Effect of feeding a by-product feed-based silage on nutrients intake, apparent digestibility, and nitrogen balance in sheep

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Abstract

Background: Literature is lacking on the effects of feeding by-product feed (BF)-based silage on rumen fermentation parameters, nutrient digestion and nitrogen (N) retention in sheep. Therefore, this study was conducted to determine the effect of replacing rye straw with BF-based silage as a roughage source on ruminal parameters, total-tract apparent nutrient digestibility, and N balance in sheep.

Methods: The by-product feed silage was composed of spent mushroom substrate (SMS) (45 %), recycled poultry bedding (RPB) (21 %), rye straw (11 %), rice bran (10.8 %), corn taffy residue (10 %), protected fat (1.0 %), bentonite (0.6 %), and mixed microbial additive (0.6 %). Six sheep were assigned randomly to either the control (concentrate mix + rye straw) or a treatment diet (concentrate mix + BF-based silage).

Results: Compared with the control diet, feeding a BF-based silage diet resulted in similar ruminal characteristics (pH, acetate, propionate, and butyrate concentrations, and acetate: propionate ratio), higher (p < 0.05) ruminal NH3-N, higher (p < 0.05) ether extract digestibility, similar crude protein digestibility, lower (p < 0.05) dry matter, fiber, and crude ash digestibilities, and higher (p < 0.05) N retention (g/d).

Conclusion: The BF-based silage showed similar energy value, higher protein metabolism and utilization, and lower fiber digestion in sheep compared to the control diet containing rye straw.

Keywords: Spent mushroom substrate, By-product feed, Silage, Digestibility, Sheep

Background

Imported roughages such as rye straw, tall fescue hay, and timothy hay are expensive, however cheap, good quality silage can be developed using the feed ingredients which are abundant and easily available in many Asian countries. Previously in our laboratory the good quality by-product feed (BF)-based roughage was successfully manufactured by ensiling spent mushroom substrate (SMS), recycled poultry bedding (RPB), rice bran, and a minimal amount of straw, with added molasses and highly cellulolytic microbes [1], which were isolated from SMS [2, 3]. The silage exhibited favorable ensiling characteristics and had a higher degradability of dry matter (DM) and crude protein (CP) than did rice straw or rye straw [1].

Despite the fact that feeding BF-based silage as a roughage source to growing or fattening beef steers improved the intakes of DM and neutral detergent fiber (NDF) [4–6]. However, to our knowledge, it is unknown how rumen fermentation parameters, nutrient digestion, and N retention are affected by feeding BF-based silage in ruminants. Therefore, this study was performed to determine the effect of replacing rye straw (a conventional roughage source) with BF-based silage on ruminal parameters, total-tract apparent nutrient digestibility, and N balance in sheep.

Methods

Manufacture of BF-based silage
The SMS was collected fresh from a local mushroom (Pleurotus eryngii) farm. The original mushroom substrate consisted of 70 % sawdust, 15 % corn cobs, and 15 % rice bran. The by-product feed-based silage...
was manufactured as described in Kim et al. [1] at the experimental farm of Konkuk University located in Chung-Ju city in Chung-Buk province. The SMS (45 %) was mixed with RPB (21 %), rye straw (11 %), rice bran (10.8 %), corn taffy residue (10 %), protected fat (1.0 %), bentonite (0.6 %), and microbial additive (0.6 %), and ensiled in two folds of polyvinyl bags that were placed in a 40-kg capacity plastic bag for 30 d. The microbial inoculants used in this experiment were isolated and identified previously in our laboratory [2, 3], and included *Enterobacter ludwigii* KU201-3, *Bacillus cereus* KU206-3, *Bacillus subtilis* KU201-7, *Saccharomyces cerevisiae*, and *Lactobacillus plantarum*. The mixture was inoculated with the strains (each added at 0.17 % [v/w] to ensure supplying $1 \times 10^9$ cfu/g fresh biomass). *Bacillus* sp. and *Enterobacter* sp. were cultured in plate count broth (5 g casein, 2.5 g yeast extract, and 1 g/L dextrose) at 36 °C for 24 h, *Saccharomyces* sp. was cultured in yeast malt broth (0711, Difco Laboratories Inc., Detroit, MI, USA) at 30 °C for 48 h, and *Lactobacillus* sp. was cultured in MRS broth (0881, Difco Laboratories Inc.) at 36 °C for 24 h.

