# Chapter 5

# The effect of fish oil in the diet on clinical response in weanling piglets challenged with a pathogenic *Escherichia coli*

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**Abstract**

Weaned piglets were used to determine the effect of fish oil in the diet on the clinical response to an infection with a pathogenic *E. coli* O149:K91:K88. The piglets were divided into two groups of 8 animals each. One group was fed the control diet containing 5% corn oil.
The test piglets were fed a diet with 0.5% corn oil and 4.5% fish oil. Piglets were orally infected with the challenge strain on days 6 and 7 after weaning. The experimental period lasted 14 days, during which no piglets died. Feed intake and weight gain, faecal and condition scores were measured daily. Faecal samples were collected for bacteriological analysis. Blood samples were taken for analysis of the fatty acid composition of erythrocyte membranes.

The average daily feed intake (ADFI) and average daily gain (ADG) after infection tended to be higher in the test group than in the control group. There were no significant differences in the condition scores between the two groups. The faecal excretion of O149:K91:K88 tended to be lower for test than control piglets. This experiment indicates a possible positive effect of fish oil on the clinical response in weaned piglets to a pathogenic *E. coli*.

## Introduction

Weaned piglets often suffer from post-weaning diarrhoea (PWD) or oedema disease (OD), causing impaired growth performance and high mortality. PWD occurs mainly during the first week after weaning and is associated with the proliferation of enterotoxigenic *E. coli* (ETEC) and toxins produced by these bacteria, like heat labile enterotoxin (LT). OD is associated with the proliferation of enterotoxemic *E. coli* (ETEEC) and release of their Shiga-like toxins (Van Beers-Schreurs et al. 1992; Nabuurs et al. 1993; Nagy and Feteke 1999; Bertschinger 1999). Other factors, such as changes in the flora, function and morphology of the intestine also are involved in the development of PWD and OD (Nabuurs, 1998).

Various measures are taken to improve feed intake and health of piglets after weaning. Amongst these measures is the addition of specific substances to the weaner pig diet. One of the potential substances under study is fish oil which is rich in the n-3 polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3). Apart from their role as precursors for eicosanoids, n-3 and n-6 PUFAs are incorporated into cell membranes where they influence membrane fluidity, receptor function and enzyme activity (Burns et al., 1979). In addition, n-3 PUFAs have a positive effect on the immune response (Wu and Meydani, 1998). Thus, adding fish oil to the diet of weaned piglets might contribute to preventing PWD and OD.

The oral challenge of piglets with pathogenic *E. coli* is used as a model of PWD or OD (Sarmiento et al. 1988; Nagy and Feteke, 1992; Deprez et al. 1996; Meijer et al. 1997; Jeyasingham et al. 1999; McDonald et al. 1999; Nollet et al. 1999). In many cases, clinical signs cannot be provoked by an oral challenge alone, and a stressor such as cold stress is introduced in the model (Sarmiento et al. 1988; McDonald et al. 1999). Generally, the number of bacteria used for challenge are counted in faeces after the infection in order to quantify the impact of the challenge strain. The colony forming units (CFU) of a specific pathogenic *E. coli* strain in faeces of diarrhoeic weaned piglets was positively related with the proliferation of that specific strain in the small intestine (Nabuurs et al. 1993). Resistance to colonisation of pathogenic bacteria is reflected by low bacterial counts in faeces (Bovee-Oudenhoven et al. 1997). Moreover, large numbers of pathogenic bacteria in faeces increase the shedding of these pathogens to the environment, causing a higher risk for the disease.

The aim of the present experiment was to determine whether fish oil in the diet of piglets challenged with pathogenic *E. coli* could reduce the faecal excretion of the bacterium challenged with. Apart from assessing the degree of colonisation of the pathogenic bacteria, growth performance, faeces consistency and body condition were quantified. We used a challenge model more severe than that was

used by Nollet et al. (1999). Our piglets were pre-treated with colistin (Meijer et al. 1997), received a double instead of single oral challenge, and were exposed to moderate cold stress.

