rccp Revista Colombiana de Ciencias Pecuarias

Rumen fermentation and digestibility of spent mushroom (*Pleurotus ostreatus*) substrate inoculated with *Lactobacillus brevis* for Hanwoo steers¤

Fermentación ruminal y digestibilidad de la cama de champiñón (Pleurotus ostreatus) desechada inoculada con Lactobacillus brevis en novillos Hanwoo

Fermentação ruminal e digestibilidade de resíduo de substrato de cogumelo (Pleurotus ostreatus) inoculado com Lactobacillus brevis en novilhos Hanwoo

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(Received: July 29, 2016; accepted: May 16, 2017)

doi: 10.17533/udea.rccp.v30n4a02

Abstract

Background: Ensiling spent mushroom substrate (SMS) generally increases its nutrient digestibility and quality. **Objective:** To determine the feed quality and digestibility of SMS from *Pleurotus ostreatus* (SMSP) inoculated with lactic acid bacteria (LAB: *Lactobacillus brevis*) in Hanwoo steers. **Methods:** Ruminal disappearance of SMSP and inoculated SMSP (ISMSP) were evaluated in three rumen-fistulated Hanwoo steers (408 ± 13.0 Kg body weight). Further, three healthy Hanwoo steers (336 ± 69.0 Kg body weight) were randomly allotted to one of three dietary treatments (control: 25% straw, 75% concentrates; treatments: 25% straw, 60% concentrates, and 15% of either SMSP or ISMSP) in a 3×3 Latin square design. **Results:** The

¤ To cite this article: Baek YC, Kim MS, Reddy KE, Oh YK, Jung YH, Yeo JM, Choi H. Rumen fermentation and digestibility of spent mushroom (*Pleurotus ostreatus*) substrate inoculated with *Lactobacillus brevis* for Hanwoo steers. Rev Colomb Cienc Pecu 2017; 30:267-277.

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chemical composition of the ISMSP diet did not differ from that of the control or the SMSP diets. In the ISMSP diet, the rate of decrease of pH of ruminal fluid and the increase in storage period was greater than with the SMSP diet. Ruminal disappearance of dry matter, crude protein, neutral detergent fiber, and acid detergent fiber were slightly higher in steers fed ISMSP than those fed SMSP. Furthermore, the degradation rate and effective degradability of crude protein was greater in the ISMSP diet than in the SMSP diet. Effective ruminal fermentation characteristics and total nutrients digestibility were not affected by SMSP nor ISMSP diet. **Conclusion:** The SMSP and ISMSP diets could replace formulated concentrate without adverse effects and be a cost-effective feed for Hanwoo steers. Furthermore, LAB inoculation improved the SMSP preservation.

Keywords: *agricultural by-products ensilage, feed cost, nylon bag technique, total-tract digestibility, volatile fatty acid.*

Resumen

Antecedentes: El ensilado de cama de champiñón desechada (SMS) generalmente aumenta la digestibilidad y la calidad de sus nutrientes. **Objetivo:** Determinar la calidad del alimento y digestibilidad del SMS a partir de *Pleurotus ostreatus* (SMSP) inoculado con bacterias ácido-lácticas (LAB: *Lactobacillus brevis*) en bueyes Hanwoo. **Métodos:** La desaparición ruminal del SMSP y ISMSP (SMSP inoculado) fue evaluada en tres bueyes Hanwoo fistulados en el rumen (408 ± 13,0 Kg peso corporal). Igualmente, tres bueyes Hanwoo sanos (336 ± 69,0 Kg peso corporal) fueron asignados al azar a uno de los tres tratamientos dietéticos (control: 25% de heno, 75% de concentrados; tratamientos: 25% de heno, 60% de concentrados y 15% de SMSPo ISMSP) en un diseño cuadrado latino 3×3. **Resultados:** La composición química de la dieta ISMSP no difirió de la del control o de la dieta SMSP. En la dieta ISMSP, la tasa de disminución del pH del fluido ruminal y el incremento del tiempo de almacenamiento fueron mayores que los de la dieta SMSP. La desaparición ruminal de la materia seca, proteína cruda, fibra detergente neutra y la fibra detergente ácida fue ligeramente superior en los bueyes alimentados con el ISMSPque en aquellos alimentados con SMSP. Además, la tasa de degradación y la degradabilidad efectiva de la proteína cruda fueron mayores en la dieta ISMSPque en la dieta SMSP. Las características de la fermentación ruminal efectiva y la digestibilidad total de nutrientes no fueron afectadas por la dieta SMSP ni por la de ISMSP. **Conclusión:** Las dietas SMSPe ISMSP podrían reemplazar el concentrado formulado sin efectos adversos y ser una alimentación económica para los bueyes Hanwoo. Además, la inoculación con LAB mejoró la conservación del SMSP.

