Potential of Fermented Sweet Corn Stover as a Substitute for Corncob in Mushroom (*Flammulina velutipes*) Substrate

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***Abstract*—In the present study, with a final goal of using spent mushroom substrates as roughage for cattle, mushroom cultivation experiment has been conducted on *Flammulina velutipes* using fermented sweet corn stover (FS) cut at the length of 13mm and 30mm as a substrate material (FS13 and FS30, respectively), and productivity and eating quality of fruiting bodies were evaluated. A total of nine substrates were prepared: control (composed mainly of ground corncob and rice bran), four FS13 groups (24, 48, 73 and 100% of rate of replacement with corncob, on dry matter basis) and four FS30 groups (the same replacement rates as in FS13 groups). The cultivation period increased in FS13 groups comparing with FS30 groups (P<0.05) and did with increasing replacement rate (P<0.01). Fruiting body yield was not affected by the length of FS, but increased gradually with increasing replacement rate, although decreased values were found when the rate was reached to 100%. Eating quality of the fruiting bodies was affected neither by length of FS nor by replacement rate. In conclusion, corncob can be replaced up to 73% with FS without showing any negative effect on productivity of *F. velutipes*.**

***Index Terms*—agricultural waste, corncob, *Flammulina velutipes*, mushroom, mushroom substrate, sweet corn stover**

I. Introduction

Edible mushrooms are produced worldwide: *Pleurotus ostreatus* (Oyster mushroom) is one of the most eaten mushrooms in western countries, but *Flammulina velutipes* in Japan [1]. Generally, substrates for *F. velutipes* are composed of a base material, such as corncob and sawdust, and a nutritional material, such as rice bran and wheat bran. However, in Japan, most of the materials, except rice bran, are dependent on imports and the Spent Mushroom Substrate (SMS) is discarded as a waste material, which indicates that sustainable production has not yet been achieved. So far, several studies have been conducted to use SMS as a substitute cattle feed, but practical feed made of SMS has not yet been developed, because of the poor nutritional value as concentrate feed [2]. In this context, we started a project to use SMS as roughage for cattle. It has been reported that the silage containing SMS can be used as a roughage for ruminants [3]-[5], although some adverse effects, such as reduced rumination and chewing activity, were observed [6], which may be because of the shorter fiber length of SMS used in this study: it is well known that there is a positive relationship between fiber length and ruminating time or total chewing time [7], [8]. If this assumption holds true, the above problem may be solved when materials having longer fiber length are used as base materials.

In this context, sweet corn stover may be a promising base material, because this has long fiber length and easily available with low-cost due to an unused by-product: usually, this is plowed back to the soil after sweet corn is harvested. In addition, this material has good water absorbency, which is necessary for a mushroom substrate and corn stem in the substrate of *P. ostreatus* showed a little adverse effect on its growth [9], [10]. On the minus side of this material, this has poor preservability, and hence ensiling is needed for the practical use.

The aim of the present study was to evaluate productivity and eating quality of fruiting bodies of *F. velutipes* cultivated on substrates contained fermented sweet corn stover (FS) as a base material and to discuss the maximal level of FS in conjunction with using spent mushroom substrates as roughage for cattle.

II. Materials and Methods

A. Preparation of Mushroom Substrate and Cultivation

1) Preparation of fermented sweet corn stover

The sweet corn stover was harvested in Nagano, Japan from August to September, 2015. They were pre-dried for 2 to 3 days in the shade, hammered to give flexibility and then cut into 13mm or 30mm length pieces (Fig. 1). The cut stover was mixed with a commercial silage starter (Si-

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Master AC, *Lactobacillus paracasei* SBS0003 + *Lactococcus lactis* SBS0001, Snow Brand Seed Co. Ltd., Japan). The stover was vacuum-packed into plastic bags (400mm×550mm). The bags were opened six months later and content (FS) was used as a material of mushroom substrate.

