Essential-fatty acid supply of weanling piglets

De voorziening van gespeende biggen met essentiële vetzuren

(met een samenvatting in het Nederlands)

Proefschrift

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door

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Voorwoord

In de jaren dat ik werkte aan dit proefschrift, ben ik met veel mensen en nog meer dieren in aanraking gekomen. Elk op hun eigen wijze hebben ze niet alleen bijgedragen aan de totstandkoming van dit proefschrift, maar vooral ook aan mijn groei en ontwikkeling, hetzij ‘wetenschappelijk’ of meer persoonlijk. Ik wil graag iedereen bedanken.

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Scope of the thesis

Introduction

This thesis describes research on the essential-fatty acid supply of weanling piglets. Prior to outlining the scope of the thesis, the polyunsaturated fatty acids and their functions are briefly summarised.

Nomenclature of polyunsaturated fatty acids

Table 1 gives the names and shorthand notations of selected polyunsaturated fatty acids.

Table 1. Names, shorthand notation and abbreviations of selected polyunsaturated fatty acids

Fatty acid	Shorthand notation	Abbreviation
Linoleic acid	C18:2 n-6	LA
α-Linolenic acid	C18:3 n-3	ALA
γ-Linolenic acid	C18:3 n-6	GLA
Dihomo- γ-linolenic acid	C20:3 n-6	DGLA
Arachidonic acid	C20:4 n-6	AA
Eicosapentaenoic acid	C20:5 n-3	EPA
Docosahexaenoic acid	C22:6 n-3	DHA

Polyunsaturated fatty acids (PUFAs) are classified by the length of the carbon chain, the number of double bounds and the location of the first double bound. Holman (1964) introduced the 'omega' (ω or n) nomenclature for the identification of PUFAs. The omega number refers to the position of the first double bound as counted from the methyl end of the carbon chain. For example, linoleic acid, in short hand notation C18:2 n-6, has 18 carbons and 2 double bounds with the first double bound at the sixth carbon atom from the methyl end of the chain. All metabolic conversions in vertebrates, i.e. desaturation and elongation, occur beyond the ninth carbon atom from the methyl end of the chain. Therefore, the omega nomenclature classifies PUFAs into two families with fixed structure at the methyl end of the molecule. Vertebrates lack the enzymes to introduce double bounds within the first 9 carbons from the methyl end, and thus require dietary sources of the essential fatty acids linoleic acid (LA, C18:2 n-6) and α-linolenic acid (ALA, C18:3 n-3) which are considered the parent compounds of the n-6 and n-3 families of PUFAs, respectively. The parent fatty acids can be elongated and desaturated to other PUFAs, like arachidonic acid (AA, C20:4 n-6) or

eicosapentaenoic acid (EPA, C20:5 n-3) (Fig. 1). However, the two parent fatty acids and their metabolites compete with each other for the desaturase and elongase enzymes, which generally have more affinity for n-3 than for n-6 PUFAs.

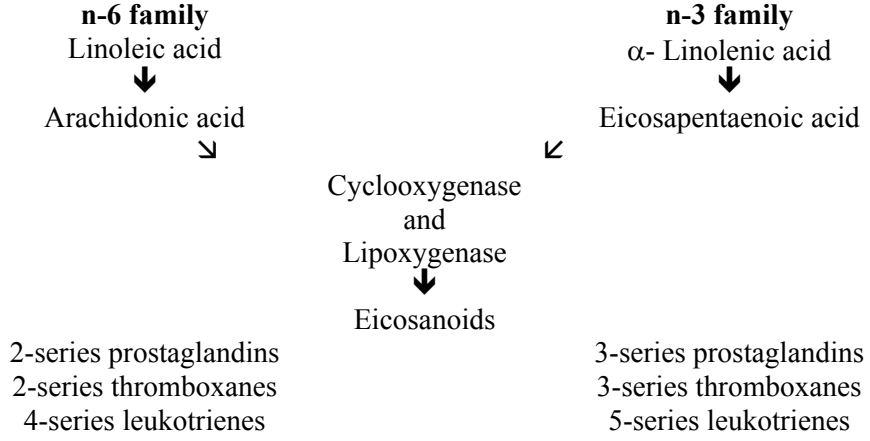

Fig 1. Simplified scheme of fatty acid metabolism and the production of eicosanoids (prostaglandins, thromboxanes and leukotrienes).

Functions of polyunsaturated fatty acids

Essential fatty acids have two main functions. First, PUFAs, especially AA, are structural components of cellular membranes. In retina and brain, EPA and docosahexaenoic acid (DHA, C22:6 n-3) are essential structural components. These PUFAs render to the cell membranes their fluid nature. Without the availability of PUFAs, membranes will incorporate more saturated fatty acids, resulting in less fluid and instable membranes. As a result, the tissue permeability increases and leads to nutrient and water loss, change of receptor function, enzyme activity and altered cytokine production (Wan et al., 1988). Secondly, PUFAs play an important role in the immune response. The fatty acids with 20 carbons, AA and EPA are precursors for the eicosanoids, i.e. the prostaglandins, thromboxanes and leukotrienes (Fig. 1). Eicosanoids affect processes such as immunity, platelet aggregation and vasoconstriction. In general, the eicosanoids derived from the n-6 PUFAs have effects opposite to those derived from the n-3 PUFAs. Due to the competition between n-3 and n-6 PUFAs for the desaturases and elongages, the net effects of the eicosanoids depend on the amounts and on the ratio of n-3 and n-6 PUFAs present in the diet.

Scope of the thesis

Weanling piglets are prone to the development of the so-called post-weaning syndrome which is associated with atrophy of the villi, inflammation of the gut (Cera et al., 1988; Hall and Byrne, 1989; Hampson, 1986; Kenworthy, 1976; Nabuurs, 1991) and depressed performance (Jahn and Uecker, 1987; Svedsen et al., 1974; Svensmark et al., 1989). There is evidence that dietary n-3 PUFAs may antagonize atrophy of villi and have anti-inflammatory activity. In growing chicks, the intake of extra n-3 PUFAs has been shown to improve performance and decrease the inflammatory response to LPS from *S. typhimurium* and *S. aureus* (Korver and Klasing, 1997). In young mice with hypoxia-induced bowel necrosis, supplementation with n-3 PUFAs reduced the degree of necrosis (Akisu et al., 1998). Mucosal damage in food-sensitive enteropathy in mice was prevented by supplementation of the diet with n-3 PUFAs (Ohtsuka et al., 1997).

The general scope of the research in the present thesis was to investigate the influence of n-3 and n-6 PUFAs on performance and health of weanling piglets. It was anticipated that the information thus obtained would provide clues as to the ideal fatty acid composition of the diet for weanling piglets. The specific objectives were as follows:

1. To gain information as to the fatty acid supply and status of piglets from birth to two weeks after weaning.
2. To investigate the effect of supplemental n-3 PUFAs and the n-3:n-6 ratio on small intestinal morphology and growth performance.
3. To study whether the dietary fatty acid composition influences the response to a challenge with *Escherichia coli*.
4. To describe the determinants of essential-fatty acid status of piglets at weaning.
5. To put into perspective the effects of dietary fatty acid composition and the intake of dietary dry matter.

References

Akisu, M., Baka, M., Coker, I., Kultursay, N. and Huseyinov, A. (1998) Effect of dietary n-3 fatty acids on hypoxia-induced necrotizing enterocolitis in young mice. n-3 fatty acids alter platelet-activating factor and leukotriene B4 production in the intestine. Biolog. Neonate. 74: 31-38.

Cera, K.R., Mahan, D.C., Cross, R.F., Reinhart, G.A. and Whitmoyer, R.E. (1988) Effect of age, weaning and postweaning diet on small intestinal growth and jejunal morphology in young swine. J. Anim. Sci. 66: 574-584.

Jahn, S. and Uecker, E. (1987) Ökonomische Untersuchingen zur Kolienterotoxamie des Schweines. Mh. Vet. Med. 42: 769-771.

Hall, G.A. and Byrne, R.F. (1989) Effects of age and diet on small intestinal structure and function in gnotobiotic pigs. Res. Vet. Sci. 47: 387-392.
Hampson, D.J. (1986) Alterations in piglets small intestinal structure at weaning. Res. Vet. Sci. 40: 32-40.
Holman, R. T. (1964) Nutritional and metabolic interrelationships between fatty acids. Fed. Proc. 23: 1062-1067.
Kenworthy, R. (1976) Observations on the effects of weaning in the young pig. Clinical and histopathological studies of intestinal function and morphology. Res. Vet. Sci. 21: 69-75.
Korver, D.R. and K. C. Klasing, K.C. (1997) Dietary fish oil alters specific and inflammatory responses in chicks. J. Nutr. 127: 2039-2046.
Nabuurs, M.J.A. (1991) Etiologic and pathogenic studies on postweaning diarrhoea. Ph.D. thesis, Utrecht University, Utrecht, The Netherlands.
Ohtsuka, Y., Yamashiro, Y, Shimizu, T., Nagata, S., Igarashi, J., Shinohara, K., Oguchi, S. and Yabuta, K. (1997) Reducing cell-membrane n-6 fatty acids attenuate mucosal damage in food-sensitive enteropathy in mice. Pedriatr. Res. 42: 835-839.
Svedsen, J., Larsen, J.L. and Bille, N. (1974) Outbreaks of post weaning *Escherichia Coli* infections in weaned pigs. Nord. Vet. Med. 26: 314-322.
Svensmark, B., Nielsen, K., Willeberg and P., Jorsal, S.E. (1989) Epidemiological studies of piglet diarrhoea in intesively managed Danish sow herds. II. Postweaning diarrhoea. Acta Vet. Scand. 30: 55-62.
Wan, J.M.F, Haw, M.P. and Blackburn, G.L. (1989) Nutrition, immune function, and inflammation: an overview. Proc. Nutr. Soc. 48: 315-335.

Chapter 1

Fatty acid supply and status of piglets from birth to two weeks post weaning

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Abstract

We had reasoned that, around weaning, piglets would experience a decrease in the intake of n-3 polyunsaturated fatty acids (PUFAs). A low essential fatty acid status, and especially a low ratio of n-3:n-6 PUFAs, might play a role in the development of the syndrome of post-weaning diarrhoea. The intake of n-3 and n-6 PUFAs was assessed in piglets kept under practical conditions from birth to two weeks postweaning. In addition, the fatty acid composition of erythrocyte membranes, liver fat and lymph nodular fat tissue was determined. It was found that between weaning and one week post weaning there was no clear difference in the intake of n-3 and n-6 PUFAs. Likewise, the fatty acid composition of erythrocyte membranes remained constant around weaning. Weaning was associated with a drop of plasma total cholesterol, HDL cholesterol and phospholipid concentrations as well as a decrease in heparin-released plasma lipoprotein lipase activity. The changes in plasma lipid metabolism around weaning are explained by the decrease in fat intake at that moment. It is concluded that this study does not point at a lowering of the status of n-3 and n-6 PUFAs in piglets at the stage around weaning. However, it is stressed that the outcome of this study is determined by the fatty acid compositions of the commercial lactation diet, creep feed and weaner diet that were used.

Introduction

Weaning of piglets is associated with an abrupt change in the nature of nutrition, i.e. a change from milk to solid food. Amongst others, there is a shift in the main energy source from fat to carbohydrates. In addition, for a period of about 4 days after weaning, feed intake is very low (Pluske et al., 1996). The low fat content of the post-weaning diet and the low feed intake result in a low intake of PUFAs, including the essential fatty acids, linoleic acid (LA, C18:2 n-6) and α-linolenic acid (ALA, C18:3 n-3). The two essential fatty acids are precursors for eicosanoids, affecting a variety of biological functions, including immunity (Wu and Meydani 1998). In general, the eicosanoids produced from the n-6 and n-3 families of PUFAs have antagonistic activities (Vaughn et al., 1994), and it has been suggested that the optimum ratio of n-3:n-6 PUFAs in the human diet is 0.2 (Aggett et al., 1991). 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).

Piglets just before weaning drink approximately 1 kg milk per day, resulting in a daily intake of 1.8 g n-3 and 10 g n-6 PUFAs (Taugbol et al., 1993) so that the n-3:n-6 ratio of the milk is 0.18. When compared with sow milk, the post-weaning, dry diet not only is low in fat, but the fat fraction generally contains a relatively large proportion of LA and small proportion of n-3 fatty acids, resulting in a n-3:n-6 ratio of about 0.09 to 0.12 (unpublished results). Thus, after weaning the absolute intake of LA and ALA is low as well as the n-3:n-6 ratio. Newly weaned piglets often suffer from atrophy of small intestinal villi and inflammation of the intestine (Kenworthy 1976; Hampson 1986; Cera et al., 1988; Hall and Byrne 1989; Nabuurs 1991), these disorders being associated with the syndrome of post-weaning diarrhoea and oedema disease (Nabuurs 1991; Van Beers-Schreurs et al., 1992). It could be suggested that the low intake of PUFAs and the low n-3:n-6 ratio, through influencing membrane integrity and immune function, play a role in the development of post-weaning disorders. In an attempt to substantiate the idea of inappropriate supply and intake of PUFAs by newly weaned piglets, we have quantified both the ingestion and status of polyunsaturated fatty acids in piglets from birth to two weeks post weaning. In order to describe the time course of plasma lipid metabolism, the concentrations of plasma lipids and lipoproteins, and the plasma activity of heparin-released lipoprotein lipase were measured.

Materials and methods

Animals, feed and housing

Six sows with their litters (F2: Finish GY slaughterline x [GY sow-line x Dutch Landrace]) were used. The sows were housed in farrowing pens and litter

size was 11 to 13. A total of 72 piglets was used. During the experimental period six piglets died. Three piglets died of unknown cause, one was euthanised because of a too low birth weight, one was crushed by the sow and one died during the blood sampling procedure. The piglets were studied from the day of birth until 42 days of age. Piglets were weighed at birth (day 0) and at the age of 14, 28, 35 and 42 days. All piglets were weaned at the age of 28 days by removing the sows. The farrowing pens (2.40 x 1.80 m) had a combination of a slatted (1/3) and concrete (2/3) floor. The temperature in the room was set at 20 °C on the day of birth and additional heat was provided by lamps. On the day of weaning the ambient temperature was set at 25 °C. After 14 days the lamps were removed. Daylight could enter the rooms.

Table 1. Analysed composition of the commercial lactation diet, creep feed and weaner diet.

	Lactation diet	Creep feed	Weaner diet
Chemical analysis (g/kg)			
Dry matter	907.4	934.7	914.5
Crude protein	174.5	198.8	184.3
Crude fat	65.4	106.0	64.6
Crude fiber	82.4	17.3	33.6
Ash	64	48.1	50.5
Analysed fatty acids[1] (g/100 g methylesters)			
C18:2 n-6	23.23	28.02	46.69
C18:3 n-3	2.54	2.30	2.80
C20:2 n-6	0.19	0.07	0.07
C20:4 n-6	0.15	0.17	0.12
C20:5 n-3	0.00	1.16	0.76
C22:6 n-3	0.07	1.58	1.39
n-3[2]	2.61	5.04	4.94
n-6[3]	23.57	28.26	46.88
n-3:n-6 ratio	0.11	0.18	0.11

[1]Fatty acids indicated in shorthand notation: number of carbon atoms before colon and number of double bonds after colon; n-6 and n-3 refers to the first double bond at carbon atom 6 or 3 as counted from the methyl end of the fatty acid.
[2] Σ of C18:3 n-3, C20:5 n-3 and C22:6 n-3.
[3] Σ of C18:2 n-6, C20:2 n-6 and C20:4 n-6.

The sows were fed with a commercial lactation diet and had ad libitum access to tap water. At the age of 14 days, the piglets received about 100 g of a commercial creep feed per animal. The creep feed was put in plastic bowls. When the creep feed was consumed, a commercial weaner pellet was provided in a self feeder. Water was freely available to the piglets. No medications were used. The analysed compositions of the diets are given in Table 1.

Sample collection and analyses

The sows were milked weekly after intravenous injection of oxytocine (1.5 – 2.0 ml, oxytocine-s®, Intervet, Boxmeer, The Netherlands), starting on the day they gave birth and finishing at weaning of the piglets. The milk was stored at –20 °C until analysis. Feed samples of the lactation diet, creep feed and weaner pellets were taken for chemical analysis. On days 0, 14, 28, 35 and 42, blood samples were collected by vena cava puncture from one piglet chosen at random of each litter for the analysis of the fatty acid composition of erythrocyte membranes and the concentrations of blood lipids and lipoproteins. On days 14, 28, 35 and 42 another piglet chosen at random of each litter was sampled for the analysis of lipoprotein lipase activity in plasma after intravenous heparin injection. On days 0, 28 and 42 the piglet with body weight nearest to the mean weight of the litter was anesthesized with intramuscular administration of ketamine (200 mg/kg, ketamine 10%, Alfasan, Woerden, The Netherlands) and xylazine (1 mg/kg, Sedamun, Eurovet, Bladel, The Netherlands). The liver (days 0, 28 and 42) and fat near and far from the lnn popliteus (days 28 and 42) were removed and stored at – 80 °C until further analysis. The fatty acid composition of fat near to the lnn popliteus is considered to be particularly important as this fat provides the precursors for eicosanoid synthesis (Pond 1996).

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 crypt 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, a mean of all 10 values was calculated and used for further analysis.

Crude fat and fatty acids in sows' milk and the pelleted 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 analysis. 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. For the analysis of plasma lipids and lipoproteins, blood was taken into heparinized tubes. Plasma triglycerides, phospholipids, total cholesterol and HDL cholesterol were measured enzymatically using commercial test combinations (Boehringer-Mannheim GmbH, Mannheim, Germany). Lipoproteins were isolated according to (Terpstra et al., 1982). For measuring lipoprotein lipase activity, one piglet of each litter was injected intravenously with heparin (50 IE/kg, heparine, Leo, Weesp, The Netherlands) followed by blood sampling through vena cava puncture 10 min. later. The blood was collected in EDTA-containing tubes. Total and hepatic lipase activities were determined according to Nilsson-Ehle and Schotz (1976) in the presence of a low and high concentration of NaCl, respectively. Lipoprotein lipase activity was calculated as the difference. The fatty acid composition of the liver and lymph node fat was determined as described above.

Statistical analyses

Time-dependent differences in the various variables were evaluated using Student's t test with Bonferroni's adaptation to take into account the increased risk of a type I error due to multiple comparisons. The level of statistical significance was pre-set at $P < 0.05$.

Results

The lactation diet contained 13.7 g LA and 1.5 g ALA per kg, when assuming that on a weight basis crude fat contains 90 % of fatty acids. Long-chain n-3 PUFAs other than ALA were essentially absent in the lactation diet (Table 1). The pelleted creep feed and weaner diet had similar relative percentages of ALA, but the former had a high fat content and thus contained more ALA per unit of weight. Due to the high relative percentage of LA in the weaner diet, the creep feed and weaner diet had similar contents of LA. The n-3:n-6 ratio in the creep feed was higher than in the weaner diet.

