Effect of purified docosahexaenoic acid supplementation on production performance, meat quality, and intestinal microbiome of finishing pigs

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KEY WORDS: DHA, faecal microbial count, finishing pig, growth performance, meat quality, nutrient digestibility

ABSTRACT. This study examined the effects of docosahexaenoic acid (DHA) purified from fish oil on the growth performance, nutrient digestibility, faecal microbial count, faecal score, and meat quality of finishing pigs. A total of 160 crossbred finishing pigs [(Yorkshire × Landrace) × Duroc] with an average body weight of 70.51 ± 2.23 kg were randomly assigned to 1 of 4 diets [5 pigs per pen (2 barrows and 3 gilts); 8 pens per treatment]. Dietary treatments were: CON – basal diet, TRT1 – CON + 0.10% DHA, TRT2 – CON + 0.25% DHA, TRT3 – CON + 0.50% DHA. DHA supplementation resulted in a linear increase \( (P = 0.046) \) in final body weight of finishing pigs. DHA supplementation increased average daily gain (ADG) in the TRT3 group compared to the CON group at week 6. In addition, the gain to feed ratio (G:F) was increased in the TRT3 group compared to the CON group. Increasing dietary DHA levels linearly improved ADG \( (P = 0.046) \) and G:F \( (P = 0.021) \). DHA supplementation did not influence nutrient digestibility. The pH, water holding capacity, cooking loss, and meat colour were not affected by the supplementation with graded DHA levels. On day 7, drip loss was reduced in the TRT2 and TRT3 groups compared to CON. However, faecal microbial and faecal score measurements remained unaffected among the treatments. In short, powdered DHA supplementation improved growth performance in finishing pigs without affecting nutrient digestibility, intestinal microorganisms and faecal score.

Received: 10 June 2021
Revised: 30 March 2022
Accepted: 13 May 2022

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Introduction

Fats are an important source of energy in the diet of pigs. Fish oil is one of the main sources of fat that contains omega 3 fatty acid components. Sun et al. (2019) and Zhang et al. (2020) reported improved growth performance in pigs as a result of supplementing fatty acids from fish oil. Fats and oils play an important role in feed ingredients due to their high energy value and ability to absorb certain vitamins and minerals. They are also essential for maintaining body temperature and insulating vital organs. Fat can be obtained from various plant and animal sources. Dietary fat has a very variable composition. Diets rich in long-chain fatty acids have been linked to a lower incidence of atherosclerosis and coronary heart disease, as reported by Simopoulos (1991), and Øverland et al. (2015). Pig meat products are rich in fat, with relatively high amounts of saturated fats, low amounts of polyunsaturated n-3 fatty acids, and a high ratio of n-6 to n-3 fatty acids. Pork and other pig meat products have an unhealthy reputation. The inclusion of omega-3 polyunsaturated fatty acids (n-3 PUFA) in pigs’ diets may reduce the inflammation associated with stress occurring at weaning, allowing for optimal growth and health. Fish oil supplementation leads to reduced levels of pro-inflammatory cytokines in peripheral blood and
spleen in both animals and humans (Endres et al., 1989; Kew et al., 2003), as well as enhanced cardiovascular function (Ruxton et al., 2004). Docosahexaenoic acid (DHA) can be synthesized by mammals from alpha-linolenic acid (ALA), but its conversion to long-chain PUFA is limited in pigs (Smink et al., 2013). Weaner pigs would benefit from dietary supplements rich in DHA and eicosapentaenoic acid (EPA), such as fish oil or microalgae. Husby (1938) claimed that a diet high in fish oil caused pigs fed rations containing oil above a certain level to develop softer fat, as well as a fishy taste to the fat and meat. A method to prevent this problem is to eliminate the dietary inclusion of fish oil during the finishing phase. Vestal et al. (1945) found that 1% and 2% fish oil addition resulted in fishy flavour occurrence, while 0.5% and 0.125% did not produce fishy smell in pork. Generally, the fattening period starts between 9 and 14 weeks of age, depending on different production systems. Studies by Irie and Sakimato (1992) and Taugbol (1993) aimed to determine how polyunsaturated n-3 fatty acids integrated into the tissues of growing and finishing pigs. There has been a slight improvement in polyunsaturated fatty acid levels in pig tissues without significant adverse effects on accompanying physico-chemical properties (Whittington et al., 1986; Rhee et al., 1988), including organoleptic characteristics (Oldfield and Anglemier, 1957).

