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Effects of Persimmon (*Diospros kaki* L.) Vinegar as a Dietary Supplement on Feed Intake, Digestibility, and Ruminal Fermentation Indices in Sheep

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ABSTRACT : This study estimated the effect of fermented persimmon (*Diospros kaki* L.) extract (FPE) supplement on feed intake, digestibility, nitrogen (N) balance, and rumen fermentation characteristics in sheep. Five male sheep (Corriedale×Polwarth) with average body weight of 48.6±1.3 kg were housed in metabolism crates and assigned to a 5×5 Latin square design with five consecutive 20-d periods which consisted of 14-d adaptation and 6-d data collection. The sheep were fed *ad libitum* a diet containing concentrate and rice straw (3:7). The five treatments were FPE supplemented at 0 (Control), 5, 10, 20, and 30 g/kg of concentrate. Intakes of dry matter (DM, $p<0.01$), organic matter (OM, $p<0.01$), neutral detergent fiber (NDF, $p<0.05$), acid detergent fiber (ADF, $p<0.05$), and nitrogen-free extract (NFE, $p<0.01$) increased quadratically with increasing intake of FPE supplement and maximized ($p<0.05$) at 10 g/kg FPE. The digestibilities of DM ($p<0.05$), OM ($p<0.05$), crude protein ($p<0.01$), and NFE ($p<0.01$) increased quadratically with increasing amount of FPE supplement, and sheep fed 5 and 10 g/kg diets had greater ($p<0.05$) DM, OM, and NFE digestibilites than the Control treatment. By increasing FPE supplement concentration, N intake ($p<0.01$) and fecal N ($p<0.05$) increased linearly, whereas retained N ($p<0.05$) and retained N ratio ($p<0.05$) increased quadratically. The retained N was maximized ($p<0.05$) in sheep fed 5 and 10 g/kg diets. The mean rumen pH was not affected by FPE supplement, but there was a quadratic increase ($p<0.05$) of mean rumen ammonia N concentration and a linear increase ($p<0.01$) in mean rumen total volatile fatty acid (VFA) and acetate concentrations. The mean concentration of rumen propionate in sheep fed all FPE supplemented diets was greater ($p<0.05$) than the Control, but the mean ratios of rumen acetate to propionate in sheep fed 5 and 10 g/kg diets were lower ($p<0.05$) than that of Control sheep. In conclusion, FPE supplemented at 5-10 g/kg of concentrate improved feed intake, the digestibilites of OM and NFE, N metabolism, and rumen fermentation indices of sheep. (**Key Words :** Fermented Persimmon Extract, Digestibility, Nitrogen Balance, Sheep)

INTRODUCTION

The use of antibiotic has been prohibited in European Union since 2006. Some herbs and plant extract had verified the antimicrobial effects against bacteria, yeast, and molds (Thompson, 1986). Accordingly, the interest of using plant and plant extract increased. Korean persimmon (*Diospyros kaki* L.) has great concentrations of sugar (11-15 g/kg) and vitamin C (10-20 mg/g; NRLSI, 1996). The fermented persimmon extract (FPE) is a byproduct of the persimmon industry in South Korea which is producing by the aerobic fermentation of persimmon for 1-2 years, and it contained 3-5 g/dl of acetate, 180-221 mg/dl of amino acids, and various minerals (Moon et al., 1997; Jeong et al., 1999). The FPE has been used for food safety and flavor due to the great acetate concentration and low pH which can minimize the effect of off-flavor, lipid oxidation, and growth of microbes i.e. *E. coli*, *S. aureus*, *M. luteus* (Kittelmann et al., 1989; Woo et al., 2004; Sakanaka and Ishihara, 2008). The chemical compositions of FPE produced by fermented substrates are variable, but it mainly has great acetate content (2-15 g/dl) and low pH (2.5-3.7). The acetate in FPE is usually produced by glucose fermentation, theoretically, 1 g of glucose can produce 0.67 g of acetate (Wood et al., 1998). Recently, some diluted FPE is on sale as a health drink based on the observations of its bioactive effects by phenolic compounds, organic acids, and various minerals (Shimoji et al., 2002). In general, the presence of acetate in diets improved aerobic stability by inhibition of

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mold and yeast growth (Weinberg et al., 2002; Adesogan et al., 2004). Reynolds et al. (1979) reported that the infusion of acetate into the ventral rumen of cows fed a high concentrate diet decreased fiber digestibility, but did not affect digestibility by cows fed a high forage diet. Walt and Loerch (1986) also observed that 6 g/kg of acetate supplementation did not increase the fiber digestibility of lamb, but nitrogen (N) retention was in an intermediate of increasing. In their *in vitro* study using soybean meal, ammonia N accumulation rate was not affected by 25 g/kg of acetate supplement, but decreased when 50 g/kg of acetate supplement was used. Kook and Kim (2003) reported that dry matter intake (DMI) by Hanwoo (Korean native cattle) cows increased when bamboo vinegar was supplemented at 30 g/kg of dietary dry matter (DM), but decreased by supplementation at 60 g/kg of dietary DM. Therefore, this study was investigated to estimate the effect of FPE supplement on feed intake, nutrient digestibility, N balance, and rumen fermentation indices in sheep.