**Animal treatment, feeding regime and experimental design**

All animal care protocols were approved by the Konkuk University Institutional Animal Care and Use Committee. Six crossbred ram lambs (a mean body weight of $55 \pm 2$ kg) were randomly allotted to two dietary treatments: a control diet (formulated concentrate mix + rye straw) and a treatment diet (formulated concentrate mix + BF-based silage). The chemical composition of concentrate mix, rye straw, and BF-based silage is shown in Table 1. The BF-based silage contained about 4-times higher CP and lower neutral detergent fiber (NDF) than rye straw. The formulated concentrate mix was composed of 23 % corn grain, 17.5 % wheat bran, 14 % coconut meal, 10 % wheat grain, 7 % palm meal, 6% rapeseed meal, 5.8 % molasses, 5 % distillers grain, 5% corn gluten feed, 1.5 % wheat flour, 1.4 % limestone, 1.3 % tapioca, 1 % bentonite, 0.5 % dehydrate salt, 0.45 % red clay, 0.25 % calcium phosphate, 0.2 % calcium sulfate, and 0.1 % vitamin-mineral premix. Ingredients and chemical composition of diets fed to sheep are shown in Table 2. Control or treated diet was fed in equal portions in the amount of 850 g dry matter (DM, approximately 1.5% of body weight) at 07:00 and 18:00 h. The amount of diet DM corresponded to the requirements of growing ram lambs [7].

During the experiment, sheep always had free access to fresh water. Diets were randomly assigned to sheep at the start of the trial provided that sheep would not receive the same diet in two consecutive trials. The experiment consisted of 5 d transition, 10 d preliminary, and 7 d collection periods. The number of observations per treatment was 6. During the experiment, the animals were kept in individual metabolism crate (1.6 m × 0.5 m) that permitted separate collection of feces and urine. Daily fecal output during the collection period was dried at 60 °C. Feces were thoroughly mixed at the end of the collection period to obtain a composited sample, which was subsequently ground through a 2 mm screen prior to storage. Daily urine output was collected in plastic bottles containing 15 ml of 13.5 N H$_2$SO$_4$. After weighing, 2 % of the urine volume was refrigerated and bulked for the collection period.

On the last day of the experiment (d 7), 2 hours after the morning feeding, rumen fluid (250 ml) was collected with a stomach tube using an electric

| Item                  | Concentrate mix (%) | Rye straw (%) | BF-based silage (%) |
|-----------------------|---------------------|---------------|---------------------|
| Dry matter            | 87.9                | 90.0          | 58.3                |
| Organic matter        | 91.7                | 95.4          | 88.5                |
| Crude protein         | 16.4                | 4.2           | 16.9                |
| Ether extract         | 2.5                 | 0.2           | 3.9                 |
| Neutral detergent fiber | 32.7               | 67.7          | 50.6                |
| Acid detergent fiber  | 19.5                | 41.7          | 38.9                |
| Hemicellulose         | 13.2                | 25.9          | 11.7                |
| Crude fiber           | 11.3                | 37.8          | 31.2                |
| Nitrogen free extracts | 61.5              | 53.3          | 36.6                |
| Crude ash             | 8.3                 | 4.6           | 11.5                |

$^1$On a dry matter basis

$^2$BF-based silage was by-product feed-based silage, which was composed of 45 % spent mushroom substrate, 21 % recycled poultry bedding, 11 % rye straw, 10.8 % rice bran, 10 % corn taffy residue, 1.0 % protected fat, 0.6 % bentonite, and 0.6 % microbial additive, and ensiled for 30 d.
vacuum pump. The fluid was strained through four layers of cheesecloth prior to the determination of pH (HI9321, Hanna Instrument, Portugal). Each 5 ml of fluid was transferred to a tube containing two drops of concentrated H₂SO₄ and another 5 ml to a tube containing 1 ml of 25% (w/v) of metaphosphoric acid plus 5 ml isocaproic acid for the determination of NH₃-N and volatile fatty acids (VFA), respectively.

