



Effect of Eugenol and Cinnamaldehyde on the Growth Performance, Nutrient Digestibility, Blood Characteristics, Fecal Microbial Shedding and Fecal Noxious Gas Content in Growing Pigs

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ABSTRACT: A 5-wk trial with 96 ((Landrace×Yorkshire)×Duroc) pigs (BW = 26.56±0.42 kg) was conducted to investigate the effect of eugenol and cinnamaldehyde as feed additive in growing pigs. Pigs were assigned to 1 of 3 treatments in a randomized complete block design according to their sex and BW. Each treatment contained 8 replications with 4 pigs (2 gilts and 2 barrows) per pen. Treatments included: control (basal diet; CON); (basal diet+1,000 mg eugenol/kg; ET); (basal diet+1,000 mg cinnamaldehyde/kg; CT). Administration of eugenol and cinnamaldehyde did not affect ($p>0.05$) the growth performance and apparent total tract digestibility. Dietary CT and ET led to a higher ($p<0.05$) lymphocyte concentration compared with CON. The inclusion of CT and ET decreased ($p<0.05$) the fecal *E. coli* concentration ($p>0.05$). Pigs fed the diets supplemented with eugenol and cinnamaldehyde had reduced ($p<0.05$) NH₃ and H₂S concentration throughout the experiment. In conclusion, results obtained in the present study indicated that supplementation of eugenol and cinamaldehyde had no effect on growth performance of pigs but exhibited lymphocyte-enhancing activity and decreased the fecal *E. coli* concentration and fecal noxious gas content (NH₃ and H₂S). (**Key Words:** Cinamaldehyde, Eugenol, Herb, Pigs)

INTRODUCTION

Recently, phytogetic feed additives have been widely used as an antibiotics alternative due to their plant derived property and growth-promoting effects (Wang et al., 2007; Wang et al., 2008; Ao et al., 2011). Among these, essential oils (EO) are generally referred as volatile or aromatic oily liquids acquired from plant materials, which have been well documented because of its stimulate effect on appetite and secretion of digestive enzymes (Wenk, 2003; Cho et al., 2006; Huang et al., 2010; Yan et al., 2010; Yan et al., 2011a, b; Yan et al., 2012).

Eugenol is an important chemical constituent in essential oils of many aromatic plants, such as *Corton zehntneri* and *Occimum gratissimum*, which have been used in folk medicine for a long time (Craveiro et al., 1977). Cinnamaldehyde is the major component comprising 85% in the essential oils of *Cinnamomum verum* and *Cinnamomum Cassia*, which is well known as a traditional

medicine herb worldwide (Ooi et al., 2006). Recently, both eugenol and cinnamaldehyde have gained renewed interest for application in animal feed because of its antimicrobial properties (Michiels et al., 2005; Michiels et al., 2007; Michiels et al., 2010). In those studies, they demonstrated that eugenol and cinamaldehyde could inhibit the *E. coli* and coliform bacteria in the pig gut flora, and showed clearly less inhibitory activity toward lactobacilli *in vitro*. Therefore, it is logical to conclude that eugenol and cinnamaldehyde in the small intestine can result in a shift in the microbial ecology in favor of lactic acid producing bacteria and reducing the number of pathogenic bacteria, which offers an alternative to antibiotics to maintain gut health and performance of young animals.

However, to the best of our knowledge, the effects of the eugenol and cinnamaldehyde have not yet been vigorously evaluated in pigs. Thus, our study was conducted to assess the effect of eugenol and cinnamaldehyde on growth performance, nutrient digestibility, blood characteristics and fecal microbial shedding and noxious gas content in growing pigs.

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MATERIALS AND METHODS

The experiment was conducted at the Experimental Unit of the Dankook University (Anseodong, Cheonan, Choongnam, Korea). The protocol for the current experiment was approved by the Animal Care and Use Committee of Dankook University.