MATERIALS AND METHODS

Animals and diets

This experiment was conducted according to the animal care and use guidelines of the National Livestock Research Institute of South Korea. Five male sheep (Corriedale× Polwarth) with average body weight of 48.6±1.3 kg were used in a 5×5 Latin square design with 5 consecutive 20-d periods. Each period consisted of 14-d of adaptation for a new diet, 5-d of total fecal and urinary output collection for feed intake, nutrient digestibility, and nitrogen balance measures, and 1 d of rumen fluid collection for measure of rumen fermentation indices. The sheep were individually housed in metabolism crates and fed twice daily (0800 h and 1700 h) at 20 g/kg of body weight (DM basis). The basal diet, concentrate and rice straw, was formulated to meet their maintenance energy requirements (NRC, 1985). Concentrate and rice straw were fed separately using two feed bunks for each sheep, and consisted of concentrate and rice straw in a 3:7 ratio (DM basis). The treatments represented FPE supplementation at 0, 5, 10, 20 and 30 g/kg of concentrate (DM basis). The FPE was donated by Sachun Agricultural Station, Sachun, South Korea. The FPE contains mainly 4.6, 0.6, and 0.23 g/100 ml of acetate, lactate, and amino acid (AA), respectively, and had low pH (2.51). Just before the morning feeding, the FPE was applied to the concentrate. The rice straw used in this study offered from a Animal Research Unit at Gyeongsang National University (Jinju, South Korea), and chopped to a 3-5 cm length. Sheep had free access to fresh water and a mineral block (Na, 380 g/kg; Mg, 5 g/kg; I, 150 mg/kg; P, 10,000 mg/kg; Ca, 10,000 mg/kg; Mn, 200 mg/kg; Fe, 150 mg/kg; Vit-A, 60,000 IU/kg; Vit-D_3, 60,000 IU/kg).

Sampling

The basal feedstuffs were collected at the beginning of each period and stored at -20°C. Feed refused, feces and urine were collected daily just before the morning feed during the collection periods. Feces (200 g) and urine (100 ml) were sub-sampled daily and stored at -20°C until analyses. Urine was collected into a bucket, covered by cheese cloth, containing 15 ml of 4 M sulfuric acid to prevent ammonia evaporation. On day 20 of each period, rumen fluid was collected at 0 (just before morning feed), 0.5, 1, 2, 4, and 8 h after the morning feeding by aspiration via stomach tube. The collected rumen fluid was filtered immediately through two layers cheese cloth and measured for pH using an electric pH meter with a glass probe (Orion 520A, USA). Saturated mercuric chloride solution (3 ml) was added to prevent further fermentation and stored at -20°C until analysis.

Chemical analysis

Approximately 10 g of feeds, orts, and fecal samples were dried at 105°C for 24 h to measure DM concentration. Approximately 100 g of the feeds and fecal samples were oven-dried at 65°C for 48 h and ground to pass through a 1-mm screen of a Wiley mill to use for chemical composition analysis. The concentrations of N and ether extract (EE) were analyzed by the Kjeldahl method (AOAC, 1990) and the soxhlet method (AOAC, 1965), respectively. The concentrations of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed by the methods of Van Soest et al. (1991). Amylase and sodium sulphite were used in the NDF procedure and the results were not expressed on an ash-free basis. Nitrogen free extract (NFE) was calculated by the difference from the sum of the other nutrients. Urinary N was determined with the Kjeldahl procedure previously described. The frozen rumen fluid samples were thawed at room temperature, after then, centrifuged for 10 minutes at 3,000 rpm (1,465×g), and the supernatant used for ammonia N and volatile fatty acids (VFA) analyses. The concentrations of rumen ammonia N and total VFA were measured by colorimetry (Chaney and Marbach, 1962) and steam distillation (Fenner and Elliot, 1963) procedures, respectively. The analysis of individual VFAs was done using a gas chromatograph (GC; Hewlett Packard GC-5890 series II) by the method of Erwin et al. (1961). The GC was equipped with a flame ionization detector and a HP-Innowax column (Crosslinked polyethylene glycol; 30 m×0.32 mm×0.5 um). The carrier gas was N, and the injector and detector temperatures were 250°C and 280°C. Initial oven temperature was 120°C for 1 min. Following injection of sample, the oven temperature

was increased by 10°C/min up to 180°C and held for 10 min. Individual VFAs were identified by comparison of retention times to those of pure standards.

