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Effects of Spent Mushroom Substrates Supplementation on Rumen Fermentation and Blood Metabolites in Hanwoo Steers

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ABSTRACT : This study was designed to investigate the effects of supplementation of spent mushroom substrates (SMS) on rumen fermentation and blood metabolites in Hanwoo steers. The experiment was conducted as a duplicated Latin square design with six Hanwoo steers (600±47 kg), each permanently fitted with a ruminal cannula. There were three treatments; i) control (concentrates 4.8 kg +rice straw 1.2 kg/d), ii) *Pleurotus eryngii*a (PE) treatment (concentrates 4.8 kg+rice straw 0.73 kg+*Pleurotus eryngiia* 1.20 kg/d) and iii) *Pleurotus osteratus* (PO) treatment (concentrates 4.8 kg+rice straw 0.73 kg+*Pleurotus osteratus* 1.20 kg/d). There were no major effects of different dietary treatments on rumen parameters such as pH, ammonia-N, individual and total VFA production. Parameters of N utilization, including blood urea nitrogen (BUN), total protein and albumin levels, were not significantly different among the treatments, except for creatinine. Thus, the present results indicated that protein utilization was mostly unaffected by SMS treatments such as PE and PO, even though creatinine concentration was lower in PE compared with control and PO treatments ($p<0.05$). The present results indicate that *Pleurotus eryngii* and *Pleurotus osteratus* could be used as a forage source to replace 40% of rice straw without any negative effects on rumen fermentation and blood metabolites in Hanwoo steers. (**Key Words :** Spent Mushroom, Rumen Fermentation, Blood metabolites, Hanwoo)

INTRODUCTION

Similarly to the global situation, mushroom production has been increased in Korea in response to trends in human health and well-being. The majority of the spent mushroom substrates (SMS) comprises the remains of the compost in which mushrooms are produced. The mushroom industry has been considering problems with SMS from an environmental standpoint concerning its effective disposal and recycling. Previous studies have shown the feasibility of using these kinds of waste to produce animal feed (Calzada et al., 1987; Zhang et al., 1995; Adamovic et al., 1998; Bae et al., 2006), because SMS is a nutrient-rich organic by-product of the mushroom industry. In addition, feed expenses comprise about 40% of the total product cost in fattening Hanwoo in Korea (KOSIS, 2010). Thus, dietary use of SMS in animal feed could be feasible from an economic point of view. Xu et al. (2010) recommended a SMS level of 6.5% dietary DM in a silage-based total mixed ration for wethers. Also, Kim et al. (2010) suggested that ensiled SMS could be used as an appropriate forage source in maintenance rations for ruminants, possibly due to high levels of fiber.

Therefore, this study was conducted to investigate effects of dietary SMS supplementation such as *Pleurotus ostreatus* or *Pleurotus eryngiia* on rumen fermentation and blood metabolites in Hanwoo steers.

MATERIALS AND METHODS

Animals and treatments

Six ruminally-cannulated Hanwoo steers of 600±47 kg body weight received the three experimental diets in a duplicated 3×3 Latin square design. There were three treatments with different SMS content: control (concentrates 4.8 kg+rice straw 1.20 kg/d), *Pleurotus eryngii*a (PE) treatment (concentrates 4.8 kg+rice straw

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0.73 kg+*Pleurotus eryngiia* 1.20 kg/d) and *Pleurotus osteratus* (PO) treatment (concentrates 4.8 kg+rice straw 0.73 kg+*Pleurotus osteratus* 1.20 kg/d). The formulation and chemical analysis of experiment feeds are shown in the Table 1. Water and trace mineral salt were available free-choice. Each experimental period lasted 14 d, with a 13 d period of adaptation to the diets, followed by a 1 d sampling period. Rice straw and/or SMS were offered at 09:00 h and concentrates were offered in two equal feeds at 09:00 and 16:00 h. Refusals were removed daily at 08:30 h and recorded.

