



## Effects of Supplementing Microbially-fermented Spent Mushroom Substrates on Growth Performance and Carcass Characteristics of Hanwoo Steers (a Field Study)

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**ABSTRACT:** This study evaluated the effect of dietary supplementation of microbially-fermented spent mushroom substrates (MFSMS) on weight gain, carcass characteristics, and economic efficiency of Hanwoo steers. Highly cellulolytic bacteria (*Enterobacter* spp. and *Bacillus* spp.) isolated from spent mushroom substrates (SMS) stacks were inoculated (1% v/v) into the SMS, which was anaerobically fermented and fed to the steers for 12.6 months during the growing and fattening periods. Growing Hanwoo steers were assigned to the control group without supplementation of Microbially-fermented SMS (MFSMS), to a treatment group with 50% of MFSMS (1/2 of the *ad libitum* group), and to a treatment group with *ad libitum* access to SMS (the *ad libitum* group). All the groups were fed the formulated feed and rice straw. The voluntary intake (DM basis) of MFSMS was 1.6 kg/d during the growing period and 1.4 kg/d during the fattening period. The voluntary rice straw intake decreased by 6 to 11%, but the total voluntary DMI increased by 7 to 15% with MFSMS fed. The increased DMI with MFSMS supplementation resulted in a tendency of increased ( $p = 0.055$ ) live weight gain by 8 to 12% compared with the control group. At slaughtering, the supplementation of MFSMS increased ( $p < 0.05$ ) the ribeye area by an average of 10 cm<sup>2</sup>. In conclusion, feeding MFSMS improved growth performance and carcass traits of Hanwoo steers and could successfully replace a part of conventional roughage such as rice straw commonly used in Asian countries. (**Key Words:** Spent Mushroom Substrates, Spent Mushroom Compost, By-product Feed, Beef Cattle)

### INTRODUCTION

Annually about 660,000 tons of spent mushroom (*Pleurotus eryngii*) substrates are produced in Korea. It is usually composed of sawdust, corn cob, and rice bran. Its chemical composition was 73.6% neutral detergent fiber (NDF), 55.0% acid detergent fiber (ADF), 8.1% crude protein (CP), 2.1% ether extract (EE), 9.8% non-fibrous carbohydrate (NFC), and 6.4% crude ash (Kim et al., 2007a), and based on this, the spent mushroom substrates (SMS) has potential as a feed resource for livestock.

For the effective use of SMS as animal feed, there is need to improve its preservative quality as it typically contains about 60% moisture (Kim et al., 2007a) and thus,

putrefies rapidly (Kwak et al., 2008). Ensiling the material under anaerobic conditions prevents putrefication (Kwak et al., 2008) and improves the preservative quality (Kim et al., 2007b; Kim et al., 2008b). In previous studies Kim et al. (2007b; 2008a; 2008b) have identified the presence of highly cellulolytic bacteria in the SMS. Furthermore, it was found that SMS exhibited good preservative quality for eight weeks after inoculation with the identified bacteria and fermenting agents. The change in the nutritional constituents was found to be slight, and after eight weeks of anaerobic storage, the number of microorganisms such as yeast and lactobacillus remained high at 10<sup>8</sup> cfu/g. These results suggested that the microbes inoculated into SMS could be used also as direct-fed microbes (DFM) for livestock.

In the aspect of DFM, Krebiel et al. (2003) clearly described the benefit of supplementation with DFM such as improved weight gain and feed efficiency in ruminants. Currently available commercial DFM are expensive, making them impractical to feed to livestock. It was

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hypothesized that if low-priced DFM produced from SMS were fed to Hanwoo steers, their effect could be maximized. In addition, such low-priced DFM culture could partly replace conventional roughage (rice straw) because of the high amount of cellulose contained in SMS. As fresh SMS is mostly used as an ingredient of total mixed rations (TMR), more diverse usages need to be explored. At present, there have been no attempts to use Microbially-fermented SMS (MFSMS) as a component of the ration for growing and fattening beef steers.