Statistical analysis

The data were analyzed using the GLM procedure of SAS (2000) and diet and period were included in the model. Polynomial contrasts were used to estimate the effect of FPE supplement level. The IML procedure of SAS was used to generate coefficients for testing linear and quadratic effects of treatments with unequal spacing. Rumen pH, ammonia N, and VFAs were analyzed at each sampling time using the PROC MIXED procedure of SAS with a repeated measures statement. The model included treatment, time and the treatment×time interaction. Tukey Test was used to identify differences ($p<0.05$) between means.

RESULTS

The ingredients of concentrate and the chemical compositions of basal diets are shown in Table 1. The contents of CP, NDF, and ADF of concentrate and rice straw were 133 and 52 g/kg, 216 and 663 g/kg, and 126 and 469 g/kg, respectively.

No period effects were found at any measurements. The intakes of DM ($p<0.01$), OM ($p<0.01$), NDF ($p<0.01$), ADF ($p<0.01$), and NFE ($p<0.05$) increased quadratically with increasing intake of FPE, while CP intake increased ($p<0.01$) linearly (Table 2). These measurements maximized ($p<0.05$) in sheep fed 10 g of FPE/kg of concentrate diet (828, 710, 418, 288, and 428 g/d of DM, OM, NDF, ADF, and NFE, respectively). The apparent digestibilities of DM ($p<0.05$), OM ($p<0.05$), CP ($p<0.01$), EE ($p<0.01$), and NFE ($p<0.01$) increased quadratically with increasing intake of FPE. The apparent digestibilities of DM, OM, and NFE by sheep fed 5 and 10 diets were greater ($p<0.05$) than those fed Control diet (DM = 52.2 and 52.3 vs. 49.3%; OM = 55.4 and 55.7 vs. 51.7%; NFE = 60.0 and 60.2 vs. 55.3%). Digestibility of EE followed a quadratic pattern, peaking at 5 and 10 g of FPE per kg of concentrate although diet 20 g of FPE per kg of concentrate had higher EE digestibility than the control as well. However, effects of FPE supplement on the digestibilities of NDF and ADF were not detected.

With increasing of FPE, N intake ($p<0.01$) and fecal N ($p<0.05$) increased linearly, whereas N digestibility ($p<0.01$), retained N ($p<0.05$), and retained N ratio ($p<0.05$) increased quadratically (Table 3). The N intakes by sheep fed 20 and 30 diets were greater ($p<0.05$) than that of sheep fed Control diet (11.2 and 11.5 vs. 9.9 g/d), but there was no difference among FPE supplemented treatments. The retained N in sheep fed FPE supplemented diets was greater ($p<0.05$) than that of Control sheep, particularly in 5 and 10 treatments (1.97 and 2.00 g/d), but urinary N was not affected by FPE supplement.

Table 1. Ingredients of the concentrate and the chemical compositions of experimental diets (g/kg DM)

Items	Concentrate	Rice straw
Ingredients		
Yellow corn, ground	220	
Wheat grain	55	
Molasses	55	
Wheat bran	190	
Rice bran	35	
Corn gluten meal	76	
Oat bran	14	
Rapeseed oil meal	23	
Coconut meal, extracted	130	
Palm meal	165	
Tricalcium phosphate, 18%	3	
Calcium sulfate	7	
Limestone, 1 mm	25	
Vitamin premix[1]	1	
Mineral premix[2]	1	
Chemical composition		
Dry matter	881	878
Organic matter	895	831
Crude protein	133	52
Ether extract	34	20
Neutral detergent fiber	216	663
Acid detergent fiber	126	469
Nitrogen free extract	677	430

[1] Vitamin premix: Vit. A, 28,500 IU; Vit. D_3 45,000 IU; Vit. E, 15,000 IU; Vit. K_3, 800 mg; Vit. B_1, 500 mg; Vit B_2, 2,000 mg; Vit B_6, 900 mg; Vit B_{12}, 10 mg; Pantothenic acid, 6,300 mg; Niacin, 15,000 mg; Biotin, 230 mg; Folic acid, 260 mg; Anti-oxidation 6,200 mg per kg of DM.

