



## Influences of Phytoncide Supplementation on Growth Performance, Nutrient Digestibility, Blood Profiles, Diarrhea Scores and Fecal Microflora Shedding in Weaning Pigs

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**ABSTRACT:** A total of 140 weaning pigs ((Landrace×Yorkshire)×Duroc, BW = 6.47±0.86 kg) were used in a 5-wk growth trail to determine the effects of phytoncide supplementation on growth performance, nutrient apparent total tract digestibility (ATTD), blood profiles, diarrhea scores and fecal microflora shedding. Pigs were assigned randomly by BW into 5 treatments, dietary treatments were: i) NC, basal diet; ii) PC, NC+0.05% tylosin; iii) EO, NC+0.1% essential oil; iv) PP, NC+0.2% PP (phytoncide with 2% citric acid), and v) PA, NC+0.2% PA (phytoncide). Each treatment had 7 replicate pens with 4 pigs per pen. All pigs were housed in pens with a self-feeder and nipple drinker to allow *ad libitum* access to feed and water throughout the experimental period. During 0 to 2 wks, supplementation with essential oil and PA decreased ( $p<0.05$ ) G/F compared with the other treatments. During 2 to 5 wks, supplementation with PA led to a higher ( $p<0.05$ ) G/F than the other treatments. At 2 wk, ATTD of dry matter (DM) and gross energy (GE) in EO treatment were decreased ( $p<0.05$ ) compared with NC treatment. Dietary PC treatment improved ( $p<0.05$ ) ATTD of DM and E compared with the CON group, and PA and PP treatments showed a higher ( $p<0.05$ ) ATTD of E than that in NC treatment. Pigs fed phytoncide (PA and PP) had a greater ( $p<0.05$ ) ATTD of DM than those of NC and EO treatments at 5 wk. Moreover, supplementation with phytoncide elevated ( $p<0.05$ ) the concentration of immunoglobulin (IgG) in blood at 2 wk. The inclusion of EO, PP and PA treatments showed a greater ( $p<0.05$ ) amount of fecal *Lactobacillus* compared with CON group. However, no difference ( $p>0.05$ ) was observed in diarrhea scores among treatments. In conclusion, phytoncide can elevate feed efficiency, nutrient digestibility, and improve the fecal *Lactobacillus* counts in weaning pigs. Our results indicated that the phytoncide could be used as a good antibiotics alternative in weaning pigs. (**Key Words:** Blood Profiles, Growth Performance, Nutrient Digestibility, Phytoncide, Phytogetic Feed Additives, Weaning Pigs)

### INTRODUCTION

Early weaning of piglets is often accompanied by a severe growth deterioration and diarrhea. Indeed, post-weaning gastrointestinal tract (GIT) disorders in pigs result not only from alterations in GIT architecture and function, but also from major changes in the adapting enteric microbiota (Konstantinov et al., 2004b) and immune responses (Stokes et al., 2004; Bailey et al., 2005). For many years, both therapeutic and growth-promoter antibiotics (AGP) have been effective in improving the performance of the piglets through a decrease in the detrimental effects caused by microbiota (Visek, 1978). Currently, because of concerns about residues in animal

products and bacterial resistance to antibiotics, a ban on most AGPs in animal nutrition has been enacted in some developed countries. In this context, phytogetic feed additives as a class of alternative to AGP has recently gained increasing interest, evident as the increasing number of scientific publications since 2000. Most of these studies have demonstrated that phytogetic feed additives enhance growth performance and nutrient digestibility, improve immune actives, and reduce fecal gas emission and the frequency of diarrhea (Hong et al., 2004; Huang et al., 2011).

Phytoncide are antimicrobial allelochemic volatile organic compounds derived from plants. They play a crucial role in phytogetic feed additives. Li et al. (2006) reported that phytoncide significantly enhance human natural killer activity. Kohei et al. (2004) reported phytoncide mediated reduction of stress responses in stroke-prone spontaneously hypertensive rats. Significant antioxidant and antimicrobial

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activities have been described (Abe et al., 2008). Korean pines and other conifers release relatively large amounts of phytoncide into the environment as a mean of suppressing the activity of microorganisms, including bacteria and fungi (Peciulyte et al., 2010). However, knowledge is still rather limited regarding the effects of pine-derived phytoncide on weaning pigs.

