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Do varied dietary omega-6 to omega-3 ratios affect the performance, nutrient digestibility, immune status and faecal microbiota of weaner pigs?

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Abstract. The present study tested the hypothesis that altering the ratio of omega-6 (n-6) to omega-3 (n-3) fatty acids (FAs) in the diet will improve growth performance, nutrient digestibility and blood parameters of weaner pigs. In total, 90 crossbred weaner pigs ((Yorkshire × Landrace) × Duroc, 28 days old), with an average bodyweight (BW) of 6.53 ± 0.71 kg were used in a 6-week experiment. Pigs were blocked on the basis of BW and sex and randomly allotted to one of three dietary treatments (5 pigs per pen (2 barrows and 3 gilts); 6 pens per treatment). Treatments consisted of plant-derived n-6 and n-3 FAs (15 : 1, 10 : 1 and 5 : 1). The experimental period was divided into three phases: Phase 1; 28–35 days of age, Phase 2; 36-49 days of age and Phase 3; 50–70 days of age. Supplementation of varied n-6 : n-3 FA ratios in the diet of weaned pigs showed linear increases ($P < 0.05$) in BW and average daily gain during Phases 1 and 2. The feed : gain ratios reduced linearly ($P < 0.05$) during Phase 1 and tended to reduce ($P = 0.08$) during Phase 2 as the ratio of n-6 : n-3 FA decreased from 15 : 1 to 5 : 1. However, the overall performance of weaners was not affected by the n-6 : n-3 FA ratio. Dry matter and nitrogen (N) digestibility increased linearly ($P < 0.05$) and energy digestibility tended ($P = 0.089$) to increase linearly with the reduction of n-6 : n-3 FA ratio from 15 : 1 to 5 : 1 during Week 3 and, in Week 6, dry-matter digestibility increased ($P < 0.05$) linearly, whereas energy digestibility tended to increase with a decreasing n-6 : n-3 FA ratio in the diet. The high-density lipid cholesterol showed a significant ($P < 0.05$) linear increase at Week 3 and a tendency to increase at Week 6, but no significant effects on other serum lipids were observed. The dietary n-6 : n-3 FA ratio did not have a significant effect on white blood cell, lymphocyte, immunoglobulin G and tumour necrosis factor-α concentrations or faecal microbial counts. In conclusion, the reduction of n-6 : n-3 FA ratio from 15 : 1 to 5 : 1 in the diet did not affect the overall performance of weaning pigs. However, it showed a positive effect on the growth performance of pigs during Phases 1 and 2.

Additional keywords: growth performance, linseed oil.

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Introduction

Weaning is considered a challenging period in a piglet's life because it causes nutritional, environmental and immunological stresses. These stressors can result in reduced feed intake, depressed growth and altered gut integrity of piglets, which may lead to increased inflammation (Le Dividich and Seve 2000; Montagne *et al*. 2007; Li *et al*. 2014). So as to help combat the negative effects of stress at the time of weaning, piglets are often fed diet containing antibiotics. However, the use of antibiotics in animal production is facing increased restriction and scrutiny owing to concerns of microbial antibiotic resistance. Thus, finding alternate strategies to help piglets cope during weaning is important, and nutritional modulation for this purpose is a growing area of interest. 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-3 FAs; Palmquist 2009). These FAs are known to have many different health benefits in humans (Freeman 2000; MacLean *et al*. 2006; Medeiros *et al*. 2007; Theodoratou *et al*. 2007). Intake of n-3 FA has been reported to reduce tumour necrosis factor-α (TNF-α) concentrations in humans and pigs (de Batlle *et al*. 2012; Li *et al*. 2014). Noriega *et al*. (2016) demonstrated that an n-3-rich diet is capable of producing significant changes in the gut microbiota in human beings, but n-3 FA supplementation did not have a detectable impact on faecal microbiota in swine (Holman *et al*. 2014). The supplementation of n-3 FAs in starter pigs may improve the health status of weaned pigs who are faced with different stressors due to their effects on the immune system (Turek *et al*. 1996; Gaines *et al*. 2003; Liu *et al*. 2003; Li *et al*. 2014) and, thus, producers may include n-3 FA into piglet diets, with the aim of reducing the use of antibiotic for prophylactic treatment in starter feeds. However, in swine diets, the n-3 research has mostly investigated improving sow reproductive performance and subsequent piglet viability. The result of n-3 FA supplementation in the pig diet is not equivocal and may reflect the different n-6 : n-3 FA ratios used (Eastwood *et al*. 2014). Since there is a direct competition

