Journal of Pre-Clinical and Clinical Research, 2012, Vol 6, No 2, 98-104
www.jpccr.eu

ORIGINAL ARTICLE

The effect of fish oil supplementation of pigs maternal diet during pregnancy and lactation, and the effect of fish oil addition to formula milk on gene expression and fatty acids composition in small intestine, liver and muscle of offspring

Katarzyna Gaca, Maciej Firląg, Bożena Bałasińska

Department of Physiological Science, Faculty of Veterinary Medicine, Warsaw University of Live Science, Warsaw, Poland

Gaca K, Firląg M, Bałasińska B. The effect of fish oil supplementation of pigs maternal diet during pregnancy and lactation, and the effect of fish oil addition to formula milk on gene expression and fatty acids composition in small intestine, liver and muscle of offspring. J Pre-Clin Clin Res. 2012; 6(2):98-104.

Abstract

Introduction: The supplementation of preterm infant formulas with n-3 fatty acids is now well accepted, while the benefits of adding n-3 fatty acids to formula milk for term infants remains controversial.

Objective: To investigate the effect of fish oil supplementation of maternal diet during pregnancy and lactation, and the effect of fish oil addition to formula milk on gene expression and fatty acids composition in the small intestine, liver, and quadriceps femoris muscle of offspring in the first month of life.

Materials and method: Four groups of piglets were used: PC – piglets fed milk from control sows from birth for 28 days, PS – piglets fed milk from sows supplemented with 10 mL/d menhaden oil from birth for 28 days, PF – piglets fed formula from control sows from day 7 after birth for 28 days, and PFS – piglets fed formula with 1 mL/d menhaden oil from control sows from day 7 after birth for 28 days. On day 28, all piglets were slaughtered and samples of plasma, small intestine, liver and muscle were collected.

Results: The results provided evidence that maternal dietary supplementation with fish oil significantly increased the expression of PPARα and FABP genes involved in fatty acids transport and metabolism, in comparison to the levels observed in offspring fed the supplemented formula milk.

Conclusions: The differences in genes' expression, as well as n-3 LP-PUFA concentration in plasma and tissue between PS and PFS groups of piglets were insignificant. We suggest that fish oil supplementation of formula milk for term infants constitutes a sufficient way to increase piglets' plasma and tissue concentrations of n-3 fatty acids, producing a similar effect to maternal diet supplementation with these fatty acids.

Key words

fish oil, piglets, formula milk, PUFA

INTRODUCTION

Various studies have demonstrated a significant effect of maternal diet composition on the pre- and post-natal development of offspring [1]. The appropriate nutrition of children in the prenatal and postnatal periods may have an impact on health status in adult life. However, nutrients involved in foetal 'metabolic programming' are not yet known. Therefore, identifying the possible involvement of specific nutritional components is an important step in the preparation of a diet for infants.

Long-chain polyunsaturated fatty acids (LC-PUFA) belong to the macronutrients required for the proper development of a growing foetus. LC-PUFA maintain fluidity and permeability of cell membranes, are ligands for receptors, and transcription factors that regulate gene expression, and constitute precursors of important bioactive compounds [2]. Adverse amounts of fatty acid during foetal and infant development alter fatty acid composition of tissue lipids, which may cause changes in cell structure and functions, particularly in tissues such as the brain, where lipids contribute more than 50% of the dry weight [3]. Large cohort studies have shown that the children of women with low n-3 fatty acids intakes during pregnancy are at increased risk of poor cognitive and behavioral outcome, as well as lower visual acuity [4].

Mammalian tissues, especially the liver, intestine and brain may synthesize long-chain polyunsaturated fatty acids by the reactions of desaturation and elongation of shorter essential fatty acids (EFAs), although in infants the activity of desaturase is low and therefore unable to supply sufficient PUFA to meet their demand [5]. Thus, to obtain an adequate amount of DHA and EPA, the growing neonate receives EPA and DHA via breast milk. Studies have shown that maternal dietary intakes of n-3 fatty acids differ, and this leads to a wide range of n-3 fatty acid levels in milk. Many authors have shown that the diet of lactating women is of essential importance for providing a sufficient amount of essential fatty acids to meet the needs of the mother, required for the support of optimal growth and development of the infant [6].