Palabras clave: *ácido graso volátil, costo de alimentación, digestibilidad total del tracto digestivo, ensilado de subproductos agrícolas, técnica de la bolsa de nylon.*

Resumo

Antecedentes: Ensilagem de resíduo de substrato de cogumelo (SMS) geralmente aumenta a digestibilidade e a qualidade dos nutrientes. **Objetivo:** Determinar a qualidade da alimentação e digestibilidade do RSC apartir do *Pleurotus ostreatus* (SMSP) inoculado com bactérias de ácido láctico (LAB: *Lactobacillus brevis*) nos novilhos Hanwoo. **Métodos:** Desaparecimento ruminal do SMSP e ISMSP (SMSP inoculado) foram avaliados em três novilhos Hanwoo fistulados no rúmen (408 ± 13.0 Kg peso corporal). Além disso, três novilhos Hanwoo (336 ± 69.0 Kg peso corporal) foram aleatoriamente distribuídos para um dos três tratamentos dietéticos (controle: 25% palha, 75% concentrado; tratamentos: 25% palha, 60% concentrado, e 15% ambos SMSP e ISMSP) em um quadrado Latino de 3×3. **Resultados:** A composição química da dieta ISMSP não diferiu do controle ou das dietas SMSP. Na dieta ISMSP, a taxa de diminuição do pH do fluido ruminal e o aumento no período de armazenamento foram melhores do que com a dieta SMSP. O desaparecimento ruminal de matéria seca, proteína bruta, fibra em detergente neutro, e fibra detergente ácido foi ligeiramente superior em novilhos alimentados com ISMSP, do que aqueles alimentados com SMSP. Além disso, a taxa de degradação e degradabilidade da proteína bruta foi maior na dieta ISMSP, do que na dieta SMSP. As características efetivas de fermentação ruminal e a digestibilidade total de nutrientes não foram afetadas pela dieta SMSP, nem pela dieta ISMSP. **Conclusão:** Dietas com SMSP e ISMSP podem ser utilizados em substituição ao concentrado formulado sem causar efeitos adversos e ser um alimento rentável em novilhos Hanwoo. Além disso, a inoculação com LAB melhorou a qualidade conservante da SMSP.

Palabras chave: *ácidos graxos voláteis, custo de alimentação, digestibilidade total do trato, ensilados de subprodutos agrícolas, técnica de saco de nylon.*

Introduction

The cost of feed accounts for approximately 70% of the total cost of beef production (Liu *et al*., 2000); thus, various approaches are being developed to reduce feed cost and improve feed efficiency using by-products, as they contribute to the reduction of both feed and waste cost (Eastridge, 2006; Lee *et al*., 2010). Mushroom production has increased rapidly over the past few decades (Fazaeli and Massodi, 2006) and millions of tons of SMS have become available for other uses (Rinker, 2002). Spent mushroom substrate has been used as a feed resource for sheep, buffalo and beef cattle due to its contents of crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF; Kakkar *et al*., 1990; Kakkar and Dhanda 1998; Rinker, 2002; Fazaeli and Masoodi, 2006; Ayala *et al*., 2011). In addition, as SMS can be obtained cheaply from mushroom-processing plants, its utilization as a ruminant feed could be cost-effective, particularly in countries where animal feeds are heavily dependent on imports. Although SMS putrefies rapidly due to its high moisture content, ensiling SMS under anaerobic conditions helps prevent putrefaction (Kwak *et al*., 2008; Kim *et al*., 2012).

Under anaerobic conditions, lactic acid bacteria (*Lactobacillus brevis;* LAB) in silage converts water-soluble carbohydrates (WSC) into organic acids and decreases silage pH, therefore preventing moist forage crops from putrefying (Rowghani and Zamiri, 2009). In addition, previous studies showed that *Lactobacillus* spp. inoculated into SMS can improve fermentation, preservation and digestibility of SMS (Muck *et al*., 2007; Kim *et al*., 2014). However, to the best of our knowledge, no study has examined the efficacy of LAB inoculation into SMS on ruminal fermentation and digestibility in Korean native Hanwoo cattle. Still, nitrogenous compounds in silage are rapidly degraded due to low microbial protein synthesis in the rumen (Rooke *et al*., 1983; Bosch *et al*., 1988); thus, supplementary enzymes do not consistently increase nutrient digestibility and quality of feeds (Weinberg *et al*., 2008). Therefore, the efficacy of ensiling SMS to maintain its nutrient quality and reduce feed costs remains to be investigated in ruminant animals.

The objective of this study was to determine the effect of SMS from *Pleurotus ostreatus* (SMSP) inoculated with LAB on feed quality, ruminal fermentation and total-tract digestibility in Hanwoo steers.

