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Effects of Fruit By-product Extracts Supplementation on Growth Performance and Nutrient Digestibility in Growing Pigs

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ABSTRACT

A total of 96 pigs [(Landrace × Yorkshire) × Duroc] with an initial BW of 27.94 ± 0.92 kg were used in a 6-week experiment to determine the effects of dietary supplementation with fruit by-product extracts on growth performance and nutrient digestibility in growing pigs. Pigs were randomly allotted to four treatments : 1) CON (basal diet), 2) PRO (CON + 0.5% procyanidin), 3) HES (CON + 0.5% hesperidin), 4) TAN (CON + 0.5% tannin). There were six replications per treatment with four pigs per pen. Supplementation had no effect (p>0.05) on average daily gain, average daily feed intake, and G/F. The apparent total tract digestibility (ATTD) of dry matter and nitrogen was increased (p<0.05) in the HES treatment relative to the PRO treatment. Pigs fed the HES and TAN diets had greater (p<0.05) ATTD of energy than pigs fed PRO diet. The ATTD of ash was greatest (p<0.05) in HES treatments. In addition, the ATTD of calcium was greater (p<0.05) in HES treatments than in CON and PRO treatments. Overall, the results of this study indicated that dietary supplementation with 0.5% fruit by-products did not affect growth performance, but inclusion of 0.5% hesperidin increased nutrient digestibility in growing pigs.

(**Key words :** Growth performance, Growing pigs, Nutrient digestibility)

INTRODUCTION

Large quantities of grapes, citrus fruits and persimmons are produced in South Korea during summer. Flavonoids found in grape extract and citrus peels are known to exert health-promoting effects in humans and animals (Lien et al., 2007). Persimmon also contains many medicinally bioactive compounds, such as carotenoids, tannins and flavonoids (Mallavadhani et al., 1998). The concentrations of carotenoids and polyphenols are higher in the peels than in the pulp (Kawase et al., 2003). As a result, fruit by-products are considered a good alternative for antibiotics (Goñi et al., 2007; Yan and Kim, 2011). However, the results of their effects in animal studies have been inconsistent. For example, dietary supplementation with 30 g/kg grape seed extract decreased growth performance in chickens (Hughes et al., 2005), whereas inclusion of 15% defatted grape seed meal in the basal diet improved growth performance in finishing rabbits (Garcia et al., 2002).

Therefore, this study was conducted to investigate the effects of procyanidin, hesperidin and tannin supplementation on growth performance and nutrient digestibility in growing pigs.

MATERIALS AND METHODS

1. Preparation of fruit by-products

The fruit by-product extracts used in this study were supplied by SINE-BIO Inc. (Seongman, Korea). Briefly, fruit by-product extracts were collected and sterilized as described by Hwang et al. (2011). The procyanidin was then extracted using a mixture of acetone/water/acetic acid (v/v/v 70/29.5/0.5), after which it was filtered and dried under reduced pressure using a rotary evaporator at 40°C. The procyanidin was further dried using a freeze drier and the 70% acetone extracts were finally obtained. The extracts were then separated using n-hexane followed by open column chromatography with a silica gel packed column. During chromatography, the stationary phase consisted of sephadex LX-20 and the mobile phase consisted of a mixture of methanol and water (v/v 20/80).

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Citrus peel was ground and then soaked in methanol for one day. The methanol extracts were subsequently filtered and dried under reduced pressure at 40°C using a rotary evaporator. Samples were then applied to a reversed phase C18 column (Phenomenex, Torance, CA, 250 × 4.6 mm) with a mixture of acetonitrile and water (20:80 v/v) as the mobile phase. The flow rate was 1.0 ml/min and samples were detected at 283 nm.

Persimmon peel was ground and soaked in 50% aqueous methanol (water: methanol = 50/50) for one day. The 50% aqueous methanol extracts were then filtered and dried under reduced pressure using a rotary evaporator at 40°C. Next, samples were applied to a reversed phase C18 column (Phenomenex, Torance, CA, 250 × 4.6 mm) with a mixture of methanol and 0.2% phosphate solution (10:90 v/v) as the mobile phase. The flow rate was 1.0 ml/min and the sample was detected at 280 nm.

2. Animal management and experimental diets

The experimental protocols used in this study were approved by the Animal Care and Use Committee of Dankook University.

