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Effects of Applying Microbial Additive Inoculants to Spent Mushroom Substrate (*Flammulina velutipes*) on Rumen Fermentation and Total-tract Nutrient Digestibility in Hanwoo Steers

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팽이버섯 부산물 발효에 따른 한우 거세우 반추위 성상 및 소화율에 미치는 영향

백열창 · 정진영 · 오영균 · 김민석 · 이성대 · 이현정 · 도윤정 · Farhad Ahmadi · 최혁

We inoculated a spent mushroom substrate from *Flammulina velutipes* (SMSF) with a microbial additive and assessed the effects on chemical composition, ruminal fermentation parameters, and total-tract nutrient digestibility. In Exp. 1, three cannulated Hanwoo steers were used in an *in situ* trial to determine the degradation kinetics of dry matter (DM) and crude protein (CP). In Exp. 2, three Hanwoo steers were randomly assigned to experimental diets according to a 3 × 3 Latin square for a 3-week period (2 weeks for adaptation and 1 week for sample collection). Experimental diets included the control diet (3.75 kg/d formulated concentrate mixture + 1.25 kg/d rice straw), SMSF diet (3.19 kg/d formulated concentrate mixture + 1.25 kg/d rice straw + 0.56 kg/d SMSF), and inoculated SMSF (ISMSF) diet (3.19 kg/d formulated concentrate mixture + 1.25 kg/d rice straw + 0.56 kg/d ISMSF). The chemical composition of ISMSF did not differ from that of SMSF. Microbial additive inoculation decreased pH ($P<0.05$) and

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improved preservation for SMSF. The percentages of DM, neutral detergent fiber (NDF), and acid detergent fiber (ADF) in ISMSF were slightly lesser than those in SMSF. Ruminal fermentation characteristics and total-tract nutrient digestibility were not affected by diet. Overall, microbial additive inoculation improved preservation for SMSF and may allow improved digestion in the rumen; however, the total digestible nutrients (TDN) of SMSF and ISMSF diets were slightly lesser than the control diet. The ISMSF can be used as an alternative feedstuff to partially replace formulated concentrate feed.

Key words : *agriculture by-products, feed cost, feed preservative, silage, total tract digestibility, volatile fatty acid*

Ⅰ. Introduction

Feed is the most crucial factor in the total cost of beef production, and reducing feed costs therefore increases the profit margin for beef cattle farm (Liu et al., 2000). The use of food by-products as animal feed may contribute to decreased feed costs (Oishi et al., 2011). Spent (used) mushroom substrate (SMS) is an abundant and cheap by-product of mushroom cultivation that has a potential application as animal feed (Phan and Sabaratnam, 2012; Kim et al., 2015a; Kim et al., 2015b).

The spent mushroom substrate is a rich source of protein, plant residues, and nutrients that can be used as ruminant feed (Fazaeli and Masoodi, 2006; Bae et al., 2006). Because enzymatic conversion processes can modify the fiber structure of raw materials during mushroom cultivation (e.g., a 50% reduction in cellulose and a 30% reduction in lignin), SMS is more digestible for the animals (Adamovieć et al., 1998; Phan and Sabaratnam, 2012). Although SMS putrefies quickly due to its high moisture content, but ensiling SMS under anaerobic conditions improves preservation (Kwak et al., 2008).

A previous study in our laboratory showed that microbial inoculation improved the preservative quality of SMS from *Pleurotus ostreatus,* and increased *in situ* rumen degradation of DM, CP, NDF, and ADF compared with uninoculated SMS (unpublished). Similarly, Kwak et al. (2009) reported that molasses and microbial inoculants improve the silage fermentation quality of cotton-waste-based spent mushroom substrate.

Although *Flammulina velutipes* is one of the most cultivated edible mushrooms in Korea, to our knowledge, few studies have investigated the nutrient quality, ruminal fermentation, and digestibility of spent mushroom substrate from *F. velutipes* (SMSF). Therefore, the present study investigated the effects of a microbial additive on feed quality and ruminal fermentation of SMSF.

