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Ensiling Characteristics and the *In situ* Nutrient Degradability of a By-product Feed-based Silage

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ABSTRACT: This study was conducted to evaluate the ensiling characteristics and the *in situ* degradability of a by-product feed (BF)-based silage. Before ensilation, the BF-based mixture was composed of 50% spent mushroom substrate, 21% recycled poultry bedding, 15% ryegrass straw, 10.8% rice bran, 2% molasses, 0.6% bentonite, and 0.6% microbial inoculant on a wet basis and ensiled for up to 4 weeks. The BF-based silage contained on average 39.3% moisture, 13.4% crude protein (CP), and 52.2% neutral detergent fiber (NDF), 49% total digestible nutrient, and 37.8% physically effective $NDF_{1.18}$ on a dry matter (DM) basis. Ensiling the BF-based silage for up to 4 weeks affected ($p<0.01$) the chemical composition to a small extent, increased ($p<0.05$) the lactic acid and NH_3-N content, and decreased ($p<0.05$) both the total bacterial and lactic acid bacterial counts from 10^9 to 10^8 cfu/g when compared to that before ensiling. These parameters indicated that the silage was fermented and stored well during the 4-week ensiling period. Compared with rice or ryegrass straws, the BF-based silage had a higher ($p<0.05$) water-soluble and filterable fraction, a lower insoluble degradable DM and CP fraction ($p<0.05$), a lower digestible NDF ($p<0.05$) fraction, a higher ($p<0.05$) DM and CP disappearance and degradability rate, and a lower ($p<0.05$) NDF disappearance and degradability rate. These results indicated that cheap, good-quality BF-based roughage could be produced by ensiling SMS, RPB, rice bran, and a minimal amount of straw. (**Key Words:** Spent Mushroom Substrate, By-product Feed, Silage, Degradability, Ruminant)

INTRODUCTION

The recent increase in the feed cost has exerted considerable pressure on the beef cattle industry. Many Asian countries including Korea import expensive sources of roughage from other nations. Providing good quality roughage to beef cattle is a general practice adopted to produce well-marbled beef. Accordingly, there is a need to develop a cheap, high quality by-product-based roughage source to relieve the problem of roughage scarcity faced by certain countries.

Among the by-product feeds (BF) in Korea, cheap spent mushroom substrates (SMS) are produced on a large scale and are available nationwide in any season. The primary fiber sources in mushroom substrates include sawdust, cotton waste, corncobs, or straw. Previous research (Bae et al., 2006) from our laboratory revealed that sawdust-based SMS contained too much moisture (over 60%) and neutral detergent fiber (NDF, 78.2%), with a too low crude protein (CP, 7.2%) and energy content. Therefore, to improve the nutritive quality of sawdust-based SMS, it should be mixed with other complementary feed sources. Furthermore, the storage of SMS is problematic, as this putrefies quickly due to its high moisture content (Kwak et al., 2008).

In comparison to SMS, recycled poultry bedding (RPB) is easily available and is a cheap proteinaceous feed (CP 22%) with a low fiber content (NDF 53%) (Kwak et al., 2008). When RPB was used to supplement sawdust-based SMS and ensiled, it improved the quality of SMS (with respect to storage and nutritive value) by increasing the CP

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content while decreasing the fiber and lignin levels (Kwak et al., 2008). Moreover, processed RPB has been successfully used as a cattle feed (McCaskey et al., 1994; Fontenot, 2001).

Rice bran is produced on a large scale in most Asian countries. It is a cheap energy source containing a large total digestible nutrient (TDN) (77.7%) fraction and a moderate level of ether extract (EE) (17.2%) (NIAS, 2012).

Microbial inoculation is efficient in improving the ensiling characteristics of straw (Gao et al., 2008). A previous study conducted in our lab found that molasses and microbial inoculants could improve the fermentability and silage quality of SMS (Kwak et al., 2009). Since SMS contains low levels of readily fermentable carbohydrates, it was better to enrich it with molasses to stimulate the growth of lactic acid bacteria (LAB) and yeast (Weinberg et al., 2008). The inclusion of bentonite in a cattle diet formulation is an environmentally friendly practice as it considerably reduces manure gases. This practice also resulted in an increase in the mean body weight of Hanwoo steers by enhancing mineral availability (Lee et al., 2010b) and improved the meat quality of beef cattle (Cho et al., 2001; Kang et al., 2002). A minimal amount of straw containing large particles was used to provide the fiber required for normal ruminant activity. Conventionally, the anaerobic ensiling of high moisture-containing, by-product-based feedstuffs is effective in improving storage characteristics and resulted in enhanced nutrient conservation (Schneider et al., 1995; Fontenot, 2001; Weinberg et al., 2008).