Address for correspondence: Katarzyna Gaca, Department of Physiological Science, Faculty of Veterinary Medicine, Warsaw University of Live Science, Nowoursynowska 161E, 02-787 Warszawa, Poland
E-mail: kasia_wojcieszak@wp.pl

Received: 20 October 2012; accepted: 19 December 2012

The highest foetal accretion of LC-PUFA occurs during the last trimester of gestation; therefore, the administration of these fatty acids seems to be important at this time. This hypothesis is supported by the results of studies showing that premature infants are born with minimal LC-PUFA reserves [7]. Many reports also indicate that premature infants fed formula milk, compared with maternal milk, have a lower level of (n-3) PUFA in plasma, which is associated with a lower neural maturation in infancy and later childhood [8]. These findings formed the basis for the suggestion that formulas may not support optimal rates of essential fatty acids. Therefore, LC-PUFA enrichment of formulas for premature infants is desirable, but for term infants remains controversial. Available nutritional requirements for term infants do not provide any specific recommendations concerning the amount and ideal source of n-3 fatty acids added to the formula.

In recent years, the direct involvement of n-3 fatty acids in the regulation of gene expression has been established. *In vitro* binding and cell culture studies have identified many transcription factors, including peroxisome proliferator-activated receptors (PPARs), sterol regulatory element binding proteins (SREBPs), fatty acid binding proteins (FABPs), among others. FABPs are a family of carrier proteins for fatty acids and other lipophilic substances. PPARα is expressed in tissues with high rates of fatty acid oxidation, such as the liver and skeletal muscle, and its physiological role is to up-regulate genes involved in all aspects of fatty acid catabolism. SREBPs are a major group of transcription factors that control the expression of genes involved in cholesterol biosynthesis and uptake of fatty acid, as well as their biosynthesis [9].

Nowadays, research aimed at the improvement of the quality of formula milk is not necessarily focused on mimicking the exact composition of human milk, but rather on achieving the functional effects that are observed in breastfed infants. Therefore, the purpose of the presented study was to compare the effect of fish oil supplementation of maternal diet during pregnancy and lactation, and the effect of fish oils addition to milk formula for term infants on mRNA expression of genes involved in fatty acids metabolism in the small intestine, liver, and quadriceps femoris muscle of the offspring in the first month of life.

The intestinal epithelial cells were chosen for analysis since this is the first tissue exposed to the fatty acids from milk; the liver is the organ to which fatty acids are delivered from the blood as lipoprotein and are utilized or re-synthesized, whereas muscles constitute the recipient of the majority of fatty acids assigned through the liver. Due to the similarities in the fatty acids metabolism in humans and pigs, and for obvious ethical reasons, a piglet model of such treatments has been successfully developed in this study.

MATERIALS AND METHOD

Animals. Twelve nulliparous pregnant sows were used in this study (Polish Landrace x Pietrain,). On day 80 of pregnancy all sows were assigned into two groups: control and fish oil-supplemented. Control animals were fed a standard diet for pregnant sows (DM 87.6%, ME 11.35MJ/kg, CP 13.1%), and during lactation the diet was adjusted to the energy requirements for females (DM 87.3%, ME 12.93MJ/kg, CP 15.4%). During the experimental period, all sows from the supplemented group received the same standard diet to which 10 ml of menhaden oil was added as a top-dressing. Animals had free access to water during the experimental period.

Twelve sow-milk-fed piglets, each from a different litter (PC – piglets from control sows, PS – piglets from supplemented sows), were kept with their mothers and fed colostrum and milk until day 28 of age. The formula-fed piglets were taken from the control sow (two piglets of average body weight) on day 7 after birth and fed the formula until day 28. One piglet from each pair was assigned to one of the two groups – PF and PFS. These piglets were fed the same formula milk, but PFS piglets additionally received 1 ml/d of fish oil directly into the mouth. Piglets were housed together in a light-dark cycle controlled room, with free access to water and fed by bottle (fresh formula milk was prepared daily and refrigerated) twice a day (08:00 and 16:00) for a 21-day period.

Milk samples were taken from sows on day 28 of lactation. The samples were collected before feeding, following intramuscular administration of 2 mL oxytocin (10 IU/ml) and stored at -20°C. On the same day, sow-milk-fed piglets and formula-fed piglets were anaesthetized and 5 mL blood collected by cardiac puncture, with 15% EDTA/L in 9g/L NaCl/L as the anticoagulant. Piglets were immediately euthanized by intracardiac injection of 0.4mL/kg morbital. The small intestine, liver and quadriceps femoris muscle were immediately removed and frozen at -80°C. Plasma was obtained by blood centrifugation at 2,000g for 10 min and stored at -20°C before fatty acids analysis.

