDF decreased during the cultivation of *Agaricus bisporus* (p<0.05).

**Table 4.** In situ fractions (g/kg) of the DM, NDF, CP, and degradabilities of raw and spent rice straws at 0.05/h of passage rate  $(K_nB)^1$ 

Item	Rice straw	Spent rice straw	SEM	p value
Dry matter fractions				
Water-soluble and 50 µm-filterable	112 <sup>b</sup>	417 <sup>a</sup>	41.4	0.0003
Insoluble degradable	528 <sup>a</sup>	272 <sup>b</sup>	42.6	0.0009
Undegradable	360 <sup>a</sup>	311 <sup>b</sup>	5.4	< 0.0001
NDF fractions				
Degradable	555 <sup>a</sup>	443 <sup>b</sup>	8.4	< 0.0001
Undegradable	445 <sup>b</sup>	557 <sup>a</sup>	8.4	< 0.0001
CP fractions				
Water-soluble and 50 µm-filterable	320 <sup>b</sup>	574 <sup>a</sup>	11.1	< 0.0001
Insoluble degradable	227	316	27.7	0.0581
Undegradable	457 <sup>a</sup>	110 <sup>b</sup>	20.4	< 0.0001
Degradability				
Dry matter	$0.287^{b}$	$0.451^{a}$	0.0223	0.0003
Neutral detergent fiber	$0.305^{a}$	$0.238^{b}$	0.0042	< 0.0001
Crude protein	0.341 <sup>b</sup>	$0.607^{a}$	0.0121	< 0.0001

<sup>&</sup>lt;sup>1</sup> Means of four observations

 $<sup>^{</sup>a,b,c}$  Means with different superscripts within the same row are significantly different (p<0.05).

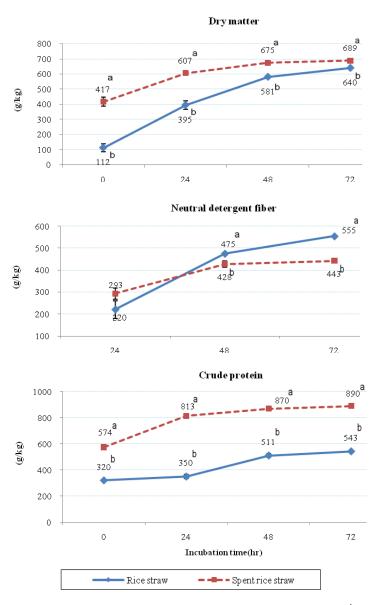
<sup>&</sup>lt;sup>a,b</sup> Means with different superscripts within the same row are significantly different.

Compared with raw rice straw CP, spent rice straw CP had much higher water-soluble, filterable fraction and much lower undegradable fraction (p<0.0001). The increase in the water-soluble fraction of spent rice straw CP was primarily due to the chemical treatment effect by non-protein N components in urea and broiler litter added to the raw substrates. As a result, the degradable DM and CP fractions in rice straw increased, but the degradable NDF fraction decreased during the cultivation of *Agaricus bisporus*. These changes in fractions affected predicted degradability of spent rice straw nutrients. Predicted degradabilities of DM and CP in spent rice straw were much higher (p<0.001) and those of NDF were rather lower (p<0.0001) than for raw rice straw.

# In situ ruminal disappearance of spent rice straw nutrients

In situ ruminal disappearances of DM, NDF and CP of spent rice straw at any given incubation time are presented in Figure 2. At the early phase of ruminal incubation, DM disappearance of spent rice straw was much higher than that of raw rice straw (p<0.05); however, the difference diminished with ruminal incubation time elapsed. The cultivation process of Agaricus bisporus considerably increased the early disappearance of spent rice straw DM (p<0.05).

The ruminal disappearance of spent rice straw NDF was not different at 24 h (p>0.05), slightly lower at 48 h (p<0.05), and rather lower at 72 h (p<0.05) than for raw rice straw NDF.



**Figure 2.** The *in situ* ruminal disappearance of spent rice straw nutrients of *Agaricus bisporus*. <sup>ab</sup> Means with different superscripts within the same incubation time are significantly different (p<0.05).

The ruminal disappearances of spent rice straw CP were 79.4%, 132.3%, 70.3% and 63.9% higher than those of raw rice straw CP at 0, 24, 48 and 74 h of incubation, respectively (p<0.05).

#### **DISCUSSION**

#### Yield of the spent Agaricus bisporus substrates

Compared with about 210,000 tons (M/T) of annual yield of spent Agaricus bisporus substrates, those of spent substrates of king oyster mushrooms, oyster mushrooms, and collvibis velutipes were about 210,000 tons (M/T), 450,000 tons (M/T), and 310,000 tons (M/T), respectively, in South Korea (Kim et al., 2007). Williams et al. (2001) reported that 5 kg of spent Agaricus bisporus substrates was produced per kg of mushrooms grown on the vinyl bag type cultivation system. But, in the present study, 23.5 kg of spent Agaricus bisporus substrate was produced per kg of mushrooms on the bed type cultivation system. The yield of spent Agaricus bisporus substrates appeared to vary considerably by the cultivation system. In addition, it was recommended that a separation screen (or net) be placed between the upper soil layer and the lower substrate layer, and that most of the soil be removed, to promote the effective use of spent Agaricus bisporus substrates as animal feed.

