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Supplemental effects of coated omega-3 polyunsaturated fatty acids to basal diet on improving growth performance, nutrients digestibility, fecal lactobacillus count and fecal score in weaning pigs

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ABSTRACT

Effects of dietary supplementation of coated omega-3 fatty acids (n-3FAs) on the growth performance, apparent total tract digestibility (ATTD) of nutrients, fecal scores, serum IgG and haptoglobin concentrations, and selected faecal microbial in weaning pigs was evaluated in the present study In total, 160 piglets [(Landrace x Yorkshire) x Duroc, body weight =7.42 ± 1.21 kg] were randomly assigned in a 6-week trial to four treatments: Treatments consisted of basal diet and basal diet supplemented with graded levels (5g/kg, 10g/kg and 15g/kg) of coated n-3FAs, The supplementation of basal diet with increasing levels of coated n-3FAs linearly increased the average daily gain of pig during week 1, 3 and during overall experiment period, the gain feed ratio (G/F) was linearly increased during the overall experimental period, the ATTD of dry matter (DM) and nitrogen (N) was also linearly increased at the end of experiment ($P < 0.05$). Besides, the fecal lactobacillus counts in pigs fed diet supplemented with graded level of coated n-3FAs showed trends in increment ($P = 0.055$ and $P = 0.053$, respectively) at week 3 and 6. There were no differences in serum haptoglobin and IgG concentrations, and fecal *E.coli* counts in pigs offered diet with coated n-3FAs treatment. In conclusion, the coated n-3FAs into the diet exerted positive effects in improving the growth performance, increasing the digestibility of dry matter and nitrogen, and enhanced the fecal lactobacillus counts in weaning pigs.

1. Introduction

Immature digestive and immune system are the major reasons that make the young pigs susceptible to pathogenic bacteria. In addition, nutritional, environmental and immunological stresses faced by the young pigs impair digestion and absorption of nutrients thereby reducing growth performance after weaning (Blecha et al., 1983; Lalles et al., 2007). Immature immune system exacerbated the progression of the intestinal tissue damage such as various immune-mediated hypersensitivity reactions (Dréau and Lallès, 1999; Burrin et al., 1985). Moreover, piglets are especially susceptible to infections disease. Oedema disease, caused by the Shiga-like toxin type II variant from some *Escherichia coli* strains, is prevalent during the weaning phase (Niewold et al., 2000). Usually, antibiotics or

Abbreviations: ADG, average daily body gain; ADFI, average daily feed intake; ATTD, apparent total tract digestibility; BW, body weight; DM, dry matter; N, nitrogen; GE, gross energy; n-3FAs, coated omega-3 fatty acids; TNF-α, tumor necrosis factor-α

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some metals (zinc and copper) are the regarded as the best choice for improving pig's management to keep away from stress factors. However, increased bacterial resistance to antibiotics and environmental problems caused by metals has led the European Union to enforce ban on in-feed antibiotics in 2006 and since then a drastic reduction in the levels of incorporation of copper and zinc (Ren et al., 2009; Zhang et al., 2019) has come into effect. Thus, more desirable alternative is required for weaning pigs for the enhancement of nutritional absorption.

Plant-based oils from flaxseed (also known as linseed), marine seaweed extract and fish oils are a rich source of omega-3 fatty acids (n-3FAs, Palmquist, 2009). It has a variety of anti-inflammatory and immune- modulating effects. For instance, in taking of n-3FAs have been reported to reduce inflammation in farm animal (Zhan et al., 2009; Gabler et al., 2009). Although exact mechanisms by which dietary n-3 polyunsaturated fatty acids (PUFA) modulate immune and metabolic functions in pigs are yet to be fully elucidated. A study by Li et al. (2014) indicated that dietary n-3FAs help the piglets to mitigate the immune stress at weaning.

More recently, it was hypothesized that coated n-3FAs have more advantages than traditional n-3FAs. For instance, coating may efficiently prevent the oxidation and the deterioration of the unsaturated fatty acid by the dissolution in stomach. Moreover, it also maintains effects of orally administering the unsaturated fatty acid and derivative particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) because of easy dissolution of the capsule in intestine (Sunohara et al., 2003). The aim of this study was to evaluate the effects of dietary supplementation of coated n-3FAs which were derived from refined fish oil, on growth performance, nutrient digestibility, selected blood profile, and selected fecal microbial and fecal score in weaning pigs. We anticipated that the coated n-3FAs derived from fish oil in powdered form in lower levels ranging from 5 g/kg to 10 g/kg could be effective in reducing the risk of oxidation, reducing fishy flavor and may have positive effects in improving growth performance, nutrients digestibility, and immune status of piglets.