The protocol of the presented study was approved by the Local Ethical Committee in Warsaw.

Fatty acids analysis. Total lipids in menhaden oil, milk, formula milk, serum and tissues were extracted using a chloroform/methanol mixture (2:1 v/v), according to the Folch method [10]. 200 µl of plasma, menhaden oil or milk was extracted with 6 ml of chloroform/methanol mixture. Tissues (100 mg) were earlier homogenized with 6 ml of the mixture. After extractions, NaCl aqueous solution (0.9%) was added to organic fractions. The chloroform phase was then filtered through anhydrous magnesium sulphate and evaporated to dryness. Next, fat extract was converted to fatty acids methyl esters, determined by Perkin-Elmer gas chromatograph. The preparation of fatty acids esters comprised of hot saponification with 1 mL of 3 M KOH in MeOH, for 35 min at 85°C, followed by methylation of fatty acids in 1 mL of 0,5M HCl in MeOH for 45 min at 85°C in Teflon-lined screw-capped vials. Fatty acids methyl esters (FAME) were separated on a 100 m × 0.25 mm capillary column filled with 0.2 µm C-Sil 88 stationary phase gas chromatograph equipped with an autosampler and flame isolation detector. Column temperature was kept at 170°C for the first 42 min, then raised to 240°C at the rate of 5°C/min, and finally maintained at this temperature for 31 min. Identification of peaks corresponding to FAME was accomplished by means of a standard mixture of 37 FAMEs (Supelco, Belafonte USA).

RT-PCR analysis. Total RNA was prepared from a tissue sample (100 mg) using Total RNA kit (A&A Biotechnology, Poland), according to the manufacturer's protocol. RNA quantity was measured using a NanoDrop ND-1,000 Spectrophotometer (NanoDrop Technologies, Wilmington,

DE, USA), and quality examined using a RNA 6,000 Nano LabChip® Kit on 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). cDNA was prepared from 1 µg of total RNA by reverse transcription using Transcriptor First Stand cDNA Synthesis Kit (Roche Diagnostic, Warsaw, Poland). Reactions were incubated at 50°C for 60 min, and at 85°C for 5 min to inactivate the reverse transcriptase.

The mRNA expression level of PPARα (peroxisome proliferator-activated receptor α), FABP-1 (fatty acid binding protein 1), and SREBP (sterol regulating element binding protein) genes were analyzed by real-time PCR, using Light Cycler FastStart DNA Master SYBR Green I kit (Roche Applied Science, Basel, Switzerland). Each PCR mixture, with a final volume of 10 µl, contained 0.5 µM of each gene-specific primer (Tab. 2), and 1 µl cDNA in each reaction. The mRNA levels of genes were normalized according the mRNA level of the b-actin RNA. Mixtures were incubated in a LightCycler termocycler (Roche Diagnostic, Warsaw, Poland). Quantitation of the transcripts was performed using a standard curve with 10-fold serial dilutions of cDNA. A melting curve was constructed to ensure that only a single PCR product was amplified. Samples were assayed in triplicate, and each experiment was repeated at least twice.

Statistical analysis. Data are expressed as means ± standard deviation. Statistical analysis was performed by one-way ANOVA. When significant differences were found, a Tukey test was performed at $P \leq 0.05$.

RESULTS

Profiles of fatty acids in menhaden oil, milk from control and supplemented sows, and formula milk are shown in Table 1.

Table 1. Fatty acid composition (% of total fatty acids) of menhaden oil, milk from control and supplemented sows and formula milk used in the experiment

Fatty acid	Menhaden oil	Milk from control sows	Milk from supplemented sows	Formula milk
C14:0	8.43	4.99	3.23	4.62
C16:0	15.63	30.19	31.48	16.49
C16:1	11.32	9.83	7.03	0.22
C18:0	2.91	4.1	5.63	5.2
C18:1	10.46	27.45	29.09	26.72
C18:2 n-6 (LA)	1.2	15.71	13.47	17.36
C18:3 n-3 (ALA)	1.24	1.26	1.13	1.2
C20:4 n-6 (AA)	1.13	0.55	0.26	nd.
C20:5 n-3 (EPA)	6.54	0.21	1.38	nd.
C22:6 n-3 (DHA)	4.32	0.13	1.23	nd.
∑ n-3	12.1	1.6	3.74	1.2
∑ n-6	2.33	16.26	13.73	17.36

nd. – not detected.
Milk samples from sows were collected on 28 days of lactation.