Ⅱ. Materials and methods

1. Inoculation of SMSF with microbial additive

The original mushroom substrate consisted of 36.6% rice bran, 35.1% corn cob, 8.6% beet pulp, 6.9% wheat bran, 5.2% cotton seed hull, 3.8% dried tofu cake, and 3.8% pearl. The SMSF was inoculated with a microbial additive according to a modified procedure (Kim et al., 2014). Briefly, 99.0% SMSF was mixed with microbial additive (1% v/w to supply 1.0×10^5 cfu/g, consisting of two strains of *Bacillus* sp. UJ03 and *Saccharomyces cerevisiae* UJ14, friendly donated by Dr. S. J. Cho, Gyeongnam National University of Science and Technology; Gal and Cho, 2011). Next, approximately 5 kg of the mixture was packed into polyvinyl bags and sealed. The inoculated SMSF (ISMSF) was fermented at 20℃ for 5 days and stored at room temperature, and then the inoculated SMSF (total bacteria count, 1.0×10^8 to 10^9 cfu/g) was used to experiments. Visual inspections and pH measurements were made at 0, 1, 24, 48, 72, 96 and 120 h. The chemical compositions of test diets (SMSF and ISMSF) are shown in Table 1.

Table 1. Chemical composition of test diets

Item (%)	Diets[1]	
	SMSF	ISMSF
Dry matter	41.6	41.6
Crude protein	9.9	10.4
Ether extract	5.2	5.7
Crude fiber	23.5	22.8
Crude ash	11.8	11.9
Nitrogen free extract	49.5	49.2
Neutral detergent fiber	52.3	50.1
Acid detergent fiber	29.3	28.6
Gross energy (Kcal/g)	4.7	4.9

[1] SMSF, spent mushroom substrate from *Flammulina velutipes*; ISMSF, SMSF inoculated with microbial additive.

2. Exp. 1: *In situ* experiments

All procedures involving animals were approved by the Animal Ethics Committee of the National Institute of Animal Science. Three ruminally cannulated Hanwoo steers (mean body

weight = 408 ± 13.0 kg) were housed in individual tie-stalls (127 × 250 × 200 cm). The steers were fed a diet consisting of 3.75 kg/d of a formulated concentrate mixture and 1.25 kg/d of rice straw twice daily (at 0900 and 1700 h). The diet was formulated to meet the recommendations of the NRC (2001) for beef cattle. Prior to the start of experiments, all Hanwoo steers were acclimated to tie-stall barns and the basal diet for 14 days. Animals had free access to water and mineral blocks. The chemical composition and ingredient of the formulated concentrate diet is shown in Table 2.

Table 2. Chemical and ingredient composition of formulated concentrate diet

Composition, % of dry matter	Concentrate[1]
Ground corn	47.8
Wheat bran	41.0
Soybean meal	5.0
Rape seed meal	2.0
Molasses	2.0
Calcium phosphate	1.5
Salt	0.4
Vitamin-mineral mixture[2]	0.2
Lasalocid	0.1
Total	100.0

[1] Concentrate, formulate concentrates mixture.

[2] Vitamin-mineral premix components: Vitamin A, 2,650,000 IU; Vitamin D3, 530,000 IU; Vitamin E, 1,050 IU; Niacin, 10,000 mg; Mn, 4,400 mg; Fe, 13,200 mg; I, 440 mg; Co, 440mg.