### Chemical analysis

Representative samples of the test feeds that supplied to the sheep were collected and stored at −20 °C. Immediately before the analysis, all the samples were dried and ground to pass through a 1-mm filter using a sample mill (Cemotec, Tecator, Sweden). The DM fraction was quantified by drying the samples at 60°C for 48 h until constant weight. The CP, ether extract (EE), and crude ash contents were determined by the AOAC method [8]. Neutral detergent fiber (NDF, heat-stable α-amylase), acid detergent fiber (ADF) were determined using the method of Van Soest et al. [9]. Ruminal NH₃-N was determined according to Chaney and Marbach [10]. Ruminal VFA was determined according to Erwin et al. [11] using a Trace GC Ultra gas chromatograph (Trace GC ultra, Thermo, Italy).

### Statistical analysis

Data were analyzed as a completely randomized design and subjected to one-way analysis of variance (ANOVA) using the general linear model procedure. Means separation was performed using t-test (Statistix7, 2000) [12] and significance was declared at p ≤ 0.05. Data are presented as least squares means and standard errors of the means.

### Results and discussion

#### Ruminal fermentation parameters

Ruminal parameters of sheep fed the different diets is shown in Table 3. The ruminal pH of both treatments was 6.6. Ruminal pH above 6.2 means adequate rumen fermentation [13]. Additionally, feeding BF-based silage did not affect ruminal acetate, propionate, and butyrate concentrations, and acetate: propionate ratio in sheep, but increased ruminal NH₃-N (p < 0.05). The NH₃-N is used as a nitrogen source by ruminal microbes which can degrade both nonstructural and structural carbohydrates [14]. It was found that BF-based silage had a higher non-protein nitrogen portion than that of rye straw [1]. In the present study, feeding BF-based silage increased dietary CP intake by 44%, resulting in 71% higher ruminal NH₃-N concentration. Adequate ruminal NH₃-N concentrations for ruminal microbial synthesis were in 5 ~ 23 mg/dl [15, 16]. The ruminal NH₃-N concentration for the treated group were in the upper normal range. The increased ruminal NH₃-N could increase ruminal VFA concentration [17], but feeding BF-based silage in the present study did not affect ruminal VFA concentrations.

### Table 2 Ingredients and chemical composition of diets fed to sheep

| Item | Diet with |  |
|------|-----------|  |
| Concentrate mix | 60.0 | 60.0 |
| Rye straw | 40.0 | - |
| BF-based silage | - | 40.0 |

| Item | Diet with |  |
|------|-----------|  |
| Dry matter | 88.7 | 76.1 |
| Organic matter | 93.2 | 90.4 |
| Crude protein | 11.5 | 16.6 |
| Ether extract | 1.6 | 3.1 |
| Neutral detergent fiber | 46.7 | 39.9 |
| Acid detergent fiber | 28.4 | 27.3 |
| Hemicellulose | 18.3 | 12.6 |
| Crude fiber | 21.9 | 19.2 |
| Nitrogen-free extracts | 58.2 | 51.5 |
| Crude ash | 6.8 | 9.6 |

### Table 3 Ruminal parameters of sheep fed the different diets

| Item | Diet with | SE |
|------|-----------|----|
| Ruminal pH | 6.6 | 6.6 |
| Ruminal VFA (mM/ℓ) | 105.6 | 107.5 |
| Acetate | 16.9 | 22.9 |
| Butyrate | 4.5 | 4.7 |
| Acetate/propionate | 5.9 | 4.7 |
| NH₃-N (mg/dl) | 13.2 | 22.6 |

| Diet with | SE |
|-----------|----|
| Rye straw | 0.1 |
| BF-based silage | |

| Item | Diet with | SE |
|------|-----------|----|
| Acetate | 105.6 | 107.5 |
| Propionate | 16.9 | 22.9 |
| Butyrate | 4.5 | 4.7 |
| Acetate/propionate | 5.9 | 4.7 |
| NH₃-N (mg/dl) | 13.2 | 22.6 |

| Diet with | SE |
|-----------|----|
| Rye straw | 0.1 |
| BF-based silage | 1.0 |