[2] Mineral premix: $FeSO_4$, 41,000 mg; $CoSO_4$, 165 mg; $CuSO_4$, 65,000 mg; $MnSO_4$, 21,050 mg; $ZnSO_4$, 40,000 mg; Se(Na), 100 mg per kg of DM.

The mean rumen pH and the mean concentrations of butyrate and valerate were not affected by FPE supplement (Table 4). The mean concentrations of total VFA and acetate increased ($p<0.01$) linearly by increasing FPE supplements, whereas the mean concentration of rumen ammonia N ($p<0.05$) decreased quadratically. All sheep fed FPE had greater ($p<0.05$) mean propionate concentration than sheep fed Control (2.18, 2.27, 2.20, and 2.21 vs. 1.78 mM/dl), but sheep fed 5 and 10 diets had lower ($p<0.05$) A/P ratio.

The treatment, sampling time, and their interaction affected ($p<0.05$) rumen pH, A/P ratio, and the concentrations of ammonia N, total VFA, acetate, and propionate (Figure 1 and 2). Rumen pH and A/P ratio dropped ($p<0.05$) rapidly between 0.5-1 h of morning feeding, and then gradually increased ($p<0.05$). As opposed to rumen pH and A/P ratio, the concentrations of total VFA, ammonia N, acetate, and propionate had shown the rapid

Table 2. Effects of the fermented persimmon (*Diospros kaki* L.) extract supplement on nutrient intake and digestibility in sheep

Items	Fermented persimmon extract levels[1]					SEM	Effects[2]
	0	5	10	20	30		
Feed intake (g/d)							
Dry matter	747^{b}	804^{a}	828^{a}	791^{ab}	767^{ab}	27.9	**, Q
Organic matter	639^{b}	689^{a}	710^{a}	682^{ab}	665^{ab}	21.1	**, Q
Crude protein	62^{b}	67^{ab}	69^{ab}	70^{a}	72^{a}	4.1	**, L
Ether extract	19	20	20	20	19	0.9	NS
Neutral detergent fiber	364^{b}	403^{a}	418^{a}	394^{ab}	378^{ab}	16.0	**, Q
Acid detergent fiber	250^{b}	277^{ab}	288^{a}	271^{ab}	259^{ab}	6.9	**, Q
Nitrogen free extract	393^{b}	418^{a}	428^{a}	412^{a}	402^{ab}	9.5	*, Q
Apparent digestibility (% DM)							
Dry matter	49.3^{b}	52.2^{a}	52.3^{a}	51.8^{ab}	50.6^{b}	1.2	*, Q
Organic matter	51.7^{b}	55.4^{a}	55.7^{a}	54.3^{ab}	54.2^{ab}	1.4	*, Q
Crude protein	53.5^{b}	57.0^{a}	56.5^{ab}	56.6^{ab}	55.2^{ab}	1.3	**, Q
Ether extract	71.6^{d}	78.5^{ab}	79.1^{a}	75.9^{bc}	73.9^{cd}	1.2	**, Q
Neutral detergent fiber	46.7	46.5	45.5	47.4	46.4	1.4	NS
Acid detergent fiber	36.8	35.6	37.1	35.7	36.7	1.4	NS
Nitrogen free extract	55.3^{b}	60.0^{a}	60.2^{a}	58.4^{ab}	57.7^{ab}	1.3	**, Q

[1] Addition of 0, 5, 10, 20, and 30 g/kg of concentrate with the fermented persimmon extract, respectively.
[2] NS = Not significant; L = Linear effect; Q = Quadratic effect; * $p<0.05$; ** $p<0.01$.
[a-d] Means with different superscripts in the same row differ significantly ($p<0.05$).

Table 3. Effects of the fermented persimmon (*Diospros kaki* L.) extract supplement on nitrogen balance in sheep

Items	Fermented persimmon extract levels[1]					SEM	Effects[2]
	0	5	10	20	30		
N intake (g/d)	9.9^{b}	10.7^{ab}	11.0^{ab}	11.2^{a}	11.5^{a}	0.66	**, L
Fecal N (g/d)	4.61	4.61	4.79	4.86	5.17	0.32	*, L
Urinary N (g/d)	4.25	4.13	4.22	4.59	4.66	0.29	NS
Retained N (g/d)	1.06^{c}	1.97^{a}	2.00^{a}	1.74^{ab}	1.71^{b}	0.23	*, Q
Retained N ratio	0.15	0.18	0.18	0.16	0.15	0.01	*, Q

[1] Addition of 0, 5, 10, 20, and 30 g/kg of concentrate with the fermented persimmon extract, respectively.
[2] NS = Not significant; L = Linear effect; Q = Quadratic effect; * $p<0.05$; ** $p<0.01$.
[a-c] Means with different superscripts in the same row differ significantly ($p<0.05$).