The spent mushroom substrate from *F. velutipes* and inoculated SMSF were dried, milled, and passed through a 2-mm mesh screen in a Wiley mill (Model 4, Thomas Scientific, Swedesboro, NJ, USA) before rumen incubation. Each 5-g feed sample (DM basis) was placed in a nylon bag (8 × 15 cm; pore size = 45 μm; surface area = 41.67 mg/cm^2; NL 130-030/330PW, NBC, Inc., Tokyo, Japan), tied with a rubber band, and incubated in triplicate in the rumen of Hanwoo steers for 0, 3, 6, 9, 12, 24, 48, 72, 96 and 120 h. All nylon bags were suspended in the rumen in a polyester mesh bag (25 × 40 cm; 3 mm pore size) and removed at the intervals above. Upon removal, bags were rinsed in cold water to remove ruminal contents, then washed with cold tap water for 30 min. Additional bags were also prepared and machine-rinsed without

ruminal incubation, thereby creating a zero-hour incubation group. After rinsing, the residues were dried at 60℃ in a forced-air oven for 48 h and weighed to determine residual DM.

Parameters of ruminal disappearance were calculated using the following nonlinear model (Ørskov and McDonald, 1979):

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

where P is rumen degradation (%), a is the 45-μm filterable and soluble fraction (%), b is the degradable fraction (%), c is the degradation rate of fraction b (h^{-1}), and t is the incubation time (h).

Effective degradability (ED) of CP and DM was calculated using the following equation:

$$ED\ (\%) = a + [bc/\ (c + k)], \quad (2)$$

where a, b, and c are constants from the nonlinear model described above, and k is the measured particle passage rate of 0.02 and 0.05 h^{-1} (Denham et al., 1989).

3. Exp. 2: Feeding trial and total-tract digestibility of nutrients

A previous study recommended that SMS level of 6.5% dietary DM in a silage-based total mixed ration for wethers (Xu et al., 2010). In addition, Oh et al. (2010) suggested that SMS could be used as a forage source to replace up to 40% of rice straw without any negative effects on Hanwoo steers. Three healthy Hanwoo steers (mean body weight = 336 ± 69.0 kg) were randomly assigned one of three dietary treatments sequenced in a duplicated 3 × 3 Latin square design. The treatments consisted of control diet (3.75 kg/d formulated concentrate mixture + 1.25 kg/d rice straw; rice straw and concentrates in ratio of 1:3 on a DM base; as fed basis: Table 2), SMSF diet (3.19 kg/d formulated concentrate mixture + 1.25 kg/d rice straw + 0.56 kg/d SMSF; 15% of concentrates replaced by SMSF on a DM base), and ISMSF diet (3.19 kg/d formulated concentrate mixture + 1.25 kg/d rice straw + 0.56 kg/d ISMSF; 15% of concentrates replaced by ISMSF on a DM base). Diets were provided twice daily at 0900 and 1700 h. The chemical composition of experimental diets is shown in Table 3.

Table 3. Chemical compositions of experimental diets

Chemical composition, % of dry matter	Treatments[1]		
	Control	SMSF	ISMSF
Crude protein	10.5	10.2	10.2
Ether extract	2.7	2.9	2.9
Crude fiber	12.6	14.6	14.5
Crude ash	6.9	7.7	7.7
Nitrogen free extract	67.4	64.6	64.6
Neutral detergent fiber	33.8	37.2	36.9
Acid detergent fiber	13.8	16.6	16.5
Hemicellulose	20.0	20.6	20.4
Non-fibrous carbohydrates	46.1	42.0	42.3
Gross energy (Kcal/g)	4.3	4.4	4.4

[1] Treatments: Control, formulated concentrate mixture 3.75 kg + rice straw 1.25 kg/d; SMSF, formulated concentrate mixture 3.19 kg + rice straw 1.25 kg + SMSF 0.56 kg/d; ISMSF, formulated concentrate mixture 3.19 kg + rice straw 1.25 kg + ISMSF 0.56 kg/d.

Each experimental period consisted of a 2-week adaptation period, followed by a 1-week sample collection period. Once weighed, fecal subsamples (5%) were collected during the collection period (twice daily at 0930 and 1730 h) and frozen at -20℃ until further analyses. Fecal samples were dried for 48 h in a forced-air oven at 65℃, ground through a 2-mm mesh screen, and analyzed.

