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Supplemental effects of fish oil and powdered/coated docosahexaenoic acid on the growth performance, nutrient digestibility, blood profile and fecal coliform and lactic acid bacteria counts in weaner pigs

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ABSTRACT

The present study was conducted to evaluate the effect of supplemental unrefined fish oil and refined fish oil enriched in docosahexaenoic acid (DHA) in powdered and coated forms on the performance and immune status of weaner pigs. In total, 140 crossbred weaner pigs [(Yorkshire × Landrace) × Duroc, 28 days old] with an average body weight (BW) of 7.47 ± 1.27 kg were used in a 6-week experiment trial in three phases. Pigs were blocked based on BW and sex and randomly allotted to 1 of 4 dietary treatments [5 pigs per pen (2 barrows and 3 gilts); 7 pens per treatment]. Treatments consisted of pigs fed basal diet or basal diet supplemented with 5 g/kg unrefined fish oil (FO), 1.73 g/kg powdered DHA enriched oil (pDHA), and 2.99 g/kg coated DHA enriched oil (cDHA). The supplementation of unrefined FO or DHA enriched FO to the diet of weaner pigs significantly increased body weight (BW) at days 7, 21, and 42 compared with pigs fed control diet. The average daily gain (ADG) increased ($P < 0.05$) during day 1−7 and day 22−42 as well as the overall experiment period (day1−42); and the average daily feed intake (ADFI) during day 1−7 and gain: feed (G:F) during overall experiment period were higher ($P < 0.05$) in pigs receiving FO or DHA supplemented diet as compared with pigs fed control diet. The BW during days 7 and 42, ADG during day 1−7 and overall and G:F during overall experiment were higher ($P < 0.05$) in pigs receiving DHA than the pigs fed with FO supplemented diet. A higher ($P < 0.05$) co-efficient of apparent total tract digestibility (CATTD) of fat was observed in pigs receiving cDHA and pDHA supplemented diet compared with the pigs fed basal or FO supplemented diet at day 42 of the experiment. The C-reactive protein concentration was reduced in fish oil and DHA supplemented groups compared with control. However, the fecal lactic acid bacteria and coliform counts as well as fecal noxious gases emission remained unaffected among the treatments. Thus, the supplementation of DHA in coated or powdered form has beneficial effect on the performance and immunity and it may help to overcome the stress faced by the weaner pigs during the transition of liquid to solid feed intake.

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; BW, body weight; CATTD, co-efficient of apparent total tract digestibility; DHA, ocosahexaenoic acid; DM, dry matter; EPA, eicosapentaenoic acid; FO, fish oil; GE, gross energy; G:F, gain:feed; N, nitrogen; PUFA, polyunsaturated fatty acids.

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1. Introduction

Fats and oils are important feed ingredients due to their high energy value as well as their role in the absorption of certain vitamins and minerals, maintenance of body temperature, and insulation of the body's vital organs. Fats can be derived from plant and animal sources. The fatty acid composition of dietary fat varies greatly. For example, pork lard and beef fat contain high amounts of saturated fatty acids (SFA) whereas fish oil is highly enriched with polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) which are essential for cell development. Although mammals are capable of synthesizing DHA from its precursor, alpha linolenic acid (ALA), its conversion to long chain PUFA is limited in pigs (Smink et al., 2013). As such, dietary supplementation with DHA and EPA rich sources such as fish oil or microalgae would be more effective for improving the performance and health of weaner pigs. Studies in animals and human subjects revealed that fish oil supplementation led to the reduction in pro-inflammatory cytokines in peripheral blood and spleen (Endres et al., 1989; Kew et al., 2003) and supports a healthy heart by enhancing cardiovascular function (Ruxton et al., 2007). Addition of 3–5 % fish oil to the diet of sows has been reported to improve the growth of suckling piglet (Rooke et al., 2000; Zhao et al., 2005).

Despite some advantages, there are some limitations with unrefined fish oil supplementation. For instance, the non-delayed significant release of lipids from unrefined fish oil in the stomach can lead to digestive problems. Moreover, PUFA are highly susceptible to oxidation, and the end-products of oxidation such as 4-hydroxy-2-alkenals may result in metabolic oxidative stress and inflammation (Russell, 2003; Bauer et al., 2005; Marschall and Beuers, 2013). Regardless of the presence of peroxidation products, fish oils generally do not have a very pleasant odor for the consumer. At industrial scale, handling of fish oil is difficult and quite expensive considering the cost of transportation, handling and manipulation of these oils.