We hypothesised that DHA supplementation from purified fish oil would improve growth performance in finishing pigs without affecting their meat quality, intestinal bacterial population and faecal score.

Material and methods

The Animal Care and Use Committee of Dankook University, Cheonan, South Korea approved the research protocol (DK-1-1964) outlined for this study. The experiment was conducted at the swine experimental unit of Dankook University, Cheonan, South Korea.

Source of fish oil/ DHA

DHA used in this study (Table 1) was obtained from a commercial company (Morningbio Co., Ltd., Cheonan, Korea). According to the supplier’s information, DHA was produced by purifying fish oil through transesterification and molecular distillation processes according to the method described by Hoque et al. (2011). The purified product, which is a DHA-rich liquid oil was powdered and absorbed onto a silica-containing carrier. The main fatty acid composition of the product was as follows: C18:0 (1.19%), C18:1 (4.49%), C18:3n6 (1.03%), C20:1 (9.49%), C20:4n6 (1.59%), C22:1n9 (1.84%), C20:5n3 (23.08%), C20:3n6 (2.78%), C22:2 (1.37%), C24:0 (6.57%) and C22:6n3, DHA (39.18%).

| Table 1. Composition of finishing pig diets (as-fed basis) |
|-----------------|----------------|
| **Item**        | **Experimental diet** |
|                 | **CON** | **T1** | **T2** | **T3** |
| Ingredients, %  |         |        |        |        |
| corn            | 76.73   | 76.67  | 76.58  | 76.45  |
| soybean meal (48%) | 15.32  | 15.32  | 15.34  | 15.36  |
| tallow (Beef)   | 2.52    | 2.48   | 2.40   | 2.26   |
| molasses        | 2.00    | 2.00   | 2.00   | 2.00   |
| di calcium phosphate | 1.15   | 1.15   | 1.15   | 1.15   |
| limestone       | 0.81    | 0.81   | 0.81   | 0.81   |
| salt            | 0.30    | 0.30   | 0.30   | 0.30   |
| methionine (99%)| 0.07    | 0.07   | 0.07   | 0.07   |
| lysine (HCl)    | 0.48    | 0.48   | 0.48   | 0.48   |
| threonine (99%) | 0.14    | 0.14   | 0.14   | 0.14   |
| tryptophan (99%)| 0.05    | 0.05   | 0.05   | 0.05   |
| mineral mix1    | 0.20    | 0.20   | 0.20   | 0.20   |
| vitamin mix2    | 0.20    | 0.20   | 0.20   | 0.20   |
| choline (25%)   | 0.03    | 0.03   | 0.03   | 0.03   |
| DHA             | 0.10    | 0.25   | 0.50   |        |
| Total           | 100.00  | 100.00 | 100.00 | 100.00 |
| Calculated value |        |        |        |        |
| crude protein, %| 14.00   | 14.00  | 14.00  | 14.00  |
| Ca, %           | 0.60    | 0.60   | 0.60   | 0.60   |
| total P, %      | 0.55    | 0.55   | 0.55   | 0.55   |
| lysine, %       | 1.00    | 1.00   | 1.00   | 1.00   |
| methionine, %   | 0.30    | 0.30   | 0.30   | 0.30   |
| threonine, %    | 0.65    | 0.65   | 0.65   | 0.65   |
| tryptophan, %   | 0.20    | 0.20   | 0.20   | 0.20   |
| metabolizable energy, kcal/kg | 3300 | 3300 | 3300 | 3300 |
| fat, %          | 5.38    | 5.42   | 5.46   | 5.52   |
| fiber, %        | 2.34    | 2.34   | 2.34   | 2.33   |
| ash, %          | 3.26    | 3.26   | 3.26   | 3.26   |
| omega-3, %      | 0.06    | 0.08   | 0.12   | 0.18   |
| omega-6, %      | 1.61    | 1.61   | 1.61   | 1.60   |