Experimental design, animals, housing and diets

A total of 96 growing pigs of mixed sex (initial BW: 26.56±0.42 kg) were randomly allotted to 3 treatments on the basis of BW. There were 8 replicates in each treatment with 4 pigs (2 gilts and 2 barrows per replicate per replicate). Dietary treatments were basal diet without any additive (CON), basal diet+1,000 mg eugenol/kg (ET); basal diet+1,000 mg cinnamaldehyde/kg (CT). Experimental diets were fed for 5 wks. Diets used in this experiment were formulated to meet or exceed NRC (1998) recommendations for all nutrients (Table 1). Additive was added to the diet by replacing the same amount of corn. Pigs were housed in an environmental controlled, slatted-floor facility in 25 adjacent pens and were allowed *ad libitum* access to feed and water through a self-feeder and nipple drinker throughout the experimental period.

Sampling and measurements

Body weight was measured at the beginning and the end (wk 5) of the experimental period, and feed consumption was recorded on a pen basis during the experiment to calculate the average daily gain (ADG), average daily feed intake (ADFI) and gain to feed ratio (G:F). To evaluate the effect of dietary treatments on ATTD of nutrients, 0.20% chromic oxide (an inert and indigestible indicator) was included in each diet for 7 days prior to fecal collection during 6th week. Pooled fecal grab samples were collected at random from 1 gilt and 1 barrow in each pen. All feed and feces samples were stored immediately at -20°C until analysis. Fecal samples were freeze-dried and finely ground to pass through a 1-mm screen. Analysis of DM, N and gross energy content of experimental diets and excreta was done according to AOAC (2000). Chromium concentration was determined with an UV absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan) and the ATTD of DM, N and energy were calculated using indirect methods as described by Fenton and Fenton (1979).

For the analysis of blood profiles, 2 pigs from each pen were randomly selected and bled by anterior vena cava puncture at the beginning of the experiment and wk 5. Blood samples of the pig were collected into vacuum tubes containing K₃EDTA (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) to obtain blood, after which serum samples were centrifuged (2,000×g) for 30 min at 4°C. The red blood cells (RBC), white blood cells (WBC) and

Table 1. Composition of the basal experimental diets (as-fed basis)³

Items	Content
Ingredients (g/kg)	
Corn	553.8
Soybean meal	334.3
Molasses	25.0
Animal fat	53.3
Difluorinated phosphate	19.3
Limestone	7.8
L-lysine HCl	1.7
Trace mineral premix ¹	1.0
Vitamin premix ²	1.2
Salt	2.0
DL-methionine	0.3
Choline chloride	0.3
Chemical composition	
ME (kcal/kg)	3,360
Crude protein (%)	20.00
Lysine (%)	1.30
Calcium (%)	0.90
Phosphorus (%)	0.80
Analyzed composition	
GE (kcal/kg)	4,112
Crude protein (%)	19.65
Calcium (%)	0.87
Phosphorus (%)	0.76

¹ Provided per kg of complete diet: 12.5 mg Mn, 179 mg Zn, 140 mg Cu, 0.5 mg I and 0.4 mg Se.

² Provided per kg of complete diet: 20,000 IU of vitamin A; 4,000 IU of vitamin D₃; 80 IU of vitamin E; 16 mg of vitamin K₃; 4 mg of thiamine, 20 mg of riboflavin; 6 mg of pyridoxine; 0.08 mg of vitamin B₁₂; 120 mg of niacin; 50 mg of Ca-pantothenate; 2 mg of folic acid and 0.08 mg of biotin.

³ The additive was included in the diets by replacing the same amount of corn.

lymphocyte counts of the whole blood samples were determined using an automatic blood analyzer (ADVIA 120, Bayer, Tarrytown, NY, USA).

