Chapter 4

Growth performance of weanling piglets fed diets with different contents of fish oil

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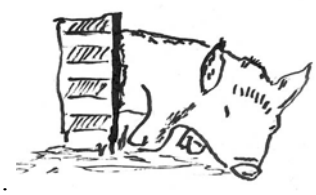


Abstract

To find out whether the intake of fish oil has a positive effect on growth performance of weanling piglets, a total of 480 piglets was fed diets without fish oil or with either 13 or 22 g fish oil/kg. Fish oil was added to the diets at the expense of the corn-oil component. The diets were fed ad libitum from weaning until 14 days post weaning. Fish oil feeding did neither affect feed intake nor weight gain and feed conversion efficiency. The fatty acid composition of erythrocyte membranes reflected fish oil consumption and pointed at inhibition of desaturation and elongation of linoleic acid by fish oil feeding. Piglets fed the diets with fish oil had higher erythrocyte concentrations of eicosapentaenoic acid and lower concentrations of arachidonic acid while linoleic acid contents were not affected. It is concluded that under the conditions of this study, the addition of fish oil to a weaner diet adequate in α-linolenic acid does not enhance growth performance, faeces consistency and body condition of weanling piglets.

Introduction

A weaner diet containing fish oil, and having a ratio of n-3:n-6 polyunsaturated fatty acids (PUFAs) of 0.3, on average produced 27% more weight gain in piglets during 0-10 days post weaning than did a diet containing linseed oil, but with identical n-3:n-6 ratio (Chapter 3). It was reasoned that eicosapentaenoic acid (EPA, C20:5 n-3) in fish oil had improved immunity and thus improved condition of the weanling piglets, this effect being reflected by more rapid growth (Chapter 3). The experiment involved a small number of piglets and did not provide information as to the optimum amount of fish oil in the weaner diet. The inclusion levels of fish oil were 20 and 26 g/kg diet (Chapter 3), but such high levels might negatively affect palatability (Kolanowski et al., 1999). Thus, this experiment was carried out with diets containing no fish oil or either 13 or 22 g fish oil/kg diet and having n-3:n-6 ratios of 0.04, 0.10 and 0.18 respectively. Fish oil was added to the diets at the expense of the corn-oil component. The diets were fed to as many as 480 piglets to obtain sufficient statistical power. To assess the efficacy of fish oil, one group of piglets was fed the fish-oil-free diet, but containing 40 ppm of the growth promoter, salinomycine. The fatty acid composition of erythrocyte membranes was determined to verify essential fatty acid status of the piglets.

Materials and Methods

Animals, feed and housing

Four hundred and eighty weanling pigs (F2 cross-bred: GY x [Finnish X Dutch Landrace]), weighing on average 8.7 ± 1.0 kg and aged 21 days, were used. They were housed in pens containing 10 piglets each. According to a randomised complete block design the pens were allocated to one of the four dietary treatments on the basis of weight, gender and ancestry of the piglets. The experiment was carried out in the form of four cohorts of 120 piglets each. The pens (2.60 x 1.20 m) were climate controlled and had a combination of a slatted and concrete (1.10 x 1.20 m) floor. The piglets had ad libitum access to feed and water. Each pen was equipped with a single-space self-feeder and a water nipple. The room temperature was 26 °C on the first day after weaning, gradually declining to 23 °C after 35 days. Daylight could enter the pens.

There were 3 experimental diets without fish oil or with either 13 or 22 g fish oil/kg. The extra control diet contained no fish oil, but was fortified with 40 ppm salinomycine. Fish oil was added to the diets at the expense of the corn-oil component. Table 1 shows the ingredients and analysed composition of the experimental diets. With increasing contents of fish oil the amount of α-linolenic acid (ALA, C18:3 n-3) did not alter much, whereas the amount of EPA and