Table 4. Effects of the fermented persimmon (*Diospros kaki* L.) extract supplement on mean rumen pH and mean concentrations of rumen ammonia N and volatile fatty acid in sheep

Items	Fermented persimmon extract levels[1]					SEM	Effects[2]
	0	5	10	20	30		
pH	6.55	6.62	6.62	6.66	6.55	0.94	NS
Ammonia N (mg/dl)	6.8^{b}	7.5^{ab}	8.2^{a}	7.4^{ab}	7.5^{ab}	0.52	*, Q
Total VFA (mM/dl)	7.77^{c}	8.38^{bc}	9.08^{ab}	9.11^{ab}	9.16^{a}	0.41	**, L
Acetate (mM/dl)	5.25^{b}	5.56^{ab}	5.90^{ab}	6.02^{ab}	6.03^{a}	0.27	**, L
Propionate (mM/dl)	1.78^{b}	2.18^{a}	2.27^{a}	2.20^{a}	2.21^{a}	0.16	NS
Butyrate (mM/dl)	0.58	0.51	0.71	0.69	0.71	0.11	NS
Valerate (mM/dl)	0.16	0.12	0.20	0.20	0.21	0.06	NS
A/P ratio[3]	2.94^{a}	2.55^{b}	2.60^{b}	2.74^{ab}	2.73^{ab}	0.12	NS

[1] Addition of 0, 5, 10, 20, and 30 g/kg of concentrate with the fermented persimmon extract, respectively.
[2] NS = Not significant; L = Linear effect; Q = Quadratic effect; * $p<0.05$; ** $p<0.01$.
[3] Acetate to Propionate ratio.
[a-c] Means with different superscripts in the same row differ significantly ($p<0.05$).