CON – basal diet; TRT1 – CON + 0.10% DHA; TRT2 – CON + 0.25% DHA; TRT3 – CON + 0.50% DHA; DHA – docosahexaenoic acid; 1 provided per kg of diet: mg: Fe 115 (as ferrous sulphate), Cu 70 (as copper sulphate), Mn 20 (as manganese oxide), Zn 60 (as zinc oxide), 10.5 (as potassium iodide), Se 0.3 (as sodium selenite); 2 provided per kilograms of diet: IU: vit. A 13 000, vit. D3 1 700, vit. E 60, mg: vit. K, 5, vit. B6 4.2, vit. B, 19, vit. B6 6.7, vit. B2 0.05, biotin 0.34, folic acid 2.1, niacin 55, D-calcium pantothenate 45.
DHA in finishing pig

0.50% DHA. All nutrients in the diets (Table 2) met or exceeded the recommendations of the NRC (2012). Pigs were housed in an environmentally controlled facility with a slatted plastic flooring and a mechanical ventilation system. Each pen contained a single self-feeder and a nipple drinker to ensure continuous access to feed and water.

Growth performance

Body weights of individual pigs and feed intake were recorded at the beginning of the experiment and on day 42. The gain to feed ratio (G:F) was determined by estimating average daily gain (ADG), and average daily feed intake (ADFI).

Nutrient digestibility

In order to determine the apparent total tract digestibility (ATTD) of dry matter (DM), nitrogen (N), and energy (E), pigs were fed diets containing 0.2% chromium oxide (7 days before faecal sample collection at the beginning and end of week 6). The experimental diets were mixed with chromium oxide for 15 min at 200 revolutions per minute (RPM) using a feed mixture (DDK-801F/M, Daedong tech, Gyeongbuk, South Korea). Direct rectal massage was applied to 16 pigs per treatment at the end of the initial phase of the experiment and on week 6 to collect fresh faces from 2 pigs per pen (1 gilt and 1 barrow); one mixed sample was collected for analysis from each pen. For analysis, faecal samples from each pen were thoroughly mixed and kept at −20 °C. After drying at 70 °C for 72 h, the faecal and feed samples were ground and sieved through a 1-mm sieve before analysis. DM and N samples of from feed and faeces were tested according to the recommendations of AOAC International (2005). UV absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan) was used for chromium analysis. Gross energy was measured using an oxygen bomb calorimeter (Parr 6100, Parr Instrument Co., Moline, IL, USA). The apparent total tract digestibility of dry matter (DM), nitrogen (N), and energy were calculated following the procedures described by Williams et al. (1962).

Faecal microbial analysis

Two pigs (1 gilt and 1 barrow) from each pen, were randomly sampled on day 1 and 42 by direct rectal massage, followed by pooling the collected stool samples. One mixed sample per pen and 8 samples per treatment were collected. Immediately after collection, the samples were placed on ice for transport to the laboratory, and direct microbial analysis was conducted. To homogenize the pooled faecal sample, 1 gram from each pen was diluted and dissolved in 9 ml of 1% peptone broth (Becton, Dickinson, and Co., Franklin Lakes, NJ, USA). MacConkey agar plates (Difco Laboratories, Detroit, MI, USA), incubated for 24 h at 37 °C, and Lactobacillus medium III agar plates (Medium 638, DSMZ, Braunschweig, Germany), incubated for 48 h at 39 °C under anaerobic conditions, were used to isolate *Escherichia coli* and *Lactobacillus*, respectively, and viable bacteria ins stool samples were counted by plating them in serial 10-fold dilutions (in 1% peptone solution). *E. coli* and *Lactobacillus* colonies were counted as soon as they were removed from the incubator.