between the 18-carbon n-3 and omega-6 (n-6) FAs for the enzymes involved in the synthesis of their longer-chain derivatives, the relative proportions of precursor fatty acids determine the net rate of conversion to their long-chain FA (Liou *et al*. 2007). Thus, the dietary ratio may be important to maximise the benefits of inclusion of 18-carbon n-3 FAs into diets (Palmquist 2009). The conversion of α-linolenic acid into eicosapentaenoic acid and docosahexaenoic acid depends more on the ratio of n-3 to n-6 FA than on the absolute intake (Harnack *et al*. 2009). Therefore, it is important to consider the relative proportions of n-6 FA and n-3 FA in animal diets. With the decrease in the ratio of n-6 : n-3 FA in the diet, there will be an increased ability in the animals to convert the 18-carbon α-linolenic acid into its longer-chain counterparts such as eicosapentaenoic acid and docosahexaenoic acid that are biologically more active (Eastwood *et al*. 2014). Simopoulos (1991, 2002) indicated that the balance between n-6 and n-3 FA is a vital factor for health and longevity. So far, there is limited information on the effects of varied ratios of plant-based n-6 : n-3 FA on growth performance, nutrient digestibility, blood profiles and faecal microflora of weaned piglets.

Thus, the objective of the present experiment was to determine whether reducing n-6 : n-3 FA ratios (15 : 1, 10 : 1 and 5 : 1) of diets improves the performance, nutrient digestibility, blood characteristics, faecal *E.coli* and *Lactobacillus* counts in weaner pigs.

Materials and methods

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

Source of n-3 fatty acid

The protected n-3 FA derived from linseed oil was provided by a commercial company (Morningbio Co. Ltd, Cheonan, Korea). The spray-drying method as previously described by Watanabe *et al*. (2002) was used for coating. According to the information provided by the suppliers, the linolenic acid (n-3) and linoleic acid (n-6) concentrations in the linseed were 56.71% and 14.79% respectively.

Animals and diets

In total, 90 crossbred weaner pigs ((Yorkshire × Landrace) × Duroc, 28 days old), with an average bodyweight (BW) of 6.53 ± 0.71 kg, were used in a 6-week experiment. Pigs were blocked on the basis of BW and sex and randomly allotted to one of three dietary treatments (5 pigs per pen (2 barrows and 3 gilts), 6 pens per treatment). Treatments consisted of plant-based FA, with n-6 : n-3 ratios 15 : 1, 10 : 1 and 5 : 1. The experiment was divided into the following three phases: Phase 1 (28–35 days of age), Phase 2 (36–49 days of age) and Phase 3 (50–70 days of age). All nutrients in the diets were formulated to meet or exceed the recommendation of NRC (2012) for weaner pigs fed in mash form (Table 1). The additive was supplemented into the diet by replacing the same amount of soy oil in the basal diet at different phases. All piglets were housed in an environmentally controlled room, maintained at 30°C, and this was reduced 1°C for each week of the experiment. An area of 0.26 × 0.53 m^2 was provided to each pig. Each pen was equipped with a stainless-steel feeder and a nipple drinker, with *ad libitum* access to feed and water throughout the experiment. Ventilation was provided by a mechanical system. Lighting was automatically regulated to provide 12 h of artificial light per day.

Table 1. Composition of basal diet (as-fed basis, g/kg, unless otherwise indicated)
Diets were in mash form

Item	Phase 1 (Days 0–7)	Phase 2 (Days 8–21)	Phase 3 (Days 22–42)
Ingredients			
Corn	–	119.1	281.4
Extruded corn	329.7	280.5	219.1
Soybean meal (48% CP)	232.3	298	365
Soy oil	48.4	37	45
Lactose	100	80	–
Whey	250	150	50
Mono calcium phosphate	15.4	12	14
L-lysine HCl	5.3	5.4	5.3
DL-methionine	2.2	1.4	3.1
L-threonine	2	2.2	2.1
Vitamin premix[A]	1	1	1
Mineral premix[B]	2	2	2
Limestone	9.7	9.4	9
Salt	2	2	3
Calculated nutritional content			
Metabolisable energy (MJ/kg)	15.7	15.49	15.28
Crude protein	225	217	205
Lysine	16	14	13.5
Calcium	7	6.5	6.2
Total phosphorus	5.5	5.2	5
Crude fat	97.5	84.9	75
Crude fibre	14.8	20.1	25.5
Analysed nutritional content			
Crude protein	225	217	205
Lysine	16.2	13.9	13.6
Calcium	7.2	6.8	6.4
Total phosphorus	5.5	5.3	5.1
Crude fat	97.8	85	75
Crude fibre	14.9	20.1	25.6

[A]Provided per kg 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 B_{12}, 33 μg.

[B]Provided per kg of complete diet: copper (as $CuSO_4 \cdot 5H_2O$), 12 mg; zinc (as $ZnSO_4$), 85 mg; manganese (as MnO_2), 8 mg; iodine (as KI), 0.28 mg; and selenium (as $Na_2SeO_3 \cdot 5H_2O$), 0.15 mg.

