

## Effects of dietary *Forsythia suspensa* on feed utilization, rumen fermentation, and immune response of Korean native goats (*Capra hircus*)<sup>□</sup>

*Efectos de la adición dietaria de Forsythia suspensa sobre utilización del alimento, fermentación ruminal y respuesta inmune en cabras nativas coreanas (Capra hircus)*

*Efeitos na dieta pela utilização de Forsythia suspensa nos alimentos, fermentação ruminal e resposta imune em caprinos nativos coreanos (Capra hircus)*

No Seong Park<sup>1</sup>, BSc, MSc; Chi Hyun Cho<sup>1</sup>, BSc, MSc; Jung Min Heo<sup>1</sup>, BSc, PhD; Minho Song<sup>1</sup>, BSc, PhD; Byung Mo Yang<sup>1</sup>, BSc; Hyung Suk Lee<sup>2</sup>, BSc, PhD; Soo Kee Lee<sup>1\*</sup>, BSc, PhD.

<sup>1</sup>Department of Animal Science and Biotechnology, Chungnam National University, Daejeon 305-764, South Korea.

<sup>2</sup>Department of Pet-animal Science, Woosong Information College, Daejeon 300-715, South Korea.

(Received: September 2, 2013; accepted: April 26, 2014)

doi: 10.17533/udea.rccp.v28n2a05.

### Summary

**Background:** plant extracts of *Forsythia suspensa*, which contain pinoresinol, have been proposed as an alternative to antibiotics due to their antioxidant, anti-inflammatory, and microflora modulating effects. **Objective:** to evaluate the effects of dietary *F. suspensa* on feed utilization, rumen fermentation, blood parameters and immune response of Korean native goats (*Capra hircus*). **Methods:** a total of nine Korean native goats were assigned to one of three dietary treatments: 1) a basal diet without *F. suspensa*, 2) a basal diet added with 0.25% *F. suspensa*, or 3) a basal diet added with 0.50% *F. suspensa*. A change-over design was used in three consecutive periods to give 9 replicates per treatment. Each period lasted 18 days, including 15 days of adaptation to feed and environmental conditions and three days of total collection of feces and urine. Rumen fluid and blood samples were also collected. Nutrient digestibility, nitrogen retention, ruminal content, and blood metabolites—including immunoglobulins—were measured. **Results:** *F. suspensa* supplementation had no effect on nutrient digestibility, whole body nitrogen retention rate, ruminal pH, acetate, propionate, isobutyrate, butyrate, isovalerate, or valerate content ( $p > 0.05$ ). However, *F. suspensa* supplementation decreased total volatile fatty acids (VFA) concentration compared with the control diet, regardless of *F. suspensa* concentrations ( $p < 0.05$ ). Goats fed a diet containing 0.25% *F. suspensa* had greater alkaline phosphatase (ALP) concentration than those fed a control or a diet with 0.50% *F. suspensa* ( $p < 0.05$ ). Feeding different concentrations of dietary *F. suspensa* did not influence plasma IgA and IgM levels ( $p > 0.05$ ), although goats receiving both 0.25% and 0.50% *F. suspensa* diets had greater plasma IgG than those fed the control diet ( $p < 0.05$ ). **Conclusions:** supplementation of 0.25% *F. suspensa* significantly decreased total VFA concentrations and increased plasma IgG in Korean native goats, compared with those fed the control diet.

**Keywords:** antibiotic replacement, immunoglobulin, plant extract.

□ To cite this article: Park NS, Cho CH, Heo JM, Song M, Yang BM, Lee HS, Lee SK. Effects of dietary *Forsythia suspensa* on feed utilization, rumen fermentation, and immune response of Korean native goats (*Capra hircus*). Rev Colomb Cienc Pecu 2015; 28:165-173.

\* Corresponding author: Soo Kee Lee. Department of Animal Science and Biotechnology, Chungnam National University, Daejeon 305-764, South Korea. Tel: 82-42-821-5775, Fax: 82-42-825-9754. E-mail: leeskk@cnu.ac.kr