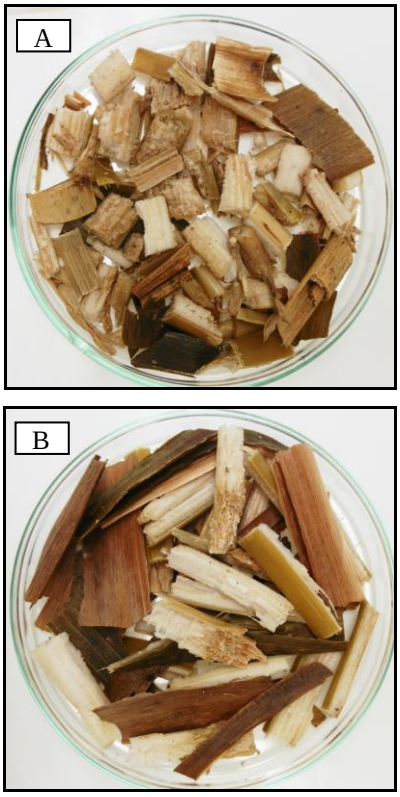

Figure 1. A: 13mm length pieces of fermented sweet corn stover, B: 30mm length pieces of fermented corn stover

2) Substrate preparation

The mushroom substrate composition in each group was given in Table I. Briefly, substrate in control group was composed of 67.3g of corncob and 124.5g of the common ingredients (rice bran, beet pulp, cotton seed hull, wheat bran, sorghum powder, oyster shell and dried tofu residue) on Dry Matter (DM) basis (Table I). The four substrates containing FS of 13mm (FS13) were made in substitution for corncob at the rate of 24, 48, 73 and 100% on DM basis. Another four substrates containing FS of 30mm (FS30) were made in the same manner as given above. Finally, a total of nine substrates were prepared, and all substrates contained 124.5g of the common ingredients (Table I). C/N ratio of ingredients of the mushroom substrate were measured using a CHNS/O Elemental Analyzer (2400 Series II, PerkinElmer, USA). The substrates were moistened with water to be the final moisture content of 60-70%, mixed well, and then filled into mushroom culture bottles (850ml). The surface of substrate was covered by 40.0g (fresh basis) of the mixture of corncob and rice bran at 3:1 ratio to make it flat. All bottles were autoclaved at 121 ℃ for 2h, cooled to 17 ℃. A inoculation hole was made in the center of substrate, and with 10.0g of spawn (*F. velutipes*) was applied in the hole.

TABLE I. COMPOSITION OF SUBSTRATES IN A BOTTLE[1]

Length of FS[2] (mm)	Replacement rate (%)	Corncob (g)[3]	FS (g)[3]	Amount of substrate (g)[4]	C/N ratio
Control	0	67.3	0.0	588.2	33
13 or 30	24	50.5	15.5	584.5	30
13 or 30	48	33.7	31.1	580.7	28
13 or 30	73	16.8	46.6	577.0	26
13 or 30	100	0.0	62.1	573.3	24

[1] Each bottle contained 63.4g of rice bran, 19.2g of beet pulp, 9.6g of cotton seed hull, 9.6g of wheat bran, 9.6g of sorghum powder, 7.3g of oyster shell and 5.8g of dried tofu residue (DM basis) and 40.0g mixture of corncob and rice bran at 3:1 ratio (fresh basis); [2] FS=fermented sweet corn stover; [3] DM basis; [4] Fresh basis.

3) Incubation and cultivation of mushroom

Mushroom cultivation was performed according to the standard method [1] with a slight modification. A total of 45 bottles (five bottles in each group) were incubated at 17 ℃ with relative humidity of 70-80% in dark condition. When 4 out of 5 bottles in each group were fully colonized, the surface mycelial layer was removed and the bottles were moved to a chamber set at 14 ℃ with relative humidity of 95% in dark condition. After primordium initiation, the bottles were moved to a chamber set at 8 ℃ with relative humidity of 95% for 2-3 days to acclimate them to low temperature in dark condition. After that, they were moved to a chamber set at 5 ℃ with relative humidity of 95% to retard their growth and to uniform their stem length and kept for about 10 days. During this period, illumination (300 lux) was performed for 30 min per day. Just after the end of this period, a plastic collar was fitted in the mouth of each bottle to elongate and straighten the mushroom stem and then the bottles were moved to a chamber set at 6 ℃ with relative humidity of 95% in dark condition. When fruiting body of *F. velutipes* grew up to about 12.5cm length, they were harvested. At the harvesting day, fruiting body weights were measured and Biological Efficiency (BE) was calculated: BE is defined as the grams of fruiting body yield (flesh basis) which produced per 100g of mushroom substrate (DM basis).