Sampling and measurement

Individual BW and pen feed consumption 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 feed : gain (F : G) ratio. Chromium oxide was added to the diet as an indigestible marker at 0.20% of the diet for 7 days before faecal collection at the 3rd and 6th week to calculate apparent dry matter (DM), nitrogen (N), and energy digestibility. Faecal samples were collected randomly

from at least two pigs (1 barrow and 1 gilt) from each pen, mixed and pooled and a representative sample was stored in a freezer at −20°C until analysed. All feed and faecal samples were freeze-dried and finely ground to pass through a 1-mm screen. DM and N digestibility were determined using methods established by Association of Official Analytical Chemists (AOAC 2000). Chromium concentrations were determined via UV-absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan). Apparent total tract digestibility of DM and N were calculated using indirect methods described by Williams *et al*. (1962). 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). The FA concentrations of the experimental diets were the mean of two replicates, and the FAs were analysed by using an HP 5890 gas chromatography with a flame-ionisation detector (Hewlett Packard 5890 Series II, Palo Alto, CA, USA). The FAME were separated using an 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. 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. The peaks of fatty acid were identified by comparing the retention time and peak area of each fatty acid standard respectively. The FA profiles of experimental diet are presented in Table 2.

Eight pigs (4 barrows and 4 gilts, i.e. 2 pigs (1 barrow and 1 gilt) each from 4 pens) from each treatment (n = 8) were randomly selected to be bled via venepuncture using a sterile needle. Blood samples were added into either a 5-mL tube or a K_3EDTA tube on Day 21 and Day 42 for subsequent analysis (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA). White blood-cell (WBC) and lymphocyte counts of whole blood samples were determined using an automatic blood analyser (ADVIA 120; Bayer, Tarrytown, NY, USA). Serum was separated by centrifugation at 3000*g* for 15 min at 4°C and stored at −4°C until determination of serum concentrations of immunoglobulin G (IgG), cortisol (Rodent Cortisol ELISA Kit, Endocrine Technologies, Minneapolis, MN, USA) and TNF-α by ELISA (R and D Porcine ELISA Kit, R and D Systems, Minneapolis, MN, USA). High-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol in serum samples were analysed with an auto analyser (Automatic Biochemical Analyzer, RA-1000; Bayer Corporation, Tarrytown, NY, USA), using colorimetric methods. At 0800 hours and 2000 hours on Days 7, 21, and 42, faecal score was evaluated and recorded. The faecal score was determined as the average value of five pigs of each pen using a 5-grade score system (Hu *et al.* 2012), with grades 1, 2, 3 and 4 representing dry, soft, moist and extra soft watery faeces. Scores were recorded on a pen basis, following the observations of individual pig and signs of stool consistency in the pen.

At Days 21 and 42, faecal samples were collected via rectal massage from eight (4 barrows and 4 gilts) randomly selected pigs from 4 pens per treatment. Faecal samples were pooled on a pen basis and placed on ice for transportation to the laboratory where analysis was immediately performed. The composite faecal sample (1 g) from each pen was diluted with 9 mL of 1% peptone broth (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) and homogenised. Viable counts of bacteria in faecal samples were then determined by plating serial 10-fold dilutions (in 1% peptone solution) onto MacConkey agar plates (Difco Laboratories, Detroit, MI, USA) and lactobacilli medium III agar

Table 2. Analysed fatty acid profile of the experimental diet

Item	Dietary treatment (n-6 : n-3 ratio)								
	Phase 1			Phase 2			Phase 3		
	15 : 1	10 : 1	5 : 1	15 : 1	10 : 1	5 : 1	15 : 1	10 : 1	5 : 1
C14:0	1.01	1.02	1.05	0.82	0.83	0.86	0.04	0.05	0.10
C16:0	8.97	9.88	12.60	9.67	10.76	14.02	9.48	10.93	14.92
C16:1	0.16	0.16	0.16	0.16	0.16	0.16	0.10	0.10	0.10
C18:0	3.02	3.65	5.52	2.94	3.69	5.94	2.07	3.07	5.82
C18:1	16.91	17.11	17.73	18.88	19.12	19.86	20.39	20.72	21.63
C18:2	33.63	33.79	34.28	40.19	40.38	40.96	49.70	49.96	50.67
C18:3	2.29	3.17	5.81	2.63	3.68	6.85	3.23	4.64	8.51
C18:4	0.45	0.45	0.45	0.55	0.55	0.55	0.69	0.69	0.69
C20:0	0.03	0.04	0.07	0.02	0.03	0.07	0.03	0.04	0.08
C20:1	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
C20:4	0.00	0.00	0.00						
C20:5	0.00	0.01	0.04	0.00	0.01	0.05	0.00	0.02	0.06
C22:0	0.00	0.00	0.00						
C22:1	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
C22:5	0.00	0.01	0.06	0.00	0.02	0.07	0.00	0.02	0.09
C22:6	0.00	0.03	0.10	0.00	0.03	0.12	0.00	0.04	0.16
C24:0	0.02	0.02	0.02	0.01	0.01	0.01	0.02	0.02	0.02
Saturated fatty acid	13.00	14.54	19.17	13.43	15.28	20.82	11.59	14.06	20.84
Monounsaturated fatty acid	17.07	17.27	17.89	19.03	19.28	20.02	20.50	20.83	21.73
Polyunsaturated fatty acid	35.92	36.99	40.23	42.82	44.11	47.99	52.93	54.66	59.40
n-3	2.29	3.22	6.01	2.63	3.74	7.09	3.23	4.72	8.82
n-6	33.66	33.83	34.34	40.21	40.42	41.03	49.73	50.00	50.75