Thus, to overcome these problems, technological solutions such as making a powdered form of pharmaceutical composition of oily material source of DHA or coating DHA after the purification of fish oil could be a viable strategy to reduce the rancidity of unsaturated fat as well as for controlled release of this fatty acid into the active site of absorption in the gut. To enrich fish oil with DHA, unrefined fish oil is purified using different techniques so as to make it acceptable for human and animal consumption (Crexi et al., 2010).

We hypothesized that purified fish oil enriched in DHA may be more effective compared with unrefined fish oil in improving performance in weaner pigs Therefore, the objective of the present study was to investigate the effects of fish oil or coated/silica impregnated powdered DHA on the growth performance, nutrient digestibility, blood profile, fecal coliform and lactic acid bacteria counts and fecal noxious gas emission of weaner pigs.

Table 1
Fatty acid profile of unrefined fish oil, silica impregnated powdered DHA and coated DHA (g/100 g FA).

Item	FO	pDHA	cDHA
C14:0	9.28	0.02	0.03
C14:1	0.15	0.00	0.00
C15:0	0.47	0.00	0.00
C16:0	20.18	0.00	25.81
C16:1	11.06	0.13	0.07
C17:0	1.72	0.12	0.07
C18:0	0.92	1.19	16.86
C18:1	1.90	4.49	2.61
C18:1	0.00	0.18	0.10
C18:2	5.61	0.57	0.33
C18:2n6	1.22	0.02	0.01
C18:3n6	1.14	1.03	0.60
C18:3n3	2.86	1.17	0.68
C20:0	0.00	0.30	0.17
C20:1	0.00	9.49	5.51
C20:2	0.00	0.48	0.27
C22:0	0.10	0.36	0.21
C20:3n3	0.11	0.29	0.17
C20:4n6	0.78	1.59	0.93
C22:1n9	1.26	1.84	1.07
C20:5n3	19.03	23.08	13.41
C20:3n6	5.08	2.78	1.61
C22:2	0.63	1.37	0.80
C23:0	0.06	0.45	0.26
C24:0	2.55	6.57	3.81
C22:6n3, DHA	13.45	39.18	22.76
C24:1	0.00	3.16	1.84
unknown	0.26		
Total	99.82	99.86	99.99

Abbreviation: FO, fish oil; pDHA, powdered docosahexaenoic acid; cDHA, coated docosahexaenoic acid.
Values are means of duplicate analyses.

2. Materials and methods

The experiment was conducted at the swine experimental unit of Dankook University (Cheonan, Choongnam, Korea). The study protocol was approved by the Animal Care and Use Committee of Dankook University (DK-2-1828).

2.1. Source of fish oil/ DHA

The unrefined fish oil and the DHA used in this study (Table 1) were obtained from a commercial company (Morningbio Co., Ltd., Cheonan, Korea).

As per the supplier's information, DHA was produced through the purification of fish oil through transesterification and molecular distillation process according to the method described by Hoque et al. (2011). The purified product which is liquid oil rich in DHA was powdered and absorbed on a support comprising silica or it was coated in lipid matrix using spray drying method.

Table 2
Ingredients and nutrients composition of experimental diets (as fed-basis; g/kg).