## Materials and methods

*Animals*

Sixteen piglets from the closed herd of the research station 'Laverdonk', Veghel were used. The piglets (F2 cross-bred: GY x [Finnish X Dutch Landrace]) were females and castrates aged 19 days. The piglets did not receive creep feed during the lactation period. The piglets were divided into two groups of 8 animals each that were group housed. The piglets in each group were randomly selected from the litters of 8 different sows so that litter origin distributions were identical. Each group was randomly assigned to one of two dietary treatments: the control or fish-oil containing diet. The average weight of the piglets at the beginning of the experiment was 7.61 kg for the control group and 7.75 kg for the fish-oil group.

*Housing*

The groups were housed in pens (2.33 x 3.65 m) with concrete floors covered with sawdust bedding. The 2 pens were located in one environmentally regulated room in an isolated stable. The two pens were separated from each other by an empty space of 100 cm so that physical contact between piglets of the two groups was excluded. The person that entered the pens used separate boots for each pen. The piglets had free access to feed and water. Each pen was equipped with a water nipple and a one-hole self-feeder. Room temperature was kept at 24 $^{o}$C to induce moderate cold stress.

*Bacteria*

The challenge strain used in this experiment was an *E. coli* O149:K91:K88 isolated from a clinical case with PWD. Strains of O serogroup 149 have a well-established association with both OD and PWD (Van Beers-Schreurs et al. 1992; Bertschinger 1999). The strain was haemolytic and was resistant to chloramphenicol. The bacteria were grown in brain heart infusion broth (Oxoid CM225) at 37 $^{o}$C during 24 h. Bacteria were harvested by centrifugation, washed with 0.20 M sodium phosphate buffered saline (PBS), pH 7.0, and resuspended in PBS at a concentration of 1 x $10^4$ bacteria.ml$^{-1}$.

*Diets*

The composition of the two experimental diets is shown in Table 1. The diets were formulated to meet the requirements of growing pigs as set by the

Table 1. Ingredient and analysed composition of the experimental diets

| | Control diet | Fish oil diet |
|---|---|---|
| Ingredient composition | | |
| Constant components[1] | 950 | 950 |
| Corn oil | 50 | 5 |
| Fish oil | 0 | 45 |
| Chemical analysis | | |
| Dry matter (g/kg) | 889 | 886 |
| Crude protein (g/kg dm[2]) | 189 | 188 |
| Crude fat (g/kg dm) | 66 | 67 |
| Crude fiber (g/kg dm) | 28 | 33 |
| Ash (g/kg dm) | 49 | 49 |
| Analysed fatty acids (g/100 g methylesters) | | |
| C16:0 | 17.43 | 18.85 |
| C18:0 | 2.32 | 3.00 |
| C18:1 n-9 | 24.81 | 23.47 |
| C18:2 n-6 | 47.36 | 34.88 |
| C18:3 n-3 | 1.65 | 2.31 |
| C20:3 n-6 | ND[3] | ND |
| C20:4 n-6 | ND | 0.05 |
| C20:5 n-3 | ND | 1.44 |
| C22:4 n-6 | ND | ND |
| C22:5 n-3 | ND | ND |
| C22:6 n-3 | 0.19 | 1.99 |
| n-3[4] | 1.84 | 5.74 |
| n-6[5] | 47.36 | 34.94 |
| n-3:n-6 ratio | 0.04 | 0.16 |

[1] Constant components: 20.41 g soya beans, extracted, 10.21 serolat 15 (Cehave, Veghel, The Netherlands), 0.93 g monocalcium phosphate (Ca (H2PO4)2.H2O), 0.24 g sodium chloride (NaCl), 2.04 g molasses, 0.09 g DL-methionine, 0.56 g threonine, 15.31 g barley, 48.72 g wheat, 1.02 g Mer biggenspeen (Cehave, Veghel, The Netherlands), 0.45 biolysine (Cehave, Veghel, The Netherlands)
[2] dm = dry matter
[3] ND = Non Detectable
[4] Σ C18:3 n-3 + C20:5 n-3 + C22:6 n-3
[5] Σ C18:2 n-6 + C20:4 n-6

National Research Council (1998). The control diet contained 5% corn oil and had a ratio of n-3:n-6 PUFAs of 0.04. The test diet contained 0.5% corn oil and 4.5%

fish oil and had a n-3:n-6 ratio of 0.16. Crude fat and fatty acids were determined according to the methods of Folch et al. (1957) and Metcalfe et al. (1966), respectively. Crude protein, crude fiber and ash were measured by the Weende analysis.