Based on these factors, we devised a method for the development and manufacture of a cheap, high quality BF-based roughage by ensiling SMS with rice bran, RPB, straws, molasses, bentonite, and microbes. This study was conducted to evaluate the ensiling and storage characteristics and to determine *in situ* nutrient fractionation, disappearance rate, and degradability of the BF-based silage.

MATERIALS AND METHODS

Manufacture of BF-based silage

The SMS was collected fresh from a local oyster mushroom (*Pleurotus osteratus*) farm. The original mushroom substrate consisted of 47% sawdust, 18% kapok meal, 18% beet pulp, 13% corncobs, and 4% cottonseed meal. The SMS (50%) was mixed with RPB (21%), cut ryegrass straw (15%), rice bran (10.8%), molasses (2%), bentonite (0.6%), and microbial additives (0.6%), and ensiled for up to 4 weeks. The microbial inoculants used in this experiment were isolated and identified previously in our lab (Kim et al., 2007b; Kim et al., 2008a) and include *Enterobacter ludwigii* KU201-3, *Bacillus cereus* KU206-3, *Bacillus subtilis* KU3, *Saccharomyces cerevisiae*, and *Lactobacillus plantarum*. The mixture was inoculated with the strains (each added at 0.12% [v/w]). *Bacillus* sp. and *Enterobacter* sp. were cultured in plate count broth (casein 5 g, yeast extract 2.5 g, and dextrose 1 g/L) at 36°C for 24 h, *Saccharomyces* sp. in yeast male broth (0711, Difco Laboratories Inc., Detroit, MI, USA) at 30°C for 48 h, and *Lactobacillus* sp. in MRS broth (0881, Difco Laboratories Inc., Detroit, MI, USA) at 36°C for 24 h. As presented in Table 1, the SMS and ryegrass straw were used as a fiber source; the RPB, a protein source; and the rice bran and molasses, an energy source. The factors considered in feed formulation were moisture (40±5%), CP (over 12%), physically effective NDF (peNDF; over 25%), and cost.

About 1.2-kg lots of the BF-based mixture sealed in two folds of poly-vinyl bags were prepared (with 10 replicates per treatment) and ensiled for a period of 0, 3, 5, 7, 14, or 28 d at room temperature (25°C). Samples were collected at each of the different ensiling periods and were stored at –20°C for later analysis.

In situ dacron bag test

All animal care protocols were approved by the Konkuk

Table 1. The chemical composition of feed ingredients[1]

Item	SMS[2]	Ryegrass straw	RPB[3]	Rice bran	Molasses	Bentonite
	%					
Dry matter	27. 7	90.8	81.5	89. 7	60.7	91.0
Crude protein[4]	12. 2	5.4	22.8	15.2	3.9	0.2
TP/CP	82. 3	67.0	53.9	75.0	9.5	24.3
NPN-CP/CP	17. 7	33.0	46.1	25.0	90.5	75.7
Ether extract	0.5	0.5	0.6	19.7	0.7	0
Crude ash	5.3	3.5	27.4	10.8	10.1	96.6
Neutral detergent fiber	70.4	80.3	34.4	36.4	1.9	0
Acid detergent fiber	62.0	65.2	29.4	18.4	0.6	0
Hemicellulose	8.4	15.1	5.0	18. 0	1.3	0
Acid detergent lignin	33.5	22.6	13.4	5.1	0.2	0
Non-fibrous carbohydrate	11.6	10.2	16.9	18.3	84.3	0

[1] On a dry matter basis. [2] Spent mushroom substrates (SMS). [3] Recycled poultry bedding. [4] TP = True protein, NPN = Non-protein nitrogen.

