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Effects of dietary supplementation with graded levels of omega-3 fatty acids on growth performance, nutrients digestibility, blood profile, faecal microbial in weaning pigs

Jian Ying Zhang[a,b], Xue Bin Wang[b], Jing Hu[a,b] and In Ho Kim[a]

[a]Department of Animal Resource and Science, Dankook University, Cheonan, Republic of Korea; [b]College of Life Science, Linyi University, Linyi, People's Republic of China

ABSTRACT

This experiment was conducted to determine effects of dietary supplementation with coated omega-3 fatty acids (n-3FA) on growth performance, nutrients digestibility, blood profile and faecal microflora in weaning pigs. A total of 200 weaning pigs with initial body weight (IBW, 6.97 ± 1.22 kg) were randomly assigned to four treatments: CON, basal diet; during phase 1, 2 and 3, 0.3FA, basal diet + 0.3%, 0.2%, 0.1% coated n-3FA; 0.6FA, basal diet + 0.6%, 0.4% 0.2% coated n-3FA; 0.9FA, basal diet + 0.9%, 0.6% and 0.3% coated n-3FA. When the graded level of n-3FA supplementation was added in the diet, a linearly increased average daily gain and gain/feed of the pig was observed in every phase ($p < .05$), the digestibility of dry matter (DM) was linearly increased, whereas nitrogen (N) digestibility showed a trend ($p = .0594$), the concentration of serum IgG ($p = .0886$) as well as the faecal lactobacillus count showed a linearly increase at the end of the experiment. However, there were no differences in the concentrations of serum haptoglobin and faecal microflora on among treatment. In conclusion, the coated n-3FA diet showed positive effects in improving growth performance, digestibility of DM and N in weaning pigs. Furthermore, it has a trend improvement on the count of lactobacillus in weaning pigs.

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Fatty acid; coated n-3 fatty acids; weaning pigs; growth performance; nutrients digestibility; blood profiles

Introduction

Extensive research about the importance of omega-3 fatty acids (n-3FA) has been conducted in recent years. As polyunsaturated fatty acids, n-3FA provides important health benefits to humans (Trautwein 2001; Cottin et al. 2011; Tur et al. 2012). However, it is unconvertible freely between α-linolenic acid (18:3 n-3, ALA), eicosapentaenoic acid (20:5 n-3, EPA) and docosahexaenoic acid (22:6 n-3, DHA) in the animal cell as the three major compositions of n-3FA were indicated. The majority of the research is focused on the direct intake of EPA and DHA which were thought to have more important contributions, by enrichment in animal production from flaxseed, fish oil or other marine products (Nain et al. 2012; Twining et al. 2018).

The functions of n-3FA on improving growth performance and keeping the animal healthy are noteworthy. As a novel form, powdered coated n-3FA particles are derived from plant or fish oil, which overcomes many physiology drawbacks, physical characteristics and fishery resources. For example, it reduces the influences of anti-nutrients factors and mucilage in plant seeds on growth performance and intestinal health of farm animal (Alzueta et al. 2003; Harris et al. 2009; Goyal et al. 2014). Furthermore, it eliminates dependencies on fish oil because fishery resources are becoming increasingly scarce (Jaturasitha et al. 2007). Eventually, the maximum content limits of it in diet were broken which have a positive proportional relationship with animal production enriched with n-3FA since oil characteristics influences the palatability and storage of dietary products, which were assigned <10% in general, such as 2.4% canola oil (Nain et al. 2012) and 5% flaxseed oil (Ehr et al. 2017).

A major challenge of post-weaning is that weaning pigs are readily challenged by pathogenic bacteria and have to face nutritional, environmental and immunological stresses (Montagne et al. 2007; Li et al. 2014). Diarrhoea mostly caused by *enterotoxigenic Escherichia coli* (ETEC) is the most common enteric disease in piglets, accounting for 50% of mortality in piglets (Owusu-Asiedu et al. 2003). Improving the health condition of piglets is not only cost-effective but also suitable for sustainable pork production. Thus, the effects of coated n-3FA by graded level supplementation in the present experiment were to determine the optional content in the diet. We anticipated that the coated n-3FA in powder form could have positive effects for the improvement of growth performance, nutrients digestibility and health status of weaning pigs. Therefore, the aim of this study was to evaluate the effects of dietary supplementation of coated n-3FA on growth performance, nutrient digestibility, blood profile and faecal microbial in weaning pigs (Table 1).