2. Material and methods

The experiment was conducted at the swine experimental unit of Dankook University (Anseodong, Cheonan, Chungnam, South Korea). The protocol for the current experiment was approved by the Animal Care and Use Committee of Dankook University (Anseodong, Cheonan, Chungnam, South Korea).

The coated n-3FAs was obtained from a commercial company (Morningbio Co., Ltd, Cheonan, Korea), which derived from refined fish oil using method as previously described by (Watanabe et al., 2002). The coated n-3FAs contains the Eicosapentaenoic acid (EPA, 106 g/kg), and docosahexaenoic acid (DHA, 71.8 g/kg). The composition of coated n-3FAs was shown on Table 1.

2.1. Experimental Design, animals, and diets

A total of 160 crossbred weaning pigs [(Landrace × Yorkshire) × Duroc] with an average initial body weight of 7.42 ± 1.21 kg were randomly allotted in feeding trial by BW and gender according to a randomized complete design for 6 weeks. Pigs were randomly allotted to four experiment treatments as follows: CON (Basal diet); FA5 (CON + 5 g/kg coated n-3FAs); FA10 (CON +10 g/kg coated n-3FAs); FA15 (CON + 15 g/kg coated n-3FAs). There were 8 replicates per treatment with 5 pigs (three gilts and two barrows) per replicate. All diets were provided in mash form and formulated to meet or exceed the NRC - National Research Council (2012) recommendations for all nutrients (Table 2). The experiment was divided into the following three phases: Phase 1

Table 1
Coated Fatty acid composition.

Fatty acid	Content, g/kg
Caprylic acid, C8:0	0.2
Capric acid, C10:0	0.9
Lauric acid, C12:0	1.0
Tridecylic acid, C13:0	0.4
Myristic acid, C14:0	100.7
Tetradecadienoic acid, C14:2	0.2
Pentadecylic acid, C15:0	4.3
Palmitic acid, C16:0	383.5
Margaric acid, C17:0	8.8
Stearic acid, C18:0	0.3
Oleic acid, C18:1w9	204.7
Linoleic acid, C18:2w6	22.2
alpha-Linolenic acid, C18:3w3	8.6
Eicosenoic acid, C20:1w9	18.3
Docosanoic acid, C22:0	25.3
Arachidonic acid, C20:4w6	6.8
Eicosapentaenoic acid (EPA)	105.9
Docosahexaenoic acid (DHA)	71.8
Omega-3 fatty acids	186.4
Omega -6 fatty acids	29.0
Omega -9 fatty acids	223.0
Omega -6: omega-3 ratio	1:6.4

Table 2
Composition of weaning pig diets (as fed-basis).

Item	Basal diet		
	Phase 1	Phase 2	Phase 3
Ingredients, g/kg			
Extruded corn	402.9	519.1	594.8
Soybean meal (Dehulled)	97.4	170.5	224.6
Fermented soybean meal	100	55	31
LT- Fish meal	74.5	20	–
Soybean oil	23	29.1	28
Monocalcium phosphate	6.6	11	12.3
Limestone	5.8	10.2	12.3
Sugar	30	20	20
Whey protein	110	70	30
Lactose	134.6	77.8	31.8
L-Lysine – HCL	5.6	7.1	6.3
DL-Methionine	1.5	1.3	0.9
Threonine	2.1	2.9	2
Choline Chl (50 %)	1	1	1
Salt	1	1	1
Vitamin premix[1]	2	2	2
Mineral premix[2]	2	2	2
Calculated value			
Metabolizable Energy, MJ/kg	14.4	14.2	14.0
Dry matter, g/kg	910	880	990
Crude protein, g/kg	200	180	180
Crude fat, g/kg	46.7	52.5	52.6
Neutral detergent fiber, g/kg	92.2	90.8	91.4
Acid detergent fiber, g/kg	35.8	33.6	34.5
Ash, g/kg	58	58	59
Lysine, g/kg	16	15	14
Methionine, g/kg	4.8	4.0	3.5
Calcium, g/kg	8.0	8.0	8.0
Phosphorus, g/kg	6.0	6.0	6.0
Lactose, g/kg	200	120	50

[1] Provided per kilogram of complete diet: vitamin A, 11,025 IU; vitamin D_3, 1103 IU; vitamin E, 44 IU; vitamin K, 4.4 mg; riboflavin, 8.3 mg; niacin, 50 mg; thiamine, 4 mg; d-pantothenic, 29 mg; choline, 166 mg; and vitamin B12, 33 g.