University Institutional Animal Care and Use Committee. Two Holstein cows (having an average body weight of 626 kg) fitted with ruminal cannula were used for the evaluation of *in situ* ruminal fractionation, disappearance rate, and degradability of BP-based silage by examining the dry matter (DM), NDF, and CP levels and comparing it to that of two most widely used, conventional, poor-quality roughage sources such as rice straw and ryegrass straw. Two trials were performed and four observations were made per treatment. The test animals were fed 4 kg of a formulated concentrate mix, 1 kg of rice straw, 1 kg of ryegrass straw, and 3 kg of BF-based silage on a daily basis to meet the nutritional requirements for their maintenance (NRC, 2001). The animals had free access to water. The BF-based silage used in this bag test was sampled from mixtures ensiled for 14 d.

The *in situ* bag test was carried out according to the method by Ø rskov et al. (1980). Samples were ground using a Wiley mill (Thomas Scientific, Model 4, NJ, USA). Particles in a size range of 63 μm to 2 mm that were filtered using an experimental sieve were used for this test. The specimen (approximately 10 g) was weighed and placed in a dacron bag (R1020, Ankom Technology, NY, USA) (bag size, 10×25 cm; mean pore size, 45 μm). The proportion of the wet sample weight to the dacron bag surface area was 20 mg/cm^2, which satisfies the optimal proportion recommended by Nocek (1985).

Two hours after feeding, the bags containing test samples were incubated in the ruminal ventral sac for 0, 24, 48, or 72 h. After the incubation was completed, bags were retrieved, washed in cold, running water for 24 h until clear water emerged from the bag, and were dried at 60°C in a drying oven for 48 h. The DM was classified into water-soluble and 45-μm filterable, insoluble degradable, and non-degradable fractions (Armentano et al., 1986). It is assumed that the digestible 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 indigestible fraction. The CP was classified into water-soluble and 45-μm filterable, insoluble degradable, and non-degradable fractions. The portion of CP that remained after 48 h of incubation was considered the non-degradable fraction. Excluding the non-degradable fraction, residues at each incubation time were converted into a percentage, transformed into the natural logarithmic form, and subjected to a linear regression (Armentano et al., 1986). The degradation rate of degradable fraction was derived from the slope of the regression line. Computed degradation rates were calculated using Equation 1 to predict the ruminal degradability of DM, NDF, and CP fractions in the tested feeds (Orskov et al., 1983), and the assumed passage rates (K_pB) were 0.025 and 0.05/h (Miller, 1982).

$$\text{Degradability} = A+(K_dB\times B)/(K_dB+K_pB) \qquad \text{Equation 1}$$

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

The ruminal disappearance rates of DM, NDF, and CP were calculated for all incubation times (0, 24, 48, or 72 h).

Chemical analysis

Immediately before analysis, all samples were dried and ground to pass through a 1-mm filter using a sample mill (Cemotec, Tecator, Sweden). The dry matter fraction was quantified by drying the samples at 60°C for 48 h to reach a constant weight. The CP, EE, and crude ash content were determined by the AOAC method (2000). The NDF and acid detergent fiber (ADF) content were analyzed using the method described by Van Soest et al. (1991). The non-fibrous carbohydrate (NFC) content was calculated as 100−(NDF%+CP%+EE%+ash%). The true protein (TP) content was measured by evaluating the nitrogen fractions precipitated in a 5% trichloroacetic acid solution. The non-protein nitrogen (NPN)-CP fraction was calculated as CP minus TP. The indigestible protein (ADF-CP) content was determined by the method described by Goering and Van Soest (1970).

The pH was measured using a pH meter (HI9321, Hanna Instrument, Portugal). The water-soluble carbohydrate (WSC), lactic acid, and NH_3-N contents were determined by the Dubois (1956) method, the Barker and Summerson (1941) method, and the Chaney and Marbach (1962) method using a UV spectrometer (S-1100, Scinco, Korea), respectively. The amount of volatile fatty acids such as acetic and butyric acids was assessed by the method described by Erwin et al. (1961) using gas chromatography (Thermo Electron Corporation, OH, USA).

Microbial analyses of the samples (sample size, 25 g) were conducted according to the AOAC (2000) method as follows: total bacterial count was determined on plate count agar (PCA, Difco Laboratories Inc., Detroit, MI, USA) incubated at 30°C for 48 h. LAB were determined on MRS agar plate (Difco Laboratories Inc., Detroit, MI, USA) incubated at 36°C for 24 h.