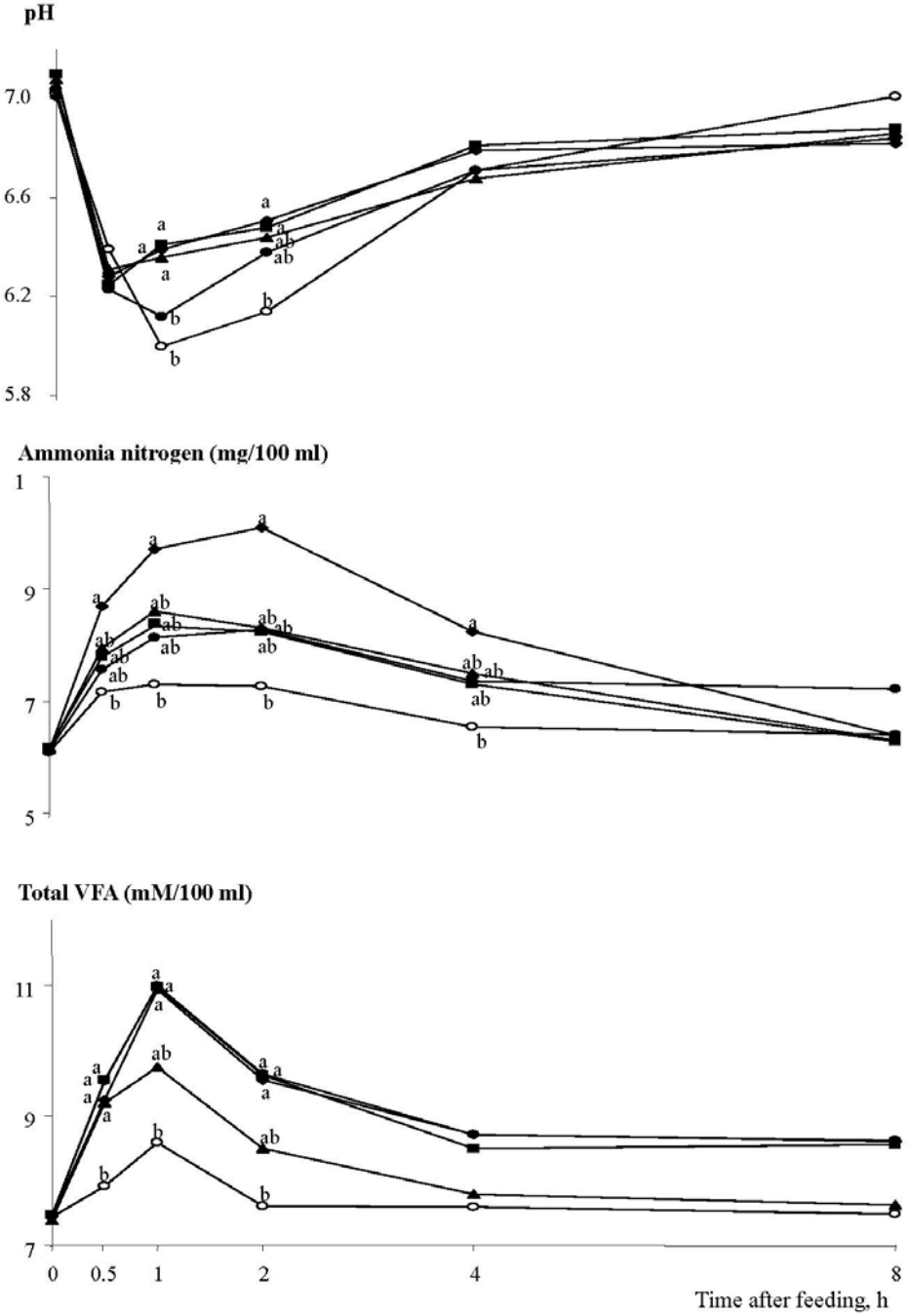

Figure 1. Changes in rumen pH, ammonia N (mg/100 ml), and total volatile fatty acid (mM/100 ml) in sheep fed the diets in which the fermented persimmon (*Diospros kaki* L.) extract supplemented for 0 (o), 5 (▲), 10 (♦), 20 (■), and 30 g/kg DM (●) of concentrate. The symbol with different letters differ ($p<0.05$) at the same time after feeding. The respective significance levels for treatments, time and time×treatment and SEM for rumen pH, ammonia N and total VFA concentration are $p = 0.038$, $p<0.001$, $p = 0.425$, 0.031; $p = 0.007$, $p<0.001$, $p<0.001$, 0.352; $p<0.001$, $p<0.001$, $p = 0.026$, 0.308.