Materials and methods

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

CONTACT In Ho Kim inhokim@dankook.ac.kr Department of Animal Resource and Science, Dankook University, Cheonan, Choongnam 330-714, South Korea

Table 1. Fatty acid composition of coated n-3 fatty acids.

Fatty acids	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
n-3 fatty acids	18.64
n-6 fatty acids	2.90
n-9 fatty acids	22.30
n-6:n-3 ratio	1:6.4

The coated n-3FA derived from fish oil were provided by a commercial company (Morningbio Co. Ltd, Cheonan, Korea). According to the information provided by the suppliers, the Docosahexaenoic acid and Eicosapentaenoic acid concentrations in the coated n-3FAs were 7.18% and 10.60%, respectively. The ratio of n-6 to n-3 is 1:6.4.

Animals and diets

A total of 200 weaning pigs [(Landrace × Yorkshire) × Duroc] with an average initial body weight (IBW) of 6.97 ± 1.22 kg were randomly assigned by BW and gender according to a randomized complete block design in a 6-week feeding trial with three phases. Pigs were randomly allotted to four experiment treatments as follows: (1) CON, basic diet; (2) 0.3FA, CON + phase 1 (0.3% coated n-3FA), phase 2 (0.2% coated n-3FA), phase 3 (0.1% coated n-3FA); (3) 0.6FA, CON + phase 1 (0.6% coated n-3FA), phase 2 (0.4% coated n-3FA), phase 3 (0.2% coated n-3FA); (4) 0.9FA, CON + phase 1 (0.9% coated n-3FA), phase 2(0.6% coated n-3FA), phase 3 (0.3% coated n-3FA). Phase 1 was thought during week 1; phase 2 was thought during weeks 2 and 3. Phase 3 was thought during weeks 5 and 6. Each treatment had 10 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 (2012) recommendations for all nutrients (Tables 2–4). All animals were housed in an environmentally controlled nursery facility with settled plastic flooring and mechanical ventilation system. The target room temperature and humidity were maintained at 24°C and 60%, respectively. Each pen was equipped with a self-feeder and nipple water. All the pigs had free access to their corresponding diets during the experimental period.

Sampling and measurement

Body weight (BW) was measured individually at the initial (0 day), 1st wk, 3rd wk and 6th wk of the experimental period.

Table 2. Composition of weaning pig diets.

		Phase 1		
Items	CON	0.3FA	0.6FA	0.9FA
Ingredients (%)				
Extruded corn	40.29	40.10	39.88	39.68
Soybean meal (dehulled)	9.74	9.76	9.80	9.83
Fermented soybean meal	10.00	10.00	10.00	10.00
LT fish meal	7.45	7.45	7.45	7.45
Soy oil	2.30	2.17	2.05	1.92
Coated n-3 fatty acids	–	0.30	0.60	0.90
Monocalcium phosphate	0.66	0.66	0.66	0.66
Limestone	0.58	0.58	0.58	0.58
Sugar	3.00	3.00	3.00	3.00
Whey protein	11.00	11.00	11.00	11.00
Lactose	13.46	13.46	13.46	13.46
L-Lysine – HCL	0.56	0.56	0.56	0.56
DL-methionine	0.15	0.15	0.15	0.15
Threonine	0.21	0.21	0.21	0.21
Choline Chl 50%	0.10	0.10	0.10	0.10
Salt	0.10	0.10	0.10	0.10
Vitamin premix[†]	0.20	0.20	0.20	0.20
Mineral premix[‡]	0.20	0.20	0.20	0.20
Calculated value				
Metabolizable energy, kacl/kg	3450	3450	3450	3450
Crude protein, %	20.00	20.00	20.00	20.00
Fat, %	4.67	4.78	4.90	5.01
Lysine, %	1.60	1.60	1.60	1.60
Methionine, %	0.48	0.48	0.48	0.48
Calcium, %	0.80	0.80	0.80	0.80
Phosphorus, %	0.60	0.60	0.60	0.60
Lactose, %	20.00	20.00	20.00	20.00

[†]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, 29 mg; choline, 166 mg and vitamin B12, 33 g.