In this study, we investigated the effects of phytoncide on growth performance, nutrient digestibility, blood profile, diarrhea score and fecal microflora shedding in weaning pigs.

## MATERIALS AND METHODS

### Preparation of phytoncide

The phytoncide (PHYLUS Company, Korea) used in this study was abstracted from Korean pine, which was composed with 20% active substance (Flavonoid, Phenolic compounds, Alkaloid, Tannin, Terpene, Saponin) and 80% carrier (dextrin). The essential oil was extracted from oregano, which contained 60% active substance (Cymene, Terpinene, Carvacrol) and 40% carrier (dextrin).

### Experimental animals

The protocol of animal care and use was approved by the Animal Care and Use Committee of Dankook University. A total of 140 crossbred ((Landrace×Yorkshire)×Duroc) pigs (average 21 d) with an average initial BW of 6.47±0.86 kg were randomly assigned by BW and sex according to a randomized complete block design. This experiment included 5 treatments: NC, basal diet; PC, NC+0.05% tylosin; EO, NC+0.1% essential oil; PP, NC+0.2% PP (phytoncide with 2% citric acid), and PA, NC+0.2% PA (phytoncide). Each treatment had 7 replicate pens with 4 pigs per pen. All pigs were housed in pens (0.6×1.2 m) with a self-feeder and nipple drinker to allow *ad libitum* access to enough feed and water throughout the experimental period. The photoperiod consisted of 10 h of artificial light and 14 h of darkness. Temperature was maintained at 30°C and decreased by 1°C each week of the experiment. Diets were formulated to the same concentrations of Lys, CP, ME, Ca, and P. Corn were altered to keep dietary the same ME concentration among treatments. Nutrients were provided to meet or exceed the requirements suggested by NRC (1998).

### Sampling and measurements

Individual body weight and feed consumption per pen were measured at the end of each phase of experiment to monitor the average daily gain (ADG), average daily feed intake (ADFI) and gain to feed ratio (G/F).

Chromium oxide (Cr<sub>2</sub>O<sub>3</sub>) was added to each of the diet as an inert indicator (0.20%) to calculate the apparent total

**Table 1.** The composition of experimental basal diet for weaning pigs

Ingredients (%)	
Corn	61.00
Soybean meal (44%)	24.55
Fish meal (65%)	6.00
Whey	2.50
Soya oil	3.20
Iodine salt	0.30
Limestone, pulverized	0.75
Dicalcium phosphate	1.30
Choline chloride	0.05
L-lysine HCL	0.10
Methionine	0.03
Vitamin-premix*	0.12
Mineral-premix	0.10
Calculated nutrients	
Metabolizable energy (kcal/kg)	3,200.00
Crude protein (%)	20.00
Lysine (%)	1.20
Calcium (%)	0.70
Total phosphorus (%)	0.60

\* Supplied per kg of diet: vitamin A, 15,000 IU; vitamin D<sub>3</sub>, 3,000 IU; vitamin E, 30 mg; vitamin K<sub>3</sub>, 4 mg; riboflavin, 8 mg; pyridoxine, 5 mg; vitamin B<sub>12</sub>, 20 mg; Ca-pantothenate, 19 mg; niacin, 50 mg; folic acid, 1.5 mg; biotin, 60 mg. Supplied per kg of diet: CoCO<sub>3</sub>, 0.255 mg; CuSO<sub>4</sub>·5H<sub>2</sub>O, 10.8 mg; FeSO<sub>4</sub>·H<sub>2</sub>O, 90 mg; ZnO, 68.4 mg; MnSO<sub>4</sub>·H<sub>2</sub>O, 90 mg; Na<sub>2</sub>SeO<sub>3</sub>, 0.18 mg.

