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Original article

# Effect of phytogenics on growth performance, fecal score, blood profiles, fecal noxious gas emission, digestibility, and intestinal morphology of weanling pigs challenged with *Escherichia coli* K88

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## Abstract

Phytogenic feed additives have become attractive alternatives for use in animal diets. The objective of the present study was to evaluate the effect of a phytogenic-based feed additive on growth performance, nutrient digestibility, blood profiles, fecal noxious gas emission, and intestinal morphology of weaning pigs after dietary challenge with *E. coli* K88. A total of 120 crossbred pigs [(Yorkshire × Landrace) × Duroc] with an initial body weight (BW) of  $6.09 \pm 0.96$  kg (21 d of age) were assigned randomly to 1 of the 4 dietary treatments. Each pen housed 5 pigs, and there were 6 pens/treatment. Treatments included: T1, negative control (without antibiotics); T2, T1 + antibiotic; T3, T1 + 0.05% phytogenics; and T4, T1 + 0.2% commercial mix of organic acids. Overall, the average daily gain (ADG) with the T3 treatment was higher ( $P < 0.05$ ). At wk 1, the apparent total tract digestibility (ATTD) of dry matter (DM) was increased ( $P < 0.05$ ) with T4 treatment. The ATTD of ash with T3 and T4 treatments was greater ( $P < 0.05$ ). At wk 3, pigs fed with the T4 diet had a significantly higher ( $P < 0.05$ ) ATTD of DM. The ATTD of ash and calcium (Ca) was significantly increased ( $P < 0.05$ ) with the T4 treatment. Pigs fed with the T3 diet had a higher ( $P < 0.05$ ) ATTD of phosphorus (P). At wk 6, the ATTD of ash was significantly increased ( $P < 0.05$ ) with the T1 and T3 treatments. The data indicate that phytogenics positively affect growth performance of weaning pigs, indicating that their use as an alternative in the diets of weaning pigs can significantly improve ADG, under challenge with *E. coli* K88.

**Key words:** weaning pigs, phytogenics, growth performance, digestibility, *Escherichia coli* K88

## Introduction

*Escherichia coli* is one of the most important pathogens causing neonatal and post-weaning diarrhea in pigs (Fairbrother et al. 2005). After the implementation of a complete ban on most of the antibiotic feed additives in 2006, phytogetic feed additives have become attractive alternatives for use in animal diets to prevent pathogenic microbiota (Griggs and Jacob 2005). Phytogetic feed additives, which are plant-derived products added to non-antibiotic growth promoters, such as essential oils and probiotics, increase the average daily feed intake (ADFI) and average daily gain (ADG) (Holden et al. 2002, Hong et al. 2004, Janz et al. 2007, Yan et al. 2011) thus improving feed efficiency (Valchev et al. 2009) and the immune status (Wang et al. 1998) through their antimicrobial (Aureli et al. 1992, Wenk et al. 2003, Yan et al. 2010) and antioxidant (Tanabe et al. 2002) activities. However, phytoGENICS are a relatively new class of feed additives and our knowledge is still rather limited regarding their modes of action and aspects of their application. Further complications arise because phytogetic feed additives may vary widely with respect to botanical origin, processing and composition. This experiment was conducted to evaluate the effect of a phytogetic-based feed additive on growth performance, nutrient digestibility, blood profiles, fecal noxious gas emission, and intestinal morphology of weaning pigs after dietary challenge with *E. coli* K88.

## Materials and Methods

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

### Animals and Facilities

A total of 120 crossbred pigs [(Yorkshire × Landrace) × Duroc] with an initial body weight (BW) of  $6.09 \pm 0.96$  kg (21 days of age) were assigned randomly to 1 of the 4 dietary treatments based on their sex and BW. Each pen housed 5 pigs (2 gilts and 3 barrows), and there were 6 pens/treatment. All pigs were housed in a temperature and humidity controlled room. The experiment lasted for 6 wks. Each pen was equipped with a 1-sided, stainless-steel self-feeder and a nipple drinker that allowed pigs *ad libitum* access to feed and water. Individual pig BW and feed consumption were recorded at wks 1, 3, and 6 of the experiment to determine average daily gain (ADG), average daily feed intake (ADFI), and gain/feed (G/F) ratio.