## Resumen

**Antecedentes:** los extractos de la planta *Forsythia suspensa* contienen pinosresinol. Dicha planta se ha propuesto como una alternativa a los antibióticos debido a su contenido de antioxidantes, antiinflamatorios y sus efectos moduladores de la microflora. **Objetivo:** evaluar los efectos dietarios de *F. suspensa* sobre la utilización del alimento, fermentación ruminal, parámetros sanguíneos y respuesta inmune en cabras nativas coreanas (*Capra hircus*). **Métodos:** un total de nueve cabras nativas coreanas fueron asignadas a 1 de 3 tratamientos: (1) una dieta basal sin *F. suspensa*, (2) una dieta basal con 0,25%, o (3) 0,50% de *F. suspensa* dietaria en un diseño change-over por tres periodos consecutivos con nueve réplicas por tratamiento. Cada periodo se prolongó por 18 días, incluyendo 15 días de adaptación al alimento y a las condiciones ambientales, y tres días de colección total de heces y orina. También se colectó fluido ruminal y muestras sanguíneas. Se midió la digestibilidad de nutrientes, nitrógeno retenido, contenido ruminal y metabolitos sanguíneos —incluyendo inmunoglobulinas—. **Resultados:** la suplementación con *F. suspensa* no tuvo efecto sobre la digestibilidad de los nutrientes, la tasa de retención de N, pH ruminal, ni sobre los contenidos de acetato, propionato, isobutirato, butirato, isovalerato o valerato ( $p > 0,05$ ). Sin embargo, la suplementación con *F. suspensa* disminuyó la concentración total de ácidos grasos volátiles (VFA) en comparación con la dieta control, sin tener en cuenta las concentraciones de *F. suspensa* ( $p < 0,05$ ). Las cabras alimentadas con la dieta de 0,25% de *F. suspensa* tuvieron mayor concentración de fosfatasa alcalina (ALP) que cuando fueron alimentadas con la dieta control o con la de 0,50% de *F. suspensa* ( $p < 0,05$ ). Aunque la alimentación con diferentes concentraciones de *F. suspensa* no influyó en los niveles de IgA y IgM en plasma ( $p > 0,05$ ), las cabras que recibieron 0,25 y 0,50% de *F. suspensa* tuvieron mayor concentración de IgG en plasma que aquellas alimentadas con la dieta control ( $p < 0,05$ ). **Conclusiones:** la suplementación de 0,25% de *F. suspensa* disminuyó significativamente la concentración total de VFA e incrementó la IgG en el plasma de las cabras nativas coreanas, en comparación con aquellas que consumieron la dieta control.

**Palabras claves:** extracto de planta, inmunoglobulina, reemplazo de antibióticos.

## Resumo

**Antecedentes:** a *Forsythiae suspensa* é uma planta e seu extrato contém pinosresinol. Tem sido proposta como uma alternativa aos antibióticos existentes, devido ao seu teor de antioxidantes, anti-inflamatórias e efeitos moduladores da microflora, com base em estudos feitos *in vivo* e *in vitro*. **Objetivo:** avaliar os efeitos na dieta da inclusão de *F. suspensa* no aproveitamento alimentar, fermentação ruminal, parâmetros sanguíneos e resposta imune em caprinos coreanos nativos (*Capra hircus*). **Métodos:** um total de nove cabras coreanas nativas foram assignadas a um de três tratamentos na dieta: (1) uma dieta basal sem *F. suspensa*, (2) uma dieta basal com 0,25% de *F. suspensa*, ou (3) com 0,50% de *F. suspensa* na dieta. Se fez um desenho *change-over* por três periodos consecutivos com nove repetições por tratamento. Cada período de pesquisa consistiu de 18 dias, incluindo 15 dias de adaptação às condições alimentares e ambientais, e três dias de coleta total de fezes e urina. Também foram coletadas amostras de líquido ruminal e sangue. Mediou-se a digestibilidade dos nutrientes, o nitrogênio (N) retido, o conteúdo ruminal, e os parâmetros sanguíneos —incluindo imunoglobulinas—. **Resultados:** a suplementação com *F. suspensa* não teve nenhum efeito sobre a digestibilidade dos nutrientes, a taxa de retenção de N, o pH do rúmen, nem sobre os conteúdos de: acetato, propionato, isobutirato, butirato, isovalerato e valerato ( $p > 0,05$ ). No entanto, a suplementação de *F. suspensa* diminuiu a concentração total de ácidos gordurosos voláteis (AGV) em comparação com a dieta controle, independentemente das concentrações de *F. Suspensa* ( $p < 0,05$ ). As cabras alimentadas com 0,25% *F. suspensa* na dieta tiveram maiores quantidades da enzima fosfatase alcalina (ALP) do que quando elas foram alimentadas com a dieta controle ou com 0,50% de *F. suspensa* ( $p < 0,05$ ). Ainda que a alimentação com diferentes níveis de *F. suspensa* não influenciou os níveis de IgA e IgM no plasma ( $p > 0,05$ ), as cabras que receberam na dieta 0,25 e 0,50% de *F. suspensa* tiveram uma maior concentração de IgG no plasma comparadas as cabras alimentadas com a dieta controle ( $p < 0,05$ ). **Conclusões:** a suplementação com 0,25% e 50% diminuiu significativamente a concentração de AGV e incrementou a concentração em plasma de IgG em cabras nativas coreanas comparadas com aquelas que consumiram a dieta controle.