In this context, a field study was performed to evaluate the effect of dietary supplementation of MFSMS on the weight gain, carcass characteristics, and economic efficiency of growing and fattening Hanwoo steers.

## MATERIALS AND METHODS

### Microbial fermentation of SMS

The spent substrates used to grow mushrooms (*Pleurotus eryngii*) were used as the test feed. The original substrates used to grow mushrooms consisted of 49% sawdust, 32% rice bran, and 19% corn cob. Fresh, spent substrates from a commercial mushroom farm located at the suburb of Chung-ju City, Korea were obtained on the day of mushroom harvest and transferred to the Konkuk University Experimental Station. The SMS were screened using a 20 mm×20 mm (width×length) sieve to remove lumps and foreign materials. Four strains of bacteria (*Enterobacter ludwigii*, *Bacillus cereus*, and two strains of *Bacillus subtilis*) were cultured for 12 h in plate count broth, and then inoculated (1% v/v) into the SMS. These bacterial species had been previously isolated and identified from the SMS and were found to have high CMCase and xylanase activities (Kim et al., 2007b; 2008b). The inoculated SMS was packed into plastic bags inside an empty feed sack and sealed to prevent air from getting in. The material was left to undergo fermentation for more than two weeks at the ambient temperature, and then the fermented SMS (total bacteria count,  $10^8$  to  $10^9$  cfu/g) was fed to Hanwoo steers.

### Animals and treatment

Eighteen Hanwoo steers (nine months old and  $227 \pm 20$  kg) were selected and randomly assigned to a control group (without SMS supplementation), a treatment group with 50% of SMS (the 1/2 of *ad libitum* group), and a treatment group with *ad libitum* access to SMS (the *ad libitum* group).

All of the groups were fed the same amount of formulated feed. The growing period was from nine to 13 months of age, during which the steers were fed the formulated feed at an average amount of 1.75% (as-fed basis) of the body weight. The fattening period was from 14 to 22 months of age, during which the steers were fed the formulated feed at an average amount of 1.85% of the body weight. The finishing period was from 24 to 30 months of age, during which the steers were restricted-fed the formulated feed at 9 kg/d. The formulated feed was restricted-fed during the growing and fattening period in order to prevent excessive gain and during the finishing period in order to prevent excessive back fat thickness.

During the growing and fattening periods, steers had free access to rice straw; however, during the finishing period, the steers were restricted to 1.0 kg/d (as-fed basis) of rice straw. The SMS was fed only during the growing and fattening periods by top-dressing it on the formulated feed. The SMS were not fed during the finishing period in order to meet the maximum energy requirement with high energy-containing formulated feed, which is normal feeding practice in this period. Steers had free access to fresh water during the entire feeding trial. The chemical composition of the formulated feed, rice straw and SMS is shown in Table 1. The CP and EE contents were slightly higher in SMS than in rice straw. The crude ash content of SMS was 6%, equating to about half that of the rice straw. The NDF content of SMS was similar to that of rice straw. Physically, the mean particle size of SMS was 4 mm, being much smaller than that of the rice straw.

### Sampling and analysis

Representative samples of the test feeds and the formulated feed that were fed to the Hanwoo steers were

**Table 1.** Chemical composition of feeds fed to Hanwoo steers<sup>1</sup>

Item	Formulated feed			Rice straw	MFSMS <sup>2</sup>
	Growing	Fattening	Finishing		
	----- % -----				
Dry matter	87.2	88.1	90.1	85.5	41.2
Crude protein	14.9	15.5	12.4	4.2	5.7
Ether extract	3.8	4.2	3.3	0.4	1.4
Crude ash	8.4	8.3	6.7	12.1	6.0
Neutral detergent fiber	37.6	33.8	25.2	75.7	78.8
Acid detergent fiber	20.9	20.2	13.3	44.2	66.0
Hemicellulose	16.7	13.6	11.9	31.5	12.8
Non-fibrous carbohydrate	35.3	38.2	52.4	7.6	8.1