[2] Provided per kilogram of complete diet: Fe (as $FeSO_4 \cdot 7H_2O$), 80 mg; Cu (as $CuSO_4 \cdot 5H_2O$), 12 mg; Zn (as $ZnSO_4$), 85 mg; Mn (as MnO_2), 8 mg; I (as KI), 0.28 mg; and Se (as $Na_2SeO_3 \cdot 5H_2O$), 0.15 mg.

(week 1 of experiment), Phase2 (week 2–3 of experiment) and Phase3 (week 4–6 of experiment). All the pigs were housed in an environmentally controlled room with a slatted plastic floor. Throughout all the experimental period, each pen was equipped with a 1-sided self-feeder and a nipple drinker to allow the pig's ad libitum access to feed and water.

2.2. Sampling and measurements

Body weight and feed consumption were measured at initially, week 1, 3 and 6 to monitor the average daily gain (ADG), average daily feed intake (ADFI) and gain/feed (G/F) ratio.

To determine the apparent total tract digestibility (ATTD) of dry matter (DM) and nitrogen (N), chromic oxide 2 g/kg was added as an inert indicator in the diet during 6th week. Fresh fecal grab samples was collected from 2 pigs per pen by rectal massage, and then stored immediately at −20 °C until analyzed. All the fecal samples were dried at 60 °C for 72 h and pass through a 1-mm screen. All feed and fecal samples were analyzed for DM (method 930.15) and N (method 990.03) following the procedures outlined by the Association of Official Analytical Chemists (AOAC - Association of Official Analytical Chemists, 2000). Gross energy (GE) was determined by measuring heat of combustion in the samples, using a bomb calorimeter (Parr 6100; Parr Instrument Co., Moline, IL, USA). Chromium was analyzed via UV absorption spectrophotometry (Shimadzu, UV-1201, Shimadzu, Kyoto, Japan) following the method described by Williams et al. (1962). The N content was determined and crude protein was calculated as N × 6.25. The ATTD was calculated using the following formula: ATTD = {1 - [(Nf × Cd)/(Nd × Cf)]}, Where Nf = nutrient concentration in faces (% DM), Nd = nutrient concentration in diet (% DM), Cd = chromium concentration in diet (% DM) and Cf = chromium dioxide concentration in faces (% DM).

Viable counts of bacteria in the faecal samples were counted from three steps as follow: 1) the faecal sample from each pig was diluted by plating serial 10-fold dilutions with 1% peptone broth (Becton, Dickinson and Co., Franklin Lakes, NJ, USA). 2) The diluted sample was established onto MacConkey agar plates (Difco Laboratories, Detroit, MI, USA) and Lactobacilli medium III agar plates (Medium 638, DSMZ, Braunschweig, Germany) to isolate the *E. coli* and *Lactobacillii*, respectively. 3) The MacConkey agar plates were incubated at 37 °C for 24 h. The Lactobacilli medium III agar plates were incubated at 39 °C for 48 h under anaerobic conditions. The

colonies of *E. coli* and *Lactobacillus* were counted immediately after removal from the incubator.

To assess the faecal scores, fresh faeces were scored (08:00 and 20:00) per day during the study according to Larson et al. (1977) that the score was distributed from 1 to 5. Briefly, 1, dry pellet; 2, formed faeces; 3, moist faeces that retains shape; 4, unformed faeces that assumes the shape of container; 5, watery liquid that can be poured.

At the end of the experiment, 16 pigs (1 barrow and 1 gilt) from each pen from each treatment were randomly selected to be bled via venepuncture using a sterile needle. Blood samples were added into a 5-ml Vacuum tube (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA). Serum samples were isolated (centrifuged at 3000 × g for 15 min) 2–4 h after collection, and serum samples were used in the IgG and haptoglobin with an automatic blood analyzer (HITACHI 747, Tokyo, Japan).