B. Eating Quality of the Fruiting Body

F. velutipes obtained from the present study and a mushroom company (Nagano, Japan) were used in the eating quality test. The latter was used as a standard sample. Eating quality test was performed according to the method described by Ref. [11] with a slight modification. Briefly, fruiting bodies were boiled with water for two minutes, and then placed on white plate. The panel members composed of 24 peoples of Faculty of Agriculture, Shinshu University, Japan ate standard sample and samples obtained from the present study, and then examined the following parameters: 1) texture preference, 2) umami intensity, 3) umami preference, 4) bitterness intensity, 5) sweetness intensity and 6) overall impression (Fig. 2). The scoring range was from -2 to 2, where the score 0 meant no difference in the parameter from standard sample.

Age range: (-19/20-29/30-39/40-49)
Sex: (Man/Woman)
Preference of enoki mushroom:
I (like/don't mind /dislike) to eat enoki mushroom.

	Sample name	Very -2	moderately -1	0	moderately +1	Very +2	Sample name	
Texture preference is bad	A						A	Texture preference is good
	B						B	
	C						C	
	D						D	
Umami intensity is weak	A						A	Umami intensity is strong
	B						B	
	C						C	
	D						D	
Umami preference is bad	A						A	Umami preference is good
	B						B	
	C						C	
	D						D	
Bitterness intensity is weak	A						A	Bitterness intensity is strong
	B						B	
	C						C	
	D						D	
Sweetness intensity is weak	A						A	Sweetness intensity is strong
	B						B	
	C						C	
	D						D	
Overall impression is bad	A						A	Overall impression is good
	B						B	
	C						C	
	D						D	

Figure 2. Sheet for eating quality test

C. Statistical Analysis

Statistical significances among the groups were determined with Tukey's multiple comparison tests at a significant level of 5% after one-way ANOVA. In addition, two-way ANOVA was done to test for main and interaction effects between length and replacement rate of FS.

III. Results and Discussion

A. Productivity of Mushroom

Effects of FS on productivity of *F. velutipes* are shown in Table II. All FS groups required longer cultivation period than control group ($P<0.05$). In this connection, Ref. [12] reported that *Pleurotus* sajor-*caju* grew faster on ground rice straw than on cut one. This may explain, in part, the reason why control group (composed of small particles) required shorter cultivation period than FS groups (composed of longer materials). On the other hand, longer length (FS30) groups required a little shorter cultivation period than shorter length (FS13) groups, which is contrary to the result given above. Ref. [13] also reported that *F. velutipes* grew faster on cut straws (10mm) than on ground sawdust (1.0 to 1.4mm). It is difficult to discuss this discrepancy, but it seems that the smaller particle size does not always lead to the better results.

TABLE II. Effects of Fermented Corn Stover on Productivity of *Flammulina velutipes*

Substrates		Cultivation period (days)[2]	Fruiting body yield (g)[2]	Biological Efficiency[2]
Length of FS (mm)[1]	Replacement rate (%)			
Control	0	57.2 ± 0.2^{a}	218.8 ± 6.2^{a}	89.2 ± 2.5^{abc}
13	24	64.8 ± 0.6^{bc}	204.2 ± 16.7^{ab}	99.1 ± 8.1^{abcd}
30	24	62.0 ± 1.2^{c}	206.0 ± 11.0^{ab}	90.0 ± 4.8^{abc}
13	48	67.6 ± 0.7^{bd}	219.5 ± 6.5^{a}	97.8 ± 2.9^{abcd}
30	48	64.4 ± 0.4^{cd}	233.5 ± 8.1^{a}	116.2 ± 4.0^{bde}
13	73	68.6 ± 0.5^{b}	233.1 ± 4.9^{a}	132.8 ± 2.8^{e}
30	73	66.6 ± 0.4^{bd}	238.2 ± 3.5^{a}	122.2 ± 1.8^{de}
13	100	76.8 ± 1.1^{e}	150.1 ± 16.3^{b}	74.7 ± 8.1^{ac}
30	100	75.6 ± 0.9^{e}	190.4 ± 15.7^{ab}	97.1 ± 8.0^{abcd}
Contrasts		----------Probabilities----------		
Length		*	NS	NS
Replacement rate		**	*	NS
Length × Replacement rate		NS	NS	*

[1] FS=Fermented sweet corn stover; [2] Values for each parameter represent mean ± standard error values (n=5); [a-e] Means within the same column with different superscripts are significantly different ($P<0.05$); * $P<0.05$; ** $P<0.01$; No significant.