Materials and methods

Ethical considerations

The protocol for the experimental procedures were reviewed and approved by the Animal Care and Use Committee of the National Institute of Animal Science, Republic of Korea (No. 2014-061).

Inoculation of SMS with LAB

Spent mushroom substrate from *Pleurotus ostreatus* (SMSP) was obtained from the Mushroom Research Institute (GARES, Gwangju, Korea). The original mushroom substrate consisted of 50% corn cob, 35% beet pulp, and 15% kapok meal. Regarding the microbial inoculants, LAB (donated by Dr. WS Kwak) was grown on De Man-Rogosa-Sharpe (MRS) broth (Difco Laboratories Inc., Detroit, MI, USA) at 37 °C for 24 h and then inoculated into SMS by modification of the method by Kim *et al*. (2014; 2015). Briefly, 97% SMSP was mixed with 2% rice bran, 0.5% molasses and 0.5% (v/w) LAB, and then approximately 20 Kg of the mixture was packed into two folds of polyvinyl-bags and sealed. The inoculated SMSP (ISMSP) was fermented at 30 °C for 5 d, and then stored at 4 °C until used. The test diet samples of SMSP and ISMSP were prepared using the five subsamples from each polyvinyl-bags silo, corresponding to each treatment and storage period. Visual inspections for spoilage were made at 0, 1, 24, 48, 72, 96, and 120 h, and the pH of the SMSP and ISMSP was measured at these times as well. Chemical compositions of the test diets are shown in Table 1.

Experiment 1: In situ experimental design and rumen digestibility

Three rumen-fistulated Hanwoo steers (408 ± 13 Kg body weight) were individually housed in tie-stall barns (127 × 250 × 200 cm) for an *in situ* experiment. They were all fed 1.25 Kg rice straw with 3.75 Kg formulated concentrate mixture, and had free access to water and mineral block.

Table 1. Chemical composition of the test diets.

Composition, %	Diets		SEM
	SMSP	ISMSP	
Dry matter	38.0	38.0	0.01
Crude protein	7.9	14.2	4.45
Ether extract	0.3	1.1	0.57
Crude fiber	24.2	23.0	0.85
Crude ash	4.4	6.7	1.63
Nitrogen-free extract	63.3	55.0	5.87
Neutral detergent fiber	74.8	67.3	5.30
Acid detergent fiber	49.4	43.4	4.24
Energy (Kcal/g)	4.7	4.9	0.14

SMSP: Spent mushroom substrate from *Pleurotus ostreatus*. ISMSP: SMSP inoculated with LAB. SEM: Standard error of the mean.

The diet was formulated to meet NRC requirements (2001), and animals were fed twice daily (at 09:00 and 17:00 h). The chemical and ingredient composition of the formulated concentrate mixture diet are shown in Table 2.

Table 2. Chemical and ingredient composition of the formulated concentrate mixture diet.

Composition, % of dry matter	Formulated concentrate mixture
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[1]	0.2
Lasalocid	0.1
Total	100.0

[1]Vitamin-mineral premix components: Vitamin A, 2,650,000 IU; Vitamin D_3, 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 test diet samples of SMSP and ISMSP were dried and milled to pass through a 2-mm mesh screen in a Wiley® Mill (Thomas Scientific, Swedesboro, NJ, USA) before rumen incubation. Nylon bags (NL 130-030/330PW, NBC Inc., Tokyo, Japan), each approximately 8 × 15 cm (45-µm pore size; sample size: Surface area = 41.67 mg/cm^2), were filled with 5 g of dry ground feed samples (100% SMSP and ISMSP) and tied with a rubber band. The bags containing feed samples (3 replicates for each steer) were incubated in the rumen of Hanwoo steers for 3, 6, 9, 12, 24, 48, 72, 96, and 120 h. The bags that were not incubated, representing time 0, were treated in the same manner as the incubated ones. The nylon bags were washed in a washing machine for 30 min and then dried in a forced air oven at 60 °C for 48 h until a constant weight was achieved before determination of residual dry matter (DM).The DM degradation in the rumen was calculated according to the method of Ørskov and McDonald (1979) with a passage rate of 0.02 and 0.05%/h. Parameters for ruminal disappearance were calculated according to the iterative least squares nonlinear model:

$$P = A + B \times (1 - \exp^{-ct})$$

Where:

P: Is rumen degradation (%).

A: Is rapidly degradable A fraction (%).

B: Is slowly degradable B fraction (%).

C: Is degradation rate of degradable B fraction (%/h).

T: Is time (h; Ørskov and McDonald, 1979).