2.3. Statistical processing

The data were analyzed using the GLM procedure of SAS (2001) as a randomized complete block design. Data on growth performance and nutrients digestibility was analyzed using pen served as the experimental unit, whereas blood profile, fecal microbial and fecal score incidence was analyzed using individual pig as the experimental unit. Orthogonal comparisons were conducted using polynomial regression to measure the linear, quadratic, and cubic effects of increasing the supplementation omega-3 fatty acids. Results were expressed as the least squares means and SEM. Probability values less than 0.05 were considered significant.

3. Results

3.1. Growth performance

Weaning pigs fed diet supplemented with coated n-3FAs had higher BW than pigs fed the CON diet on week 1 (P = 0.019), 3 (P = 0.003) and 6 (P = 0.018, Table 3). Moreover, the coated n-3FAs supplementation linearly improved ADG of pigs on week 1 (P = 0.025) and 3 (P = 0.014), and G/F of pig was linearly increased on overall phase (P = 0.010) with the increase in the levels of coated n-3FAs. No differences were observed in ADFI among dietary treatment during the experiment.

3.2. ATTD of nutrients

Pigs fed graded level of coated n-3FAs linearly increased DM and N digestibility at the end of experiment (P = 0.018, P = 0.044, respectively). However, no significant differences on GE digestibility were observed among treatment (Table 4).

Table 3
Effect of coated n-3 fatty acids supplementation on growth performance in weaning pigs[1].

Items[2]	CON	FA5	FA10	FA15	SEM[3]	P-value		
						Linear	Quadratic	Cubic
Body weight, kg								
Initial	7.42	7.42	7.43	7.42	0.021	0.328	0.276	0.094
week1	8.58^{b}	8.63^{ab}	8.69^{ab}	8.72^{a}	0.038	0.019	0.719	0.882
week3	12.72^{b}	12.94^{ab}	13.11^{ab}	13.31^{a}	0.125	0.003	0.943	0.874
week6	24.61^{b}	25.04^{ab}	25.32^{ab}	25.81^{a}	0.343	0.018	0.929	0.824
week 1								
ADG, g	165	173	181	185	5.9	0.025	0.777	0.878
ADFI, g	186	192	199	200	8.8	0.235	0.760	0.853
G/F	0.89	0.90	0.91	0.93	0.015	0.101	0.862	0.961
week 3								
ADG, g	296^{b}	308^{ab}	315^{ab}	328^{a}	9.2	0.014	0.961	0.837
ADFI, g	410	420	423	431	14.1	0.281	0.931	0.872
G/F	0.72	0.74	0.75	0.76	0.019	0.136	0.997	0.981
week 6								
ADG, g	566	576	582	595	13.6	0.146	0.892	0.840
ADFI, g	838	843	850	865	16.4	0.228	0.765	0.921
G/F	0.68	0.68	0.68	0.69	0.006	0.158	0.784	0.642
Overall								
ADG, g	409^{b}	419^{ab}	426^{ab}	438^{a}	8.0	0.018	0.939	0.810
ADFI, g	555	561	566	576	9.6	0.153	0.844	0.882
G/F	0.74^{b}	0.75^{ab}	0.75^{ab}	0.76^{a}	0.060	0.010	0.702	0.800

Mean values from 8 replicates of 5 pigs per replicate pen per treatment.
^{a,b}Means in the same row with different superscripts differ (P < 0.05).
[1] Note: CON, basal diet; FA5, CON + 5 g/kg coated n-3FAs; FA10, CON + 10 g/kg coated n-3FAs; FA15, CON + 15 g/kg coated n-3FAs.
[2] Abbreviation: ADG, average daily gain; ADFI, average daily feed intake; G/F, gain/ feed.
[3] Standard error of means.

Table 4
Effect of coated n-3 fatty acids supplementation on nutrient digestibility in weaning pigs[1].

