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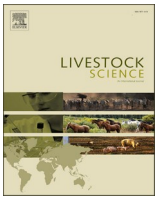

Coated refined fish oil supplementation improves growth performance and meat quality in finishing pigs

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HIGHLIGHTS

- Coated refined fish oil improves growth performance.
- Drip loss from meat reduces by coated refined fish oil addition.
- The appropriate dose of coated refined fish oil used in finishing pigs is 0.50%.

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ABSTRACT

Fish oil is rich in docosahexaenoic acid (DHA). However, uncoated fish oil is easily oxidized. Coating technique is a method for improving the stability and the availability of fish oil. This study investigated the effects of supplementing coated refined fish oil to the diet of finishing pigs on growth performance, apparent nutrient digestibility, fecal microbiota, fecal gas emission, meat quality, and carcass traits. A total of 160 crossbred finishing pigs [(Yorkshire × Landrace) × Duroc], with an initial body weight of 70.51 ± 0.56 kg, were randomly assigned to 1 of 4 dietary treatments according to the initial body weight. The experimental period was 42 days. There were 8 replicate pens per treatment and 5 pigs (three barrows and two gilts) per pen. Dietary treatments were based on a corn-soybean meal-basal diet (CON) supplemented with 0.00, 0.10, 0.25, or 0.50% coated refined fish oil. As a result, supplementing coated refined fish oil to the diet linearly increased ($P < 0.05$) final body weight (BW) and average daily gain (ADG), whereas linearly decreased ($P < 0.05$) feed conversion ratio (FCR) and the drip loss from meat on day 7 ($P < 0.01$) in a dose-dependent manner. Moreover, finishing pigs fed with 0.50% coated refined fish oil containing diet had higher final BW ($P = 0.018$) and ADG ($P = 0.029$) than those fed the control diet. The FCR ($P = 0.011$) in pigs consuming 0.10 or 0.50% coated refined fish oil supplemented diet was lower than those consuming control diet. The drip loss on day 7 ($P = 0.008$) in pigs fed the diet supplemented with 0.25 or 0.50% coated refined fish oil was lower than those fed the control diet. In brief, based on the findings from the present study, the supplementation of 0.50% coated refined fish oil was suggested to be the optimum dose in enhancing growth performance and improving drip loss of meat quality in the finishing pigs.

1. Introduction

Polyunsaturated fatty acids (PUFA) are essential for mammals, however, mammals cannot synthesize PUFA in *vivo*, and must obtain them through diet (Simopoulos, 2009). Docosahexaenoic acid (DHA) is one of the most important PUFA (Tanghe and de Smet, 2013). It has been reported that fish oil is a source of PUFA, especially DHA (Lee et al., 2019). Increasing the intake of DHA by consuming fish oil can improve the growth, immunity, and intestinal health in swine (Liu et al., 2012; Arnardottir et al., 2012; Rizliya and Mendis, 2014). These findings suggested that feeding DHA-rich fish oil to monogastric animals can bring benefits to the health and growth of animals (Lee et al., 2019).

However, the PUFAs in fish oil are highly unsaturated molecules that are easily oxidized, leading to the loss of nutritional and sensory value. In order to overcome the limitation of fish oil use, some techniques such as coating may be a feasible strategy to reduce the rancidity of PUFA and improve its stability and usability. Coating technique protects the coated material and allows it to be released under certain conditions (Champagne and Fustier, 2007). Encapsulation enables to remove the unpleasant fish oil flavours (Kralovec et al., 2012). Upadhaya and Kim

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(2021) reported that feeding weaning pigs with coated fish oil had better growth performance than those fed with raw fish oil containing diet. In addition, Zentek et al. (2012) found the richness of amylolytic bacteria and beneficial bacteria, *Lactobacillus*, in the gastrointestinal tract was higher in the group of pigs fed with coated fatty acids containing diet in comparison to those fed with normal fatty acids containing diet.

Thus, it is necessary to develop coated refined fish oil to improve animal growth performance. However, there are few reports on the use of coated refined fish oil in the diet of finishing pigs. The objective of this study was to test the effects of coated refined fish oil supplementation on growth performance, nutrient digestibility, fecal microbiota, fecal gas emission, meat quality, and carcass traits in finishing pigs. We hypothesized that the supplementation of coated refined fish oil in the diet may have a positive effect on meat quality and carcass traits, and improved nutrient digestibility by regulating fecal microbiota community, further improving growth performance and reducing fecal noxious gas emission.

2. Material and methods

The protocol (DK-1–1964) of this study has been approved by the Animal Care and Use committee of Dankook University (Cheonan, Choongnam, Korea).

2.1. Information on coated refined fish oil

The coated refined fish oil used in this study was provided from a commercial company (Morningbio Co., Ltd., Cheonan, Korea). The refined fish oil was produced through the transesterification and molecular distillation process according to the method described by Hoque et al. (2011). In addition, the powder refined fish oil was coated with joint matrix coating method to protect unsaturated fatty acid from rancidity, thereby increasing its usability.