On the final day of each collection period, rumen fluid (approximately 200 mL) was collected from each steer via stomach tubes at 0, 2, 4, 6 and 8 h after morning feeding. The pH of the rumen fluid was measured with a pH meter (Orion Star A211 bench-top pH meter; Thermo Scientific, Bremen, Germany) immediately after collection and subsequently strained through four layers of cheesecloth. The strained rumen fluid (1 mL) was acidified with 0.2 mL of HPO_3 and frozen until analysis for ammonia-N and volatile fatty acids (VFA). Concentration of VFA was determined using gas chromatography (Varian CP-3800; Varian, Walnut, Creek, CA, USA) as described by Oh et al. (2010). Ammonia-N concentration was determined using a multiplate spectrophotometer (Bio-Rad, Benchmark Plus™, Tokyo, Japan) at 630 nm as described by Chaney and Marbach (1962).

4. Chemical composition analysis

All feed samples were dried, milled using a Wiley mill, and passed through a 1-mm mesh screen. The DM was determined by drying the samples to a constant weight at 65℃. Samples were analyzed for crude protein (CP), ether extract (EE), crude fiber (CF), and crude ash (Ash) according to the methods described by the AOAC (2012). The neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were evaluated were performed by the sequential procedure of Van Soest et al. (1991) using a fiber analyzer (ANKOM^{2000}, ANKOM Technology Corporation, Macedon, NY, USA). Hemicellulose (HEM) was calculated as NDF - ADF and cellulose by ADF - ADL. The amount of non-fibrous carbohydrate (NFC) was calculated by subtracting the sum of NDF, CP, EE, and Ash from 100. The concentration of gross energy was measured using bomb calorimetry (Parr 6400 calorimeter, Parr Instrument Company, IL, USA).

5. Statistical analysis

All collected and recorded data were analyzed by one-way analysis of variance (ANOVA) using generalized linear model procedure (GLM) in SAS (Statistical Analysis Systems Institute, Inc., 2003). Experimental results are expressed as mean and standard error of mean. Mean separation was performed using *t*-test and P-values less than 0.05 were considered statistically significant.

Ⅲ. Results

1. Visual inspection and pH measurement

Data from pH changes on the SMSF and ISMSF diets are shown in Fig. 1. Mold was detected on the SMSF at day 5, however, no visible spoilage was observed in ISMSF until d 5 (data not shown). The pH of SMSF decreased slightly to 5.20 at d 2, but increased to 5.50 at d 5. However, the pH of ISMSF dropped rapidly from 5.60 to 4.50 during the ensiling period on d 2 to 5 ($P<0.05$). The pH of ISMSF was significantly lower ($P<0.05$) than that of SMSF from d 1 to 5 (Fig. 1. C).

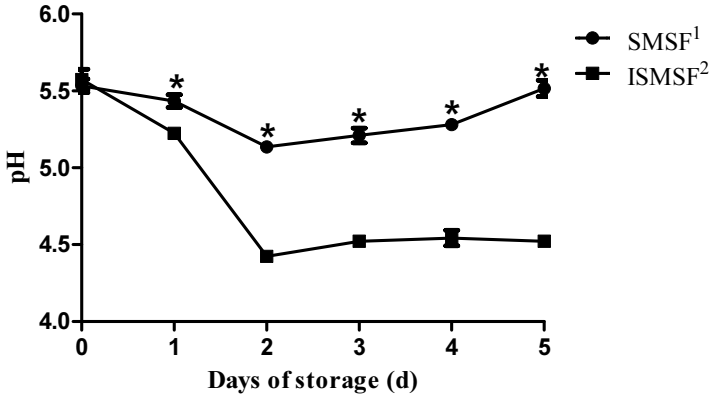

Fig. 1. The pH changes for the SMSF and ISMSF diets during 5 days of storage.

[1] SMSF, spent mushroom substrate from Flammulina velutipes; [2] ISMSF, SMSF inoculated with microbial additive; * Means differ significantly between treatments ($P<0.05$).