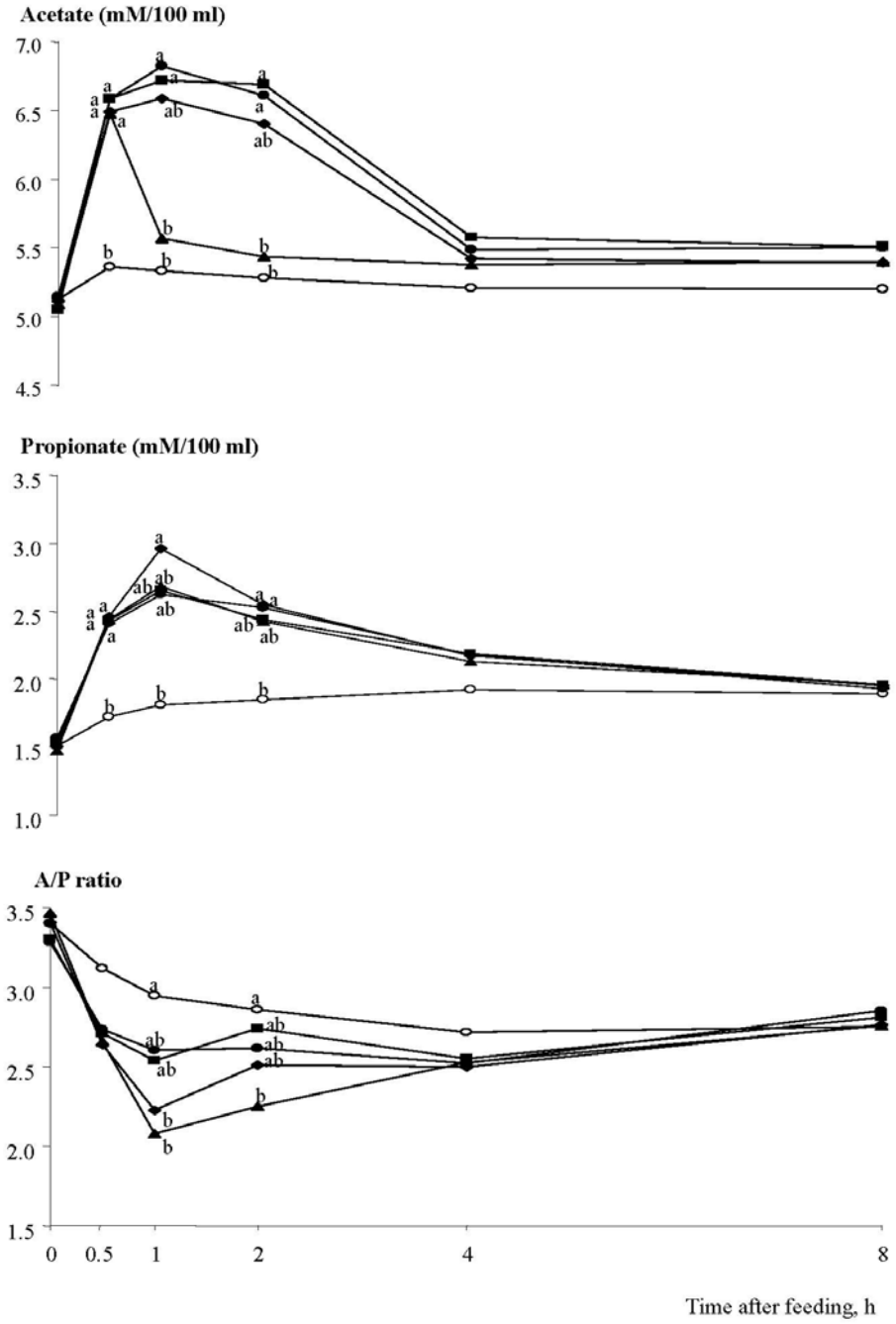

Figure 2. Changes in the concentrations (mM/100 ml) of rumen acetate and propionate and the ratio of acetate to propionate in sheep given diets in which the fermented persimmon (*Diospros kaki* L.) extract supplemented for 0 (o), 5 (▲), 10 (♦), 20 (■), and 30 g/kg DM (●) of concentrate. The symbol with different letters differ ($p<0.05$) at the same time after feeding. The respective significance levels for treatments, time and time x treatment and SEM for rumen pH, ammonia N and total VFA concentration are $p = 0.005$, $p<0.001$, $p<0.001$, 0.104; $p<0.001$, $p<0.001$, $p = 0.038$, 0.029; $p = 0.011$,$p<0.001$, $p = 0.026$, 0.047.

increases ($p<0.05$) at 0.5 of morning feeding and peak within 1-2 h, and then decreased ($p<0.05$).

DISCUSSION

The CP and NDF concentrations of concentrate and rice straw used in the present study were similar to those concentrations of concentrate and rice straw reported by Kim et al. (2006a; CP = 158 and 54 g/kg of DM; NDF = 217 and 644 g/kg of DM). Korean rice straw used in this study also had similar concentrations of CP (52 vs. 46 g/kg of DM) and NDF (663 vs. 706 g/kg of DM) compare to those reported by Nader and Robinson (2008). The chemical compositions of fermented persimmon extract are

variable by the raw fermented materials and the fermentation time period (Wood et al., 1998). Nevertheless it contains mainly acetate, and has a low pH and various amino acids (Wood et al., 1998). They noted that it normally contains 5-13 g/100 ml of acetate. The FPE used in the present study had slightly less acetate (4.6 g/dl), but Jeong et al. (1999) reported a similar concentration of acetate (4.9 g/dl) in FPE made from Korean persimmon (*Diospyros kaki* L.). Sakanaka and Ishihara (2008) also reported that two different FPE from different persimmon varieties (*Diospyros kaki* T.) in Japan had about 4.3 ml/dl of acetate. The low pH (2) and AA concentration (225 mg/dl) of FPE in the present study were in agreement with the results reported by Jeong et al. (1999) and Moon et al. (1997) that Korean FPE ranged in pH from 2.6-3.7 and in AA from 110-221 mg/dl. Concentrate was not refused during the whole trial. The DM intake across treatments in the present study was similar to that of Kim et al. (2006a,b) and Ko et al. (2006) who fed similar concentrate and Korean rice straw with a 2:8 and a 3:7 ratio to sheep (780, 812, and 793 g/d). Intake of DM also was in the range of AFRC guideline (1993) for 40-kg male lambs (600-900 g/d). When FPE was supplemented into the diet, feed intake could be increased by the presence of acetone compounds and the sour taste of acetate (Yoon et al., 1998). Kook and Kim (2003) reported that DM intake by Hanwoo (Korean native cattle) cows improved by adding bamboo extract which contains acetate mainly, at 30 g/kg of DM, but it decreased at 60 g/kg of DM. These studies may partially support the quadratic change of DM intake in the present study. The chemical composition of diets across the treatments was similar caused by using the same concentrate and rice straw. Therefore, the intakes of OM, NDF, ADF, and NFE had a similar changing pattern as DM intake. The reason for the quadratic change in EE digestibility is unclear, but it could be supported partially by the report of Wood et al. (1989) that FPE can dissolve the essential oil quickly and reduce lipid oxidation. Reynolds et al. (1979) reported that the infusion of acetic acid into the ventral rumen of cows fed a diet with an 8:2 ratio of concentrate to forage decreased fiber digestibility, whereas cow fed a 5:5 or a 2:8 ratio of concentrate to forage did not digest fiber differently. Walt and Loerch (1986) also reported a similar result that supplementation with 6 g of acetic acid per kg of DM did not increase fiber digestibility. These observations were consistent with our results of NDF and ADF digestibility. Tonroy and Perry (1974) reported that 15 g per kg of DM of VFA application did not affect starch digestibility at 1, 4, 24, and 48 h of incubation, but the mean starch digestibility at these time periods decreased slightly compared to Control (630 vs. 660 g/kg of DM). In the present study, the additional acetic acid in 30 g/kg treatment was about 0.4 g/kg of DM which is much less than their study.