At d 35, fecal samples were collected via massaging the rectum from 2 pigs randomly selected from each pen (1 gilt and 1 barrow) and pooled and placed on ice for transportation to the laboratory, where analysis was immediately carried out. The composite fecal sample (1 g) from each pen was diluted with 9 ml of 1% peptone broth (Becton, Dickinson and Co.) and homogenized. Viable counts of bacteria in the fecal samples were then conducted by plating serial 10-fold dilutions (in 1% peptone solution) onto MacConkey agar plates (Difco Laboratories, Detroit, MI, USA) and *Lactobacilli* medium III agar plates (Medium 638, DSMZ, Braunschweig, Germany) to isolate the *E. coli* and *Lactobacillus*, respectively. The *Lactobacilli* medium III agar plates were then incubated for 48 h at 39°C under anaerobic conditions. The MacConkey agar plates were

Table 2. The effects of dietary eugenol and cinnamaldehyde on growth performance in finishing pigs

Items	CON ^a	ET ^a	CT ^a	SE ^b	p-value
Initial BW (kg)	26.56	26.48	26.64	0.42	
Final BW (kg)	49.80	49.93	50.30	0.95	0.423
ADG (g)	664	670	676	8.23	0.606
ADFI (g)	1,692	1,706	1,746	110.2	0.937
FCR	0.400	0.408	0.392	0.025	0.903

^a CON = Basal diet; ET = CON+eugenol 1 g/kg; CT = CON+cinnamaldehyde 1 g/kg. ^b Pooled standard error.

incubated for 24 h at 37°C. The *E. coli* and *Lactobacillus* colonies were counted immediately after removal from the incubator.

For the analysis of the fecal NH₃ and H₂S, fresh feces were collected from 2 pigs in each pen on the last 2 d of the experiment. The total sampled feces was then thawed and homogenized, after which the stock feces were stored in 2.6-L plastic boxes with a small hole in the middle of one side that was sealed with adhesive plaster. The samples were allowed to ferment for 1 d at room temperature (25°C), after which 100 ml of the headspace air was sampled from approximately 2.0 cm above the fecal sample. Concentration of NH₃ and H₂S were measured within the scope of 5.0 to 100.0 ppm (No. 3La, detector tube; Gastec Corp. Kanagawa, Japan) and 2.0 to 20.0 ppm (4LK, detector tube; Gastec Corp.). After collection, box was resealed with adhesive plaster to measure the fecal noxious content at d 3 and 5 as aforementioned. Prior to measurement, the fecal samples were manually shaken for approximately 30 s to disrupt any crust formation on the surface of the fecal sample and to homogenize the samples.

Statistical analyses

All data were statistically analyzed by ANOVA using the GLM procedure of SAS as a randomized complete block design (SAS Inst. Inc., Cary, NC, USA), with the pen serving as the experimental unit. The initial BW was used as a covariate for ADG and ADFI, as well as the blood profile. The difference among treatments was compared using the fisher's LSD test when the treatment effect was observed with the alpha level of 0.05.

RESULTS

Growth performance and nutrient digestibility

Growth performance and ATTD of nutrients in pigs were not different ($p>0.05$) among dietary treatments (Tables 2 and 3).

Blood characteristics

Pigs fed CT and ET diets had higher ($p<0.05$) lymphocyte concentration than pigs fed CON diet (Table 4). However, there were no effects ($p>0.05$) of any dietary

Table 3. The effects of dietary eugenol and cinnamaldehyde on nutrient digestibility in finishing pigs

Items (%)	CON ^a	ET ^a	CT ^a	SE ^b	p-value
DM	82.53	82.83	83.27	1.621	0.946
N	84.43	82.13	83.73	1.426	0.525
Energy	81.78	82.60	82.93	1.764	0.895

^a CON = Basal diet; ET = CON+eugenol 1 g/kg; CT = CON+cinnamaldehyde 1 g/kg.

^b Pooled standard error.

treatment on other blood parameter evaluated in present study.