Statistical analysis

All statistical analyses were conducted using one-way analysis of variance, using the general linear model procedure (Statistix7, 2000). Tukey's multiple range test was used to compare the treatment means. Linear and quadratic trends for the samples ensiled for the different time periods were tested using polynomial contrasts. The data derived from the *in situ* bag test were processed using a randomized complete block design (Statistix7, 2000). The

Table 2. Changes in chemical composition (DM basis) of the by-product feed-based silage following different lengths of ensilation[1]

Item[2]	Ensilation period						SE	Polynomial contrast degree	
	0 d	3 d	5 d	7 d	14 d	28 d		Linear	Quadratic
				%					
Dry matter	62.2^{a}	60.6^{b}	59.8^{b}	60.8^{b}	60.3^{b}	60.7^{b}	0.45	0.264	0.038
Organic matter	88.3^{a}	87.3^{b}	87.7^{ab}	87.3^{b}	87.8^{ab}	87.6^{ab}	0.28	0.680	0.577
Crude protein (CP)	12.6^{b}	13.6^{a}	13.4^{a}	13.4^{a}	13.2^{a}	13.2^{a}	0.19	0.754	0.137
TP/CP	65.1^{a}	60.7^{ab}	60.8^{ab}	60.7^{ab}	58.5^{b}	59.8^{b}	1.68	0.026	0.027
NPN-CP/CP	34.9^{b}	39.3^{ab}	39.2^{ab}	39.3^{ab}	41.5^{a}	40.2^{a}	1.68	0.026	0.027
ADF-CP/CP	24.9^{abc}	24.0^{bc}	25.1^{abc}	26.3^{ab}	28.2^{a}	22.6^{c}	1.24	0.211	0.001
Ether extract	3.1^{d}	3.3^{cd}	3.7^{ab}	3.6^{abc}	3.5^{bc}	3.8^{a}	0.10	0.001	0.339
Crude ash	11.7^{b}	12.7^{a}	12.3^{ab}	12.7^{a}	12.2^{ab}	12.4^{ab}	0.28	0.680	0.577
NDF	53.8	52.3	52.5	52.4	52.4	51.2	0.91	0.032	0.978
ADF	41.1^{c}	42.4^{abc}	43.5^{a}	43.2^{ab}	41.1^{c}	41.9^{bc}	0.51	0.095	0.955
NFC	18.7	18.5	18.0	18.0	18.7	19.3	0.92	0.216	0.498

[1] The mean of 10 observations.

[2] TP = True protein, NPN = Non-protein nitrogen, NDF = Neutral detergent fiber, ADF = Acid detergent fiber, NFC = Non-fibrous carbohydrate.

[a,b,c,d] Means with different superscripts within the same row are significantly different (p<0.05).

model used was as follows:

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

Where Y_{ijk} is the dependent variable, μ is the overall mean, F_i is the fixed effect of the feed (i = 1 to 3), C_j is the random and block effect of the cow (j = 1 to 2), and e_{ijk} is the error term. A value of $p<0.05$ was considered to be significant.

RESULTS

Ensiling characteristics of BF-based silage

Chemical, fermentative, and microbial parameters: The changes in chemical composition of BF-based silage are presented in Table 2. The ensiling period (0 to 28 d) affected ($p<0.01$) the chemical composition and the DM, OM, CP, NPN-CP/CP, ADF-CP/CP, EE, crude ash, and ADF content in the BF-based silage. Compared with the observation before ensiling, ensiling for up to 28 d decreased the DM content but increased NPN-CP/CP and ADF-CP/CP content quadratically ($p<0.05$). Specifically, the DM and the OM content was reduced by 1.5% and 0.7%, respectively, while the CP content was increased by 0.6%; EE, by 0.7%; crude ash, by 0.7%; and ADF, by 0.8%. Generally, the ensilation-induced change in the chemical composition of the BF-based silage was very low.

The length of ensiling period (0 to 28 d) affected all the measured fermentative and microbial parameters of BF-based silage ($p<0.01$), as shown in Table 3. Ensiling for up

Table 3. The effects of the ensilation period of by-product feed-based silage on fermentation parameters and microbes[1]