## Dietary Treatments

Treatments were as follows: T1, negative control (without antibiotics); T2, T1 + antibiotic (Apramycin 150 ppm in Phase 1 + Tiamulin 39 ppm in Phase 2 and Phase 3); T3, T1 + 0.05% phytoGENICS (FRESTA® F Wean blend of Clove 5%, Cinnamon 3%, Fenugreek 16%, Delacon, Austria); and T4, T1 + 0.2% commercial mix of organic acids. The group IV commercial mixture contained organic acids (60%) including formic acid, lactic acid, fumaric acid and citric acid along with carrier (40%). All nutrients in diets were formulated to meet or exceed the recommendations of NRC (2012) for weaning pigs (Table 1). All diets were grounded through a 1-mm screen in a Wiley mill before analyzing for dry matter (DM), crude protein (CP), gross energy (GE), ash, calcium (Ca), and phosphorus (P) (AOAC 2003).

## Sampling and Measurements

Fecal scores were determined at 08:00 and 20:00 using the following fecal scoring system: 1 hard, dry pellet; 2 firm, formed stool; 3 soft, moist stool that retains shape; 4 soft, unformed stool that assumes shape of container; 5 watery liquid that can be poured. Fecal grab samples were collected randomly from two pigs in each pen (1 gilt and 1 barrow) at wks 1, 3, and 6 of the experiment. Nutrient digestibility was determined on the final day of the experiment and 0.2% chromium oxide ( $\text{Cr}_2\text{O}_3$ ) was added into the diets as an indigestible marker, and after giving chromium diet for 4 days, fresh excreta samples were collected. All fresh excreta samples were dried for 72 hours at 60°C. The digestibility of dry matter (DM), nitrogen (N), energy, ash, Ca, and P was calculated using the Wiley mill method. The chromium was analyzed via UV absorption spectrophotometry (Shimadzu UV-1201, Shimadzu, Tokyo, Japan) following the method described by Williams et al. (1962). At the beginning and the end of 1, 3, and 6 wks, two pigs were randomly chosen from each pen and bled via jugular venipuncture to obtain blood samples. All blood samples were centrifuged for 15 min at  $3000 \times g$  and 4°C to separate the serum, after which the white blood cell (WBC), red blood cell (RBC), and lymphocyte levels were assessed using an automatic biochemistry analyzer (HITACHI 747, Japan). Six pigs per treatment (1 pig per replicate) were orally challenged with 5 ml of skim milk containing  $10^8$  cfu/ml *E. coli* K88 by using a syringe attached to a polyethylene tube at d 15, 16, and 17, and they were slaughtered at the end of the experiment followed by exsanguination for collection of intestinal segments. The intestinal

Table 1. Feed compositions of control diet (as-fed basis).

Item	Phase 1 (d 1 to 7)	Phase 2 (d 7 to 21)	Phase 3 (d 22 to 42)
Ingredient, %			
Extruded corn	29.18	44.49	61.97
Soybean meal (48% CP)	6.94	16.20	25.30
Fermented soybean meal (45% CP)	10.00	5.00	2.50
Fish meal (66% CP, Brazil)	5.00	3.50	–
Soybean oil	3.65	2.55	1.05
Lactose	15.30	8.30	–
Whey	15.00	10.00	5.00
MCP	1.45	–	–
DCP	–	1.5	1.5
Sugar	5.00	3.00	–
Plasma powder (AP 920)	6.00	0.00	–
L-Lys HCl (78%)	0.29	0.39	0.46
DL-Met (50%)	0.32	0.30	0.24
L-Thr (89%)	0.13	0.19	0.20
Choline chloride (25%)	0.20	0.10	0.10
Vitamin premix <sup>1</sup>	0.10	0.10	0.10
Trace mineral premix <sup>2</sup>	0.20	0.20	0.20
Limestone	1.24	0.98	1.13
Salt	–	0.20	0.25
Calculated Nutritional Content:			
ME, kcal/kg	3,640	3,540	3,410
Analysed composition			
Crude protein, %	21.12	20.21	19.05
Lysine, %	1.57	1.48	1.37
Methionine, %	0.65	0.62	0.53
Calcium, %	1.00	0.95	0.90
Available phosphorus, %	0.64	0.53	0.44
Crude fat, %	5.61	5.04	3.99
Crude fiber, %	1.45	1.86	2.45