1Means of 6 observations

2BF-based silage was by-product feed-based silage, which was composed of 45% spent mushroom substrate, 21% recycled poultry bedding, 11% rye straw, 10.8% rice bran, 10% corn taffy residue, 1.0% protected fat, 0.6% bentonite, and 0.6% microbial additive, and ensiled for 30 d

3Means with different superscripts within the same row are significantly different (p < 0.05)
Nutrients intake and digestibility

The total feed intake are shown in Table 4. Compared to feeding rye straw, feeding BF-based silage increased CP, EE, and crude ash intake \((p < 0.05)\), but decreased fiber (NDF, ADF and CF), OM, and nitrogen free extracts (NFE) intake \((p < 0.05)\). Apparent nutrient digestibility and digestible nutrient intake are shown in Table 5. Digestible CP and EE intakes increased and digestible DM, OM, fiber (NDF, ADF, and CF), and NFE intakes decreased by replacing rye straw with BF-based silage \((p < 0.05)\). Compared to control treatment, sheep fed BF-based silage were observed to have 1.8, 2.9 and 8.8 percentage units lower apparent digestibility of DM, NDF, and crude ash, respectively \((p < 0.05)\). It appeared that about 2-fold increase in EE intake induced increased EE digestibility. The decreased DM digestibility by feeding BF-based silage was attributed to the high ash content in BF-based silage. The decreased fiber (NDF, ADF, and crude fiber) digestibility was attributed to the high lignin content in the sawdust-based mushroom substrate included in the BF-based silage [1]. It seems natural that fiber in sawdust and corn cobs constituting major source in the SMS and BF-based silage would be less digested than that in rye straw. Calculated total digestible nutrients (TDN) of both diets did not alter, indicating that BF-based silage was equivalent to rye straw in energy value for sheep. In detail, TDN content of BF-based silage was estimated to be 44.1 % when TDN content of commercial concentrate mix was assumed to be normally 79.0 %.

Nitrogen balance

The N balance of sheep fed the different diets is shown in Table 6. Because of the CP content difference between rye straw (4.2 %) and BF-based silage (16.9 %), N intake significantly increased 6.9 g/d by feeding BF-based silage \((p < 0.05)\). Feeding BF-based silage increased N excretion, absorption and retention by 6, 5.1, and 0.9 g/d \((p < 0.05)\), respectively. But N retention, expressed % intake or % absorbed, was not different between rye straw and BF-based silage, indicating that N retention efficiency was similar between rye straw N and BF-based silage N. Normally with dietary N intake increased, N absorption and total N excretion were increased [18] as shown in this study. The high ruminal NH_3-N could be available mostly as a nitrogen source for ruminal microbial growth, but excessive NH_3-N would be absorbed through the

### Table 4 Total feed and nutrient intake by sheep fed different diets

| Item                      | Diet with SE      | Rye straw | BF-based silage |
|---------------------------|-------------------|-----------|-----------------|
| Intake (g/d)              |                   | Rye straw | BF-based silage |
| Dry matter                | 850.0             | 850.0     | 0.0             |
| Organic matter            | 792.2             | 768.7     | 0.1             |
| Crude protein             | 97.9              | 141.3     | 0.1             |
| Ether extract             | 135.6             | 26.1      | 0.1             |
| Neutral detergent fiber   | 397.0             | 338.8     | 0.1             |
| Acid detergent fiber      | 241.6             | 232.0     | 0.1             |
| Hemicellulose             | 155.4             | 106.8     | 0.1             |
| Crude fiber               | 185.9             | 163.4     | 0.1             |
| Nitrogen free extracts    | 494.8             | 437.8     | 0.1             |
| Crude ash                 | 57.8              | 81.3      | 0.1             |