plates (Medium 638, DSMZ, Braunschweig, Germany) to isolate *Escherichia coli* and *Lactobacillus* respectively. Lactobacilli medium III agar plates were then incubated at 39°C under anaerobic conditions. MacConkey agar plates were incubated at 37°C for 24 h. Colonies of *E. coli* and *Lactobacillus* were counted immediately after removing the plates from the incubator.

Statistical analyses

Data were analysed as a completely randomised-block design using the GLM procedures (SAS Institute Inc., Cary, NC, USA). For all response criteria, the pen served as the experimental unit. Before conducting the statistical analysis of the microbial counts, data were log-transformed. Variability in data is expressed as standard errors of the mean (s.e.m.). Linear and quadratic polynomial contrasts were performed to determine the effects of the different n-6 : n-3 ratios in the diet, with $P \leq 0.05$ indicating significance and $P \leq 0.10$ indicating trends.

Result

Growth performance

Decreasing the n-6 : n-3 FA ratio of weaner diets (Days 1–7 post-weaning) led to a significant ($P < 0.05$) linear increase in BW and ADG during Phases 1 and 2 (28–35 days of age and 36–49 days of age respectively). The F : G ratio was also significantly ($P < 0.05$) improved in Phase 1 and tended to improve during Phase 2 with a decreasing n-6 : n-3 FA ratio, whereas ADFI was not affected in all phases of experiment. There were no effects of a decreased n-6 : n-3 FA ratio diet on the overall performance result (Table 3).

Apparent total-tract nutrient digestibility

Digestibility of DM and N significantly ($P \leq 0.05$) increased linearly and energy digestibility tended ($P = 0.059$) to increase linearly with the reduction of n-6 : n-3 FA ratios from 15 : 1 to 5 : 1 during Week 3 and, in Week 6, DM digestibility showed a significant ($P < 0.05$) linear increment, whereas energy digestibility showed a trend (linear, $P = 0.089$) for an increase with the decreasing n-6 : n-3 FA ratios (Table 4).

Blood characteristics

Immune-related markers such as WBC, lymphocyte, IgG and TNF-α were not affected with the variation in the n-6 : n-3 ratio in the diet. However, of the serum lipids, HDL cholesterol showed a significant ($P < 0.05$) linear increase during Week 3 and HDL cholesterol during Week 6 showed a trend ($P = 0.1$) in increase, but there were no effects in LDL cholesterol with the change in omega6 : omega 3 ratio in the diet (Table 5).

Faecal Escherichia coli *and Lactobacillus count and faecal score*

Supplementation of FA at a varied n-6 : n-3 ratio in the diet of weaned pigs did not have any significant ($P > 0.05$) effect on faecal *Escherichia coli* and *Lactobacillus* counts, or on faecal score (data not shown).

Table 3. Effect of n-6 : n-3 fatty acid ratios from linseed oil on growth performance in weaner pig

For 15 : 1 n-6 : n-3 ratio, the concentrations of linseed oil-derived omega-3 used in the three phases were 0% n3 for Phase 1, 0.03% n3 for Phase 2, and 0.07% n3 for Phase 3. For 10 : 1 n-6 : n-3 ratio, the concentrations of omega-3 used in the three phases were 0.27% n3 for Phase 1, 0.28% n3 for Phase 2, and 0.32% n3 for Phase 3. For 5 : 1 n-6 : n-3 ratio, the concentrations of omega-3 used in the three phases were 1.15% n3 for Phase 1, 1.12% n3 for Phase 2, and 1.13% n3 for Phase 3. ADG, average daily gain; ADFI, average daily feed intake; F : G, feed to gain ratio