tract digestibility (ATTD) for dry matter (DM), nitrogen (N) and energy during each dietary phase. After the pigs were fed diet containing the indicator for 5 d, fresh fecal grab samples were obtained from 2 pigs (one barrow and one gilt) per pen. All fecal and feed samples from each pen were then pooled and mixed, after which a representative sample was stored in a freezer at -20°C until analysis. Prior to 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 sieve. All of the feed and fecal samples were then analyzed for DM and N following the procedures outlined by the AOAC (AOAC, 1995). Chromium was analyzed using UV absorption spectrophotometry (Shimadzu, UV-1201, Kyoto, Japan) and nitrogen was determined using a Kjeltac 2300 Analyzer (Foss Tecator AB, Hoeganaes, Sweden). Gross energy was determined by measuring the heat of combustion in the samples using a Parr 6100 oxygen bomb calorimeter (Parr instrument Co., Moline, IL, USA).

Fecal consistency scoring was based on the following index used by Sherman et al. (1983): 0, normal (feces firm and well formed); 1, soft consistency (feces soft and formed); 2, mild diarrhea (fluid feces, usually yellowish); and 3, severe diarrhea (feces watery and projectile).

At the beginning of the experiment, two pigs were

**Table 2.** Effect of supplementation dietary phytoncide on growth performance in weaning pigs<sup>1</sup>

Item	NC	PC	EO	PP	PA	p-value	SE <sup>2</sup>
0 to 14 d							
ADG (kg)	0.346	0.373	0.328	0.361	0.321	0.204	0.019
ADFI (kg)	0.424	0.458	0.417	0.438	0.428	0.221	0.017
G/F	0.816 <sup>a</sup>	0.814 <sup>a</sup>	0.787 <sup>ab</sup>	0.824 <sup>a</sup>	0.750 <sup>b</sup>	0.039	0.016
15 to 35 d							
ADG (kg)	0.483	0.506	0.508	0.509	0.522	0.349	0.028
ADFI (kg)	0.739	0.779	0.768	0.777	0.733	0.358	0.035
G/F	0.654 <sup>b</sup>	0.650 <sup>b</sup>	0.661 <sup>b</sup>	0.655 <sup>b</sup>	0.712 <sup>a</sup>	0.021	0.010
0 to 35 d							
ADG (kg)	0.428	0.453	0.436	0.450	0.442	0.365	0.021
ADFI (kg)	0.613	0.651	0.628	0.641	0.611	0.336	0.025
G/F	0.698	0.696	0.694	0.702	0.723	0.124	0.009

<sup>1</sup> NC = Basal diet; PC = NC+0.05% tylosin; EO = NC+0.1% essential oil; PP = NC+0.2% PP (phytoncide with 2% citric acid), and PA = NC+0.2% PA (phytoncide).

<sup>2</sup> Standard error. <sup>a,b</sup> Means in the same row with different superscripts differ ( $p < 0.05$ ).

randomly selected from each pen, and blood samples were taken by jugular venipuncture. The same pigs were again bled at the wk 0, 2 and 5. The concentrations of red blood cell (RBC), white blood cell (WBC) and lymphocytes in the blood were measured to investigate the effect of essential oils supplementation in weaned pigs. Blood samples were collected via jugular vein into K2 vacuum tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA) from ten pigs in each treatment on the initial and final days of the feeding trial. The concentrations of RBC, WBC and lymphocytes in the blood were measured using the automatic blood analyzer (ADVIA 120, Bayer, USA). The concentrations of IgG in the blood serum were measured using the automatic biochemistry analyzer (HITACHI 747, Japan).

Total bacteria, including *Lactobacillus*, *Escherichia coli* (*E. coli*) were determined on fresh morning fecal samples at end of this experiment. Fecal microbiota was determined by serial dilution ( $10^{-1}$  to  $10^{-7}$ ) in anaerobic diluent before inoculation onto petridishes of sterile agar as described by Bryant and Burkey (1953). *Lactobacilli* and *E. coli* present in the fresh fecal samples were enumerated. The selective medium for *Lactobacilli* was Rogosa SL agar (Rogosa; Difco Laboratories, Detroit, MI, USA) and for *E. coli* was MacConkey agar, and plate count agar (Difco Laboratories) for *Bacillus* sp. After inoculation, all the dishes were inverted and incubated anaerobically at 37°C for 48 h. The colony counts were then enumerated and results were presented as log<sub>10</sub>-transformed data.