*E. coli challenge trial*

On day 1 after weaning, the piglets did not receive feed to induce maximum villus atrophy (Pluske et al. 1997), but drinking water was freely available. From day 2, the piglets were offered either the test or control diet ad libitum. From days 1 to 5 after weaning, the piglets received colistin (Dopharma, Raamsdonksveer, The Netherlands) in their drinking water at a dosage of about 5 mg/kg live-weight. Colistin pre-treatment increases the sensitivity of the piglets towards pathogenic *E. coli* (Meijer et al. 1997). On the sixth and seventh day after weaning, all piglets were perorally infected with 1 x $10^5$ CFU of the *E. coli*, suspended in 10 ml PBS.

*Methods used to assess the clinical response to E. coli challenge*

For a period of 2 weeks after weaning, each piglet was monitored daily. Faecal and condition scores were assigned by the same person who was blinded to treatment modality. Faecal scores were based on the following scale: 0 = normal, solid faeces; 1 = soft, looser than normal faeces, 2 = diarrhoeal faeces and 3 = liquid, severe diarrhoeal faeces. Condition scores were based on a scale of which the extreme values are described as: 0 = good condition (healthy appearance, short hair, shiny skin) and 3 = poor condition (unhealthy appearance, long hair, pale and dull skin). Individual body weight and feed intake per group were measured daily. Faecal samples were collected daily, if necessary by rectal stimulation with a swab. The faecal samples were immediately put into sterile plastic containers, placed on ice and transported within two hours to the laboratory where they were frozen at – 80 $^{o}$C until being processed for determination of bacterial counts.

For enumeration of *E. coli* O149:K91:K88 in faeces, the material was diluted ten times with peptone physiological salt solution (PFS). Serial dilutions were made in PFS and numbers of bacteria per gram of wet faeces were determined by surface plating techniques on blood agar (Oxoid CM271) with 7% defibrinated sheep blood (Biotrading) to which 80 mg amoxicillin (Sigma A-8523) and 40 mg spectinomycin dihydrochloride (Sigma S-4014) per litre had been added. After 20-24 h of incubation at 37°C, the colonies were counted. Randomly picked colonies were identified by slide agglutination with specific antiserum (ID-Lelystad 7432110).

*Blood samples*

Blood samples were collected in EDTA-containing tubes to analyse the fatty acid composition of erythrocyte membranes. Erythrocytes were separated by centrifugation for 5 min at 3000 rpm. The erythrocytes were haemolysed and stored at –80 $^{o}$C. From the erythrocyte membranes fatty acids were extracted, methylated (Metcalfe et al., 1966) and determined by gas chromatography (Nelson, 1975; Angelico et al., 1983; Popp-Snijders, 1985). Fatty acid methyl esters were isolated on a Chrompack 9002 gas chromatograph equipped with a CP-FFAP CB 25 m x 0.32 mm column (Chrompack, Bergen op Zoom, The Netherlands) and a flame ionization detector.

*Statistical analyses*

The individual piglet was considered to be the experimental unit. Treatments were compared with a t-test using the general linear models procedure of SAS (1988). Faecal and condition scores were compared with a proportional odds model using the logistic procedure of SAS (1988). The statistical model used was: Y = mean + diet effect + error. The level of statistical significance was pre-set at $P < 0.05$.

**Results**

During the experiment, the piglets huddled closely together when they were at rest. No piglets died during the experiment. Average daily feed intake (ADFI) per treatment group is presented in Figure 1. Feed intake in the fish oil group tended to be higher after infection than in the control group. Daily feed intake could only be determined per pen so that statistical analysis could not be performed.

The average daily gain (ADG) during the week after infection (days 7-14) was 203 % higher in the fish-oil group than in the control group ($p = 0.099$). ADG in the fish oil group was 1086 ± 154 g (mean ± SEM; n=8) and in the control group it was 535 ± 256 g.