The fatty acid composition of coated refined fish oil used in this study is shown in Table 2. The content of omega-3 PUFA in the coated refined fish oil was 18.64%; omega-6 PUFA was 2.90%. The ratio of omega-6 PUFA to omega-3 PUFA was 1:6.4.

2.2. Animals and housing

One hundred and sixty crossbred finishing pigs [(Landrace × Yorkshire) × Duroc] with an initial body weight of 70.51 ± 0.56 kg were randomly allotted to one of four dietary treatments according to the initial body weight. There were eight replicate pens per treatment and five pigs (three barrows and two gilts) per pen. Dietary treatments consisted of a corn-soybean meal-based basal diet and a basal diet supplemented with 0.10, 0.25, or 0.50% coated refined fish oil. Fish oil was added to the basal diet at the expense of tallows to balance dietary nitrogen and caloric. The experimental period was 42 days. Coated refined fish oil in different levels were added to the basal diet at the expense of corn and provided as a mash form. All diets were formulated to meet the nutrient requirements recommended by the National Research Council (NRC 2012; Table 1).

All pigs were housed in an environmentally controlled room. The one-side stainless steel self-feeder and nipple drinker were installed for pigs to give the animals free access to feed and water.

2.3. Sample collection and measurements

2.3.1. Growth performance analysis

Body weight of pigs weighed individually on the 1st and 42nd days to measure the average daily gain (ADG). Feed consumption was recorded daily on a pen basis to calculate the average daily feed intake (ADFI). The feed conversion ratio (FCR) was measured using ADG and ADFI values.

Table 1
Experimental diet compositions (as fed-basis)[1].

Items	CON	T1	T2	T3
Ingredients,%				
Corn	76.83	76.76	76.69	76.55
Soybean meal	15.30	15.32	15.32	15.34
Tallow	2.49	2.44	2.36	2.23
Molasses	2.00	2.00	2.00	2.00
Dicalcium phosphate	1.35	1.35	1.35	1.35
Limestone	0.56	0.56	0.56	0.56
Salt	0.30	0.30	0.30	0.30
Methionine (99%)	0.07	0.07	0.07	0.07
Lysine	0.48	0.48	0.48	0.48
Threonine (99%)	0.14	0.14	0.14	0.14
Tryptophan (99%)	0.05	0.05	0.05	0.05
Choline (25%)	0.03	0.03	0.03	0.03
Mineral mix[2]	0.20	0.20	0.20	0.20
Vitamin mix[3]	0.20	0.20	0.20	0.20
Refine fish oil	–	0.10	0.25	0.50
Total	100.00	100.00	100.00	100.00
Calculated value,%				
Gross energy (kcal/kg)	3300	3300	3300	3300
Crude protein	14.00	14.00	14.00	14.00
Crude fat	5.35	5.38	5.42	5.49
Fiber	2.34	2.34	2.34	2.33
Ash	4.36	4.36	4.36	4.36
Calcium	0.60	0.60	0.60	0.60
Phosphorus	0.55	0.55	0.55	0.55
Lysine	1.00	1.00	1.00	1.00
Methionine	0.30	0.30	0.30	0.30
Threonine	0.65	0.65	0.65	0.65
Tryptophan	0.20	0.20	0.20	0.20
Omega-3 PUFA,%	0.06	0.08	0.12	0.18
Omega-6 PUFA,%	1.61	1.61	1.61	1.60
Omega-6 to omega-3 ratio	26.83	20.13	13.42	8.89

[1] Dietary treatments were: CON, basal diet; T1, basal diet + 0.10% coated refined fish oil; T2, basal diet + 0.25% coated refined fish oil; T3, basal diet + 0.50% coated refined fish oil.

[2] Provided per kg diet: Fe, 115 mg as ferrous sulfate; Cu, 70 mg as copper sulfate; Mn, 20 mg as manganese oxide; Zn, 60 mg as zinc oxide; I, 0.5 mg as potassium iodide; and Se, 0.3 mg as sodium selenite.

[3] Provided per kilograms of diet: vitamin A, 13,000 IU; vitamin D_3, 1700 IU; vitamin E, 60 IU; vitamin K_3, 5 mg; vitamin B_1, 4.2 mg; vitamin B_2, 19 mg; vitamin B_6, 6.7 mg; vitamin B_{12}, 0.05 mg; biotin, 0.34 mg; folic acid, 2.1 mg; niacin, 55 mg; D-calcium pantothenate, 45 mg.