### Statistical analyses

Data were analyzed by ANOVA using the GLM procedure of SAS (SAS Institute, 1996), with pen being defined as the experimental unit. Differences among all treatments were separated by Duncan's multiple range test.

Results were expressed as the least squares means and SEM. Probability values less than 0.05 were considered significant.

## RESULTS

### Growth performance

Growth performance was showed in Table 2. During 0 to 2 wk, pigs fed with essential oil and phytoncide showed a lower G/F than that of the other pigs ( $p < 0.05$ ). Nevertheless, during 2 to 5 wk, pigs fed phytoncide presented a greater G/F to feed than that of the others ( $p < 0.05$ ). No difference in growth performance was observed during 0 to 5 wk ( $p > 0.05$ ).

### Nutrient digestibility

At 2 wk, ATTD of dry matter (DM) and energy (E) in EO treatment were decreased ( $p < 0.05$ ) compared with NC treatment (Table 3). Dietary PC treatment improved ( $p < 0.05$ ) ATTD of DM and E than the CON group, and PA and PP treatments showed a higher ( $p < 0.05$ ) ATTD of E than that in NC treatment. Pigs fed phytoncide (PA and PP) had a greater ( $p < 0.05$ ) ATTD of DM than those of NC and EO treatments. Moreover, no significant difference was found between PA and PC treatments ( $p > 0.05$ ).

### Blood profile

The concentration of serum IgG were greater in PA and PP treatments than NC, PC and EO treatments ( $p < 0.05$ ) at 2 wk (Table 4). Besides, the concentration of blood RBC in pigs fed PC and PA treatments' diets were higher than that in NC, EO and PA treatments ( $p < 0.05$ ). And, pigs fed PA and PP experimental diets showed a greater concentration of serum IgG compared with that in other treatments ( $p < 0.05$ ).

**Table 3.** Effect of supplementation dietary phytoncide on nutrient digestibility in weaning pigs<sup>1</sup>

Item (%)	NC	PC	EO	PP	PA	p-value	SE <sup>2</sup>
2 wk							
Nitrogen	83.16	83.62	82.99	83.33	83.46	0.344	0.42
Dry matter	82.91 <sup>ab</sup>	83.59 <sup>a</sup>	82.78 <sup>b</sup>	83.40 <sup>ab</sup>	83.13 <sup>ab</sup>	0.048	0.21
Energy	84.14 <sup>b</sup>	84.96 <sup>a</sup>	84.13 <sup>b</sup>	84.85 <sup>a</sup>	84.59 <sup>ab</sup>	0.041	0.20
5 wk							
Nitrogen	84.92	85.78	84.79	85.51	85.28	0.217	0.36
Dry matter	82.15 <sup>b</sup>	83.83 <sup>a</sup>	82.93 <sup>ab</sup>	83.74 <sup>a</sup>	83.30 <sup>a</sup>	0.035	0.29
Energy	84.73	85.59	84.54	85.23	85.00	0.153	0.35

<sup>1</sup> NC = Basal diet; PC = NC+0.05% tylosin; EO = NC+0.1% essential oil; PP = NC+0.2% PP (phytoncide with 2% citric acid), and PA = NC+0.2% PA (phytoncide).

<sup>2</sup> Standard error. <sup>a,b</sup> Means in the same row with different superscripts differ (p<0.05).

**Table 4.** Effect of supplementation dietary phytoncide on blood profile in weaning pigs<sup>1</sup>

Item	NC	PC	EO	PP	PA	p-value	SE <sup>2</sup>
2 wk							
RBC (10 <sup>6</sup> /μl)	5.81	5.71	5.71	5.62	5.65	0.574	0.30
WBC (10 <sup>3</sup> /μl)	15.94	14.22	15.11	14.02	14.76	0.597	1.49
Lymphocyte (%)	48.57	43.77	42.57	49.90	53.67	0.211	3.74
IgG (mg/dl)	277.0 <sup>b</sup>	278.0 <sup>b</sup>	277.7 <sup>b</sup>	332.0 <sup>a</sup>	317.7 <sup>ab</sup>	0.042	13.4
5 wk							
RBC (10 <sup>6</sup> /μl)	5.45	6.08	5.64	5.55	5.72	0.243	0.28
WBC (10 <sup>3</sup> /μl)	19.68	18.92	18.61	19.11	18.95	0.194	0.35
Lymphocyte (%)	46.29	54.15	54.81	50.62	55.28	0.259	4.26
IgG (mg/dl)	260.0	269.0	263.3	344.0	316.7	0.247	26.3