The consistency of faeces decreased after the challenge, an average score of 1.9 being reached on day 11 (4 days after challenge). Faecal scores, both before and after challenge, were more favourable for the control group than for the fish oil group, with a significant difference on day 7 ($p = 0.018$).

Both the control and fish-oil group had an increase in faecal *E. coli* O139:K82 counts after infection till day 11, followed by a decrease. The counts were generally lower in the piglets given the diet with fish oil. However, there were no significant differences between the groups (Figure 2).

The relative percentage of total n-3 PUFAs in the erythrocyte membrane between days 4 and 11 remained stable in the control group and increased

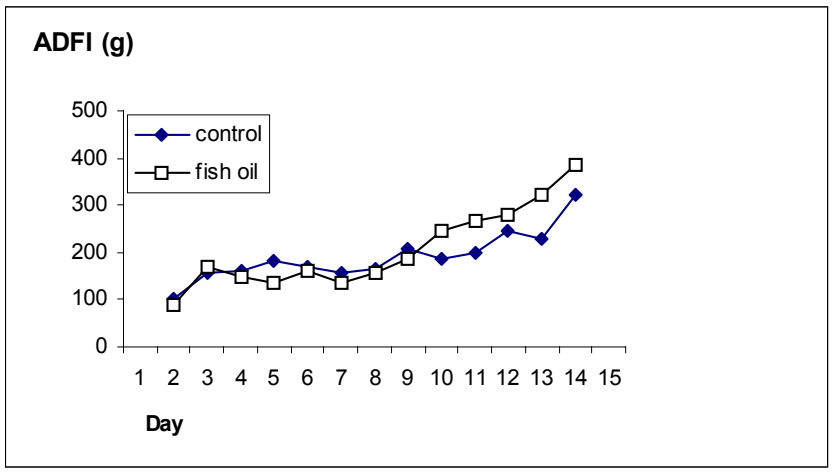


Fig. 1: Average daily feed intake (ADFI) per dietary treatment. The values are given as pen means. The piglets were weaned on day 1, witheld from feed during day 1 and challenged on both days 6 and 7.

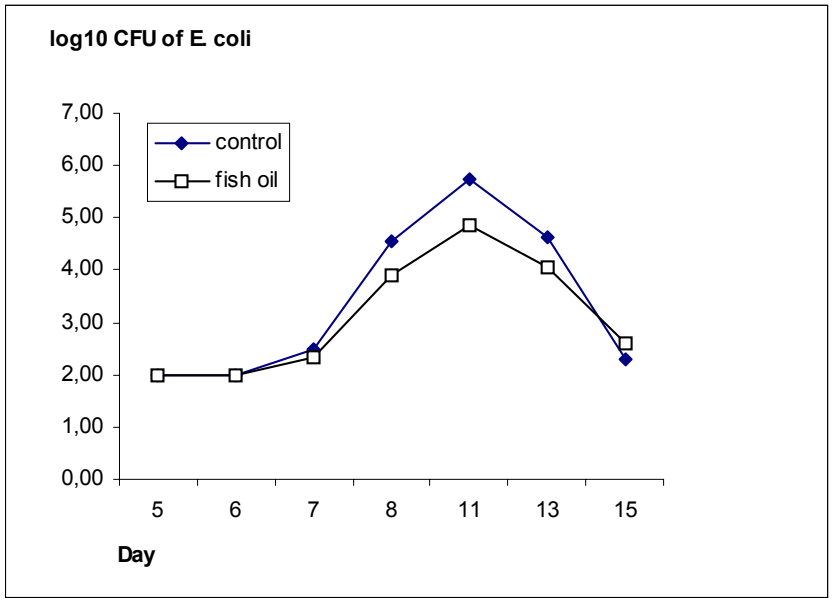


Figure 2. Faecal *E. coli* O149K91K88 counts during the experimental period. Mean values for 8 piglets per group are given.