2.4. Nutrient digestibility analysis

For evaluating the apparent total tract digestibility of dry matter (DM), nitrogen (N), and energy, 0.20% chromium oxide was supplemented to the diet of finishing pigs during days 35 to 42 of this experiment. Representative feed samples in each dietary treatment were taken after mixing. Fecal samples were collected randomly from two pigs (one barrow and one gilt) in each replicate pen on days 42, samples from the same pen were mixed. Both feed sample and fecal sample were dried in a 60 °C oven for a 72 h period. After that, feed and fecal samples were finely ground to pass through a 1 mm sieve. Following the procedure established by the Association of Official Analytical Chemists (AOAC International, 2000), diet samples were analyzed for DM (method 930.15), crude protein ($N \times 6.25$; method 968.06), crude fat (method 954.02), and ash (method 930.15). Fecal samples were also analyzed for DM (method 930.15) and N (method 988.05) following the procedures established by AOAC International (2000). The combustion heat was measured by a bomb calorimeter (Parr 6100; Parr Instrument Co., Moline, IL, USA) to determine the energy in feces and feed. The chromium levels were analyzed via UV absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan). The ATTD of DM, N, and energy were calculated using the following formula:

$$ATTD\,(\%) = \left(1 - \frac{Nf \times Cd}{Nd \times Cf}\right) \times 100$$

Table 2
Fatty acid composition of coated refined fish oil[1].

Items	Content,%
Caprylic acid, C8:0	0.0209
Capric acid, C10:0	0.0940
Lauric acid, C12:0	0.1000
Tridecylic acid, C13:0	0.0438
Myristic acid, C14:0	10.0720
Tetradecadienoic acid, C14:2	0.0227
Pentadecylic acid, C15:0	0.4337
Palmitic acid, C16:0	38.3533
Margaric acid, C17:0	0.8785
Stearic acid, C18:0	0.0284
Oleic acid, C18:1w9	20.4667
Linoleic acid, C18:2w6	2.2202
alpha-Linolenic acid, C18:3w3	0.8599
Eicosenoic acid, C20:1w9	1.8299
Docosanoic acid, C22:0	2.5253
Arachidonic acid, C20:4w6	0.6773
Eicosapentaenoic acid, C20:5w3	10.5974
Docosahexaenoic acid, C22:6w3	7.1816
Unknown	3.5943

[1] The refined fish oil was produced through the transesterification and molecular distillation process according to the method described by Hoque et al. (2011). In addition, in order to powder refined fish oil, it is coated with joint matrix coating method using silica as an adsorbent to protect unsaturated fatty acid from rancidity, thereby increasing its usability.

where Nf is the nutrient concentration in excreta (% DM), Nd is the nutrient concentration in diet (% DM), Cf is the chromium concentration in excreta (% DM), and Cd is the chromium concentration in diet (% DM).

The analysis of fatty acid of coated refined fish oil was done by using an HP 5890 gas chromatography with a flame ionization detector (Hewlett Packard 5890 Seried II, Palo Alto, CA, USA). Omegawax-320 fused silica capillary column (30 m × 0.32 mm × 0.25 μm; Supelco, Inc., Bellefonte, PA, USA), with 1.2 mL/min of helium flow was used to separate fatty acid methyl esters (FAME). The oven temperature was increased from 180 °C to 204 °C, at the rate of 1.5 °C/min. Temperatures of the injector and detector were 260 °C and 280 °C, respectively. By comparing the retention time and peak area of each fatty acid standard, the peak of fatty acids of analysed samples were identified. The content of omega-3 PUFA or omega-6 PUFA in feed were also analyzed by the method of the above.

2.5. Fecal microbiota analysis

On the 1st and 42nd day of this study, fresh fecal samples were collected via the method of rectal massage from two pigs (one barrow and one gilt) randomly selected in each pen for measuring the counts of coliform bacteria and lactic acid bacteria in feces. The samples were pooled on a pen basis and placed on ice for transportation to the laboratory, where analysis was immediately carried out. One gram of the composite fecal sample from each pen was diluted with 9 mL of 1% peptone broth (Becton, Dickinson and Co., Franklin Lakes, NJ, USA), and homogenized. The counts of microbial in the fecal samples were calculated through plating serial 10-fold dilutions (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 coliform bacteria and lactic acid bacteria, respectively. The lactobacilli medium III agar plates were incubated for 48 h at 39 °C with anaerobic conditions. Besides, the MacConkey agar plates were incubated for 24 h at 37 °C under aerobic conditions. The colonies of coliform bacteria and lactic acid bacteria were counted immediately after removal from the incubator.