Table 1. Composition of the experimental weaner diets

	Diet code[1]			
	0.04 + S	0.04	0.10	0.18
Ingredients (g/kg)				
Corn oil	50	50	37	28
Fish oil	0	0	13	22
Constant components[2]	950	950	950	950
Salinomycine (ppm)	40	0	0	0
Chemical analysis				
Dry matter (g/kg)	896	893	894	897
Crude protein (g/kg dm[3])	183	182	170	173
Crude fat (g/kg dm)	70	69	70	71
Crude fiber (g/kg dm)	29	29	32	34
Ash (g/kg dm)	52	50	49	48
Analysed fatty acids (g/100 g methylesters)				
C16:0	14.41	14.54	14.25	14.10
C18:0	1.96	1.97	1.86	1.84
C18:1 n-9	23.24	23.38	22.75	22.13
C18:2 n-6	55.91	55.37	46.89	39.47
C18:3 n-3	1.99	1.97	1.93	1.98
C20:3 n-6	ND[4]	ND	ND	ND
C20:4 n-6	ND	ND	ND	ND
C20:5 n-3	ND	ND	1.04	1.86
C22:4 n-6	ND	ND	ND	ND
C22:5 n-3	ND	ND	0.61	1.10
C22:6 n-3	ND	ND	1.28	2.32
n-3[5]	1.99	1.97	4.85	7.26
n-6[6]	55.91	55.37	46.89	39.47
n-3:n-6 ratio	0.04	0.04	0.10	0.18

[1] The n-3:n-6 ratios are given as based on analysis of the diets; S = salinomycine
[2] Constant components: 282 g wheat, 465 g barley, 130 g potato-protein, 20 g molasses, 18 g monocalciumphosphate, 21 g calcium carbonate, 2.5 g lysine 78.4%, 1.5 g methionin 50%, 5 g tryptophan, 5 g premix (Cehave, Veghel, The Netherlands).
[3] dm = dry matter
[4] ND = not detectable
[5] Σ of C18:3 n-3, C20:5 n-3 and C22:6 n-3.
[6] Σ of C18:2 n-6, C20:2 n-6 and C20:4 n-6.

docosahexaenoic acid (DHA, C22:6 n-3) increased and linoleic acid (LA, C18:2 n-6) decreased, but the analysed macronutrient levels of all four diets were similar. The diets were formulated to meet the requirements of growing pigs as set by the National Research Council (1998). The diets were fed for two weeks. This two-week period was followed by a three-week period during which all piglets received the same commercial diet (Standard pig pellet "315", Cehave, Veghel, The Netherlands). The declared composition of the commercial diet was 174 g crude protein/kg, 40 g crude fat/kg, 45 g crude fiber/kg, 62 g ash/kg and 105 g moisture/kg.

Data collection and analyses

The piglets were weighed on days 0, 2, 7, 14, 21, 28 and 35 post weaning. Amounts of feed offered were recorded and left-overs were weighed to calculate feed intake. Feed samples were taken for chemical analyses. Crude fat concentration and fatty acid composition of the diets were determined according to the methods of Folch et al. (1957) and Metcalfe et al. (1966), respectively. Crude protein, crude fiber and ash were determined by the Weende method.

The consistency of faeces was scored weekly on a scale ranging from 0 to 3 (0 = normal, solid faeces, 1 = soft, looser than normal stools, 2 = diarrhoea and 3=liquid, severe diarrhoeal faeces). The condition of the pigs was scored weekly, the scores being based on an integration of color and gloss of the skin, hair length and meat cover (0 = good, 3 = bad condition). Both faeces and body condition were scored by the same experienced person who was blinded to treatment modality.

Blood samples were collected by vena cava puncture on days 0, 7 and 14 post weaning from one pig choosen at random out of each pen. For the analysis of the fatty acid composition of erythrocyte membranes, blood was collected in EDTA-containing tubes. Erythrocytes were separated by centrifugation for 5 min at 3000 rpm. The erythrocytes were haemolysed and stored at - 80 °C until fatty acid analysis. From the erythrocyte membranes fatty acids were extracted, methylated 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

Results are presented as means ± SEM. Data were tested for normal distribution with the Kolmogorov-Smirnov test. Diet effects were evaluated for statistically significant differences with ANOVA and Bonferroni test. The body-condition and faeces-consistency scores were subjected to the Chi-square test. For daily gain and feed intake, pen was the experimental unit and for the data on the fatty acid

composition of the erythrocyte membranes the experimental unit was pen as well because one pig per pen was sampled. The model was y = mean + treatment effect + error. For all statistical analyses, the SPSS program (SPSS Inc., Chicago, IL, USA) was used. The level of statistical significance was pre-set at $p<0.05$.

Results

Performance

From days 0-2 post weaning, the addition of salinomycine to the diet produced a significant increase in feed intake. Piglets fed the diets with fish oil ingested more feed during days 0-2 than did their counterparts fed the diet without fish oil, but the increase was not statistically significant. For the intervals of days 0-7, 7-14 and 0-14 post weaning, there was no diet effect on feed intake. On day 14, the piglets were switched onto a commercial diet. There was no carry-over effect of the type of weaner diet on feed intake during days 14-35.