Sample preparation and analytical methods

On the last day of each period, fermentation characteristics were determined at 0, 1, 3, 5, 7 and 9 h post-morning feeding. Rumen contents were sampled in the area of the ventral blind sac, with the composite sample being strained through four layers of cheesecloth. The pH of ruminal fluid was immediately determined using a pH meter (Coring model 530 pH meter, Artington, UK). To determinate volatile fatty acids (VFA) and ammonia-N, 1 ml rumen fluid was treated with 0.2 ml HPO_3 for 30 min and stored at -20°C. VFA concentration of rumen fluid was analyzed with gas chromatography (Varian, CP-3800, USA) according to the method of Erwin et al. (1961) after the sample was filtered through a 0.45 μm disposable micro filter. Ammonia-N concentration was analyzed by UV-spectrophotometer (UVIKON 923, Double beam UV/VIS) at 630 nm according to the method of Chaney and Marbach (1962).

Blood samples were collected before morning feeding on Day 14 by jugular vein puncture into two 10-ml vacuum tubes containing K_3-EDTA (Vacutainer, Becton Dickinson, Franklin Lakes, NJ, USA). Samples were centrifuged (5,000×*g* for 20 min at 4°C) and frozen at -20°C until analyzed. Blood urea nitrogen, glucose, albumin, creatinine and total protein concentrations were analyzed using an automatic blood analyzer (Express Plus, Ciba-Corning, CA, USA) according to the urease method of Rocch-Ramel (1967), the hexokinase method of Farrance (1987), the bromocresol green method of Doumas et al. (1971), the picric acid method of Husdan and Rapoport (1968), and the biuret method of Flack and Woollen (1984), respectively. Chemical composition of the diet was determined by the method of AOAC (1990). The analysis of neutral detergent fibre (NDF) and acid detergent fibre (ADF) was carried out

Table 1. Ingredients and chemical composition of the diet

Item	Basal diet			
Ingredient (% of DM)				
Ground corn	47.8			
Wheat bran	41.0			
Soybean meal	5.0			
Rapeseed meal	2.0			
Molasses	2.0			
Calcium phosphate	1.5			
Salt	0.4			
Vitamin-mineral mixture[1]	0.2			
Lasalocid	0.1			
Total	100			
Chemical composition	Basal diet	Rice straw	*Pleurotus eryngii*	*Pleurotus osteratus*
Dry matter	84.22	84.22	34.15	20.58
Crude protein (%)	15.52	15.52	6.96	7.51
Ether extract (%)	4.46	1.28	2.19	0.93
Crude fiber (%)	8.14	34.56	47.84	42.95
Crude ash (%)	5.13	16.48	9.62	14.90
NFE[2] (%)	66.75	42.77	33.39	35.65
NDF[3] (%)	22.56	60.11	67.94	59.50
ADF[4] (%)	0.98	41.60	54.80	55.32
Calcium (%)	0.96	0.16	0.40	2.68
Phosphorus (%)	0.70	0.16	0.72	0.07
Energy (Kcal/kg)	4,302.8	3,829.5	4,343.0	3,447.0

[1] Provided following nutrients per kg of additive (Grobic-DC, Bayer HealthCare, Leverkusen, Germany): Vit. A, 2,650,000 IU; Vit. D_3, 530,000 IU; Vit. E, 1,050 IU; Niacin, 10,000 mg; Mn, 4,400 mg; Fe, 13,200 mg; I, 440 mg; Co, 440 mg.
[2] Nitrogen free extract. [3] Neutral detergent fiber. [4] Acid detergent fiber.

according to Van Soest et al. (1991). The energy value of the diet was determined using a calorimeter (6200 Isoperibol Calorimeter, Illinois, USA).

Statistical analysis

Statistical analyses were performed using the GLM procedure of SAS (2002) in a 3×3 Latin square design with two replicates. Means among treatments were compared by least significant difference (LSD) test.