<sup>1</sup> NC = Basal diet; PC = NC+0.05% tylosin; EO = NC+0.1% essential oil; PP = NC+0.2% PP (phytoncide with 2% citric acid), and PA = NC+0.2% PA (phytoncide).

<sup>2</sup> Standard error. <sup>a,b</sup> Means in the same row with different superscripts differ (p<0.05).

### Diarrhea score and fecal microflora shedding

The inclusion of EO, PP and PA treatments showed a greater (p<0.05) amount of fecal *Lactobacillus* compared with CON group. However, no difference (p>0.05) was observed in diarrhea scores among treatments (Table 5).

## DISCUSSION

### Growth performance and nutrient digestibility

In many plants, some of the active substances are highly odorous or may taste hot or pungent, which may restrict their use for animal feeding purposes. They produce dose related depression of palatability in pigs fed essential oils (Jugl-Chizzola et al., 2006; Schone et al., 2006). However,

in the current study, phytoncide did not affect the ADFI throughout the experimental period. This may be attributed to a lower level of gustatory properties of alkaloids. Whittemore et al. (1977) observed a negative reaction to the gustatory and aromatic properties of ergot alkaloids in pigs weighing 17 kg when dietary ergot content reached 10%. Numerous reports have documented improved feed intake through phytongenic feed additives in swine. For instance, Kong et al. (2007) reported that Chinese herb powder at 2 g/kg elevated ADG and ADFI, but didn't influence G/F ratio, during the entire period of the trial. Freitag et al. (1998) considered that this may reflect the higher consumption capacity of larger animals compared with untreated controls. Of course, the majority of experimental

**Table 5.** Effect of supplementation dietary phytoncide on diarrhea score and fecal microflora shedding in weaning pigs<sup>1</sup>

Items (log <sub>10</sub> cfu/g)	NC	PC	EO	PP	PA	p-value	SE <sup>2</sup>
<i>Lactobacillus</i>	6.82 <sup>b</sup>	6.99 <sup>ab</sup>	7.24 <sup>ab</sup>	7.39 <sup>a</sup>	7.14 <sup>ab</sup>	0.044	0.15
<i>E. coli</i>	5.66	5.72	5.70	5.97	5.82	0.201	0.17
Diarrhea scores <sup>3</sup>	0.0	0.0	0.0	0.0	0.0	-	0.00

<sup>1</sup> NC = Basal diet; PC = NC+0.05% tylosin; EO = NC+0.1% essential oil; PP = NC+0.2% PP (phytoncide with 2% citric acid), and PA = NC+0.2% PA (phytoncide).

<sup>2</sup> Standard error. <sup>a,b</sup> Means in the same row with different superscripts differ (p<0.05).

<sup>3</sup> Diarrhea score: 0, normal (feces firm and well formed); 1, soft consistency (feces soft and formed); 2, mild diarrhea (fluid feces, usually yellowish); and 3, severe diarrhea (feces watery and projectile).

results indicate reduced feed intake at largely unchanged BW gain or final BW, leading to an improved G/F when feeding phytogetic compounds (Windisch et al., 2007). Similarly, our results confirmed this situation from 3-5 weeks. Interestingly, a lower G/F was evident in EO and PP treatments compared with PA treatment during wks 0 to 2. As far as we know, the acid secreting capacity of weaning pigs is insufficient. It may be speculated that essential oil and phytoncide derived from pine elevates gastric pH. Furthermore, the first week after diet transition is a very stressful period for piglets, oftentimes resulting in a sharp decrease in digestibility. This should be considered when phytogetic feed additives are applied. However, due to limited investigations about the use of pine derived phytoncide in pigs, the specific mechanism remains unclear.