Fecal microbial shedding and fecal noxious gas content

Pigs fed the ET and CT diets had decreased ($p<0.05$) fecal *E. coli* concentration than pigs fed the CON diet (Table 5). However, there were no effects ($p>0.05$) of dietary treatments on fecal lactobacilli concentration. Pigs fed the CT and ET diets had decreased ($p<0.05$) NH₃ and H₂S concentration throughout experiment than pigs fed the CON diet (Table 6).

DISCUSSION

Growth performance and nutrient digestibility

In this study, the inclusion of eugenol and cinnamaldehyde did not affect the growth performance throughout the experiment, which is in line with our

Table 4. The effects of dietary eugenol and cinnamaldehyde on blood characteristics in finishing pigs

Items	CON ^a	ET ^a	CT ^a	SE ^b	p-value
RBC (10 ⁶ /μl)					
Initial	6.62	6.50	6.50	0.173	0.452
Final	7.49	7.37	7.35	0.185	0.842
WBC (10 ³ /μl)					
Initial	13.98	14.78	13.58	1.021	0.581
Final	19.7	19.2	19.6	1.094	0.943
Lymphocyte (%)					
Initial	47.56	51.28	49.96	3.072	0.811
Final	55.34 ^c	62.54 ^d	66.58 ^d	2.864	0.008

^a CON = Basal diet; ET = CON+eugenol 1 g/kg; CT = CON+cinnamaldehyde 1 g/kg.

^b Pooled standard error.

^{c,d} Means the significant difference among the same row ($p<0.05$).

Table 5. The effects of dietary eugenol and cinnamaldehyde on fecal microbial shedding in finishing pigs

Items (%)	CON ^a	ET ^a	CT ^a	SE ^b	p-value
<i>E. coli</i>	7.54 ^c	6.35 ^d	6.14 ^d	0.285	<0.0001
<i>Lactobacilli</i>	6.83	7.25	7.14	0.309	0.243

^a CON = Basal diet; ET = CON+eugenol 1 g/kg; CT = CON+cinnamaldehyde 1 g/kg.

^b Pooled standard error.

^{c,d} Means the significant difference among the same row (p<0.05).

previous study (Ao et al., 2011), who suggested that pigs fed the herb extract did not affect the growth performance in finishing pigs. Previously, it has been suggested that essential oils could increase the flavor and palatability of feed by stimulating the appetite of the animals (Wenk, 2003). Several studies also suggested that essential oils could improve the growth performance by increasing the nutrient digestibility because of its beneficial effect on the enzymes secretions and gut health (Cho et al., 2006; Yan et al., 2010). Therefore, it is suitable to suggest that dietary eugenol and cinnamaldehyde supplementation may affect the feed intake and feed efficiency in the current study. However, our results suggested that no significant difference was observed on the feed intake and the nutrient digestibility, indicating the eugenol and cinnamaldehyde supplementation cannot affect the growth performance and nutrient digestibility in the growing pigs. It should be noted that a well developed digestive system, improved immunity and increased resistance to intestinal disorder in older pigs may also result in the absence of increased growth performance and nutrient digestibility (Nousiainen and Setälä, 1993; Ao et al., 2011). Therefore, the increased digestive system and immunity of growing pigs may be considered as a reason for the lack of significant difference in this study.

Blood characteristics

In this study, pig fed the ET and CT treatments led to a greater lymphocyte count compared with NC treatment, which is in agreement with our recent study (Yan et al., 2011a; Yan et al., 2012), in which the inclusion of herbal extract increased the lymphocyte count in growing pigs. Previous studies have reported that the gastrointestinal system and its associated lymphoid are the largest immunologically competent organ in the body, and indicated that maturation of gastrointestinal and optimal development of the associated lymphoid depend on the composition of the indigenous microflora (Insoft et al., 2005; Michael and Marteau, 2007). Therefore, a benefited indigenous microflora environment in pigs may promote the development of gastrointestinal and its associated lymphoid. Previously, Koh et al. (1998) had suggested that the inclusion of Cinnamaldehyde could reduce the negative