[‡]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

Feed consumption was recorded on a pen basis during the experiment to calculate the average daily gain (ADG), average daily feed intake (ADFI) and gain/feed ratio (G/F).

Chromium oxide (Cr_2O_3) was added to the diet at 0.2% of the diet as an indigestible marker for 7 days prior to faecal collection on week 6 to calculate apparent total tract digestibility (ATTD) of dry matter (DM), nitrogen (N) and gross energy (GE). Faecal grab samples were collected randomly from at least two pigs in each pen (one gilt and one barrow), and all fresh faecal samples were pooled by pen and mixed, and immediately stored at −20°C until analysis. Before chemical analysis, the feed and fresh faecal samples were thawed and dried for 72 h at 60°C in a forced-air oven (model FC-610, Advantec, Toyo Seisakusho Co. Ltd, Tokyo, Japan), after which they were finely ground to a size that could pass through a 1 mm screen. Then all the feed and faecal samples were analysed for DM (method 930.15), and N (method 990.03) following the procedures outlined by the association of Official Analytical Chemists International AOAC (2010). E was determined by measuring the heat of combustion in the samples, using a bomb calorimeter (Parr 6100; Parr Instrument Co., Moline, IL, USA). Chromium was analysed via UV/VIS spectrophotometer (Optizen POP, Korea) (Williams et al. 1962). For calculating the ATTD of the nutrients, we used the following formula: Digestibility=1 − [(Nf × Cd)/(Nd × Cf)]×100, where Nf is the concentration of nutrient in faecal (% DM), Nd the concentration of nutrient in the diet, Cd the concentration of chromium in the diet and Cf the concentration of chromium in the faecal.

Table 3. Composition of weaning pig diets.

		Phase 2		
Item	CON	0.3FA	0.6FA	0.9FA
Ingredients (%)				
Extruded corn	51.91	51.76	51.61	51.48
Soybean meal (dehulled)	17.05	17.08	17.11	17.13
Fermented soybean meal	5.50	5.50	5.50	5.50
LT fish meal	2.00	2.00	2.00	2.00
Soy oil	2.91	2.83	2.75	2.66
Coated n-3 fatty acids	–	0.20	0.40	0.60
Monocalcium phosphate	1.10	1.10	1.10	1.10
Limestone	1.02	1.02	1.02	1.02
Sugar	2.00	2.00	2.00	2.00
Whey protein	7.00	7.00	7.00	7.00
Lactose	7.78	7.78	7.78	7.78
L-Lysine – HCL	0.71	0.71	0.71	0.71
DL-methionine	0.13	0.13	0.13	0.13
Threonine	0.29	0.29	0.29	0.29
Choline Chl 50%	0.10	0.10	0.10	0.10
Salt	0.10	0.10	0.10	0.10
Vitamin premix[†]	0.20	0.20	0.20	0.20
Mineral premix[‡]	0.20	0.20	0.20	0.20
Calculated value				
Metabolizable energy, kacl/kg	3400	3400	3400	3400
Crude protein, %	18.00	18.00	18.00	18.00
Fat, %	5.25	5.33	5.41	5.48
Lysine, %	1.50	1.50	1.50	1.50
Methionine, %	0.40	0.40	0.40	0.40
Calcium, %	0.80	0.80	0.80	0.80
Phosphorus, %	0.60	0.60	0.60	0.60
Lactose, %	12.00	12.00	12.00	12.00

[†]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, 29 mg; choline, 166 mg and vitamin B12, 33 g.

[‡]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.

At the end of the experiment, blood samples were collected into clot activator vacuum tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA) from two pigs (one male and one female) in each pen. Serum samples were isolated (centrifuged at $3000 \times g$ for 15 min) for 1 h after collection, and serum samples were maintained at −4°C until used. Serum IgG was determined using an automatic biochemistry analyzer (Hitachi 747, Hitachi, Tokyo, Japan). Serum haptoglobin concentrations were assayed using an enzyme-linked immunosorbent assay kit (TP801; Tri-Delta Diagnostics, Inc., Morris Plains, NJ, USA).