<sup>1</sup> On dry matter basis. <sup>2</sup> MFSMS = Microbially-fermented spent mushroom substrates.

collected and stored in a freezer at  $-20^{\circ}\text{C}$  (Lassele LOC-520F, Korea) until the analysis. The samples were thawed in the refrigerator prior to analysis. Dry matter (DM), CP, EE, crude fiber (CF), NDF, acid detergent fiber (ADF) and crude ash were analyzed according to the AOAC method (2000). Non-fibrous carbohydrate was calculated using the formula  $100 - (\text{NDF} + \text{CP} + \text{EE} + \text{crude ash})$ .

The body weight (BW) of the steers was measured monthly. The back fat thickness and the marbling degree were measured at 24 months of age using an ultrasound (Aloka SSD-500, USA). Steers were slaughtered at 31 months of age. Following a 48-h carcass chill, yield and quality grade of each carcass were measured using Korean carcass grading standards specified in Korean Livestock Enforcement Regulation (KMAF, 2007).

### Statistical analysis

The General Linear Model was used for the statistical analysis (Statistix 7, 2000). To compare the mean values, orthogonal contrast was performed as follows: the control vs the 1/2 of *ad libitum* group and the *ad libitum* group; and the 1/2 of *ad libitum* group vs the *ad libitum* group.

## RESULTS AND DISCUSSION

### Feed intake and weight gain of the Hanwoo steers

The feed intake of the growing Hanwoo steers with SMS supplementation is shown in Table 2. Under the formulated feed and rice straw feeding system, when SMS was top-dressed on formulated feed, the daily voluntary intake of SMS was 1.6 kg in the growing period and 1.4 kg

**Table 2.** Effect of dietary supplementation of microbially-fermented spent mushroom substrates (MFSMS) on feed intake of Hanwoo steers<sup>1</sup>

Item	Control	Feeding MFSMS	
		1/2 of <i>ad libitum</i>	<i>Ad libitum</i>
Growing period (kg/d)			
Formulated feed	4.53	4.53	4.53
Rice straw	2.91	2.74	2.57
MFSMS	-	0.82	1.64
Total DM intake	7.44	8.09	8.74
Total CP intake	0.80	0.84	0.88
Fattening period (kg/d)			
Formulated feed	7.31	7.31	7.31
Rice straw	2.31	2.14	1.97
MFSMS	-	0.71	1.42
Total DM intake	7.44	8.09	8.74
Total CP intake	1.23	1.26	1.30
Finishing period (kg/d)			
Formulated feed	8.29	8.29	8.29
Rice straw	0.86	0.86	0.86
Total DM intake	9.14	9.14	9.14

<sup>1</sup> Dry matter basis.

in the fattening period, which was equivalent to about 11 to 16% of the total DM intake (DMI).

When SMS was supplemented, the total DMI increased by 7 to 15%; however, rice straw intake decreased by 6 to 11% during the growing period. The Hanwoo steers showed relatively high preference for the MFSMS. The increase in total DMI was a reflection of the high SMS intake because feeding the formulated feed was equally restricted to all experimental steers during the growing period. Also, it is believed that because of the decreased intake of rice straw with long particle size and the increased intake of SMS with a relatively small particle size of 4 mm, the mean particle size of the ingested feed decreased, which probably resulted in increased DMI. Jaster and Murphy (1983) and Kato et al. (1989) reported that when steers were fed with a feed with a small particle size, total DMI increased. As the NDF content of SMS (78.8%) was 3.1% higher than that of the rice straw, the total NDF intake by the treatment groups was 5.5 kg/d, which reflects a 22.3% increase from 4.5 kg/d in the control group. Generally, if NDF intake increases, DMI decreases (Forbes, 1995; Lee et al., 2002). In the present study; however, both NDF intake and DMI increased with SMS supplementation. Although the mean particle size of SMS was 4 mm, larger than the 1.18 mm that Sniffen et al. (1992) suggested as the minimum particle size that is capable of inducing rumination, it was much smaller than that of the rice straw. It can thus be postulated that the increased DMI was due to the faster movement of the SMS to the lower intestine due to their small particle size. Supplementing with SMS increased total CP intake by 5 to 10% during the growing period and by 3 to 6% during the fattening period.