Phytogetic feed additives might improve the digestion and absorption of dietary nutrients, and may also directly regulate the metabolism of absorbed nutrients through signal transduction mechanisms, including nitric oxide signaling (Jobgen et al., 2006). Cho et al. (2006) reported that treatment with added 0.03% essential oils resulted in greater nitrogen digestibility on d 14 to 28 than other diets. In agreement with these findings, we presently observed that essential oil improved digestibility of dry matter at 5 wks. This may have been caused by flavonoid rich substances, which positively affected intestinal villi height. Sehm et al. (2007) opined that animals treated with essential oil responded with a higher villus length in the duodenum.

### Blood profiles

Huang et al. (2011) suggested that a basal diet with 0.1% essential oil increased IgG concentrations in weaning pigs. Furthermore, Cho et al. (2006) reported an increased serum IgG concentration when weaning pigs were fed a diet supplemented with 0.03% essential oil. It has been long acknowledged that some plant essential oils exhibited antimicrobial properties (Finnemore, 1926; Koedam, 1977). Besides, the gastrointestinal system and its associated lymphoid is the largest immunologically competent organ in the body, and the maturation and optimal development of the immune system depend on the development and composition of the indigenous microflora, and vice versa (de Vrese and Marteau, 2007). Consequently, we had reason to believe the effect of essential oil compounds on the animal's immune ability was due to their antimicrobial activity, because the maturation and optimal development of the immune system depends on the development and composition of the indigenous microflora (Yan et al., 2010). A salient observation from this study is that dietary supplementation with phytoncide powder affected plasma concentrations of IgG. However, essential oil did not

influence IgG concentrations. This was probably associated with the volatility of some active compounds in essential oil, such as thymol and cinnamaldehyde applied microencapsulated in carriers to reduce evaporation and to increase shelf life of the product in feed. This encapsulated product has a recovery rate exceeding 90% for both thymol and cinnamaldehyde after 6 months storage (Tiuhonen et al., 2010). Therefore, the carrier imparts better stability in feed and probably also in the animal body, and consequently improved bio-efficacy in piglets (Li et al., 2012).

### Fecal microflora shedding and diarrhea scores

Post-weaning diarrhea is one of the many interdependent factors of high mortality rate in piglets. Presently, no diarrhea was found in each treatment. This may be related to the strict hygienic conditions. Besides, Janczyk et al. (2009) reported that the microbiota in the intestine of the piglets from commercial farm seemed to be more stable and less affected by the essential oil, indicating the importance of the hygienic condition of the farm.

In general, feeding diets supplemented with plant extracts can influence the microflora in the digestive tract of early weaned piglets by increasing the ratio of *Lactobacillus* and *E. coli* in the jejunum and caecum (Manzanilla et al., 2004). Additionally, Zhu et al. (2000) suggested that the pig GIT microflora influenced growth efficiency and disease susceptibility through mechanisms, which may involve the increased content of *Lactobacillus* and *Bifidobacterium*, and decreased of *E. coli* in the intestine, or apparent selection for *Lactobacillus*, commensals known to competitively exclude potentially pathogenic species from colonizing the intestine. Li et al. (2012) confirmed this viewpoint; they described an increasing amount of *Lactobacillus* and a decreasing amount of *E. coli* in feces was found in pigs fed 50 and 100 ppm essential oil. Our results also supported this view, by documenting increased numbers of *Lactobacillus* in feces. However, results have been inconsistent. For example, Hong et al. (2012) showed that gastrointestinal tract microflora was not affected by a diet containing 125 ppm essential oil in broilers. A possible explanation for the discrepancy in findings could be intrinsic and extrinsic factors including environment, diet and nutritional status. Besides, phytogetic compounds, mainly essential oils, may specifically enhance activities of digestive enzymes and nutrient absorption, which may improve the biological values of inferior feed (Windisch et al., 2008).

In conclusion, dietary supplementation with 0.2% phytoncide or 0.2% phytoncide with 0.2% citric acid in weaning pigs can elevate feed efficiency, increase nutrient digestibility, enhance the immune function and improve the intestinal microbial population. In general, these responses indicate that phytoncide are a potential alternative to AGPs

as a means of enhancing growth and health of pigs.

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