The colostrum had a higher concentration of protein and lower amount of fat than the milk produced later (Table 2). The fat content rose during the first two

Table 2. Time course of analysed composition of sow milk

	Day				
	0	7	14	21	28
Chemical analysis (g/kg)					
Dry matter	234 ± 22[a]	195 ± 4[b]	205 ± 10[a]	183 ± 3[b]	183 ± 4[b]
Crude protein	139 ± 23[a]	56 ± 3[b]	53 ± 2[b]	52 ± 2[b]	57 ± 3[b]
Crude fat	59 ± 6[a]	92 ± 4[b]	96 ± 10[b]	73 ± 3[a]	69 ± 3[a]
Ash	6.5 ± 0.3[a]	7.5 ± 0.2[b]	7.5 ± 0.2[b]	8.0 ± 0.3[b]	8.9 ± 0.2[c]
Analysed fatty acids (g/100 g methylesters)					
C18:2 n-6	20.88 ± 0.57[a]	11.68 ± 0.29[b]	12.11 ± 0.31[b]	12.05 ± 0.23[b]	11.20 ± 0.33[b]
C18:3 n-6	0.21 ± 0.02[a]	0.14 ± 0.01[b]	0.11 ± 0.01[c]	0.10 ± 0.01[c]	0.05 ± 0.01[d]
C18:3 n-3	1.51 ± 0.04[a]	0.98 ± 0.03[b]	1.04 ± 0.02[b]	1.00 ± 0.02[b]	0.92 ± 0.03[c]
C20:2 n-6	0.46 ± 0.02[a]	0.31 ± 0.02[b]	0.33 ± 0.02[b]	0.30 ± 0.03[b]	0.30 ± 0.02[b]
C20:3 n-6	0.27 ± 0.01[a]	0.11 ± 0.01[b]	0.09 ± 0.01[b]	0.10 ± 0.01[b]	0.06 ± 0.02[c]
C20:4 n-6	0.98 ± 0.04[a]	0.59 ± 0.03[b]	0.51 ± 0.02[c]	0.48 ± 0.03[c,d]	0.42 ± 0.02[d]
C20:3 n-3	0.14 ± 0.01[a]	0.11 ± 0.01[b]	0.11 ± 0.01[b]	0.10 ± 0.01[b]	0.06 ± 0.02[c]
C20:5 n-3	0.09 ± 0.01[a]	0.05 ± 0.01[b]	0.05 ± 0.01[b]	0.06 ± 0.01[b]	0.02 ± 0.01[c]
C22:4 n-6	0.18 ± 0.01[a]	0.09 ± 0.01[b]	0.09 ± 0.01[b]	0.09 ± 0.01[b]	0.05 ± 0.01[c]
C22:6 n-3	0.19 ± 0.02[a]	0.09 ± 0.02[b]	0.09 ± 0.01[b]	0.08 ± 0.01[b]	0.04 ± 0.01[c]
n-3	1.93 ± 0.02[a]	1.23 ± 0.04[b]	1.30 ± 0.02[b]	1.24 ± 0.02[b]	1.04 ± 0.02[c]
n-6	22.98 ± 0.06[a]	12.92 ± 0.31[c]	13.24 ± 0.33[b]	13.11 ± 0.27[b,c]	12.08 ± 0.34[c]
n-3:n-6 ratio	0.08 ± 0.01[a]	0.09 ± 0.01[b]	0.10 ± 0.01[b]	0.09 ± 0.01[b]	0.09 ± 0.01[a]

Results are means ± SE for 6 sows. Means with different superscripts within a row are significantly different.
See legend to table 1.

weeks and then fell. The fatty acid pattern of the colostrum differed from that of the milk in that it had higher relative percentages of all PUFAs. The amounts of LA and ALA in milk remained stable, but the amount of arachidonic acid (AA, C20:4 n-6) dropped gradually. The concentrations of eicosapentaenoic (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3) were remarkably low on day 28. The n-3:n-6 ratio of the colostrum and milk was 0.08 to 0.10.

The piglets' liveweight at birth (day 0) was 1.64 ± 0.06 kg (mean ± SE, n=66). At the age of 14 days, body weight was 4.55 ± 0.15 At weaning (day 28) body weight was 8.00 ± 0.20 kg and at the age of 35 and 42 days, it was 9.25 ± 0.48 and 11.95 ± 0.66 kg, respectively. The average daily feed intake and feed conversion ratio during the first two weeks after weaning was 0.271 ± 0.016 kg and 1.09 ± 0.04 respectively.

Table 3 shows the time course of villus height and crypt depth for small intestinal mucosa. As would be expected (Van Beers-Schreurs et al., 1992*)*, there

Table 3. Time course of villus height and crypt depth of small intestinal mucosa

	Day								
	0			28			42		
	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum
Villus height (μm)	442 ± 47[a]	533 ± 61[a]	392 ± 46[a]	417 ± 32[a]	364 ± 29[b]	333 ± 17[a]	431 ± 28[a]	365 ± 26[b]	285 ± 31[a]
Crypt depth (μm)	94 ± 18[a]	72 ± 5[a]	71 ± 6[a]	178 ± 42[b]	175 ± 22[b]	144 ± 18[b]	222 ± 7[b]	249 ± 47[b]	191 ± 11[c]
Villus:crypt ratio	5.59 ± 0.95[a]	7.38 ± 0.74[a]	5.84 ± 0.96[a]	2.43 ± 0.24[b]	2.19 ± 0.24[b]	2.54 ± 0.40[b]	1.95 ± 0.13[b]	1.65 ± 0.24[b]	1.51 ± 0.19[b]

Results are means ± SE for 6 piglets. Means with different superscripts within gut section, but between days, are significantly diferent.

was a marked drop in the villus:crypt ratio at two weeks after weaning (day 42) when compared with the value at weaning.

The fatty acid status of the newborn piglets, as represented by the percentage fatty acid composition of the erythrocyte membranes, showed a level of AA as high as that of LA (Table 4). The level of ALA acid was very low as compared to that of DHA. With time, the amount of AA decreased. ALA showed an increase on day 14 and then fell. DHA had decreased on day 14 and then rose again. The content of EPA in erythrocyte membranes rose sharply after weaning on day 28. The n-3:n-6 ratio was 0.21 at birth, but dropped to values of 0.11 to 0.15, which was mainly due to an increase in LA.

Table 4. Time course of fatty acid composition of erythrocyte membranes from the piglets

	Day				
	0	14	28	35	42
Analysed fatty acids (g/100 g methylesters)					
C18:2 n-6	6.19 ± 0.40^{a}	13.00 ± 0.28^{b}	12.04 ± 0.65^{b}	12.15 ± 0.47^{b}	14.75 ± 0.66^{c}
C18:3 n-3	0.05 ± 0.05^{a}	0.27 ± 0.02^{b}	0.15 ± 0.07^{a}	0.17 ± 0.06^{a}	0.18 ± 0.08^{a}
C20:2 n-6	0.10 ± 0.10^{a}	0.30 ± 0.01^{b}	0.12 ± 0.06^{a}	0.26 ± 0.01^{b}	0.31 ± 0.01^{b}
C20:3 n-6	0.53 ± 0.03^{a}	0.35 ± 0.02^{b}	0.31 ± 0.02^{b}	0.31 ± 0.02^{b}	0.29 ± 0.01^{b}
C20:4 n-6	7.11 ± 0.25^{a}	5.01 ± 0.18^{b}	4.20 ± 0.10^{c}	4.28 ± 0.15^{c}	3.99 ± 0.12^{c}
C20:5 n-3	0.04 ± 0.04^{a}	0.00 ± 0.00^{a}	0.03 ± 0.03^{a}	0.15 ± 0.05^{b}	0.32 ± 0.02^{c}
C22:4 n-6	0.78 ± 0.06^{a}	0.50 ± 0.02^{b}	$0.43 \pm 0.03^{b,c}$	$0.44 \pm 0.04^{b,c}$	0.39 ± 0.02^{c}
C22:6 n-3	2.88 ± 0.21^{a}	1.83 ± 0.12^{b}	1.90 ± 0.07^{b}	$2.20 \pm 0.18^{b,c}$	2.44 ± 0.10^{c}
n-3	3.01 ± 0.13^{a}	2.10 ± 0.14^{b}	2.07 ± 0.11^{b}	$2.53 \pm 0.26^{a,b}$	2.94 ± 0.18^{a}
n-6	14.70 ± 0.57^{a}	$19.15 \pm 0.31^{b,d}$	17.10 ± 0.77^{c}	$17.44 \pm 0.58^{b,c}$	19.74 ± 0.78^{d}
n-3:n-6 ratio	0.21 ± 0.01^{a}	0.11 ± 0.01^{b}	0.12 ± 0.01^{b}	0.14 ± 0.01^{c}	0.15 ± 0.01^{c}

Results are means ± SE for 6 piglets. Means with different superscripts within a row are significantly different.
See legend to Table 1.

From birth to weaning, the relative percentages of LA and AA increased in the liver (Table 5). As to the n-3 PUFAs, ALA decreased, but EPA and DHA increased with age. The n-3:n-6 ratio in liver rose from a value of 0.17 at birth to 0.20 at weaning and increased further to 0.29 at the age of 42 days. At the age of 28 and 42 days, the fatty acid composition of fat tissue either far from or close to lymph nodes did not differ much (Table 6). The n-3:n-6 ratio in the fat tissues was 0.09.

Table 5. Neonatal, weaning and post-weaning fatty acid composition of whole liver from the piglets.

	Day		
	0	28	42
Analysed fatty acids (g/100 g methylesters)			
C18:2 n-6	10.13 ± 1.75[a]	13.26 ± 0.33[a]	14.97 ± 0.48[b]
C18:3 n-6	0.31 ± 0.04[a]	0.16 ± 0.01[b]	0.18 ± 0.01[b]
C18:3 n-3	0.43 ± 0.11[a]	0.24 ± 0.02[a]	0.20 ± 0.02[b]
C20:2 n-6	0.37 ± 0.05[a]	0.34 ± 0.03[a]	0.57 ± 0.09[b]
C20:3 n-6	0.57 ± 0.04[a]	0.87 ± 0.10[b]	0.71 ± 0.03[b]
C20:4 n-6	6.87 ± 0.66[a]	16.63 ± 0.43[b]	15.60 ± 0.36[b]
C20:3 n-3	0.07 ± 0.03[a]	0.07 ± 0.01[a]	0.03 ± 0.02[a]
C20:5 n-3	0.14 ± 0.02[a]	0.28 ± 0.03[a]	0.86 ± 0.12[b]
C22:4 n-6	0.38 ± 0.04[a]	0.84 ± 0.06[b]	0.56 ± 0.03[c]
C22:6 n-3	2.37 ± 0.23[a]	5.68 ± 0.33[b]	8.27 ± 0.31[c]
n-3	3.01 ± 0.21[a]	6.27 ± 0.35[b]	9.36 ± 0.37[c]
n-6	18.64 ± 2.09[a]	32.10 ± 0.29[b]	32.59 ± 0.76[b]
n-3:n-6	0.17 ± 0.01[a]	0.20 ± 0.01[a]	0.29 ± 0.02[b]

Results are means ± SE for 6 piglets. Means with different superscripts within a row are significantly different.
See legend to Table 1.

The levels of plasma total cholesterol, HDL cholesterol and phospholipids rose during the suckling period (Table 7). As from weaning on day 28, the plasma concentrations of phospholipids, total and HDL cholesterol fell, but did not reach neonatal values. The levels of triacylglycerols were highest at birth.

Table 8 shows the distribution of plasma total cholesterol between lipoprotein fractions. The recovery of lipoprotein cholesterol was on average 79 % of total plasma cholesterol. At birth, the VLDL, LDL and HDL_2 fractions carried equivalent percentages of plasma total cholesterol, but then up to weaning the HDL_2 fraction contained most cholesterol. After weaning, HDL_2 and LDL contained similar amounts of cholesterol. LDL and HDL_2 cholesterol rose from birth to weaning and fell during the post-weaning period. Table 9 shows that the activity of lipoprotein lipase increased from days 14 to 28 and decreased markedly after weaning. The pattern of hepatic triacylglycerol lipase was similar.

Table 6. Weaning and post-weaning fatty and composition of lymph nodular fat tissue from the piglets

	Day 28		Day 42	
	Close to ln	Far from ln	Close to ln	Far from ln
Analysed fatty acids (g/100 g methylesters)				
C18:2 n-6	11.12 ± 1.73[a]	10.88 ± 0.22[a]	14.79 ± 2.29[b]	14.02 ± 0.25[b]
C18:3 n-6	0.04 ± 0.02[a]	0.04 ± 0.02[a]	0.06 ± 0.02[a]	0.06 ± 0.02[a]
C18:3 n-3	0.90 ± 0.14[a]	0.88 ± 0.02[a]	0.99 ± 0.15[b]	0.95 ± 0.03[a]
C20:2 n-6	0.38 ± 0.06[a]	0.40 ± 0.02[a]	0.47 ± 0.08[b]	0.47 ± 0.03[a]
C20:3 n-6	0.15 ± 0.02[a]	0.16 ± 0.01[a]	0.14 ± 0.02[a]	0.15 ± 0.01[a]
C20:4 n-6	0.44 ± 0.07[a]	0.44 ± 0.02[a]	0.41 ± 0.06[a]	0.40 ± 0.01[a]
C20:3 n-3	0.11 ± 0.03[a]	0.11 ± 0.02[a]	0.12 ± 0.02[a]	0.11 ± 0.02[a]
C20:5 n-3	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.02 ± 0.01[a]	0.02 ± 0.01[a]
C22:4 n-6	0.14 ± 0.02[a]	0.14 ± 0.01[a]	0.13 ±0.02[a]	0.13 ± 0.01[a]
C22:6 n-3	0.08 ± 0.03[a]	0.08 ± 0.02[a]	0.29 ±0.05[b]	0.26 ± 0.01[b]
n-3	1.08 ± 0.17[a]	1.06 ± 0.04[a]	1.43 ± 0.22[b]	1.34 ± 0.02[b]
n-6	12.27 ± 1.91[a]	12.05 ± 0.26[a]	16.02 ± 2.48[b]	15.23 ± 0.23[b]
n-3:n-6	0.09 ± 0.01[a]	0.9 ± 0.01[a]	0.09 ±0.01[a]	0.09 ± 0.01[a]

Results are means ± SE for 6 piglets. Means with different superscripts within site of fat tissue, but between days, are significantly different.
See legend to Table 1.

Table 7. Plasma lipid concentrations in piglets from birth to two weeks post weaning

	Day				
Plasma lipids (mmol/l)	0	14	28	35	42
Total cholesterol	1.23 ± 0.16[a]	3.97 ± 0.46[b]	4.70 ± 0.51[c]	1.74 ± 0.15[a]	2.01 ± 0.14[a]
HDL-cholesterol	0.53 ± 0.05[a]	2.00 ± 0.10[b]	2.03 ± 0.12[b]	0.85 ± 0.08[a]	1.17 ± 0.11[c]
Triacylglycerols	0.74 ± 0.30[a]	0.42 ± 0.05[b]	0.46 ± 0.08[a]	0.41 ± 0.03[b]	0.52 ± 0.04[a]
Phospholipids	1.25 ± 0.19[a]	2.58 ± 0.13[b]	2.53 ± 0.19[b]	1.00 ± 0.09[a]	1.36 ± 0.12[a]

Results are means ± SE for 6 piglets. Means with different superscripts within a row are significantly different.
See legend to Table 1.

Table 8. Lipoprotein cholesterol concentrations in piglets from birth to two weeks post weaning

Lipoprotein cholesterol (mmol/l)	Day				
	0	14	28	35	42
VLDL	0.25 ± 0.19^{a}	0.07 ± 0.01^{b}	0.06 ± 0.01^{b}	0.03 ± 0.01^{c}	0.03 ± 0.01^{c}
IDL	0.04 ± 0.02^{a}	0.02 ± 0.01^{a}	0.03 ± 0.01^{a}	0.01 ± 0.01^{b}	0.01 ± 0.01^{b}
LDL	0.24 ± 0.03^{a}	0.35 ± 0.08^{b}	0.82 ± 0.20^{c}	0.55 ± 0.06^{d}	0.56 ± 0.05^{d}
HDL2	0.42 ± 0.04^{a}	1.74 ± 0.10^{b}	1.80 ± 0.13^{b}	0.64 ± 0.07^{c}	0.82 ± 0.07^{d}
HDL3	0.06 ± 0.01^{a}	0.30 ± 0.02^{b}	0.20 ± 0.01^{c}	0.24 ± 0.03^{c}	0.20 ± 0.02^{c}

Results are means ± SE for 6 piglets. Means with different superscripts within a row are significantly different.
See legend to Table 1.

Table 9. Heparin-released lipase activity in plasma from piglets sampled before and after weaning

Plasma lipase activity (μmol fatty acid/l.h)	Day			
	14	28	35	42
Total	6.4 ± 2.4^{a}	12.0 ± 2.6^{b}	2.8 ± 0.8^{c}	3.8 ± 0.9^{c}
Lipoprotein lipase	4.4 ± 1.4^{a}	7.5 ± 1.4^{b}	1.8 ± 0.6^{c}	2.7 ± 0.6^{d}
Hepatic lipase	2.0 ± 1.0^{a}	4.6 ± 1.2^{b}	1.0 ± 0.3^{c}	1.1 ± 0.3^{c}

Results are means ± SE for 6 piglets. Means with different superscripts within a row are significantly different.
See legend to Table 1.

Discussion

The main objective of this study was to find out whether, under practical conditions, the intake of essential fatty acids during the first week after weaning of piglets would be so low that it may diminish the status of essential fatty acids that was reached during the suckling period. It is reasonable to assume that the piglets consumed about 1 kg milk/day just before weaning (Taugbol et al., 1993). During the first two weeks after weaning, average feed intake was 271 g per day. The intake of n-3 and n-6 PUFAs with 1 kg of sow's milk was similar to that with 275 g weaner diet (Table 10). It would follow that the intake of PUFAs at weaning did not differ much from the average daily intake during two weeks post weaning. However, during the first two days after weaning average daily feed intake is

Table 10. Calculated provision of n-3 and n-6 fatty acids by sow's milk and the weaner diet

	Milk	Weaning pellet	
Fatty acids	1 kg	100 g	300 g
n-3 (g)	0.65	0.29	0.86
n-6 (g)	7.50	2.73	8.18
n-3:n –6 ratio	0.09	0.11	0.11

expected (Bruininx et al., 2001) not to reach 100 g so that essential fatty acid intake during this period was much lower than just before weaning. In spite of a different fatty acid intake around days 28 and 35, the fatty acid composition of erythrocytes, including the n-3:n-6 ratio, remained stable during this period. The fatty acid composition of erythrocytes reflects a change in n-3 and n-6 fatty acids within 7 days after diet change (unpublished results). Thus, it is concluded that, under the present conditions, essential fatty acid status of the piglets was unchanged after weaning. It should be noted that the outcome of this study depends on the fatty acid composition of the body fat of the sow and the lactation and weaner diet. Fat stores or a lactation diet with less essential fatty acids or low n-3:n-6 ratio will affect sow's milk accordingly (Taugbol et al., 1993). A large difference in fatty acid composition between the lactation and weaner diet could lead to a change in fatty acid status of piglets around weaning. However, without knowing the ideal fatty acid composition of erythrocyte membranes in relation to post-weaning health, any change is difficult to interpret. As would be expected, on the basis of the stable fatty acid composition of erythrocyte membranes there was no change around weaning in the fatty acid composition of liver fat and of lymph nodular fat tissue in this study.

The present study shows that there were many changes in lipid metabolism in the piglets from birth to two weeks postweaning. Newborn piglets have only a small amount of fat in their body (Farnworth and Kramer 1987a; Farnworth and Kramer 1987b), but were found to have a relatively high n-3:n-6 fatty acid ratio in erythrocyte membranes. The n-3:n-6 ratio of erythrocytes decreased during the suckling period, which may be explained by the low ratio in milk. The weaner diet had a n-3:n-6 ratio not much higher than that of milk. This would explain that as from day 14 the n-3:n-6 ratio of erythrocyte membranes did not reach the neonatal value again. From day 14 the creep feed was an additional source of PUFAs, but due to the low amount supplied (100 g/day/animal) this effect is very small. In the erythrocytes the proportions of AA acid and adrenic acid (C22:4 n-6) decreased with age, while the content of LA increased considerably. The supply of AA was low, indicating that the conversion of LA to AA is limited.

The fatty acid composition of the liver on the day of birth was different from that of the erythrocyte membranes and the fatty acid pattern developed in an other direction. The contents of AA, EPA and DHA rose markedly in liver fat during lactation, whereas there was a decrease in these contents of the erythrocytes. After weaning this tendency to increase continued for EPA and DHA, but not for AA. The lymph nodular fat differed strongly from liver and erythrocytes. The percentage of n-3 and n-6 PUFAs were lower in the fat tissue and the levels of all PUFAs were low except for LA. These data confirm earlier work (Innis, 1991; Innis, 1993; Goustard-Langelier et al., 1999) that there is a selective process of storage of individual fatty acids in the different types of tissue.

The changes in plasma total cholesterol, HDL cholesterol and phospholipids during suckling and after weaning can be explained by the supply of a large amount of fat with the milk and subsequent low feed intake after weaning. The high fat intake during suckling had caused high levels of total cholesterol, HDL cholesterol and phospholipids at weaning. The low fat intake after weaning produced a decrease in the lipid concentrations. The effects of fat intake on total cholesterol, HDL cholesterol and phospholipids have been described extensively (Herman and Beynen, 1989; Geelen et al., 1995; Salter et al, 1998; Roche and Gibney, 2000). Analogously, it would have been anticipated (Herman and Beynen, 1989) that plasma triacylglycerols were relatively low at weaning and would rise thereafter. However, no such time course of plasma triacylglycerols was observed, pointing at an interaction between diet and age effects. The increase in LDL cholesterol from birth until weaning corroborates the high fat supply during the suckling period. The observed activity of lipoprotein lipase can also be explained by fat intake. The enzyme is upregulated by fat feeding (Beisiegel, 1998), which explains the rise in activity until weaning and the fall thereafter.