<sup>1</sup> Provided per kg of complete diet: vitamin A, 11,025 IU; vitamin D<sub>3</sub>, 1,103 IU; vitamin E, 44 IU; vitamin K, 4.4 mg; riboflavin, 8.3 mg; niacin, 50 mg; thiamine, 4 mg; d-pantothenic, 29 mg; choline, 166 mg; and vitamin B<sub>12</sub>, 33 µg.

<sup>2</sup> Provided per kg of complete diet: Cu (as CuSO<sub>4</sub> · 5H<sub>2</sub>O), 12 mg; Zn (as ZnSO<sub>4</sub>), 85 mg; Mn (as MnO<sub>2</sub>), 8 mg; I (as KI), 0.28 mg; and Se (as Na<sub>2</sub>SeO<sub>3</sub> · 5H<sub>2</sub>O), 0.15 mg.

segments were stored in 10% neutral buffered formalin for 24 h, after which they were cut and histological slides were prepared. Three cross sections (5 µm thick) of each intestinal segment were processed in low-melt paraffin and stained with hematoxylin and eosin. Intestinal morphological measurements included the following criteria: villus height, crypt depth, and villus height: crypt depth ratio. These criteria were quantified by using a digitized board coupled to a video monitor receiving output from a video camera mounted on a binocular microscope. The 10 longest and straightest villi and their associated crypts were measured. Mean values of villus height, crypt depth, and villus height: crypt depth ratio within each segment were calculated for statistical analyses.

Fecal ammonia (NH<sub>3</sub>), hydrogen sulphide (H<sub>2</sub>S), total mercaptans and acetic acid emission was measured at wk 6. Three hundred grams of excreta was collected in a plastic box (polyvinyl, W25×L35 cm).

Gas was determined after 5 d fermentation using Gastec gas sampling pumps (Gastec, GV-100S, Japan) during 1 minute.

## Statistical analysis

Data were analyzed by using GLM procedures of SAS (1996), with each pen being used as the experimental unit. The means of the treatments were compared by Duncan's multiple range test (Duncan 1955), with a P<0.05 indicating statistical significance. In addition, the influence of sex on the analyzed traits was performed by two-way analysis of variance.

## Results

The effects of phytogenics on growth performance and fecal score are shown in Table 2 and Table 3,

Table 2. Effects of phytochemicals on growth performance in weaning pigs<sup>1</sup>.

Items	T1	T2	T3	T4	SE <sup>2</sup>	P-value	Male diet Vs supplementation	Female diet Vs supplementation
Phase1 (1-7 d)								
ADG, g	340	336	339	338	7	0.980	0.562	0.991
ADFI, g	368	363	362	362	5	0.784	0.775	0.112
G/F	0.924	0.926	0.937	0.934	0.023	0.976	0.965	0.887
Phase2 (8-21 d)								
ADG, g	432	467	456	433	15	0.303	0.661	0.342
ADFI, g	640	667	658	629	17	0.415	0.551	0.399
G/F	0.675	0.700	0.693	0.688	0.032	0.949	0.999	0.889
Phase3 (22-42 d)								
ADG, g	587	582	610	588	10	0.247	0.110	0.225
ADFI, g	900	890	887	898	24	0.976	0.723	0.899
G/F	0.652	0.654	0.688	0.655	0.022	0.575	0.455	0.511
Overall (1-42 d)								
ADG, g	494 <sup>b</sup>	503 <sup>ab</sup>	514 <sup>a</sup>	495 <sup>b</sup>	5	0.029	0.062	0.074
ADFI, g	725	728	723	719	15	0.975	0.885	0.999
G/F	0.681	0.691	0.711	0.689	0.015	0.606	0.542	0.662