The effective degradability of CP and DM was calculated using the following equation:

$$ED (\%) = A + [BC/(C + K)]$$

Where:

A, B, and C: Are constants of the nonlinear model described above.

K: Is the measured particle passage rate of 0.02 and 0.05/h (Denham *et al*., 1989).

Experiment 2: Feeding trial design and total-tract digestibility

Three healthy Hanwoo steers (336 ± 69.0 Kg body weight) were randomly allotted to one of three dietary treatments in a 3×3 Latin square design. The treatments consisted of a control (3.75 Kg/d concentrates + 1.25 Kg/d rice straw), a SMSP diet (3.19 Kg/d concentrates + 1.25 Kg/d rice straw + 0.56 Kg/d SMSP), and an ISMSP diet (3.19 Kg/d concentrates + 1.25 Kg/d rice straw + 0.56 Kg/d ISMSP; five silos were thoroughly mixed per treatment). The chemical composition of the experimental diets are shown in Table 3.

Table 3. Chemical composition of the experimental diets.

Composition, % of dry matter	Treatments			SEM
	Control	SMSP	ISMSP	
Crude protein	10.5	10.7	9.9	0.42
Ether extract	2.7	2.4	2.3	0.21
Crude fiber	12.6	14.5	14.7	1.16
Crude ash	6.9	5.5	7.5	1.03
Nitrogen-free extract	67.4	65.3	66.2	1.05
Neutral detergent fiber	33.8	38.9	39.7	3.20
Acid detergent fiber	13.8	18.2	18.8	2.73
Hemicellulose	20.0	20.7	20.9	0.47
None-fibrous carbohydrate	46.1	42.5	40.6	2.79
Energy (Kcal/g)	4.3	4.4	4.4	0.06

Control: Formulated concentrate mix 3.75 Kg + rice straw 1.25 Kg/d. SMSP diet: Formulated concentrate mix 3.19 Kg + rice straw 1.25 Kg + SMSP 0.56 Kg/d. ISMSP diet: Formulated concentrate mix 3.19 Kg + rice straw 1.25 Kg + ISMSP 0.56 Kg/d. SMSP: Spent mushroom substrate from *Pleurotus ostreatus*. ISMSP: SMSP inoculated with LAB. SEM: Standard error of the mean.

The experimental diets were provided twice daily (at 0900 and 1700 h). Each experimental period consisted of an 8-d adaptation period and a 5-d sample collection period. Once weighed, fecal subsamples (5%) were obtained during the collection period (twice daily at 09:30 and 17:30 h) and stored at -20 °C until analysis. All fecal samples were dried in a forced air oven at 60 °C for 48 h, and then ground through a 2-mm mesh screen, and analyzed. The energy value of the feed was calculated based on total digestible nutrients (TDN, %). TDN was calculated using the following equation:

$$\text{TDN} = \text{Digestible crude protein} + (\text{digestible crude fat} \times 2.25) + \text{digestible neutral detergent fiber (\%)} + \text{digestible soluble carbohydrate (\%)}$$

Rumen sample preparation and analysis

Ruminal fluid was sampled at 0, 2, 4, 6, and 8 h post-morning feeding on the last day of each period. Approximately 200 mL of ruminal fluid was collected by a stomach tube and strained through four layers of cheesecloth. After recording pH, 1 mL of the fluid was treated with 0.2 mL HPO_3 and frozen for ammonia-N concentration and subsequent volatile fatty acid (VFA) analysis. The concentration of VFA was determined by gas chromatography (CP-3800; Varian Inc., Walnut Creek, CA, USA) as previously described by Oh *et al.* (2010). Ammonia-N concentration was analyzed by a multiplate spectrophotometer (Bio-Rad, Benchmark Plus™, Tokyo, Japan) at 630 nm according to the method of Chaney and Marbach (1962).

All diet samples were dried and milled to pass through a 1-mm mesh screen in a Wiley® Mill before chemical analysis. Diet samples were analyzed for DM, CP, ether extract (EE), crude fiber (CF), crude ash (Ash), and nitrogen-free extract (NFE) according to the methods described by the AOAC (2012). NDF, ADF and acid detergent lignin (ADL) concentrations were evaluated using a fiber analyzer (ANKOM 2000, ANKOM Technology Corporation, Macedon, NY, USA). Hemicellulose (HEM) was calculated as NDF - ADF, and cellulose by ADF - ADL.

Energy (Kcal/g) was measured with an oxygen bomb calorimeter (Parr 6400 calorimeter, Parr Instruments Company, IL, USA). The amount of non-fibrous carbohydrates (NFC) was calculated by subtracting the sum of NDF, CP, EE, and Ash from 100.