2. *In situ* rumen degradation of SMSF and ISMSF diets

The digestibility of DM, CP, NDF, and ADF did not significantly differ between ISMSP and SMSP at all incubation times (Table 4).

Table 4. DM, CP, ADF, and NDF residues at each rumen incubation time in the test diets

Degradability, %	Time	Diets[1]		SEM	P-value
		SMSF	ISMSF		
Dry matter	0	28.09	33.38	1.026	0.557
	3	30.51	34.43	0.599	0.577
	6	31.89	36.23	0.689	0.538
	12	35.23	36.72	0.318	0.291
	24	42.79	43.02	0.442	0.221
	48	51.07	51.24	0.415	0.118
	72	56.47	55.98	0.221	0.182
	96	59.06	58.11	0.327	0.472
	120	61.07	59.46	0.345	0.959
Crude protein	0	53.33	52.70	0.193	0.988
	3	57.24	57.34	0.069	0.928
	6	58.90	60.70	0.301	0.620

Degradability, %	Time	Diets[1]		SEM	P-value
		SMSF	ISMSF		
Crude protein	12	60.35	61.41	0.210	0.284
	24	63.82	65.50	0.315	0.517
	48	67.94	69.61	0.325	0.821
	72	73.80	73.0	0.153	0.568
	96	74.96	75.16	0.185	0.268
	120	73.94	73.84	0.188	0.386
Neutral detergent fiber	0	4.29	5.73	0.411	0.214
	3	6.50	6.09	0.144	0.582
	6	6.66	7.98	0.343	0.581
	12	7.96	8.75	0.305	0.156
	24	18.12	16.71	0.667	0.108
	48	27.74	25.73	0.598	0.891
	72	34.79	33.33	0.344	0.473
	96	39.29	36.99	0.663	0.672
	120	42.41	40.41	0.375	0.433
Acid detergent fiber	0	3.06	4.78	0.425	0.464
	3	5.08	5.69	0.175	0.786
	6	5.52	5.94	0.330	0.179
	12	5.65	6.59	0.318	0.163
	24	15.62	12.26	0.877	0.337
	48	23.69	20.31	0.778	0.470
	72	28.87	25.76	0.606	0.664
	96	31.61	30.04	0.630	0.479
	120	29.38	33.14	3.036	0.643

[1] SMSF, spent mushroom substrate from Flammulina velutipes; ISMSF, SMSF inoculated with microbial additive.

The degradation characteristics of CP and DM in SMSF and ISMSF are shown in Table 5. The rapidly degradable *a* fraction of DM in ISMSF (32.61%) was greater ($P = 0.040$) compared with in SMSF (27.85%). The degradation rate of fraction *b* (*c*) of DM in SMSF (0.022 h^{-1}) was faster ($P = 0.012$) than in ISMSF (0.018 h^{-1}).

Table 5. The degradation characteristics of crude protein (CP) and dry matter (DM) in the test diets

Item	Diets[1]		SEM	P-value
	SMSF	ISMSF		
a (%)	54.81	54.74	0.184	0.820
b (%)	21.19	19.99	0.489	0.901
a + *b* (%)	76.01	74.73	0.580	0.725
c (h^{-1})	0.020	0.030	0.003	0.114
Effective degradability of CP	61.81	62.79	0.312	0.112
a (%)	27.85b	32.61a	1.400	0.040
b (%)	36.05	31.11	1.583	0.235
a + *b* (%)	63.90	63.72	0.837	0.835
c (h^{-1})	0.022a	0.018b	0.001	0.012
Effective degradability of DM	38.69	40.80	0.648	0.310

[1]SMSF, spent mushroom substrate from *Flammulina velutipes*; ISMSF, SMSF inoculated with microbial additive. *a* = rapidly degradable fraction; *b* = slowly degradable fraction; *a* + *b* = potentially degradable fraction; *c* = degradation rate of fraction *b*. Means with different superscript letters ($^{a, b}$) within the same row are significantly different ($P < 0.05$).