<sup>1</sup> Abbreviation: T1, negative control (without antibiotics); T2, T1 + antibiotic (150ppm apramycin at Phase 1, 39ppm tiamulin at Phase 2 and Phase 3); T3, T1 + 0.05% phytochemicals; T4, T1 + 0.2% commercial mix of organic acids.

<sup>2</sup> Standard error.

a,b Means in the same row with different superscripts differ (P<0.05).

Table 3. Effects of phytochemicals on fecal scores in weaning pigs<sup>1</sup>.

Items	T1	T2	T3	T4	SE <sup>2</sup>	P-value	Male diet Vs supplementation	Female diet Vs supplementation
Fecal score <sup>3</sup>	2.76	2.81	2.77	2.83	0.05	0.732	0.881	0.925

<sup>1</sup> Abbreviation: T1, negative control (without antibiotics); T2, T1 + antibiotic (150ppm apramycin at Phase 1, 39 ppm tiamulin at Phase 2 and Phase 3); T3, T1 + 0.05% phytochemicals; T4, T1 + 0.2% commercial mix of organic acids.

<sup>2</sup> Standard error.

<sup>3</sup> Fecal score. 1 hard, dry pellet; 2 firm, formed stool; 3 soft, moist stool that retains shape; 4 soft, unformed stool that assumes shape of container; 5 watery liquid that can be poured.

respectively. There was no difference (P>0.05) in ADG, ADFI, and G/F ratio between treatments during each phase. Overall, the ADG with the T3 treatment was higher (P<0.05) than that with the T1 and T4 treatments. The fecal score was not affected (P>0.05) by any dietary treatment.

The effects of phytochemicals on nutrient digestibility are shown in Table 4. At wk 1, the ATTD of DM was increased (P<0.05) with T4 treatment compared to that with T1 treatment. The ATTD of ash with T3 and T4 treatments was greater (P<0.05) than that with T2 treatment. At wk 3, pigs fed the T4 diet had significantly higher (P<0.05) ATTD of DM than those fed the T1 and T2 diets. The ATTD of ash and Ca was

significantly increased (P<0.05) with the T4 treatment than with the T1 treatment. Pigs fed the T3 diet had a higher (P<0.05) ATTD of P than those fed the T1 and T4 diets. At wk 6, the ATTD of ash was significantly increased (P<0.05) with the T1 and T3 treatments than with in the T4 treatment. The ATTD of N and energy was unaffected (P>0.05) by dietary treatments throughout the experimental period.

The effects of phytochemicals on blood profiles are shown in Table 5. No difference (P>0.05) was noted in the concentrations of white blood cells (WBC) and red blood cells (RBC), and lymphocyte percentage between dietary treatments throughout the experimental period. The effects of phytochemicals on fecal

Table 4. Effects of phytogenics on nutrient digestibility in weaning pigs<sup>1</sup>.