Intake of N increased linearly due to increased intake of FPE which contains 225 mg/dl of AA. The presence of AA in FPE also could support partially the improvement of N metabolism in the present study. Most AA in FPE may exist as rumen degradable protein (RDP) or in a water-soluble form due to persimmon fermentation during FPE production. Therefore, FPE supplementation might lead to an increase of RDP intake which could increase N digestibility and rumen ammonia N concentrations. Walt and Loerch (1986) reported that N retention by lambs fed 6 g of acetic acid per kg of DM was not different from Control (4.2 vs. 3.7 g/d). They also reported in an *in vitro* study that ammonia N accumulation rate was not affected by supplementing with 25 g/kg DM of acetic acid at soybean meal, but decreased when supplemented with 50 g/kg DM of acetic acid which seems to decrease RDP concentration by acetic acid supplementation. The increased intake of RDP may help excel the N metabolism results in the present study. Although there was a linear increase of N intake with measuring intake of FPE, no differences of excretion of urinary N seem due to the quadratic change of N digestibility. The retained N in the present study was similar to that reported by Ko et al. (2006) which fed similar concentrate and rice straw as basal diets. And, the changing patterns of retained N (g/d) and ratio (g/kg of N intake) by FPE supplement were consistent with N digestibility.

The mean rumen pH was not affected by FPE supplement, but shown the quadratic change across sampling time agrees with the observations of Kim et al. (2006b) and Ko et al. (2006) who fed similar concentrate and rice straw basal diets in an 8:2 and a 7:3 ratios. Tonroy and Perry (1974) also reported no effect on *in vitro* rumen pH when 15 g/kg of VFA were added, consisting 57 g/100 g of acetic acid, 40 g/100 g of propionic acid, and 3 g/100 g of water. The great rice straw proportion (700 g/kg of DM) in the basal diets may accelerate salivation which could increase rumen pH (Ko et al., 2006). The greater DM intake by sheep fed 5 and 10 diets versus Control sheep reflects greater rice straw intake, because no concentrate was refused in the present study. Therefore, even though a FPE supplement having a low pH was fed, the greater rumen pH at 1-2 h after feeding in 5, 10, or 20 treatments may be caused by greater rice straw intake. This result also could be explained partially by the increase in rumen ammonia N concentration, observed at 0.5-4 h after feeding FPE (Figure 1). The quadratic decrease of mean ammonia N concentration followed the change of N digestibility with increasing intake of FPE. The linear increase of mean VFA concentration with increasing FPE supplementation was likely due to its great concentration of acetate, as well as

NFE digestibility which can be converted to propionate during rumen fermentation, and the greater mean propionate concentration in all FPE supplemented treatments versus Control might be due to increase of NFE intake as well as digestibility (Table 2). By reflection of acetate and propionate concentrations, A/P ratio had shown a quadratic change and a lowest ratio at 1-2 h after feeding which is consistent to the observation of Ko et al. (2006) and Kim et al. (2006a,b).

CONCLUSION

By the fermented persimmon extract supplementing at 5-10 g/kg of concentrate, feed intake, the digestibility of organic matter and neutral detergent fiber, and nitrogen balance were improved. And mean concentrations of rumen volatile fatty acid and acetate increased linearly by increase of the fermented persimmon extract supplemented level, while ammonia nitrogen decreased quadratically. These results suggest that the optimum the fermented persimmon extract supplement rate might be 5-10 g/kg of concentrate.

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