**Palavras chave:** extrato de planta, imunoglobulina, uso alternativo de antibióticos.

## Introduction

Plants produce a huge variety of secondary compounds for protection against microbial and insect attacks (Wallace, 2004). Some of these compounds,

such as essential oils (Patra *et al.*, 2006; Yang *et al.*, 2007; Agarwal *et al.*, 2009; Sallam *et al.*, 2009; Wang *et al.*, 2009a), saponins (Lovett *et al.*, 2006; Pen *et al.*, 2007; Singer *et al.*, 2008; Mao *et al.*,

2010), tannins (Hervas *et al.*, 2003; Mueller-Harvey, 2006; Beauchemin *et al.*, 2007; Waghorn, 2008), and organosulfurs (Bampidis *et al.*, 2005; Yang *et al.*, 2007) are used as dietary additives to selectively modulate rumen microbial populations, improve nitrogen metabolism and decrease methane production in the rumen. Overall ecology modulation of rumen microflora can also influence the gastrointestinal health of the host animal. Several experiments showed *Forsythia fructus* has antioxidant effects in rats (Lu *et al.*, 2010), *in vitro* anti-inflammatory effects (Lim *et al.*, 2008), *in vitro* prevention of unfavorable-microorganisms (Bae *et al.*, 2005; Qu *et al.*, 2012), effects against allergic reactions to soybeans in weaned pigs (Hao *et al.*, 2010), and can regulate blood pressure in rabbits (Moon and Ha, 1977).

However, there is little information available about the effects of *F. fructus* or *Forsythia suspensa* supplementation on ruminants. Therefore, the objective of this study was to evaluate the effects of dietary *F. suspensa* on feed utilization, rumen fermentation, blood parameters, and immune responses in Korean native goats.

## Materials and methods

The experimental protocol used in this study was approved by the Animal Care and Use Committee of Chungnam National University in South Korea.

A total of nine Korean native goats (average body weight:  $26.1 \pm 0.42$  kg) were used. Goats were individually housed in metabolism crates (50 cm × 100 cm × 70 cm). Crates were equipped with wire-mesh flooring, a nipple drinker and a metal feeder. Goats had *ad libitum* access to water and experimental diets throughout the experiment. Diets were fed as a total mixed ration (TMR). The test supplement (i.e., *F. suspensa*) was ground using a laboratory hammer mill (Glen Creston Ltd., London, UK) through a 1 mm screen before top-dressed to the control TMR. Experimental diets were formulated to meet or exceed nutrient requirements of goats (NRC, 1985). Composition and nutrient contents of the experimental diets are presented in Tables 1 and 2. The experiment was conducted as a change-over design with three

consecutive periods and nine observations per treatment. Goats were fed once per day (0900 h) to meet or exceed daily energy requirements based on body weight. Each period lasted 18 days, including 15 days of adaptation to feed and environmental conditions, and 3 days of total collection of feces and urine. The ambient temperature was maintained at 25 °C to 30 °C throughout the study. Feed and water intake were measured daily. Fecal samples were collected daily in sample bags and immediately stored at -20 °C for further analysis. Urine was collected daily in sample containers added with 50 mL HCl (10%, v/v) to minimize N losses. Urine was weighed and a sub-sample (10% of the total weight) was strained through glass wool and stored at -20 °C.

**Table 1.** Chemical composition of *Forsythia suspensa*.

Chemical composition	DM basis, %
Crude protein	7.2
Crude fat	21.5
NDF <sup>1</sup>	47.7
ADF <sup>2</sup>	41.8
ADL <sup>3</sup>	24.1
Ash	8.1

<sup>1</sup>Neutral detergent fiber.

<sup>2</sup>Acid detergent fiber.

<sup>3</sup>Acid detergent lignin.

Fecal samples were thawed and pooled for each goat in each period, then subsampled and oven dried. The dried samples of feces and diet were ground with a laboratory hammer mill (through 1 mm screen; Glen Creston Ltd., London, UK) and thoroughly mixed prior to chemical analysis. Fecal and diet samples were analyzed for DM, ether extract, nitrogen (N), neutral detergent fiber (NDF), and acid detergent fiber (ADF). Dry matter was determined according to the method of AOAC (1990; method925.09). Nitrogen content was determined by combustion (method 990.03; AOAC 1990) using a combustion analyzer (model CNC-2000; Leco Corporation, St. Joseph, MI, USA). Ether extract was determined using the AOAC method920.39 (1990). NDF and ADF content were analyzed according to the method of Goring and Van Soest (1970). Total tract apparent digestibility (TTAD) was calculated using the following equation [(nutrient intake – fecal excretion)/nutrient intake] x 100.

**Table 2.** Ingredients and chemical composition of TMR (as-fed basis)<sup>1</sup>.

Items	Control diet
<i>Ingredients (%)</i>	
Hay	40.0
Corn	19.0
Soybean meal	6.4
By-products of grains <sup>2</sup>	25.2
Molasses	4.5
By-products of food processing <sup>2</sup>	4.4
Salt	0.2
CaCO <sub>3</sub> <sup>3</sup>	0.1
Vitamin-mineral mixture <sup>4</sup>	0.2
<i>Calculated nutrient composition</i>	
Crude protein, %	12.5
Crude fat, %	4.3
NDF <sup>5</sup> , %	60.9
ADF <sup>6</sup> , %	32.8
Ash, %	8.8
<i>Analyzed nutrient composition</i>	
Crude protein, %	13.1
NDF, %	62.8
ADF, %	31.2

<sup>1</sup>Total mixed ration.<sup>2</sup>Fusion Bio Co., Ltd. South Korea.<sup>3</sup>Calcium carbonate.<sup>4</sup>Vitamin and mineral premix supplied the following per kilogram of complete diet: 3,800 IU of vitamin A, 400 IU of vitamin D<sub>3</sub>, 20 IU of vitamin E, 50 mg of iron, 0.15 mg of cobalt, 7 mg of copper, 24 mg of manganese, 30 mg of zinc, 0.6 mg of iodine, 0.15 mg of selenium.<sup>5</sup>Neutral detergent fiber.<sup>6</sup>Acid detergent fiber.

