Chapter 3

Influence of dietary n-3 polyunsaturated acids, in the form of either linseed or fish oil, on growth performance, small intestinal morphology and essential fatty acid status of weanling piglets

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Abstract

We addressed the question whether in weanling piglets the feeding of eicosapentaenoic acid, in the form of fish oil, would be more beneficial as to growth performance and gut integrity than the feeding of α-linolenic acid in the form of linseed oil. Weaner diets were formulated that contained two levels each of either fish oil or linseed oil, each level having similar ratios of n-3:n-6 polyunsaturated fatty acids. The fish-oil diets on average increased post-weaning growth by 27%, when compared with the linseed-oil diets. Feed intake was not affected by the experimental diets. There was no systematic influence of diet on the villus:crypt ratio of small intestinal mucosa. The highest group mean ratio was seen with the control diet having a n-3:n-6 ratio of 0.1, and the lowest group mean ratio was found in the piglets fed the linseed-oil diet with a n-3:n-6 ratio of 0.3. The diets containing fish oil produced higher n-3:n-6 ratios in erythrocytes, liver fat, storage fat and lymph nodular fat than did the diets containing linseed oil and having similar n-3:n-6 ratios. It is concluded that dietary fish oil might positively affect growth of weanling piglets, this effect not being mediated by counteracting the weaning-induced decrease in villus height.

Introduction

The weaning transition in piglets is associated with low feed intake and atrophy of small intestinal villi (Cera et al., 1988; Hall and Byrne, 1989; Hampson, 1986; Kenworthy, 1976; Nabuurs, 1991). The low feed intake by definition causes low intakes of nutrients, including n-3 polyunsaturated fatty acids (PUFAs). There is suggestive evidence that enrichment of the weaner diet with α-linolenic acid (ALA, C18:3 n-3), in the form of linseed oil, had positive effects on growth performance and body condition of weanling piglets (Chapter 2). The metabolic basis for the effect of ALA is unknown, but it could relate to the conversion into eicosanoids which have regulatory activity as to immunity and membrane function (Wu and Meydani, 1998; Burns et al., 1979; Shinitzky and Sourojon, 1979; Traill and Wick, 1984). Through desaturation and elongation, ALA can be converted into eicosapentaenoic acid (EPA, C20:5 n-3), which is the immediate precursor of eicosanoids. However, weanling piglets have low desaturase activity in fat tissue (Bee, 2000), so that the formation of EPA may be depressed. It could be suggested that fortification of the weaner diet with EPA is more effective than that with ALA.

In this study, weanling piglets had free acces to diets containing different concentrations of ALA and EPA. The experimental diets were formulated by the addition of either linseed oil or fish oil to the control diet. Linseed oil is rich in ALA and fish oil is rich in EPA. Formulation of the diets was done so that the ratios of n-3 to n-6 PUFAs in the linseed and fish-oil diets were comparable, i.e. either about 0.2 or 0.3 while there were two inclusion levels of linseed and fish oil each. The control diet had a ratio of 0.1. Thus, there were five dietary groups. In the piglets, growht performance, villus height and crypt depth of small intestinal mucosa, and the fatty acid composition of erythrocyte membranes, liver and lymph nodular tissue were determined.

Materials and Methods

Animals, feed and housing

Thirty six piglets (F2: Finish GY slaughterline x [GY sow-line x Dutch Landrace]) were used. During lactation the pigs received no creep feed. They were weaned at 22-23 days of age. Two piglets were euthanised and sampled on the day of weaning of each cohort. Thirty piglets, in three cohorts of 10 piglets each, were individually housed in cages placed in one room with a temperature of 25 °C. Daylight could enter the room. The cages (1.20x0.50x1.00 m) had a slatted floor, wich was half covered with a rubber mat. The piglets received food and water ad libitum. The feeds were in meal form. The control diet contained corn oil and had a n-3:n-6 ratio of 0.10. The other four diets contained either linseed or fish oil, each with n-3:n-6 ratios of 0.20 or 0.30. The analysed compositions of the diets are given in Table 1.