Table 2. Specific primers used for real-time PCR

Gene Name	Sequence	Ta (°C)	Product size (bp)
PPARα	5′ CCAGTATTGTCGTTTCCACA 3′	59	139
FABP-1	5′ CAGGAAAGTCAAGAGCACCA 3′	60	227
SREBP-1	5′ GAGGCGAAGCTGAATAAATC 3′	61	133

INFLUENCE OF PIGLETS' DIET ON FATTY ACIDS COMPOSITON IN TISSUES

Plasma. The fatty acids composition of piglets' plasma in the 4 experimental groups is presented in Table 3. Fish oil in the diet of sows and formula milk altered the n-3 and n-6 fatty acids content in the plasma of piglets from groups PS and PFS, compared with the content observed in piglets from control sows (PC) and piglets receiving formula milk without fish oil (PF). Concentration of α-linolenic acid (18:3 n-3; ALA) was 4 times higher in plasma of PS piglets than in piglets fed formula milk and receiving fish oil (PFS). Piglets from PFS group had a significantly higher EPA and DHA content in comparison to the piglets fed formula milk without fish oil (PF), and piglets from control sows (PC). In addition, concentration of EPA increased in plasma of PS piglets compared with PC and PF piglets. In the case of long chain n-6 fatty acids, administration of fish oil to the sows diet and formula milk resulted in significant differences in the concentration of arachidonic acid (20:4 n-6; AA) in plasma of all piglets from these experimental groups – PS and PFS. No significant changes were observed in linoleic acid (18:2 n-6; LA) content in plasma of PS vs. PFS group; however, comparison of LA concentration between these 2 groups and the other experimental groups of piglets (PC and PF) showed that the diets induced significant changes in these fatty acids.

Table 3. Fatty acids composition (% of total fatty acids) of total lipids in plasma of piglets receiving milk from sows (PC and PS) and piglets receiving formula milk (PF and PFS)

	Plasma			
Fatty acid	PC	PS	PF	PFS
C14:0	1.83±0.56	1.58±0.27	1.63±0.15	2.05±0.44
C16:0	31.02±1.01^{bc}	29.53±1.49^{ab}	28.75±0.51^{a}	32.48±0.37^{c}
C16:1	3.86±0.37^{a}	3.86±0.34^{a}	1.55±0.03^{b}	1.86±0.02^{b}
C18:0	15.11±1.53^{a}	15.64±1.57^{a}	26.18±0.68^{b}	24.36±0.6^{b}
C18:1	18.78±0.88^{a}	18.97±2.93^{a}	14.91±0.85^{b}	16.19±0.92^{ab}
C18:2 n-6 (LA)	17.36±0.71^{a}	15.43±0.61^{b}	18.73±0.22^{c}	15.33±0.25^{b}
C18:3 n-3 (ALA)	0.81±0.16^{ab}	0.91±0.07^{a}	nd.	0.24±0.02^{b}
C20:4 n-6 (AA)	4.42±0.92^{a}	2.16±0.42^{b}	14.31±0.09^{c}	12.48±0.07^{d}
C20:5 n-3 (EPA)	2.72±0.66^{a}	4.37±0.59^{b}	2.5±0.23^{a}	4.71±0.27^{b}
C22:6 n-3 (DHA)	0.16±0.02^{a}	0.33±0.18^{ab}	0.14±0.01^{a}	0.46±0.05^{b}
∑ n-3	3.69	5.61	2.64	5.41
∑ n-6	21.78	17.59	33.04	27.81

Values for fatty acids are expressed as the percentage of total fatty acids (mean ± S.D.).
a, b, c, d – means in a row with different letters are significantly different; $P \leq 0.05$.
nd – not detected; PC – piglets fed milk from control sows; PS – piglets fed milk from supplemented sows; PF – piglets fed formula milk; PFS – piglets fed formula milk and additionally received 1 ml/d fish oil.