Rumen fluid was collected using a stomach tube 2 h prior to daily feeding. Rumen contents were filtered through four layers of cheesecloth and pH was immediately determined using a pH electrode (pH meter 440, Corning, NY, USA). Volatile fatty acid (VFA) concentrations were determined in duplicate samples using methods described by Fenner and Elliot (1963).

Blood samples were collected from the jugular vein in heparinized vacuum tubes (BD Vacutainer, Becton, Dickinson and Company, NJ, USA) before the morning feeding at the end of each experimental period. Samples were immediately centrifuged at 2000 × g for 30 min, and plasma was stored at -70 °C until analysis. Plasma samples were analyzed using an automatic analyzer (SPOTCHEM EZ SP-4430, Arkray Inc., Kyoto, Japan) to measure albumin, alkaline phosphatase (ALP), blood urea nitrogen (BUN), calcium (Ca), creatine, glucose, glutamic-oxaloacetic transaminase (GOT), glutamate pyruvate transaminase (GPT), total Bilirubin, total cholesterol, and total protein. Plasma IgA, IgM, and IgG were measured using commercially available ELISA kits (Blue Gene Biotech Co. Ltd., Shanghai, China). Goat plasma was dispensed in each well and incubated together with IgA, M and G-HRP conjugate in a pre-coated plate at 37 °C for 1 h. Thereafter, the plates were decanted and washed five times with washing solution, and then substrate solutions were added and kept at 37 °C for 15 min. Finally, a stop solution was added to the reaction and measured at 450 nm in a microplate reader (Thermo-Max, Molecular Devices, Sunnyvale, CA, USA).

Data were analyzed using the GLM procedure of ANOVA (SAS software release 9.1, SAS Inst. Inc., Cary, NC, USA). Individual goats were the experimental unit for all measured variables. Pair-wise comparisons between means were made when appropriate using Fisher's-protected LSD analysis when a significant treatment effect was observed. Statistical significance and tendency were considered at  $p < 0.05$  and  $0.05 \leq p < 0.10$ , respectively.

## Results

Dietary treatments had no effects ( $p > 0.05$ ) on feed or water intakes, nor on urine, or feces excretion (Table 3).

**Table 3.** Effect of dietary *Forsythia suspensa* on feed and water intakes, urine, and feces excretion in Korean native goats<sup>1</sup>.

Items	<i>Forsythia suspensa</i> addition, %		
	0	0.25	0.5
Feed intake, DM, g/d	962 ± 39.8	938 ± 32.0	962 ± 27.6
Water intake, mL/g feed, DM	2.7 ± 0.10	2.8 ± 0.15	2.4 ± 0.13
Urine excretion, mL/d	1108.1 ± 135.93	1132.4 ± 145.07	1076.8 ± 142.88
Feces excretion, DM, g/d	312.1 ± 14.20	291.6 ± 12.57	302.7 ± 11.61

<sup>1</sup>Values are Mean ± SEM. All measured variables were not significant ( $p > 0.05$ ).

Supplementation of *F. suspensa* tended to increase total tract apparent digestibility of dry matter ( $p < 0.10$ ), while total tract apparent crude protein digestibility tended to decrease with *F. suspensa* supplementation (Table 4).

No effects were observed of *F. suspensa* addition on total N intake and excretion through feces and urine, N retention, or whole body N retention rate (Table 5).

**Table 4.** Effect of dietary *Forsythia suspensa* on total tract apparent nutrient digestibilities in Korean native goats<sup>1</sup>.

Total tract apparent digestibility, %	<i>Forsythia suspensa</i> addition, %		
	0	0.25	0.5
Dry matter	67.6 ± 0.42 <sup>a</sup>	68.9 ± 0.39 <sup>b</sup>	68.5 ± 0.52 <sup>b</sup>
Ether extract	76.2 ± 0.82	78.0 ± 0.31	77.5 ± 0.52
Crude protein	68.5 ± 0.49 <sup>a</sup>	67.7 ± 0.51 <sup>b</sup>	67.5 ± 0.54 <sup>b</sup>
NDF <sup>2</sup>	62.7 ± 0.36	64.2 ± 0.66	64.4 ± 0.74
ADF <sup>3</sup>	48.6 ± 0.66	50.8 ± 0.62	50.2 ± 0.82

<sup>1</sup>Values are Mean ± SEM.

<sup>2</sup>Neutral detergent fiber.

<sup>3</sup>Acid detergent fiber.

<sup>a-b</sup>Means within a row with different letters are different ( $p < 0.10$ ).