3. Ruminal fermentation parameters

The changes of pH and ammonia-N concentration in the rumen are shown in Fig. 2. No significant differences were observed in ruminal pH and ammonia-N concentration among the treatments at every sampling time ($P > 0.05$). The rumen pH in all treatments decreased from 0 to 4 h of post-feeding, and then it was increased again (Fig. 2A). The rumen ammonia-N concentration in all treatments increased till 2 h of post-feeding, and it was declined gradually thereafter (Fig. 2B).

There were no significant differences in the concentrations of total or individual VFA between treatments (Table 6). The concentrations of total and individual VFA in all treatments increased at 2 h and then decrease from 4 to 8 h. The acetate : propionate ratio was not affected by the treatments.

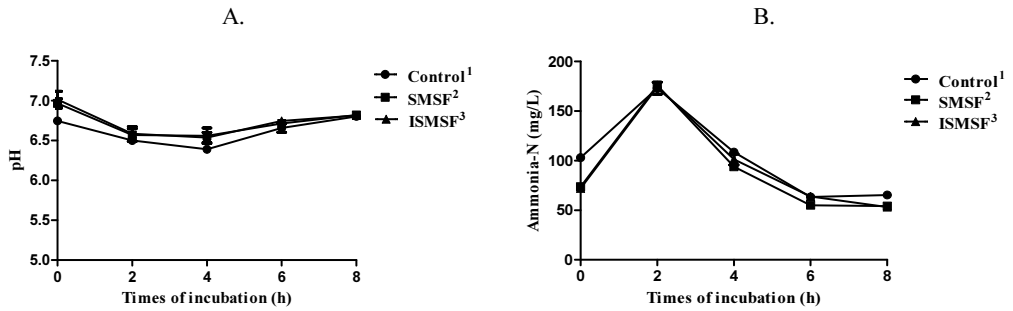

Fig. 2. The changes in pH and ammonia-N concentration in the rumen for the SMSF and ISMSF diets.

[1]Control, formulated concentrate mixture 3.75 kg + rice straw 1.25 kg/d; [2]SMSF, formulated concentrate mixture 3.0 kg + rice straw 1.25 kg + SMSF 0.75 kg/d; [3]ISMSF, formulated concentrate mixture 3.0 kg + rice straw 1.25 kg + ISMSF 0.75 kg/d.

Table 6. Effects of experimental diets on ruminal VFA

Time	Item	Treatments[1]			SEM	P-value
		Control	SMSF	ISMSF		
0	Total VFA, mM	69.2	66.8	65.0	3.320	0.887
	Acetate (A), %	46.4	45.3	43.6	2.013	0.868
	Propionate (P), %	10.9	10.7	10.1	0.669	0.887
	Isobutyrate, %	0.7	0.6	0.7	0.052	0.747
	Butyrate, %	9.5	8.5	8.7	0.551	0.772
	Isovalerate, %	1.2	1.1	1.3	0.087	0.576
	Valerate, %	0.5	0.5	0.6	0.051	0.824
	A : P	4.3	4.3	4.5	0.100	0.729
2	Total VFA, mM	97.5	85.8	80.2	5.577	0.458
	Acetate (A), %	62.3	54.5	50.7	3.546	0.419
	Propionate (P), %	18.0	16.3	15.0	0.983	0.489
	Isobutyrate, %	0.4	0.3	0.3	0.046	0.527
	Butyrate, %	14.1	12.3	11.9	0.960	0.613
	Isovalerate, %	1.3	1.2	1.2	0.083	0.835
	Valerate, %	1.3	1.1	1.0	0.054	0.237
	A : P	3.4	3.3	3.5	0.065	0.778