2.6. Fecal noxious gas emission analysis

On the 1st and 42nd day, fresh fecal samples were collected from two pigs (one barrow and one gilt) randomly selected in each pen via the method of rectal massage to measure fecal ammonia (NH_3), hydrogen sulfide (H_2S), total mercaptan (R-SH), carbon dioxide (CO_2), and acetic acid emission. Fecal samples from the same pen were mixed and stored into 2.6-L sealed plastic boxes, which had a small hole in the middle of one sidewall that was sealed by adhesive plaster, in duplicates and fermented 24 h at 25 °C. After the fermentation process, a gas-sampling pump (GAS Detector, GV-100S; Gastec Corp., Kanagawa, Japan) was used to detect the levels of NH_3, H_2S, R-SH, and acetic acid within the scope of 5.0–100.0 ppm (No. 3La, detector tube; Gastec Corp.), 2.0–20.0 ppm (No. 4LK, detector tube; Gastec Corp.), 0.5–120.0 ppm (No.70 and 70-L, detector tubes; Gastec Corp.), and 2.0–50.0 ppm (No. 81 L, detector tube; Gastec Corp.), respectively. The concentration of carbon dioxide (CO_2) was calculated by a multi-gas meter (MultiRAE Lite model PGM-6208, RAE, USA). Before measurement, fecal samples were homogenized manually for 30 s. Air samples (100 mL) were taken from the head-space above the surface of feces through the small hole and the sampling height was about 2.0 cm. Two samples from each pen were measured and the average was calculated.

2.7. Carcass traits analysis

At the end of the study, all pigs were slaughtered in a commercial slaughterhouse. Carcass weight was recorded after exsanguination and evisceration. A real-time ultrasound instrument (Piglot 105; SFK Technology, Herlev, Denmark) was used to measure the carcass back-fat thickness after mortem 45 min.

2.8. Meat quality analysis

After chilling at 2 °C for 24 h. Two pigs (one barrow and one gilt) from each pen were randomly selected and a sample of the right loin was removed between the 10th and 11th ribs. Sensory evaluation (color, marbling, and firmness score) was conducted according to the National Pork Producers Council Standards (NPPC 2000) at ambient temperature immediately. After the subjective tests, the lightness (L*), redness (a*), and yellowness (b*) values were measured by a Model CR-410 Chroma meter (Konica Minolta Sensing, Inc., Osaka, Japan) at three locations of each sample surface. The pH values of each sample were directly measured using a pH meter (Pittsburgh, PA, USA). Two different positions were measured and recorded with each sample. Thereafter, a 0.3 g sample was pressed at 3000 psi for 3 min on a 125-mm-diameter piece of filter paper. The areas of the pressed sample and the expressed moisture were delineated and then determined using a digitizing area-line sensor (MT-10S; M.T. Precision Co. Ltd., Tokyo, Japan) to calculate the water-holding capacity (WHC). The plastic bag method was used to calculate the drip loss, about 4 g of meat sample was used to measure the drip loss at the 1, 3, 5, and 7 days after chilled. During the measurement period, meat samples were stored in the fridge. When measuring its weight, removed sample from the plastic bag and clean the surface water by absorbent paper at different timepoints. The drip loss was calculated as:

$$drip\ loss = \frac{sample\ weight\ at\ measuring\ timepoint\ -\ initial\ weight\ of\ sample}{initial\ weight\ of\ sample} \times 100$$

After that, 5 g of meat sample was heat-treated in plastic bags separately in a water bath (100 °C) for 5 min to measure cooking loss. The samples were cooled at room temperature (25 °C). Cooking loss was calculated as:

$$Cooking\ loss = \frac{sample\ weight\ before\ Cooking\ -\ sample\ weight\ after\ Cooking}{sample\ weight\ before\ Cooking} \times 100$$

2.9. Statistical analysis

All data were subjected to statistical analysis in a randomized completely block design using the General Linear Model procedure (SAS Inst. Inc., Cary, NC, USA). The replicate pen was used as the experimental unit. Orthogonal contrasts were used to examine the linear, quadratic, and cubic effects in response to increasing the dietary supplementation of coated refined fish oil. Variability in the data was expressed as the standard error of means (SEM), $P < 0.05$ was considered to be statistically significant.

3. Results

The experimental dietary ingredients used in this study are shown in Table 1. Supplemented diets with coated refined fish oil, were characterised by a consequent increase of total omega-3 polyunsaturated fatty acid (PUFA) and crude fat, when compared to the non-supplemental diets. Moreover, the content of omega-6 PUFA was similar among all dietary treatments. Therefore, the differences in crude fat and omega-3 PUFA contents detected in all dietary treatments were related to the supplementation of coated refined fish oil.

Finishing pigs fed the diet supplemented with 0.10, 0.25, or 0.50% coated refined fish oil linearly increased final body weight ($P < 0.05$) and average daily gain ($P < 0.05$), whereas linearly decreased feed conversion ratio ($P < 0.05$) with the dose of coated refined fish oil increased in the diet. In addition, the final body weight ($P = 0.018$) and average daily gain ($P = 0.029$) in finishing pigs fed with 0.50% coated refined fish oil containing diet were higher than those fed with control diet. Finishing pigs fed the diet supplemented with 0.10 or 0.50% coated refined fish oil had lower feed conversion ratio ($P = 0.011$) compared with those fed the control diet. However, the addition of coated refined fish oil had no effects on the average daily feed intake (Table 3).