The n-3:n-6 ratio of the diet is reflected by that in erythrocytes (Arbuckle and Innis, 1993; Alessandri et al., 1996; Ward et al., 1998; Rooke et al., 1998; Goustard-Langelier et al., 1999). Contrary to our expectation, this study shows that the status of n-3 and n-6 PUFAs in piglets was unaltered around weaning. The status of essential fatty acids in piglets is determined by the fatty acid composition of the sow milk, which in turn is determined by the fatty acid composition of the fat stores of the sow and that of the lactation diet (Arbuckle and Innis 1993; Fritsche et al., 1993; Taugbol et al., 1993). In addition, the fatty acid compositions of the creep feed and weaner diet play a role. Thus, it is stressed that the outcome of the present study is determined by the fatty acid composition of the commercial lactation diet, creep feed and weaner diet that were used. This study does not point at a lowering of the status of essential fatty acids in piglets around weaning. It cannot be excluded however, that an increased intake of n-3 PUFAs with the weaner diet can have a protective effect with regard to post-weaning diarrhoea.

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References

Aggett, P.R., Haschke, R., Heine, W., Hernell, O., Koletzko, B., Launiala, K., Rey, J., Rubino, A., Schoch, G., Senterre, J. and Tormo, R. (1991) Comment on the Content and Composition of Lipids in Infant Formulas, ESPGAN Committee on Nutrition. Acta Pediatr. Scand. 80: 887-896.

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 either 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.

Beisiegel, U. (1998) Lipoprotein metabolism. Eur. Heart J. 19 suppl A: A20-23.

Bruininx, E.M.A.M., Van der Peet-Schwering, C.M.C., Schrama, J.W., Vereijken, P.F.G., Vesseur, P.C., Everts, H., Den Hartog, L.A. and Beynen, A.C. (2001) Individually measured feed intake characteristics and growth performance of group-housed weanling pigs: effect of gender, initial body weight, and body weight distribution within groups. J. Anim. Sc. 79: 301-308.

Burns, C.P., Luttenegger, D.G., Dudley, D.T., Buettner, G.R. and Spector, A.A. (1979) Effect of modification of plasma membrane fatty acid composition on fluidity and methotrexate transport in L1210 murine leukemia cells. Cancer Res. 39: 1726-1732.

Cera, K.R., Mahan, D.C., Cross, R.F., Reinhart, G.A. and Whitmoyer, R.E. (1988) Effect of age, weaning and postweaning diet on small intestinal growth and jejunal morphology in young swine. J. Anim. Sci. 66: 574-584.

Farnworth, E.R. and Kramer, J.K.G. (1987a) Fat metabolism in growing swine: a review. Can. J. Anim. Sci. 67: 301-318.

Farnworth, E.R. and Kramer, J.K.G. (1987b) Fetal pig development in sows fed different diets. Can. J. Anim. Sci. 68: 249-256.

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.

Fritsche, K.L., Huang, S.-C., Cassity and Nancy A. (1993) Enrichment of omega-3 fatty acids in suckling pigs by maternal dietary fish oil supplementation. J. Anim. Sci. 71: 1841-1847.

Geelen, M.J.H., Schoots, W.J., Bijleveld, C. and Beynen, A.C. (1995) Dietary medium-chain fatty acids raise and (n-3) polyunsaturated fatty acids lower hepatic triacylglycerol synthesis in rats. J. Nutr. 125: 2449-2456.

Goustard-Langelier, B., Guesnet, P., Durand, G.A.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.

Hall, G.A. and Byrne, R.F. (1989) Effects of age and diet on small intestinal structure and function in gnotobiotic pigs. Res. Vet. Sci. 47: 387-392.

Hampson, D.J. (1986) Alterations in piglets small intestinal structure at weaning. Res. Vet. Sci. 40: 32-40.

Herman, S. and Beynen, A.C. (1989) Hypotriglyceridemic effect of dietary linseed oil in rats. Internat. J. Vit. Nutr. Res. 59: 417-418.
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.
Kenworthy, R. (1976) Observations on the effects of weaning in the young pig. Clinical and histopathological studies of intestinal function and morphology. Res. Vet. Sci. 21: 69-75.
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.
Nabuurs, M.J.A. (1991) Etiologic and pathogenic studies on postweaning diarrhoea. Ph.D. thesis, Utrecht University, Utrecht, The Netherlands.
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.
Nilsson-Ehle, P. and Schotz, M.C. (1976) A stable, radioactive substrate emulsion for assay of lipoprotein lipase. J. Lipid Res. 17: 536-541.
Pluske, J.R., Williams, I.H. and Aherne, F.X. (1996) Maintenance of villous height and crypt depth in piglets by providing continuous nutrition after weaning. Anim. Sci. 62: 131-144.
Pond, C.M. (1996) Interactions between adipose tissue and the immune system. Proc. Nutr. Soc. 55: 111-126.
Popp-Snijders, C. (1985) Preparation of lipid extracts H 2.2.1 in n-4 polyunsaturated fatty acids and erythrocyte membranes: 18-20.
Roche, H.M. and Gibney, M.J. (2000) Effect of long-chain n-3 polyunsaturated fatty acids on fasting and postprandial triacylglycerol metabolism. Am. J. Clin. Nutr. 71(suppl): 232S-237S.
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.
Salter, A.M., Mangiapane, E.H., Bennett, A., Bruce, J.S., Billett, M.A., Anderton, K.L., Marenah, C.B., Lawson, N. and White, D.A. (1998) The effect of different dietary fatty acids on lipoprotein metabolism: concentration-dependent effects of diets enriched in oleic, myristic, palmitic and stearic acids. Br. J. Nutr. 79: 195-202.
Taugbol, O., Framstad, T. and Saarem, K. (1993) Supplements of cod liver oil to lactating sows. Influence on milk fatty acid composition and growth performance of piglets. Zentralbl. Veterinarmed. A40: 437-443.
Terpstra, A.H.M., Sanchez-Muniz, F.J., West, C.E. and Woodward, C.J.H. (1982) The density profile and cholesterol concentration of serum lipoproteins in domestic and laboratory animals. Comp. Biochem. Physiol. 71b: 669.
Van Beers-Schreurs, H.M.G., Vellenga, L., Wensing, T.H. and Breukink, H.J. (1992) The pathogenesis of the post-weaning syndrome in weaned pigs; a review. Vet. Quarterly 14: 29-34.
Vaughn, D.M., Reinhart, G.A. and Swain, S.F. (1994) Evaluation of dietary n-6 to n-3 fatty acids ratios on leukotriene B synthesis in dog skin and neutrophils. J. Vet. Med. 8: 155.
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.
Wu, D. and Meydani, S.N. (1998) n-3 Polyunsaturated fatty acids and immune function. Proc. Nutr. Soc. 57: 503-509

Chapter 2

Effect of increasing intakes of α-linolenic acid on growth performance, essential fatty acid status and plasma lipids in weanling piglets

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Abstract

Weanling piglets (n = 360) were fed diets with different levels of α-linolenic acid (ALA, C18:3 n-3), the levels being 0.22, 0.47, 0.77 and 1.13 % of metabolizable energy. The experimental diets were formulated by the addition of various amounts of linseed oil at the expense of corn oil. The experimental diets were fed for two weeks followed by a three-week period during which all piglets received the same commercial diet. Intakes of ALA above 0.22 energy % tended to increase growth during the first two weeks post weaning and tended to reduce feed conversion during the first week. The average increase in weight gain was 9% and the decrease in feed conversion was 14%. The diet with 1.13 energy % ALA produced a significantly better body condition after two weeks than did the diet with 0.22 energy % ALA. The good condition persisted after the pigs had been transferred to the commercial diet. Increasing amounts of ALA in the diets stimulated the desaturation and elongation into eicosapentaenoic (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3) and the incorporation of these fatty acids into erythrocyte membranes. The piglets showed a post-weaning decrease in total cholesterol, HDL cholesterol and phospholipids, but the intake of various amounts of linseed oil did not influence the concentration of plasma lipids. The requirement of ALA by weanling piglets to display maximum growth is not known, but this study indicates that it may be above 0.22 energy %.

Introduction

Weanling piglets are prone to the development of the so-called post-weaning syndrome which is associated with atrophy of the villi, inflammation of the gut (Cera et al., 1988; Hall et al., 1989; Hampson, 1986; Kenworthy, 1976; Nabuurs, 1991) and depressed performance (Jahn and Uecker, E., 1987; Svedsen et al., 1974; Svensmark et al., 1989). There is evidence that dietary n-3 polyunsaturated fatty acids (PUFAs) may antagonize atrophy of villi and have anti-inflammatory activity. In growing chicks, the intake of extra n-3 PUFAs has been shown to improve performance and decrease the inflammatory response to LPS from *S. typhimurium* and *S. aureus* (Korver and Klasing, 1997). In young mice with hypoxia-induced bowel necrosis, supplementation with n-3 PUFAs reduced the degree of necrosis (Akisu et al., 1998). Mucosal damage in food-sensitive enteropathy in mice was prevented by supplementation with n-3 PUFAs (Ohtsuka et al., 1997).

The beneficial effects of n-3 PUFAs probably relate to their conversion into eicosanoids which have a variety of biological functions, including stimulation of immunity (Wu and Meydani, 1998; Fritsche et al., 1993). The parent compound of the eicosanoids is α-linolenic acid (ALA, C18:3 n-3), which can be converted by piglets (Clandinin et al., 1985) into the direct precursor, eicosapentaenoic acid (EPA, C20:5 n-3). The piglet is also able to further desaturate and elongate EPA into docosahexaenoic acid (DHA, C22:6 n-3) which is abundant in brain and retina (Bourre et al., 1993; Ward et al., 1998; Clandinin, 1999; Arbuckle et al., 1994; Arbuckle and Innis, 1992). The requirement of ALA by piglets is not exactly known (Innis, 1993), but it could be suggested that extra intake of n-3 PUFAs is beneficial. Diets for weanling piglets typically contain 0.20 – 0.30 % (w/w) n-3 PUFAs in the form of the sum of ALA, EPA and DHA (unpublished results).

In this study, weanling piglets were fed experimental diets with increasing contents of linseed oil which is rich in ALA. Three levels of linseed oil were added to the diets at the expense of the corn oil component which is rich in linoleic acid (LA, C18:2 n-6). The control diet without linseed oil had a nutrient composition similar to that of common diets for weanling piglets. The following three questions were addressed. (i) Does the consumption of increasing amounts of linseed oil affect weight gain, feed conversion, body condition and consistency of the faeces? In the light of the above-mentioned, it was anticipated that extra ALA in the form of linseed oil would have beneficial effects. (ii) Does linseed oil ingestion raise the status of n-3 PUFAs as mirrored by the fatty acid composition of erythrocyte membranes? The intake of PUFAs is generally reflected by the fatty acid composition of erythrocytes (Alessandri et al., 1996; Arbuckle and Innis, 1993; Goustard-Langelier et al., 1999; Rooke et al., 1998; Ward et al., 1998), and it was expected that this study would add to knowledge of quantitative relationships. (iii)

Does the intake of linseed oil influence the concentrations of plasma lipids? In rats, diets containing linseed oil instead of corn oil have been shown to lower plasma triglyceride and cholesterol concentrations (Herman and Beynen, 1989). It was expected that the answer to the first question would provide information as to the optimum level of ALA in diets for weanling piglets. Answers to the second and third question would give more insight in fundamental aspects of fatty acid and lipid metabolism in weanling piglets.

Animals, materials and methods

Animals, feed and housing

Three hundred and sixty weanling pigs (F2 cross-bred: GY x [Finnish X Dutch Landrace]), weighing on average 8.5 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 three 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. During the experiment one piglet died due to an internal bleeding caused by vene puncture. No medicines were used.

There were 4 experimental diets with an increasing amount of linseed oil. Table 1 shows the ingredients and analysed composition of the experimental diets. With increasing contents of linseed oil the amount of ALA was higher and that of LA lower, but the analysed macronutrient levels of all four diets were similar. The diets, which were in meal form, 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, 7, 14 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

Table 1. Ingredients, chemical composition and fatty acid composition of the experimental diets.

	α-linolenic acid (% of ME)[1]			
	0.22	0.47	0.77	1.13
Ingredients (g/kg)				
Corn oil	50	46	41	36
Linseed oil	0	4	9	14
Constant components[2]	950	950	950	950
Chemical analysis				
Dry matter (g/kg)	906	905	907	906
Crude protein (g/kg dm[3])	191	193	187	194
Crude fat (g/kg dm)	54	55	55	55
Crude fiber (g/kg dm)	27	26	27	27
Ash (g/kg dm)	42	43	47	47
Analysed fatty acids (g/100 g methylesters)[4]				
C16:0	12.87	12.90	12.94	12.71
C18:0	2.30	2.38	2.53	2.83
C18:1 n-9	24.95	25.00	24.67	24.63
C18:2 n-6	51.92	48.11	44.26	41.17
C18:3 n-3	1.66	3.37	5.59	8.17
n-3:n-6 ratio	0.03	0.07	0.13	0.20

[1] ME = metabolizable energy. The ME content of the diets was calculated on the basis of the ingredient composition and feed tables (National Research Council, 1998).
[2] Constant components: 505 g barley, 231.5 g dextrose, 152.5 g casein, 20 g molasses, 15 g rukanaphos, 12.5 g $CaCO_3$, 5 g vitamin-mineral premix (Cehave, Veghel, The Netherlands), 5 g tryptophan (purity 5%), 1.75 g threonine (purity 10%) and 1.75 g methionine (purity 50%). FAME: fatty acid methyl esters
[3] dm = dry matter
[4] C20:3 n-6, C20:4 n-6, 20:5 n-3, 22:4 n-6, 22:5 n-3 and 22:6 n-3 were not detectable.

al. (1957) and Metcalfe et al. (1966), respectively. Crude protein, crude fiber and ash were determined by the Weende analysis.

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 diarrheal 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 (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. To analyse plasma lipids and lipoproteins, blood was taken in heparinized tubes. Plasma triglycerides, phospholipids, total cholesterol and HDL cholesterol were measured enzymatically using test combinations (Boehringer-Mannheim GmbH, Mannheim, Germany). Lipoproteins were isolated according to Terpstra et al (1982).

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. The correlation between body condition and either the α-linolenic acid to linoleic acid (18:2 n-6) or the ratio of eicosapentaenoic acid to arachidonic acid (C20:4 n-6) was calculated. The correlations were based on group-mean values which can be considered the best estimates for each diet. 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 animal 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

There were no significant differences in average daily gain (ADG) and feed conversion ratio for the four experimental diets (Table 2). ADG during weeks 1 + 2 rose with increasing intakes of ALA and during weeks 1 and 2 it was on average 9% higher for the diets containing linseed oil than for the control diet without linseed oil. The overall average daily feed intake (ADFI) during weeks 1 + 2 was 246 g and there were no significant differences for the four diets. Feed conversion during the first week was consistently lower for the diets with linseed oil, the lowering being 14%, which failed to reach statistical significance. After

Table 2. Growth performance of weanling piglets fed increasing amounts of linseed oil.

	α-linolenic acid (% of ME)				Pooled	
Item	0.22	0.47	0.77	1.13	SEM	P value
Weight, day 0 (g)	8521	8429	8424	8502	473	0.998
ADG, week 1 (g)	138	172	155	172	93	0.230
ADG, week 2 (g)	272	271	292	279	116	0.795
ADG, week 1 + 2 (g)	205	221	224	226	185	0.679
ADFI, week 1 (g)	256	266	253	276	93	0.614
ADFI, week 2 (g)	421	440	444	452	158	0.814
ADFI, week 1 + 2 (g)	339	353	348	364	236	0.768
Feed conversion, week 1	1.93	1.59	1.70	1.71	0.12	0.272
Feed conversion, week 2	1.56	1.66	1.52	1.66	0.08	0.485
Feed conversion, week 1 + 2	1.68	1.63	1.57	1.67	0.08	0.771

Results are means for 9 pens, containing 10 piglets each, per dietary treatment.

two weeks on the experimental diets, all piglets were switched to the same commercial diet. There was no significant carry-over effect of ALA intake. After three weeks on the commercial diet, body weights were 22.61 ± 0.84, 23.17 ± 0.54, 22.84 ± 0.75 and 23.09 ± 0.89 kg (mean ± SE, n = 90) for the piglets with increasing post-weaning intakes of ALA.

All diets induced solid faeces and there was no diet effect on faeces-consistency scores. The median of the scores for days 7 and 14 was 0.5 and 0.0 respectively. There were diet-related differences in body-condition of the pigs. The diet with 1.13 % ALA had produced a significantly better condition after two weeks than had the diet with 0.22 energy % ALA. The improved condition persisted after the pigs had been switched to the commercial diet. After 21, 28 and 35 days, the body-condition scores for the pigs earlier fed the diet with 1.13 energy % ALA were 0.6, 0.9 and 1.3, respectively, whereas for the pigs weaned onto the control diet the scores were 0.8, 1.1 and 1.4. Such a carry-over effect on body condition was also seen for the diet containing 0.47 energy % ALA, but it lasted only until day 28, the score at that time point being 0.9.

The increasing amounts of ALA in the diets were reflected by the amounts of this fatty acid in the erythrocyte membranes (Table 3). The percentage of EPA in the erythrocytes was highest for the diet with the highest amount of ALA. At two weeks after weaning only the level of DHA was elevated in piglets fed the diet with the highest amount of ALA. The amounts of LA, dihomo-γ-linolenic acid

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

	α-linolenic acid (% of ME)				Pooled	
	0.22	0.47	0.77	1.13	SEM	P value
Analysed fatty acids (g/100 g methylesters)						
C16:0	22.00	22.31	21.89	21.81	0.222	0.579
C18:0	11.46	11.57	11.70	12.00	0.231	0.234
C18:1 n-9	30.37	29.26	29.20	29.39	0.549	0.705
C18:2 n-6	12.50	13.72	13.17	12.98	0.501	0.633
C18:3 n-3	0.17	0.23	0.35	0.46	0.129	0.000
C20:3 n-6	0.27	0.26	0.29	0.27	0.013	0.414
C20:4 n-6	4.39	4.36	4.38	4.13	0.123	0.367
C20:5 n-3	0.07	0.06	0.10	0.19	0.059	0.001
C22:4 n-6	0.44	0.44	0.40	0.36	0.041	0.012
C22:5 n-3	ND[1]	ND	ND	ND		
C22:6 n-3	1.74	1.65	1.83	2.04	0.170	0.002
n-3	1.98	1.93	2.29	2.69	0.363	0.001
n-6	17.61	18.77	18.24	17.74	0.535	0.651
n-3:n-6 ratio	0.11	0.10	0.13	0.15	0.023	0.000

Results are means for 9 pigs per dietary treatment.
[1] ND = non detectable

(DGLA, C18:3 n-6) and AA in the erythrocyte membranes were similar for all four diets. There was significantly more adrenic acid (C22:4 n-6) in the erythrocyte membranes of piglets fed on the diet with 0.22 energy % ALA when compared to that seen after feeding the diet with 1.13 energy % ALA. The changes in fatty acid composition of erythrocyte membranes resulted in significantly different ratios of ALA to LA for all four diets, the ratio increasing with increasing amounts of ALA (Fig. 1). A similar pattern was seen for the ratio of EPA to AA in erythrocyte membranes (Fig. 2).