Small intestine. The relative fatty acids content in the small intestine of piglets from the different experimental groups is shown in Table 4. ALA and EPA content in the intestinal epithelium of piglets from PS group was higher than in piglets from PC and PF groups. The ALA and EPA concentration also increased in the small intestine of piglets from PFS group, compared with PF and PC, but EPA content only was higher in PFS group vs. PS group. The amount of DHA increased in PFS piglets compared with PC piglets. With regard to long chain n-6 fatty acids, no significant difference was observed in the concentration of LA in piglets' small intestine. The AA content decreased in PFS group of piglets when compared with PF. In addition, the concentration of AA was lower in the small intestine of PS piglets than in PC and PF piglets.

Table 4. Fatty acids composition (% of total fatty acids) of total lipids in small intestine of piglets receiving milk from sows (PC and PS) and piglets receiving formula milk (PF and PFS)

	Small intestine			
Fatty acid	PC	PS	PF	PFS
C14:0	2.31±0.5	2.65±0.62	2.08±0.18	2.95±0.59
C16:0	28.59±1.72^{a}	31.32±1.3^{b}	28.41±0.29^{a}	29.99±0.39^{ab}
C16:1	5.04±0.8^{a}	3.44±1.1^{b}	1.15±0.07^{c}	0.77±0.04^{c}
C18:0	12.5±1.89^{a}	14.33±0.89^{b}	16.22±0.56^{c}	12.31±0.25^{a}
C18:1	19.05±0.85	20.58±2.44	21.07±0.58	21.68±0.46
C18:2 n-6 (LA)	15.43±1.74	15.22±1.99	18.31±0.46	16.63±1.34
C18:3 n-3 (ALA)	0.23±0.09^{a}	0.53±0.14^{b}	0.22±0.02^{a}	0.49±0.02^{b}
C20:4 n-6 (AA)	6.32±0.51^{a}	4.02±0.57^{b}	8.1±0.23^{c}	6.47±0.45^{d}
C20:5 n-3 (EPA)	0.28±0.1^{a}	1.45±0.11^{b}	0.3±0.02^{a}	1.98±0.05^{c}
C22:6 n-3 (DHA)	0.13±0.02^{a}	0.33±0.08^{ac}	0.19±0.03^{ac}	0.26±0.04^{bc}
∑ n-3	0.64	2.31	0.71	2.73
∑ n-6	21.75	19.24	26.41	23.1

Values for fatty acids are expressed as the percentage of total fatty acids (mean ± S.D.).
a, b, c, d - means in a row with different letters are significantly different; P ≤ 0.05.
PC - piglets fed milk from control sows; PS - piglets fed milk from supplemented sows;
PF - piglets fed formula milk; PFS - piglets fed formula milk and additionally received 1 ml/d fish oil.

Liver. The fatty acids profiles in piglets' liver samples are shown in Table 5. The results demonstrated that PFS piglets had increased ALA content in the liver when compared to PF, PC and PS groups. Piglets from PS group had significantly higher EPA and DHA concentration than piglets from PC and PF group. The EPA content was elevated in the liver of PFS piglets when compared with PF, PC, as well as PS piglets. In the case of DHA content, animals from PFS group had a higher concentration of this fatty acid in the liver than PF piglets, whereas comparison of this experimental group with PS piglets showed that the content was lower. Increased n-3 fatty acids concentration in piglets' liver was accompanied by a decreased level of n-6 fatty acids. The LA concentration was lower in the liver samples of PFS piglets, compared with piglets from supplemented and control sows (PS and PC). Also, the AA content was decreased in piglets from PFS group when compared with the piglets from PC and PF groups. In piglets from PS group, the amount of AA was lower than in piglets from PC group.

Muscle. The fatty acids composition of piglet muscle in the 4 experimental groups is presented in Table 6. In the case of muscle samples, PS piglets showed a higher ALA content than PC and PF piglets. In addition, the concentration of ALA increased in piglets from PFS group, compared with piglets from PF and PC groups. With regard to the EPA content, there was a different response depending on the type of diet. Fish oil added to the diet of sows, and used as an addition to the formula milk for offspring, caused the appearance of DHA, although no significant differences between groups of piglets were observed. In piglets' muscle, the diets enriched with fish oil resulted in a significant decrease in n-6 fatty acids concentrations. Piglets from PF group had a higher level of LA than piglets from PFS, PC, and PS groups. The AA concentration was lower in piglets fed supplemented formula milk than in piglets from PF, PC, and PS groups. Also, in muscle of PS piglets the amount of AA decreased when compared with PC and PF piglets.