Finishing pigs fed with coated refined fish oil containing diet had no effects on the apparent total tract digestibility of dry matter, nitrogen, and energy (Table 4).

The emission of ammonia, hydrogen sulfide, total mercaptan, carbon dioxide, and acetic acid in feces were not affected by the addition of coated refined fish oil (Table 5).

Dietary supplementation of coated refined fish oil did not affect the counts of lactic acid bacteria and coliform bacteria in feces (Table 6).

Regarding the effects of supplementing coated refined fish oil to the diet on meat quality and carcass traits, with the increase of coated refined fish oil dosage, the drip loss on day 7 ($P < 0.01$) showed a linear decrease, but the lightness, redness, yellowness, color, marbling, firmness, pH, cooking loss, longissimus muscle area, carcass weight, and carcass back-fat thickness were not affected. Moreover, finishing pigs fed with 0.25 or 0.50% coated refined fish oil containing diet had lower drip loss on day 7 ($P = 0.008$) compared with those fed the control diet (Table 7).

4. Discussion

DHA is the active ingredient in fish oil, and belongs to PUFA (Chen and Yeh, 2003). PUFA is essential for improving the health and growth of animals (Zhang et al., 2010). Studies of Liu et al. (2003) reported that weaning pigs fed a diet containing 7% fish oil (6.30% DHA) increased body weight gain and feed intake. Like fish oil, microalgae are also the source of DHA (Lee et al., 2019). Meadus et al. (2011) reported that supplementing 1.60% algae (18% DHA) to the diet of finishing pigs increased average daily gain and decreased feed conversion ratio. Samuel et al. (2014) noted that weaning pigs fed with 0.50 or 1.00% algal (27% DHA) containing diet increased average daily gain and average daily feed intake, but decreased feed conversion ratio. To sum up, DHA-rich substance is beneficial to the growth and development of pigs. Similar to the above studies, this study observed that the final body weight and average daily gain increased, and feed conversion ratio decreased, in finishing pigs fed the diet supplemented with coated refined fish oil. As the studies reported by Liu et al. (2020), the growth performance of pigs could be regulated by improving the nutrient digestibility or intestinal microbiota. Sun and Kim (2020) reported the strategy for ameliorating growth performance by improving nutrient digestibility. In addition, Dang et al. (2020) noted that increasing the counts of beneficial bacteria and decreasing the counts of harmful bacteria in the intestine were beneficial to intestinal health, which would lead to a high feed efficiency and thus improving growth performance. However, in this study, the supplementation of coated refined fish oil had no effects on nutrient digestibility and fecal microbiota. As mentioned by Gaines et al. (2003) and Liu et al. (2003), feeding fish oil to pigs can partially improve growth performance by regulating the immune system. In addition, Lee et al. (2019) mentioned that the mechanisms in improving the growth performance of animals by feeding with a PUFA-containing diet were related to the enhancement of glucose intake. Moreover, Fair et al. (2014) and Tran et al. (2016) mentioned that feeding animals with a diet supplemented with DHA activated the metabolic pathway of energy production, thus increasing levels of insulin growth factor-1. Therefore, DHA-riched fish oil has the potential to be used as the growth promoter in finishing pigs. However, in terms of the results obtained in this study, the mechanism of growth performance promotion in finishing pigs fed the diet supplemented with coated refined fish oil was not related to the improvement of nutrient digestibility and fecal microbiota. Fat is essential in the diet to increase the calorific density of feeds (Lee et al., 2019). In this study, the crude fat in the experimental diet was increased gradually with the increase of coated refined fish oil contents in the diet (5.35 vs. 5.38 vs. 5.42 vs. 5.49%). Therefore, the crude fat increased in the diet partially benefited the improvement of growth performance. In addition, with the contents of coated refined fish oil increased in the experimental diet, the contents of omega-3 PUFA was increased gradually (0.06 vs. 0.08 vs. 0.12 vs. 0.18%) whereas the omega-6 PUFA was similar. It is worth mentioning that the omega-6 PUFA to the omega-3 PUFA ratio in the diet decreased with the content of omega-3 PUFA increased. Duan et al. (2014) and Konieczka et al. (2015) investigated the varying inclusion of fish oil in the diet and found that the variation of the omega-6 PUFA to the omega-3 PUFA ratio appears to be influential for improving growth

Table 3
Effect of dietary supplementation of coated refined fish oil on growth performance in finishing pigs[1].