There was a linear relation between the group-mean fatty acid composition of erythrocyte membranes and group-mean body condition scores. The regression line between the body condition score on day 14 and the ALA to LA ratio can be described by $y = -18.29x + 1.6552$ with $R^2 = 0.8686$ (Fig. 3). The regression between the body condition on day 14 and the EPA to AA ratio can be described by $y = -13.373x + 1.5584$ with $R^2 = 0.9533$ (Fig. 4). Thus, high amounts of both the parent compound and direct eicosanoid precursor of the n-3 family of PUFAs

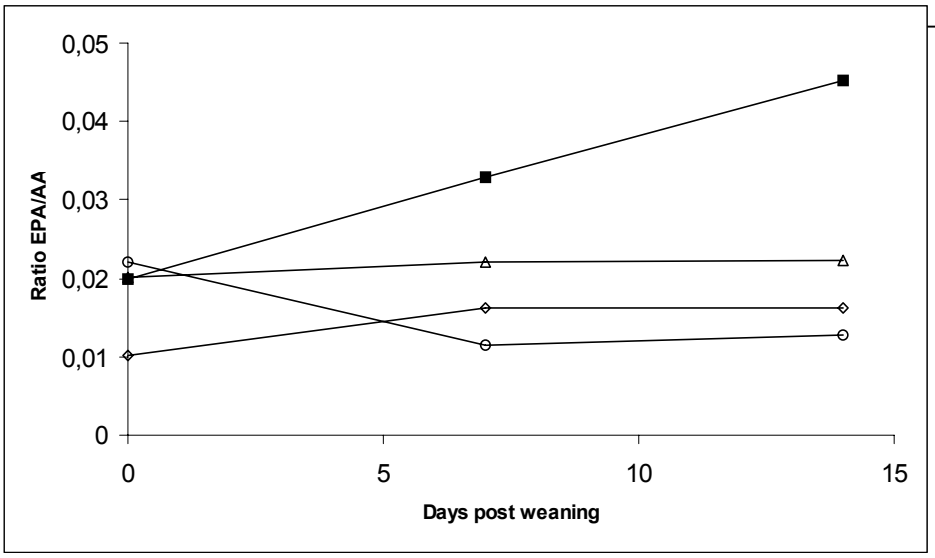

fig. 1. Time course of the ratio of α-linolenic acid (ALA) to linoleic acid (LA) in erythrocyte membranes from weanling piglets fed the experimental diets. Symbols: ○, 0.22 energy % ALA; ●, 0.47 energy % ALA; □, 0.77 energy % ALA; ■, 1.13 energy % ALA.

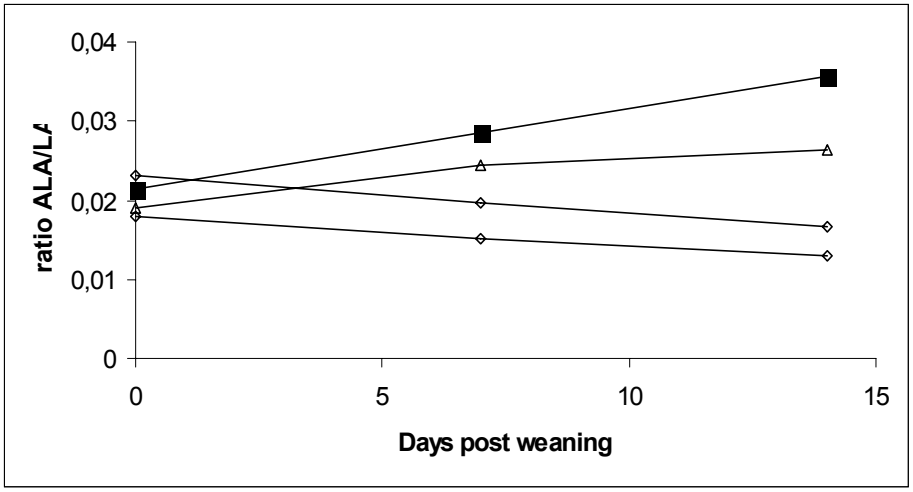

Fig. 2. Time course of the ratio of eicosapentaenoic acid (EPA) to arachidonic acid (AA) in erythrocyte membranes from weanling piglets fed the experimental diets. Symbols: ○, 0.22 energy % ALA; ●, 0.47 energy % ALA; □, 0.77 energy % ALA; ■, 1.13 energy % ALA.

Effect of increasing intakes of α-linolenic acid

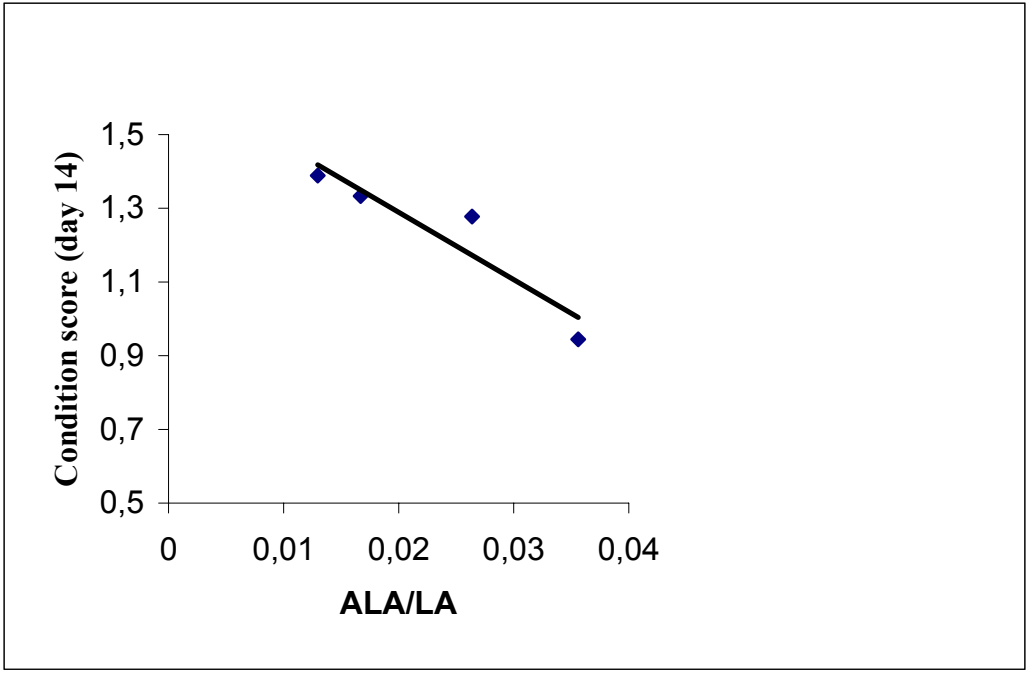
Condition score (day 14)
1,5
1,3
1,1
0,9
0,7
0,5
0
0,01
0,02
0,03
0,04
ALA/LA

Fig. 3. Relationship between group mean values on day 14 post weaning for the ratio of α-linolenic acid (ALA) to linoleic acid (LA) in erythrocyte membranes and body condition scores. Symbols: ○, 0.22 energy % ALA; ●, 0.47 energy % ALA; □, 0.77 energy % ALA; ■, 1.13 energy % ALA.

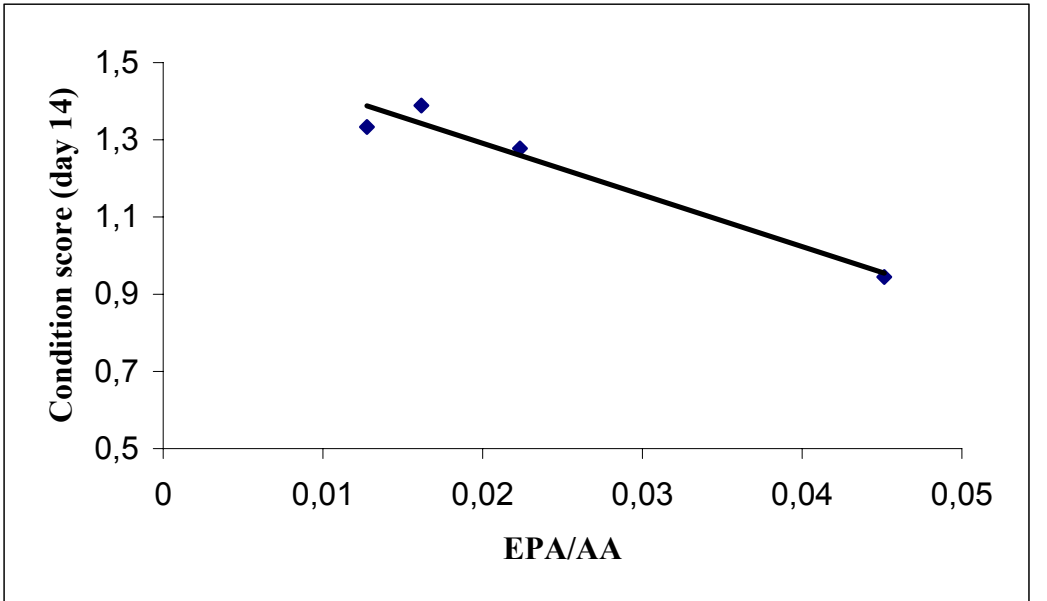
Condition score (day 14)
1,5
1,3
1,1
0,9
0,7
0,5
0
0,01
0,02
0,03
0,04
0,05
EPA/AA

Fig. 4. Relationship between group mean values on day 14 post weaning for the ratio of eicosapentaenoic acid (EPA) to arachidonic acid (AA) in erythrocyte membranes and body condition scores. Symbols: ○, 0.22 energy % ALA; ●, 0.47 energy % ALA; □, 0.77 energy % ALA; ■, 1.13 energy % ALA.

Table 4. Plasma lipid concentrations in weanling piglets fed increasing amount of linseed oil

		α-linolenic acid (% of ME)				Pooled	
	Day	0.22	0.47	0.77	1.13	SEM	P value
Total cholesterol	0	5.80	5.82	5.48	5.79	0.72	0.957
(mmol/l)	7	1.73	1.69	1.65	1.92	0.15	0.648
	14	2.06	2.20	2.23	1.97	0.23	0.848
HDL cholesterol	0	2.36	1.73.	1.67	1.74	0.25	0.640
(mmol/l)	7	0.79	0.77	0.73	0.68	0.05	0.505
	14	0.90	1.25	0.96	0.89	0.17	0.446
Triacylglycerols	0	0.32	0.36	0.34	0.33	0.04	0.999
(mmol/l)	7	0.33	0.30	0.29	0.27	0.03	0.592
	14	0.39	0.41	0.31	0.41	0.04	0.374
Phospholipids	0	2.74	2.58	2.53	2.68	0.22	0.993
(mmol/l)	7	1.10	1.06	1.03	1.04	0.07	0.915
	14	1.06	1.42	1.24	1.25	0.11	0.662

Resultes are means for 9 pigs per dietary treatment.

Table 5. Lipoprotein cholesterol concentrations in weanling piglets fed increasing amounts of linseed oil

Lipoprotein		α-linolenic acid (% of ME)				Pooled	
cholesterol	Day	0.22	0.47	0.77	1.13	SEM	P value
VLDL (mmol/l)	0	0.21	0.22	0.21	0.16	0.44	0.808
	7	0.05	0.03	0.05	0.05	0.05	0.502
	14	0.06	0.06	0.05	0.07	0.07	0.838
LDL (mmol/l)	0	4.13	4.29	3.65	4.24	4.57	0.528
	7	0.97	1.07	1.02	1.62	0.88	0.171
	14	1.17	1.23	1.51	1.06	1.18	0.539
HDL (mmol/l)	0	1.92	1.64	1.81	1.66	1.10	0.789
	7	0.71	0.67	0.76	0.65	0.25	0.150
	14	0.87	0.84	0.97	0.81	0.52	0.652

Results are means for 9 pigs per dietary treatment.

relative to those of the n-6 family of PUFAs were associated with low body-condition scores, i.e. good appearance.

The levels of total cholesterol, HDL cholesterol and phospholipids in plasma showed a decrease during the first week after weaning and generally rose again during the second week without reaching the levels at weaning (Table 4). Plasma concentrations of triacylglycerols were generally higher at 14 days after weaning than at weaning. There was no sytematic effect of the dietary ALA concentration on plasma lipids. Lipoprotein-cholesterol concentrations were not significantly affected by the amount of ALA in the diet (Table 5). The recovery of lipoprotein cholesterol was on average 116% of total plasma cholesterol. At weaning, the VLDL, LDL and HDL fractions carried 3, 65 and 28% of plasma total cholesterol, respectively. After 14 days, the proportion of total cholesterol in the VLDL, LDL and HDL fractions was 3, 51 and 36%. Lipoprotein-cholesterol concentrations at weaning were higher than those seen on day 14.

Discussion

In this study with weanling piglets, experimental diets were fed in which the corn-oil component was replaced by increasing amounts of linseed oil. Thus, diets were obtained with increasing levels of ALA at the expense of LA. The dietary concentrations of ALA ranged between 0.22 and 1.13 % of ME. The experimental diets produced no significant differences in weight gain and feed conversion. However, intakes of ALA higher than 0.22 energy % increased group-mean ADG during the first two weeks post weaning in a dose-dependent fashion. In addition, extra ALA in the diet, when compared with the linseed-oil free diet, reduced FCR during the first week. There were statistically significant diet effects on body condition of the pigs. The pigs fed on the diet with the highest amount of ALA had a significantly better condition than those given the diet with the lowest amount of ALA. Thus, high intake of ALA had a positive effect on the integrated measure of color and gloss of the skin, hair length and meat cover. For group means, high ratios of ALA to LA and EPA to AA in erythrocyte membranes were associated with low body-condition scores, i.e. good condition. It appears that high intakes of ALA, which is reflected by high concentrations of n-3 PUFAs in erythrocyte membranes, tended to have positive effects on ADG, FCR and body condition.

Possibly, the conditions of the present study were not suitable to demonstrate clear effects of increasing intakes of ALA on growth performance. Dietary ALA can be converted into EPA which is the direct substrate for the synthesis of eicosanoids. The status of EPA, as based on its concentration in the erythrocyte membranes, did not fall with time in the linseed-oil free, control group and was only slightly affected by the intake of ALA. It could be suggested that in

all piglets the status of EPA was optimal so that no significant effects of ALA intake on growth performance could be shown. This suggestion would imply that the initial fatty acid status of weanling piglets may affect their sensitivity to the fatty acid composition of the diet onto which they are weaned. Moreover, it cannot be excluded that the diets used in this study had ALA levels well above the requirement of weanling piglets. If this were the case, no effect of ALA would be expected. The diet with the lowest amount contained 1.10 g ALA/kg which is equivalent to 0.22 energy %. The requirement of n-3 PUFAs by piglets is not known. Recommended intakes for humans are 0.2 to 0.4 energy % (Bjerve et al., 1987; Bjerve, 1989), but a value of 1 energy % had also been put forward (Bjerve, 1989). For growing rats, 0.4 energy % had been suggested (Bourre et al., 1989b) and for adult rats an ALA intake of 0.26 energy % has been recommended (Bourre et al., 1993, Bourre et al, 1996). Although different criteria were used to arrive at the recommended intakes for the different species the range of ALA intakes in this study may be considerd appropriate to demonstrate effects, if any, on growth performance. There is evidence that apart from the absolute amount of ALA in the diet the ratio of n-3:n-6 PUFAs is important (Innis, 1991). The two types of PUFA have inhibitory effects on each others conversion into eicosanoids, whereas the eicosanoids produced from the n-6 and n-3 families of PUFAs have antagonistic activities (Calder ,1996). In this study the dietary concentration of LA was relatively high. It cannot be excluded that the high concentration of LA in the diet had influenced the observed effects on weight gain and body condition.

As expected, ingestion of increasing amounts of linseed oil did increase the status of n-3 PUFAs. The levels of EPA and DHA in erythrocyte membranes were increased in piglets fed the diets with high levels of ALA. Thus, the intake of extra ALA stimulates its desaturation and elongation. It is unlikely that ALA inhibits the catabolism of EPA and DHA. Although the intake of LA decreased with increasing intakes of linseed oil, there was no change in the LA content of erythrocyte membranes. This could indicate that LA intake with all four experimental diets was close to or above the requirement. Indeed, the LA requirement of piglets is 0.3 energy % (National Research Council, 1998), while the diet with the lowest level contained 0.22 energy %. A surprising finding emerged in that the diets with lowest amounts of ALA, and thus the highest amounts of LA, produced a somewhat higher content of adrenic acid in erythrocyte membranes, whereas the content of AA remained stable. Adrenic acid is formed by elongation of AA. Possibly, a low intake of n-3 PUFAs leads to a diminished transformation of adrenic acid. Rats fed a diet with 6 instead of 130 mg ALA/100 g showed an increase in the amount of docosapentaenoic acid (C22:5 n-6) and adrenic acid in liver, while the content of AA remained stable (Bourre et al., 1993).

During the first week after weaning, plasma levels of total cholesterol, HDL cholesterol and phospholipids decreased. This observation can be explained by the change from milk as sole source of nutrition to dry feed. Sow milk is rich in fat, where as the dry feed was lower in fat and richer in carbohydrates. It is well-known that a decrease in fat intake in favour of carbohydrates leads to a fall in plasma total cholesterol and HDL cholesterol (Herman and Beynen, 1989; Geelen et al, 1995; Salter et al, 1998; Roche and Gibney, 2000). However, the invariably associated rise of plasma triacylglycerol concentrations (Herman and Beynen, 1989) was not seen in the piglets. The intake of various amounts of linseed oil did not influence the concentrations of plasma lipids. The type of fat in the diet of rats can affect plasma lipid concentrations (Geelen et al., 1995; Van Lith et al., 1992). It seems that in this study the variation in fatty acid composition was not sufficiently extreme to elicit plasma lipid responses or that the background composition of the diet had masked any effects.

In conclusion, this study shows that a diet containing 0.22 energy % of ALA sustains growth in weanling piglets and that extra ALA had only a minor impact. This study does present suggestive evidence that extra ALA in the diet may have positive effects on ADG, FCR and body condition of weanling piglets. Further studies on the intake of n-3 PUFAs and growth performance of weanling piglets appear to be relevant. At least three important questions can be raised since the requirement of n-3 PUFAs by weanling piglets is not known. Do dietary levels lower than 0.22 energy % of ALA depress growth performance? Will higher levels be beneficial in weanling piglets kept under stressful conditions such as infectious pressure? Is the feeding of the product of ALA transformation, EPA, more effective in stimulating growth performance than is the feeding of ALA feeding?

References

Akisu, M., Baka, M., Coker, I., Kultursay, N. and Huseyinov, A. (1998) Effect of dietary n-3 fatty acids on hypoxia-induced necrotizing enterocolitis in young mice. N-3 fatty acids alter platelet-activating factor and leukotriene B4 production in the intestine. Biolog. Neonate. 74: 31-38.

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. (1992) Docosahexaenoic acid in developing brain and retina of piglets fed high or low alpha linolenate formula with and without fish oil. Lipids 27: 89-93.

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.

Arbuckle, L.D., Murray, M.J. and Innis, S.M. (1994) Formula 18:2(n-6) and 18:3(n-3) content and ratio influence long-chain polyunsaturated fatty acids n the developing piglet liver and central nervous system. J. Nutr. 124: 289-298.

Bjerve, K.S., Fisher, S. and Alme, K. (1987) Alpha-linolenic acid deficiency in man: effect of ethyl linolenate on plasma and erythrocyte fatty acid composition and biosythesis of prostanoids. Am. J. Clin. Nutr. 46: 570-576.

Bjerve, K.S. (1989). n-3 fatty acid deficiency in man. J. Intern. Med. 225, Suppl 1: 171-175.

Bourre, J. M., M. Francois, M., Youyou, A., Dumont, O., Piciotti, M., Pascal, G. and Durand, G. (1989b) The effect of dietary alpha-linolenic acid on the composition of nerve membranes, enzymatic activity, amplitude of electrophysiological parameters, resistance to poisons and performance of learning tasks in rats. J. Nutr. 119: 1880-1892.

Bourre, J.M., Dumont, O., Pascal, G. and Durand, G. (1993) Dietary alpha linolenic acid at 1.3 g/kg maintains maximal docosahexaenoic acid concentration in brain, heart and liver of adult rats. J. Nutr. 123: 1313-1319.

Bourre, J. M., O. Dumont, and Durand, G (1996) Does an increase in deitary linolenic acid modify tissue concentrations of cervonic acid and consequently alter alpha-linolenic acid requirements? Minimal requirement of linoleic acid in adult rats. Biochem. Mol. Biol. Int. 39: 607-619.

Calder, P.C. (1996) Effects of fatty acids and dietary lipids on cells of the immune system. Proc. Nutr. Soc. 55: 127-150.

Cera, K.R., Mahan, D.C., Cross, R.F., Reinhart, G.A. and Whitmoyer, R.E. (1988) Effect of age, weaninig and postweaning diet on small intestinal growth and jejunal morphology in young swine. J. Anim. Sci. 66: 574-584.

Clandinin, M.T., Wong, K. and Hacker, R.R. (1985) Delta 5-desaturase activity in liver and brain microsomes during development of the pig. J. Biochem. 227: 1021-1023.

Clandinin, M.T. (1999) Brain development and assesing the supply of polyunsaturated fatty acid. Lipids 34: 131-137.

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.