	Phase 1				Phase 2				Phase 3			
Ingredients	CON	FO	pDHA	cDHA	CON	FO	pDHA	cDHA	CON	FO	pDHA	cDHA
Extruded corn	402.9	403	399.8	400.81	519.1	519	517.9	519.1	595	595	594	595
Soybean meal	97.4	97.4	101.5	101.2	170.5	171	170.7	170.5	225	225	225	225
Fermented soybean meal	100	100	100	100	55	55	55	55	31	31	31	31
LT Fish meal	74.5	74.5	72	72	20	20	20	20	0	0	0	0
Soy oil	23	17.8	22.5	20.5	29.1	24	28.4	26.11	28	22.5	27.2	25
Fish oil	0	5	0	0	0	5	0	0	0	5	0	0
Powdered DHA	0	0	1.73	0	0	0	1.73	0	0	0	1.73	0
Coated DHA	0	0	0	2.99	0	0	0	2.99	0	0	0	2.99
MCP	6.6	6.7	6.7	6.7	11	11	11	11	12.3	12.3	12.3	12.3
Limestone	5.8	5.8	6	6	10.2	10	10.2	10.2	12.3	12.3	12.3	12.3
Sugar	30	30	30	30	20	20	20	20	20	20	20	20
Whey protein	110	110	110	110	70	70	70	70	30	30	30	30
Lactose	134.6	134.6	134.6	134.6	77.8	78	77.8	77.8	31.8	31.8	31.8	31.8
L-Lysine – HCL	5.6	5.6	5.6	5.6	7.1	7.1	7.1	7.1	6.3	6.3	6.3	6.3
DL-Met	1.5	1.5	1.5	1.5	1.3	1.3	1.3	1.3	0.9	0.9	0.9	0.9
Threonine	2.1	2.1	2.1	2.1	2.9	2.9	2.9	2.9	2	2	2	2
Choline 50 %	1	1	1	1	1	1	1	1	1	1	1	1
Salt	1	1	1	1	1	1	1	1	1	1	1	1
Vitamin premix[1]	2	2	2	2	2	2	2	2	2	2	2	2
Mineral premix[2]	2	2	2	2	2	2	2	2	2	2	2	2
Calculated values, g/kg												
Crude protein	200	200	200	200	180	180	180	180	180	180	180	180
ME, MJ/kg	14.44	14.44	14.44	14.44	14.24	14	14.24	14.24	14	14	14	14
Crude fat	46.7	46.5	47.2	47	52.5	52	53	52.5	52.6	52.1	53	52.6
Lysine	16	16	16	16	15	15	15	15	14	14	14	14
Methionine	4.8	4.8	4.8	4.8	4	4	4	4	3.5	3.5	3.5	3.5
Calcium	8	8	8	8	8	8	8	8	8	8	8	8
Phosphorous	6	6	6	6	6	6	6	6	6	6	6	6
Lactose	200	200	200	200	120	120	120	120	50	50	50	50
DHA	0	0.7	0.7	0.7	0	0.7	0.7	0.7	0	0.7	0.7	0.7
Analyzed values, g/kg												
GE, MJ/kg	18.25	18.25	18.28	18.23	18.01	18.00	18.04	18.02	17.80	17.78	17.82	17.80
DM	643.9	643.7	644.1	644	735.5	736	735.7	735.6	811	811	811	812
Crude protein	197.8	198	197.9	197.8	180.2	180	180	180.1	181	181	181	181
Crude fat	46.9	46.7	47.8	47.1	52.2	52	53.1	52.2	52.1	51.6	53	52.1
Ash	54.8	54.9	54.6	54.9	53.5	54	53.5	53.4	52.6	52.5	52.6	52.7
aNDF	48.7	48.8	48.6	48.7	62.4	62	62.3	62.5	72.6	72.7	72.5	72.5
ADF	17.2	17.3	17.3	17.4	24.1	24	24	24.2	29	29.1	29	28.9

Abbreviation: CON, Basal diet; FO, CON + 5 g/kg Fish oil; pDHA, CON + 1.73 g/kg Silica impregnated powdered docosahexaenoic acid; cDHA, CON + 2.99 g/kg coated docosahexaenoic acid, DHA; docosahexaenoic acid, LT Fish meal; low temperature fish meal, MCP; Mono-calcium phosphate, aNDF; neutral detergent fiber, ADF; acid detergent fiber, ME; metabolisable energy, DE; Digestible energy; DM; dry matter.

[1]Provided per kilogram of complete diet: vitamin A, 11,025 IU; vitamin D3, 1103 IU; vitamin E, 44 IU; vitamin K, 4.4 mg; riboflavin, 8.3 mg; niacin, 50 mg; thiamine, 4 mg; d-pantothenic acid, 29 mg; choline, 166 mg and vitamin B12, 33 μg.

[2]Provided per kilogram of complete diet: Fe (as $FeSO_4 \times 7H_2O$), 80 mg; Cu (as $CuSO_4 \times 5H_2O$), 12 mg; Zn (as $ZnSO_4$), 85 mg; Mn (as MnO_2), 8 mg; I (as KI), .28 mg and Se (as $Na_2SeO_3 \times 5H_2O$), 0.15 mg.

[3]Diets were in mash form. Phase 1 diet was fed from day1 to day7; phase 2 diet was fed from day 8 to day 21 and phase 3 diet was fed from day 22 to day 42 of experiment period.

2.2. Animals and diets

One hundred and forty crossbred weanling pigs [(Yorkshire × Landrace) × Duroc, 28 days old] with an initial body weight (BW) of 7.47 ± 1.27 kg were randomly allotted to 1 of 4 dietary treatments [5 pigs per pen (2 barrows and 3 gilts); 7 pens per treatment] based on BW and sex. Treatments consisted of pigs fed basal diet or basal diet supplemented with 5 g/kg fish oil or 1.73 g/kg powdered DHA absorbed in silica or 2.99 g/kg coated DHA. The experiment was divided into 3 phases: day 1 to day 7 (Phase 1), day 8 to day 21 (Phase 2) and day 22 to day 42 (Phase 3) in a 6-week trial. All nutrients in diets were formulated to meet or exceed the recommendation of National Research Council (2012) for weanling pigs and fed in mash form (Table 2). The additive was supplemented into the diet by partially replacing, soy oil in different phase's basal diet. All pigs were housed in an environmentally-controlled room having mechanical ventilation system and an area of 0.26 × 0.53 m^2 were allotted per pig/pen. Each pen was equipped with stainless steel feeder and a nipple drinker and pigs were offered *ad libitum* feed and water throughout the experiment. Automatic regulation of lighting was done to provide 12 h of artificial light per day. The initial ambient temperature within the room was approximately 30 °C and was gradually reduced by 1 °C during each week of the experiment.

