Growth performance, nutrient digestibility, blood lipid profile and faecal Escherichia coli and Lactobacillus counts on growing pigs fed with de-oiled lecithin emulsifier

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ABSTRACT
The aim of this study was to determine the effect of dietary supplementation of de-oiled lecithin (DOL) emulsifier on growth performance, nutrient digestibility, blood lipid profile and faecal Escherichia coli (E. coli) and Lactobacillus count in growing pigs. A total of 75 crossbred growing pigs [(Landrace × Yorkshire) × Duroc] with an average initial body weight (BW) of 24.97 ± 1.42 kg (54 days of age) were used in a 6-week feeding trial. Pigs were randomly allotted to one of three treatments [five pigs per pen (three barrows and two gilts); five pens per treatment] based on BW and sex. Treatments consisted of CON, basal diet; TRT1 basal diet + 0.1% DOL – 60; TRT2 basal diet + 0.1% DOL – 97. The DOL – 60 and DOL – 97 contents were 60% and 97% of DOL, respectively. Results indicated that the increased contents of DOL lead to higher average daily gain, gain-to-feed ratio, digestibility of dry matter, nitrogen and gross energy and faecal Lactobacillus counts. Meanwhile, blood triglyceride and total cholesterol were trended to reduce. In conclusion, the supplementation of DOL in growing pig diets could improve the growth performance, nutrient digestibility and intestinal Lactobacillus count, thereby contributing to improved growth rate.

HIGHLIGHTS
- Dietary supplementation of DOL improved growth performance and nutrient digestibility of growing pigs.
- Dietary supplementation of DOL modulated gut health by altering intestine Lactobacillus count of growing pigs.
- Dietary supplementation of DOL improved health status through decreasing blood triglyceride and total cholesterol of growing pigs.

Introduction
The modern livestock husbandry, especially pigs, needs diets with high energetic concentrations to meet their nutritional requirements. Fats and oils have been incorporated in commercial diets as the most valuable method to achieve these requirements. Digestibility and absorption of lipids are critical to ensure the maximum potential growth of pigs. Emulsification of dietary lipid occurs under the action of bile salts and lipase as endogenous emulsifiers in small intestine (Gu and Li 2003). It was reported that many factors could influence lipids digestion including animal age, genetic, lipase activity and microbiota status and diet composition (Tancharoenrat et al. 2013, 2014; Zampiga et al. 2016). Thus, exogenous emulsifier supplementation as a kind of nutritional bioactive additives may be helpful in growing pigs.

Lecithin, an excellent emulsifier, was first isolated in 1845 by the French chemist (Gobley 1846). It is usually available from sources such as soybeans, eggs, milk and marine sources. The main phospholipids of commercial soybean derived lecithin are: 20–21% of inositol phosphatides; 19–21% of phosphatidylcholine; 8–20% phosphatidylethanolamine and 5–11% other phosphatides (Scholfield 1981). By modifying or combining these phospholipid molecules, desirable behaviour such as emulsification can be achieved (Gu and Li 2003). It was also reported that for a better nutrient absorption and utilisation, emulsifier could help to lower the surface tension, increase the concentration

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of monoglycerides in the intestine and promote the nutrient transport through the membrane (Aguilar et al. 2013). Previous studies have reported the positive effects of additional lecithin as emulsifier on digestibility and utilisation of nutrients (Xing et al. 2004; Smulders 2009). Recently, Attia et al. (2018) investigated that supplementation with 1% or 1.5% soya lecithin improved rabbit growth performance and fat digestibility in both summer and winter. Additionally, Akit et al. (2018) reported that lecithin improved feed efficiency in finishing pigs without impacting pork quality.

It was hypothesised that dietary supplementation of de-oiled lecithin (DOL) as exogenous emulsifier may be helpful in emulsification, consequently improving the nutrient absorption and growth of growing pigs. Therefore, in order to extend these previous studies and provide new evidence for the use of emulsifier in growing pigs, the purpose of the present study was to investigate the effect of dietary supplementation of DOL as emulsifier on growth performance, nutrition digestibility, blood lipid profiles and faecal Escherichia coli and Lactobacillus count in growing pigs.