Item	15:1	10:1	5:1	s.e.m.	*P*-value	
					Linear	Quadratic
			Bodyweight (kg)			
Initial	6.43	6.43	6.43	0.004	0.761	0.861
Phase 1	8.13	8.2	8.24	0.03	0.016	0.636
Phase 2	13.4	13.5	13.8	0.04	0.0001	0.19
Phase 3	25.19	25.35	25.45	0.18	0.335	0.896
			Phase 1			
ADG (g)	244.0	253.5	259.3	3.5	0.012	0.682
ADFI (g)	286.2	282.9	287.1	2.7	0.805	0.269
F : G	1.173	1.116	1.107	0.01	0.006	0.195
			Phase 2			
ADG (g)	376.9	379.3	394.0	2.8	0.002	0.107
ADFI (g)	534.3	535.0	542.9	5.2	0.273	0.589
F : G	1.420	1.410	1.380	0.01	0.080	0.471
			Phase 3			
ADG (g)	561.1	563.8	556.7	7.5	0.686	0.612
ADFI (g)	842.5	838.7	840.0	4.8	0.717	0.675
F : G	1.502	1.488	1.508	0.02	0.861	0.484
			Overall			
ADG (g)	446.9	450.6	452.9	4.2	0.334	0.896
ADFI (g)	647.1	644.8	648.8	3.3	0.713	0.457
F : G	1.448	1.431	1.433	0.01	0.364	0.663

Table 4. Effect of n-6 : n-3 fatty acid ratios from linseed oil on co-efficient of apparent nutrient digestibility in weaner pigs

For 15 : 1 n-6 : n-3 ratio, the concentrations of linseed oil-derived omega-3 used in the three phases were 0% n3 for Phase 1, 0.03% n3 for Phase 2, and 0.07% n3 for Phase 3. For 10 : 1 n-6 : n-3 ratio, the concentrations of omega-3 used in the three phases were 0.27% n3 for Phase 1, 0.28% n3 for Phase 2, and 0.32% n3 for Phase 3. For 5 : 1 n-6 : n-3 ratio, the concentrations of omega-3 used in the three phases were 1.15% n3 for Phase 1, 1.12% n3 for Phase 2, and 1.13% n3 for Phase 3

Item	15 : 1	10 : 1	5 : 1	s.e.m.	*P*-value	
					Linear	Quadratic
			Week 3			
Dry matter	0.771	0.786	0.794	0.078	0.051	0.714
Nitrogen	0.779	0.793	0.806	0.066	0.014	0.946
Energy	0.773	0.789	0.795	0.074	0.059	0.578
			Week 6			
Dry matter	0.768	0.776	0.781	0.034	0.019	0.722
Nitrogen	0.777	0.778	0.782	0.11	0.724	0.886
Energy	0.77	0.777	0.781	0.043	0.089	0.809

Table 5. Effect of n-6 : n-3 fatty acid ratios from linseed oil on blood profile in weaner pigs

For 15 : 1 n-6 : n-3 ratio, the concentrations of linseed oil-derived omega-3 used in the three phases were 0% n3 for Phase 1, 0.03% n3 for Phase 2 and 0.07% n3 for Phase 3. For 10 : 1 n-6 : n-3 ratio, the concentrations of omega-3 used in the three phases were 0.27% n3 for Phase 1, 0.28% n3 for Phase 2, and 0.32% n3 for Phase 3. For 5 : 1 n-6 : n-3 ratio, the concentrations of omega-3 used in the three phases were 1.15% n3 for Phase 1, 1.12% n3 for Phase 2, and 1.13% n3 for Phase 3. HDL, high-density lipoprotein; LDL, low-density lipoprotein; IgG, immunoglobulin G; WBC, white blood cell; TNF-α, tumour necrosis factor-α

Item	15 : 1	10 : 1	5 : 1	s.e.m.	*P*-value	
					Linear	Quadratic
		Week 3				
HDL cholesterol (mg/dL)	25.3	30.8	32.8	1.5	0.013	0.383
LDL cholesterol (mg/d)L	39.8	43.5	39.5	2.8	0.951	0.299
WBC (μg dL)	14.6	16.3	16.2	1.4	0.436	0.633
Lymphocyte concentration (%)	45.8	52.7	52.1	3.8	0.28	0.449
IgG (mg/dL)	220	225	234.5	14.4	0.503	0.903
TNF-α (pg/mL)	203.5	184.3	180	29.3	0.591	0.841
		Week 6				
HDL cholesterol (mg/dL)	29.5	33.5	37.3	3	0.116	0.974
LDL cholesterol (mg/dL)	53	52	57.8	4.9	0.523	0.598
WBC (μg/dL)	17.8	17.7	16	2.1	0.561	0.755
Lymphocyte concentration (%)	40.9	43.1	45.8	5.3	0.547	0.966
IgG (mg/dL)	225	239	244.5	15.4	0.404	0.829
TNF-α (pg/mL)	201	195	181.8	11.1	0.265	0.798