All pigs were housed in an environmentally controlled facility with slatted plastic flooring and a mechanical ventilation system. Each pen was equipped with a 1-sided,

Table 1. Compositions of experimental diets (as-fed basis)

Item	CON[1)]	PRO[1)]	HES[1)]	TAN[1)]
Ingredients, %				
Corn	47.01	46.71	46.71	46.71
Wheat	11.15	11.15	11.15	11.15
Soybean meal	23.10	22.90	22.90	22.90
DDGS	8.00	8.00	8.00	8.00
Rice bran	1.00	1.00	1.00	1.00
Tallow	3.23	3.23	3.23	3.23
Molasses	3.00	3.00	3.00	3.00
Dicalcium phosphate	0.25	0.25	0.25	0.25
Limestone	1.68	1.68	1.68	1.68
Salt	0.30	0.30	0.30	0.30
Choline chloride	0.08	0.08	0.08	0.08
L-Lysine	0.83	0.83	0.83	0.83
DL-Methionine	0.05	0.05	0.05	0.05
L-Threonine	0.02	0.02	0.02	0.02
Vitamin premix[2)]	0.20	0.20	0.20	0.20
Trace mineral premix[3)]	0.10	0.10	0.10	0.10
Procyanidin	−	0.50	−	−
Hesperidin	−	−	0.50	−
Tannin	−	−	−	0.50
Chemical analysis value				
DE, kcal/kg	3380	3350	3360	3350
Crude protein, %	19.00	18.87	18.91	18.85
Crude fat, %	6.49	6.33	6.40	6.37
Lys, %	0.94	0.92	0.93	0.91
Ca, %	0.80	0.80	0.80	0.80
Total P, %	0.63	0.63	0.63	0.63

1) Abbreviations: CON, basal diet; PRO, CON + 0.5% procyanidin; HES, CON + 0.5% tannins; TAN, CON + 0.5% tannins.

2) Provided per kg of complete diet: vitamin A, 4,000 IU; vitamin D_3, 800 IU; vitamin E, 171 IU; vitamin K, 2 mg; riboflavin, 4 mg; niacin, 20 mg; thiamine, 4 mg; d-pantothenic, 11 mg; choline, 166 mg; biotin, 0.08 mg; and vitamin B_{12}, 16 µg.

3) Provided per kg of complete diet: Cu (as $CuSO_4 \cdot 5H_2O$), 15 mg; Fe (as $FeSO_4 \cdot 7H_2O$), 80 mg; Zn (as $ZnSO_4$), 56 mg; Mn (MnO_2), 74 mg; I (as KI), 0.3 mg; Co (as $CoSO_4 \cdot 5H_2O$), 0.5 mg; and Se (as $Na_2SeO_3 \cdot 5H_2O$), 0.4 mg.

stainless-steel self-feeder and a nipple drinker that provided feed and water *ad libitum*. All constituents of diets were formulated to meet or exceed the nutrient requirements (NRC, 1998) for 20 to 50 kg BW growing pigs fed in a mash form (Table 1).

(1) Experimental animals and design

A total of 96 pigs [(Landrace × Yorkshire) × Duroc, BW = 27.94 ± 0.92 kg] were used in a 6-week experiment. Pigs were randomly allotted to four treatments with six replications (four pigs per replication) per treatment according to their initial BW. The treatments were: 1) CON, basal diet; 2) PRO, CON + 0.5% procyanidin; 3) HES, CON + 0.5% hesperidin; 4) TAN, CON + 0.5% tannin.

(2) Sampling and measurements

Individual pig BW and feed disappearance were recorded at the beginning and the end of the 6-week period to determine the ADG, ADFI, and gain/feed (G/F) ratio.

The apparent total tract digestibility (ATTD) of DM and N was determined using chromic oxide (0.2%) as an inert indicator (Fenton and Fenton, 1979). Briefly, pigs were fed diets mixed with chromic oxide on d 35. On d 42, fresh fecal grab samples were collected from two pigs per pen, mixed and pooled, and representative samples was stored in a freezer at −20°C until analysis. Before chemical analysis, the fecal samples were thawed and dried at 70°C for 72 h, after which they were finely ground to a size that could pass through a 1-mm screen. Allfeed and fecal samples were analyzed for DM, N, ash, Ca, and P following the procedures outlined by the Association of Official Analytical Chemists (1995). Chromium was analyzed via UV absorption spectrophotometry (Shimadzu UV-1201, Shimadzu, Kyoto, Japan) following the method described by Williams et al. (1962).

(3) Statistical analysis

All data were analyzed by ANOVA using the General Linear Models (GLM) procedure of SAS (SAS Institute, 2008), with the pen being defined as the experimental unit. Differences among treatments were separated by Duncan's multiple range tests. The results were expressed as the least square means ± SEM. Probability values < 0.05 were considered significant.