Items	T1	T2	T3	T4	SE <sup>2</sup>	P-value	Male diet Vs supplementation	Female diet Vs supplementation
1 wk								
DM	81.31b	81.96 <sup>ab</sup>	81.80 <sup>ab</sup>	82.48 <sup>a</sup>	0.28	0.044	0.066	0.061
N	81.14	80.82	80.75	81.13	0.51	0.921	0.889	0.951
Energy	82.86	82.83	82.76	82.88	0.55	0.999	0.911	0.882
Ash	63.10 <sup>ab</sup>	62.22 <sup>b</sup>	64.84 <sup>a</sup>	64.80 <sup>a</sup>	0.63	0.017	0.074	0.063
Ca	43.25	48.96	43.59	43.84	2.67	0.401	0.552	0.814
P	49.04	44.13	47.69	45.03	2.56	0.509	0.411	0.520
3 wk								
DM	81.80 <sup>b</sup>	81.90 <sup>b</sup>	82.35 <sup>ab</sup>	82.70 <sup>a</sup>	0.20	0.005	0.075	0.066
N	80.79	80.77	80.63	81.00	0.34	0.887	0.811	0.901
Energy	84.62	84.31	84.27	84.50	0.18	0.495	0.564	0.521
Ash	64.44 <sup>b</sup>	64.99 <sup>ab</sup>	65.05 <sup>ab</sup>	66.14 <sup>a</sup>	0.49	0.034	0.081	0.065
Ca	41.54 <sup>b</sup>	45.75 <sup>ab</sup>	44.83 <sup>ab</sup>	49.76 <sup>a</sup>	2.13	0.041	0.060	0.077
P	42.36 <sup>b</sup>	46.48 <sup>ab</sup>	49.99 <sup>a</sup>	40.73 <sup>b</sup>	1.91	0.011	0.100	0.992
6 wk								
DM	80.05	80.64	80.22	79.87	0.29	0.308	0.112	0.321
N	78.39	79.29	78.99	78.55	0.41	0.408	0.521	0.422
Energy	80.19	81.19	81.14	80.70	0.33	0.137	0.100	0.201
Ash	64.44 <sup>a</sup>	63.91 <sup>ab</sup>	65.05 <sup>a</sup>	62.64 <sup>b</sup>	0.51	0.020	0.061	0.074
Ca	41.26	47.77	44.22	49.35	3.36	0.347	0.231	0.322
P	45.75	43.31	48.50	46.58	1.70	0.216	0.199	0.211

<sup>1</sup> Abbreviation: T1, negative control(without antibiotics); T2, T1 + antibiotic (150 ppm apramycin at Phase 1, 39 ppm tiamulin at Phase 2 and Phase 3); T3, T1 + 0.05% phytogenics; T4, T1 + 0.2% commercial mix of organic acids.

<sup>2</sup> Standard error.

a,b Means in the same row with different superscripts differ ( $P < 0.05$ ).

Table 5. Effects of phytogenics on blood profiles in weaning pigs<sup>1</sup>.

Items	T1	T2	T3	T4	SE <sup>2</sup>	P-value	Male diet Vs supplementation	Female diet Vs supplementation
WBC, 10 <sup>3</sup> /μl								
Initial	12.58	12.74	12.77	12.58	1.83	0.999	0.886	0.995
1 wk	14.89	15.42	15.03	14.93	1.56	0.995	0.991	0.856
3 wk	14.31	14.82	14.93	14.34	1.48	0.986	0.886	0.990
6 wk	19.24	20.29	20.47	20.65	1.71	0.937	0.788	0.995
RBC, 10 <sup>6</sup> /μl								
Initial	5.90	5.97	6.04	5.97	0.14	0.933	0.885	0.997
1 wk	6.01	6.10	6.06	6.03	0.14	0.971	0.995	0.871
3 wk	5.90	5.91	5.93	5.91	0.10	0.996	0.884	0.774
6 wk	6.27	6.48	6.41	6.54	0.21	0.825	0.880	0.952
Lymphocyte, %								
Initial	58.27	58.88	59.48	58.57	2.12	0.980	0.955	0.966
1 wk	57.00	59.30	59.80	57.10	2.45	0.787	0.655	0.881
3 wk	56.08	57.32	57.07	56.38	2.07	0.971	0.995	0.663
6 wk	54.52	57.98	58.32	57.15	4.50	0.930	0.999	0.846

<sup>1</sup> Abbreviation: T1, negative control(without antibiotics); T2, T1 + antibiotic (150 ppm apramycin at Phase 1, 39 ppm tiamulin at Phase 2 and Phase 3); T3, T1 + 0.05% phytogenics; T4, T1 + 0.2% commercial mix of organic acids.