2.3. Sampling and measurement

The individual BW and feed consumption on pen basis were determined at the start of the experiment and at the end of each phase to calculate the average daily gain (ADG), average daily feed intake (ADFI), and gain/feed ratio (G/F). To determine dry matter (DM), nitrogen (N), and energy digestibility, chromium oxide was added to the diet as an indigestible marker at 2 g/kg of the diet for 7 days prior to fecal collection. Fecal samples were collected randomly from at least two pigs (one barrow and one gilt) from each pen and were mixed and pooled, and a representative sample was stored in a freezer at −20 °C until analyzed. All feed and fecal samples were freeze-dried and finely ground to pass through a 1 mm screen. Following the procedure established by Association of Official Analytical Chemists (AOAC International, 2000), diet samples were analyzed for DM (method 930.15,), crude protein (N × 6.25; method 968.06;), crude fat (method 954.02;) ash (method 942.05), acid detergent fiber (ADF, method 973.18). The neutral detergent fiber (NDF) assayed with heat stable amylase (aNDF) was determined using the method established by Van Soest et al. (1991). Fecal samples were also analyzed for DM (method 930.15), N (method 988.05) following the procedures established by AOAC International (2000). Gross energy was determined by measuring the heat of combustion in the samples using a Parr 6100 oxygen bomb calorimeter (Parr instrument Co., Moline, IL, USA). Apparent total tract digestibility of DM, N, fat and energy were calculated using indirect methods described by Williams et al. (1962).

The analysis of FA profile of unrefined fish oil, DHA (powdered and coated forms)was done by using an HP 5890 gas chromatography with a flame ionization detector (Hewlett Packard 5890 Series 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 analyzed samples were identified.

Two pigs each pen (1 barrow and 1 gilt) from randomly selected four pens per treatmentwere bled via jugular venipuncture using a sterile needle. Blood samples from the same pigs per treatment on days 7, 21 and day 42 were collected into a 5 mL vacuum tubes containing no additive and tubes containing K_3EDTA (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA) to obtain serum and whole blood respectively. Serum was separated by centrifugation at 3000 × *g* for 15 min at 4 °C. Automatic blood analyzer (ADVIA 120; Bayer, Tarrytown, NY, USA) was used to determine the concentrations of White blood cell (WBC), Red Blood cells (RBC) and lymphocyte from whole blood samples. The lymphocytes were expressed as a percentage of total WBC. For the determination of serum C - reactive protein (CRP), commercial enzyme linked immune- sorbent assay (ELISA) kits (IBL International GmbH, Hamburg, Germany) was used.

At days 7, 21 and 42, fecal samples were collected via massaging the rectum 8 pigs per treatment (1barrow and 1 gilt each) from randomly selected 4 pens/treatment. They were the same piglets used for nutrient digestibility analysis. The samples were pooled on a pen basis, placed on ice and immediately transported to the laboratory for microbial analysis. For the dilution of microbes in the faeces sample, 1 g of fecal sample was mixed with 9 mL of 10 g kg^{-1} peptone broth (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) and homogenized properly. Counts of viable bacteria in the fecal samples were determined by plating serial 10-fold dilutions (10^{-1} to 10^{-8}) into MacConkey agar plates (Difco Laboratories, Detroit, MI, USA) and Lactobacilli Medium III agar plates (Medium 638; DSMZ, Braunschweig, Germany) for the isolation and enumeration of coliform and lactic acid bacteria respectively. The Lactobacilli Medium III agar plates were then incubated for 48 h at 39 °C under anaerobic conditions, while the MacConkey agar plates were incubated for 24 h at 37 °C. The colonies of coliform and lactic acid bacteria were counted immediately after removal from the incubator. The colonies were identified based on morphology, color and lactose fermentation ability.