Items	Coated refined fish oil,%				SEM	*P*-value		
	0.00	0.10	0.25	0.50		Linear	Quadratic	Cubic
Initial body weight, kg	70.51	70.51	70.51	70.51	0.555	1.000	0.998	0.995
Final body weight, kg	103.41^{b}	105.08^{ab}	104.66^{ab}	105.98^{a}	0.669	0.018	0.790	0.204
ADG, g	783.22^{b}	823.16^{ab}	812.84^{ab}	844.59^{a}	16.865	0.029	0.810	0.231
ADFI, g	2571.43	2616.85	2601.55	2649.82	37.567	0.201	0.970	0.466
FCR	3.29^{a}	3.18^{b}	3.21^{ab}	3.14^{b}	0.034	0.011	0.577	0.139

Abbreviation: ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio; SEM, standard error of the mean.
[1] Values represent the means of eight pens ($n = 8$) per treatment.^{a,b}Different superscripts within a row indicate a significant difference ($P < 0.05$).

Table 4
Effect of dietary supplementation of coated refined fish oil on apparent total tract digestibility in finishing pigs[1].

Items,%	Coated refined fish oil,%					*P*-value		
	0.00	0.10	0.25	0.50	SEM	Linear	Quadratic	Cubic
Dry matter	71.48	72.87	72.67	73.05	1.404	0.480	0.725	0.733
Nitrogen	69.42	70.53	70.40	70.83	1.438	0.529	0.814	0.781
Energy	71.82	72.49	71.68	72.26	1.463	0.941	0.975	0.662

Abbreviation: SEM, standard error of the mean.
[1] Values represent the means of eight pens with 2 pigs per replicate pen ($n = 16$) per treatment.

Table 5
Effect of dietary supplementation of coated refined fish oil on fecal gas emission in finishing pigs[1].

Items, ppm	Coated refined fish oil,%					*P*-value		
	0.00	0.10	0.25	0.50	SEM	Linear	Quadratic	Cubic
Ammonia								
Initial	2.33	2.28	2.18	2.58	0.517	0.784	0.671	0.816
Final	1.78	2.15	1.33	1.45	0.387	0.318	0.752	0.237
Hydrogen sulfide								
Initial	6.20	5.30	5.78	5.33	0.651	0.474	0.735	0.445
Final	8.15	7.48	8.08	6.15	0.787	0.151	0.443	0.302
Total mercaptan								
Initial	6.18	6.03	6.05	5.98	1.085	0.908	0.973	0.956
Final	7.53	7.48	6.03	5.88	0.787	0.094	0.950	0.458
Carbon dioxide								
Initial	1500.00	1325.00	1475.00	1425.00	244.204	0.946	0.802	0.639
Final	1900.00	1550.00	1625.00	1500.00	226.270	0.288	0.628	0.548
Acetic acid								
Initial	5.43	6.65	6.90	5.00	1.003	0.823	0.145	0.798
Final	6.58	6.30	7.58	7.20	0.864	0.431	0.955	0.424

Abbreviation: SEM, standard error of the mean.
[1] Values represent the means of six pens with 2 pigs per replicate pen ($n = 12$) per treatment.

Table 6
Effect of dietary supplementation of coated refined fish oil on fecal microbiota in finishing pigs[1].

Items, log_{10}cfu/g	Coated refined fish oil,%					*P*-value		
	0.00	0.10	0.25	0.50	SEM	Linear	Quadratic	Cubic
Coliform bacteria								
Initial	7.10	7.14	7.13	7.13	0.166	0.938	0.905	0.967
Final	7.35	7.28	7.31	7.27	0.156	0.746	0.934	0.810
Lactic acid bacteria								
Initial	9.18	9.21	9.17	9.21	0.166	0.948	0.971	0.848
Final	9.36	9.38	9.41	9.45	0.118	0.554	0.907	0.980

Abbreviation: SEM, standard error of the mean.
[1] Values represent the means of eight pens with 2 pigs per replicate pen ($n = 16$) per treatment.

performance in animals. Optimizing the omega-6 PUFA to the omega-3 PUFA ratio in the diet of pigs can induce favourable partitioning of dietary nutrients toward body weight gain, that is improved feed efficiency by reducing maintenance energy expenditure (Gaines et al., 2003; Liu et al., 2003). It is reported that the reduction of the ratio of omega-6 PUFA to the omega-3 PUFA in the diet had positive effects on the growth performance in pigs (Duan et al., 2014; Li et al., 2015; Upadhaya et al., 2019). Moreover, growing evidence suggested that dietary modulation of the balance between omega-6 PUFA and omega-3 PUFA may affect behavioral functions and emotions (Clouard et al., 2015), including anxiety-related reactions (Ng and Innis, 2003), thus potentially increasing the well-being of pigs in familiar and unfamiliar/stressful environments, which was conducive to the improvement of growth performance. However, the optimal ratio of omega-6 PUFA to omega-3 PUFA has not yet been affirmed (Lee et al., 2019). Further experiments are needed to evaluate the effects of the dietary balance between omega-6 PUFA and omega-3 PUFA on the growth and development of pigs. In this study, the growth performance improved in the finishing pigs fed the diet supplemented with graded level of coated refined fish oil was seems to be related to the reduction of omega-6 PUFA to omega-3 PUFA ratio by increasing the dose of coated refined fish oil.