The effect of SMS supplementation on the growth performance of Hanwoo steers is shown in Table 3. During the growing period, supplementing SMS did not affect weight gain ( $p > 0.05$ ). During the fattening period, the 1/2 of *ad libitum* group gained more than the *ad libitum* group ( $p < 0.05$ ). During the finishing period, all the SMS-fed groups tended ( $p = 0.061$ ) to have approximately 24 to 40 kg more weight gain compared to the control group. As a result, the BW of the animals over the whole period was 8 to 12% (approximately 36 to 52 kg/head) more in the SMS-supplemented groups than in the control group. These results were attributed to the increased DM and CP intakes and possibly to the favorable effect of the microbes fed. Huck et al. (2000) reported that the weight gain of feedlot cattle with added direct-fed microbes (DFM) increased by 2.5 to 3.0%. Galyean et al. (2000) also reported that dietary addition of DFM increased the body weight of feedlot cattle. It is believed that feeding MFSMS favorably affected the growth of Hanwoo steers because it functions as sources of roughage as well as DFM.

**Table 3.** Effect of dietary supplementation of microbially-fermented spent mushroom substrates (MFSMS) on growth performance of Hanwoo steers<sup>1</sup>

Item	Control (A)	Feeding MFSMS		SE	Significance of contrast	
		1/2 of <i>ad libitum</i> (B)	<i>Ad libitum</i> (C)		A vs. B+C	B vs. C
----- kg -----						
Growing period						
Initial BW (9.6 mo old)	227.0	226.8	228.3	12.3	0.9600	0.8991
Final BW (14.6 mo old)	356.3	361.0	354.3	13.8	0.9102	0.6329
Gain	129.3	134.3	125.9	7.6	0.9005	0.2880
ADG	0.84	0.88	0.82	0.05	0.9010	0.2907
Fattening period						
Initial BW (14.6 mo old)	356.3	361.0	354.3	13.8	0.9102	0.6329
Final BW (22.2 mo old)	555.8	584.5	553.3	21.4	0.4918	0.1666
Gain <sup>a</sup>	203.3	223.5	199.1	9.8	0.3612	0.0252
ADG	0.88	0.97	0.86	0.04	0.3595	0.0755
Finishing period						
Initial BW (22.2 mo old)	555.8	584.5	553.3	21.4	0.4918	0.1666
Final BW (31.0 mo old)	659.8	712.2	697.5	26.4	0.0656	0.5820
Gain	104.0	127.7	144.2	18.1	0.0610	0.3779
ADG	0.41	0.46	0.53	0.06	0.1437	0.3287
Whole period						
Initial BW (9.6 mo old)	227.0	226.8	228.3	12.3	0.9600	0.8991
Final BW (31.0 mo old)	659.8	712.2	697.5	26.4	0.0656	0.5820
Gain	433.4	485.4	469.2	24.2	0.0551	0.5132
ADG	0.68	0.73	0.71	0.04	0.2030	0.5902

<sup>1</sup> Least square means of 6 observations.<sup>a</sup> 1/2 of *ad libitum* group differs from *ad libitum* group.

### Carcass characteristics

An ultrasound scanning test was conducted when the Hanwoo steers were 24 months of age. The back fat thickness and marbling score are shown in Table 4. Supplementing SMS did not affect the back fat thickness ( $p>0.05$ ) with the fat thickness of all steers being in the range of 7.1 to 9.8 mm. Kim et al. (2003) reported a similar level ( $9.3\pm 0.4$  mm) at 24 months of age. Supplementing MFSMS did not affect marbling score at 24 months of age ( $p>0.05$ ).