At the last day of week 6, faecal samples were collected directly via massaging the rectum from eight (four barrows and four gilts) randomly selected pigs from four pens per treatment and then pooled them on a pen basis and placed on ice for transportation to the laboratory, where analysis was immediately carried out. One gram of the composite fresh faecal sample from each pen was diluted with 9 mL of 1% peptone broth (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) and then homogenized. Viable counts of bacteria in the faecal samples were then conducted by plating serial 10-fold dilutions (in 1% peptone solution) onto MacConkey agar plates (Difco Laboratories, Detroit, MI, USA) and Lactobacilli medium III agar plates (Medium 638; DSMZ, Braunschweig, Germany) to isolate the *E. coli*, *Clostridium perfringens*, *Salmonella* and *Lactobacillus*, respectively.

The lactobacilli medium III agar plates were then incubated for 48 h at 39°C under anaerobic conditions. The MacConkey agar plates were incubated for 24 h at 37°C. The *E. coli*, *C. perfringens*, *Salmonella* and *Lactobacillus* colonies were counted immediately after removal from the incubator.

Statistical methods

The data were analysed using the GLM procedure of SAS as a randomized complete block design. Pen served as the experimental unit. Linear and quadratic polynomial contrasts were used to examine the effect of dietary treatment with coated n-3FA in the basal diet. Variability in the data was expressed as the standard error of means (SEM) and $p<.05$ was considered to be statistically significant.

Results

Growth performance

The effects of growth performance are shown in Table 5. When the pig was fed coated n-3FA, the ADG was linearly increased in weeks 1, 3, 6 and overall phases ($p < .05$). Meanwhile, the G/F of pigs was linearly improved in week 6 and the overall phase for pigs fed coated n-3FA ($p < .05$). There are trends increasing in G/F at week 3 and ADF at week 1 for pig feeding coated n-3FA ($p = .0552$ and $p = .0514$, respectively).

Nutrients digestibility

The results of nutrients digestibility are shown in Table 6. Pigs fed a diet supplemented with coated n-3FA linearly improved their digestibility of DM ($p < 0.05$) and tended to improve the digestibility of N compared with pigs fed a diet without any supplements ($p = .0594$). No significant differences on the digestibility of GE were observed among treatment.

Blood profiles

The results of blood profiles are shown in Table 7. Supplementation of coated n-3FA tended to increase the concentration of IgG on weeks 1 ($p = 0.0918$) and 6 ($p = 0.0886$) compared with pigs that did not receive additional n-3FA. However, the concentration of haptoglobin was not influenced by coated n-3FA in the diet.

Faecal microbial

The effects on faecal microbial are shown in Table 8. The diet supplement of coated n-3FA linearly increased the lactobacillus count compared with pigs fed a diet without any supplements at the end of the experiment ($p < .05$). There were no significant changes on the count of *E. coli*, clostridium and salmonella in relation to coated n-3FA among different treatments at every experiment stage.

Discussion

As described above, the use of n-3FA has increased considerably in recent years as growth promoter because of the reported beneficial health and performance effects. In the

Table 5. Effect of coated n-3FA supplementation on growth performance in weaning pigs[†].

Items	CON	0.3FA	0.6FA	0.9FA	SEM[‡]	*p*-value	
						Linear	Quadratic
Body weight, kg							
Initial	6.97	6.97	6.98	6.97	0.00	1.0000	.5863
Week 1	8.04[ab]	8.10[ab]	8.16[ab]	8.20[a]	0.04	.0098	.7945
Week 3	12.04[b]	12.20[ab]	12.45[a]	12.53[a]	0.13	.0055	.7306
Week 6	24.18[b]	24.63[ab]	24.97[ab]	25.41[a]	0.27	.0024	.9867
Week 1							
Average daily gain, g	153[b]	162[ab]	168[ab]	175[a]	6	.0100	.8826
Average daily feed intake, g	166	175	182	189	8	.0514	.9433
Gain/feed	0.931	0.929	0.935	0.926	0.024	.9286	.8937
Week 3							
Average daily gain, g	286	292	307	310	9	.0421	.8343
Average daily feed intake, g	381	386	391	391	13	.5421	.8676
Gain/feed	0.750	0.758	0.787	0.794	0.018	.0552	.9955
Week 6							
Average daily gain, g	574	589	597	614	14	.0457	.9197
Average daily feed intake, g	866	870	874	882	19	.5399	.9234
Gain/feed	0.663[b]	0.678[ab]	0.683[ab]	0.697[a]	0.008	.0080	.6439
Overall							
Average daily gain, g	409[b]	421[ab]	428[ab]	439[a]	6	.0022	.9441
Average daily feed intake, g	560	563	567	571	10	.4052	.9804
Gain/feed	0.732[b]	0.748[ab]	0.755[ab]	0.769[a]	0.008	.0036	.9576