Materials and methods

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

Preparation of de-oiled lecithin

The de-oiled lecithin used in the current experiment is a commercial product manufactured by company (FeedBEST CO., LTD., Cheonan, Korea). The commercial DOL powder was obtained from soybean oil. The DOL contents in DOL–60 and DOL–97 were 61.80% and 97.16%, respectively. The active phospholipid composition of the product is presented in Table 1.

Experimental design, animals, diets and housing

A total of 75 female and castrated male crossbred growing pigs ([Landrace × Yorkshire] × Duroc; 54 days of age) with an average initial body weight (BW) of 24.97 ± 1.42 kg were used in a 6-week feeding trial. Pigs were randomly allotted to three treatments [five pigs per pen (three barrows and two gilts); five pens per treatment] based on BW and sex. Treatments were: (1) CON (basal diet), (2) TRT1 (basal diet + 0.1% DOL–60) and (3) TRT2 (basal diet + 0.1% DOL–97). All diets were formulated to meet or exceed the NRC (2012) requirement for growing pigs (Table 2). Throughout the experiment, all pigs were provided with ad libitum access to feed and water through a self-feeder and nipple drinker, respectively. Target room temperature and humidity were 25 °C and 60%, respectively.

Sampling and measurements

Pigs were weighed on a pen basis initially and finally, and feed consumption was recorded throughout the

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Table 1. Phospholipid composition of DOL-60 and DOL-97.

| Items (%)        | DOL-60 | DOL-97 |
|------------------|--------|--------|
| Phosphatidyl choline | 31.71  | 38.24  |
| 1-lysophosphatidyl choline | 0.25   | 0.53   |
| 2-lysophosphatidyl choline | 2.53   | 3.33   |
| Phosphatidyl inositols | 21.04  | 21.75  |
| Phosphatidyl serines-Na | 1.03   | 0.91   |
| Phosphatidyl ethanolamines | 1.44   | 2.15   |
| Lysophosphatidyl ethanolamines | 4.77   | 1.22   |
| Phosphatidyl glycerol | 1.97   | 1.83   |
| Diphosphatidyl glycerols | 1.74   | 1.12   |
| Phosphatidic acid | 9.67   | 5.39   |
| Lysophosphatic acid | 0.53   | 0.57   |
| Other phosphatidyl inositols | 0.83   | 2.50   |

DOL: De-oiled lecithin.

Table 2. Ingredient composition of experimental diets (as-fed basis).

| Items (%) | CON          | TRT1         | TRT2         |
|-----------|--------------|--------------|--------------|
| Ingredient (%) |         |              |              |
| Corn       | 60.01        | 60.01        | 60.01        |
| Soybean meal (45% CP) | 16.07 | 16.07 | 16.07 |
| DDGSa      | 6.50         | 6.50         | 6.50         |
| Rapeseed meal | 2.50      | 2.50         | 2.50         |
| Wheat      | 6.00         | 6.00         | 6.00         |
| Tallow     | 3.00         | 3.00         | 3.00         |
| Molasses   | 3.00         | 3.00         | 3.00         |
| Dicalcium phosphate | 1.08 | 1.08 | 1.08 |
| Limestone  | 0.65         | 0.65         | 0.65         |
| Salt       | 0.30         | 0.30         | 0.30         |
| L-Lysine   | 0.19         | 0.19         | 0.19         |
| Vitamin premixb | 0.20   | 0.20         | 0.20         |
| Mineral premixc | 0.10    | 0.10         | 0.10         |
| Choline chloride | 0.04 | 0.04         | 0.04         |
| DOL-60     |              | –            | 0.10         |
| DOL-97     | –            | –            | 0.10         |
| Calculated value (%) |         |              |              |
| MEa (kcal/kg) | 3428        | 3428         | 3428         |
| Crude protein | 15.50       | 15.50        | 15.50        |
| Crude fat  | 5.78         | 5.78         | 5.78         |
| Crude fibre | 3.43         | 3.43         | 3.43         |
| Crude ash  | 4.59         | 4.59         | 4.59         |
| Calcium    | 0.65         | 0.65         | 0.65         |
| Phosphorus | 0.55         | 0.55         | 0.55         |

CON, Basal diet; TRT1, Basal diet + 0.1% DOL-60; TRT2, Basal diet + 0.1% DOL-97.