Item	Ensilation period						SE	Polynomial contrast degree	
	0 d	3 d	5 d	7 d	14 d	28 d		Linear	Quadratic
				%					
Fermentation									
pH	5.11^{ab}	5.19^{ab}	5.19^{ab}	5.22^{a}	5.13^{ab}	5.10^{b}	0.040	0.049	0.042
Acetic acid (mM/g)	206.8^{bcd}	226.9^{bc}	45.0^{d}	127.9^{cd}	309.3^{ab}	449.6^{a}	58.38	0.001	0.046
Butyric acid (mM/g)	2.3^{a}	1.6^{a}	ND[2]	ND	1.0^{b}	1.1^{ab}	0.42	0.101	0.001
Lactic acid (%)	0.28^{b}	1.98^{a}	1.99^{a}	1.97^{a}	2.03^{a}	2.02^{a}	0.028	0.001	0.001
Water-soluble carbohydrate (%)	1.76^{a}	1.02^{b}	1.06^{b}	1.04^{b}	0.96^{b}	0.97^{b}	0.038	0.001	0.001
NH_3-N (ppm)	169.5^{a}	193.2^{b}	194.0^{b}	201.8^{b}	204.3^{b}	227.9^{c}	5.60	0.001	0.028
Microbes									
Total bacteria (Log_{10} cfu/g)	9.22^{a}	9.16^{ab}	9.10^{ab}	9.03^{b}	8.76^{c}	8.85^{c}	0.056	0.001	0.001
Lactic acid bacteria (Log_{10} cfu/g)	9.25^{a}	9.18^{a}	9.17^{a}	8.99^{b}	8.93^{b}	8.88^{b}	0.058	0.001	0.002

[1] Means of 10 observations. [2] ND = Not detected.

[a,b,c,d] Means with different superscripts within the same row are significantly different (p<0.05).

to 28 d produced only small alterations in pH while producing quadratic decreases in the butyric acid and WSC content ($p<0.05$) and increases in the acetic acid, lactic acid, and NH_3-N content ($p<0.05$). The lactic acid content increased about 7-fold after ensiling ($p<0.0001$). The butyric acid levels remained very low (below 2 mM/g) during the entire ensiling period. This implies that the silage was not aerobically deteriorated.

Counts of total bacteria and LAB in the BF-based silage decreased quadratically with prolonged ensiling ($p<0.005$). The bacterial count ($p<0.05$) decreased from the second week of ensiling, as did the LAB counts from the first week of ensiling ($p<0.05$). Thereafter, counts of total bacteria and LAB remained unchanged (about 10^8 cfu/g) until day 28 of ensilation.

The chemical composition, *in situ* fractionation, and ruminal disappearance rate

The BF-based silage used in the *in situ* bag test was that from the 14-d ensiling period (Table 2) and its chemical composition, *in situ* fractionation, and ruminal disappearance rate were compared with that of rice straw and ryegrass straw. The rice straw used in this test contained 73.9% DM, 3.6% CP (84.1% TP/CP), 1.1% EE, 9.3% crude ash, 72.8% NDF, 47.1% ADF, and 13.3% NFC, while the ryegrass straw contained 90.8% DM, 5.4% CP (67% TP/CP), 0.5% EE, 3.5% crude ash, 80.3% NDF, 65.2% ADF, and 10.2% NFC on a DM basis (data not shown).

The DM, NDF, and CP fractions of the feedstuffs tested in the *in situ* bag test are shown in Table 4. The water-soluble and filterable DM fraction was increased in the BF-based silage, while the insoluble degradable DM fraction in this silage was lower than that in rice or ryegrass straw ($p<0.05$). The non-degradable DM fraction of this silage was lower than that of rice straw ($p<0.05$), while digestible and indigestible NDF fractions were lower and higher, respectively, compared to that of rice or ryegrass straw ($p<0.05$). This silage had higher water-soluble and filterable, and lower insoluble degradable and lower non-degradable CP fractions compared to that in the rice straw or ryegrass straw ($p<0.05$).

The DM disappearance rate from the silage in the early stage of ruminal incubation was higher than that of rice straw and ryegrass straw ($p<0.05$); however, as the ruminal incubation progressed, the difference between the silage and rice straw decreased gradually (data not shown). The NDF disappearance rate from the silage after 72 h of incubation was 15% and 18% lower than that of rice straw or ryegrass straw ($p<0.05$), respectively. The CP disappearance rate from the silage following 24 h of ruminal incubation was 95.9% that of the total CP disappearance. The CP disappearance rate from the silage was consistently much higher throughout the incubation period, and at 48 h of incubation, it was 39% and 24% higher than that of rice straw and ryegrass straw ($p<0.05$), respectively.