RESULTS

Growth performance was not affected ($p>0.05$) by supplementation of the diet with any fruit by-product (Table 2). The ATTD of DM and N in the HES treatment were higher ($p<0.05$) than those in the PRO treatment (Table 3). Pigs fed the HES and TAN diets had higher ATTD of energy than those fed the PRO treatment ($p<0.05$). The HES treatment had the highest ($p<0.05$) ATTD of ash among dietary treatments. A greater ATTD of calcium was noted in the HES treatment than the CON and PRO treatments ($p<0.05$). No difference ($p>0.05$) was observed in the ATTD of P among treatments.

DISCUSSION

In our study, fruit by-products supplementation had no effect on growth performance of growing pigs. In agreement with our results, dietary supplementation with 1% ground grape seed had no effect on the growth performance of broilers (Jang et al., 2007). However, Garcia et al. (2002) reported that inclusion of 15% defatted grape seed meal improved the growth performance and feed efficiency of rabbits. Similarly, the growth performance of finishing pigs was improved by application of 30 g/kg fermented grape pomace product to the diet (Yan and Kim, 2011). Additionally, pervious *in vivo* and *in vitro* experiments have shown that flavonoids (catechin, epicatechin, procyanidins and anthocyanins), phenolic acids (gallic acid and ellagic acid) and stilbenes (resveratrol and piceid) in fruit by-products have health-functional activities such as enhanced antioxidant

Table 2. Effects of fruit by-product extracts on growth performance in growing pigs[1)]

Items	CON	PRO	HES	TAN	SEM[2)]
ADG, g	539	501	515	505	27
ADFI, g	1,231	1,204	1,232	1,263	28
G/F	0.438	0.417	0.419	0.400	0.023

[1)] Abbreviations: CON, basal diet; PRO, CON + 0.5% procyanidin; HES, CON + 0.5% hesperidin; TAN, CON + 0.5% tannin.
[2)] Standard error of the means

Table 3. Effects of fruit by-product extracts on nutrient digestibility in growing pigs[1)]

Items, %	CON	PRO	HES	TAN	SEM[2)]
DM	73.23[ab]	68.98[b]	76.61[a]	73.90[ab]	2.01
N	71.04[ab]	66.45[b]	74.63[a]	72.15[ab]	2.10
Energy	73.46[ab]	69.73[b]	77.28[a]	76.22[a]	1.95
Ash	36.74[b]	39.43[b]	48.39[a]	40.41[b]	2.48
Ca	40.73[b]	42.19[b]	49.66[a]	43.56[ab]	2.10
P	25.99	28.68	30.18	25.01	5.28

1) Abbreviations: CON, basal diet; PRO, CON + 0.5% procyanidin; HES, CON + 0.5% hesperidin; TAN, CON + 0.5% tannin.
2) Standard error of the means
a,b Means in the same row with different superscripts differ (p<0.05).

activity via a reduction of lipid oxidation and/or inhibition of the production of free radicals (Bouhamidi et al., 1998; Bagchi et al., 1998). However, different antioxidant activity has been found in response to different extract methods, conditions (temperature and time) and physical conditions of grape seeds (Jayaprakasha et al., 2003), which may explain the inconsistent effects on animal production among studies.

In our study, the apparent total tract digestibility (ATTD) of DM, N, energy, ash and Ca were decreased in the PRO treatment relative to the HES treatment. *In vitro* digestibility depression has been reported when procyanidins were intubated with food (Artz et al., 1986). Additionally, El-Sayed et al. (2010) confirmed that nutrients digestibility improved in Nile Tilapia fed diets containing 5% dried citrus pulp. Previous studies have also demonstrated that procyanidin showed stability under gastric and duodenal digestion conditions, and that it could bind with digestive enzymes and proteins located on the luminal side of the intestinal tract (Serra et al., 2010). Decreasing digestive enzyme binding sites may suppress nutrients diffusion across the epithelial cells, which would explain the decreased nutrients digestibility after intake of procyanidin (Baba et al., 2002).

CONCLUSION

The results of this study indicated that dietary supplementation with 0.5% fruit by-products did not affect growth performance, but that inclusion of 0.5% hesperidin increased nutrient digestibility in growing pigs.

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REFERENCES

AOAC. 1995. Official Method of Analysis 16th ed. Assoc. Off. Anal. Chem., Washington, DC.

Artz, W. E., Swanson, B. G., Sendzicki, B. J., Rasyid, A. and Birch, R. E. W. 1986. Protein-Procyanidin Interaction and Nutritional Quality of Dry Beans. Plant Proteins: Applications, Biological Effects, and Chemistry Chapter 11:126-137.