Faecal score

Using the five-grade score system described by Hu et al. (2012), faecal scores were determined by averaging the values of five pigs from each pen. In this system, 1 represents hard, dry pellets; 2 represents hard, formed stool that remains firm and soft; 3 represents soft, formed, moist stool that retains its shape; 4 represents soft, unformed stool that takes the shape of a container; and 5 represents watery, liquid stool. Pig scores were recorded based on the observations of individual pigs and signs of stool consistency in each pen.

Meat quality

One barrow and one gilt from each pen (16 pigs per treatment) were slaughtered at a local commercial slaughterhouse at the end of the experiment. The process of electrical stunning (automatic system) was carried at 240 V (1.25–1.3 A) for 3 s.
to render the pig unconscious, and was followed by bleeding and evisceration. Subsequently, carcasses were placed at 4 °C and refrigerated. After cooling for 24 h, meat from the longissimus muscle was taken for quality analysis. A Minolta CR-410 chroma meter (Konica Minolta Sensing Inc., Osaka, Japan) was used to perform reflectance spectrometry measurements of lightness (L*), redness (a*), and yellowness (b*). Colour, marbling, and firmness scores, required for sensory evaluation, were determined according to the standards of the National Pork Producers Council (NPPC, 2000). The plastic bag method described by Honikel (1998) was used to measure drip loss. Approximately 40-g samples were weighed and put in a plastic bag which was placed in an 80 °C water bath. After reaching an internal temperature of 75 °C, the samples were cooled and weighed again. The difference in weight before and after boiling was recorded as cooking loss (%). A glass-electrode pH meter (WTW pH 340-A, WTW Measurement Systems Inc., Ft. Myers, FL, USA) was used to measure duplicate pH values from each sample 24 h after slaughtering. The method described by Kauffman et al. (1986) was used to determine the water-holding capacity of meat. A 0.3-gram test sample was placed on a piece of filter paper (125 mm diameter) and pressed at 3000 psi for three minutes at 26 °C. A digital area-line sensor (MT-10S; M.T. Precision Co. Ltd., Tokyo, Japan) was used to take measurements of the surface area of the pressed sample and the wet moisture area. The surface area of the longissimus muscle (LMA) sample was measured using a digitizing area-line sensor mentioned above.

Table 3. Effect of dietary supplementation with purified docosahexaenoic acid (DHA) on growth performance in finishing pigs

| Items          | CON   | TRT1  | TRT2  | TRT3  | SEM   | P-value |
|---------------|-------|-------|-------|-------|-------|---------|
| Body weight, kg |       |       |       |       |       |         |
| initial       | 70.51 | 70.51 | 70.51 | 70.51 | 0.004 | 0.8784  |
| finish        | 103.41| 105.08| 104.66| 105.98| 0.77  | 0.0466  |
| Overall       |       |       |       |       | 18    | 0.0468  |
| ADG, g        | 783b  | 823ab | 813ab | 845ab | 37    | 0.0033  |
| ADFI, g       | 2571  | 2617  | 2602  | 2650  | 37    | 0.2033  |
| G:F           | 0.304b| 0.315ab| 0.312ab| 0.319a| 0.004 | 0.2025  |

Systems Inc., Ft. Myers, FL, USA) was used to measure duplicate pH values from each sample 24 h after slaughtering. The method described by Kauffman et al. (1986) was used to determine the water-holding capacity of meat. A 0.3-gram test sample was placed on a piece of filter paper (125 mm diameter) and pressed at 3000 psi for three minutes at 26 °C. A digital area-line sensor (MT-10S; M.T. Precision Co. Ltd., Tokyo, Japan) was used to take measurements of the surface area of the pressed sample and the wet moisture area. The surface area of the longissimus muscle (LMA) sample was measured using a digitizing area-line sensor mentioned above.

Table 4 lists the effects of dietary purified DHA supplementation on nutrient digestibility in finishing pigs. DHA supplementation at different levels did not result in any improvement in dry matter, nitrogen, and energy digestibility. In addition, no linear or quadratic responses to nutrient digestibility were observed in pigs fed diets with increasing DHA levels.