Plant-based oils from flaxseed/linseed are rich in omega-3 PUFA, just like fish oil (Palmquist, 2009). Holman et al. (2014) reported that extruded flaxseed/linseed supplementation in the diet of growing-finishing pigs had no measurable effects on the fecal microbiota. However, it has been reported that the PUFA from fish oil can reduce the population of *Frimicutes* in rats (Yu et al., 2014; Kaliannan et al., 2015). The omega-3 PUFA rich diet has the capacity to produce significant changes in the gut microbiota of human beings also has been demonstrated by Noriega et al. (2016). It seems that DHA modulated microbiota in the gut is variable among different species. The mechanisms of improving intestinal microbiota by adding fish oil are not clear. In this study, we found that supplementing coated refined fish oil to the diet of finishing pigs did not improve the intestinal microbiota.

It is reported that the apparent nutrient digestibility is regulated by the intestinal microbiota (Wu et al., 2019). In this study, the addition of coated refined fish oil had no effects on the nutrient digestibility and fecal microbiota. Studies of Upadhaya et al. (2017) reported that finishing pigs fed the diet supplemented with 0.75% omega-3 PUFA derived from linseed oil had no effects on the apparent total tract digestibility of dry matter, nitrogen, and energy. However, they did not

Table 7
Effect of dietary supplementation of coated refined fish oil on meat quality and carcass traits in finishing pigs1.

Items	Coated refined fish oil,%					*P*-value		
	0.00	0.10	0.25	0.50	SEM	Linear	Quadratic	Cubic
Meat color								
Lightness	56.56	56.14	56.21	56.86	0.538	0.689	0.326	0.967
Redness	14.76	14.93	14.72	14.86	0.217	0.910	0.947	0.468
Yellowness	7.67	7.57	7.64	7.56	0.206	0.776	0.965	0.736
Sensory evaluation								
Color	3.45	3.49	3.49	3.46	0.034	0.805	0.361	0.935
Marbling	2.38	2.41	2.40	2.43	0.034	0.367	0.854	0.565
Firmness	2.80	2.89	2.95	2.91	0.056	0.118	0.270	0.766
Cooking loss,%	23.55	22.14	21.65	21.06	1.285	0.175	0.749	0.859
pH	6.39	6.33	6.25	6.30	0.103	0.453	0.614	0.747
WHC,%	41.46	41.92	42.76	43.80	1.648	0.294	0.861	0.980
Drip loss,%								
Day 1	2.69	2.41	2.40	2.57	0.225	0.706	0.317	0.933
Day 3	5.86	5.44	5.10	5.15	0.347	0.123	0.501	0.849
Day 5	11.06	11.51	11.04	11.61	0.430	0.540	0.883	0.316
Day 7	19.95[a]	18.87[ab]	18.56[b]	18.45[b]	0.384	0.008	0.217	0.751
LMA, cm^2	61.05	63.05	62.70	63.78	1.733	0.319	0.795	0.629
Carcass weight, kg	89.20	90.13	89.50	90.38	1.223	0.597	0.984	0.578
Carcass back-fat thickness, mm	18.23	18.95	18.70	19.05	0.490	0.312	0.703	0.474

Abbreviation: WHC, water-holding capacity; LMA, longissimus muscle area; SEM, standard error of the mean.
[1]Values represent the means of eight pens with 2 pigs per replicate pen ($n = 16$) per treatment for meat color, sensory evaluation, cooking loss, pH, WHC, and drip loss; eight pens with 5 pigs per replicate pen ($n = 40$) per treatment for carcass weight and carcass back-fat thickness.a,bDifferent superscripts within a row indicate a significant difference ($P < 0.05$).

evaluate the relationship between apparent nutrient digestibility and fecal microbiota community. As the report of Holman et al. (2014), we speculated that omega-3 PUFA has limited ability to regulate the intestinal microbiota community in pigs, which seemed to be the reason why fish oil supplementation has no effects on nutrient digestibility. However, more studies are needed to explore the effects of supplementing fish oil to the diet on nutrient digestibility and intestinal microbiota.

The harmful bacteria especially coliform bacteria in the intestine can degrade the nitrogenous ingredients in undigested nutrient compounds correspondingly produce noxious gasses (Ferket et al., 2002). Therefore, reducing harmful bacteria in the intestine and improving nutrient digestibility are strategies to reduce harmful gas emissions (Dang et al., 2020; Liu et al., 2020). However, the supplementation of coated refined fish oil did not affect the nutrient digestibility, fecal microbiota, and fecal gas emission. The emission of noxious gas in feces based on fish oil supplementation in pigs has not been reported, thus, no comparisons could be made. In this study, the fecal noxious gas emission unlimited by coated refined fish oil supplementation was considered as due to the apparent nutrient digestibility and fecal microbiota unaffected.