<sup>2</sup> Standard error.

noxious gas emission are shown in Table 6. Dietary supplementation with phytogenics had no effect ( $P > 0.05$ ) on the fecal NH<sub>3</sub>, H<sub>2</sub>S, total mercaptans, and acetic acid emission. The effects of phytogenics on

intestinal morphology are shown in Table 7. The villi height, crypt depth, and villi/crypt ratio of the small intestine were not affected ( $P > 0.05$ ) by dietary treatments at the end of the experiment.

Table 6. Effects of phytoGENICS on fecal noxious gas emission in weaning pigs<sup>1</sup>.

Items	T1	T2	T3	T4	SE <sup>2</sup>	P-value	Male diet Vs supplementation	Female diet Vs supplementation
NH <sub>3</sub>	24.2	20.0	17.9	21.5	2.5	0.380	0.211	0.166
Total mercaptans	8.7	8.0	7.8	8.2	0.8	0.870	0.998	0.854
H <sub>2</sub> S	6.4	5.9	5.9	6.1	0.4	0.742	0.778	0.655
Acetic acid	2.9	2.5	2.2	2.6	0.4	0.674	0.586	0.741

<sup>1</sup> Abbreviation: T1, negative control(without antibiotics); T2, T1 + antibiotic(150ppm apramycin at Phase 1, 39ppm tiamulin at Phase 2 and Phase 3); T3, T1 + 0.05% phytoGENICS; T4, T1 + 0.2% commercial mix of organic acids.

Table 7. Effects of phytoGENICS on intestinal morphology in weaning pigs<sup>1</sup>.

Items	T1	T2	T3	T4	SE <sup>2</sup>	P-value	Male diet Vs supplementation	Female diet Vs supplementation
Villi height, $\mu$ m	619	632	670	606	53	0.874	0.778	0.894
Crypt depth, $\mu$ m	463	457	451	463	20	0.954	0.655	0.788
Villi/Crypt ratio	1.34	1.39	1.49	1.31	0.16	0.886	0.995	0.961

<sup>1</sup> Abbreviation: T1, negative control(without antibiotics); T2, T1 + antibiotic (150 ppm apramycin at Phase 1, 39 ppm tiamulin at Phase 2 and Phase 3); T3, T1 + 0.05% phytoGENICS; T4, T1 + 0.2% commercial mix of organic acids.

<sup>2</sup> Standard error.

## Discussion

The data indicate that phytoGENICS positively affect growth performance of weaning pigs. The negative effect of challenge with *E. coli* K88 was not more evident because of comparably better conditions than those in commercial farms and partial challenge among the experimental pigs. Nonetheless, given the antimicrobial trait of the phytoGENICS, there is no doubt that they act on harmful bacteria such as *E. coli* and therefore they may be a viable alternative to Animal Growth Promoters (AGP). Yan et al. (2012) observed that dietary supplementation of *Taraxacum officinale*, a medical herb, significantly increased ( $P<0.05$ ) the ADG and G:F in weaning pigs. In the study by Li et al. (2012), the addition of EO containing thymol and cinnamaldehyde at 100 and 150 g/tonne improved ( $P<0.05$ ) the daily weight gain and feed conversion ratio (FCR) of weaner pigs. Likewise, Ouwehand et al. (2010) reported that the mixture of thymol and cinnamaldehyde would have the best potential to control the proliferation of pathogenic bacteria and contribute to better gut health. However, Janz et al. (2007) found that growth performance of pigs was not affected by essential oils. The main mode of action of growth-promoting feed additives results from stabilizing feed hygiene, and even more from beneficially influencing the environment of gastrointestinal microbiota through the control of latent pathogens (Roth and Kirchgessner 1998). This applies especially to crucial stages of an animal's production

cycle characterized by high susceptibility to digestive disorders, such as the weaning phase of piglets. Hayden et al. (1998) proposed that psyllium improves enterotoxic *E. coli* (ETEC)-induced diarrhea and hampers the enhanced secretory responses to calcium-mediated agonists that occur in ETEC-infected piglet jejunum. However, there was no difference between treatments in fecal score despite the challenge with *Escherichia coli* K88 in this study.