All the experimental steers were slaughtered at 31 months of age; the carcass characteristics are shown in Table 5. Supplementing SMS did not affect the cold carcass weight, yield index and yield grade ( $p>0.05$ ). However, the ribeye area increased ( $p<0.05$ ) by 9.5 to 10.3 cm<sup>2</sup> by supplementing MFSMS. The growth of ribeye area has

been known to be affected by age, body weight and feed. The active growth of Hanwoo ribeye area reaches a peak at around 11 months of age and its growth continues until 18 months of age (HFMC, 2009). Okumura et al. (2007) reported that the ribeye area of Japanese Black steers (twins) was not different between 24 and 30 months of age. Thus, muscle growth seems complete before maturity. The feeding management during the growing and early fattening period seems to be important to maximize ribeye area. Ribeye area has been found to be correlated positively with weight gain (Kang et al., 1995), slaughter weight up to 24 months of age of Hanwoo steers (Moon et al., 2003) and cold carcass weight of Hanwoo steers (Park et al., 2002) and goats (Palmieri et al., 2012). Dietary protein levels may or may not affect ribeye area depending upon experimental conditions. Feeding high levels of protein in the early

**Table 4.** Effect of dietary supplementation of microbially-fermented spent mushroom substrates (MFSMS) on back fat thickness and marbling score of Hanwoo steers measured by ultrasound scanning<sup>1,2</sup>

Item	Control (A)	Feeding MFSMS		SE	Significance of contrast	
		1/2 of <i>ad libitum</i> (B)	<i>Ad libitum</i> (C)		A vs B+C	B vs C
Back fat thickness (mm)	9.8	9.5	7.1	1.6	0.3040	0.1420
Marbling score	2.4	2.2	2.3	0.5	0.7188	0.7550

<sup>1</sup> Means of 6 observations. <sup>2</sup> Ultrasound scanning was measured at 24 months of age.

**Table 5.** Carcass characteristics of Hanwoo steers supplemented with microbially-fermented spent mushroom substrates (MFSMS)<sup>1</sup>

Item	Control (A)	Feeding MFSMS		SE	Significance of contrast	
		1/2 of <i>ad libitum</i> (B)	<i>Ad libitum</i> (C)		A vs B+C	B vs C
Marketing weight (kg)	659.8	712.2	697.5	26.4	0.0656	0.5820
Cold carcass weight (kg)	396.6	414.5	400.7	16.9	0.4649	0.4263
Yield traits <sup>2</sup>						
Back fat thickness (mm)	17.4	17.3	11.5	4.0	0.4002	0.1639
Ribeye area (cm <sup>2</sup> ) <sup>a</sup>	77.4	86.8	87.7	4.1	0.0150	0.8420
Yield index	61.1	61.9	66.0	2.8	0.2568	0.1712
Yield grade (A:B:C)	1:2:2	0:4:2	1:4:1	-	-	-
Quality traits <sup>3</sup>						
Marbling score	5.2	4.5	5.7	0.8	0.8656	0.1575
Meat color	4.6	5.0	5.0	0.2	0.0502	-
Fat color	3.0	2.8	3.0	0.1	0.5057	0.2564
Texture	1.6	2.0	2.0	0.3	0.0761	-
Maturity	2.0	2.0	2.0	-	-	-

<sup>1</sup> Least square means of 6 observations. <sup>2</sup> Ribeye areas and back fat thickness were measured at 13th rib.

<sup>3</sup> Marbling score ranges are 1 to 9 (1 = devoid, 9=abundant); meat color (1 = brightly cheery red, 7 = extremely dark red); fat color (1 = white, 7 = dark yellow); Texture (1 = soft, 3 = firm); Maturity (1 = youthful, 9 = mature).