The model used to analyze fermentation measures was

$$Y_{ijkl} = \mu+\alpha_i+\beta_j+\gamma_k+\delta_l+e_{ijkl}$$

where Y_{ijk} = observation, μ = mean, α_i = treatment effect, β_j = animal effect, γ_k = sampling time effect, δ_l = period effect and e_{ijkl} = residual error.

The model used to analyze blood metabolites was

$$Y_{ijk} = \mu+\alpha_i+\beta_j+\gamma_k+e_{ijk}$$

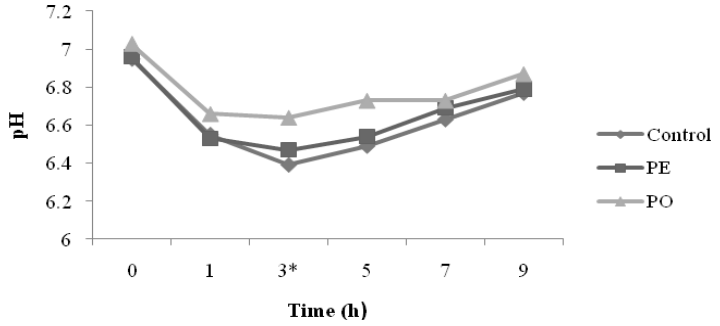
where Y_{ijk} = observation, μ = mean, α_i = treatment effect, β_j = animal effect, γ_k = period effect, and e_{ijk} = residual error.

The effects included replicate, treatment, animal and period. Significance was declared at $p<0.05$.

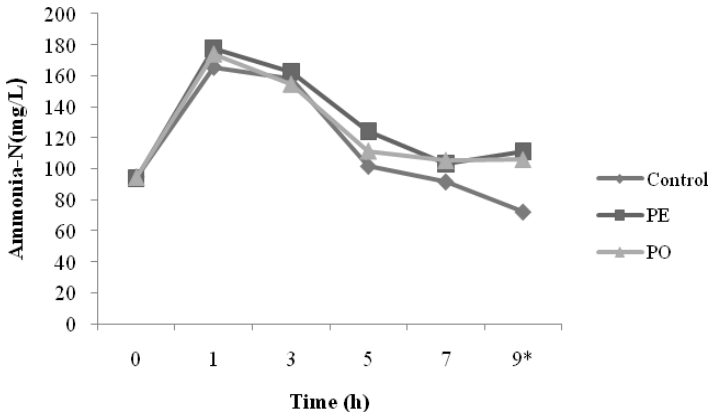
RESULTS AND DISCUSSION

Rumen parameters

SMS from PE and PO had no major influence on rumen fermentative parameters such as pH, ammonia-N, total and individual fatty acid concentrations (Figures 1 and 2). On every sampling time post-feeding, rumen pH values were not significantly different among the treatments (Figure 1a) except at 3 h post-feeding when the rumen pH was higher in the order of 6.64 Control>6.47 PE>6.39 PO ($p<0.05$). The rumen pH tended to decrease from 0 to 3 h post feeding, and tended to increase from 3 h up to 9 h post- feeding. Overall, rumen pH values among the treatments ranged between 6.4 and 7.0, which were above the low pH limiting value for microbial fermentation (Figure 1a) (Cardozo et al., 2000, 2002), and indicated that SMS from PE or PO supplementation was an alternative to forage in maintaining a stable rumen pH.



(a)



(b)

Figure 1. (a) Changes in ruminal pH by dietary PE and PO post-morning feeding. (b) Changes in ruminal ammonia-N concentration by dietary PE and PO post-morning feeding. Control = concentrates 4.8 kg+rice straw 1.2 kg/d; PE = concentrates 4.8 kg+rice straw 0.73 kg +*Pleurotus eryngiia* 1.20 kg/d; PO = concentrates 4.8 kg+rice straw 0.73 kg+*Pleurotus osteratus* 2.1 kg/d; * Means differ significantly between treatments ($p<0.05$).