Table 1. Composition of the experimental weaner diets

	Diet code[1]				
	Control 0.1	LO 0.2	LO 0.3	FO 0.2	FO 0.3
Ingredients (g/kg)					
Corn oil	44	39	34	30	24
Linseed oil	6	11	16	0	0
Fish oil	0	0	0	20	26
Constant components[2]	950	950	950	950	950
Chemical analysis (g/kg)					
Dry matter	895	899	897	897	898
Crude protein	183	183	184	182	183
Crude fat	68	68	68	69	69
Crude fiber	32	33	35	34	35
Ash	47	47	48	48	49
Fatty acids (g/100g methylesters)					
C16:0	12.45	12.07	11.59	14.83	15.85
C18:0	2.30	2.38	2.51	2.17	2.18
C18:1 n-9	25.39	24.70	23.82	24.18	23.34
C18:2 n-6	49.10	46.74	43.80	37.49	32.35
C18:3 n-3	5.74	8.51	11.81	2.11	2.17
C20:3 n-6	ND[5]	ND	ND	ND	ND
C20:4 n-6	ND	ND	ND	0.16	0.21
C20:5 n-3	ND	ND	ND	2.50	3.14
C22:4 n-6	ND	ND	ND	ND	ND
C22:5 n-3	ND	ND	ND	ND	ND
C22:6 n-3	ND	ND	ND	4.35	5.45
n-3[3]	5.74	8.51	11.81	8.97	10.76
n-6[4]	49.10	46.74	43.80	37.87	33.82
n-3:n-6 ratio	0.12	0.18	0.27	0.24	0.32

[1] LO = linseed oil; FO = fish oil; the numbers refer to the aimed n-3:n-6 ratios.
[2] The constant components consistent of 152.5 g casein, 505 g barley, 215.05 g dextrose, 20 g molasses, 45.2 g dicalcium phosphate, 10 g premix, 0.875 g threonine 100%, 0.875 g methionin 100% and 0.5 g tryptophane 100%.
[3] Σ C18:3 n-3 + C20:5 n-3 + C22:6 n-3
[4] Σ C18:2 n-6 + C20:4 n-6
[5] ND = not detectable

Sample collection and analyses

The piglets were weighed on the day of weaning (day 0) and at the end of the experiment (day 10). 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.

On days 0 and 10, blood samples were collected by vena cava puncture for the analysis of the fatty acid composition of erythrocyte membranes and the concentrations of blood lipids and lipoproteins. For the analysis of fatty acids in 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. 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. Lipoproteins were isolated according to Terpstra, et al. (1982).

On day 10, the piglets were euthanised and liver, subcutaneous fat and lymph nodes (lnn cervicale) were removed and stored at – 80 °C until further analysis. The fatty acid composition of liver, fat and lymph nodes were determined as described above.

For histology measurements, samples were taken at 20, 50 and 80% of the total length of the small intestine, representing duodenum, jejunum and ileum, respectively. The samples were rinsed in saline, pinned to a piece of dental wax, fixed in 10% phosphate-buffered formaline, and embedded in parafine wax. Villous height and crypth depth were measured at 100x magnification by means of the TEA Image Manager System (Difa Measuring Systems B.V., Breda, The Netherlands). The height of the villus was taken as the distance from the crypt opening to the tip of the villus. The crypt depth was determined from the base of the crypt to the level of the crypt opening. All measurements were made in 10 well oriented villi and crypts per section per animal. The means of the sets of 10 values was calculated and used for statistical analysis.

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. 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

Analysed composition of the diets

The analysed macronutrient compostion of the five diets was similar (Table 1). The incorporation into the diets of either linseed oil or fish oil at the expense of corn oil raised the levels of ALA and EPA, respectively, and lowered the concentration of linoleic acid (LA, C18:2 n-6). The n-3:n-6 ratios as based on fatty acid analysis corresponded reasonably well with the calculated ones used in the diet codes.

Feed intake and growth

Table 2 shows that post-weaning feed intake did not differ significantly between the dietary groups. Group-mean body-weight gain was highest for the piglets given the diets containing fish oil, but the increase did not reach statistical significance.