The *in situ* degradability of nutrients

The estimated DM, CP, and NDF degradabilities at two rates of passage (K_pB 0.025 and 0.05) are shown in Table 5. In general, the rate of DM and CP degradation was higher in the BF-based siliage, while that of NDF was lower, at both passage rates ($p<0.05$), when compared to that in rice straw and ryegrass straw. At the passage rate ($K_pB = 0.05$) of animal production level, the DM degradability in the silage was about 1.8-fold and 1.6-fold higher than that in rice straw and ryegrass straw ($p<0.05$). Additionally, compared to the rice or ryegrass straw values, NDF degradability in the BF-based silage was 28% and 32% lower, while the CP degradability was 2.3-fold and 2.1-fold higher, respectively ($p<0.05$).

DISCUSSION

The ensiling characteristics of BF-based silage

Chemical, fermentative, and microbial parameters: The level of ensilation-induced change in the chemical

Table 4. *In situ* fraction of the DM, NDF and CP content of different feedstuffs[1]

Item	Rice straw	Ryegrass straw	BF-based silage[2]	SE	p value
Dry matter fraction	----------------	%	----------------		
Water-soluble and 45-μm filterable	17.4^b	18.6^b	44.5^a	1.05	<0.0001
Insoluble degradable	37.2^b	44.2^a	20.2^c	1.75	<0.0001
Non-degradable	45.4^a	37.2^b	35.3^b	1.15	<0.0001
NDF fraction					
Digestible	53.5^a	56.6^a	38.7^b	2.11	<0.0001
Indigestible	46.5^b	43.4^b	61.3^a	2.11	<0.0001
CP fraction					
Water-soluble and 45-μm filterable	28.8^b	27.7^b	73.0^a	2.01	<0.0001
Insoluble degradable	18.1^b	33.0^a	8.2^c	2.69	<0.0001
Non-degradable	53.1^a	39.4^b	18.8^c	2.86	<0.0001

[1] Means of 4 observations. [2] By-product feed-based silage.

[a,b,c] Means with different superscripts within the same row are significantly different ($p<0.05$).

Table 5. The *in situ* dry matter (DM), crude protein (CP) and neutral detergent fiber (NDF) degradabilities at 2 rates of passage[1, 2]

Item	Rice straw	Ryegrass straw	BF-based silage[3]	SE	p value
DM degradability at K_pB					
0.025	31.7^c	35.9^b	47.8^a	0.60	<0.0001
0.05	26.2^c	29.4^b	46.3^a	0.59	<0.0001
NDF degradability at K_pB					
0.025	37.6^a	39.7^a	27.0^b	1.46	<0.0001
0.05	29.0^a	30.6^a	20.8^b	1.11	<0.0001
CP degradability at K_pB					
0.025	33.2^c	40.1^b	73.8^a	2.03	<0.0001
0.05	31.3^b	35.3^b	73.4^a	1.95	<0.0001

[1] The mean of 4 observations. [2] The rate of passage (K_pB) was assumed to be 0.025 and 0.05/h. [3] By-product feed-based silage.

[a,b,c] Means with different superscripts within the same row are significantly different ($p<0.05$).

composition of the BF-based silage was very low, as reported in other studies using SMS silage (Kim et al., 2008b; Kwak et al., 2009). Since mixing and packing of the mixture when ensiling become difficult with the blending of a large quantity of straw, a minimal amount of straw was used to provide a normal level of physically effective NDF. The $\text{peNDF}_{1.18}$ (37.8%) of BF-based silage in the present study was a little higher than the value (35.1%) reported by Lee et al. (2010a). About 20% of eNDF in dry diet is required to maintain the rumen pH at more than 6.2, and 25% eNDF is required to maintain the pH necessary for maximum forage digestion and microbial growth (NRC, 2000).

The ensilation of SMS with microbial inoculants and molasses could reduce pH and WSC and increase the lactic acid content compared with that prior to ensilation (Kim et al., 2008b; Kwak et al., 2009). The increase in the lactic acid content in the present study is attributed to the conversion of WSC into lactic acid by LAB, as suggested by Wang et al. (2003). The decrease in the WSC level indicated that the rate of WSC conversion into microbial biomass or other metabolites exceeded that of carbohydrate breakdown into WSC. Generally, the anaerobic fermentation of SMS reduced the pH of the silage (Kwak et al., 2009). However, in the present study, the silage pH was not reduced, probably due to the added RPB, which would produce alkaline ammonia. A pH of 4 to 5 is desired when using fermented feed ingredients, because below pH 4, voluntary feed intake is decreased and over pH 5, microbial spoilage is likely to occur (Lee et al., 2004). The pH of the ensiled RPB alone usually remained above 4.5 due to the buffering capacity of ammonia in RPB, and a pH above 5.5 still effectively destroyed coliforms (Duque et al., 1978). In the present study, although the silage pH was slightly more than 5, the silage showed an appropriate appearance and fermentative odor without any fungal occurrence.