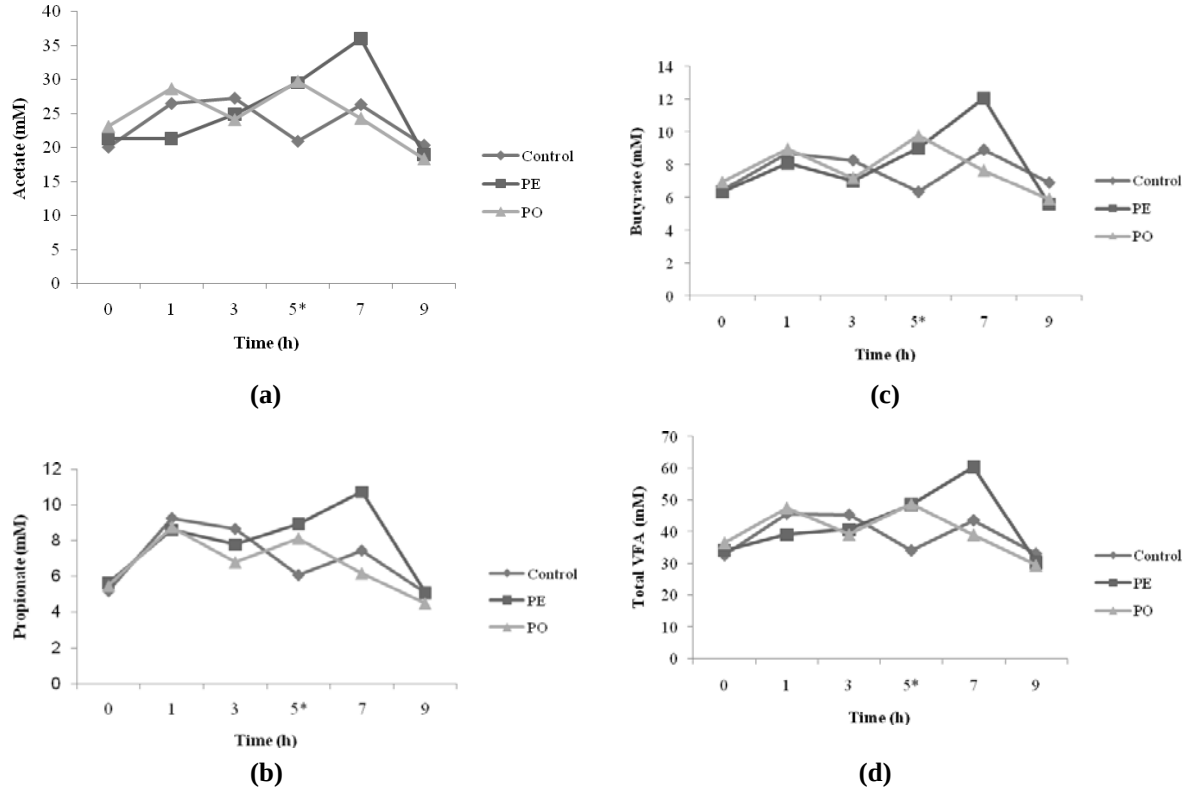


Figure 2. (a) Changes in ruminal acetate concentration by dietary PE and PO post-morning feeding. (b) Changes in ruminal propionate concentration by dietary PE and PO post-morning feeding. (c) Changes in ruminal butyrate concentration by dietary PE and PO post-morning feeding. (d) Changes in ruminal total VFA concentration by dietary PE and PO post-morning feeding. Control = concentrates 4.8 kg+rice straw 1.2 kg/d; PE = concentrates 4.8 kg+rice straw 0.73 kg +*Pleurotus eryngiia* 1.20 kg/d; PO = concentrates 4.8 kg+rice straw 0.73 kg+*Pleurotus osteratus* 2.1 kg/d; * Means differ significantly between treatments ($p<0.05$).