DDGS: distillers dried grains with soluble; ME: metabolisable energy.

bProvided per kg of complete diet: 11,025 IU vitamin A; 1,103 IU vitamin D3; 11 IU vitamin E; 4.4 mg vitamin K; 8.3 mg riboflavin; 50 mg niacin; 4 mg thiamine; 29 mg d-pantothenic; 166 mg choline; 33 μg vitamin B12.

cProvided per kg of complete diet: 44 IU vitamin A; 1,103 IU vitamin D3; 11 IU vitamin E; 4.4 mg vitamin K; 8.3 mg riboflavin; 50 mg niacin; 4 mg thiamine; 29 mg d-pantothenic; 166 mg choline; 33 μg vitamin B12.

Provided per kg of complete diet: 8 mg of Mn (as MnO2); 60 mg of Zn (as ZnSO4); 5 mg of Cu (as CuSO4·5H2O); 40 mg of Fe (as FeSO4·7H2O); 0.3 mg of Co (as CoSO4·5H2O); 1.5 mg of I (as KI); and 0.15 mg of Se (as Na2SeO3·5H2O).
experiment. Average daily gain (ADG), average daily feed intake (ADFI) and gain-to-feed ratio (G/F) were then calculated. From Days 35 to 42, pigs were fed diets mixed with 0.2% chromic oxide as an indigestible marker to determine apparent total tract digestibility (ATTD) of dry matter (DM), nitrogen (N) and gross energy (GE).

At the end of the experiment (week 6), two pigs (one barrow and one gilt) were randomly selected from each pen (10 pigs per treatment). Blood samples were taken from the pigs by jugular venipuncture using vacuum tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). After collection, the serum samples from tubes were centrifuged (2000 × g) for 30 min at 4 °C and stored until further analysis. At the same time, faecal samples were collected from the pigs by via rectal massage. For nutrient digestibility analysing, fresh faecal samples from per pen were mixed and pooled, and were stored together with feed samples in a freezer at −20 °C until analysis. For faecal E. coli and Lactobacilli counts analysing, faecal samples were pooled and placed on ice for transportation to the laboratory and analysis was immediately carried out.

**Chemical analysis**

Before chemical analysis for nutrient digestibility, the feed faecal samples were thawed and dried at 70 °C for 72 h and finely ground to pass through a 1-mm screen. DM and N digestibility were determined using methods established by Association of Official Analytical Chemists (AOAC 2000). Chromium concentrations were determined via UV-absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan). 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). The ATTD was then calculated using the following formula:

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\text{Digestibility} \% = \left\{1 - \frac{[(\text{Nf} \times \text{Cd})/(\text{Nd} \times \text{Cf})]]}{100}
\]

where \( \text{Nf} \) = nutrient concentration in faeces (% DM), \( \text{Cd} \) = chromium concentration in diet (% DM), \( \text{Nd} \) = nutrient concentration in diet (% DM) and \( \text{Cf} \) = chromium concentration in faeces (% DM).

Blood lipid profiles, including total cholesterol, high-density lipoprotein cholesterol (HDLC), low-density lipoprotein cholesterol (LDLC) and triglyceride were analysed by using an automatic biochemistry analyser (Hitachi 747; Hitachi Ltd., Tokyo, Japan) with commercial kits (MAK043, MAK045, and TR0100, Sigma Diagnostics, MO, USA) according to the manufacturer’s protocol.

One-gram faecal sample from each pen for faecal E. coli and Lactobacillus counts was diluted with 9 ml of 1% peptone broth (Becton, Dickinson) and then homogenised. Viable count of bacteria in the faecal samples was then conducted by plating serial 10-fold dilution (in 1% peptone solution) onto MacConkey agar plates (Difco Laboratories, Detroit, MI) and lactobacilli medium III agar plates (Medium 638; DSMZ, Braunschweig, Germany) to isolate 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 incubated for 24 h at 37 °C. The E. coli and Lactobacillus colonies were counted immediately after removing from the incubator.

**Statistical analysis**

Data were subjected to analysis of variance as a completely randomised design using the general linear models (GLM) procedure of SAS (SAS Institute, Inc. Cary, NC, USA). The pen was used as the experimental unit. Before conducting the statistical analysis of the microbial counts, data were log-transformed. Orthogonal polynomials were used to assess the linear and quadratic effects of increasing dietary composition of DOL. Variability in the data was expressed as the pooled SEM and a p value of less than .05 was considered statistically significant and a p value of .05 to .10 was considered tendency.