The counts of total bacteria and LAB started to reduce from 10^9 to 10^8 cfu/g level between the first and second week of ensilation in the present study. These trends were also observed in the study of Kim et al. (2008b), when sawdust-based SMS inoculated with mixed microbes was ensiled for 8 weeks.

In situ fractionation and the ruminal disappearance rate

The high water-soluble and filterable DM fraction of the BF-based silage was attributed to high CP and NFC components of RPB and rice bran, as shown in Table 1. Thus, the non-degradable DM fraction of the BF-based silage was 10% lower than that of rice straw.

In the present study, the digestible NDF fraction of the BF-based silage was lower than that of the straws. Makela et al. (2002) reported that, during mushroom cultivation, lignocellulose in SMS was decomposed by the mycelium, which preferentially utilizes digestible cellulose and hemicellulose. Fazaeli and Masoodi (2006) reported that the lignin content of SMS was increased during mushroom cultivation. Bonnen et al. (1994), and Ball and Jackson (1995) reported that the lignin content negatively correlated with digestibility. These phenomena must be related with the low digestible NDF fraction of the BF-based silage.

The water-soluble and filterable CP fraction in the BF-based silage was higher than that of rice straw and ryegrass straw; this was attributed to the high NPN-CP/CP content in SMS (41.5%, Table 2) and RPB (46.1%, Table 1). The relatively low digestible NDF fraction of the silage was attributed to the relatively less digestible sawdust fiber, which is the main fibrous component in SMS. The NDF fraction and its ruminal disappearance rate in rice straw in the present study were similar (within 1 to 2% points) to the values reported by Kim et al. (2011). Compared with that of raw rice straw, the rate of disappearance of DM and CP from spent rice straw (SMS of *Agaricus bisporus*) was higher, while that of NDF was lower (Kim et al., 2011), similar to the results of the present study.

The *in situ* degradability of nutrients

The estimated degradabilities of DM, NDF, and CP in the rice straw in the present study were similar to the values (28.7, 30.5, and 34.1%, respectively) reported by Kim et al. (2011). In the present study, the rate of DM or CP degradation in the BF-based silage was higher than that of rice straw and ryegrass straw. Kim et al. (2010) also reported that NDF digestibility was decreased by 4.8% when they replaced 50% of the rice straw in a sheep diet with ensiled SMS. The major fiber source in the SMS and RPB used in the present study were sawdust and rice hull respectively, both of which generally had a low amount of digestible fiber (Kim et al., 2007c; NIAS, 2012). For sawdust-based SMS-derived DM and NDF, the rate of ruminal disappearance or degradability was lower than that of corncobs- or cotton waste-based SMS (Bae et al., 2006). These results indicate that the relatively low NDF degradability of the BF-based silage could be attributable to the sawdust content, as this material contains a high level of indigestible fiber. The relatively high degradabilities of DM and CP and the low degradability of NDF in the BF-based silage were attributed to the high water-soluble and filterable fractions of DM and CP and the low digestible fraction of NDF, respectively.

CONCLUSION

The examination of chemical, fermentative, and microbial parameters indicated that the BF-based silage was fermented and could be stored well up to the fourth week of ensilation. The estimated rate of degradation of the BF-based silage DM and CP were much higher than that of rice straw and ryegrass straw, and vice versa for NDF. The BF-based silage, which showed high CP solubility and low NDF degradability, may be more suitable when used in combination with conventional roughage, which contains low-quality proteins and shows high NDF degradability. Thus, BF-based silage can be used in combination with conventional roughage to improve forage quality. The low ruminal NDF digestion of the BF-based silage would be increased when the sawdust-based SMS is substituted with corncobs-based SMS. These results show that it may be possible to manufacture cheap good quality BF-based roughage for use in Asian countries facing a roughage scarcity by ensiling SMS, RPB, rice bran, and a minimal amount of straw.

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