**Table 5.** Effect of dietary *Forsythia suspensa* on nitrogen retention in Korean native goats<sup>1</sup>.

Items	<i>Forsythia suspensa</i> addition, %		
	0	0.25	0.5
Total N intake, g/d	11.9 ± 0.47	12.0 ± 0.51	11.8 ± 0.52
Loss, g/d			
Feces	3.9 ± 0.20	3.9 ± 0.13	3.9 ± 0.16
Urine	3.9 ± 0.47	4.1 ± 0.45	3.8 ± 0.52
Total	7.8 ± 0.62	7.9 ± 0.53	7.7 ± 0.61
Retention, g/d	4.0 ± 0.24	4.1 ± 0.41	4.9 ± 0.40
Retention rate, %	34.0 ± 2.39	34.1 ± 3.17	35.3 ± 3.30

<sup>1</sup>Values are Mean ± SEM. All measured variables were not significant ( $p > 0.05$ ).

*F. suspensa* supplementation decreased ( $p < 0.05$ ) total VFA concentration in ruminal digesta compared with the control diet, regardless of *F. suspensa* concentrations (Table 6). However, ruminal pH, acetate, propionate, iso-butyrate, butyrate, iso-valerate, and valerate contents, and acetate:propionate ratio were not affected ( $p > 0.05$ ) by different concentrations of dietary *F. suspensa* supplementation (Table 6).

Goats fed the 0.25% *F. suspensa* diet had greater ( $p < 0.05$ ) ALP concentration than those fed the control

or 0.50% *F. suspensa* diet (Table 7). Nonetheless, dietary treatments had no effect ( $p > 0.05$ ) on blood parameters such as albumin, BUN, Ca, creatine, glucose, GOT, GPT, total bilirubin, total cholesterol, and total protein (Table 7). Although feeding different concentrations of dietary *F. suspensa* did not influence ( $p > 0.05$ ) plasma IgA and IgM contents, goats receiving both 0.25% and 0.50% *F. suspensa* had greater ( $p < 0.05$ ) plasma IgG than those fed the control diet (Table 8).

**Table 6.** Effect of dietary *Forsythia suspensa* on ruminal parameters in Korean native goats<sup>1</sup>.

Items	<i>Forsythia suspensa</i> addition, %		
	0	0.25	0.5
pH	5.8 ± 0.12	5.7 ± 0.14	5.7 ± 0.08
Total VFA, mM/L	106.1 ± 5.19 <sup>a</sup>	92.8 ± 4.34 <sup>b</sup>	90.5 ± 4.15 <sup>b</sup>
C <sub>2</sub> , molar % <sup>2</sup>	56.1 ± 1.71	58.9 ± 2.24	56.9 ± 1.07
C <sub>3</sub> , molar %	21.5 ± 2.14	21.1 ± 1.72	21.1 ± 1.85
i-C <sub>4</sub> , molar %	1.5 ± 0.06	1.6 ± 0.11	1.5 ± 0.14
C <sub>4</sub> , molar %	16.0 ± 2.71	13.8 ± 1.68	15.0 ± 1.14
i-C <sub>5</sub> , molar %	2.1 ± 0.20	2.0 ± 0.24	1.6 ± 0.25
C <sub>5</sub> , molar %	2.9 ± 0.32	2.7 ± 0.22	2.8 ± 0.36
C <sub>2</sub> /C <sub>3</sub>	2.61 ± 0.09	2.65 ± 0.13	2.69 ± 0.11

<sup>1</sup>Values are Mean ± SEM.

<sup>2</sup>Acetate, propionate, iso-butyrate, butyrate, iso-valerate, and valerate are abbreviated as: C<sub>2</sub>, C<sub>3</sub>, i-C<sub>4</sub>, C<sub>4</sub>, i-C<sub>5</sub>, and C<sub>5</sub>, respectively.

<sup>a-b</sup>Means within a row with different letters are different (p<0.05).

**Table 7.** Effect of dietary *Forsythia suspensa* on blood parameters in Korean native goats<sup>1</sup>.

Items	<i>Forsythia suspensa</i> addition, %		
	0	0.25	0.5
Albumin, g/dL	3.6 ± 0.08	3.7 ± 0.09	3.8 ± 0.03
ALP, IU/L	264.2 ± 16.10 <sup>ab</sup>	308.4 ± 14.20 <sup>a</sup>	252.2 ± 16.94 <sup>b</sup>
BUN, mg/dL	12.7 ± 0.96	13.0 ± 1.01	12.6 ± 0.67
Ca, mg/dL	9.2 ± 0.23	9.2 ± 0.35	9.8 ± 0.23
Creatine, mg/dL	1.2 ± 0.05	1.2 ± 0.14	1.3 ± 0.07
Glucose, mg/dL	69.3 ± 2.02	72.3 ± 1.99	73.4 ± 2.22
GOT <sup>2</sup> , IU/L	76.1 ± 3.12	76.2 ± 3.00	72.1 ± 3.20
GPT <sup>3</sup> , IU/L	16.0 ± 1.38	17.3 ± 1.11	15.8 ± 1.28
Bilirubin, mg/dL	0.5 ± 0.03	0.5 ± 0.03	0.5 ± 0.02
Cholesterol, mg/dL	68.9 ± 3.88	72.7 ± 3.82	70.6 ± 2.95
Protein, g/dL	6.5 ± 0.22	6.6 ± 0.20	6.7 ± 0.14

<sup>1</sup>Values are Mean ± SEM.