Baba, S., Osakabe, N. and Natsume, M. 2002. Absorption and urinary excretion of procyanidin B2 [epicatechin-(4β-8)-epicatechin] in rats. Free. Radic. Biol. Med. 33:142-148.

Bagchi, D., Garg, A., Krohn, R. L., Bagchi, M., Bagchi, D. J., Balmoori, J. and Stohs, S. J. 1998. Protective effects of grape seed proanthocyanidins and selected antioxidants against TPA-induced hepatic and brain lipid peroxidation and DNA fragmentation, and peritoneal macrophage activation in mice. Gen Pharmacol. 30:771-776.

Bouhamidi, R., Prevost, V. and Nouvelot, A. 1998. High protection by grape seed proanthocyanidins (GSPC) of polyunsaturated fatty acids against UV-C induced peroxidation. C R Acad Sci III. 321:31-38.

El-Sayed, S. A., El-Kholy, M. E., Eleraky, W. A. and Soliman, M. H. 2010. Effect of Partial Replacement of Yellow Corn With Dried Citrus Pulp in Nile Tilapia Diets on Growth Performance, Nutrient Digestibility and Immune Status. Aquaculture Nutrition. 10:35-42.

Fenton, T. W. and Fenton, M. 1979. An improved procedure for the determination of chromic oxide in feed and feces. Can. J. Anim. Sci. 59:631-634.

Garcia, J., Nicodemus, N., Carabano, R. and De Blass, J. C. 2002. Effect of inclusion of defatted grape seed meal in the diet on digestion and performance of growing rabbits. J. Anim. Sci. 80:162-170.

Goñi, I., Brenes, A., Centeno, C., Viveros, A., Saura-Calixto, F., Rebolé, A., ArijaI, I. and Estévez, R. 2007. Effect of dietary grape pomace and vitamin E on growth performance, nutrient digestibility and susceptibility to meat lipid oxidation in chickens. Poult. Sci. 86:508-516.

Hughes, R. J., Brooker, J. D. and Smyl, C. 2005. Growth rate of broiler chickens given condensed tannins extracted from grape seed. Aust. Poult. Sci. Symp. 17:65-68.

Hwang, D. S., Shin, S. Y., Lee, Y. G., Hyun, J. Y., Yong, Y. J., Park, J. C., Lee, Y. H. and Lim, Y. H. 2011. A compound isolated from *Schisandra chinensis* induces apoptosis. Bioorg. Med. Chem. Lett. 21:6054-6057.

Jang, I. S., Ko, Y. H., Kang, S. Y., Moon, Y. S. and Sohon, S. H. 2007. Effect of dietary supplementation of ground grape seed on growth performance and antioxidant status in the intestine and liver in broiler chickens. Korean J. Poult. Sci. 34:1-8.

Jayaprakasha, G. K., Tamil, S. and Sakariah, K. K. 2003. Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extracts. Food Res Int. 36:117-122.

Kawase, M., Motohashi, N., Satoh, K., Sakagami, H., Nakashima, H., Tani, S., Shirataki, Y., Kurihara, T., Spengler, G., Wolfard, K. and Molnár, J. 2003. Biological activity of persimmon (Diospyros kaki) peel extracts. Phytother Res. 17:495-500.

Lien, T. F., Yeh, H. S. and Su, W. T. 2007. Effect of adding extracted hesperetin, naringenin and pectin on egg cholesterol, serum traits and antioxidant activity in laying hens. Arch Ani Nutr. 62:33-43.

Mallavadhani, U. V., Panda, A. K. and Rao, Y. R. 1998. Pharmacology and chemotaxonomy of Diospyros. Phytochemistry. 49:901-951.

NRC. 1998. Nutrient Requirement of Pigs. 10th ed. National Research Council. Academy Press, Washington, DC.

SAS Institute, 2008. SAS user's guide, Statistical Analysis System Inst. Inc Cary NC.

Serra, A., Macia, A., Romero, M., Valls, J., Blade, C., Arola, L. and Motilva, M. 2010. Bioavailability of procyanidin dimers and trimers and matrix food effects in *in vitro* and *in vivo* models. Br. J. Nutr. 103:944-952.

Williams, C. H., David, D. J. and Iismaa, O. 1962. The determination of chromic oxide in faeces samples by atomic absorption spectrophotometry. J. Agric. Sci. 59:381-385.

Yan, L. and Kim, I. H. 2011. Effect of dietary grape pomace fermented by *Saccharomyces boulardii* on the growth performance, nutrient digestibility and meat quality in finishing pigs. Asian-Aust. J. Anim. Sci. 24:1763-1770.

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