Weight gain was not significantly influenced by the type of weaner diet. There was no tendency towards a dose-response effect of fish oil on weight gain. Feed conversion during the first week post weaning was lowest for the piglets that received salinomycine, and this continued throughout the experiment. The amount of fish oil in the diet did not have a systematic effect on feed conversion (Table 2).

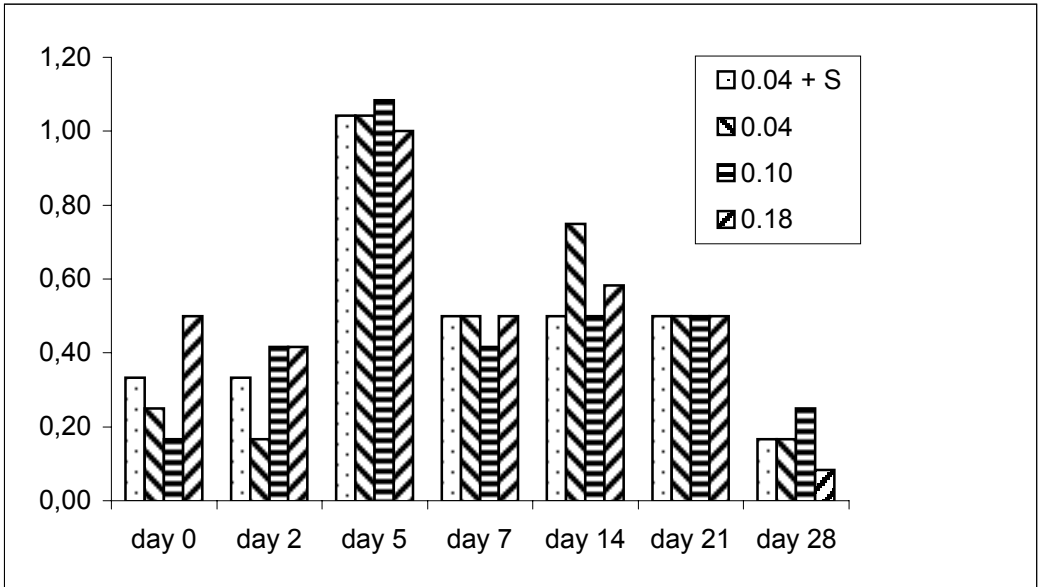
Table 2. Growth performance of weanling piglets fed the experimental weaner diets

	Diet code[1]				Pooled	
	0.04 + S	0.04	0.10	0.18	SED	P value
Feed intake (g/day/pen)						
Days 0-2	578	420	528	498	14	0.999
Days 0-7	1255	1182	1189	1266	680	0.648
Days 7-14	2576	2560	2705	2617	102	0.815
Days 0-14	1916	1871	1947	1941	1416	0.814
Days 14-35[2]	5728	5552	6128	5418	591	0.571
Weight gain (g/day/pig)						
Days 0-2	37	-16	32	-11	44	0.360
Days 0-7	421	361	338	384	18	0.935
Days 7-14	1126	1085	1165	1000	16	0.920
Days 0-14	1547	1447	1503	1384	17	0.909
Days 14-35	8917	8599	8724	8891	29	0.784

[1] The n-3:n-6 ratios are given as based on analysis of the diets; S = salinomycine

Faeces consistency and body condition

Fig. 1. shows the time course of the faeces consistency scores, with 0 representing normal, solid faeces and 3 pointing at liquid, diarrhoeal faeces. Faeces consistency was least on day 3 post weaning and reached stable scores again after another 4 days. There was no diet effect on faeces scores. Body condition scores were considered good on day 2 post weaning (median score for all piglets = 0.0) and remained stable until day 4, but was inferior between days 5 and 7 (median score = 1.0). Diet had no effect on body condition scores.


1,20
1,00
0,80
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0,00
0.04 + S
0.04
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0.18
day 0
day 2
day 5
day 7
day 14
day 21
day 28

Fig. 1. Faecal consistency scores (0 = normal, solid faeces, 1 = soft, looser than normal stools, 2 = diarrhoea and 3 = liquid, severe diarrheal faeces) for piglets fed the experimental diets during 7 days after weaning (= day 0).