The replacement rate affected negatively the cultivation period, which increased with increasing replacement rate in both FS13 and FS30 groups ($P<0.01$) and reached maximum when 100% of corncob was replaced with FS (Table II). The longest cultivation period was found in group of FS13-100% which was 19.6 days longer than control group ($P<0.05$). In this respect, Ref. [14] reported that lactic acid reduced mycelial growth of *Lentinula edodes* and *Hypsizygus tessellatus*. If the same is true in *F. velutipes*, lactic acid in FS may be a possible factor to delay the mycelial growth and hence elongate the cultivation period. To prove this hypothesis, cultivation experiment on *F. velutipes* using FS containing reduced level of lactic acid by washing it is needed.

In the present study, the length of FS affected little on the fruiting body yield, which is similar to the observation in *P. sajor-caju*: Ref. [12] found length of cut

straw (25mm and 50mm) had no significant effect on the fruiting body yield of this mushroom.

Interestingly, the replacement rate affected the fruiting body yield of *F. velutipes* significantly: the yield increased with the increasing replacement rate, although the best yield was obtained in FS30-73% group and the yield decreased in 100% groups (Table II). The reason for this decrease in the yield in 100% groups is obscure, but it may be worth investigating whether C/N ratio, pH and lactic acid level in FS can be the factors, because C/N ratio and pH reached minimum and lactic acid level may reach maximum in 100% groups. In addition, the best BE value was obtained in 73% groups, suggesting that FS can be better base material for *F. velutipes* than corncob.

B. Eating Quality of the Fruiting Body

The length and replacement rate affected little all parameters although overall impression was tended to increase in control group (Table III), suggesting that FS in the substrate had little effect on eating quality of fruiting bodies of *F. velutipes*.

TABLE III. EFFECTS OF FERMENTED CORN STOVER ON EATING QUARITY OF THE FRUITING BODY OF *FLAMMULINA VELUTIPES*

Substrates		Texture preference[2]	Umami Intensity[2]	Umami preference[2]	Bitterness intensity[2]	Sweetness intensity[2]	Overall Impression[2]
Length of FS[1] (mm)	Replacement rate (%)						
Control	0	0.08 ±0.10	0.26 ±1.07	0.09 ±1.02	-0.08 ±0.10	0.21 ±1.00	0.29 ±1.02
13	24	-0.19 ±0.68	-0.23 ±0.80	-0.15 ±0.95	-0.35 ±0.92	-0.08 ±0.92	-0.12 ±1.07
30	24	0.08 ±0.92	-0.04 ±1.06	-0.12 ±1.12	-0.27 ±1.16	0.12 ±1.28	0.08 ±1.02
13	48	0.20 ±1.13	-0.36 ±0.84	0.20 ±0.69	-0.20 ±0.94	-0.04 ±0.96	0.24 ±1.03
30	48	-0.23 ±0.93	-0.19 ±0.88	0.04 ±0.81	-0.20 ±1.06	0.13 ±0.90	-0.04 ±1.02
13	73	0.08 ±0.98	-0.36 ±0. 2	-0.36 ±0.97	-0.48 ±0.98	0.04 ±1.08	0.04 ±1.18
30	73	0.42 ±0.88	0.32 ±0.97	-0.12 ±1.03	-0.04 ±1.09	0.08 ±0.92	-0.08 ±0.93
13	100	0.12 ±0.82	0.00 ±0.98	0.04 ±0.72	-0.36 ±0.79	-0.20 ±0.85	-0.08 ±0.74
30	100	0.00 ±0.78	-0.23 ±0.80	0.15 ±0.72	-0.04 ±0.85	0.27 ±0.94	0.12 ±0.89

[1] FS=fermented corn stover; [2] Values for each parameter represent mean ± standard error values (n=24).

IV. CONCLUSION

The results obtained here suggest that corncob can be replaced up to 73% with FS without negative effect on the fruiting body yield of *F. velutipes*, although the cultivation period increased with increasing replacement rate. On the other hand, length of FS had no effect on productivity except cultivation period: FS30 groups required a little shorter cultivation period than FS13 groups. In addition, FS in the substrate had little effect on eating quality of fruiting bodies. In conclusion, these finding may be worth to use spent mushroom substrate as roughage for cattle, if the negative effects of FS on cultivation period can be eliminated.

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