Items	CON	FA5	FA10	FA15	SEM[2]	P-value		
						Linear	Quadratic	Cubic
week 6								
Dry matter	0.82	0.83	0.84	0.85	0.812	0.018	0.895	0.852
Nitrogen	0.80	0.81	0.81	0.82	0.688	0.044	0.792	0.807
Gross Energy	0.81	0.82	0.82	0.82	0.646	0.127	0.567	0.879

Mean values from 8 replicates of 5 pigs per replicate pen per treatment.
[1] Note: CON, basal diet; FA5, CON + 5 g/kg coated n-3FAs; FA10, CON + 10 g/kg coated n-3FAs; FA15, CON + 15 g/kg coated n-3FAs.
[2] Standard error of means.

3.3. Blood profiles

Pigs fed increasing levels of coated n-3FAs supplemented diet had no effects on serum haptoglobin and IgG (Table 5).

3.4. Fecal microbial and scores

On the inclusion of graded level of coated n-3FAs showed tendency in linear increment of lactobacillus counts at week 3 and 6 (P = 0.055, P = 0.056, respectively). However, *E.coli* counts and fecal score remained unaffected (Tables 6 and 7).

4. Discussion

It is well known that all weaning pigs are challenged with different stress which consequently affects performance, nutrient absorption and health of pigs negatively (van Beers-Schreurs et al., 1998; Huang et al., 2004; Wijtten et al., 2011; Li et al., 2014). To overcome this issue, researches are focused to minimize the weaning challenges and enhance the performance and health of pigs. The present study was also focused to evaluate effects of coated n-3FAs on the growth performance, nutrients digestibility and fecal microbial and fecal score in weaning pigs. We hypothesized that the diet supplemented with increasing level of coated n-3FAs may have positive effects on growth performance, nutrients digestibility and fecal microbial as well as health benefits in weaning pigs.

Several studies indicated that the addition of n-3FAs from fish oil, marine seaweed or linseed oil in the diet of weaning pigs could improve the growth performance of piglets. For instance, a study by Liu et al. (2003) noted that 7% fish oil could lead to improved pig performance although under an immunological challenge. Another research from Zhan et al. (2009) noted that diet containing 10 % linseed oil as a source of n-3FAs may stimulate growth in growing-finishing barrows. Currently results are shown that the coated n-3FAs had beneficial effects on improving body weight and ADG of piglets during first two weeks after weaning. Similarly, in another study, dietary supplementation with 0.75 % protected n-3FAs which were derived from linseed oil promoted ADG (Upadhaya et al., 2017a). Here a beneficial mechanism is that n-3FAs could modulate immune reactions to prevent the animal suffering from an antigen overload. Such an antigen overload may result in an excessive activation of the immune system when the pigs are subjected to their new environment. We speculate that increase in the growth performance of weaning pigs in the present study may be associated with reduction in antigen overload and improvement in the digestibility of feed nutrients. Webel et al. (1997) reported that in immune system–challenged pigs, there is increase in the release of proinflammatory cytokines which cause reduced feed intake and growth performance and deterioration in feed efficiency due to altered nutrient partitioning towards infection responses. Liu et al.

Table 5
Effect of coated n-3 fatty acids supplementation on blood profile in weaning pigs[1].

Items, mg/dL	CON	FA5	FA10	FA15	SEM[2]	P-value		
						Linear	Quadratic	Cubic
week 1								
IgG	213.0	217.3	222.0	229.5	11.86	0.335	0.895	0.967
Haptoglobin	18.8	17.8	14.5	11.8	3.57	0.203	0.830	0.880
week 3								
IgG	230.5	239.0	240.5	245.0	13.81	0.485	0.888	0.875
Haptoglobin	23.5	22.0	19.3	17.8	3.54	0.290	1.000	0.891
week 6								
IgG	249.8	250.8	255.3	265.3	12.61	0.391	0.730	0.973
Haptoglobin	30.3	27.5	26.5	23.0	5.66	0.403	0.950	0.873

10 g/kg coated n-3FAs; FA15, CON + 15 g/kg coated n-3FAs.
Mean values from 8 replicates of 5 pigs per replicate pen per treatment.
[1] Note: CON, basal diet; FA5, CON + 5 g/kg coated n-3FAs; FA10, CON +.
[2] Standard error of means.

Table 6
Effect of coated n-3 fatty acids supplementation on fecal microbial in weaning pigs[1].