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

	Diet code[1]				
	Control 0.1	LO 0.2	LO 0.3	FO 0.2	FO 0.3
Feed intake (g)					
Days 0-3	433 ± 128	411 ± 198	366 ±135	426 ± 61	441 ± 200
Days 0-7	1192 ± 320	1168 ± 521	1219 ± 393	1185 ± 269	1263 ± 408
Days 0-10	1853 ± 629	1703 ± 620	1822 ± 682	1879 ± 551	1916 ± 569
Weight gain (g)					
Days 0-10	433 ± 524	550 ± 753	417 ± 471	683 ± 479	650 ± 568

[1]See legend to Table 1.
Results are presented as means ± SD for 6 piglets per dietary group.
Means within a row are not significantly different.

Table 3. Villus height and crypt depth of small intestinal mucosa at weaning and in piglets fed the experimental diets for 10 days

		Diet code				
	At weaning	Control 0.1	LO 0.2	LO 0.3	FO 0.2	FO 0.3
Villus height (μm)	445 ± 170^{a}	229 ± 76^{b}	214 ± 8^{b}	168 ± 66^{b}	225 ± 78^{b}	248 ± 81^{b}
Crypt depth (μm)	148 ± 62^{a}	245 ± 79^{b}	290 ± 100^{b}	291 ± 70^{b}	280 ± 86^{b}	253 ± 53^{b}
Villus:crypt ratio	3.69 ± 2.47^{a}	$1.07 \pm 0.57^{b,c}$	$0.82 \pm 0.45^{b,c}$	0.62 ± 0.31^{b}	0.90 ± 0.45^{bc}	1.05 ± 0.48^{c}

See legend to Table 2.
Means within a row not sharing a common superscript are significantly different.

Table 4. Fatty acid composition of erythrocyte membranes at weaning and in piglets fed the experimental diets for 10 days

		Diet code				
	At weaning	Control 0.1	LO 0.2	LO 0.3	FO 0.2	FO 0.3
Fatty acid (g/100g methylesters)						
C16:0	22.78 ± 1.20[a]	21.56 ± 0.76[a]	21.95 ± 1.01[a]	21.30 ± 0.54[a]	22.17 ± 0.35[b]	22.72 ± 1.31[b]
C18:0	10.49 ± 0.65	12.05 ± 0.41	11.84 ± 0.42	12.02 ± 0.59	11.80 ± 0.29	12.19 ± 0.54
C18:1 n-9	30.15 ± 2.86	28.33 ± 3.13	30.46 ± 3.38	28.73 ± 2.40	28.69 ± 2.69	29.03 ± 3.51
C18:2 n-6	13.52 ± 1.91	14.29 ± 2.35	13.09 ± 2.47	14.20 ± 2.25	13.22 ± 1.84	11.69 ± 1.42
C18:3 n-3	0.27 ± 0.08[a]	0.34 ± 0.12[a]	0.38 ± 0.16[a]	0.58 ± 0.19[b]	0.22 ± 0.09[a]	0.18 ± 0.06[c]
C20:2 n-6	0.24 ± 0.08	0.29 ± 0.05	0.27 ± 0.05	0.27 ± 0.04	0.24 ± 0.02	0.16 ± 0.08
C20:3 n-6	0.33 ± 0.06	0.32 ± 0.06	0.30 ± 0.05	0.33 ± 0.04	0.32 ± 0.05	0.29 ± 0.03
C20:4 n-6	4.70 ± 0.46	5.13 ± 0.42	4.76 ± 0.79	4.83 ± 0.49	4.37 ± 0.37	3.92 ± 0.30
C20:5 n-3	0.10 ± 0.06[a]	0.08 ± 0.06[a]	0.13 ± 0.03[a]	0.19 ± 0.04[a]	0.62 ± 0.17[b]	0.79 ± 0.17[b]
C22:4 n-6	0.53 ± 0.10	0.60 ± 0.14	0.52 ± 0.05	0.51 ± 0.11	0.43 ± 0.07	0.36 ± 0.06
C22:5 n-3	ND[1]	ND	ND	ND	ND	ND
C22:6 n-3	1.94 ± 0.31[a]	2.12 ± 0.22[a]	2.14 ± 0.23[a]	2.05 ± 0.33[a]	3.10 ± 0.33[b]	3.07 ± 0.41[b]
n-3	2.31 ± 0.36	2.54 ± 0.39	2.65 ± 0.37	2.81 ± 0.40	3.94 ± 0.43	4.04 ± 0.35
n-6	19.32 ± 2.18	20.62 ± 2.67	18.93 ± 3.20	20.14 ± 2.23	18.57 ± 2.16	16.42 ± 1.53
n-3:n-6 ratio	0.12 ± 0.02[a]	0.12 ± 0.01[a]	0.14 ± 0.02[a]	0.14 ± 0.01[a]	0.21 ± 0.03[b]	0.25 ± 0.03[b]