<sup>2</sup>Glutamic-oxaloacetic transaminase.

<sup>3</sup>Glutamate pyruvate transaminase.

<sup>a-b</sup>Means with different superscript letters within each column are significantly different (p<0.05).

**Table 8.** Effect of dietary *Forsythia suspensa* on immune responses in Korean native goats<sup>1</sup>.

Items	<i>Forsythia suspensa</i> addition, %		
	0	0.25	0.5
IgA, mg/mL	0.19 ± 0.04 <sup>1</sup>	0.19 ± 0.03	0.22 ± 0.04
IgM, mg/mL	0.99 ± 0.16	1.02 ± 0.19	0.94 ± 0.16
IgG, mg/mL	16.21 ± 0.77 <sup>b</sup>	18.72 ± 0.78 <sup>a</sup>	18.88 ± 0.86 <sup>a</sup>

<sup>1</sup>Values are Mean ± SEM.<sup>a,b</sup>Means with different superscript letters within each column are significantly different (p<0.05).

## Discussion

The main purpose of this study was to determine whether supplementation of either 0.25% or 0.50% *F. suspensa* alters total tract apparent digestibility, rumen fermentation, and immune response in Korean native goats. There are increasing concerns associated with the use of antimicrobial compounds in animal feed due to possible transfer of antibiotic resistance to humans. *F. suspensa* has been proposed as a possible alternative to antimicrobial compounds along with other plant extracts.

No dietary effects were observed on feed intake, water consumption, urine, or feces excretion in the current study. This indicates that *F. suspensa* odor does not affect diet palatability. According to Devendra and Burns (1983), feed intake by goats varies from 1.7% to 4.8% of the body weight, depending on the breed, which is in agreement with our result. In addition, there were no effects of dietary treatments on water or dry matter intake. According to Silanikove (1992), water intake is closely related to DM intake. There was no significant difference in urine or feces output, and this appears to be due to intake of feed and water.

No significant differences were found on digestibility of dry matter, crude protein, crude fat, NDF, or ADF in response to dietary supplementation of *F. suspensa*. There is limited evidence showing a relationship between *F. suspensa* supplementation and total tract apparent nutrient digestibility in ruminants. However, *F. suspensa* triterpenes may affect nutrient digestibility. Although Pen *et al.* (2007) reported that supplementation of *Yucca schidigera* or *Quillaja saponaria* extracts containing

triterpenes did not alter total tract apparent nutrient digestibility, it decreased upon supplementation of an extract containing triterpene (Holtshausen *et al.*, 2009). On the other hand, high fiber and lignin contents in the plant extract are known to affect total tract apparent nutrient digestibility in ruminants. For example, Mekasha *et al.* (2002) reported that high fiber and lignin diets decrease nutrient digestibility. However, despite the high lignin in *F. suspensa* (24.1%), *F. suspensa* supplementation up to 0.5% did not affect total tract apparent nutrient digestibility in this study.

No effects of *F. suspensa* addition were observed on total nitrogen intake, discharged nitrogen through feces and urine, or nitrogen retention. Rumen ammonia concentration was reduced and nitrogen retention rate was increased by intra-ruminal supplementation of 60 g *Yucca schidigera* extract (Hristov *et al.*, 1999) or 120 ppm *Yucca schidigera* extract containing a triterpene (Santoso *et al.*, 2004). Santoso *et al.* (2007) also reported that triterpene saponin decreases nitrogen excreted through urine. However, *F. suspensa* containing triterpene did not affect nitrogen retention in this study, indicating that *F. suspensa* does not have the ability to alter nitrogen metabolism in the rumen.

According to Wang *et al.* (2009b), increases feed ration concentration dramatically increased VFA production and decreases ruminal pH. When VFA production exceeds VFA absorption rate into the rumen wall, ruminal pH decreases sharply, causing acidosis. Therefore, ruminal pH and VFA concentration were measured to examine whether *F. suspensa* has any effect on VFA metabolism. Total VFA production decreased significantly with *F. suspensa* supplementation, while no changes in the proportion of acetic and propionic acids were observed. This could be due to the strong antibacterial activity of phillyrin, a main component of *F. suspensa* extract. Similar results were demonstrated by Pen *et al.* (2006) who reported that *Yucca schidigera* or *Quillaja saponaria* supplementation decreased protozoa, resulting in total VFA reduction, while there was no difference in acetic:propionic acid ratio. Therefore, it is conceived that *F. suspensa* supplementation can affect the rate of carbohydrate fermentation in the rumen.