Discussion

The nutritional needs for weaner pigs are unique both in terms of sources of nutrients and concentrations of nutrients in the diet. Weaner pig diets must be high in nutrient density to provide adequate amounts of energy, protein, vitamin and minerals because these pigs typically have low feed intake due to the sudden change from sow's milk to solid feed. The caloric intake can be increased by the supplemental fat but the type of fat may affect efficiency of energy utilisation. Soybean oil, a long-chain, highly unsaturated fat source, is well utilised by pigs. It has been reported that 3–5% soy oil addition is beneficial to starter-pig performance (Thaler *et al*. 1986). It is true that n-6 : n-3 FA ratio is vital for the elongation and desaturation of their respective biologically active long-chain fatty acid. However, numerous studies have also suggested that providing long-chain n-3 FA will be of more immediate benefit overall to humans (Calder 2010, 2013), swine (Carroll *et al*. 2003; Liu *et al*. 2003; Mateo *et al*. 2009) and poultry (Korver and Klasing 1997). The current study tested the hypothesis that nutritional management strategies could positively influence the overall wellbeing of weaned pigs. Thus, we examined the effects of varied ratios of dietary n-6 : n-3 FA ratios (15 : 1, 10 : 1 and 5 : 1) by partially replacing soy oil with linseed oil, which contains n-3 FA on growth performance, nutrient digestibility, immune status and faecal microflora of weaned pigs.

Our findings showed that the supplementation of varied n-6 : n-3 FA ratios (15 : 1, 10 : 1 and 5 : 1) in the diet led to a linear increase in BW and ADG during Phase 1 and Phase 2. The F : G ratio also increased linearly during phase1 and tended to increase during phase 2 as the ratio of n-6: n-3 decreased from 15 : 1 to 5 : 1. However, ADFI was not affected by treatment. In contrast, Li *et al*. (2014) suggested that inclusion of 3% n-3 PUFA in the weaner piglet's diet did not significantly improve ADG, ADFI or G : F ratio. A study by Eastwood *et al*. (2009) also indicated that there were no linear or quadratic effects of dietary flax seed meal rich n-3 PUFA at different levels (0, 100, 200 and 300 g of flax seed meal/kg of diet) on basal BW gain, feed intake or feed efficiency in growing pigs. The inconsistent finding regarding growth performance in pigs could be due to the oversupply of n-6 in the diet, different feed ingredients or age of pigs.

The digestibility of DM, N and energy increased linearly with the reduction of the *n*-6 : *n*-3 ratio from 15 : 1 to 5 : 1 during Week 3. In Week 6, DM digestibility showed a significant linear improvement and energy digestibility tended to increase with a reducing n-6 : n-3 FA ratios. The digestibility of nutrients based on varied n-6 : n-3 FA ratio in weaner pigs has not been reported; thus, no comparisons could be made. In a recent study, finisher pigs fed 0.75% protected omega-3 fatty acid did not show improvement in the total tract digestibility of DM, N and energy (Upadhaya *et al*. 2017). However, lactating cows fed diets with 3% linseed oil had significant improvements in the digestibility of DM and organic matter (Ueda *et al*. 2003). The lack of consistency among species and studies may be related to the type of diets, age and species of animals and the dose of omega-3 FA in the diet.

Weaning has been shown to induce immune stress in piglets and this stress can be mitigated by omega-3 supplementation (Li *et al*. 2014). In the current study, the immunological mediators such as WBC, lymphocytes, IgG and TNF-α were unaffected in weaned pigs fed *n*-6 : *n*-3 FA at different ratios. However, serum HDL-cholesterol was significantly increased during Week 3 and tended to increase during Week 6 with the reduction in the ratio of *n*-6 : *n*-3 in the diet. The influence of

dietary supplementation effects of dietary *n*-6 : *n*-3 FA at different ratios (15 : 1, 10 : 1 and 5 : 1) on serum lipids has not been reported. Thus, comparisons could not be made with other studies. The linear increase in HDL-cholesterol concentrations with the reduction in the ratio of *n*-6 : *n*-3 FA in the diet suggests that decreasing n-6 : n-3 FA ratios in the piglet diet may have a beneficial effect on the animal health. In the current experiment, the *Escherichia coli* and *Lactobacillus* counts as well as faecal score were not affected by altering n-6 : n-3 FA ratios in the diet of weaner piglets indicating that the supplementation of additive had no adverse effect on faecal microbial population.