See legend to Table 2.
Means within a row not sharing a common superscript are significantly different.
[1] ND = not detectable

Table 5. Fatty acid composition of liver fat at weaning and in piglets fed the experimental diets for 10 days

		Diet code				
		Control	LO	LO	FO	FO
	At weaning	0.1	0.2	0.3	0.2	0.3
Fatty acid (g/100g methylesters)						
C16:0	18.51 ± 2.50	13.04 ± 1.00	13.48 ± 1.62	13.13 ± 0.60	14.37 ± 1.25	14.37 ± 1.28
C18:0	20.58 ± 4.35	23.56 ± 1.85	22.59 ± 3.89	23.42 ± 1.32	23.09 ± 1.32	23.35 ± 0.92
C18:1 n-9	12.85 ± 3.80	10.07 ± 0.71	11.15 ± 2.85	10.08 ± 1.26	9.34 ± 0.80	9.37 ± 0.85
C18:2 n-6	13.69 ± 1.03	17.19 ± 1.89	18.09 ± 0.94	18.02 ± 2.74	16.30 ± 1.65	14.62 ± 1.02
C18:3 n-6	0.15 ± 0.22^{a}	0.17 ± 0.17^{a}	0.13 ± 0.15^{a}	0.16 ± 0.10^{a}	0.03 ± 0.08^{b}	0.13 ± 0.10^{a}
C18:3 n-3	0.30 ± 0.19^{a}	0.36 ± 0.20^{a}	0.73 ± 0.27^{b}	0.88 ± 0.33^{b}	0.19 ± 0.10^{c}	0.19 ± 0.11^{c}
C20:2 n-6	0.34 ± 0.07^{a}	0.68 ± 0.26^{b}	0.59 ± 0.19^{c}	0.68 ± 0.31^{b}	0.56 ± 0.12^{c}	0.43 ± 0.06^{d}
C20:3 n-6	0.66 ± 0.07	0.54 ± 0.15	0.57± 0.17	0.59 ± 0.08	0.67 ± 0.13	0.74 ± 0.09
C20:3 n-3	0.01 ± 0.03	0.04 ± 0.09	0.03 ± 0.07	0.19 ± 0.16	0.00 ± 0.00	0.00 ± 0.00
C20:4 n-6	16.25 ± 3.51	18.92 ± 1.47	16.84 ± 2.17	16.97 ± 1.40	13.48 ± 1.39	12.85 ± 0.86
C20:5 n-3	0.23 ± 0.05^{a}	0.33 ± 0.06^{b}	0.55 ± 0.16^{c}	0.93 ± 0.23^{d}	2.88 ± 0.87^{e}	3.33 ± 1.07^{e}
C22:4 n-6	0.73 ± 0.13^{a}	0.72 ± 0.07^{a}	0.57 ± 0.09^{b}	0.50 ± 0.17^{b}	0.27 ± 0.03^{c}	0.25 ± 0.08^{c}
C22:5 n-3	ND	ND	ND	ND	ND	ND
C22:6 n-3	4.96 ± 1.05^{a}	6.01 ± 1.29^{a}	5.59 ± 0.86^{a}	5.76 ± 0.62^{a}	10.73 ± 1.05^{b}	11.41 ± 0.74^{b}
n-3	5.50 ± 0.92^{a}	6.74 ± 1.15^{a}	6.90 ± 0.92^{a}	7.75 ± 0.62^{a}	13.80 ± 1.56^{b}	14.93 ± 1.00^{b}
n-6	31.81 ± 2.47^{a}	38.22 ± 1.20^{b}	36.80 ± 2.81^{b}	36.91 ± 2.11^{b}	31.31 ± 2.91^{a}	29.00 ± 1.28^{a}
n-3:n-6 ratio	0.17 ± 0.02^{a}	0.18 ± 0.03^{a}	0.19 ± 0.03^{a}	0.21 ± 0.02^{a}	0.45 ± 0.09^{b}	0.52 ± 0.05^{b}

See legend to Table 2.
Means within a row not sharing a common superscript are significantly different.