Statistical analysis

Statistical analysis were conducted using the General Linear Models (GLM) procedure of SAS, version 9.1® (Statistical Analysis Systems Institute Inc., Cary, NC, USA, 2002) with a one-way ANOVA. Duncan's multiple range tests were used to compare means. All data presented are expressed as mean ± SEM from three independent measurements. Significant difference was declared at $p<0.05$.

Results

Spoilage and pH measurements

Visual evaluation and pH change are shown in Figure 1. Mold was detected on the SMSP at d 5, and severe spoilage was detected at d 3 on the SMSP (data not shown). However, no visible spoilage was observed in ISMSP until d 5, and the pH of ISMSP was lower ($p<0.05$) than that of SMSP from d 1 to d 5.

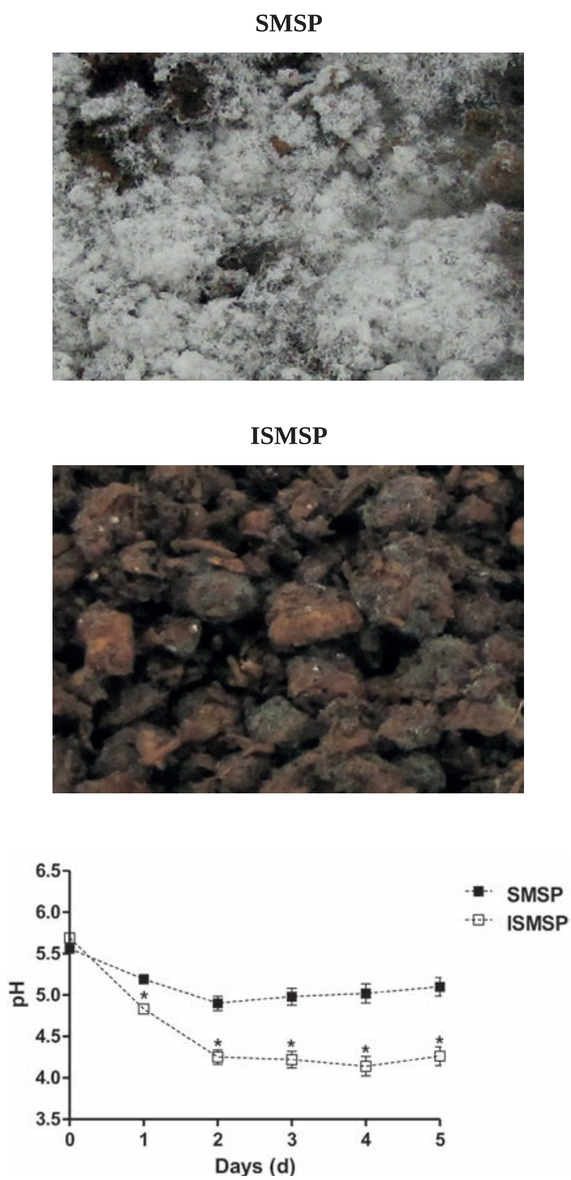


Figure 1. Visual evaluation and pH change of SMSP and ISMSP during 5 d of storage under anaerobic condition. SMSP: Spent mushroom substrate from *Pleurotus ostreatus*. ISMSP: SMSP inoculated with LAB. Asterisks (*) indicate means differ significantly between treatments (p<0.05).

In situ rumen degradation

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

The rapidly degradable A fraction of CP in ISMSP (46.5%) was higher (p<0.05) compared to the SMSP (38.3%) treatment (Table 5). The degradation rate

Table 4. Dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) digestibility at each rumen incubation time of the test diets.

Item	Time	Diets (%)		SEM
		SMSP	ISMSP	
DM digestibility	0	17.3	19.8	0.55
	3	17.7	22.1	0.66
	6	18.6	23.5	0.77
	12	20.3	25.2	0.75
	24	23.2	28.6	0.67
	48	31.2	38.7	0.90
	72	31.2	41.6	1.04
	96	38.8	44.4	1.69
	120	37.7	45.8	0.96
CP digestibility	0	38.0	44.4	1.23
	3	38.5	48.7	1.54
	6	41.1	49.9	1.33
	12	42.2	51.9	1.46
	24	39.2	49.9	1.24
	48	44.8	54.5	1.12
	72	49.3	58.0	1.04
	96	51.2	58.0	1.46
	120	48.1	55.3	0.85
NDF digestibility	0	6.4	5.5	0.37
	3	3.2	5.3	0.36
	6	3.6	7.3	0.67
	12	6.0	9.4	0.87
	24	12.3	16.7	1.06
	48	20.5	25.6	0.74
	72	21.7	29.8	1.22
	96	25.3	32.2	1.00
	120	27.5	31.7	1.84
ADF digestibility	0	4.4	3.5	0.13
	3	2.3	3.5	0.22
	6	3.8	5.1	0.37
	12	5.7	6.1	0.62
	24	11.6	12.6	0.46
	48	18.1	22.5	0.65
	72	20.1	25.5	0.83
	96	22.7	28.0	0.78
	120	22.2	31.8	1.92

