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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