Table 6. Fatty acid composition of adipose tissue at weaning and in piglets fed the experimental diets for 10 days

		Diet code				
	At weaning	Control 0.1	LO 0.2	LO 0.3	FO 0.2	FO 0.3
Fatty acids (g/100g methylesters)						
C16:0	24.81 ± 2.41	24.59 ± 2.25	24.38 ± 1.47	23.45 ± 1.64	23.71 ± 0.59	29.64 ± 0.75
C18:0	5.53 ± 0.43	6.86 ± 1.28	5.95 ± 0.05	5.77 ± 0.62	7.18 ± 0.34	6.89 ± 1.04
C18:1 n-9	38.14 ± 2.60	36.24 ± 1.68	37.39 ± 2.39	38.49 ± 1.99	37.31 ± 2.06	35.02 ± 1.41
C18:2 n-6	11.85 ± 0.65	14.42 ± 1.92	13.36 ± 0.87	12.82 ± 0.81	12.45 ± 0.69	12.36 ± 0.51
C18:3 n-6	0.03 ± 0.05	0.04 ± 0.04	0.04 ± 0.05	0.03 ± 0.04	0.03 ± 0.04	0.02 ± 0.03
C18:3 n-3	0.97 ± 0.09^{ab}	1.15 ± 0.24^{c}	1.17 ± 0.20^{c}	1.20 ± 0.26^{d}	0.88 ± 0.05^{a}	0.91 ± 0.06^{a}
C20:2 n-6	0.43 ± 0.07	0.44 ± 0.08	0.41 ± 0.05	0.45 ± 0.08	0.46 ± 0.04	0.40 ± 0.05
C20:3 n-6	0.18 ± 0.03^{b}	0.15 ± 0.03^{b}	0.16 ± 0.03^{b}	0.14 ± 0.07^{b}	0.15 ± 0.01^{b}	0.15 ± 0.02^{b}
C20:3 n-3	0.13 ± 0.03^{b}	0.08 ± 0.07^{a}	0.13 ± 0.03^{b}	0.12 ± 0.06^{b}	0.10 ± 0.05^{a}	0.09 ± 0.04^{a}
C20:4 n-6	0.44 ± 0.08^{b}	0.38 ± 0.07^{b}	0.40 ± 0.05^{b}	0.40 ± 0.05^{b}	0.41 ± 0.03^{b}	0.36 ± 0.02^{b}
C20:5 n-3	ND	ND	ND	ND	0.06 ± 0.05^{b}	0.09 ± 0.11^{b}
C22:4 n-6	0.09 ± 0.07^{b}	0.11 ± 0.06^{b}	0.12 ± 0.03^{b}	0.11 ± 0.06^{b}	0.14 ± 0.04^{b}	0.08 ± 0.06^{b}
C22:6 n-3	0.08 ± 0.07^{b}	0.09 ± 0.05^{b}	0.09 ± 0.04^{b}	0.08 ± 0.07^{b}	0.29 ± 0.10^{a}	0.44 ± 0.21^{e}
n-3	1.18 ± 0.15^{a}	1.32 ± 0.32^{a}	1.39 ± 0.20^{b}	1.40 ± 0.26^{b}	1.34 ± 0.21^{a}	1.52 ± 0.37^{e}
n-6	13.01 ± 0.79^{b}	15.54 ± 2.09^{a}	14.48 ± 0.93^{b}	13.95 ± 0.95^{b}	13.65 ± 0.71^{b}	13.36 ± 0.55^{b}
n-3:n-6 ratio	0.09 ± 0.01	0.08 ± 0.01	0.10 ± 0.01	0.10 ± 0.02	0.10 ± 0.20	0.11 ± 0.02

See legend to Table 2.
Means within a row not sharing a common superscript are significantly different.