Items, log_{10}cfu/g	CON	FA5	FA10	FA15	SEM[2]	P-value		
						Linear	Quadratic	Cubic
week 1								
Lactobacillus	7.52	7.54	7.55	7.60	0.043	0.197	0.779	0.834
E.coli	6.46	6.44	6.43	6.40	0.061	0.463	0.830	0.609
week 3								
Lactobacillus	7.46	7.51	7.53	7.58	0.038	0.055	0.909	0.775
E.coli	6.43	6.40	6.36	6.34	0.066	0.741	0.378	0.578
week 6								
Lactobacillus	7.40	7.43	7.48	7.52	0.052	0.053	0.719	0.950
E.coli	6.36	6.32	6.31	6.28	0.047	0.110	0.968	0.555

10 g/kg coated n-3FAs; FA15, CON + 15 g/kg coated n-3FAs.
Mean values from 8 replicates of 5 pigs per replicate pen per treatment.
[1] Note: CON, basal diet; FA5, CON + 5 g/kg coated n-3FAs; FA10, CON +.
[2] Standard error of means.

Table 7
Effect of coated n-3fatty acids supplementation on fecal score in weaning pigs[1].

Items	CON	FA5	FA10	FA15	SEM[2]	P-value		
						Linear	Quadratic	Cubic
Fecal score[3]								
week 1	3.44	3.38	3.39	3.42	0.044	0.833	0.234	0.674
week 2	3.38	3.33	3.29	3.37	0.048	0.637	0.161	0.570
week 3	3.30	3.20	3.22	3.27	0.080	0.821	0.354	0.752
week 4	3.27	3.15	3.21	3.24	0.051	0.907	0.136	0.617
week 5	3.19	3.18	3.17	3.18	0.036	0.839	0.843	0.919
week 6	3.15	3.17	3.13	3.14	0.041	0.739	0.934	0.657

Mean values from 8 replicates of 5 pigs per replicate pen per treatment.
[1] Note: CON, basal diet; FA5, CON + 5 g/kg coated n-3FAs; FA10, CON + 10 g/kg coated n-3FAs; FA15, CON + 15 g/kg coated n-3FAs.
[2] Standard error of means.
[3] Score, 1 = hard, dry pellets in a small, hard mass; 2= hard, formed stool that remains firm and soft; 3= soft, formed, and moist stool that retains its shape; 4= soft, unformed stool that assumes the shape of the container; 5= watery, liquid stool that can be poured.

(2003) indicated that supplementation fish of oil alters the release of proinflammatory cytokines resulting in lower interleukin-1β (IL-1β) and prostaglandin E2 (PGE2) concentrations. Also a report by Zhan et al. (2009) noted that mRNA expression of tumor necrosis factor-α and IL-6 in muscle, adipose, and spleen, as well as serum concentration of TNF-α were decreased in pigs receiving linseed oil as a source of n-3FAs. A diet supplemented with n-3FAs had fewer white blood cells and proportion of lymphocytes and IgG concentration, decreased serum concentrations of cortisol and tumor necrosis factor-α (Huber et al., 2018).

The IgG is main antibody component in serum accounting for about 75 % of immunoglobulin. The function of IgG mainly plays a protective role in the body's immunity including antibacterial and antiviral (Burrin et al., 1985; Simister, 2003). Farm animals are readily challenged by infectious agents which comprise a number of physiologic changes, collectively known as the acute phase response at early stages of the host response. During this phase, some acute phase proteins, such as haptoglobin show rapid increase in the serum concentration (Godson et al., 1996). In the present study, the serum concentration of IgG and haptoglobin were not influenced by coated n-3FAs although a slight increment in IgG and slight reduction in haptoglobin concentrations were observed. In line with our findings, Upadhaya et al. (2017a) also demonstrated that 0.75 % protected n-3FAs had no significant effects on IgG in finishing pigs. Another study has also shown that increasing n-3FAs had no significant effects on IgG of weaning pigs (Upadhaya et al., 2017b). The slight increment and reduction in IgG and haptoglobin respectively during week 1, 3 and 6 of the experiment gives an indication that coated n-3FAs has the potential to improve the immune status of young pigs. Probably when the level of coated n-3 FA is increased, there may be significant increase in IgG and reduction in haptoglobin concentrations.