SMSP: Spent mushroom substrate from *Pleurotus ostreatus*. ISMSP: SMSP inoculated with LAB. DM: Dry matter; CP: Crude protein; NDF: Neutral detergent fiber; ADF: Acid detergent fiber. SEM: Standard error of the mean.

of degradable B fraction of CP in ISMSP (4%/h) was higher ($p<0.05$) than in SMSP (1%/h), and the effective degradability of CP in ISMSP (50.9) was higher ($p<0.05$) than in SMSP (41.5). The degradation rate of degradable B fraction of DM in ISMSP (2%/h) was higher ($p<0.05$) than in SMSP (1%/h), and the effective degradability of DM in ISMSP (27.8) was higher ($p<0.05$) than in SMSP (22.5).

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

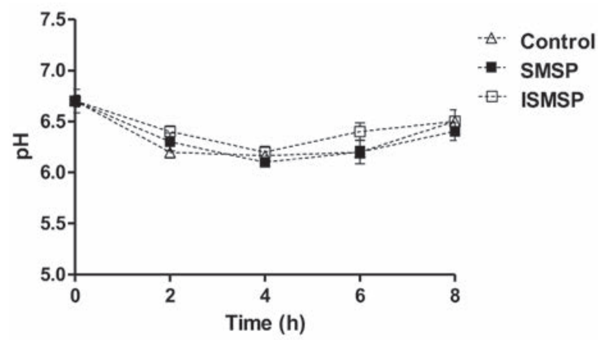
Item	Diets		SEM
	SMSP	ISMSP	
Rapidly degradable A fraction (%)	38.3^{b}	46.5^{a}	2.37
Slowly degradable B fraction (%)	14.5	10.6	1.29
Potential degradability: A + B (%)	52.8	57.2	1.40
Degradation rate of degradable B fraction (%/h)	0.01^{b}	0.04^{a}	0.01
Effective degradability of CP[3]	41.5^{a}	50.9^{b}	2.70
Rapidly degradable A fraction (%)	16.5	19.9	1.02
Slowly degradable B fraction (%)	27.4	30.2	1.26
Potential degradability: A + B (%)	43.9	50.1	2.11
Degradation rate of degradable B fraction (%/h)	0.01^{b}	0.02^{a}	0.01
Effective degradability of DM	22.5^{b}	27.8^{a}	1.53

SMSP: Spent mushroom substrate from *Pleurotus ostreatus*. ISMSP: SMSP inoculated with LAB. Effective degradability of CP and DM: Calculated according to Ørskov and McDonald (1979), and using a rumen outflow rate of 5%. Means with different superscript letters ($^{a,\,b}$) within the same row are significantly different ($p<0.05$). SEM: Standard error of the means.

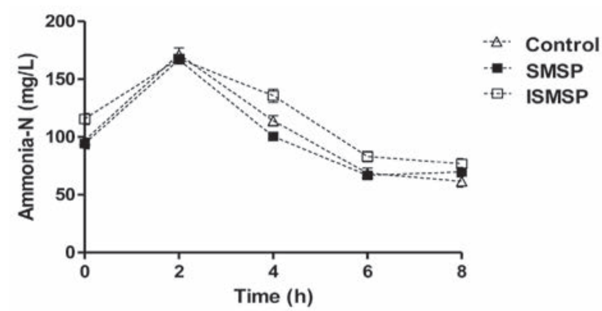
Changes in pH, ammonia-N, and VFA concentrations in the rumen

At every sampling time, rumen pH and ammonia-N concentrations were not different between treatments (Figure 2). The rumen pH in all treatments tended to decrease from 0 to 4 h post-feeding and then to increase again (Figure 2A). Rumen ammonia-N concentration in all treatments increased until 2 h post-feeding and declined gradually thereafter (Figure 2B).

There were no significant differences in the concentration of total or individual VFA between treatments, except for those of butyrate at 8 h (Table 6). Concentration of butyrate in the ruminal fluid of control (11.5) was significantly higher ($p<0.05$) than that in SMSP (8.8) and ISMSP (8.1). Concentration



A.



B.