Table 7. Fatty acid composition of lymph nodular fat at weaning and in piglets fed the experimental diets for 10 days

		Diet code				
	At weaning	Control 0.1	LO 0.2	LO 0.3	FO 0.2	FO 0.3
Fatty acids (g/100g methylesters)						
C16:0	24.88 ± 1.56	23.82 ± 1.58	24.33 ± 1.47	23.77 ± 1.28	23.82 ± 0.48	25.60 ± 1.43
C18:0	7.49 ± 0.35	8.03 ± 1.10	6.78 ± 0.06	6.90 ± 0.83	8.35 ± 0.99	8.21 ± 1.02
C18:1 n-9	34.04 ± 2.28	33.83 ± 2.91	35.94 ± 1.97	36.05 ± 1.96	34.31 ± 2.48	32.64 ± 2.60
C18:2 n-6	11.54 ± 0.70	13.82 ± 1.93	13.74 ± 1.04	13.30 ± 0.68	13.00 ± 0.77	12.20 ± 0.56
C18:3 n-6	0.03 ± 0.05	0.03 ± 0.05	0.04 ± 0.06	0.02 ± 0.05	ND	0.01 ± 0.04
C18:3 n-3	0.85 ± 0.07[a]	1.01 ± 0.29[b]	1.20 ± 0.20[d]	1.27 ± 0.16[d]	0.83 ± 0.06[a]	0.83 ± 0.06[a]
C20:2 n-6	0.45 ± 0.06	0.49 ± 0.08	0.45 ± 0.07	0.47 ± 0.06	0.47 ± 0.03	0.43 ± 0.04
C20:3 n-6	0.26 ± 0.07[a]	0.22 ± 0.10[a]	0.18 ± 0.03[b]	0.19 ± 0.02[b]	0.21 ± 0.06[a]	0.21 ± 0.03[a]
C20:3 n-3	0.04 ± 0.07[a]	0.11 ± 0.03[b]	0.13 ± 0.03[b]	0.15 ± 0.02[b]	0.07 ± 0.06[a]	0.05 ± 0.05[a]
C20:4 n-6	2.95 ± 1.37[a]	2.48 ± 2.47[a]	1.05 ± 0.22[c]	1.32 ± 0.39[d]	1.82 ± 0.96[a]	1.65 ± 0.55[a]
C20:5 n-3	ND	ND	ND	ND	0.13 ± 0.07[a]	0.16 ± 0.11[a]
C22:4 n-6	0.50 ± 0.23[a]	0.45 ± 0.39[a]	0.23 ± 0.04[c]	0.27 ± 0.05[c]	0.30 ± 0.11[c]	0.29 ± 0.07[c]
C22:6 n-3	0.37 ± 0.17[a]	0.30 ± 0.21[a]	0.17 ± 0.02[c]	0.21 ± 0.04[c]	0.63 ± 0.21[d]	0.69 ± 0.25[d]
n-3	1.27 ± 0.23[a]	1.42 ± 0.18[b]	1.50 ± 0.22[b]	1.62 ± 0.15[c]	1.66 ± 0.20[c]	1.74 ± 0.36[d]
n-6	15.72 ± 1.79[a]	17.49 ± 2.16[c]	15.68[a] ± 1.28	15.57 ± 0.84[a]	15.80 ± 1.74[a]	14.78 ± 0.70[a]
n-3:n-6 ratio	0.08 ± 0.01	0.08 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.12 ± 0.02

See legend to Table 2.
Means within a row not sharing a common superscript are significantly different.

Villus height and crypt depth

Villus heigth fell and crypt depth rose after weaning, and as a result, the villus:crypt ratio showed a marked decrease (Table 3). The fat type and n-3:n-6 ratio of the weaner diet did not significantly affect small intestinal morphology, as based on villus height and crypt depth. However, the fish-oil diet with n-3:n-6 ratio of 0.3 had produced a significantly higher villus:crypt ratio than the linseed-oil diet with n-3:n-6 ratio of 0.3. The highest group-mean villus:crypt ratio was observed in piglets weaned onto the control diet with n-3:n-6 ratio of 0.1.

Fatty acid composition of erythrocytes and tissues

The fatty acid composition of the weaner diets was reflected in the fatty acid composition of erythrocyte membranes (Table 4). The linseed-oil containing diet induced a dose-dependent increase in the relative percentage of ALA. The diets containing fish oil raised the contents of EPA and docosahexaenoic acid (DHA, C22:6 n-3) in erythrocyte membranes. The total amounts of n-3 PUFAs in erythrocytes were significantly higher after feeding the diets containing fish oil instead of linseed oil. The n-3:n-6 ratios were also highest for the fish-oil diets.