No significant differences were detected in most blood parameters, regardless of the addition of *F. suspensa* to ruminant diets (Table 8). However, ALP increased significantly in goats fed 0.25% *F. suspensa*. According to Kaneko *et al.* (1989), ALP is an indirect indicator for liver, gall bladder and bone diseases. It is present in liver, bone, intestine, placenta, and kidney as an enzyme; the main cause of its increase is hepatic cell damage due to infiltration. ALP level is generally high in animals on liver fluke medication or growing animals. As ALP level in the present study remained within the normal range of 27 to 387 IU/L, it may have had no effect. Generally, Ca increases because of hyperparathyroidism, excessive vitamin D, multiple myeloma, acidosis, or plant intoxications. However, there was no significant effect on Ca level in the present study. These results indicate there is no negative effect on blood parameters associated with the dietary addition of *F. suspensa* extract to ruminants.

Plasma IgA and IgM were not affected by the *F. suspensa* addition. However, plasma IgG concentration was significantly increased in goats fed 0.25% or 0.50% *F. suspensa* compared with goats fed the control diet. According to Bose (2000) and Woof and Kerr (2006), immunoglobulins play key roles in immune protection against bacteria and viruses. Although disease-challenge studies would be needed, this result indicates that supplementation of *F. suspensa* may have immune-modulatory effects in goats and possibly in other ruminants.

In conclusion, 0.25% or 0.5% *F. suspensa* supplementation significantly decreased total VFA concentrations and increased plasma IgG in Korean native goats.

### Acknowledgements

This research was financially supported by the research fund of Chungnam National University LINC.

### Conflicts of interest

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

### References

- Agarwal N, Shekhar C, Kumar R, Chaudhary LC, Kamra DN. Effect of peppermint (*Mentha piperita*) oil on *in vitro* methanogenesis and fermentation of feed with buffalo rumen liquor. *Anim Feed Sci Technol* 2009; 148:321-327.
- AOAC. Official method of analysis. 15th ed. Washington, DC: Association of official analytical chemists; 1990.
- Bae JH, Son KH, Lee EJ. Synergistic antimicrobial effect of *Patrinia scabiosaefolia* and *Forsythiae fructus* extracts on food-borne pathogens. *Kor J Microbiol Biotechnol* 2005; 33:130-135.
- Bampidis VA, Christodoulou V, Christaki E, Florou- Paneri P, Spais AB. Effect of dietary garlic bulb and garlic husk supplementation on performance and carcass characteristics of growing lambs. *Anim Feed Sci Technol* 2005; 121: 273-283.
- Beauchemin KA, McGinn SM, Martinez TF, McAllister TA. Use of condensed tannin extract from quebracho trees to reduce methane emissions from cattle. *J Anim Sci* 2007; 85:1990-1996.
- Devendra C, Burns M. Goat production in the tropics. London (UK): commonwealth agricultural bureau;1983.
- Fenner H, Elliot JM. Quantitative method for determining the steam volatile fatty acids in rumen fluid by gas chromatography. *J Anim Sci* 1963; 22:624-627.
- Goering HK, Van Soest PJ. Forage fiber analyses (apparatus, reagents, procedures, and some applications). Washington, DC: U.S. Agricultural Research Service; 1970.
- Hao Y, Li D, Piao X, Piao X. *Forsythia suspensa* extract alleviates hypersensitivity induced by soybean beta-conglycinin in weaned piglets. *J Ethnopharmacol* 2010; 128:412-418.
- Hervas G, Perez V, Giraldez FJ, Mantecon AR, Almar MM, Frutos P. Intoxication of sheep with quebracho tannin extract. *J Comp Pathol* 2003; 129:44-54.
- Heuer OE, Hammerum AM, Collignon P, Wegener HC. Human health hazard from antimicrobial-resistant enterococci in animals and food. *Clin Infect Dis* 2006; 43, 911-916.
- Holtshausen L, Chaves AV, Beauchemin KA, McGinn SM, McAllister TA, Odongo NE, Cheeke PR, Benchaar C. Feeding saponin-containing *Yucca schidigera* and *Quillaja saponaria* to decrease enteric methane production in dairy cows. *J Dairy Sci* 2009; 92:2809-2821.
- Hristov AN, McAllister TA, Van Herk FH, Cheng KJ, Newbold CJ, Cheeke PR. Effect of *Yucca schidigera* on ruminal fermentation and nutrient digestion in heifers. *J Anim Sci* 1999; 77: 2554-2563.
- Kaneko JJ, Harvey JW, Bruss ML. Clinical biochemistry of domestic animals. San Diego (US): Academic press; 1989.
- Lim H, Lee JG, Lee SH, Kim YS, Kim HP. Anti-inflammatory activity of phylligenin, a lignan from the fruits of *Forsythia koreana*, and its cellular mechanism of action. *J Ethnopharmacol* 2008; 118:113-117.