The meat quality indicators including color, sensory evaluation, cooking loss, drip loss, or water-holding capacity directly affected the acceptance of consumers (Chang et al., 2010). The pH is a direct reflection of muscle acid content, which affects meat color (Hoffman et al., 2005; Yu et al., 2010), sensory traits (Arkfeld, 2016), cooking loss (Zhang et al., 2011), water-holding capacity (Zhang et al., 2011), and drip loss (Castellini et al., 2002). However, in the present study, supplementing coated refined fish oil to the diet of finishing pigs had no effects on the pH, meat color, sensory traits, cooking loss, and water-holding capacity, but decreased the drip loss on day 7. Jaturasitha et al. (2002) noted that finishing pigs fed the diet supplemented with 1, 2, or 3% tuna oil had no effects on the pH of meat, thus did not affect the color, water-holding capacity, drip loss, and cooking loss. Ribeiro et al. (2013, 2014) found that supplementing 7.4% marine *Schizochytrium* algae extract to the diet had no effects on pH, cooking loss, and sensory traits of breast muscle in broilers. Therefore, due to the addition of coated refined fish oil had no effects on the pH, thus did not affect the meat color, sensory traits, cooking loss, and water-holding capacity. Apparently, the reduction of drip loss was not related to the variation of pH in meat. Water is a dipolar molecule, which is attracted to charged species including proteins (Arkfeld, 2016). The drip loss depends on the proteins and structures of binding and retaining water, especially myofibrillar proteins (Dang and Kim, 2020). Therefore, drip loss thereby depends on the protein content in muscle. Wei et al. (2013) found that feeding the growing pigs with 7.5% DHA-containing product can enhance muscle protein synthesis by increasing the expression of muscle IGF-1 and the activation of the insulin receptors. A *vitro* study showed that PUFA could stimulate skeletal muscle cell hypertrophy via a cyclooxygenase-2-dependent pathway (Markworth and Cameron--Smith, 2013). Indeed, dietary omega-3 PUFA improved muscle protein synthesis in the elderly, healthy young, and middle-aged adults (Zhao et al., 2004; Smith et al., 2011). Liu et al. (2013) also reported that dietary treatment of fish oil has beneficial effects on increasing muscle protein content. Therefore, the decrease of drip loss seems to be due to the enhancement of muscle protein biosynthesis related to the supplementary coated refined fish oil. A reduction of drip loss limits the moisture content in the packaging bag during sale and storage, thus improving the acceptance of the meat of consumers (Ngapo et al., 2007).

Moran et al. (2018a) noted that growing-finishing pigs fed the diet supplemented with 0.25 or 0.50% unextracted microalgae (17.6% DHA) did not affect backfat thickness. Meadus et al. (2011) reported that supplementing 1.60% algae (18% DHA) to the diet had no effect on the carcass weight of pigs. Moran et al. (2018b) found that finishing pigs fed with 1% unextracted *Aurantiochytrium limacinum* algae (18.0% DHA) containing diet did not affect the backfat thickness. Studies of Sardi et al. (2006) demonstrated that finishing pigs fed with 0.25 or 0.50% dried marine algae containing diet had no effects on the backfat thickness. Jaturasitha et al. (2002) also reported that 1, 2, or 3% tuna oil supplementation in the diet of finishing pigs did not affect the carcass weight and backfat thickness. These data suggested that the effects of DHA-rich substance on carcass traits are minimal. The results of the above studies are similar to those of this study. Supplementing coated refined fish oil had no positive effects on carcass weight and carcass backfat thickness of finishing pigs, but it also had no adverse effects.

5. Conclusion

The results observed in this study partially confirmed our hypothesis, in which supplementing coated refined fish oil to the diet significantly decreased drip loss from meat in finishing pigs. In addition, the growth

performance was improved by coated refined fish oil addition, but did not affect the fecal microbiota and nutrient digestibility. The application of fish oil seems to modulate the balance of omega-6 PUFA and omega-3 PUFA in the diet, where the variation of the ratio of omega-6 PUFA to omega-3 PUFA in the diet may positively influence the growth performance. Therefore, the production of pork with low drip loss by increasing the levels of coated refined fish oil is desirable for the swine industry and consumers. Moreover, the coated refined fish oil has the potential to be used as a growth promoter in finishing pigs, and the suitable dosage is 0.50%.

CRediT authorship contribution statement

De Xin Dang: Writing – original draft, Investigation, Writing – review & editing, Formal analysis, Investigation. **In Ho Kim:** Conceptualization, Methodology, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

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