Fritsche, K.L., Alexander, D.W., Cassity, N.A. and Huang, S.-C. (1993) Maternally-supplied fish oil alters piglet immune cell fatty acid profile and eicosanoid production. Lipids 28: 677-682.

Geelen, M.J.H., Schoots, W.J., Bijleveld, C. and Beynen, A.C. (1995) Dietary medium-chain fatty acids raise and (n-3) polyunsaturated fatty acids lower hepatic triacylglycerol synthesis in rats. J. Nutr. 125: 2449-2456.

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.

Hall, G.A. and Byrne, R.F. (1989) Effects of age and diet on small intestinal structure and function in gnotobiotic pigs. Res. Vet. Sci. 47: 387-392.

Hampson, D.J. (1986) Alterations in piglets small intestinal structure at weaning. Res. Vet. Sci. 40: 32-40.

Herman, S. and Beynen, A.C. (1989) Hypotriglyceridemic effect of dietary linseed oil in rats. Internat. J. Vit. Nutr. Res. 59: 417-418.

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.

Jahn, S. and Uecker, E. (1987) Okonomische Untersuchingen zur Kolienterotoxamie des Schweines. Mh. Vet. Med. 42: 769-771.

Kenworthy, R. (1976) Observations on the effects of weaning in the young pig. Clinical and histopathological studies of intestinal function and morphology. Res. Vet. Sci. 21: 69-75.
Korver, D. R. and Klasing, K.C. (1997) Dietary fish oil alters specific and inflammatory responses in chicks. J. Nutr. 127: 2039-2046.
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.
Nabuurs, M.J.A. (1991) Etiologic and pathogenic studies on postweaning diarrhoea. Ph.D. thesis, Utrecht University, Utrecht, The Netherlands.
National Research Council (1998) Nutrient Requirements of swine. Tenth revised Edition, National Academic Press, Washington.
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.
Ohtsuka, Y., Yamashiro, Y., Shimizu, T., Nagata, S., Igarashi, J., Shinohara, K., Oguchi, S. and Yabuta, K. (1997) Reducing cell-membrane n-6 fatty acids attenuate mucosal damage in food-sensitive enteropathy in mice. Pediatr. Res. 42: 835-839.
Popp-Snijders, C. (1985) Preparation of lipid extracts H 2.2.1 in n-4 polyunsaturated fatty acids and erythrocyte membranes: 18-20.
Roche, H. M. and Gibney, M.J. (2000) Effect of long-chain n-3 polyunsaturated fatty acids on fasting and postprandial triacylglycerol metabolism. Am. J. Clin. Nutr. 71(suppl): 232S-237S.
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.
Salter, A.M., Mangiapane, E.H., Bennett, A., Bruce, J.S., Billett, M.A., Anderton, K.L., Marenah, C.B., Lawson, N. and White, D.A. (1998) The effect of different dietary fatty acids on lipoprotein metabolism: concentration-dependent effects of diets enriched in oleic, myristic, palmitic and stearic acids. Br. J. Nutr. 79: 195-202.
Svedsen, J., Larsen, J.L. and Bille, N. (1974) Outbreaks of post weaning Escherichia Coli infections in weaned pigs. Nord. Vet. Med. 26: 314-322.
Svensmark, B., Nielsen, K., Willeberg, P. and Jorsal, S.E. (1989) Epidemiological studies of piglet diarrhoea in intesively managed Danish sow herds. II. Postweaning diarrhoea. Acta Vet. Scand. 30: 55-62.
Terpstra, A.H.M., Sanchez-Muniz, F.J., West, C.E. and Woodward, C.J.H. (1982) The density profile and cholesterol concentration of serum lipoproteins in domestic and laboratory animals. Comp. Biochem. Physiol. 71B: 669.
Van Lith, H.A., Haller, M., Van Tintelen, G., Van Zutphen, L.F.M. and Beynen, A.C. (1992) Plasma esterase-1 (ES-1) activity in rats is influenced by the amount and type of dietary fat, and butyryl cholinesterase activity by the type of dietary fat. J. Nutr. 122: 2109-2120.
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.
Wu, D. and Meydani, S.N. (1998) n-3 Polyunsaturated fatty acids and immune function. Proc. Nutr. Soc. 57: 503-509.

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.

References

Akisu, M., Baka, M., Coker, I., Kultursay, N. and Huseyinov, A. (1998) Effect of dietary n-3 fatty acids on hypoxia-induced necrotizing enterocolitis in young mice. N-3 fatty acids alter platelet-activating factor and leukotriene B4 production in the intestine. Biolog. Neonate. 74(1): 31-38.

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.

Burns, C.P., Luttenegger, D.G., Dudley, D.T., Buettner, G.R. and Spector, A.A. (1979) Effect of modification of plasma membrane fatty acid composition on fluidity and methotrexate transport in L1210 murine leukemia cells. Cancer Res. 39: 1726-1732.

Cera, K.R., Mahan, D.C., Cross, R.F., Reinhart, G.A. and Whitmoyer, R.E. (1988) Effect of age, weaning and postweaning diet on small intestinal growth and jejunal morphology in young swine. J. Anim. Sci. 66: 574-584.

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.

Hall, G.A. and Byrne, R.F. (1989) Effects of age and diet on small intestinal structure and function in gnotobiotic pigs. Res. Vet. Sci. 47: 387-392.

Hampson, D.J. (1986) Alterations in piglets small intestinal structure at weaning. Res. Vet. Sci. 40: 32-40.

Kenworthy, R. (1976) Observations on the effects of weaning in the young pig. Clinical and histopathological studies of intestinal function and morphology. Res. Vet. Sci. 21: 69-75.

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.

Korver, D.R. and Klasing, K.C. (1997) Dietary fish oil alters specific and inflammatory responses in chicks. J. Nutr. 127: 2039-2046.

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

Nabuurs, M.J.A. (1991) Etiologic and pathogenic studies on postweaning diarrhoea. Ph.D. thesis, Utrecht University, Utrecht, The Netherlands.

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.

Ohtsuka, Y., Yamashiro, Y., Shimizu, T., Nagata, S., Igarashi, J., Shinohara, K., Oguchi, S. and Yabuta, K. (1997) Reducing cell-membrane n-6 fatty acids attenuate mucosal damage in food-sensitive enteropathy in mice. Pediatr. Res. 42: 835-839.

Pluske, J.R., Williams, I.H. and Aherne, F.X. (1996) Maintenance of villous height and crypt depth in piglets by providing continuous nutrtion after weaning. Anim. Sci. 62: 131-144.

Pond, C.M. (1996) Interactions between adipose tissue and the immune system. Proc. Nutr. Soc. 55: 111-126.
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.
Shinitzky, M. and Sourojon, M. (1979) Passive modulation of blood-group antigens. Proc. Nat. Acad. Sci., USA 76: 4438-4440.
Terpstra, A.H.M., Sanchez-Muniz, F.J., West, C.E. and Woodward, C.J.H. (1982) The density profile and cholesterol concentration of serum lipoproteins in domestic and laboratory animals. Comp. Biochem. Physiol. 71B: 669.
Traill, K.N. and Wick, G. (1984) Lipids and lymphocyte function. Immunol. Today 5: 70-76.
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.
Wu, D. and Meydani, S.N. (1998) n-3 Polyunsaturated fatty acids and immune function. Proc. Nutr. Soc. 57: 503-509.

Chapter 4

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

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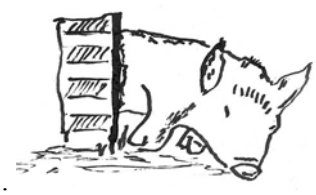

Abstract

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.

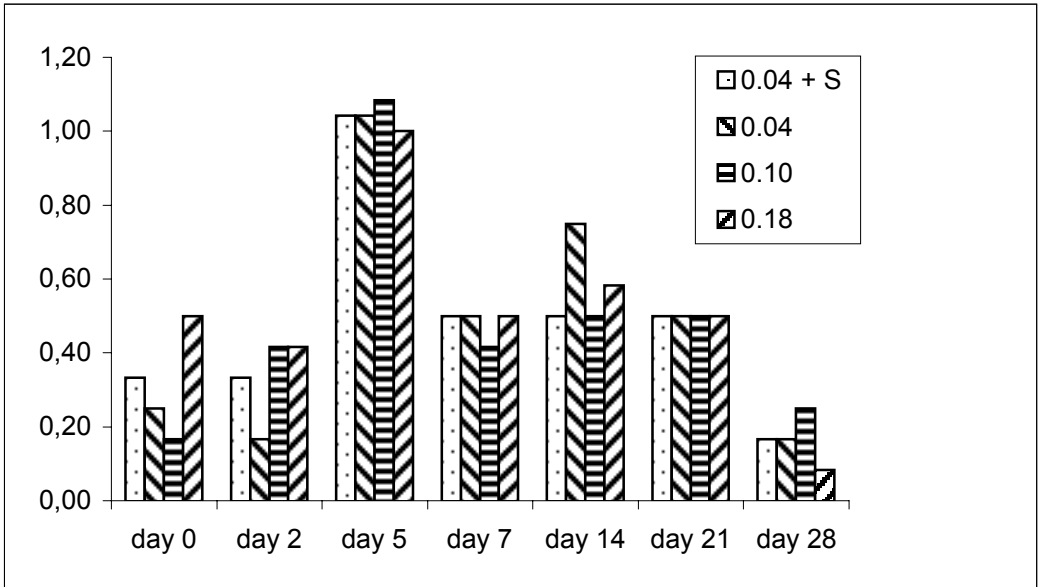
1,20
1,00
0,80
0,60
0,40
0,20
0,00
0.04 + S
0.04
0.10
0.18
day 0
day 2
day 5
day 7
day 14
day 21
day 28

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.

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

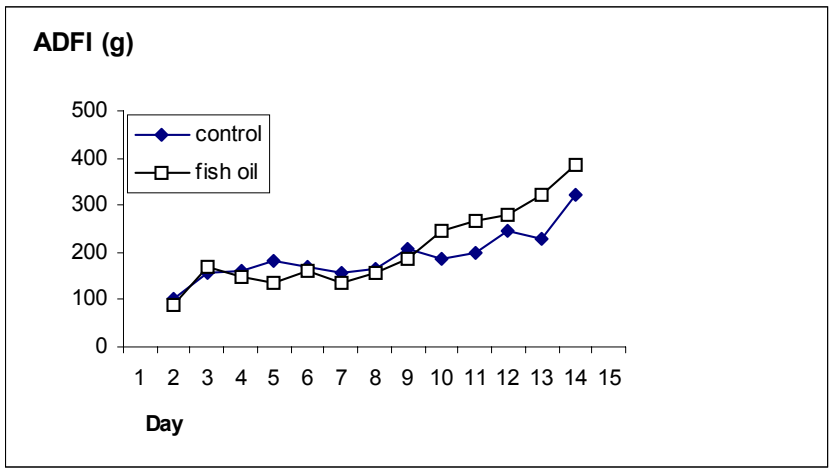

Fig. 1: Average daily feed intake (ADFI) per dietary treatment. The values are given as pen means. The piglets were weaned on day 1, witheld from feed during day 1 and challenged on both days 6 and 7.

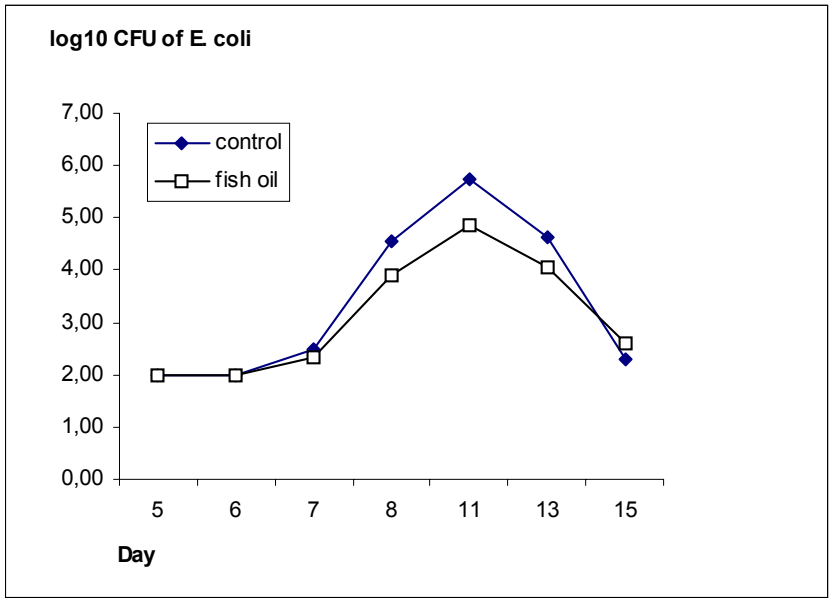

Figure 2. Faecal *E. coli* O149K91K88 counts during the experimental period. Mean values for 8 piglets per group are given.

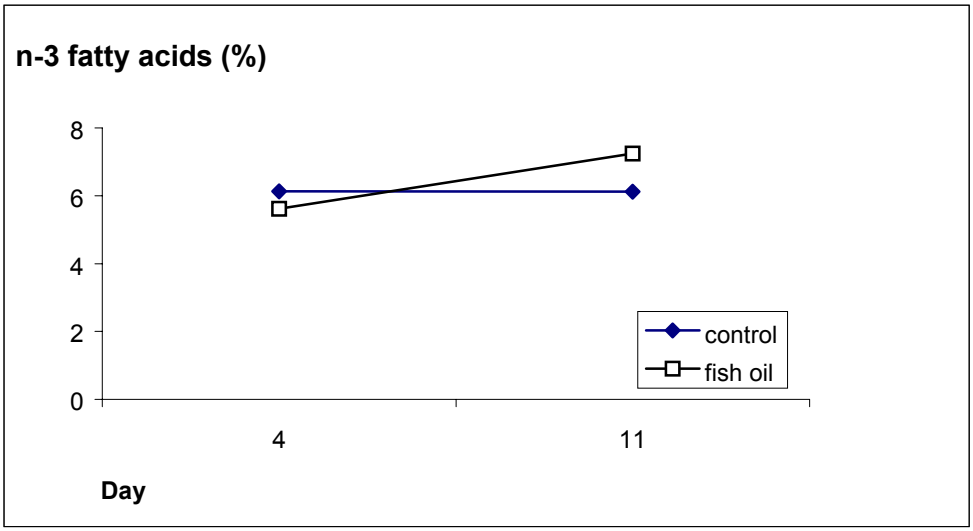

Figure 3. Total amount of n-3 polyunsaturated fatty acids (g/100g methylesters) in erythrocyte membranes. Mean values for 8 piglets per dietary group are given.

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).

References

Aggett, P.R., Haschke, R., Heine, W., Hernell, O., Koletzko, B., Launiala, K., Rey, J., Rubino, A., Schoch, G., Senterre, J. and Tormo, R. (1991) Comment on the Content and Composition of Lipids in Infant Formulas, ESPGAN Committee on Nutrition. Acta Pediatr. Scand. 80: 887-896.

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

Bertschinger, H.U. (1999) Postweaning Escherichia coli diarrhoea and edema disease In: Straw, B.E. et al. (Ed.). Diseases of Swine. Ames, Iowa, USA: Iowa State University press: 441-446.

Blok, W.L., Katan, M.B. and Van der Meer, J.W.M. (1996) Modulation of inflammation and cytokine production by dietary (n-3) fatty acids. J. Nutr. 126: 1515-1533.

Bovee-Oudenhoven, I,M., Termont, D.S., Heidt, P.J. and Van der Meer, R. (1997) Increasing the intestinal resistance of rats to the invasive pathogen Salmonella enteritidis: additive effects of dietary latulose and calcium. Gut 40: 497-504.

Burns, C.P., Luttenegger, D.G., Dudley, D.T., Buettner, G.R. and Spector, A.A. (1979) Cancer Research 39: 1726-1732.

Deprez, P., Nollet, H., Van Driessche, E. and Muylle, E. (1996) The use of swine plasma components as adhesin inhibitors in the protection of piglets against Escherichia coli enterotoxemia. Proceedings of the 14th IPVS Congress, Bologna, Italy 276.

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.

Jeyasingham, M.D., Butty, P., King, T.P., Begbie, R. and Kelly, D. (1999) Escherichia coli K88 receptor expression in intestine of disease susceptible weaned pigs. Vet Microbiol 68: 219-234.

McDonald, D.E., Pethick, D.W., Pluske, J.R. and Hampson, D.J. (1999) Adverse effects of soluble non-starch polysaccharide on piglets growth and experimental colibacillosis immediately after weaning. Res. Vet. Sci. 67: 245-250.

Meijer, J.C., Van Gils, B., Bokhout, B. De Geus, M., Harmsen, M., Nabuurs, M.J.A. and Van Zijderveld, F.G. (1997) Weaned piglets orally challenged with E. coli following stress as a model of postweaning diarrhoea. Proceedings symposium Gastro-intestinal disorders in juveniles ID-DLO, Lelystad, The Netherlands.

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.

Nabuurs, M.J.A., Van Zijderveld, F.G. and De Leeuw, P.W. (1993) Clinical and microbiological field studies in the Netherland of diarrhoea in pigs at weaning. Res. Vet. Sci. 55: 70-77.

Nabuurs, M.J.A. (1998) Weanling piglets as a model for studying pathophysiology of diarrhoea. Vet. Quarterly 20, Supplement 3: 42-45.

Nagy, B. and Feteke, P.Z. (1999) Enterotoxigenic Escherichia coli (ETEC) in farm animals. Vet. Res. 30: 259-284.

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.

Nollet, H., Deprez, P., Van Driessche, E. and Muylle, E. (1999) Protection of just weaned pigs against infection with F18+ Escherichia coli by non-immune plasma powder. Vet. Microbiol. 65: 37-45.

Pluske, J.R., Hampson, D.J. and Williams, I.H., (1997) Factors influencing the structure and function of the small intestine in the weaned pig, a review. Livest. Prod. Sci. 51: 215-236.

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

Sarmiento, J.I., Casey, T.A. and Moon, H.W. (1988) Postweaning diarrhoea in swine: Experimental model of enterotoxigenic *Escherichia coli* infection. Am. J. Vet. Res. 49: 1154-1159.

Van Beers-Schreurs, H.M.G., Vellenga, L., Wensing, T.H. and Breukink, H.J. (1992) The pathogenesis of the post-weaning syndrome in weaned pigs; a review. Vet. Quarterly 14: 29-34.

Van Dijk, A. J., Enthoven, P.M.M., Van den Hoven, S.G.C., Van Laarhoven, M.M.H., Niewold, T.A., Nabuurs, M.J.A. and Beynen, A.C. (2002) The effect of dietary spray-dried porcine plasma on clinical response in weaned piglets challenged with a pathogenic *Escherichia coli*. Vet. Microbiol. 84: 207-218.

Vaughn, D.M., Reinhart, G.A., Swain, S.F., et al. (1994) Evaluation of dietary n-6 to n-3 fatty acids ratios on leukotriene B synthesis in dog skin and neutrophils. J. Vet. Med. 8: 155.

Wu, D. and Meydani, S.N. (1998) n-3 Polyunsaturated fatty acids and immune function. Proc. Nutr. Soc. 57: 503-509.

Chapter 6

Essential-fatty acid status of piglets at weaning in relation to post-weaning health: a brief review

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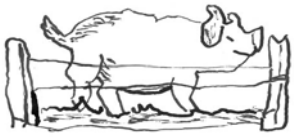

Abstract

From the outset, it is assumed that a sufficient status of polyunsaturated fatty acids (PUFAs) in combination with a high ratio of n-3:n-6 PUFAs will be beneficial for piglets so as to coping with the multiple stressors at weaning. The fatty acid status at weaning is essentially determined by the fatty acid composition of the sow's milk which is in turn determined by the fatty acid composition of fat mobilized by the sow and that of the lactation diet.