Fatty acid composition of erythrocytes

The feeding of fish oil raised the content of EPA in a dose-dependent fashion. The relative percentage of DHA was increased only when the diet with the highest amount of fish oil was fed. Fish oil feeding reduced the group mean percentages of arachidonic acid (AA, C20:4 n-6) and adrenic acid (C22:4 n-6) in erythrocytes.

Table 3. Fatty acid composition of erythrocyte membranes at weaning and from piglets fed the experimental diets for 14 days

		Diet code[1]				Pooled	P
	At weaning	0.04 + s	0.04	0.10	0.18	SEM	value
Analysed fatty acids (g/100 g methylesters)							
C16:0	25.07	23.39	23.63	23.31	23.54	0.146	0.838
C18:0	10.73	11.75	11.59	11.64	11.59	0.075	0.846
C18:1 n-9	29.4	28.11	29.67	29.88	29.02	0.797	0.611
C18:2 n-6	12.91	14.54	13.32	12.88	12.62	0.849	0.194
C18:3 n-6	ND[2]	ND	ND	ND	ND		
C18:3 n-3	0.29	0.21	0.10	0.12	0.17	0.049	0.469
C20:2 n-6	ND	0.12	0.12	0.12	0.04	0.041	0.361
C20:3 n-6	0.24	0.24	0.18	0.26	0.28	0.043	0.148
C20:3 n-3	ND	ND	ND	ND	ND		
C20:4 n-6	3.57	4.36	4.25	3.74	3.81	0.311	0.000
C20:5 n-3	0.18	0.04	0.02	0.45	0.80	0.370	0.000
C22:4 n-6	0.10	0.29	0.30	0.17	0.14	0.080	0.021
C22:5 n-3	1.18	1.25	1.33	1.31	1.41	0.064	0.497
C22:6 n-3	2.53	2.38	2.47	2.42	2.77	0.174	0.152
n-3[3]	4.18	3.88	3.93	4.30	5.14	0.584	0.002
n-6[4]	16.82	19.54	18.17	17.18	16.89	1.200	0.067
n-3:n-6 ratio	0.25	0.20	0.22	0.25	0.30	0.046	0.000

[1] The n-3:n-6 ratios are given as based on analysis of the diets; S = salinomycine
[2] ND = not detectable
[3] ∑ of C18:3 n-3, C20:3 n-3, C20:5 n-3, C22:5 n-3 and C22:6 n-3.
[4] ∑ of C18:2 n-6, C18:3 n-6, C20:2 n-6, C20:3 n-6, C20:4 n-6, C22:4 n-6.

Discussion

It is clear from this study that the addition of fish oil to the weaner diet did not significantly influence growth performance. This outcome does not agree with an earlier study in which fish oil feeding raised weight gain by on average 27%, but this effect not reaching statistical significance (Chapter 3). In this study, statistical power was considerable. With the observed variance, an increase in body-weight gain by 7 % during days 0 - 7 post weaning would have been detected (p=0.05) at a power of 80%. Thus, it is reasonable to conclude that under the conditions of the experiment, fish oil did not influence weight gain. This conclusion is supported by the observed lack of a tendency towards a dose-response relationship. In the

previous study (Chapter 3), the stimulatory effect of fish oil was found when compared with linseed oil, but not when compared with corn oil. Likewise, in this study extra fish oil at the expense of corn oil did not influence weight gain. Moreover, in this study essential-fatty acid status at weaning was higher than that in our previous study. The n-3:n-6 ratio of erythrocyte membranes at weaning was 0.25 (Table 3), whereas in the previous study it was 0.12 (Chapter 3). Possibly, the high status at weaning dampened any effect of fish oil in the weaner diet. It is also relevant to note that the diets containing fish oil contained somewhat less protein than the control diet.

The lack of effect of fish oil feeding on feed intake, growth and feed conversion was associated with absence of an effect on faeces consistency and body condition. These data indicate that the control diet without fish oil was sufficient in n-3 PUFAs. The control diet contained no detectable EPA and DHA, but the level of ALA was 0.34% of metabolizable energy. This level may be considered just adequate (Innis, 1993), which would explain that extra n-3 PUFAs did not further stimulate growth. The erythrocyte membranes of piglets fed the control diet contained a considerable percentage of DHA, which must been formed from its precursor, ALA. When compared with weaning however, the status of n-3 PUFAs of the control piglets had dropped at 14 days post weaning. In the erythrocyte membranes the contents of ALA and EPA fell markedly after weaning, whereas the DHA remained stable. Possibly, DHA was maintained at the expense of ALA and EPA.