For the evaluation of noxious gases in pigs slurry, fresh fecal and urine samples were collected at the end of the experiment from 8 pigs per treatment (1barrow and 1 gilt each) from randomly selected 4 pens/treatment and analyzed according to the method described by Cho et al. (2008). Briefly, the faeces sample was collected via massaging the rectum and urine was collected in a bucket via a funnel below the pen after moving them in individual crate. After the collection, faeces as well as urine samples were pooled on pen basis. Thereafter, 150 g faeces and 150 g of urine were mixed well; 1:1 on the wet weight basis) and stored in 2.6-L plastic boxes having a small hole in the middle of one side wall and sealed with adhesive plaster. The samples were then allowed to ferment for 1 day at room temperature (25 °C) and the concentration of gases was determined after 24 h of fermentation. For the detection of gases, a gas sampling pump (Model GV-100; Gastec Corp., Ayase, Japan) and(Gastec detector tube No. 3La for NH3, No. 4 L K for H2S, and No. 70

for mercaptans; Gastec Corp.) were used. The slurry samples were shaken manually for approximately 30 s to disrupt any crust formation on the surface of the slurry sample and to homogenise them before the measurement. The adhesive plasters were punctured, and 100 mL of headspace air was sampled in duplicates approximately 2.0 cm above the slurry surface and then the average was calculated.

2.4. Statistical analysis

Data were analyzed as a completely randomized block design using GLM procedures (SAS Institute Inc, 2002). For all response criteria, the pen served as the experimental unit. Differences among the treatment means were determined by using the Tukey's multiple-comparison test. Before carrying out statistical analysis of the microbial counts, logarithmic conversion of the data was performed. Variability in data was expressed as standard error of means (SEM). Pre-planned comparisons were used to test the main effects of PUFA i) Control/basal diet group vs Fish oil supplemented group, powdered DHA absorbed in silica or coated DHA group (Con vs FO, pDHA, cDHA), ii) Fish oil supplement vs powdered DHA absorbed in silica supplemented group (FO vs pDHA) and iii) powdered DHA vs coated DHA (pDHA vs cDHA). The significance level was set at $P < 0.05$.

3. Results

3.1. Fatty acid profile

The analyzed DHA, EPA and other fatty acid contents of unrefined fish oil and refined fish oil enriched in DHA (in two different forms; silica impregnated powdered DHA and coated DHA is presented in Table 1.

3.2. Growth performance

The effect of fish oil and DHA on growth performance of weaner pigs is presented in Table 3. Addition of fish oil or DHA to the diet of weaner pigs significantly increased ($P < 0.05$) BW on day 7, 21, and 42 compared with the pigs fed control diet. The ADG was increased ($P < 0.05$) during days 1−7and 22−42, and the overall experiment period (days 1−42) as well; and the ADFI during day 1−7 and G:F during the overall experiment period were higher ($P < 0.05$) in pigs receiving the diets supplemented with fish oil or DHA as compared with the pigs fed the control diet. Among the omega- 3 fatty acid source, the BW during days 7 and 42, ADG during day 1−7 and the overall experimental period and G:F during the overall experimental period were higher ($P < 0.05$) fin pigs fed with the diet containing DHA than the pigs fed with the fish oil supplemented diet. The ADG, ADFI and G:F values were comparable between coated DHA and DHA impregnated in silica.

Table 3
Effect of fish oil and docosahexaenoic acid (DHA) supplementation on growth performance in weaner pig[1].

Items	CON	FO	pDHA	cDHA	SEM	*P* - Value			
						Overall	CON vs others	FO vs DHAs	pDHA vs cDHA
Body weight, kg									
Initial	7.47	7.46	7.46	7.46	0.005	0.396	0.101	0.731	0.843
Day 7	8.86^{c}	9.00^{b}	9.09^{ab}	9.13^{a}	0.036	<0.001	<0.001	0.028	0.483
Day 21	13.59^{b}	13.9^{ab}	14.05^{a}	14.18^{a}	0.145	0.052	0.014	0.239	0.529
Day 42	23.72^{c}	24.09^{bc}	24.58^{ab}	24.92^{a}	0.250	0.016	0.011	0.047	0.353
Day 1−7									
ADG, g	198^{c}	219^{b}	232^{ab}	238^{a}	5.52	<0. 001	<0.001	0.032	0.460
ADFI, g	216^{b}	233^{ab}	246^{a}	248^{a}	8.96	0.078	0.024	0.199	0.868
G:F	0.923	0.944	0.951	0.957	0.024	0.799	0.337	0.737	0.855
Day 8−21									
ADG, g	338	350	355	361	9.51	0.377	0.120	0.527	0.638
ADFI, g	417	431	426	431	12.08	0.828	0.384	0.834	0.792
G:F	0.810	0.813	0.834	0.84	0.010	0.123	0.103	0.061	0.667
Day 22−42									
ADG, g	482^{b}	485^{ab}	501^{ab}	511^{a}	8.63	0.095	0.002	0.127	0.471
ADFI, g	682	685	694	699	11.12	0.694	0.412	0.411	0.782
G:F	0.707^{b}	0.709^{b}	0.722^{ab}	0.732^{a}	0.007	0.068	0.119	0.058	0.337
Overall (Day 1−42)									
ADG, g	387^{c}	396^{bc}	408^{ab}	416^{a}	5.99	0.016	0.011	0.049	0.339
ADFI, g	516	525	530	534	8.25	0.461	0.167	0.509	0.744
G:F	0.750^{c}	0.754^{bc}	0.769^{ab}	0.778^{a}	0.006	0.019	0.023	0.021	0.316