Ammonia-N concentration was not significantly different among the treatments at 0, 1, 3, 5 and 7 h, but the concentration was lower ($p<0.05$) in the control compared with PE and PO at 9 h post-feeding (Figure 1b). In addition, the ammonia-N concentrations of all treatments increased within 1 h of feeding and then declined gradually. The present results showed that there was no major effect of dietary SMS from PE or PO on ammonia-N concentration. The concentrations of total and individual volatile fatty acids were similar across the treatments, but were decreased in the control compared to both PE and PO treatments at 5 h ($p<0.05$) post-feeding. According to Fazaeli and Talebian Masoodi (2006), inclusion of spent compost straw up to 20% of the diet did not affect the digestibility of nutrients which may reflect the absence of a negative effect of dietary inclusion SMS on rumen fermentation. Although the present study did not investigate digestibility of nutrients, we could conclude that 40% SMS from PE or PO of rice straw does not decrease rumen microbial fermentative activity.

Therefore, based on the results of rumen fermentation parameters, the present study suggests that dietary supplementation with SMS, such as PE and PO, as a forage source could replace 40% of rice straw. Further research will be needed to determine growth performance and digestibility of nutrients with SMS supplements from PE or PO.

Blood metabolites

Effects of dietary SMS from PE or PO on blood metabolites are shown in Table 2. Blood glucose concentration was not significantly different across the treatments. Blood glucose is one of the most common metabolites used to assess the energy status of cattle (Ndlovu et al., 2007). Propionate derived from rumen fermentation is considered to be the major gluconegenic precursor in fed ruminants. Therefore, blood glucose concentration shows a similar trend to ruminal propionate concentration. Usually, albumin and total protein have low variability in blood (Ndlovu et al., 2007). As reported in other studies (Chumpawadee et al., 2006; Javaid et al., 2008), BUN concentration shows a similar trend to ruminal ammonia-N. Thus, the present results indicate that protein utilization is mostly unaffected by SMS from PE or PO. Creatinine concentration was lower in PE compared with control and PO treatments ($p<0.05$). Previous studies

Table 2. Effects of dietary spent mushroom substrates on blood metabolites

Metabolites	Control	PE	PO	SEM
Glucose (mg/dl)	65.83	69.33	65.25	2.93
Total protein (g/dl)	6.73	6.63	6.33	6.43
Urea nitrogen (mg/dl)	11.15	10.03	9.63	1.50
Albumin (g/dl)	3.77	3.59	3.86	0.23
Creatinine (mg/dl)	1.55[a]	1.28[b]	1.53[a]	0.12

Means with the different superscripts within a row are significantly ($p<0.05$) different.
Control = Concentrates 4.8 kg+rice straw 1.2 kg/d. PE = Concentrates 4.8 kg+rice straw 0.73 kg+*Pleurotus eryngiia* 1.2 kg/d.
PO = Concentrates 4.8 kg+rice straw 0.73 kg+*Pleurotus osteratus* 2.1 kg/d. SEM = Standard error of mean.

reported that blood creatinine concentrations vary with diet, breed, muscle mass, sex and season (Otto et al., 2000; Miller et al., 2004; Grunwaldt et al., 2005; Hammond, 2006). The reduced concentration of creatinine in the present study may be explained by prolonged active tissue protein catabolism.

CONCLUSION

The present study showed that there were no major differences in rumen fermentative parameters such as pH, ammonia-N concentration and volatile fatty acids, and blood metabolites such as glucose, urea-N, total protein, albumin and creatinine among the treatments. Thus, it should be possible to replace 40% of rice straw as a forage source with SMS from PE or PO for ruminant feed without any severe negative metabolic effects, which could thereby economically reduce feed expense and product cost in the Hanwoo industry.

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