Conclusions

In conclusion, reduction of n-6 : n-3 FA ratios in the diet of newly weaned piglets had a positive effect on the growth of the piglets during Phases 1 and 2 but no effect on the overall growth performance. Feeding diets with lower n-6 : n-3 FA ratios increased HDL cholesterol concentrations at Week 3 post-weaning, but did not influence other lipid profiles as well as immune markers such as TNF α, IgG and lymphocyte. The faecal *E. coli* and *Lactobacillus* counts and faecal scores were also not affected by altering the n-6: n-3 FA ratios in the diet of weaning pigs.

Conflicts of interest

The authors declare no conflicts of interest.

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References

AOAC (2000) 'Official methods of analysis.' 17th edn. (Association of Official Analytical Chemists: Washington, DC)

Calder PC (2010) Omega-3 fatty acids and inflammatory processes. *Nutrients* **2**, 355–374. doi:10.3390/nu2030355

Calder PC (2013) Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology. *British Journal of Clinical Pharmacology* **75**, 645–662. doi:10.1111/j.1365-2125.2012.04374.x

Carroll JA, Gaines AM, Spencer JD, Allee GL, Kattesh HG, Roberts MP, Zannelli ME (2003) Effect of menhaden fish oil supplementation and lipopolysaccharide exposure on nursery pigs. I. Effects on the immune axis when fed diets containing spray-dried plasma. *Domestic Animal Endocrinology* **24**, 341–351. doi:10.1016/S0739-7240(03)00017-1

de Batlle J, Sauleda J, Balcells E, Gomez FP, Mendez M, Rodriquez E, Barreiro E, Ferrer JJ, Romieu I, Gea J, Anto JM, Garcia-Aymerich J PAC–COPD Study Group (2012) Association between Ω3 and Ω6 fatty acid intakes and serum inflammatory markers in COPD. *The Journal of Nutritional Biochemistry* **23**, 817–821. doi:10.1016/j.jnutbio.2011.04.005

Eastwood L, Kish PR, Beaulieu AD, Leterme L (2009) Nutritional value of flaxseed meal for swine and its effects on the fatty acid profile of the carcass. *Journal of Animal Science* **87**, 3607–3619. doi:10.2527/jas.2008-1697

Eastwood L, Leterme P, Beaulieu AD (2014) Changing the omega-6 to omega-3 fatty acid ratio in sow diets alters serum, colostrum, and milk fatty acid profiles, but has minimal impact on reproductive performance. *Journal of Animal Science* **92**, 5567–5582. doi:10.2527/jas.2014-7836

Freeman MP (2000) Omega-3 fatty acids in psychiatry. A review. *Annual Review of Clinical Psychology* **12**, 159–165. doi:10.3109/10401230009147106

Gaines AM, Carroll JA, Yi GF, Allee GL, Zannelli ME (2003) Effect of menhaden fish oil supplementation and lipopolysaccharide exposure on nursery pigs. II. Effects on the immune axis when fed simple or complex diets containing no spray-dried plasma. *Domestic Animal Endocrinology* **24**, 353–365. doi:10.1016/S0739-7240(03)00016-X

Harnack K, Andersen G, Somoza V (2009) Quantitation of alpha-linolenic acid elongation to eicosapentaenoic and docosahexaenoic acid as affected by the ratio of n6/n3 fatty acids. *Nutrition & Metabolism* **6**, 8–18. doi:10.1186/1743-7075-6-8

Holman DB, Baurhoo B, Chenier MR (2014) Temporal analysis of the effect of extruded flaxseed on the swine gut microbiota. *Canadian Journal of Microbiology* **60**, 649–659. doi:10.1139/cjm-2014-0317

Hu CH, Gu LY, Luan ZS, Song J, Zhu K (2012) Effects of montmorillonite–zinc oxide hybrid on performance, diarrhea, intestinal permeability and morphology of weanling pigs. *Animal Feed Science and Technology* **177**, 108–115. doi:10.1016/j.anifeedsci.2012.07.028

Korver DR, Klasing KC (1997) Dietary fish oil alters specific inflammatory immune responses in chicks. *The Journal of Nutrition* **127**, 2039–2046.

Le Dividich J, Seve B (2000) Effects of under feeding during the weaning period on growth, metabolism, and hormonal adjustments in the piglet. *Domestic Animal Endocrinology* **19**, 63–74. doi:10.1016/S0739-7240(00)00067-9

Li Q, Brendemuhl JH, Jeong KC, Badinga L (2014) Effects of dietary omega-3 fatty acid on growth and immune response of weanling pigs. *Journal of Animal Science and Technology* **56**, 7. doi:10.1186/2055-0391-56-7

Liou YA, King DJ, Zibrik D, Innis SM (2007) Decreasing linoleic acid with constant alpha-linolenic acid in dietary fats increases (n-3) eicosapentaenoic acid in plasma phospholipids in healthy men. *The Journal of Nutrition* **137**, 945–952.