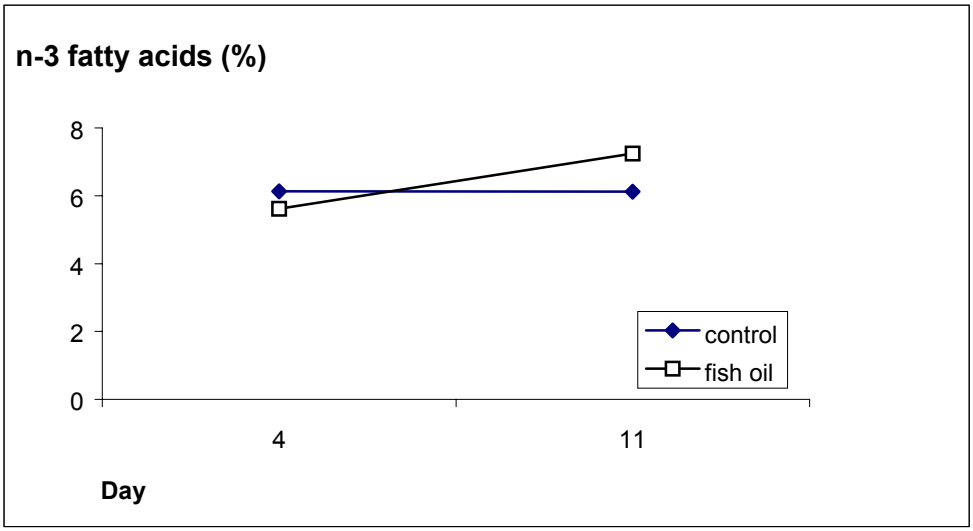


Figure 3. Total amount of n-3 polyunsaturated fatty acids (g/100g methylesters) in erythrocyte membranes. Mean values for 8 piglets per dietary group are given.

significantly in the fish-oil group (Figure 3). The fish-oil diet used in this experiment had a lower analysed content of n-3 PUFAs than the high fish-oil diet used in a previous feeding trial (Chapter 4), but the amount of total n-3 PUFAs in erythrocyte membranes was higher.

**Discussion**

The clinical response of the piglets to the challenge was similar to that in an earlier study (Van Dijk et al. 2002). This study indicates that fish oil in the diet of weaned piglets challenged with *E. coli* tended to have beneficial effects on feed intake and weight gain. The experimental model of PWD showed intestinal colonisation by the *E. coli* and produced diarrhoea in both the control and fish oil group. The faecal excretion of the .*E coli* strain the piglets were challenged with, was lower in the fih-oil group. The present data thus indicate that fish oil feeding may have a protective effect against a challenge with *E. coli*. In agreement with this study, there are data in mice showing that fish oil in the diet protects against challenges with either *Klebsiella* or *Murine AIDS* (Blok et al. 1996). The mechanism by which fish oil might protect against infectious pressure is unknown.

As shown by the fatty acid composition of the erythrocyte membranes, adding fish oil to the diet of weaned piglets led to higher status of the n-3 PUFAs.

When an inflammatory response is necessary, the n-3 PUFAs are released from the cell membrane and metabolised to different eicosanoids. In general, the eicosanoids produced from the n-3 family of PUFAs have less potent inflammatory activities (Vaughn et al., 1994). The eicosanoids synthesized from n-3 and n-6 PUFAs generally have opposite activities (Vaughn, et al., 1994) so that there may be an optimum ratio of n-3:n-6 PUFAs in the diet. For humans this optimum ratio is suggested to be 0.2 (Aggett, et al., 1991). In this experiment the n-3:n-6 ratio in the diet with fish oil was 0.16. A higher ratio might have produced a more clear protective effect against *E. coli*.

In this experiment, the feeding of fish oil to weaned piglets tended to reduce the colonisation and excretion of a pathogenic *E. coli*. Thus, based on this experiment it could be suggested that on swine farms with a history of PWD, the addition of fish oil to the weaner piglets' diet may improve post-weaning growth performance. Two points should be noted. First, the design of the present experiment without non-infected controls does not allow a solid conclusion as to fish oil providing protection against *E. coli*. Secondly, a controlled study with weanling piglets kept in a relatively clean environment did not show a growth-enhancing effect of fish oil (Chapter 4).

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