Time	Item	Treatments[1]			SEM	P-value
		Control	SMSF	ISMSF		
4	Total VFA, mM	87.7	87.7	96.4	3.412	0.583
	Acetate (A), %	60.1	56.0	63.2	2.125	0.664
	Propionate (P), %	16.8	16.3	17.5	0.738	0.826
	Isobutyrate, %	0.3	0.3	0.4	0.029	0.996
	Butyrate, %	8.3	12.3	12.7	1.012	0.134
	Isovalerate, %	1.1	1.1	1.3	0.081	0.531
	Valerate, %	1.2	1.1	1.2	0.042	0.701
	A : P	3.6	3.6	3.7	0.051	0.639
6	Total VFA, mM	81.3	78.0	75.6	2.935	0.751
	Acetate (A), %	56.0	53.2	52.5	2.254	0.817
	Propionate (P), %	15.0	14.6	14.1	0.844	0.920
	Isobutyrate, %	0.4	0.3	0.4	0.017	0.786
	Butyrate, %	8.1	8.1	6.6	1.097	0.836
	Isovalerate, %	1.0	1.0	1.1	0.073	0.531
	Valerate, %	0.9	0.8	0.9	0.039	0.819
	A : P	3.7	3.8	3.7	0.078	0.763
8	Total VFA, mM	74.0	73.3	72.6	4.535	0.993
	Acetate (A), %	49.0	48.8	47.6	2.783	0.979
	Propionate (P), %	12.6	12.4	12.3	0.853	0.988
	Isobutyrate, %	0.3	0.3	0.3	0.028	0.969
	Butyrate, %	10.5	10.3	10.7	0.877	0.985
	Isovalerate, %	0.8	0.9	1.0	0.063	0.654
	Valerate, %	0.7	0.7	0.7	0.038	0.987
	A : P	3.9	4.0	4.0	0.103	0.948

[1]Control, formulated concentrate mixture 3.75 kg + rice straw 1.25 kg/d; SMSF, formulated concentrate mixture 3.19 kg + rice straw 1.25 kg + SMSF 0.56 kg/d; ISMSF, formulated concentrate mixture 3.19 kg + rice straw 1.25 kg + ISMSF 0.56 kg/d.

4. Nutrients digestibility of SMSF and ISMSF diets

Total-tract digestibility of all nutrients that are CP, EE, ash, NFE, NDF, ADF, HEM, NFC, energy and TDN did not significantly differ between SMSP and ISMSP (Table 7).

Table 7. Total-tract nutrient digestibility of experimental diets

Item	Treatments[1]			SEM	P-value
	Control	SMSF	ISMSF		
Nutrient digestibility (%)					
Crude protein	69.8	63.6	66.1	3.28	0.575
Ether extract	63.1	56.1	60.1	3.97	0.972
Crude fiber	82.5	83.6	85.7	3.31	0.362
Crude ash	45.4	41.4	43.9	4.84	0.684
Nitrogen free extract	13.7	8.2	12.6	7.19	0.784
Neutral detergent fiber	80.6	75.5	77.5	2.72	0.856
Acid detergent fiber	56.5	50.8	53.8	3.77	0.484
Hemicellulose	41.0	39.2	40.8	3.65	0.736
Non-fibrous carbohydrates	76.2	60.1	64.3	4.75	0.752
Gross energy (Kcal/g)	2.9	2.7	2.8	0.16	0.303
TDN (%)[2]	67.6	62.8	65.1	5.52	0.335

[1]Control, formulated concentrate mixture 3.75 kg + rice straw 1.25 kg/d; SMSF, formulated concentrate mixture 3.19 kg + rice straw 1.25 kg + SMSF 0.56 kg/d; ISMSF, formulated concentrate mixture 3.19 kg + rice straw 1.25 kg + ISMSF 0.56 kg/d. [2]Total digestible nutrients (TDN), [digestible crude protein + digestible neutral detergent fiber + digestible soluble carbohydrates + (digestible crude fat × 2.25)]

Ⅳ. Discussion

Although our previous study showed the benefits of inoculating SMS with LAB for cattle feed (unpublished), it did not provide a comprehensive evaluation of SMS utility for cattle. We conducted the present study to provide new information regarding the feed quality and digestibility of SMS from *Flammulina velutipes* inoculated with a microbial additive for Hanwoo steers.