Table 6. The effects of dietary eugenol and cinnamaldehyde on noxious gas content in feces of finishing pigs

Items	CON ^a	ET ^a	CT ^a	SE ^b	p-value
NH ₃					
d 1	0.74 ^c	0.35 ^d	0.42 ^d	0.13	0.017
d 3	4.8 ^c	2.1 ^d	2.9 ^d	0.92	0.021
d 5	10.1 ^c	5.3 ^d	6.9 ^d	1.29	0.014
H ₂ S					
d 1	5.2 ^c	3.3 ^d	3.0 ^d	1.02	0.036
d 3	8.4 ^c	5.6 ^d	5.3 ^d	1.31	0.012
d 5	9.6 ^c	6.1 ^d	5.8 ^d	1.62	0.007

^a CON = Basal diet; ET = CON+eugenol 1 g/kg; CT = CON+cinnamaldehyde 1 g/kg.

^b Pooled standard error.

^{c,d} Means the significant difference among the same row (p<0.05).

effect of LPS challenge in moun. Michiels et al. (2007) and Michiels et al. (2005) also demonstrated that eugenol and cinamaldehyde could inhibit the *E. coli* and coliform bacteria in pig gut *in vitro*, and showed less inhibitory effect on the growth of *lactobacilli*. Therefore, we hypothesized the shift microbial ecology may promote the gut health and its associated lymphoid, which subsequently enhance the lymphocyte proliferation in pigs. Moreover, our results suggested that pigs fed the ET and CT treatment decreased the fecal *E. coli* concentration compared with the CON group, which confirmed the inhibitory effect of eugenol and cinamaldehyde on the *E. coli* (Michiels et al., 2007). Therefore, the effect of eugenol and cinamaldehyde on the fecal microbial shedding may reflect the beneficial effect of ET and CT on the gut health in the current study and explain the enhanced lymphocyte proliferation in this study.

Fecal noxious gas content

Previously, several studies have suggested that administration of the essential oils could decrease the fecal noxious gas content in pigs such as NH₃ and H₂S concentration, which are the main components of pig manure that contributing to the air pollution (Zahn et al., 1997). For example, Yan et al. (2010) have demonstrated that essential oils supplementation can decrease the fecal noxious gas content in finishing pigs by increasing the nutrient digestibility. Wenk (2003) also suggested that the inclusion of herbs or herbs extract could reduce the fecal noxious gas content in growing pigs by manipulating the microflora in the gastrointestinal tract of pigs. In the present study, pig fed the ET and CT treatment led to a NH₃ and H₂S concentration compared with NC group throughout the experiment. It should be noted that the inclusion of ET and CT did not affect the nutrient digestibility in the present study, whereas the fecal *E. coli* were decreased by the dietary supplementation. Therefore, we hypothesized that the reason for the reduction in fecal noxious gas content

may not be the results of increased nutrient digestibility, but the results of benefited microflora in the gastrointestinal tract of pigs. This conclusion is supported by Visek (1978) and Ferket et al. (2002), who have demonstrated that the ultimately fecal noxious gas emission of animals is related to intestinal microflora, especially the harmful intestinal bacteria populations. Moreover, Varel and Miller (2004) had previously suggested that eugenol supplementation stimulated the lactate accumulation and decrease the pH value in swine slurry. They also suggested a rapid decreased (with 4 d) pH value could also inhibit the ammonia emission from swine waste. Thus, the accumulated lactate results from eugenol may be considered as another reason for the reduced ammonia emission in this study.

CONCLUSION

In conclusion, results obtained in the present study indicated that supplementation of eugenol and cinnamaldehyde had no effect on growth performance of pigs but exhibited lymphocyte-enhancing activity and decreased the fecal *E. coli* concentration and fecal noxious gas content (NH₃ and H₂S).

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