[†]CON, Basal diet; 0.3FA, CON + phase 1 (0.3% coated n-3FA), phase 2 (0.2% coated n-3FA), phase 3 (0.1% coated n-3FA); 0.6FA, CON + phase 1 (0.6% coated n-3FA), phase 2 (0.4% coated n-3FA), phase 3 (0.2% coated n-3FA); 0.9FA, CON + phase 1 (0.9% coated n-3FA), phase 2 (0.6% coated n-3FA), phase 3 (0.3% coated n-3FA).
[‡]Standard error of means.
[a,b]Means in the same row with different superscripts differ ($p < .05$).

current study, dietary supplementation with coated n-3FA could improve the growth performance of pigs even though n-3FA composited in a different source. A diet supplemented with n-3FA in a study by Upadhaya et al. (2015) indicated that pigs improved their ADG despite linseed oil as a source of n-3FA. Liu et al. (2003) demonstrated in a weaned pig study that fish oil-derived n-3FA improved ADG and ADFI. Moreover, Zhan et al. (2009) showed that increased duration of feeding linseed-derived n-3FA linearly improved ADG and feed efficiency. In the present study, coated n-3FA showed positive effects on improving the growth performance of weaned pigs, including ADG and feed efficiency. Similarly, in a recent study by Upadhaya et al. (2017), they noted that increasing the content of the coated n-3FA in weaned diets led to an increase in BW, ADG and feed:gain ratio, whereas the ADFI was not affected.

It was speculated that this improvement in performance could be associated with the improved health condition of pigs. Under commercial conditions of pig production, pigs undergo disease challenges leading to the production of numerous immune system-related compounds such as acute-phase proteins and cytokines (Wright et al. 2000; Paradis 2012; Rakhshandeh and Lange 2012). Moreover, Upadhaya et al. (2015) noted that the increased release of proinflammatory cytokines in the immune system-challenged pigs is an important reason for reducing feed intake and growth performance and deterioration in feed efficiency. Fish oil supplementation could alter the release of proinflammatory cytokines that was demonstrated by Liu et al. (2003), which might lead to improved pig performance. In his research, fish oil-derived n-3FA decreased prostaglandin E2 (PGE2) production following lipopolysaccharide (LPS)-induced immune system stimulation in a weaned pig study. Moreover, Zhan et al. (2009) showed that increased duration of feeding linseed-derived n-3FA linearly decreased gene expression of inflammatory cytokines such as tumour necrosis factor α (TNF-α).

Our experiment demonstrated that coated n-3FA had little effect on the increasing concentration of serum IgG and had no effect on serum haptoglobin. A study by Huber et al. (2018) demonstrated that anti-OVA IgG tended to be reduced with increasing fish oil supplementation. After LPS challenge, the n-3FA supplementation independently reduced the numbers of white blood cells, serum concentrations of cortisol and the TNF-α (Upadhaya et al. 2015). Among LPS-treated pigs, pigs fed fish oil had lower IL-1β and PGE2, and higher IGF-I compared with those fed basal diet (Liu et al. 2003). Pigs received diets supplemented with 5% fish oil had a lower rectal

Table 6. Effect of coated n-3FA supplementation on nutrient digestibility in weaning pigs[†].

Items, %	CON	0.3FA	0.6FA	0.9FA	SEM[‡]	*p*-value	
						Linear	Quadratic
Week6							
Dry matter	83.59[b]	84.38[ab]	85.13[ab]	85.89[a]	0.54	.0049	.9810
Nitrogen	81.79	82.38	82.96	83.64	0.69	.0594	.9478
Energy	82.45	83.36	83.87	84.53	0.94	.1230	.8943

[†]CON, basal diet; 0.3FA, CON + phase 1 (0.3% coated n-3FA), phase 2 (0.2% coated n-3FA), phase 3 (0.1% coated n-3FA); 0.6FA, CON + phase 1 (0.6% coated n-3FA), phase 2 (0.4% coated n-3FA), phase 3 (0.2% coated n-3FA); 0.9FA, CON + phase 1(0.9% coated n-3FA), phase 2 (0.6% coated n-3FA), phase 3 (0.3% coated n-3FA).
[‡]Standard error of means.
[a,b]Means in the same row with different superscripts differ ($p < .05$).