[1]Abbreviation: CON, Basal diet; FO, CON + 5 g/kg Fish oil; pDHA, CON + 1.73 g/kg Silica impregnated powdered docosahexaenoic acid; cDHA, CON + 2.99 g/kg coated docosahexaenoic acid' ADG; average daily gain, ADFI; average daily feed intake; G:F; gain : feed ratio, SEM; standard error of means.
[a,b]Means in the same row with different superscripts differ ($P < 0.05$).

3.3. Nutrient digestibility

The effect of fish oil and DHA on the co-efficient of nutrient digestibility of weaner pigs is presented in Table 4. A significant increase ($P < 0.05$) in fat digestibility was observed in pigs receiving coated and silica impregnated powdered DHA supplemented diet compared with the pigs fed basal diet at day 42 of the experiment. However, dietary treatments did not have any effects on digestibility of DM, N and energy.

3.4. Fecal coliform and lactic acid bacteria counts

The results showed that supplementing the diets with fish oil or coated DHA/silica impregnated DHA did not have any effects ($P > 0.05$) on the fecal coliform and lactic acid bacteria counts as compared with the pigs fed with control (Table 5).

3.5. Fecal noxious gases emission

Feeding the pigs with the diets containing fish oil or coated DHA/silica impregnated DHA did not affect the emission of fecal NH_3, total mercaptans and H_2S ($P > 0.05$) compared with the pigs fed with control diet (Table 6).

3.6. Blood profile

Analysis of blood sample (Table 7) showed that the C-reactive protein values were significantly lower ($P < 0.05$) in the pigs fed with the diet supplemented with fish oil or coated DHA/silica impregnated DHA compared with the pigs fed control diet during days 7, 21 and 42. There was not any significant effect ($P > 0.05$) on RBC and WBC counts and lymphocyte percentage.

4. Discussion

The inclusion of fish oil in the diet of weaner pig has been reported to have significant beneficial effects on growth and immunity compared to the diets with lower n-3 PUFA content but with same caloric value (Huber et al., 2018). A study by Matsunaga et al. (2009) noted that supplementing the diet with ingredients rich in n-3 PUFA reduced the inflammation of gut that can occur during the weaning period, and enhanced epithelial resistance and membrane integrity in weaner pigs and overcome the challenge of transition from liquid milk to solid feed. The present study investigated the effects of unrefined fish oil and refined fish oil enriched with DHA in silica impregnated powdered form or lipid matrix coated form on growth performance, nutrient digestibility and immune status of weaner pigs. Dietary inclusion of 5 g/kg unrefined fish oil had a significant effect on BW and ADG, and overall G: F ratio of weaner pigs compared to the control diet. In agreement to this study, previous studies (Leskanich et al., 1997; Schellingerhout, 2002) also demonstrated that addition of fish oil improved ADG and feed efficiency in pigs and suggested that the degree of unsaturation of experimental diet compared with control diet was the reason for improved performance. In contrast, non-significant effects of fish oil addition in pigs on growth performance were reported in some other studies (Overland et al., 1996; Lauridsen et al., 1999; Lee et al., 2019). In addition, coated DHA as well as silica impregnated powdered DHA supplementation increased BW, overall ADG and feed efficiency in weaner pigs compared with the pigs fed fish oil indicating that DHA enriched fatty acid is more effective in improving the performance of animals. In our previous study, supplementation of 0.75 % coated linseed oil as a source of omega-3 fatty acid in the diet improved overall growth rate in growing-finishing pigs (Upadhaya et al., 2016). Preventing from rancidity by coating and the gradual release of DHA in the gut and reaching to the appropriate site of absorption and making it more bioavailable for absorption and metabolism might be the probable reason for improved growth performance in the present study.