Introduction

In current swine production, piglets are weaned at the age of 21-28 days. The weaning transition is associated with abrupt changes in social, physical, microbial and chemical environment, including the diet. For a period of a few days after weaning, feed intake is below maintenance requirement (Pluske et al., 1996) which causes atrophy of small intestinal villi and oedema disease (Nabuurs, 1998)**.** The low feed intake after weaning causes a low intake of polyunsaturated fatty acids (PUFAs), including the essential fatty acids, linoleic acid (LA, C18:2 n-6) and α-linolenic acid (ALA, C18:3 n-3). LA and ALA are the parent compounds of the so-called n-6 and n-3 families of PUFAs, respectively. The two essential fatty acids and their metabolic products of desaturation and elongation are important structural components of membranes, influencing membrane fluidity, receptor function and enzyme activity (Burns et al., 1979). In addition, the metabolites arachidonic acid (AA, C20:4 n-6) and eicosapentaenoic acid (EPA, C20:5 n-3) are the precursors of eicosanoids affecting a variety of biological functions, including immunity (Wu and Meydani, 1998; Fritsche et al., 1993a) There is evidence that dietary n-3 PUFAs may antagonize atrophy of villi and have anti-inflammatory activity. In growing chicks, the intake of extra n-3 PUFAs has been shown to improve performance and decrease the inflammatory response to LPS from *S. typhimurium* and *S. aureus* (Korver and Klasing, 1997). In young mice with hypoxia-induced bowel necrosis, supplementation of the diet with n-3 PUFAs reduced the degree of necrosis (Akisu et al., 1998). Mucosal damage in food-sensitive enteropathy in mice was prevented by fortification of the diet with n-3 PUFAs (Ohtsuka et al., 1997).

We have addressed the question whether the fatty acid composition of the diet of weanling piglets affects their villus:crypt ratio of small intestinal mucosa and growth performance. Weanling piglets were given access to diets containing different amounts of LA and ALA, in the form of corn oil and linseed oil, so that the n-3:n-6 ratio of dietary PUFAs ranged between 0.03 and 0.32. Corn oil contains about 55% LA whereas linseed oil contains 55% ALA. The different n-3:n-6 ratios did not affect growth performance (Chapter 2). Fish oil may contain 20% EPA plus docosahexaenoic acid (DHA, C22:6 n-3), and it was hypothesized (Chapter 3) that fish-oil feeding would be more effective than linseed-oil feeding because weanling piglets might have low capacity to convert ALA into EPA (Bee, 2000). However, there was no consistent effect of fish oil in the diet on either the villus:crypt ratio of small intestinal mucosa or growth performance (Chapters 3 and 4).

Just before weaning at the age of about 21 days, piglets consume about 1 kg of milk/day (Taugbol et al.,1993). We have calculated that an intake of 300 g weaner diet/day provides a similar amount of n-3 and n-6 PUFAs as does an intake of 1 kg of sow milk (Chapter 1). Clearly, the outcome of our calculations is

dependent on the fatty acid composition and fat content of both the weaner diet and sow milk. It takes about 12 days after weaning before piglets reach an intake of 300 g of dry feed (Bruininx et al., 2001). The period of low PUFA intake may not affect fatty acid status of the piglets. Indeed, we have shown that fatty acid status of weanling piglets, as represented by the fatty acid composition of erythrocytes, subcutaneous adipose tissue and lymph nodular fat, does not alter within 7 days (Chapter 1). Thus, it is likely that the observed (Chapters 3 and 4) lack of effect of fatty acid composition of the weaner diet on villus:crypt ratio of small intestinal mucosa and growth performance of weanling piglets is explained by sufficient body stores of n-6 and n-3 PUFAs at the time of weaning. If PUFAs indeed influence gut integrity, then the status of essential fatty acids of piglets at the time of weaning may determine their susceptibility to post-weaning disorders.

The fatty acid status of suckling piglets is determined by the fatty acid content of sow milk. In the case that the piglets have access to creep feed, the intake of creep feed will be too low (Bruininx et al., 2002) to influence fatty acid status. New-born piglets weighing 1.5 kg have a body-fat content of only 1%, whereas weanling piglets weighing about 8 kg have a fat content of 5% (Murry et al., 1999). It follows that the fatty acid status of new-born piglets, which is related to the sow diet fed during gestation (Rooke et al., 1999; Rooke et al., 1998; Farnworth and Kramer, 1989; Arbuckle and Innis, 1993), has no impact on the fatty acid composition of the piglets' carcass at weaning. This paper summarizes the literature data on the influence of the fatty acid composition of the lactation diet on the fatty acid status of piglets at weaning. It is assumed that a sufficient status of PUFAs in combination with a high n-3:n-6 ratio in erythrocyte membrane PUFAs will be beneficial so as to cope with the multiple stressors at weaning. Prior to describing the relation between the fatty acid composition of the lactation diet and fatty acid status of the weanling piglet, the conditions of deficient and optimum fatty acid status are discussed.

Essential-fatty acid deficiency

In order to evaluate the fatty acid status of weanling piglets, as mirrored by the fatty acid composition of selected blood components or tissues, it is helpful to know when the condition of deficiency occurs. Unfortunately, there is limited information on essential-fatty acid deficiency in suckling and weanling piglets. To study the effects of deficiency, suckling piglets have to be fed on artificial diets, because, obviously, sow milk is not deficient in essential fatty acids. Overt deficiency is associated with signs such as impaired growth. Daveloose et al. (1993) fed 10-day old piglets semipurified diets containing 7% fat either high or low in LA and ALA. Table 1 shows the fatty acid compositions of the two diets. The diets were fed for 6 weeks. Weight gain of piglets fed the high-PUFA diet was

Table 1. Fatty acid composition of the diet and that of plasma phospholipids in piglets fed either sufficient or deficient in LA

	Diet	
Fatty acids	Sufficient	Deficient
	% of total fatty acids	
LA	50.9	4.5
ALA	0.6	0.0
	Plasma phospholipids	
	Sufficient	Deficient
LA	16.0	6.9
ALA	0.2	0.1
AA	11.0	5.4
EPA	1.6	1.0
DHA	4.1	0.8
EA	0.3	4.3

Based on Daveloose et al. (1993)

236 ± 15 g/day (mean ± SEM, n=6) and for those fed the low-PUFA diet it was 137 ± 15 g/day. Thus, the low-PUFA diet can indeed be considered deficient. Table 1 documents the fatty acid composition of the PUFA-deficient piglets. It is clear that PUFA deficiency is associated with various changes in the fatty acid profile of plasma phospolipids. PUFA deficiency elicited an increase in the percentages of oleic acid (OA, C18:1 n-9) and eicosatrienoic acid (EA, C20:3 n-9). As would be expected, the percentages of LA, AA, ALA, EPA and DHA were lowered after feeding the PUFA-deficient diet (Table 1). The study of Daveloose et al. (1993) illustrates that PUFA deficiency impairs growth of piglets and alters their fatty acid status. It should be stressed that the feeding trial started when the piglets were aged 10 days and ended when they were older than 7 weeks. In the context of this paper the main interest is in new-born piglets fed variable amounts of PUFAs until the age of about 3 weeks.

LA and ALA levels in tissues vary with LA and ALA intakes and therefore the levels by themselves do not readily indicate the status of deficiency. In other words, the tissue levels can be considered deficient only when they cause metabolic aberrations. It is generally accepted that PUFA deficiency in various animal species causes an increase in the levels of EA. The study described above showed a similar effect (Table 1). The increase in n-9 fatty acids with a

simultaneous decrease in n-6 and n-3 PUFAs may maintain membrane fluidity and thereby membrane-dependent functions (Beynen et al., 1984). Thus, it is reasonable to use the tissue level of OA and EA acid as an indicator of essential-fatty acid deficiency, which may even point at a deficient intake earlier than does impaired growth. The implication would be that with varying intakes of PUFA, essential-fatty acid deficiency is about to develop when OA and EA begin to increase. The condition of deficiency may then be defined as the tissue level of LA and/or ALA at which OA and EA is about to increase. In three studies (Goustard-Langelier et al. 1999; Huang and Craig-Schmidt 1996; Alessandri et al. 1996) new-born piglets have been fed diets with different fatty acid compositions. However, the results of these studies do not provide information as to conditions that elicit essential-fatty acid deficiency.

Optimum n-3:n-6 ratio in tissues

Clearly, a condition of essential-fatty acid deficiency in weanling piglets does not readily occur under practical conditions. An important issue is to define the fatty acid status that provides optimum disease resistance. Fritsche (1993 a) fed sows a diet containing either 7% menhaden fish oil or lard from day 107 of gestation until farrowing and studied the effect on immune cell fatty acid composition and eicosnanoid production in the nursed piglets. The fatty acid profile of total diets and sow milk were not given. Fatty acid compositions of serum, liver, thymus, splenocytes and alveolar macrophages in weanling piglets were affected by the sows diet. The sow's diet influenced prostaglandin production by alveolar macrophages isolated from weanling piglets, but it cannot be concluded which effect should be considered advantageous.

Fatty acid status of piglets at weaning

Rooke (1998) fed sows diets with either soyabean oil or tuna oil (30 g/kg diet) for the last three weeks of gestation. Shortly after birth piglets from tuna-oil-fed sows had a higher amount of n-3 polyunsaturated fatty acids in their tissues compared to the soyabean-oil fed other group. Feeding sows with different oil supplements for the last three weeks of pregnancay did not cause differences in litter seize or piglet weight. However, the piglets from the tuna-oil-fed sows had a lower viability score, being based on heart rate, onset of breathing and first attempt to stand. This was probably due to the induced farrowing on day 113-114, because intake of n-3 fatty acids may prolong gestation time, so the sows fed soyabean oil were closer to their natural farrowing time. The sows diet influenced plasma fatty acids and the fatty acid composition of the milk (Table 2).

Fritsche (1993 b) fed sows from day 107 of gestation experimental diets in which fish oil was substituted for lard at 0, 3.5 or 7% of the diet. The fatty acid

Table 2. Fatty acid composition of the diet and that of milk fat and of plasma of piglets

	Diet	
Fatty acid (% FAME[1])	Soyabean oil	Tuna oil
LA	51.1	2.2
ALA	7.2	3.0
EPA	0.2	3.9
DHA	0.4	17.6
	Plasma	
	Soyabean oil	Tuna oil
LA	12.9	10.0
ALA	ND[2]	ND
EPA	1.3	5.2
DHA	3.6	7.7
	Milk fat	
	Soyabean oil	Tuna oil
LA	25.9	14.4
ALA	2.9	1.6
EPA	0.6	1.7
DHA	2.1	10.1

Based on Rooke et al. (1998)
[1] FAME = Fatty Acid MethylEsters
[2] ND = not detectable

composition of milk fat was influenced by the diet. The fatty acid composition of the experimental diets was not given, but the fatty acid composition of milk fat and that of total serum of piglets differed significantly. The suckling piglets had an increase in serum n-3 levels within 24 hours after birth when the mothers were fed 3.5 or 7% fish oil. This increase lasted throughout the lactation period (Table 3). The increasing amount of fish oil at the expense of lard from day 107 from gestation did not result in differences among the number of live piglets born per litter, their birth and weaning weights. In conclusion, manipulation of fatty acid supply in the diet of the sow from day 107 of gestation up to weaning can modify the fatty acid composition of the milk fat and the piglets fatty acid composition of plasma or serum.

Table 3. Fatty acid composition of milk fat and fat of serum of piglets (mean value over total lactation period)

	Milk fat		
Fatty acid (% FAME)[1]	7% Lard	3.5% Lard 3.5% Fish oil	7% Fish oil
LA	13.1	12.6	11.3
ALA	0.6	0.8	0.9
EPA	0.5	2.3	3.3
DHA	0.6	2.4	3.5
	Serum		
	7% Lard	3.5% Lard 3.5% Fish oil	7% Fish oil
LA	23.0	19.1	17.1
ALA	0.5	0.5	0.5
EPA	0.3	7.6	12.4
DHA	1.2	4.6	5.6

Based on Fritsche et al (1993b)
[1] FAME = Fatty Acid MethylEsters

Conclusion

The major determinant of the fatty acid status of the weanling piglet is the fatty acid composition of the pregnancy and lactation diet fed to the sow. There is insufficient data to suggest the optimum composition of these diets.

References

Akisu, M., Baka, M., Coker, I., Kultursay, N. and Huseyinov, A. (1998) Effect of dietary n-3 fatty acids on hypoxia-induced necrotizing enterocolitis in young mice. n-3 fatty acids alter platelet-activating factor and leukotriene B4 production in the intestine. Biol. Neonate 74: 31-38.

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.

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.

Beynen, A.C., Schouten, J.A. and Popp-Snijders (1984) Compensatory changes in the lipid composition of the erythrocyte membrane. Tr. Biochem. Sci. 9: 474.

Bruininx, E.M.A.M., Van der Peet-Schwering, C.M.C., Schrama, J.W., Vereijken, P.F.G., Vesseur, P.C., Everts, H., Den Hartog, L.A. and Beynen, A.C. (2001) Individually measured feed intake characteristics and growth performance of group-housed weanling pigs: effect of gender, initial body weight, and body weight distribution within groups. J. Anim. Sci. 79: 301-308.

Bruininx, E.M., Binnendijk, G.P., van der Peet-Schwering, C.M., Schrama, J.W., den Hartog, L.A., Everts, H. and Beynen, A.C. (2002) Effect of creep feed consumption on individual feed intake characteristics and performance of group-housed weanling piglets. J. Anim. Sci.80: 1413-1418.

Burns, C.P., Luttenegger, D.G., Dudley, D.T., Buettner, G.R. and Spector, A.A. (1979) Effect of modification of plasma membrane fatty acid composition on fluidity and methotrexate transport in L1210 murine leukemia cells. Cancer Res. 39: 1726-1732.

Daveloose, D., Linard, A., Arfi, T., Viret, J. and Christon, R. (1993) Simultanous changes in lipid composition, fluidity and enzyme activity in piglet intestinal brush border membrane as affected by dietary polyunsaturated fatty acid deficiency. Biochim. Biophys. Acta 1166: 229-237.

Farnworth, E.R. and Kramer J.K.G. (1989). Changes in the lipid composition of the internal organs of fetal pigs from sows fed different dietary fats. Can. J. Anim. Sci. 69: 441-448.

Fritsche, K.L., Alexander, D.W., Cassity, N.A. and Huang, S.-C. (1993a) Maternally-supplied fish oil alters piglet immune cell fatty acid profile and eicosanoid production. Lipids 28: 677-682.

Fritsche, K.L., Huang, S.-C. and Cassity, N.A. (1993b) Enrichment of omega-3 fatty acids in suckling pigs by maternal dietary fish oil supplementation. J. Anim. Sc. 71: 1841-1847.

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.

Huang, M.C. and Craig-Schmidt, M.C. (1996) Arachidonate and docosahexaenoate added to infant formula influence fatty acid composition and subsequent eicosanoid production in neonatal pigs. J. Nutr. 126: 2199-2208.

Korver, D.R. and Klasing, K.C. (1997) Dietary fish oil alters specific and inflammatory responses in chicks. J. Nutr. 127: 2039-2046.

Murry, A. C., Gelaye, S., Casey, J.M., Foutz, T.L., Koukakou, B. and Arora, D. (1999) Type of milk consumed can influence plasma concentrations of fatty acids and minerals and body composition in infant and weanling pigs. J. Nutr. 129: 132-138.

Nabuurs, M.J.A. (1998) Weanling piglets as a model for studying pathophysiology of diarrhoea. Vet. Quarterly 20, Supplement 3: 42-45.

Ohtsuka, Y., Yamashiro, Y., Shimizu, T., Nagata, S., Igarashi, J., Shinohara, K., Oguchi, S. and Yabuta, K. (1997) Reducing cell-membrane n-6 fatty acids attenuate mucosal damage in food-sensitive enteropathy in mice. Pediatr. Res. 42: 835-839.

Pluske, J. R., Williams, I.H. and Aherne, F.X. (1996) Maintenance of villous height and crypt depth in piglets by providing continuous nutrtion after weaning. Anim. Sci. 62: 131-144.

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: 273-80.

Rooke, J.A., Bland, I.M. and Edwards, S.A. (1999) Relationships between fatty acid status of sow plasma and that of umbilical cord, plasma and tissues of newborn piglets when sows were fed on diets containing tuna oil or soyabean oil in late pregnancy. Br. J. Nutr. 82: 213-221.

Taugbol, O., Framstad, T. and Saarem, K. (1993) Supplements of cod liver oil to lactating sows. Influence on milk fatty acid composition and growth performance of piglets. Zentralbl. Veterinarmed. A40: 437-443.

Wu, D. and Meydani, S.N. (1998) n-3 Polyunsaturated fatty acids and immune function. Proc. Nutr. Soc. 57: 503-509.

Chapter 7

High water content of feed raises dry matter intake by weanling piglets

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Abstract

The weaning of piglets is associated with a drop of nutrient and energy intake which is generally considered to render the animals prone to the development of post-weaning diarrhoea. In an attempt to increase post-weaning feed intake, piglets were fed diets with increasing water contents. There were three dietary treatments that were studied in three different experiments with 12 or 18 piglets. Control treatment (D) was a dry feed, and the test treatments were the same dry feed, but water added to a water; feed ratio of 1.5 : 1 (SL) or 2.5 : 1 (L). Diets and separate drinking water were freely available for a period of 7 days after weaning. The water content of the diet raised dry matter intake, total water intake and body weight gain in a dose-dependent fashion. For individual piglets there were direct relationships between dry matter intake and weight gain and also between total water intake and dry matter intake. This study does not show that a high water intake is essential for a high dry matter intake in weanling piglets, but it does indicate that the level of post-weaning feed intake is maintained better when a liquid diet instead of a dry diet is fed.

Introduction

The common practice of weaning piglets at an age of three to four weeks is associated with considerable changes for the young animal. Apart from changes in housing and social hierarchy after weaning, there is an abrupt change in nutrient supply to the piglet (Everts et al., 1999). A less digestible dry diet rich in starch now replaces a fat-rich mixture of highly digestible nutrients in the form of milk. The dietary change after weaning often coincides with a low feed intake. A shortage of nutrients and energy can result in a decreased villous height and increased crypt depth in the intestinal wall as has been shown during the first week after weaning (Pluske et al., 1996; Van Beers-Schreurs et al., 1998). The morphological changes of the intestine are often associated with post-weaning diarrhoea (PWD). It is generally accepted that in order to minimize the incidence of PWD, feed intake after weaning should be stimulated.

Given the change from liquid to dry feed at weaning, it could be suggested that immediate post-weaning feed intake could be improved by the use of liquid feed, possibly leading to a quick onset of feeding, a gradual increase in feed consumption and a sufficient water intake. Indeed, Russell et al. (1996) reported that provision of a liquid diet (water: feed = 2.5: 1 ; w/w) instead of a dry feed improved feed intake, daily gain and water consumption in piglets during the first week after weaning. Moreover, Deprez et al. (1987) observed lesser morphological changes in the distal jejunum and in the ileum of weanling pigs when a liquid diet (water: feed = 2: 1 ; w/w) instead of a dry feed was offered. Also gruel feeding both pre and post weaning had positive effect on gut integrity (Blanchard et al., 2000).

The water: feed ratio of the post-weaning gruel may be critical. Geary et al. (1996) showed that liquid diets with less than 200 g dry matter per kg have negative effects on dry matter intake, daily gain and feed conversion ratio.

From the above-mentioned literature data it is concluded that liquid feeds can be beneficial for the weaning pig. However, there is no detailed information on the daily increase in dry matter intake and water consumption by piglets during the first week after weaning. The aim of the present experiments was to describe, for gruels with different ratios of water to feed, the pattern of intake of dry matter and water during the first week after weaning. It was anticipated that the results thus obtained could be used to select the optimum water: feed ratio of liquid diets for weanling pigs.

Materials and methods

Animals

Three separate experiments were carried out for each of which piglets were selected from the litters of three or four lactating sows. At the farm of the Faculty of Veterinary Medicine, the sows are nursing their piglets for about 29 days. During this suckling period the piglets have free access to a dry creep feed and water. Within each experiment, the piglets were allocated to one of the three treatments so that the distributions within treatments were similar with respect to piglet body weight and nursing mother. The number of piglets in experiments I, II and III was 18, 12 and 12, respectively. The piglets were weighed at weaning (day 1) and at the end of the experiment (day 7).

Treatments

The feed used for all treatments was a commercially available diet for piglets aged 3 to 5 weeks. Approximate analysis of the diet is given in Table 1. Piglets subjected to the control treatment (D) received dry feed. Piglets in the group "semi-liquid" feed (SL) and the "liquid" feed (L) received the same amount of dry feed as the control piglets, but were offered it as a mixture with tap water. The water to feed ratio (w/w) was 1.5:1 and 2.5:1 for treatments SL and L, respectively. All piglets were fed twice daily at 8.00 h and 20.00 h. At each feeding time a clean trough with fresh feed was offered to the piglets and the leftovers were weighed and analyzed for dry matter. All animals had free access to tap water through reservoirs with nipples. The amount of water consumed by drinking was measured daily. When total water intake was calculated the evaporation of water from the troughs was not taken into account.