The fatty acid composition of erythrocyte membranes not only reflects the two intake levels of fish oil, but also points to an interaction between the metabolism of n-3 and n-6 PUFAs. As would be expected (Alessandri et al., 1996; Arbuckle and Innis, 1993; Goustard-Langelier et al., 1999; Rooke et al., 1998; Ward et al., 1998), fish oil feeding raised the erythrocyte content of EPA. The percentage of DHA was not raised after fish oil feeding which may relate to compensatory synthesis of this fatty acid as mentioned above. There was no diet effect on the concentration of LA in erythrocyte membranes. However, the level of the products of desaturation and elongation of LA, AA and adrenic acid tended to be lowered by fish oil feeding. It may be suggested that extra intake of n-3 PUFAs had inhibited the conversion of LA into AA and adrenic acid. There is evidence for inhibition of LA desaturation and elongation by n-3 PUFAs (Innis, 1991).

The outcome of this study indicates that the inclusion of fish oil in weaner diets with adequate content of ALA does not affect growth performance, faeces consistency and body condition of weanling piglets. Possibly, the high status of n-3 PUFAs at weaning had masked any effect of fish oil feeding. In contrast to earlier suggestions (Bee, 2000), weanling piglets appear to have sufficient capacity to convert ALA into EPA, which is the direct precursor for eicosanoids.

References

Alessandri, J.M., Goustard, B., Guesnet, P. and Durand, G. (1996) Polyunsaturated fatty acid status in blood, heart, liver, intestine, retina and brain of newborn piglets fed eighter sow milk or a milk replacer diet. Reprod. Nutr. Dev. 36: 95-109.

Angelico, F. et al. (1983) Plasma and erythrocyte fatty acids. A methodology for evaluation of hypocholestolemic dietary intercentions Prev. Med. 12: 124-127.

Arbuckle, L. D. and Innis, S.M. (1993) Docosahexaenoic acid is transferred through maternal diet to milk and to tissues of natural milk-fed piglets. J. Nutr. 123: 1668-1675.

Bee, G. (2000) Dietary conjugated linoleic acid consumption during pregnancy and lactation influences growth and tissue composition in weaned pigs. J. Nutr. 130: 2981-2989.

Folch, J., Lees, M. and Sloane Stanley, G.H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226: 497-509.

Goustard-Langelier, B., Guesnet, P., Durand, G., Antoine, J.M. and Alessandri, J.M. (1999) n-3 and n-6 fatty acid enrichment by dietary fish oil and phospholipid sources in brain cortical areas and nonneural tissues in formula-fed piglets. Lipids 34: 5-16.

Innis, S.M. (1991) Essential fatty acids in growth and development. Prog. Lipid Res. 30: 39-103.

Innis, S.M. (1993) The colostrum-deprived piglet as a model for study of infant lipid nutrition. J. Nutr. 123: 386-390.

Kolanowski, W., Swiderski, F. and Berger, S. (1999) Possibilities of fish oil application for food products enrichment with omega-3 PUFA. Int. J. Food Sci. Nutr. 50: 39-49.

Metcalfe, L.D., Schmitz, A.A. and Pelka, J.R. (1966) Rapid preparation of fatty acid esters from lipids for gaschromatographic analysis. Anal. Chem. 318: 514-515.

National Research Council, 1998. Nutrient Requirements of Swine. Tenth revised Edition, National Academic Press, Washington D.C., U.S.A..

Nelson, G.J. (1975) Isolation and purification of lipids from animal tissues In: Perkins EG (ed) Analysis of lipids and lipoproteins. Am. Oil Chem. Soc. Champaign III, USA: 1-22.

Popp-Snijders, C. (1985) Preparation of lipid extracts H 2.2.1 in n-4 polyunsaturated fatty acids and erythrocyte membranes: 18-20.

Rooke, J.A., Bland, I.M. and Edwards, S.A., (1998) Effect of feeding tuna oil or soyabean oil as supplements to sows in late pregnancy on piglet tissue composition and viability. Br. J. Nutr. 80: 273-280.

Ward, G.R., Huang, Y.S., Bobik, E., Xing, H.C., Mutsaers, L., Aestad, N., Montalto, M. and Wainwright, P. (1998) Long-chain polyunsaturated fatty acids levels in formulae influence deposition of docosahexaenoic acid and arachidonic acid in brain and red blood cells of artificially reared neonatal rats. J. Nutr. 128: 2473-2487.