In the present study, a slight improvement in the digestibility of fat was observed in the pigs fed the diet supplemented with fish oil and a significant improvement in fat digestibility was observed in pigs fed DHA containing diets either in powdered or coated forms compared with control. Different studies suggested that the digestion, absorption and metabolism of fat is highly influenced by gut microenvironment and gut microbiota (Li and Chiang, 2015; Niot et al., 2009). Generally there is an uneven distribution of fatty acids along the gut and the alterations in their metabolism may cause mucosal inflammation and other gastrointestinal disorders. The gastrointestinal function depends on the bioavailability of fatty acids which can eventually improve gut function via interaction with

Table 4
Effect of fish oiland docosahexaenoic acid (DHA) supplementation on co-efficient of apparent total tract nutrient digestibility in weaner pig[1].

Items	CON	FO	pDHA	cDHA	SEM	*P* - Value			
						Overall	CON vs others	FO vs DHAs	pDHA vs cDHA
Dry Matter	0.812	0.822	0.816	0.823	0.0042	0.271	0.103	0.620	0.332
Nitrogen	0.797	0.806	0.798	0.811	0.0043	0.138	0.148	0.758	0.062
Energy	0.813	0.817	0.814	0.821	0.0032	0.305	0.253	0.915	0.135
Fat	0.715[b]	0.726[ab]	0.738[a]	0.739[a]	0.0067	0.090	0.032	0.153	0.895

[1]Abbreviation: CON, Basal diet; FO, CON + 5 g/kg Fish oil; SDA, CON + 1.73 g/kg Silica impregnated powdered docosahexaenoic acid; CDA, CON + 2.99 g/kg coated docosahexaenoic acid, SEM; standard error of means.
[a,b] Means in the same row with different superscripts differ ($P < 0.05$).

Table 5
Effect of fish oil and docosahexaenoic acid (DHA) supplementation on fecal coliform and lactic acid bacteria counts in weaner pig[1].

Items, log_{10}cfu/g	CON	FO	pDHA	cDHA	SEM	*P* - Value			
						Overall	CON vs others	FO vs DHAs	pDHA vs cDHA
Day 7									
Lactic acid bacteria	7.32	7.38	7.4	7.43	0.058	0.619	0.248	0.663	0.727
Coliform	5.96	5.93	5.92	5.89	0.099	0.969	0.690	0.835	0.877
Day 21									
Lactic acid bacteria	7.38	7.41	7.48	7.51	0.055	0.344	0.192	0.234	0.687
Coliform	6.06	5.97	6.02	5.95	0.067	0.691	0.347	0.895	0.498
Day 42									
Lactic acid bacteria	7.45	7.49	7.53	7.54	0.040	0.422	0.157	0.438	0.798
Coliform	6.12	6.09	6.14	6.04	0.051	0.573	0.573	0.937	0.217

[1]Abbreviation: CON, Basal diet; FO, CON + 5 g/kg Fish oil; pDHA, CON + 1.73 g/kg Silica impregnated powdered docosahexaenoic acid; cDHA, CON + 2.99 g/kg coated docosahexaenoic acid, SEM; standard error of means.

Table 6
Effect of fish oil and docosahexaenoic acid (DHA) supplementation on gas emission in weaner pig[1].

Items, ppm	CON	FO	pDHA	cDHA	SEM	*P* - Value			
						Overall	CON vs others	FO vs DHAs	pDHA vs cDHA
Total mercaptans	3.30	3.13	3.16	3.08	0.198	0.876	0.460	0.887	0.805
Ammonia	7.35	7.32	7.38	7.27	0.045	0.465	0.655	0.878	0.144
H_2S	5.17	4.59	4.82	4.24	0.318	0.271	0.123	0.876	0.226
Acetic acid	1.78	2.45	2.08	2.20	0.325	0.552	0.246	0.453	0.325

[1]Abbreviation: CON, Basal diet; FO, CON + 5 g/kg Fish oil; pDHA, CON + 1.73 g/kg Silica impregnated powdered docosahexaenoic acid; cDHA, CON + 2.99 g/kg coated docosahexaenoic acid, SEM; standard error of means.

Table 7
Effect of fish oil and docosahexaenoic acid (DHA) supplementation on blood profiles in weaner pig[1].