**Results and discussion**

**Growth performance and nutrient digestibility**

As shown in Table 3, increased content of DOL led to a significant \( p < .05 \) linear increase in ADG during the experiment period. The G/F ratio was improved (linear effect, \( p < .05 \)) during the 6-week experiment. However, there was no significant \( p > .05 \) difference in ADFI. During the last week of experiment, the digestibility of DM, N and GE significantly increased (linear effect, \( p < .05 \)) with the increase in the content of DOL from 60% to 97%. In agreement with this study, Kim et al. (2008) reported that addition of 2.5% and 5% lecithin improved 15.6% and 16.2% of body weight gain and decreased the feed conversion ratio (FCR) by 7.2% and 14.3%, respectively compared with diets without lecithin. Additionally, Todorova et al. (2011) observed lecithin supplementation enhanced ADG and FCR by 10.6% and 7.6%, respectively in weaned pigs. However, it has been reported by Akit et al. (2014) that addition of different levels (4, 20 and 80 g/kg) of lecithin in finishing pig diets did not affect final body weight, feed intake and FCR.
Recently, Meng et al. (2018) investigated that with 4 g/kg lecithin supplementation, there was no influence in ADG, ADFI and G/F in growing-finishing pigs. The inconsistent findings related to growth performance in pigs may be due to the different amount of emulsifier added, feed ingredients or lipid types and ages of pigs. Moreover, the diets of this study met or exceeded NRC (2012) recommendations for nutrients and energy for pigs and the FI of pigs had no differences among treatments; thus, the improved growth performance in DOL supplementation groups was not because of the increased feed consumption, but due to the improved digestibility of nutrient in pig.

Blood lipid profiles and faecal E. coli and Lactobacilli counts

As described in Table 4, different levels of DOL of the diet did not influence the concentration of blood HDL-C and LDL-C. However, total cholesterol and

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**Table 3. Effect of dietary supplementation of de-oiled lecithin on growth performance and apparent total tract digestibility in growing pigs**

| Items                | CON | TRT1 | TRT2 | SEM | Linear | Quadratic |
|----------------------|-----|------|------|-----|--------|-----------|
| Overall growth performance |    |      |      |     |        |           |
| ADG, g               | 720 | 743  | 755  | 7.04| .0037  | .5124     |
| ADFI, g              | 1658| 1675 | 1661 | 17.12| .9163  | .4575     |
| G/F                  | 0.44| 0.44 | 0.46 | 0.07| .0420  | .8735     |
| Apparent total tract digestibility on week 6 |    |      |      |     |        |           |
| Dry matter, %        | 77.14| 79.12| 80.92| 0.80| .0032  | .9276     |
| Nitrogen, %          | 74.56| 76.82| 77.45| 0.89| .0315  | .4624     |
| Gross Energy, %      | 76.26| 77.64| 78.91| 0.87| .0436  | .9598     |

*There were five replicated pens of five pigs/pen per treatment.

Note: CON, Basal diet; TRT1, Basal diet + 0.1% DOL-60; TRT2, Basal diet + 0.1% DOL-97.

ADG: Average daily gain; ADFI: Average feed intake; G/F: gain-to-feed ratio; SEM: Standard error of means.

**Table 4. Effect of dietary supplementation of de-oiled lecithin on blood lipid profile and faecal E. coli and Lactobacilli count in growing pigs**

| Items                                      | CON | TRT1 | TRT2 | SEM  | Linear | Quadratic |
|--------------------------------------------|-----|------|------|------|--------|-----------|
| Blood lipid profile on week 6               |     |      |      |      |        |           |
| HDL-C, mg/dL                               | 44.25| 47.25| 46.25| 1.42 | .3442  | .2787     |
| LDL-C, mg/dL                               | 37.50| 37.50| 34.00| 1.45 | .9163  | .4575     |
| Total cholesterol, mg/dL                   | 117.75| 109.75| 108.75| 3.12 | .7171  | .3835     |
| Triglyceride, mg/dL                        | 53.75| 46.50| 43.50| 3.28 | .0543  | .6094     |
| Faecal E. coli and Lactobacilli count on week 6 |     |      |      |      |        |           |
| Lactobacillus, log10 cfu/g                 | 7.49 | 7.61 | 7.63 | 0.03 | .0022  | .1256     |
| Escherichia Coli, log10 cfu/g              | 6.10 | 6.01 | 5.99 | 0.05 | .2043  | .6336     |

*There were five replicated pens of five pigs/pen per treatment.