<sup>a</sup> Control group differs from 1/2 of *ad libitum* and *ad libitum* groups.

fattening stage did not affect ribeye area of Japanese Black heifers (Okumura et al., 2008). Feeding 12 to 18% dietary CP from 10 to 20 months old did not affect ribeye area of Japanese Black steers (Iwamoto et al., 2010). Although feeding 11.5 to 14.5% dietary CP levels did not affect ribeye area, CP source (urea or cottonseed meal) affected ribeye area of finishing beef steers (Gleghorn et al., 2004). However, feeding more concentrate feed with high CP during the growing period has been shown to increase ribeye area of British breed beef steers (Sainz et al., 1995). In the present study, the SMS-fed steers had a higher dietary CP intake, heavier weight and greater ribeye area ( $p < 0.05$ ). The increased ribeye area of SMS-fed steers was attributed to the increased CP intake under the low dietary CP (10.4%) and restricted concentrates feeding situation in the growing and early fattening stage of Hanwoo steers.

The back fat thickness of the *ad libitum* group steers was 6 mm less than the 1/2 of *ad libitum* group steers, although the difference was not significant ( $p > 0.05$ ) due to the large variations between animals. When compared with the ultrasound results (Table 4), the increase in the back fat thickness at 31 months of age (seven months after the body weight measurement using the ultrasound test) differed considerably between individuals. The mean increase in the back fat thickness was 6.66 mm. During the same period, the marbling score increased by an average of 2.9 grades although the difference was not significant among treatments.

Meat color, fat color, texture and maturity were not affected by supplementing SMS ( $p > 0.05$ ). These scores of both the control and the treatment groups were in the

normal range. Ware et al. (1988) also reported that although supplementation with DFM increased the BW of steers, it did not have any influence on the marbling score.

#### Economic efficiency

The effect of supplementation with SMS on the economic efficiency of Hanwoo steers is shown in Table 6. The mean gross income from selling a Hanwoo steer in the treatment groups was 4.1 to 4.8% higher than that in the control group. The feed cost per 1 kg weight gain of Hanwoo steers was 13.9% lower for the 1/2 *ad libitum* group and 9.6% lower for the *ad libitum* group than that for the control group ( $p < 0.05$ ). The mean net income per Hanwoo steer in the treatment groups was 84 to 65% higher than that of the control group. These results were attributed to both the feed cost savings and the increased BW by SMS supplementation.

#### IMPLICATION

Microbial fermentation seemed to improve palatability and stable storage of fresh SMS. The MFSMS would be able to effectively replace not only a part of conventional low-quality roughage such as rice straw but also microbial additives for beef cattle. Either restricted or *ad libitum* feeding of SMS showed similar growth performance and carcass traits in Hanwoo steers. The MFSMS may be used as a cheap ingredient of TMR for ruminants. The MFSMS can be successfully recycled as an animal feed in mushroom-cultivating and feed-lacking Asian countries.

**Table 6.** Effect of dietary supplementation of microbially-fermented spent mushroom substrates (FMSMS) on economic profit of Hanwoo steers<sup>1</sup>

Item	Control (A)	Feeding FMSMS		SE	Significance of contrast	
		1/2 of <i>ad libitum</i> (B)	<i>Ad libitum</i> (C)		A vs B+C	B vs C
----- 1,000 won -----						
Gross receipts <sup>2</sup>	5,621	5,893	5,850	205	0.3356	0.8846
Operating cost	5,159	5,043	5,089	-	-	-
Calf <sup>3</sup>	2,319	2,319	2,319	-	-	-
Feed	2,840	2,724	2,770	-	-	-
Net income <sup>4</sup>	462	850	761	205	0.1938	0.7626
Feed cost/gain (kg) <sup>a</sup>	6.57	5.66	5.94	0.31	0.0124	0.3807

<sup>1</sup> On the basis of concentrate 450 won/kg, rice straw 290 won/kg, and microbially-fermented spent mushroom substrates 80 won/kg.

<sup>2</sup> Selling price of carcass by grade and by-product. <sup>3</sup> Average purchased price of calf in the livestock market.

<sup>4</sup> Net income = Gross receipts-operating cost.

<sup>a</sup> Control group differs from 1/2 of *ad libitum* and *ad libitum* groups.

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