Items	CON	FO	pDHA	cDHA	SEM[2]	*P* - Value			
						Overall	CON vs others	FO vs DHAs	pDHA vs cDHA
Day 7									
WBC, $10^3/\mu\ell$	16.97	19.98	18.22	20.45	1.231	0.235	0.103	0.678	0.233
RBC, $10^6/\mu\ell$	1.59	1.69	1.75	1.80	0.200	0.890	0.512	0.733	0.871
Lymphocyte, %	30.67	31.73	31.50	31.03	1.717	0.971	0.713	0.829	0.852
C-reactive protein, mg/L	22.9	20.0	19.3	19.0	1.320	0.205	0.047	0.591	0.856
Day 21									
WBC, $10^3/\mu\ell$	18.46	20.37	19.89	20.79	1.794	0.812	0.385	0.989	0.732
RBC, $10^6/\mu\ell$	1.64	1.80	1.90	1.87	0.167	0.707	0.295	0.684	0.910
Lymphocyte, %	34.29	35.38	35.71	35.77	2.190	0.958	0.611	0.897	0.984
C-reactive protein, mg/L	24.2	22.1	21.4	21.0	1.014	0.186	0.047	0.458	0.774
Day 42									
WBC, $10^3/\mu\ell$	19.6	21.5	20.3	21.7	1.620	0.771	0.434	0.813	0.538
RBC, $10^6/\mu\ell$	1.8	1.9	2.0	1.9	0.154	0.791	0.398	0.700	0.755
Lymphocyte, %	36.6	37.2	37.4	38.0	1.951	0.965	0.689	0.841	0.825
C-reactive protein, mg/L	26.5	24.4	23.6	23.3	0.977	0.163	0.040	0.431	0.847

[1]Abbreviation: CON, Basal diet; FO, CON + 5 g/kg Fish oil; pDHA, CON + 1.73 g/kg Silica impregnated powdered docosahexaenoic acid; cDHA, CON + 2.99 g/kg coated docosahexaenoic acid, SEM; standard error of means, RBC; red blood cell, WBC; white blood cell.

the gut microbiota (Yu et al., 2014). Thus, the increase in fat digestion may be associated with the reduction in rancidity and increase in the bioavailability of DHA enriched fatty acid in the gut and their interaction with gut microbiota and hence improved growth performance.

Some studies have demonstrated that omega-3 FA can modify the microbiota composition, and PUFA from fish oil appeared to lead to the reduction in the population of *Frimicutes* in rats (Kaliannan et al., 2015; Yu et al., 2014). The supplementation of DHA has been shown to be effective in treating oral and gastrointestinal diseases in middle aged elderly women (Tabbaa et al., 2013) but n-3 FA supplementation from linseed did not have a detectable impact on fecal microbiota in swine (Holman et al., 2014). In the present study, inclusion of fish oil or DHA rich FA in the diet of weaner pigs however did not have significant effect on fecal coliform and lactic acid bacteria counts and fecal gases emissions compared with the pigs fed control diet. It seems that DHA modulates some specific types of micro-organisms in the gut and the modulation of microbiota by DHA is variable among different species.

Taking into account the blood profile, the WBC and RBC counts as well as lymphocyte percentage remained unaffected with the

supplementation of fish oil or DHA in the present study. In our previous study, the immunological mediators such as WBC and lymphocytes were also unaffected in the weaner pigs fed the diet containing linseed oil as a source of n-3 FA (Upadhaya et al., 2017). However, C-reactive protein (CRP) was significantly reduced in pigs receiving fish oil or DHA supplemented diet in the present study. In response to increased levels of inflammatory cytokines, a major acute phase reactant; C-reactive protein is secreted by the liver (Calabro et al., 2003) which is also is a sensitive marker for low-grade systemic inflammation (Koenig et al., 1999). In agreement to our findings, increased intake of EPA and DHA from marine source has been reported to reduce serum CRP levels (Niu et al., 2006; Darshan et al., 2009) which suggested that DHA or PUFA have anti-inflammatory properties. However, some other researches could not find detectable differences in CRP when fish oil was supplemented to the diet compared with the control diet (Madsen et al., 2003; Geelen et al., 2004). Lack of consistency in CRP concentrations among different studies with supplemental fish oil or purified DHA may be due to the differences in the amount and duration of the fish oils used, the ratio between EPA and DHA, the composition of basal diet, as well as the inflammatory status of the animals (Darshan et al., 2009).

5. Conclusions

The supplementation of fish oil as a source of poly unsaturated fatty acid as well as docosahexaenoic enriched fish oil led to beneficial effects by improving the body weight, average daily gain and feed efficiency, and enhanced fat digestibility in weaner pigs fed docosahexaenoic enriched diet compared with pigs fed basal diet. Furthermore, the C - reactive protein which is a marker of inflammation was reduced in pigs fed the diets supplemented with fish oil or docosahexaenoic indicating that the additive had positive immune effects in weaner pigs. Thus, the supplementation of docosahexaenoic enriched fish oil may contribute in reducing the problems of growth inhibition, depressed feed intake and gut disorders resulting as a consequence of different stressors faced by the weaner pigs during the transition period including the switch from liquid to solid feed intake.

CRediT authorship contribution statement

Santi Devi Upadhaya: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing - original draft. **In Ho Kim:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Validation, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing interests.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.anifeedsci.2021.114885.

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