1) On a dry matter basis
2) BF-based silage was by-product feed-based silage, which was composed of 45 % spent mushroom substrate, 21 % recycled poultry bedding, 11 % rye straw, 10 % rice bran, 10 % corn taffy residue, 1 % protected fat, 0.6 % bentonite, and 0.6 % microbial additive, and ensiled for 30 d

### Table 5 Digestible nutrient intake and apparent nutrient digestibility of different diets fed to sheep (1,2)

| Item                               | Diet with SE      | Rye straw | BF-based silage |
|------------------------------------|-------------------|-----------|-----------------|
| Digestible nutrient intake (g/d)   |                   | Rye straw | BF-based silage |
| Dry matter                         | 575.3             | 560.1     | 6.3             |
| Organic matter                     | 553.7             | 536.7     | 5.8             |
| Crude protein                      | 67.4              | 99.1      | 1.1             |
| Ether extract                       | 10.4              | 23.3      | 0.6             |
| Neutral detergent fiber            | 223.0             | 180.5     | 4.1             |
| Acid detergent fiber               | 124.7             | 113.2     | 3.5             |
| Hemicellulose                      | 98.4              | 67.3      | 1.4             |
| Crude fiber                        | 91.0              | 74.7      | 2.3             |
| Nitrogen free extracts             | 384.9             | 339.7     | 3.6             |
| Crude ash                          | 21.6              | 23.3      | 2.2             |
| Apparent digestibility (%)         |                   | Rye straw | BF-based silage |
| Dry matter                         | 67.7              | 65.9      | 0.8             |
| Organic matter                     | 69.9              | 69.8      | 0.7             |
| Crude protein                      | 68.8              | 70.1      | 0.9             |
| Ether extract                       | 76.8              | 89.2      | 2.8             |
| Neutral detergent fiber            | 56.2              | 53.3      | 1.1             |
| Acid detergent fiber               | 51.6              | 48.8      | 1.5             |
| Hemicellulose                      | 63.3              | 63.0      | 1.1             |
| Crude fiber                        | 48.9              | 45.7      | 1.3             |
| Nitrogen free extracts             | 77.8              | 77.6      | 0.8             |
| Crude ash                          | 37.5              | 28.7      | 2.7             |
| Total digestible nutrients (%)     | 66.7              | 66.6      | 0.7             |

1) On a dry matter basis
2) By-product feed-based silage

### Notes

- a,bMeans with different superscripts within the same row are significantly different \((p < 0.05)\).
Table 6 Nitrogen balance of sheep fed the different diets1)

| Item          | Diet with       | SE  |
|---------------|-----------------|-----|
| Intake (g/d)  | Rye straw       | BF-based silage2) |     |
|               | 15.7a           | 22.6b | 0.1 |
| Excretion (g/d) |                 |     |     |
| Fecal         | 4.9a            | 6.8b | 0.2 |
| Urinary       | 7.9a            | 12.1b | 0.2 |
| Total         | 12.8a           | 18.8b | 0.3 |
| Absorption (g/d) | 10.8a        | 15.9b | 0.2 |
| Retention     |                 |     |     |
| g/d           | 2.9a            | 3.8b | 0.3 |
| % intake      | 18.4            | 16.8 | 1.6 |
| % absorbed    | 26.7            | 24.0 | 2.1 |

1)Means of 6 observations  
2)BF-basedsilage was by-product feed-based silage, which was composed of 45% spent mushroom substrate, 21% recycled poultry bedding, 11% rye straw, 10.8% rice bran, 10% corn taffy residue, 1% protected fat, 0.6% bentonite, and 0.6% microbial additive, and ensiled for 30 d.  
A,bMeans with different superscripts within the same row are significantly different (p < 0.05)

rumen wall or flow into the lower digestive tract and further absorbed, resulting in increased N retention for the BF-based silage-fed sheep.

Conclusions

The present results indicate that BF-based silage showed low fiber digestion and high N retention by sheep compared to rye straw. The BF-based silage had similar energy value to rye straw. These characteristics of BF-based silage could be used as basic data for total mixed ration formulation in ruminants. Using BF-based silage could help reduce feed cost in many countries importing hays.