Statistical analysis

All data were analysed with GLM procedure of SAS (SAS Institute). Duncan's multiple test range was performed for group difference. To evaluate the effect of increasing doses of DHA orthogonal polynomial contrast was performed. P < 0.05 was considered as the level of significance.

Results

Growth performance

The effect of dietary supplementation with purified DHA on growth performance in finishing pigs is presented in Table 3. DHA supplementation resulted in a linear increase (P = 0.046) in final body weight of finishing pigs. DHA supplementation increased ADG in the TRT3 group compared to the CON group at the end of the experiment. In addition, the gain to feed ratio (G:F) was increased in the TRT3 group compared to the CON group. Continuous addition of DHA to the diets linearly improved ADG (P = 0.046) and G:F (P = 0.021). The ADFI did not show any differences between treatments.

Nutrient digestibility

Table 4 lists the effects of dietary purified DHA supplementation on nutrient digestibility in finishing pigs. DHA supplementation at different levels did not result in any improvement in dry matter, nitrogen, and energy digestibility. In addition, no linear or quadratic responses to nutrient digestibility were observed in pigs fed diets with increasing DHA levels.

Meat quality

The effect of dietary supplementation with purified DHA on meat quality in finishing pigs is shown in Table 5. On day 7, drip loss was reduced in the TRT2 and TRT3 groups compared to the CON group. Moreover, increasing doses of DHA linearly decreased (P = 0.015) drip loss on day 7. The pH, water holding capacity, cooking loss, meat colour, longissimus muscle area, and sensory parameters were not affected by DHA supplementation.
The effect of dietary supplementation with purified DHA on faecal microbial counts in finishing pigs is shown in Table 6. Supplementation with graded levels of DHA did not exert any effect on the abundance of faecal microorganisms in any treatments.

| Items       | CON | TRT1 | TRT2 | TRT3 | SEM | P-value   |
|-------------|-----|------|------|------|-----|-----------|
|             |     |      |      |      |     | linear    |
| Initial     |     |      |      |      |     | quadratic |
| dry matter  | 72.25 | 73.92 | 73.82 | 74.11 | 0.76 | 0.8987    |
| nitrogen    | 70.57 | 71.51 | 70.72 | 71.48 | 0.92 | 0.4081    |
| energy      | 71.71 | 72.89 | 71.77 | 72.76 | 0.67 | 0.3097    |
| Finish      |     |      |      |      |     | cubic     |
| dry matter  | 71.49 | 72.87 | 72.67 | 73.06 | 1.47 | 0.5022    |
| nitrogen    | 69.42 | 70.53 | 70.40 | 70.83 | 1.49 | 0.5463    |
| energy      | 71.82 | 72.49 | 71.67 | 72.25 | 1.57 | 0.9461    |

**CON** – basal diet, TRT1 – **CON + 0.10% DHA**, TRT2 – **CON + 0.25% DHA**, TRT3 – **CON + 0.50% DHA**, SEM – standard error of the mean; P > 0.05

Faecal score

The effect of purified DHA addition to the diets on the faecal score in finishing pigs is presented in Table 7. DHA supplementation had no effect on the faecal score in any treatments.
Dietary supplementation with 0.5%, 0.10%, and 0.15% purified DHA resulted in improvement in BW, ADG and G:F of pigs, which was similar to the report of Zhang et al. (2020). Upadhaya et al. (2016) demonstrated that application of 0.75% n-3 fatty acid from linseed oil increased ADG in pigs. A study by Upadhaya and Kim (2021) reported that supplementation with 0.29% coated DHA increased ADG and G:F with a partial increase in ADFI in weaning pigs. Application of 1% n-3 fatty acid from fish oil improved BW, ADG, and ADFI, as reported by Sun et al. (2019). Huber et al. (2018) found that BW, ADG, and ADFI were increased by using 2.5% fish oil supplementation. In our study, the improved G:F ratio could likely be the reason for increased ADG. Previous studies have suggested some other possible reasons such as increased fat digestibility (Upadhaya and Kim, 2021) and increased energy digestibility (Sun et al., 2019). Zhang et al. (2020) argued that a positive response in growth performance to the use of n-3 fatty acid supplementation could be associated with a reduced antigen overload and improved digestibility. In contrast, Overland et al. (2015) using 1% fish oil, Upadhaya et al. (2016) using 0.75% n-3 fatty acid, and Rodriguez et al. (2017), using 1.5% fish oil, reported no effect on ADG, ADFI, and G:F. Possible reasons for these inconsistent results could be differences in animal age, dose, and composition of the test product.