Figure 2. pH and ammonia-N concentrations changes in rumen by experimental diets. Control: Formulated concentrate mix 3.75 Kg + rice straw 1.25 Kg/d. SMSP diet: Formulated concentrate mix 3.19 Kg + rice straw 1.25 Kg + SMSP 0.56 Kg/d. ISMSP diet: Formulated concentrate mix 3.19 Kg + rice straw 1.25 Kg + ISMSP 0.56 Kg/d. SMSP: Spent mushroom substrate from *Pleurotus ostreatus*. ISMSP: SMSP inoculated with LAB.

of total and individual VFA in all treatments increased at 2 h and then tended to decrease from 4 to 8 h. The A:P ratio was not affected by the treatments.

Nutrients digestibility of SMSP and ISMSP diets in Hanwoo steers

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

Discussion

Muck *et al.* (2007) reported that LAB inoculation seemed to affect *in vitro* ruminal fermentation characteristics. Kim *et al.* (2014) also indicated

Table 6. Effects of experimental diets fed on ruminal volatile fatty acid (VFA) changes.

Time	Item	Treatments			SEM
		Control	SMSP	ISMSP	
0	Total VFA (mM)	77.3	82.0	68.3	5.10
	Acetate (A, %)	47.6	56.8	47.7	3.95
	Propionate (P, %)	12.0	13.5	11.1	0.87
	Isobutyrate (%)	1.0	1.0	0.8	0.05
	Butyrate (%)	9.5	8.5	7.0	0.57
	Isovalerate (%)	1.6	1.5	1.2	0.08
	Valerate (%)	0.7	0.7	0.5	0.04
	A:P	4.0	4.2	4.3	0.12
2	Total VFA (mM)	103.0	103.7	98.2	7.90
	Acetate (A, %)	64.8	66.8	64.8	5.97
	Propionate (P, %)	20.6	20.4	19.1	1.67
	Isobutyrate (%)	1.0	1.0	0.8	0.12
	Butyrate (%)	13.0	10.6	11.8	0.62
	Isovalerate (%)	1.9	1.9	1.5	0.14
	Valerate (%)	1.7	1.7	1.5	0.12
	A:P	3.1	3.3	3.4	0.10
4	Total VFA (mM)	93.0	87.7	75.3	4.47
	Acetate (A, %)	61.8	56.4	48.7	3.78
	Propionate (P, %)	19.0	17.3	14.7	1.01
	Isobutyrate (%)	0.9	0.8	0.7	0.05
	Butyrate (%)	8.0	10.1	8.9	0.79
	Isovalerate (%)	1.7	1.6	1.3	0.10
	Valerate (%)	1.5	1.4	1.2	0.07
	A:P	3.3	3.3	3.3	0.10
6	Total VFA (mM)	84.6	56.8	74.4	4.27
	Acetate (A, %)	57.1	59.2	51.3	3.55
	Propionate (P, %)	16.0	17.2	14.1	0.94
	Isobutyrate (%)	0.9	0.8	0.6	0.08
	Butyrate (%)	8.0	6.9	6.2	1.00
	Isovalerate (%)	1.5	1.6	1.2	0.10
	Valerate (%)	1.2	1.2	0.9	0.07
	A:P	3.6	3.4	3.6	0.67
8	Total VFA (mM)	85.2	77.2	68.2	5.42
	Acetate (A, %)	55.0	51.7	45.9	3.97
	Propionate (P, %)	15.0	13.9	11.9	1.03
	Isobutyrate (%)	0.8	0.8	0.6	0.07
	Butyrate (%)	11.5^{a}	8.8^{ab}	8.1^{b}	0.60
	Isovalerate (%)	2.0	1.2	1.0	0.22
	Valerate (%)	1.0	0.9	0.7	0.06
	A:P	3.7	3.7	3.9	0.08

Control: Formulated concentrate mix 3.75 Kg + rice straw 1.25 Kg/d. SMSP diet: Formulated concentrate mix 3.19 Kg + rice straw 1.25 Kg + SMSP 0.56 Kg/d. ISMSP diet: Formulated concentrate mix 3.19 Kg + rice straw 1.25 Kg + ISMSP 0.56 Kg/d. SMSP: Spent mushroom substrate from *Pleurotus ostreatus*; ISMSP: SMSP inoculated with LAB. Means with different superscript letters ($^{a,\ b}$) within the same row are significantly different ($p<0.05$). SEM: Standard error of the mean.

Table 7. Effects of experimental diets on total-tract nutrient digestibility in Hanwoo steers.