Competing interests

The authors declare that they have no competing interests.

Authors’ contributions

JS made the experimental design and detailed chemical and statistical analysis of the study. YI drafted the manuscript. YH assisted the manuscript writing and standard form of the journal, and DY performed the statistical analysis. WS made substantial contributions to the study conception and design, and revised the manuscript as the corresponding author.

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References

1. Kim YI, Oh YK, Park KK, Kwak WS. Ensiling characteristics and the in situ nutrient degradability of a by-product feed-based silage. Asian-Aust J Anim Sci. 2014;27:201–8.
2. Kim YL, Jung SH, Seok JS, Yang SY, Huh JW, Kwak WS. Isolation and identification of high cellulolytic bacteria from spent mushroom substrate and determination of optimal medium conditions for the growth. Kor J Microbial Biotechnol. 2010;3:255–60.
3. Kim YL, Jeong SH, Seok JS, Yang SY, Huh JW, Kwak WS. Isolation and identification of hydrolytic enzyme-producing bacteria from spent mushroom substrate. Korean J Anim Sci Technol. 2008;50:713–20.
4. Kim YL, Lee SM, Lee YH, Lee M, Choi DW, Kwak WS. Effects of by-product feed-based silage on feeding, rumination, and excretion in growing Hanwoo heifers. Korean J Anim Sci Technol. 2015;57:3.
5. Kim YL, Lee SM, Park KK, Kwak WS. Effect of feeding by-product feeds-based silage (Biosilage) on behavior pattern of growing Hanwoo steers. J Kor Grassl Forage Sci. 2013;33:290–7.
6. Kim YL, Park JM, Lee YH, Lee M, Choi DY, Kwak WS. Effect of by-product feed-based silage feeding on the performance, blood Metabolites, and carcass characteristics of Hanwoo steers (a field study). Asian-Aust J Anim Sci. 2015;2:180–7.
7. NRC. Nutrient requirements of sheep. Washington, DC: National Academy Press; 1985.
8. AOAC (Association of Official Analytical Chemists). Official methods of analysis. 17th ed. Washington DC: Association of Analytical Chemists; 2000.
9. Van Soest PJ, Robertson JB, Lewis BA. Methods of dietary fiber, neutral detergent fiber, nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci. 1991;74:3583–97.
10. Chaney AL, Marbach EP. Modified reagents for the determination of urea. Clin Chem. 1962;8:130–2.
11. Erwin ES, Naro GJ, Emery EM. Volatile fatty acid analysis of blood and rumen fluid by gas chromatography. J Dairy Sci. 1961;44:1768.
12. Statistix7. User's manual. Analytical Software, Tallagasee, FL, USA; 2000.
13. Russell JB, Schcarp WM, Baldwin RL. The effect of pH on maximum bacterial growth rate and of bacterial competition in the rumen. J Anim Sci. 1979;48:251.
14. Satter LD, Slyter LL. Effect of ammonia concentration on rumen microbial protein production in vitro. Br J Nutr. 1974;32:199.
15. Mehrez AZ, Orskov ER, McDonald I. Rates of rumen fermentation in relation to ammonia concentration. Br J Nutr. 1977;8:447.
16. Hailand GL, Tyrell HF, Moe PW, Wheeler WE. Effect of crude protein level and limestone buffer in diets fed at two levels of intake on rumen pH, ammonia-nitrogen, buffering capacity and volatile fatty acid concentration of cattle. J Anim Sci. 1982;55:943–50.
17. Kwak WS, Yoon JS, Jung KH. Nutrient utilization of broiler litter and bakery by-product ration in Sheep. J Anim Sci Technol. 2005;46:607–16.
18. Ko YD, Ryu YU, Kang HS, Kim JH, Yoo SO, Kang IR. Study on the digestibility, nitrogen retention and rumen parameters in sheep fed whole crop cm–cage layer excreta silage I. Nutrition quality, digestibility and nitrogen retention of the whole crop cm–cage layer excreta. Kor J Anim Nutr Feed. 1996;20:453–8.