Table 7. Effect of coated n-3FA supplementation on blood profile in weaning pigs[†].

Items, mg/dL	CON	0.3FA	0.6FA	0.9FA	SEM[‡]	*p*-value	
						Linear	Quadratic
Week 1							
IgG	191.0	207.0	219.8	224.5	13.4	.0918	.6850
Haptoglobin	19.0	14.3	10.8	9.5	5.6	.2353	.7628
Week 3							
IgG	247.5	266.8	267.3	286.0	14.3	.1041	.9865
Haptoglobin	16.3	15.5	16.3	13.0	2.7	.4672	.6485
Week 6							
IgG	300.8	320.5	332.8	340.8	15.5	.0886	.7132
Haptoglobin	22.0	17.0	16.5	17.3	3.5	.3765	.4384

[†]CON, basic diet; 0.3FA, CON + phase 1 (0.3% coated n-3FA), phase 2 (0.2% coated n-3FA), phase 3 (0.1% coated n-3FA); 0.6FA, CON + phase 1 (0.6% coated n-3FA), phase 2 (0.4% coated n-3FA), phase 3 (0.2% coated n-3FA); 0.9FA, CON + phase 1 (0.9% coated n-3FA), phase 2 (0.6% coated n-3FA), phase 3 (0.3% coated n-3FA).
[‡]Standard error of means.
[a,b]Means in the same row with different superscripts differ ($p < .05$).

temperature after LPS challenge (Huber et al. 2018). Although inflammatory cytokines were not measured in the current study, it is likely that the blunted acute-phase and body temperature responses observed for pigs that received diets supplemented with fish oil was due to reduced inflammatory cytokine production. Previous studies have demonstrated that pigs receiving diets supplemented with 5–7% fish oil had lower concentrations of plasma cytokines (Carroll et al. 2003).

Divergence results are that Huber et al. (2018) indicated that moderated the release of the acute-phase protein haptoglobin by increasing the level of n-3FA. There is some inconsistency in the findings associated with n-3FA supplementation. For instance, some immune-related markers, such as white blood cell, lymphocyte, IgG and TNF-a, were not found to be affected with increasing n-3FA ratio in the diet (Upadhaya et al. 2017). The beneficial effect of coated n-3FA in the present study is that we applied the novel form of fish-derived coated n-3FA in the diet which is less investigated. This may be a possible reason for having contrasting findings compared with those of Liu et al. (2012) that fish oil supplementation is associated with inhibition of (toll-like receptors 4) TLR4 and nucleotide-binding oligomerization domain proteins 2 (NOD2) signalling pathways and concomitant improvement of intestinal integrity under injection LPS. Further studies are needed to elucidate the mechanism of action and optimal dose and duration of coated n-3FA supplementation although the mechanisms involved are not fully understood.

The enhanced digestibility of nutrients is another possible reason why coated n-3FA had positive effects on growth performance. An increase in the digestibility of DM and N with the increase in n-3FA ratios was observed in a study by Upadhaya et al. (2017). These results corroborated with our results. In addition, a study by Huber et al. (2018) also indicated that increasing content of n-3FA improved the apparent ileal digestibility of organic matter.

A healthy intestine is an important reason for improving the digestibility of nutrients. The protective role of n-3FA in the intestine is closely related to their inhibitory effects on the over-release of intestinal proinflammatory cytokines (Calder, 2008). Liu et al. (2012) found that intestinal health in n-3FA supplementation experiment was by the alleviation of the intestinal inflammatory response similar to TNF-α and PGE2 regulating the production of proinflammatory cytokines by influencing TLR and NOD signalling pathways. Some trials indicated on clinical outcomes that the dose of n-3FA (included in fish oil) averaged about 4.5 g/day, which improved clinical score and gut mucosal histology (Andoh et al. 2003; Liu et al. 2012). Jejunal villus height was increased (at 5% inclusion level of

Table 8. Effect of coated n-3FA supplementation on faecal microbial in weaning pigs[†].