Due to immune-mediated hypersensitivity reactions (Stokes et al., 1987; Dréau and Lallès, 1999), intestinal morphology alteration inducing villous atrophy has been reported to be the main reason for maldigestion and malabsorption when pigs were weaned (Hampson and Smith, 1986; Cera et al., 1988; Hall et al., 1989). Besides, inflammatory cell infiltration to ileum is also a reason for maldigestion (Ralph et al., 2004).

Present study showed that there was significant increase in the ATTD of DM and nitrogen although energy digestibility was not affected by the addition of coated n-3FAs from fish oil. The reason for increase in digestibility of DM and nitrogen could be due to the changes in intestinal morphology, although our study did not analyze the intestinal morphology of the animals, which is a limitation of this study. Several studies suggested that n-3FAs could improve intestinal structural integrity such as higher villi and lower crypt

cell production rates to reduce the stress of piglets (Pell et al., 1994; Ralph et al., 2004). Meanwhile, pretreatment with fish oil emulsion improved the integrity of microscopic structures in the intestine through activated Adenosine 5′-monophosphate-activated protein kinase–sirtuin1 (AMPK/SIRT1) pathway against macrophage inflammation (Jing et al., 2014). Similarly, it has been reported that dietary supplementation with linseed oil can improve DM digestibility but no influence on GE digestibility in swine (Upadhaya et al., 2017a). Dietary supplementation with 3% linseed oil has shown improvement in the digestibility of dry matter and organic matter (Ueda et al., 2003). Those results corroborated with our results. In addition, a study by Huber et al. (2018) indicated that increasing content of n-3FAs improved the apparent ileal digestibility of organic matter. Moreover, improve in immunity contributed by n-3FAs helps to maintain the intestinal wall integrity (Costantini et al., 2017).

Weaning is a critical period during which marked changes may occur due to the alteration of liquid sow milk to solid feed (Rist et al., 2013). Microbial changes in the gastrointestinal tract (GIT) included Lactobacilli as well as potential pathogenic bacteria, for example, *Escherichia coli* (Etheridge et al., 1984; Pluske et al., 1997). A study of human adults supplementation with n-3FAs from Costantini et al. (2017) research that it can exert a positive action by reverting the microbiota composition, and increase anti-inflammatory compounds. A decrease in Helicobacter, Clostridiales bacterium, Sphingomonadales bacterium and Pseudomonas species Firmicutes was also reported in a mouse trial feeding fish oil (Santoru et al. (2017). Our results showed that faecal *lactobacillus* counts was increased when pig fed diet with n-3FAs supplementation, but no influence on fecal *Escherichia coli* counts. The mechanism between the gut microbiota and fatty acids is still unclear. Increased fat in diet is implicated in dysbiosis, furthermore, a decrease of microbiota richness considering the species counts per sample (Hildebrandt et al., 2009; Graham et al., 2015; Devkota et al., 2012; Costantini et al., 2017).

Meanwhile, our results suggested that the fecal score remained unaffected with coated n-3FAs supplementation. A study has shown that the short-chain fatty acids is involved in the absorption of electrolytes (particularly of sodium) and water (Hirsch et al., 1985), which might have facilitated to prevent the amount of fluid entering the large intestine, Thus, these compounds may help to increase the percentage of dry matter in the contents of the large intestine thereby reducing the incidence of diarrhea (Argenzio and Whipp, 1979; Roediger and Moore, 1981).

There are some inconsistent results such growth performance and nutrient digestibility, which is likely associated with differences in the source and levels of n-3FAs and in the age of the pigs used across different studies.

5. Conclusion

The coated n-3FAs supplementation diet had positive effects in improving growth performance, digestibility of dry matter and nitrogen during earlier stage of weaning. Furthermore, it has enhanced the faecal lactobacillus count significantly and resulted in slight improvement in serum IgG and haptoglobin in weaning pigs fed graded level of n-3FAs supplemented diets. The current study suggested that dietary supplementation with coated n-3FAs at least 15 g/kg offer an ideal nutritional strategy in piglets' performance.

CRediT authorship contribution statement

Jian Ying Zhang: Conceptualization, Formal analysis, Investigation, Methodology, Data curation, Writing - original draft. **Jing Hu:** Formal analysis, Investigation, Methodology, Data curation. **In Ho Kim:** Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

We further confirm that any aspect of the work covered in this manuscript that has involved either experimental animals or human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

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