Table 5 illustrates that the feeding of linseed oil and fish oil raised the concentrations in liver fat of ALA and those of EPA and DHA, respectively. The diets enriched with fish oil produced higher n-3:n-6 ratios in hepatic fat than did the diets high in linseed oil. The diets with fish oil induced lower percentages of both LA and arachidonic acid (AA, C20:4 n-6) in liver fat.

Table 6 and 7 illustrate the incorporation of dietary n-3 PUFAs into either subcutaneous adipose tissue and lymph nodular fat. The concentrations of ALA were highest in fat tissue from piglets fed the diets with linseed oil. Only when the diets contained fish oil, there were detectable amounts of EPA in the fat tissues. Fish oil feeding raised the concentrations of DHA in fat tissue. The n-3:n-6 ratio in either subcutaneous or lymph nodular fat was higher when fish oil instead of linseed oil was fed.

Discussion

We hypothesised that fish oil incorporation into the weaner diet would enhance performance of piglets when compared to the incorporation of linseed oil. Indeed, body-weight gain during days 0 - 10 post weaning was on average 27 % higher when the diets contained fish oil instead of linseed oil. The fish-oil-induced increase in weight gain was not statistically significant. It should be noted that the statistical power in this experiment was relatively low. In order to obtain statistical significance ($p=0.05$) of the detected increase in weight gain at a power of 80%, and with the observed variance of weight gain, 69 piglets per dietary group would have to be used. If and when the positive effect of fish oil seen in this experiment is

reproducible, it will be statistically significant only in an experiment using a sufficiently large number of animals. In any event, an increase in weight gain by 27% is practically relevant.

Post-weaning feed intake is an important determinant of villus height in piglets (Pluske et al., 1996). The experimental diets did not differently influence feed intake. However, when considering specific diet comparisons, the villus:crypt ratio was affected by diet composition. The highest ratio was seen for the control diet with n-3:n-6 ratio of 0.1, and the lowest ratio was found in the piglets fed the linseed oil diet with n-3:n-6 ratio of 0.3. The tendency towards more weight gain when the diets contained fish oil was associated with higher villus:crypt ratios when the diets containing linseed oil were used as comparison, but not when the control diet was considered. It can be concluded that fish oil did not improve growth through higher feed intake and more absorptive capacity as indicated by a higher:villus crypt ratio. Possibly, the anti-inflammatory activity of n-3 PUFAs in fish oil have played a role. Weanling piglets often show inflammation of the gut, and their is indirect evidence (Akisu et al., 1998; Korver and Klasing, 1997; Ohtsuka et al., 1997) that fish oil feeding might decrease the inflammatory response to the weaning process.

As would be anticipated (Alessandri et al., 1996; Arbuckle and Innis, 1993; Goustard-Langelier et al., 1999; Rooke et al., 1998; Ward et al., 1998; Pond, 1996), the dietary concentrations of ALA and EPA were generally reflected in the fatty acid composition of erythrocyte membranes, liver fat, storage fat and lymph nodular fat. The n-3:n-6 ratios of the weaner diets were not reflected in the various tissues in a dose-dependent manner. The diets containing fish oil induced higher n-3:n-6 ratios in erythrocyte membranes and liver fat than did the diets containing linseed oil, but having similar n-3:n-6 ratios. This observation indicates that ALA is either less efficiently incorporated in the tissues or that ALA is partially oxidized and not quantitatively converted into EPA and DHA. The implication is that for the production of eicosanoids the feeding of EPA is more effective than the feeding of ALA.

In conclusion, this study provides suggestive evidence for a positive effect of fish oil on growth of weanling piglets. This effect of fish oil may not be mediated by counteracting the weaning-induced decrease in villus height, but perhaps rather by improving immunity. The incorporation of fish oil into the weaner diet may negatively affect palatability (Kolanowski et al., 1999), this effect not being observed in the present study, but it should be stressed that feed intake was low. Post-weaning feed intake determines the extent of villus atrophy in piglets. A highly palatable diet containing fish oil might be advantageous in preventing post-weaning disorders as associated with the current practice of management of weanling piglets.

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