Item	Treatments			SEM
	Control	SMSP	ISMSP	
Nutrient digestibility, %				
Crude protein	67.3	65.5	53.7	0.09
Ether extract	64.1	59.7	60.9	1.39
Crude fiber	83.5	86.8	87.0	0.88
Crude ash	52.6	52.4	50.3	1.32
Nitrogen-free extract	9.2	12.6	7.5	2.09
Neutral detergent fiber	76.8	75.3	73.3	0.83
Acid detergent fiber	56.0	55.2	51.9	1.38
Hemicellulose	30.8	45.9	41.0	2.22
None-fibrous carbohydrate	73.2	63.7	61.4	2.15
Energy (Kcal/g)	2.9	2.8	2.8	1.05
TDN (%)	67.3	64.8	63.3	1.38

Control: Formulated concentrate mix 3.75 Kg + rice straw 1.25 Kg/d. SMSP diet: Formulated concentrate mix 3.19 Kg + rice straw 1.25 Kg + SMSP 0.56 Kg/d. ISMSP diet: Formulated concentrate mix 3.19 Kg + rice straw 1.25 Kg + ISMSP 0.56 Kg/d. SMSP: Spent mushroom substrate from *Pleurotus ostreatus*; ISMSP: SMSP inoculated with LAB. SEM: Standard error of the mean.

that LAB inoculation improved *in situ* ruminal fermentation. However, our *in vivo* study did not show ruminal fermentation improved by LAB inoculation. This discrepancy may result from variation among *in vitro, in situ* and *in vivo* methods. In addition, LAB (e.g. *L. plantarum or L. buchneri*) used in the previous studies (Muck *et al.*, 2007; Kim *et al*. 2014) was different from LAB used in our study. The use of different LAB inoculants may lead to different results in ruminal fermentation characteristics.

In Experiment 1, pH was lower in the ISMSP than in the SMSP during the fermentation period for 5 d, and, in contrast to SMSP, no visible spoilage was observed in the ISMSP for 5 d (Figure 1), indicating that LAB inoculation of SMS could extend its storage period. Similarly, Kim *et al.* (2012) reported that microbial fermented SMS had lower pH and exhibited less putrefaction. Jones *et al.* (1992) found that bacterial inoculation of silage alters the composition of cell-wall carbohydrates into organic acids and rapidly decreases its pH during fermentation. Therefore, LAB inoculates can be considered as a good strategy for improving the storage capacity of SMS.

The results of the *in situ* experiment showed that digestibility of DM, CP, NDF, and ADF were slightly higher for ISMSP than for SMSP (Table 4). Interestingly, this study showed that the degradation rate of degradable B fraction of CP and DM was faster in ISMSP than in SMSP (Table 5), and these results are similar to those reported by Nowak *et al.* (2004). Keady and Steen (1994) found that inoculation of silage improves *in vivo* total digestibility. However, some reports estimate that silage fermentation and silage additives may have little or no effect on digestibility and silage fermentation characteristics (McDonald *et al*., 1991; Cushnahan *et al*., 1995).

In Experiment 2, the changes in pH, ammonia-N and VFA concentrations were unaffected by the SMSP and ISMSP (Figure 2 and Table 6). Similarly, Nadeau and Buxton (1997) found that inoculants had no effect on the ammonia-N concentration in alfalfa silage. When Keady and Steen (1994) added *L. plantarum* bacteria at 10^9 cfu/g to silage at the time of feeding, they found no response in terms of intake, digestibility, or rumen fermentation. However, many studies have shown that LAB use increases DM degradability and the level of fermentable substrate in silage (Jaakkola *et al*., 1991; Stokes, 1992; Yahaya *et al*., 2004). Inoculant-treated silage significantly increases the nutrient digestibility of organic matter (OM), NDF, and ADF (Yahaya *et al*., 2004), suggesting increased microbial activity in the rumen. The results of total-tract digestibility analysis in this study showed that SMSP and ISMSP diets did not affect all nutrients digestibility compared to the control (Table 7). Thus, 0.5% LAB inoculants did not affect all nutrients digestibility of SMSP in this experiment.

These results indicate that LAB inoculation of SMSP exhibited the highest *in situ* rumen digestibility of DM, CP, NDF, and ADF residues compared with SMSP without inoculation. However, LAB inoculants did not affect ammonia-N and VFA concentration, DM degradability, or total-tract digestibility. In conclusion, 0.5% LAB inoculation improved the preservation of SMSP. Nevertheless, the TDN of SMSP and ISMSP diets was slightly lower than that of the control suggesting that the recommended level of dietary supplementation of SMSP and ISMSP should be less than 15%. The results of the present study showed that SMSP and ISMSP could be used as a feed source to replace formulated concentrates in Hanwoo cattle.

Acknowledgements

This work was carried out with the support of the "Cooperative Research Program for Agriculture Science & Technology Development (Project title: A Study on Metabolism Regulation for Optimum Vitamin A and Ca:P Ratio in Hanwoo project; Project No. PJ01002302)" Rural Development Administration, Republic of Korea. We thank Dr. W. S. Kwak, from Konkuk University who kindly donated LAB.

Conflicts of interest

This authors declare they have no conflicts of interest with regard to the work presented in this report.

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