Items (log_{10}cfu/g)	CON	0.3FA	0.6FA	0.9FA	SEM[‡]	*p*-value	
						Linear	Quadratic
Week 1							
Lactobacillus	7.44[ab]	7.50[ab]	7.56[ab]	7.64[a]	0.06	.0216	.9223
Escherichia coli	6.42	6.34	6.31	6.30	0.06	.3264	.5629
Clostridium perfringens	6.29	6.24	6.20	6.18	0.07	.2277	.8879
Salmonella	4.38	4.34	4.31	4.28	0.09	.4210	.9405
Week 3							
Lactobacillus	7.51	7.46	7.44	7.43	0.06	0.2041	.9621
Escherichia coli	6.40	6.36	6.33	6.27	0.04	0.1541	.7037
Clostridium perfringens	6.20	6.19	6.16	6.15	0.06	0.5331	.9615
Salmonella	4.26	4.23	4.23	4.21	0.10	0.7652	.9614
Week 6							
Lactobacillus	7.22[b]	7.27[ab]	7.33[ab]	7.35[a]	0.04	0.0223	.6997
E. coli	6.27	6.24	6.21	6.20	0.06	0.2225	.9294
Clostridium	6.14	6.12	6.09	6.06	0.06	0.3479	.9504
Salmonella	4.07	4.06	4.04	3.98	0.13	0.6139	.8346

[†]CON, basic diet; 0.3FA, CON + phase 1 (0.3% coated n-3FA), phase 2 (0.2% coated n-3FA), phase 3 (0.1% coated n-3FA); 0.6FA, CON + phase 1 (0.6% coated n-3FA), phase 2 (0.4% coated n-3FA), phase 3 (0.2% coated n-3FA); 0.9FA, CON + phase 1 (0.9% coated n-3FA), phase 2 (0.6% coated n-3FA), phase 3 (0.3% coated n-3FA).
[‡]Standard error of means.
[a,b]Means in the same row with different superscripts differ ($p < .05$).

fish oil) and crypt depth decreased with increasing supplementation of fish oil. Accordingly, increasing the inclusion level of fish oil protects the microstructure of the jejunum during the early weaning phase resulting in a superior absorptive surface of the jejunum (Huber et al. 2018).

It is also possible that diet composition influenced the diversity of the gut microbiome which may impact apparent nutrient digestibility. In the current research, we found that coated n-3FA could increase faecal *Lactobacillus* counts. However, *E. coli*, *C. perfringens* and *Salmonella* counts were not influenced which is in agreement with the findings of Upadhaya et al. (2017) who demonstrated that increasing coated n-3 ratio in the diet of weaned pigs did not have any significant effect on faecal *E. coli* or on faecal. Other studies suggested that the maturation and optimal development of the immune system are highly related to the development and composition of the indigenous microflora and vice versa (De Vrese and Offick, 2010; Sopková et al. 2017). An alternative explanation for increasing *Lactobacillus* counts was that coated n-3 fatty acid promoted the production of prebiotics through the organic matter broken down in the intestine, such as β-glucan (O'shea et al. 2010). Previous studies demonstrated that the use of β-glucan as a substrate could induce an increase in the population of *Lactobacillus* spp. (Pieper et al. 2008). Furthermore, this result might be attributable to the ability of β-glucan to activate the immune system by increased production of proinflammatory cytokines (i.e. CD4+, CD8+, interleukin-1β, tumour necrosis factor-α and interferon-γ) (Zhou et al. 2013).

In conclusion, nursery diets containing coated n-3FA in powder form obtained from refined fish oil has positive effects on improving growth performance, digestibility of DM and nitrogen during the weaning phase. Furthermore, it has a trend on enhancing the lactobacillus count in weaning pigs faecal. Therefore, increasing coated n-3FA is a potential nutritional method to improve feed efficiency and moderate immune reactions after weaning. The benefit of n-3FA on immune-modulatory nutrients during common and multi-factorial disease challenges that occur in commercial farms should be examined in future studies. Furthermore, sources of coated n-3FA other than plant seed and oil, or fish oil that is more advanced must be investigated.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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