Table 1. Approximate analysis (g/kg) of the diet used

Chemical Analysis	
Dry matter	50
Crude protein	174
Crude fat	53
Crude fiber	34

Housing

In experiment I, nine pairs of piglets were kept separately in clean farrowing pens. Ambient temperature was maintained at circa 25 °C. In experiments II and III, twelve piglets were kept individually in cages placed in one room. The cages (1.20x0.50x1.00) had a slatted floor, half covered with a rubber mat. Ambient temperature in experiment II was about 18 ^{o} C and in experiment III it was 27^{o} C. The low ambient temperature in experiment II was not intended, but due to inappropriate temperature control.

Statistical analysis

First, the data from each experiment were analyzed separately using analysis of variance and the paired t-test. Irrespective of the different housing conditions, the results of each experiment pointed to the same conclusions. Therefore, an analysis of variance was done with the model: $Y = \mu + \text{Experiment} + \text{Treatment} + \text{Experiment x Treatment} + \varepsilon$. Additionally, a paired t-test was used. In the model used, the mean value of two piglets of each pair in experiment I had the same weight as the individual values for the piglets in experiments II and III. Only for two parameters (dry matter intake on day 2 and drink water consumption on day 3) there was a significant effect of experiment, while there was no significant interaction between experiment and treatment. Thus, the overall analysis is used to present the results. The level of statistical significance was pre-set at $P<0.05$.

Results

Course of the experiments

During experiment I, there were no problems. In experiment II the ambient temperature was too low and the piglets reacted with a somewhat higher feed consumption. It was decided not to increase the temperature in order to maintain the settled intake pattern. No health problems were observed. In trial III, one piglet in the control group refused to eat and to drink during several days for unknown reasons; this piglet was excluded.

Dry matter intake

The dry matter intake of the piglets on the first day after weaning was low for all treatments. During the following days, dry matter intake was highest for treatment L. On day 7, treatments L and SL showed a daily intake above 300g dry matter per day. Significant treatment differences in dry matter intake were only observed for treatments D and L on days 2, 3 and 5 (Table 2). The general lack of

Table 2. Dry matter intake (g/piglet/day) for the three dietary treatments, pooled for the three experiments

Day	Diet			SED	P- values		
	D	SL	L		Experiment	Diet	Interaction
1	17	60	45	26	0.278	0.290	0.842
2	84[a]	121[ab]	197[b]	46	0.033	0.064	0.952
3	117[a]	150[ab]	237[b]	47	0.659	0.048	0.837
4	189	181	290	57	0.386	0.129	0.962
5	154[a]	233[ab]	283[b]	49	0.695	0.046	0.712
6	218	299	286	61	0.718	0.378	0.608
7	241	315	346	51	0.386	0.134	0.346
1-7	1020[a]	1360[ab]	1683[b]	290	0.440	0.095	0.883

D = dry feed, SL = semi-liquid feed, L = liquid feed
SED = standard error of difference, different superscripts in the row indicate a significant difference

Table 3. Total water intake (g/piglet/day) for the three dietary treatments, pooled for the three experiments

Day	Diet			SED	P- values		
	D	SL	L		Experiment	Diet	Interaction
1	17	60	45	26	0.278	0.290	0.842
2	84[a]	121[ab]	197[b]	46	0.033	0.064	0.952
3	117[a]	150[ab]	237[b]	47	0.659	0.048	0.837
4	189	181	290	57	0.386	0.129	0.962
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D = dry feed, SL = semi-liquid feed, L = liquid feed
SED = standard error of difference , different superscripts in the row indicate a significant difference

statistically significant treatment effects was due to low statistical power as a result of the large variation in dry matter intake between piglets. Nevertheless, the diet effect tended to be significant (P = 0.095) for the entire feeding period. The piglets subjected to treatment SL on average consumed 33% more dry matter than the

control piglets. For the L group, the increase in dry matter intake was even 65%. The effect of experiment was significant on day 2 only, when the piglets in experiment II consumed relatively more dry matter due to the low ambient temperature.

Water intake

Total water intake, including obligatory water ingested with feed was significantly higher for treatment L than for treatments D and SL, except for day 1 (Table 3). For treatment L, total water intake on day 7 was about twice that for treatment D. The amount of drinking water consumed by the piglets in group D increased gradually during the first week (Table 3). The amount of drinking water consumed during treatment L had stabilized after two days at a level of about 1000 ml per animal per day.

Performance

The data combined for the three experiments indicated that treatment L had produced a marked increase in live weight gain, but this effect was not significant (Table 4). There was a tendency (P = 0.202) that piglets given treatment L gained more live weight than did their counterparts given treatment D. The feed conversion ratio did not differ significantly between treatments, but was lowest for treatment L. The mean ratio of total ingested water to dry matter was about 4.5 for all treatments.

Table 4. Animal performances for the three dietary treatments, pooled for the three experiments

	Diet			SED	P- values		
	D	SL	L		Experiment	Diet	Interaction
Liveweight at d0 (kg)	8.14	8.16	8.20	0.27	0.546	0.972	0.439
Live weight gain (kg)	0.61	0.88	1.14	0.29	0.621	0.202	0.477
DM / gain (kg/kg)	3.78	3.40	1.59	2.20	0.249	0.577	0.752
Water / DM (kg/kg)	4.55	4.30	5.06	0.56	0.212	0.395	0.566

D = dry feed, SL = semi-liquid feed, L = liquid feed
SED = standard error of difference , different superscripts in the row indicate a significant difference

Discussion

The present results are based on combining the data from three experiments. The outcome supports earlier work (Russel et al., 1996) in that liquid feeding of weanling pigs improves dry matter intake. There were significant

Figure 1

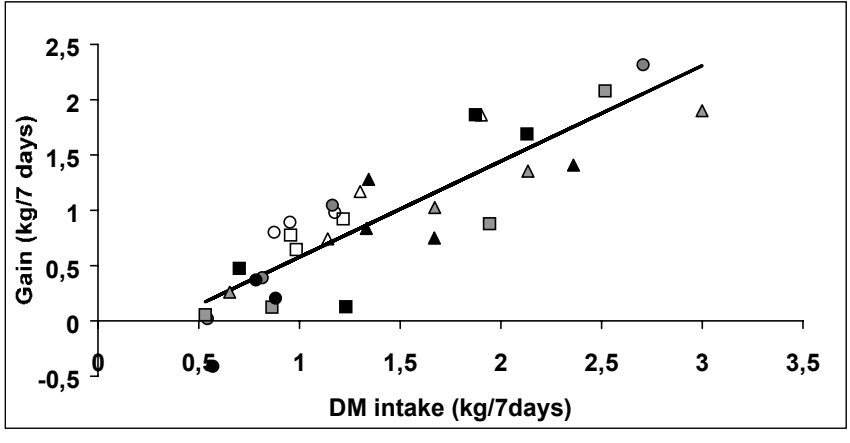


Figure 1
Relationship between dry matter intake and body weight gain for individual piglets as pooled for the three treatments and the three experiments. Open symbols: experiment I ; grey-filled symbols : experiment II; black-filled symbols : experiment III. Circles : treatment D; squares : treatment SL; triangles : treatment L. The regression equation of line is: $Y = -0.288(\pm 0.134) + 0.866\ (\pm 0.088)\ X$ ($R^2_{adj.} = 0.76$, DF= 31, $P < 0.001$)

differences in dry matter intake between the treatments L and D on various days after weaning. The dry matter intake increased fastest in the first days after weaning in the piglets given treatment L. Piglets that had access to dry feed showed the lowest intakes of dry matter. Figure 1 shows that there was a direct relation between dry matter intake and live weight gain during the first seven days after weaning. In the light of the concept that weanling pigs with high post-weaning feed intake and weight gain are less prone to PWD, it is clear that provision of nutrients in the form of gruel is more beneficial than in the form of a dry feed.

There was no significant treatment effect on feed conversion ratio (FCR) even though treatment L had caused a clear lowering of the ratio. Piglets with a low live weight gain had a major impact on the mean FCR and also increased the variation. As a result, aberrant values were obtained and statistical power became low. If the FCR was calculated with the overall group means for dry matter intake (Table 2) and live weight gain (Table 4) the values were 1.67, 1.55 and 1.47 for the

treatments D, SL and L. Thus, a trend towards a lower FCR with increasing water: feed ratio was seen. The dry matter content of the diets used, was apparently high enough to prevent an increase in FCR as observed by Geary et al., (1996) when using diets with a dry matter content less than 200 g /kg. In this study, the water: dry matter ratio of the total ingested matter was about 4.5. It would appear that over the first 7 days after weaning piglets select a rather narrow range of water: dry matter ratio. In any event, the observed ratio would be equivalent to a feed with a dry matter content of about 215 g /kg. The preferred dry matter content is comparable to that of sow milk. For weanling piglets to realize the preferred water: dry matter ratio, it took less time for treatment L than for treatment D.

Figure 2

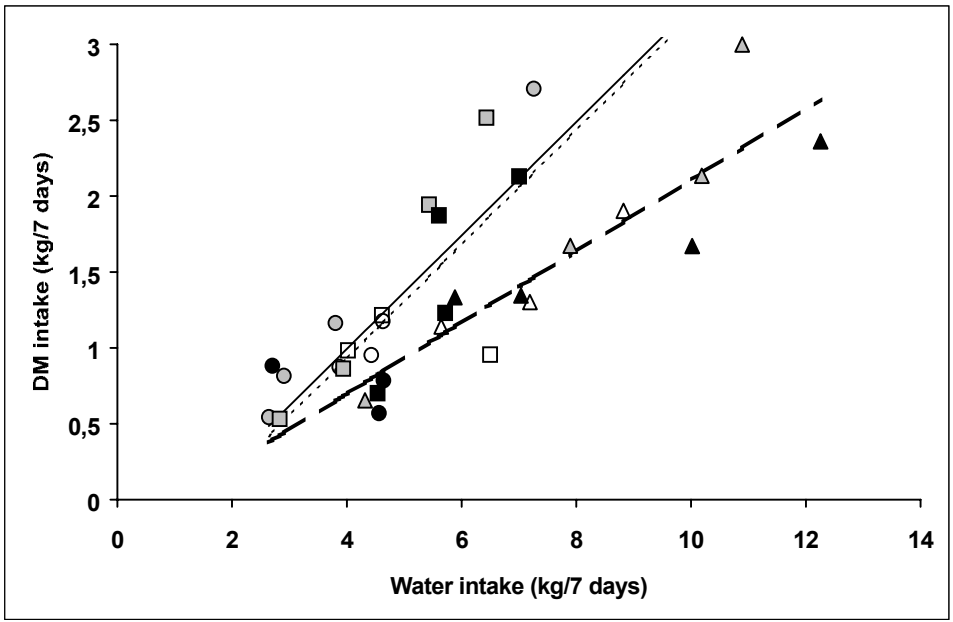

DM intake (kg/7 days)
Water intake (kg/7 days)
0
0,5
1
1,5
2
2,5
3
0
2
4
6
8
10
12
14

Figure 2

Relationship between dry matter intake and water intake for individual piglets as pooled for the three treatments and the three experiments. Open symbols: experiment I ; grey-filled symbols : experiment II; black-filled symbols : experiment III. Circles : treatment D; squares : treatment SL; triangles : treatment L. The regression equations are:

——— : diet D : $Y = -0.502 (\pm 0.405) + 0.374 (\pm 0.093) X$ ($R^2_{adj.} = 0.63$, DF=9, P<0.05)

..... : diet SL : $Y = -0.574 (\pm 0.608) + 0.376 (\pm 0.115) X$ ($R^2_{adj.} = 0.49$, DF=10, P<0.01)

– – – – : diet L : $Y = -0.242 (\pm 0.331) + 0.235 (\pm 0.039) X$ ($R^2_{adj.} = 0.78$, DF =10, P <0.001)

Piglets given treatment L consumed more than 1 L of water on the second day after water weaning, which was considerably more than the piglets in the other treatment groups. For treatment D, water consumption on day 7 was only 800 ml where as the piglets in treatment group SL had a water intake of 1 L. The amounts water consumed agree with those reported by others (Brooks et al., 1984; Russell et al.,1996; Geary et al., 1996). However, Fraser et al. (1993) reported water intakes of up to 3 L per day after the first week after weaning. Besides the obligatory water ingested with the feed, the piglets given treatment L only consumed a small amount of drinking water, except for the second day after weaning. When dry matter intake was plotted as function of total water consumption, the slope for treatment L appeared to be lower than for the treatments SL and D (Figure 2). It would appear that the L treatment had forced the piglets to ingest more water than they would drink voluntarily. The high intake of water was advantageous, or at least not detrimental, as the L treatment on average produced more weight gain. However, as mentioned above, a higher water intake than that observed, and thus a lower water : dry matter ratio of the total ingested matter could negatively affect growth performance.

The daily amount of water required by a weaned piglet is not well known. Generally, ad libitum access to water is recommended (ARC, 1981; NRC 1998, Mroz et al., 1995). When a consumption of 1 L milk at weaning is assumed, then water intake will be about 800 ml/day. As seen in Table 3, it took only 1 day for piglets given treatment L to reach the pre-weaning level of water intake, but for treatment SL it took 3 days and for treatment D 7 days. This indicates that after weaning the piglets given treatments D or LS were drinking less water than during the last phase of the suckling period. The consequence of low water intake after weaning is unknown. It could be speculated that osmotic disturbances in gut contents or body fluids occur, the severity depending on the level of dry matter intake, mineral content of the diet and ambient temperature.

Conclusion

The liquid diet may have been more preferable with increased dry matter and water intakes as secondary features. Clearly, the present results indicate that the transition from sow milk to post-weaning feed can be more smoothly when using a liquid instead of a dry feed. The effect of a gradual change from liquid to dry feed during the first week needs to be investigated. In addition, the consequences of fermentation in the liquid feed, if any, are unknown.

References

Agricultural Research Council (1981) The nutrient requirements of pigs. Slough., England. Commonwealth Agricultural Bureaux.

Blanchard, P.J., Miller, H.M., Perris, D., Toplis, P. (2000) Benefits of gruel feeding to the gut integrity of newly weaned pigs. BSAS Occasional Meeting " The weaner pig", University of Nottingham. 5-7 September 2000, p. 17.

Brooks P.H., Russell S.J. and Carpenter J.L. (1984) Water intake of weaned piglets from three to seven weeks old. Vet. Rec. 115:, 513-515.

Deprez, P., Deroose, P., Van den Hende, C., Muylle, E. and Oyaert, W. (1987) Liquid versus dry feeding in weaned piglets: The influence on small intestinal morphology. J. Vet. Med B. 34: 254-259.

Everts, H., Van Beers-Schreurs, H.M.G. and Vellenga, L. (1999) Voeding van jonge biggen in relatie tot speenproblemen. Tijdsch. Diergeneesk. 124: 44-46.

Fraser, D., Patience, J.F., Phillips, P.A. and McLeese, J.M. (1993) Water for piglets and lactating sows: quantity, quality and quandaries. In: Cole, D.J.A., Haresign, W. and Garnsworthy, P.C. (Eds), Recent Developments in Pig Nutrition 2. Nottingham university press, Loughborough, Leicestershire, pp 201-224.

Geary T.M., Brooks P.H., Morgan D.T., Campbell A. and Russell P.J. (1996) Performance of weaner pigs fed ad libitum with liquid feed at different dry matter concentrations. J. Sci. Food Agr. 72: 17-24.

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

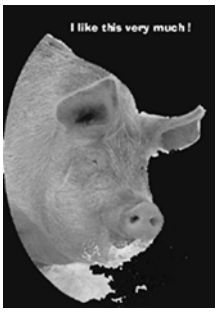
Mroz Z., Jongbloed A.W., Lenis N.P. and Vreman K. (1995) Water in pig nutrition: physiology, allowances and environmental implications. Nutr. Res. Rev. 8: 137-164.

Pluske, J.R., Williams, I.H., and Aherne, F.X. (1996) Villous height and crypt depth in piglets in response to increases in intake of cow's milk after weaning. Anim. Sci. 62: 145-158.

Russell P.J., Geary T.M., Brooks P.H. and Campbell A. (1996) Performance, water use and effluent output of weaner pigs fed ad libitum with either dry pellets or liquid feed and the role of microbial activity in the liquid feed. J. Sci. Food Agr. 72: 8-16.

Van Beers-Schreurs, H.M., Nabuurs, M.J., Vellenga, L., Kalsbeek-van der Valk, H.J., Wensing, T. and Breukink, H.J. (1998) Weaning and the weanling diet influence the villous height and crypt depth in the small intestine of pigs and alter the concentrations of short-chain fatty acids in the large intestine and blood. J. Nutr. 128: 947-953

General conclusions



General conclusions

This thesis has focussed on the dietary provision of polyunsaturated fatty acids (PUFAs) to weanling piglets in relation to growth performance and small intestinal integrity. The major results and conclusions of the various chapters above may be summarized as follows.

The status of n-3 and n-6 polyunsaturated fatty acids in piglets at weaning might determine their susceptibility to impaired growth performance and the development of post-weaning diarrhoea.

This thesis did not address the question whether the fatty acid composition of piglets at weaning affects post-weaning growth and/or the development of post-weaning disorders, but there is indirect evidence from studies in mice (Akisu et al., 1998, Ohtsuka et al., 1997) that it does. The observed lack of effect of the fatty acid composition of the weaner diet on growth performance and gut integrity might be explained by the use of piglets with sufficient fatty acid status at the time of weaning.

The lactation diet fed to the sows is a major determinant of the fatty acid status of the weanling piglet.

It is assumed that a sufficient status of PUFAs in combination with a high ratio of n-3:n-6 PUFAs will be beneficial as to coping with the multiple stressors at weaning. The fatty acid status at weaning is essentially determined by the fatty acid composition of the sow's milk which in turn is determined by the fatty acid composition of the fat mobilized by the sow and that of the lactation diet.

There may be no change of the status of n-3 and n-6 PUFAs in piglets around weaning.

The intake of n-3 and n-6 PUFAs was assessed in piglets kept under practical conditions from birth to two weeks post weaning. In addition, the fatty acid composition of erythrocyte membranes, liver fat and lymph nodular fat tissue was determined. It was found that between weaning and one week post weaning there was no clear difference in the status of n-3 and n-6 PUFAs. The low feed intake after weaning is associated with a low intake of PUFAs, but a sufficient status of PUFAs at weaning may have buffering capacity.

The requirement of ALA by weanling piglets to display maximum growth is not known, but it may be above 0.22% of metabolizable energy.

Weanling piglets were fed diets with different levels of ALA. Intakes of ALA above 0.22 energy% tended to increase growth during the first two weeks

post weaning and tended to reduce feed conversion during the first week. The average increase in weight gain was 9% and the decrease in feed conversion was 14%, but these effects did not reach statistical significance. The diet with 1.13 energy% ALA produced a significantly better body condition after two weeks than did the diet with 0.22 energy% ALA. A dietary level of 0.22 energy% is equivalent to about 0.8 g/kg air-dry diet. This level seems somewhat low in the light of the current practice of formulation of weaner diets, whereas the present evidence for this level is weak. However, Innis (1993) had suggested that ALA provision is adequate at an intake of about 0.3 energy%.

In one study dietary fish oil positively affected growth of weanling piglets, this effect not being mediated by counteracting the weaning-induced decrease in villus height. However, in another study the addition of fish oil to a weaner diet adequate in ALA did not enhance growth performance, faeces consistency and body condition of weanling piglets.

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 PUFAs. The fish-oil diets on average increased post-weaning growth by 27%, when compared with the linseed-oil diets. The effect of fish oil was not statistically significant. 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. In another feeding trial with weanling piglets there was no stimulatory effect of fish oil on growth performance. The lack of effect of fish oil in the second trial is explained by the high status of n-3 PUFAs at weaning masking any effect of fish oil consumption.

There might be a positive effect of fish oil on the clinical response in weanling piglets to a challenge with pathogenic E. Coli.

Weanling 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 average daily feed intake (ADFI) and average daily gain (ADG) after infection tended to be higher in the fish-oil group than in the control group. It appears that fish oil in the diet of weanling piglets enhances disease resistance, but given the specific conditions of the study the relevance for practice is not yet known.

Under practical conditions, the potential beneficial effect of changing the fatty acid composition of the weaner diet may be marginal and certainly is much smaller than that of increasing dry matter intake.