The improved digestive capacity of the small intestine may be considered to be an indirect side effect of feed additives stabilizing the microbial eubiosis in the gut. Such an effect has been observed in young pigs with antibiotic feed additives (Roth et al. 1999) and in broilers and swine fed with plant extracts (Jamroz et al. 2003). Cho et al. (2006) reported that the digestibility of DM and nitrogen in the weaning pigs was significantly different when compared with that in the negative control group. Regarding an improvement in nutrient digestibility with the dietary supplementation of *Taraxacum officinale* in weaning pigs, Yan et al. (2012) found that the digestibility of energy was significantly ( $P<0.05$ ) increased than that with the control treatment. In this study, mere improvement ( $P<0.05$ ) in the digestibility of P at wk 3 and numerical increase in the digestibility of ash at wk 6 with the phytoGENICS were noted.

No difference was noted in the concentrations of white blood cells (WBC) and red blood cells (RBC), and lymphocyte percentage between dietary treatments. According to Cho et al. (2006), no statistically

significant differences ( $P > 0.05$ ) were found in lymphocyte counts, total protein, and albumin, but RBC count, WBC count, and serum IgG concentration with the AGP + blended EO treatment were greater than those with the negative control ( $P < 0.05$ ). Wang et al. (1998) observed that eugenol improved immune ability by increasing the synthesis of Immunoglobulin G (IgG) in body and the synthesis of immunoglobulins A (IgA) in saliva. Essential oils improved the immune response by increasing phagocytosis. However, in this study, there was no effect of phytochemicals on the blood profiles.

Dietary supplementation with phytochemicals had no effect on the fecal  $\text{NH}_3$ ,  $\text{H}_2\text{S}$ , total mercaptans, and acetic acid emission. Ushid et al. (2002) found that ammonia concentration in feces was significantly reduced by addition of herb extracts. Sutton et al. (1992) reported that ammonia emission was decreased by 55.5% in manure from pigs fed sarsaponin extract. Also, Cho et al. (2006) observed that  $\text{NH}_3\text{-N}$  concentration in pigs fed AGP + blended EO diet was significantly lower ( $P < 0.05$ ) than that in pigs fed other diets and  $\text{H}_2\text{S}$  concentration in pigs fed diets with added EOs was significantly lower ( $P < 0.05$ ) than that in pigs fed other diets.

The villi height, crypt depth, and villi/crypt ratio in the phytochemical group were greater than those in any other groups. Michiels et al. (2011) showed that dietary supplementation of carvacrol and thymol in piglets reduced the number of intra-epithelial lymphocytes, and the ratio of villus height/crypt depth in the distal small intestine was higher. A change in morphological parameters, such as villus height, crypt depth, or number of goblet cells, was observed in several studies when birds were fed diets with supplemental phytochemicals (Demir et al. 2005, Jamroz et al. 2006). The analysis for sex and diet versus supplementation was done, but the values obtained were not significant in any of the analyzed traits. Since the animals were recruited for the experiment at the very early stage of weaning with an initial BW of  $6.09 \pm 0.96$  kg (21 day of age), the analyzed traits were not affected by sex.

Dietary phytochemicals could be used to replace AGP in the diets of weaning pigs for significantly improving the ADG without any negative effects on growth performance under challenge with *E. coli* K88 compared with AGP and organic acids. The nutrient digestibility with phytochemical treatment has been established by many scientists. However, there was no significant difference except for P digestibility at wk 3 in the study. The intestinal morphology of weaning pigs fed with phytochemicals was the best among treatments but it was not significant. Regarding blood profiles and fecal noxious gas emission, there were no significant differences.

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