Liu YL, Li DF, Gong LM, Yi GF, Gaines AM, Carroll JA (2003) Effects of fish oil supplementation on the performance and immunological, adrenal, and somatotropic responses of weaned pigs after an *Escherichia coli* lipopolysaccharide challenge. *FEMS Immunology and Medical Microbiology* **23**, 283–288.

MacLean CH, Newberry SJ, Mojica WA, Khanna P, Issa AM, Suttorp MJ, Lim YW, Traina SB, Hilton L, Garland R, Morton SC (2006) Effects of omega-3 fatty acids on cancer risk. *Journal of the American Medical Association* **295**, 403–415. doi:10.1001/jama.295.4.403

Mateo RD, Carroll JA, Hyun Y, Smith S, Kim SW (2009) Effect of dietary supplementation on n-3 fatty acids and elevated concentrations of dietary protein on the performance of sows. *Journal of Animal Science* **87**, 948–959. doi:10.2527/jas.2008-0964

Medeiros DM, Hampton M, Kurtzer K, Parelman M, Al-Tamimi E, Drouillard JS (2007) Feeding enriched omega-3 fatty acid beef to rats increases omega-3 fatty acid content of heart and liver membranes and decreases serum vascular cell adhesion molecule-1 and cholesterol levels. *Nutrition Research (New York, N.Y.)* **27**, 295–299. doi:10.1016/j.nutres.2007.04.003

Montagne L, Boudry G, Favier C, Le Huerou-Luron I, Lalles JP, Seve B (2007) Main intestinal markers associated with the changes in gut architecture and function in piglets after weaning. *British Journal of Nutrition* **97**, 45–57. doi:10.1017/S000711450720580X

National Research Council (2012) 'Nutrient requirements of swine.' 11th edn. (National Academic Press: Washington, DC)

Noriega BS, Sanchez-Gonzalez MA, Salyakina D, Coffman J (2016) Understanding the impact of omega-3 rich diet on the gut microbiota. *Case Reports in Medicine* **2016**, 3089303. doi:10.1155/2016/3089303

Palmquist DL (2009) Omega-3 fatty acids in metabolism, health, and nutrition and for modified animal products. *The Professional Animal Scientist* **25**, 207–249. doi:10.15232/S1080-7446(15)30713-0

Simopoulos AP (1991) Omega-3-fatty-acids in health and disease and in growth and development. *The American Journal of Clinical Nutrition* **54**, 438–463.

Simopoulos AP (2002) Omega-3 fatty acids in inflammation and autoimmune diseases. *Journal of the American College of Nutrition* **21**, 495–505. doi:10.1080/07315724.2002.10719248

Thaler RC, Nelssen JL, Goodband RD, Johnston ME, Allee GL (1986) 'Evaluation of soybean oil additions in starter pig diets.' pp. 57–60. (Swine Day, Manhattan, Kansas State University)

Theodoratou E, McNeil G, Cetnarskyj R, Farrington SM, Tenesa A, Barnetson R, Porteous M, Dunlop M, Campbell H (2007) Dietary fatty acids and colorectal cancer: a case-control study. *American Journal of Epidemiology* **166**, 181–195. doi:10.1093/aje/kwm063

Turek JJ, Schoenlein IA, Watkins BA, van Alstine WG, Clark LK, Knox K (1996) Dietary polyunsaturated fatty acids modulate responses of pigs to *Mycoplasma hyopneumonia* infection. *The Journal of Nutrition* **126**, 1541–1548.

Ueda K, Ferlay A, Chabrot J, Loor JJ, Chilliard Y, Doreau M (2003) Effect of linseed oil supplementation on ruminal digestion in dairy cows fed diets with different forage : concentrate ratios. *Journal of Dairy Science* **86**, 3999–4007. doi:10.3168/jds.S0022-0302(03)74011-9

Upadhaya SD, Li TS, Kim IH (2017) Effects of protected omega-3 fatty acid derived from linseed oil and vitamin E on growth performance, apparent digestibility, blood characteristics and meat quality of finishing pigs. *Animal Production Science* **57**, 1085–1090. doi:10.1071/AN15641

Watanabe Y, Fang X, Minemoto Y, Adachi S, Matsuno R (2002) Suppressive effect of saturated L-ascorbate on the oxidation of linoleic acid encapsulated with maltodextrin or gum arabic by spray-drying. *Journal of Agricultural and Food Chemistry* **50**, 3984–3987. doi:10.1021/jf011656u

Williams CH, David DJ, Lismaa O (1962) The determination of chromic oxide in faeces samples by atomic absorption spectrophotometry. *The Journal of Agricultural Science* **59**, 381–385.