It is well-known that post-weaning feed intake and the risk of development of post-weaning disorders are negatively related. In an attempt to increase post-weaning feed intake, piglets were fed diets with increasing water contents. An

increasing content of the diet raised dry matter intake, total water intake and body weight gain in a dose-dependent fashion. For individual piglets there were direct relationships between dry matter intake and weight gain and also between total water intake and dry matter intake. This study does not show that a high water intake is essential for a high dry matter intake in weanling piglets, but it does indicate that the level of post-weaning feed intake is maintained better when a liquid diet instead of a dry diet is fed. When the magnitude of the effect on feed intake seen in this study is compared with that in the previous studies using diets with different fatty acid compositions, it follows that the effect of PUFAs is relatively small.

References

Akisu, M., Baka, M., Coker, I., Kultursay, N. and Huseyinov, A. (1998) Effect of dietary n-3 fatty acids on hypoxia-induced necrotizing enterocolitis in young mice. N-3 fatty acids alter platelet-activating factor and leukotriene B4 production in the intestine. Biolog. Neonate. 74: 31-8.

Innis, S. M. (1993). "The colostrum-deprived piglet as a model for study of infant lipid nutrition." J Nutr 123: 386-90.

Ohtsuka, Y., Yamashiro, Y, Shimizu, T., Nagata, S., Igarashi, J., Shinohara, K., Oguchi, S. and Yabuta, K. (1997) Reducing cell-membrane n-6 fatty acids attenuate mucosal damage in food-sensitive enteropathy in mice. Pediatr. Res. 42: 835-839.

Summary

Summary

This thesis describes research on the essential-fatty acid supply of weanling piglets. Vertebrates require dietary sources of essential fatty acids. The polyunsaturated fatty acids (PUFAs), linoleic acid (LA, C18:2 n-6) and α-linolenic acid (ALA, C18:3 n-3) are considered the parent compounds of the n-6 and n-3 families of PUFAs, respectively. The products of desaturation and elongation arachidonic acid (AA, C20:4 n-6) and eicosapentaenoic acid (EPA, C20:5 n-3), are the precursors for eicosanoids, which play an important role in the immune response. Eicosanoids produced from n-3 PUFAs generally have effects opposite to those elicited by eicosanoids synthesized from n-6 PUFAs. Due to the competition between n-3 and n-6 PUFAs for the desaturase and elongase enzymes, the net response to eicosanoids depends on the amounts and on the ratio of n-3 and n-6 PUFAs present in the diet.

Weanling piglets are prone to the development of the so-called post-weaning syndrome which is related to a by low feed intake and is associated with atrophy of the villi, inflammation of the gut and depressed performance. To investigate the influence of PUFAs on the post-weaning syndrome, the fatty acid supply and status of piglets from birth to two weeks after weaning was measured first. In addition, the fatty acid composition of erythrocyte membranes, liver fat and lymph nodular fat tissue was determined. It was found that between weaning and one week post weaning there was no difference in the status of n-3 and n-6 PUFAs as based on their concentrations in erythrocyte membranes and tissues. Weaning was associated with a drop of plasma total cholesterol, HDL cholesterol and phospholipid concentrations as well as a decrease in heparin-released plasma lipoprotein lipase activity. The changes in plasma lipid metabolism around weaning are explained by the decrease in fat intake immediately after weaning. It was concluded that this study does not point at a lowering of the status of n-3 and n-6 PUFAs in piglets at the stage around weaning. However, it is stressed that the outcome of this study is determined by the essential-fatty acid status of the sows and the fatty acid compositions of the commercial lactation diet, creep feed and weaner diet that were used.

In a second experiment, the effect of supplemental n-3 PUFAs and the ratio of n-3:n-6 PUFAs on small intestinal morphology and growth performance was investigated. Weanling piglets (n = 360) were fed diets with different levels of ALA, the levels being 0.22, 0.47, 0.77 and 1.13 % of metabolizable energy. The experimental diets were formulated by the addition of various amounts of linseed oil at the expense of corn oil. Intakes of ALA above 0.22 energy% tended to increase growth during the first two weeks post weaning and tended to reduce feed conversion during the first week: the average increase in weight gain was 9% and

the decrease in feed conversion was 14%. Increasing amounts of ALA in the diets stimulated the desaturation and elongation into EPA and DHA and the incorporation of these fatty acids into erythrocyte membranes. The requirement of ALA by weanling piglets to display maximum growth is not known, but this study indicates that it may be above 0.22 energy %. The piglets showed a post-weaning decrease in total cholesterol, HDL cholesterol and phospholipids, but the intake of various amounts of linseed oil did not influence the concentration of plasma lipids.

The third experiment addressed the question whether in weanling piglets the feeding of EPA, in the form of fish oil, would be more beneficial as to growth performance and gut integrity than the feeding of ALA in the form of linseed oil. Weaner diets were formulated that contained two levels each of either fish oil or linseed oil. The fish-oil diets on average increased post-weaning growth by 27%, when compared with the linseed-oil diets, but this increase did not reach statistical significance. 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 villus:crypt ratio was seen with the control diet having a n-3:n-6 ratio of 0.1, and the lowest 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.

To further study whether the intake of fish oil would have positive effect on growth performance of weanling piglets, in a feeding trial with 480 piglets diets without fish oil or with either 13 or 22 g fish oil/kg were fed. 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 LA desaturation and elongation by fish oil feeding. Piglets fed the diets with fish oil had higher erythrocyte-membrane concentrations of EPA and lower concentrations of AA while LA 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 ALA did not enhance growth performance, faeces consistency and body condition of weanling piglets. However, at weaning piglets already had a high status of n-3 PUFAs which might have masked any effect of fish oil in the weaner diet on growth performance.

In the fifth experiment, weaned piglets were used to determine the effect of fish oil in the diet on the clinical response to an infection with a pathogenic *Escherichia 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 score.s 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 and average daily gain after infection tended to be higher in the test group than in the control group. The faecal excretion of O149:K91:K88 tended to be lower for the 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*. The outcome of this study is not in agreement with the second feeding trial using diets fortified with fish oil and showing a lack of effect of fish oil on growth performance. The piglets in that feeding trial were kept in a relatively clean environment which might explain the lack of effect of fish oil.

The experiments described may be interpreted in that the addition of n-3 PUFAs to the weaner diet may be beneficial, but only when the piglets have a low status of n-3 PUFAs at weaning. Thus, a literature review was made to identify the factors determining the fatty acid status at weaning. The fatty acid composition of fat mobilized by the sow and that of the lactation diet influence the fatty acid composition of the sow's milk which then determines the fatty acid status of piglets at weaning.

The risk to develop post-weaning disorders and post-weaning feed intake are negatively related. To put the potential beneficial effects of the fatty acid composition of the weaner diet in perspective the final experiment was done. In an attempt to increase post-weaning feed intake, piglets were fed diets with increasing water contents. An increase in the water content of the diet was found to raise dry matter intake, total water intake and body-weight gain in a dose-dependent fashion. When the magnitude of the effect on feed intake seen in this study is compared with that in the previous studies using diets with different fatty acid compositions, it follows that the effect of PUFAs is relatively small.

Conclusions and implications

This thesis has focussed on the dietary provision of PUFAs to weanling piglets in relation to growth performance and small intestinal integrity. The status of n-3 and n-6 PUFAs in piglets at weaning might determine their susceptibility to the development of post-weaning disorders. In agreement with this statement, it was found that fish oil, which is rich in EPA, tended to have a positive effect on the clinical response in weanling piglets to a pathogenic *E. coli*. It is suggested that the status of n-3 PUFAs at weaning relates to the risk of post-weaning growth

depression and development of diarrhoea. The status of PUFAs at weaning is determined by the fatty acid status of the sow and the fatty acid composition of the weaner diet. Depending on the fatty acid status of the piglet at weaning, there may be no change of the status after weaning in spite of the low feed intake. The requirement of ALA by weanling piglets to display maximum growth is not known, but it may be above 0.22% of metabolizable energy. Dietary fish oil might positively affect growth of weanling piglets, with low status of n-3 PUFAs, this effect not being mediated by counteracting the weaning-induced decrease in villus height. However, the addition of fish oil to a weaner diet adequate in ALA and fed to weanling piglets with high status of n-3 PUFAs
may not enhance growth performance, faeces consistency and body condition. When put in perspective, it is concluded that the potential beneficial effect of the fatty acid composition of the weaner diet, at least under practical situations, may only be marginal and certainly is much smaller than that obtained by measures that raise post-weaning feed intake.

Samenvatting

Samenvatting

Dit proefschrift beschrijft onderzoek naar de voorziening met essentiële vetzuren van gespeende biggen. Gewervelde dieren hebben in de voeding de essentiële vetzuren linolzuur (LA, C18:2 n-6) en α-linoleenzuur (ALA, C18:3 n-3), de precursors van respectievelijk de n-6 en n-3 familie van meervoudig onverzadigde vetzuren, nodig. De producten van desaturatie en ketenverlenging, arachidonzuur (AA, C20:4 n-6) en eicosapentaeenzuur (EPA, C20:5 n-3) zijn de precursors voor eicosanoiden, die een belangrijke rol spelen in de immuunrespons. Eicosanoiden gevormd uit n-3 meervoudig onverzadigde vetzuren (PUFAs) hebben over het algemeen tegengestelde effecten van de eicosanoiden gesynthetiseerd uit n-6 PUFAs. Door de competitie tussen n-3 en n-6 PUFAs voor de desaturase en elongase enzymen, hangt de netto respons op eicosanoiden af van de in het voer aanwezige ratio tussen n-3 en n-6 PUFAs.

Gespeende biggen zijn gevoelig voor de ontwikkeling van het zogenaamde post-weaning syndroom wat samenhangt met een lage voeropname en gepaard gaat met villusatrofie, darmontsteking en verminderde prestaties. Om te onderzoeken wat de invloed was van PUFAs op het post-weaning syndroom, werden eerst de vetzuurvoorziening en status van biggen vanaf de geboorte tot twee weken na het spenen bepaald. Bovendien werden de vetzuursamenstelling van erytrocytenmembranen en het vet van de lever en lymfknopen bepaald. Het bleek dat er tussen het spenen en een week na het spenen geen verschil optrad in de n-3 en n-6 PUFAs status, gebaseerd op de concentraties in de erytrocytenmembranen. Het spenen ging gepaard met een terugval in plasma totaal cholesterol, HDL cholesterol en fosfolipidenconcentraties en met een afname van de door heparine vrijgemaakte plasma lipoproteine lipase activiteit. De verandering in plasmalipidenmetabolisme rond het spenen werd verklaard door de afname in vetopname direct na het spenen. Geconcludeerd werd dat dit onderzoek niet wijst op een verlaging van de n-3 en n-6 PUFAs status bij biggen rond het moment van spenen. Benadrukt werd echter dat de uitkomst van dit onderzoek bepaald werd door de essentiële vetzuurstatus van de zeug en de vetzuursamenstelling van het gebruikte commerciële lactatievoer, melkkorrel en speenvoer.

In een tweede onderzoek werd het effect van supplementatie met n-3 PUFAs en de n-3:n-6 verhouding op de morfologie van de dunne darm en op groeiprestatie onderzocht. Gespeende biggen (n=360) kregen voeders met verschillende ALA niveaus. Deze waren 0,22, 0,47, 0,77 en 1,13% van de metaboliseerbare energie. De experimentele voeders werden samengesteld door verschillende hoeveelheden lijnzaadolie uit te wisselen tegen maïsolie. Opname van ALA boven 0,22 energie% leek de groei te verbeteren gedurende de eerste

twee weken na het spenen en leek de voederconversie in de eerste week te verlagen. De gemiddelde gewichtstoename was 9% hoger en voederconversie was 14% lager. Toenemende hoeveelheden ALA in het voer verhoogde middels de desaturatie en ketenverlenging de vorming van EPA en docosahexaeenzuur (DHA, C22:6 n-3) en de incorporatie van deze vetzuren in de erytrocytenmembranen. De behoefte van gespeende biggen aan ALA voor een maximale groei is niet bekend, maar dit onderzoek maakt het aannemelijk dat het meer dan 0,22 energie% zou moeten zijn. Bij de biggen daalde het totaal cholesterol, HDL cholesterol en fosfolipiden na het spenen, maar de opname van verschillende hoeveelheden lijnzaadolie had geen invloed op de plasmalipidenconcentraties.

In het derde experiment werd nagegaan of bij gespeende biggen het voeren van EPA, in de vorm van visolie, beter zou zijn voor de groeiprestaties en darmintegriteit dan het voeren van ALA in de vorm van lijnzaadolie. Speenvoeders werden samengesteld zodat er twee niveaus waren van zowel visolie als lijnzaadolie. De voeders met visolie verhoogden de gemiddelde groei na het spenen met 27% in vergelijking met de voeders met lijnzaadolie, maar deze toename was niet statistisch significant. De voeropname werd niet beïnvloed door de experimentele voeders. Er was geen systematische invloed van de voeding op de villus: crypt verhouding van de mucosa van de dunne darm. De hoogste villus:crypt verhouding werd gezien bij het controle voer met een n-3:n-6 verhoudig van 0,1 en de laagste verhouding werd gevonden bij de biggen gevoerd met het voer met lijnzaad en een n-3:n-6 verhouding van 0,3. De voeders met visolie gaven hogere n-3:n-6 verhoudingen in de erytrocyten, het vet van de lever, lichaamsvet en vet van lymfknopen dan de voeders met lijnzaadolie met dezelfde n-3:n-6 verhouding. Geconcludeerd werd dat visolie in de voeding mogelijk een positief effect heeft op de groei van gespeende biggen. Dit effect werd niet veroorzaakt door het tegengaan van de door het spenen geïnduceerde afname van de villushoogte.

Om verder te onderzoeken of de opname van visolie positieve effecten op de groeiprestatie van gespeende biggen had, kregen 480 biggen in een voedingsproef voeders zonder visolie of met 13 of 22 g visolie/kg. Visolie werd uitgewisseld tegen maïsolie. De voeders werden ad libitum gevoerd vanaf tot 14 dagen na het spenen. Het voeren van visolie beïnvloedde noch de voeropname noch de groei en de voederconversie. De vetzuursamenstelling van de erytrocytenmembranen weerspiegelden de opname van visolie en wezen op remming van de desaturatie en ketenverlenging van LA bij het voeren met visolie. Biggen gevoerd met voeders met visolie hadden hogere EPA en lagere AA concentraties in de erytrocyten, terwijl de hoeveelheid LA niet beïnvloed werd. Geconcludeerd werd dat onder de omstandigheden van dit onderzoek, de toevoeging van visolie aan speenvoeders met een adequate hoeveelheid ALA, de

groeiprestaties, faecesconsistentie en lichaamsconditie van gespeende biggen niet verbeterden. De biggen hadden echter op het moment van het spenen reeds een hoge n-3 PUFAs status, wat een effect van visolie in het speenvoer op de groeiprestatie gemaskeerd kan hebben.

In het vijfde onderzoek werden bij gespeende biggen het effect van visolie in het voer op de klinische respons na een infectie met een pathogene *E. coli* O149:K91:K88 onderzocht. De biggen werden verdeeld in twee groepen van elk 8 dieren. Eén groep kreeg het controlevoer met 5% maisolie. De testgroep kregen een voer met 0,5% maïsolie en 4.5% visolie. De biggen werden oraal geïnfecteerd met een challengestam op dag 6 en 7 na het spenen. Gedurende de onderzoeksperiode van 14 dagen bleven alle biggen in leven. Voeropname en groei, faeces- en conditiescores werden dagelijks bepaald. Faecesmonsters werden verzameld voor bacteriologisch onderzoek. Bloedmonsters werden genomen voor de analyse van de vetzuursamenstelling van de erytrocytenmembranen. De gemiddelde dagelijkse voeropname na infectie leek hoger te zijn voor de test dan voor de controlegroep. De uitscheiding van O149:K91:K88 in de faeces was lager bij de testgroep dan bij de controlegroep. Dit onderzoekt wees op een mogelijk positief effect van visolie op de klinische respons van gespeende biggen op een pathogene *E. coli* besmetting. De uitkomst van dit onderzoek was niet in overeenstemming met het tweede voedingsonderzoek met voeders aangevuld met visolie, dat geen effect van visolie op groeiprestaties liet zien. De biggen in dat voedingsonderzoek werden in een relatief schone omgeving gehouden, wat mogelijk het gebrek aan effect van visolie verklaarde.

De beschreven onderzoeken leken er op te wijzen dat toevoeging van n-3 PUFAs aan speenvoeders mogelijk zinvol is, maar alleen als de biggen een lage n-3 PUFAs status hebben op het moment van spenen. Daarom werd een literatuurstudie gedaan om de factoren die van invloed zijn op de vetzuurstatus op het moment van spenen te indentificeren. De vetzuursamenstelling van door de zeug gemobiliseerd vet en dat van het lactatievoer beïnvloeden de vetzuursamenstelling van de zeugenmelk, welke vervolgens de vetzuurstatus van biggen op het moment van spenen bepaalt.

Het risico op de ontwikkeling van post-weaning problemen en de voeropname na het spenen zijn negatief gerelateerd. Om de potentiële goede effecten van de vetzuursamenstelling van speenvoeders in perspectief te plaatsen, werd een laatste onderzoek uitgevoerd. In een poging om de voeropname na het spenen te verhogen, kregen biggen voeders met toenemende watergehaltes. Een verhoging van het watergehalte in de voeding verhoogde de droge stof opname, totale wateropname en groei dosisafhankelijk. Wanneer de grootte van het effect op voeropname zoals gemeten in dit onderzoek vergeleken werd met dat in voorgaande onderzoeken waarin voeders gebruikt werden met verschillende

vetzuursamenstellingen, kan geconcludeerd worden dat het effect van PUFAs relatief klein is.

Conclusies en implicaties

Dit proefschrift richtte zich met name op de PUFA voorziening aan gespeende biggen in relatie tot groeiprestatie en integriteit van de dunne darm. De n-3 en n-6 PUFAs status van biggen op het moment van spenen kan hun gevoeligheid voor het ontwikkelen van problemen na het spenen beïnvloeden. In overeenstemming met deze uitspraak werd gevonden dat visolie, wat rijk is aan EPA, een positief effect leek te hebben op de klinische respons van gespeende biggen op een pathogene *E. coli* besmetting. Gesuggereerd wordt dat de n-3 PUFAs status op het moment van spenen cruciaal is voor het risico op groeiafname en het ontwikkelen van diarrhea. Deze PUFAs status op het moment van spenen wordt bepaald door de vetzuurstatus van de zeug en de vetzuursamenstelling van het speenvoer. Afhankelijk van de vetzuurstatus van de biggen op het moment van spenen, hoeft er geen verandering van status na het spenen te zijn, ondanks de lage voeropname. De behoefte aan ALA bij gespeende biggen voor maximale groei is niet bekend, maar zal waarschijnlijk boven 0,22% metaboliseerbare energie liggen. Visolie in het voer kan mogelijk positieve effecten hebben op de groei van gespeende biggen met een lage n-3 PUFAs status, welk effect niet veroorzaakt wordt door het tegengaan van de door het spenen geïnduceerde afname van de villushoogte. De toevoeging van visolie aan speenvoer met adequate hoeveelheden ALA geeft waarschijnlijk geen verbetering van groeiprestatie, faecesconsistentie en lichaamsconditie. Geconcludeerd wordt dat de potentiële gunstige effecten van de vetzuursamenstelling van het speenvoer onder praktijkomstandigheden slechts marginaal is en veel kleiner dan methodes die de voeropname na het spenen verhogen.

Curriculum vitae

Curriculum vitae

Anneke Beatrix Schellingerhout is op 9 januari 1970 geboren te Koudekerk aan den Rijn, waar zij ook opgroeide. In 1988 behaalde zij het VWO diploma aan het Christelijk Lyceum te Alphen aan den Rijn en begon zij met de studie Diergeneeskunde aan de Universiteit Utrecht. Na haar afstuderen in 1997 was zij korte tijd werkzaam als docente in het Middelbaar Agrarisch Onderwijs. Sinds november 1997 werkte zij als beursaal en later als assistent in opleiding bij de Afdeling Voeding van de Faculteit Diergeneeskunde, waar het onderzoek beschreven in dit proefschrift werd verricht.
Sinds 1998 heeft Anneke een dierenadviesbureau waarin de holistische behandeling van dieren centraal staat. Daarnaast geeft zij voorlichting en lezingen over de diverse aspecten van holistisch werken. Na haar promotie zal zij met Aernout Dousma haar werkzaamheden voortzetten binnen Jyoti, holistische praktijk voor mens en dier.