The current study found no improvement in dry matter, nitrogen, and energy digestibility, which was consistent with the study of Upadhaya and Kim (2021). In addition, Huber et al. (2018) also did not find any change in nutrient digestibility except for increased digestibility of organic matter. Using 1% n-3 fatty acid, Sun et al. (2019) did not report any improvement in DM, nitrogen, and energy digestibility in week 5, with the exception of an increased energy digestibility score at week 10. Zhang et al. (2020) observed increased DM and nitrogen digestibility with the application of n-3 fatty acids in weaning pigs. Increased fat digestibility was recorded in the experiments of Upadhaya and Kim (2021). Positive responses in previous studies could be due to changes in intestinal morphology. In addition, improved digestibility could be the reason for the reported higher villi and lower crypt by some authors (Rodriguez et al., 2017; Zhang et al., 2020).

In the present study, we did not find any significant results regarding meat quality, except for reduced drip loss on day 7. Similar ineffectiveness of DHA supplementation on meat quality was previously reported by Overland et al. (2015), Upadhaya et al. (2016), Rodriguez et al. (2017), and Sun et al. (2019). Drip loss is highly related to the meat fibre percentage, stress-induced decrease in pH, as well as storage and thawing systems. Since we found no negative effects on pH, and water-holding capacity results were positive, thus a positive percentage of drip loss was expected. However, the reduced drip loss on day 7 could be related to storage and thawing conditions (Taylor, 2004; Guo and Dalrymple, 2017).

DHA supplementation showed no differences in the faecal microbial count and faecal score, which was consistent with findings of Zhang et al. (2020), and Upadhaya and Kim (2021). The reason for the lack of effect of DHA supplementation on microbial population and faecal score could be due to the differences in species, age, and developmental status. Since finishing pigs had developed immune systems and long-established microflora populations, DHA supplementation did not affect the bacterial population and faecal score (De Vrese and Offick, 2010; Sopková et al., 2017).

### Conclusions

Increasing the dose of DHA supplementation resulted in a positive response in terms of partial growth performance of finishing pigs without affecting meat quality and gastrointestinal bacterial population. Of the 3 doses tested, 0.50% DHA proved to have a better effect on average daily gain and feed efficiency in finishing pigs.

| Items       | CON | TRT1 | TRT2 | TRT3 | SEM | P-value |
|-------------|-----|------|------|------|-----|---------|
| Fecal score |     |      |      |      |     |         |
| initial     | 3.21| 3.20 | 3.21 | 3.16 | 0.05| 0.5257  |
|             |     |      |      |      |     | 0.7171  |
|             |     |      |      |      |     | 0.7722  |
| finish      | 3.15| 3.19 | 3.20 | 3.18 | 0.04| 0.6094  |
|             |     |      |      |      |     | 0.4825  |
|             |     |      |      |      |     | 0.9944  |

CON – basal diet, TRT1 – CON + 0.10% DHA, TRT2 – CON + 0.25% DHA, TRT3 – CON + 0.50% DHA; SEM – standard error of the mean; P > 0.05 fecal score = 1 hard, dry pellet; 2 firm, formed stool; 3 soft, moist stool that retains shape; 4 soft, unformed stool that assumes shape of container; 5 watery liquid that can be poured.
Conflict of interest

The Authors declare that there is no conflict of interest.

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