In Exp. 1, the pH of ISMSF was lower than that of SMSF during the fermentation period, and ISMSF was well preserved until d 5 (Fig. 1). Similarly, Kim et al. (2008) reported that anaerobic fermentation with mixed microbes (*Enterobacter ludwigii*, *Bacillus cereus*, 2 strains of *Bacillus subtilis*, *Saccharomyces cerevisiae* and *Lactobacillus plantarum*) inoculation improved the preservation of SMS. Well-fermented silage has a pH close to 4.0 and high lactic acid concentration, which inhibits the growth of undesirable bacteria and limits the activity of plant

enzymes (McDonald et al., 1991). Jones et al. (1992) reported that microbial inoculation alters the composition of cell wall carbohydrates during the early phases of fermentation, when pH rapidly declines. These results suggested that microbial inoculation of SMS can be an effective strategy for extending the storage period.

The results of the *in situ* rumen degradation experiment showed DM, CP, NDF, and ADF did not significantly differ between ISMSP and SMSP at all incubation times (Table 4). The rapidly degradable fraction *a* of DM in ISMSF was greater ($P = 0.040$) compared with in SMSF, whereas the slowly degradable fraction *b* of CP and DM was greater in ISMSF ($P > 0.05$) than in SMSF (Table 5). Interestingly, the degradation rate of fraction *b* (c) of DM in ISMSF ($P = 0.012$) was significantly lower than SMSF (Table 5). Adamovieć et al. (1998) suggested that inoculation significantly increased the degradability of OM, ADF, and NDF. In addition, Cushnahan and Mayne (1995) reported that extended fermentation can increase fraction *a* and reduce fractions *b* and *c*. However, applying LAB to the grass at ensiling did not result in a consistent change in silage fermentation characteristics, and bacterial inoculants and additives may have little or no effect on digestibility (McDonald et al., 1991; O'Kiely, 1996). These results can suggest that microbial inoculation of SMS might have small effects on cell components.

In Exp. 2, the changes in pH and ammonia-N and VFA concentrations did not differ among treatments (Fig. 2 and Table 6). Similarly, our preliminary investigation showed that LAB inoculation had no effect on pH, ammonia-N, or VFA concentration in SMS. Keady and Steen (1994) reported that a *Lactobacillus plantarum* bacterial inoculant had no significant effects on DM, nitrogen and fiber degradability, rumen pH, ammonia, or VFA concentration. However, other studies found that LAB-inoculated grass silage increased ruminal VFA concentration and improved the digestibility of DM, NDF, and ADF (Jaakkola et al., 1991; Stokes, 1992; Keady and Steen, 1996; Yahaya et al., 2004). In addition, Kim et al. (2012) reported that inoculation of SMS with microbial additive (*Enterobacter spp*. and *Bacillus spp*.) had higher content of NDF than that of the rice straw, and microbially-fermented SMS are attributed to the increased DM and CP intakes of Hanwoo steers.

Microbe-inoculated SMSF might, in theory, alter the rumen environment and interact with rumen microorganisms; however, our results showed no effects on the rumen environment. In this study, total-tract nutrient digestibility did not significantly differ among the SMSF, ISMSF, and control diets (Table 7). Thus, SMSF inoculated with a microbial additive had no effect on total-tract nutrient digestibility.

Ⅴ. Conclusion

The results of the present study indicate that SMSF, with or without microbial inoculation, had no effect on rumen pH, ammonia-N, VFA concentration, DM degradability, or total-tract nutrient digestibility. Overall, microbial inoculation improved the preservation of SMSF and had no effect on rumen fermentation parameters. In addition, the TDN values of the SMSF and ISMSF diets were slightly lesser than that of the control diet. A 15% dietary inclusion of SMSF and ISMSF may be recommended for Hanwoo beef cattle to supplement formulated concentrate feed and reduce feed costs in cattle production.

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