- Lovett DK, Stack L, Lovell S, Callan J, Flynn B, Hawkins, M, O'Mara FP. Effect of feeding *Yucca schidigera* extract on performance of lactating dairy cows and ruminal fermentation parameters in steers. *Livest Sci* 2006; 102:23-32.
- Lu T, Piao XL, Zhang Q, Wang D, Piao XS, Kim SW. Protective effects of *Forsythia suspensa* extract against oxidative stress induced by diquat in rats. *Food Chem Toxicol* 2010; 48:764-770.
- Mao H L, Wang J K, Zhou Y Y, Liu J X. Effects of addition of tea saponins and soybean oil on methane production, fermentation and microbial population in the rumen of growing lambs. *Livest Sci* 2010; 129:56-62.
- Mekasha Y, Tegegne A, Yami A, Umunna NN. Evaluation of non-conventional agro-industrial by-products as supplementary feeds for ruminants: *in vitro* and metabolism study with sheep. *Small Ruminant Res* 2002; 44:25-35.
- Moon YH, Ha CJ. The annual convention of the Korean society of pharmacognosy; effect of forythiae fructus water extract on the blood pressure in the rabbit. *Kor J Pharmacogn* 1977; 8:177.
- Moore PR, Evenson A, Luckey TD, McCoy E, Elvehjem CA, Hart EB. Use of sulfasuxidine, streptothricin, and streptomycin in nutritional studies with the chick. *J Biol Chem* 1946; 165:437-441.
- Mueller-Harvey I. Unravelling the conundrum of tannins in animal nutrition and health. *J Sci Food Agric* 2006; 86:2010-2037.
- NRC National Research Council. Nutrient requirement of small ruminant: sheep, goats, cervids, and new world camelids. Washington, DC (US): National Academy Press; 1985.
- Patra AK, Kamra DN, Agarwal N. Effect of plants containing secondary metabolites on *in vitro* methanogenesis, enzyme profile and fermentation of feed with rumen liquor of buffalo. *Anim Nutr Feed Technol* 2006; 6:203-213.
- Pen B, Sar C, Mwenya B, Kuwaki K, Morikawa R, Takahashi J. Effects of *Yucca schidigera* and *Quillaja saponaria* extracts on *in vitro* ruminal fermentation and methane emission. *Anim Feed Sci Technol* 2006; 129:175-186.
- Pen B, Takaura K, Yamaguchi S, Asa R, Takahashi J. Effects of *Yucca schidigera* and *Quillaja saponaria* with or without  $\beta$  1-4 galactooligo- saccharides on ruminal fermentation, methane production and nitrogen utilization in sheep. *Anim Feed Sci Technol* 2007; 138:75-88.
- Qu H, Zhang Y, Chai X, Sun W. Isoforsythiaside, an antioxidant and antibacterial phenylethanoid glycoside isolated from *Forsythia suspensa*. *Bioorg Chem* 2012; 40:87-91.
- Sallam SMA, Bueno ICS, Brigide P, Godoy PB, Vitti DMSS, Abdalla AL. Efficacy of eucalyptus oil on *in vitro* rumen fermentation and methane production. *Options Mediterraneennes* 2009; 85:267-272.
- Santoso B, Mwenya B, Sar C, Gamo Y, Kobayashi T, Morikawa R, Kimura K, Mizukoshi H, Takahashi J. Effects of supplementing galacto- oligosaccharides, *Yucca schidigera* or nisin on rumen methanogenesis, nitrogen and energy metabolism in sheep. *Livest Prod Sci* 2004; 91:209-217.
- Santoso B, Kilmaskossu A, Sambodo P. Effects of saponin from *Biophytum petersianum* klotzsch on ruminal fermentation, microbial protein synthesis and nitrogen utilization in goats. *Anim Feed Sci Technol* 2007; 137:58-68.
- Silanikove N. Effects of water scarcity and hot environment on appetite and digestion in ruminants: a review. *Livest Prod Sci* 1992; 30:175-194.
- Singer MD, Robinson PH, Salem AZM, DePeters EJ. Impacts of rumen fluid modified by feeding *Yucca schidigera* to lactating dairy cows on *in vitro* gas production of 11 common dairy feed stuffs, as well as animal performance. *Anim Feed Sci Technol* 2008; 146:242-258.
- Waghorn G. Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production—Progress and challenges. *Anim Feed Sci Technol* 2008; 147:116-139.
- Wallace RJ. Antimicrobial properties of plant secondary metabolites. *Proc Nutr Soc* 2004; 63:621- 629.
- Wang CJ, Wang SP, Zhou H. Influences of flavomycin, ropadiar, and saponin on nutrient digestibility, rumen fermentation, and methane emission from sheep. *Anim Feed Sci Technol* 2009a; 148:157-166.
- Wang YH, Xu M, Wang FN, Yu Z P, Yao JH, Zan LS, Yang FX. Effect of dietary starch on rumen and small intestine morphology and digesta pH in goats. *Livest Sci* 20096b; 122:48-52.
- Woof JM, Kerr MA. The function of immunoglobulin A in immunity. *J Pathol* 2006; 208: 270-282.
- Yang WZ, Benchaar C, Ametaj BN, Chaves AV, He ML, McAllister TA. Effects of garlic and juniper berry essential oils on ruminal fermentation and on the site and extent of digestion in lactating cows. *J Dairy Sci* 2007; 90:5671-5681.