# Chemical composition of spent *Agaricus bisporus* substrates

Crude ash content of soil-removed spent rice strawbased Agaricus bisporus substrates was 375 g/kg in the present study and similar to 351 g/kg for spent wheat straw of Agaricus bisporus reported by Fazaeli and Talebian-Masoodi (2006). The decrease in NDF and ADF contents of substrates during the Agaricus bisporus cultivation in the present study was also observed in the studies of Bakshi and Langar (1991) and Maeda et al. (1993). Seok (2009) reported that total digestible nutrients (TDN) of sawdustbased spent mushroom substrates was 30.4% and that of bed-type cultivated spent mushroom substrate, which consisted of 100% cotton waste, was 23.0%. In the present study, it was predicted that TDN of spent mushroom (Agaricus bisporus) substrates was lower than that of sawdust-based spent mushroom substrates and bed-type cultivated spent mushroom substrates due to its high ash and lignin contents. The Ca content of soil-removed spent substrates was nine times higher than for rice straw, a conventional roughage source in Asia (RDA, 2007). The high Ca content was attributed to the incorporation of 4.8% limestone (CaCO<sub>3</sub>) and 13.7% broiler litter containing high Ca levels (Kwak et al., 2008) into the substrates. The Ca and P contents in soil-removed spent substrates were much higher than those (7 and 4 g/kg, respectively) required in diets of growing cattle (NRC, 1996). In a study of Fazaeli and Talebian-Masoodi (2006), the Ca content of spent wheat straw of *Agaricus bisporus* was 54 g/kg, much higher than that of the present study. Langar et al. (1980) and Burton et al. (1994) reported that spent *Agaricus bisporus* substrates were rich in Ca, P, and K. The levels of Mn, Zn and Cu in soil-removed spent substrates were similar to those of rice straw presented in the Standard Feed Ingredients (RDA, 2007).

# In situ fractionation and degradability of spent rice straw nutrients

In the present study, the degradable DM and CP fractions in rice straw increased, but the degradable NDF fraction decreased during the cultivation of Agaricus bisporus. It is a general rule that high crude ash content depresses DM digestibility by animals. However, the watersoluble and degradable DM fractions of spent rice straw, despite their high crude ash contents, were higher than those of raw rice straw. These results were primarily due to the considerable decrease in cellulose and hemicellulose contents of spent rice straw (Table 2), which was attributable to the combination of the following: the breakdown of the ester bond between the undegradable ADL and the cellulose or hemicellulose caused by the chemical treatment effect by urea added to the substrates (Wanapat and Cherdthong, 2009); the degradation of lignocelluloses by mycelium during the cultivation (Makela et al., 2002); and mycelium's use of cellulose and hemicellulose preferentially over other ingredients. As a result, the degradable fraction of rice straw NDF decreased during the cultivation of Agaricus bisporus (p<0.05). These results were related with the increase in the proportion of undegradable ADL.

These changes in fractions affected predicted degradability of spent rice straw nutrients. Predicted degradabilities of DM and CP in spent rice straw were much higher and those of NDF were rather lower than for raw rice straw. In a similar study (Zadrazil, 1997), the *in vitro* DM digestibility of spent wheat straw of oyster mushrooms also increased by 4.4 to 8.9% compared to that of untreated straw.

### In situ ruminal disappearance of spent rice straw nutrients

Compared with raw rice straw, spent rice straw had much higher ruminal disappearances of DM and CP and a little lower disappearance of NDF. Since lignocelluloses were degraded by mycelium during the cultivation period (Makela et al., 2002), it could be expected that the fiber disappearance would be higher in the rumen. However, in the present study, the ruminal NDF disappearance of spent rice straw was lower than that of raw rice straw. These

results could explain the decreased total tract fiber digestibility of spent wheat straw of oyster mushrooms reported by Marwaha et al. (1990).

In the present study, ruminal CP disappearances at any given incubation times were consistently higher than those of raw rice straw. These results were related to the high levels of soluble and degradable fractions of spent rice straw CP. As a result, the quantity and quality of protein in mushroom substrates improved remarkably during the cultivation process of *Agaricus bisporus*.

#### **IMPLICATION**

Spent Agaricus bisporus substrates were characterized by a moderate content of protein (134 g/kg) and high contents of fiber and ash. The mycellial activity during the Agaricus bisporus mushroom cultivation increased protein contents and decreased fiber contents in the substrates. The desirable ruminal utilization of spent substrate nutrients indicated that the substrates could be used as a potential roughage source for ruminants. Spent rice straw of high CP solubility and low NDF degradability appears to be more suitable as feed for ruminants at low to medium production. Good management such as complete removal of surface soil is needed to improve the feed value of spent Agaricus bisporus substrates.

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