Note: CON, Basal diet; TRT1, Basal diet + 0.1% DOL-60; TRT2, Basal diet + 0.1% DOL-97.

HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; SEM: Standard error of means.

Xing et al. (2004) demonstrated that supplementation of lysolcithin linearly improved the digestibility of DM, crude protein and energy in nursery pigs. Moreover, Dierick and Decuypere (2004) reported that 0.3% of lecithin product enhanced growing pig ileal nutrient digestibility of DM, crude protein and energy. However, Kim et al. (2008) observed no differences in digestibility of DM, energy, protein and ether extract with supplementation of 2.5% and 5% of lecithin in finishing pigs. Saseendran et al. (2017) reported that in the digestibility coefficient of nutrients and minerals of piglets, no significant change was observed with 0.5% lecithin diet. It was reported that nutrient digestibility could be affected by fat types included in the diets (Mitchaothai et al. 2008); therefore, the inconsistency among studies may be related to the type of lecithin and fat and the age of animals.

**Blood lipid profiles and faecal E. coli and Lactobacilli counts**

As described in Table 4, different levels of DOL of the diet did not influence the concentration of blood HDL-C and LDL-C. However, total cholesterol and
triglyceride had a tendency in reduction with the increasing content of DOL. At the end of experiment, the population of E. coli was not significantly affected by dietary treatments. However, faecal Lactobacillus counts showed a significant linearly increase (p < .05) with the increasing level of DOL. It has been recognised that a lower blood total cholesterol and triglyceride levels were advantageous for humans and animals health (Zunft et al. 2003; Liu and Kim 2018). Kim et al. (2008) found that blood total cholesterol was reduced by supplementation of 2.5% and 5% of lecithin in finishing diets. Moreover, Todorova et al. (2011) reported that blood cholesterol reduced 14.8% by supplementation of 1% lecithin in weaning pigs. Similar results were reported in poultry (Huang et al. 2008). Cholesterol and triglycerides play important roles in humans and animals such as formation of cell membrane, biosynthesis of steroid hormones and bile acid and transference of adipose fat and blood glucose from the liver (Hanukoglu 1992; Olson 1998; White and Venkatesh 2011). However, the mechanism of how lecithin influenced triglyceride or cholesterol is still unclear. Huang et al. (2008) suggested that soy lecithin regulated broiler fat metabolism by altering the hormone levels and lipogenic gene expressions. The possible explanation for the lower concentration of cholesterol and triglycerides in this study may be that supplementation of DOL helped the emulsification of dietary fat into free fatty acids (Gu and Li 2003).

Diets affected the host not only through the direct intestinal absorption but also from the microbial metabolite production with downstream effects (Shen 2017). It was reported that balance of intestinal microbiota is necessary for digestive and health of animals; furthermore, imbalanced microbiome may cause poor performance, susceptible to infections and low growth rate (Czarnecki-Maulden 2008). Generally, Lactobacillus is supposed as beneficial bacteria, whereas E. coli considered harmful to animals (Halas and Nochta 2012). It has been reported that increased faecal Lactobacillus counts improved microbiota balance and enhanced growth performance and digestibility in weaning pigs which is similar with this experiment (Lan et al. 2016). Moreover, Upadhaya et al. (2016) found that the increasing population of faecal Lactobacillus enhances growth rate and feed efficiency by reducing the survival rate of enteric pathogens in growing pigs. Owing the lack of available literature, direct comparison of dietary supplementation of DOL in growing pigs is impossible. However, it could be conjectured that DOL addition may alter the intestinal Lactobacillus count which have positive effects on nutrient digestibility and growth performance in growing pigs.

Conclusions
In conclusion, dietary supplementation of increased contents of DOL improved growth performance, digestibility of DM, N and GE and Lactobacillus count; whereas, decreased the concentration of blood total cholesterol and triglyceride in growing pigs. This study provided insight for the application of DOL as an emulsifier in growing pig diets.

Disclosure statement
No potential conflict of interest was reported by the authors.

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