Table 5. Fatty acids composition (% of total fatty acids) of total lipids in liver of piglets receiving milk from sows (PC and PS) and piglets receiving formula milk (PF and PFS)

	Liver			
Fatty acid	PC	PS	PF	PFS
C14:0	1.05±0.15^{a}	1.05±0.33^{a}	1.36±0.17^{ab}	1.64±0.18^{b}
C16:0	20.84±0.61^{a}	22.05±1.9^{ab}	26.51±1.49^{a}	23.16±1.76^{c}
C16:1	3.37±0.45^{a}	2.93±0.45^{a}	1.7±0.13^{b}	2.06±0.36^{b}
C18:0	16.5±0.7^{a}	18.83±1.78^{b}	16.25±0.72^{a}	14.21±0.25^{a}
C18:1	10.09±0.86^{a}	11.86±1.51^{a}	17.82±0.56^{b}	18.31±0.33^{b}
C18:2 n-6 (LA)	16.74±1.32^{a}	15.94±1.46^{a}	14.7±1.26^{ab}	12.43±0.43^{b}
C18:3 n-3 (ALA)	0.4±0.01^{a}	0.45±0.02^{a}	0.53±0.05^{a}	0.75±0.04^{b}
C20:4 n-6 (AA)	18.76±0.68^{a}	15.32±1.63^{bc}	16.97±1.25^{ab}	13.00±2.15^{c}
C20:5 n-3 (EPA)	0.4±0.13^{a}	3.17±1.11^{b}	0.55±0.04^{a}	5.37±0.08^{c}
C22:6 n-3 (DHA)	4.58±0.53^{a}	7.38±1.29^{b}	0.5±0.11^{c}	5.14±0.11^{a}
∑ n-3	5.38	11.0	1.58	11.26
∑ n-6	35.5	31.26	31.67	25.43

Values for fatty acids are expressed as the percentage of total fatty acids (mean ± S.D.).
a, b, c, d – means in a row with different letters are significantly different; P ≤ 0.05.
PC – piglets fed milk from control sows; PS – piglets fed milk from supplemented sows; PF – piglets fed formula milk; PFS – piglets fed formula milk and additionally received 1 ml/d fish oil.

Table 6. Fatty acids composition (% of total fatty acids) of total lipids in muscle of piglets receiving milk from sows (PC and PS) and piglets receiving formula milk (PF and PFS)

	Muscle			
Fatty acid	PC	PS	PF	PFS
C14:0	2.12±0.64	1.69±0.26	1.52±0.01	2.2±0.11
C16:0	18.68±1.67^{a}	16.76±1.19^{ab}	15.88±1.04^{b}	14.92±0.83^{b}
C16:1	4.99±1.18^{a}	3.46±0.54^{bc}	4.46±0.35^{ab}	2.59±0.63^{c}
C18:0	4.62±0.54^{a}	4.04±0.75^{ab}	6.04±0.24^{c}	5.3±0.14^{bc}
C18:1	11.44±2.18	11.8±4.91	11.94±0.51	11.94±0.51
C18:2 n-6 (LA)	9.1±1.25^{a}	9.42±0.64^{a}	11.7±0.46^{b}	8.51±0.22^{a}
C18:3 n-3 (ALA)	0.24±0.02^{a}	0.45±0.05^{b}	0.22±0.02^{a}	0.39±0.02^{b}
C20:4 n-6 (AA)	2.42±0.06^{a}	1.69±0.17^{b}	2.35±0.13^{a}	1.15±0.14^{c}
C20:5 n-3 (EPA)	0.25±0.01^{a}	0.35±0.04^{b}	0.14±0.02^{c}	0.45±0.02^{d}
C22:6 n-3 (DHA)	nd	0.15±0.07^{a}	nd	0.17±0.01^{a}
∑ n-3	0.49	0.95	0.36	1.01
∑ n-6	11.52	11.11	14.05	9.66

Values for fatty acids are expressed as the percentage of total fatty acids (mean ± S.D.).
a, b, c, d – means in a row with different letters are significantly different; P ≤ 0.05.
nd – not detected; PC – piglets fed milk from control sows; PS – piglets fed milk from supplemented sows; PF – piglets fed formula milk; PFS – piglets fed formula milk and additionally received 1 ml/d fish oil.

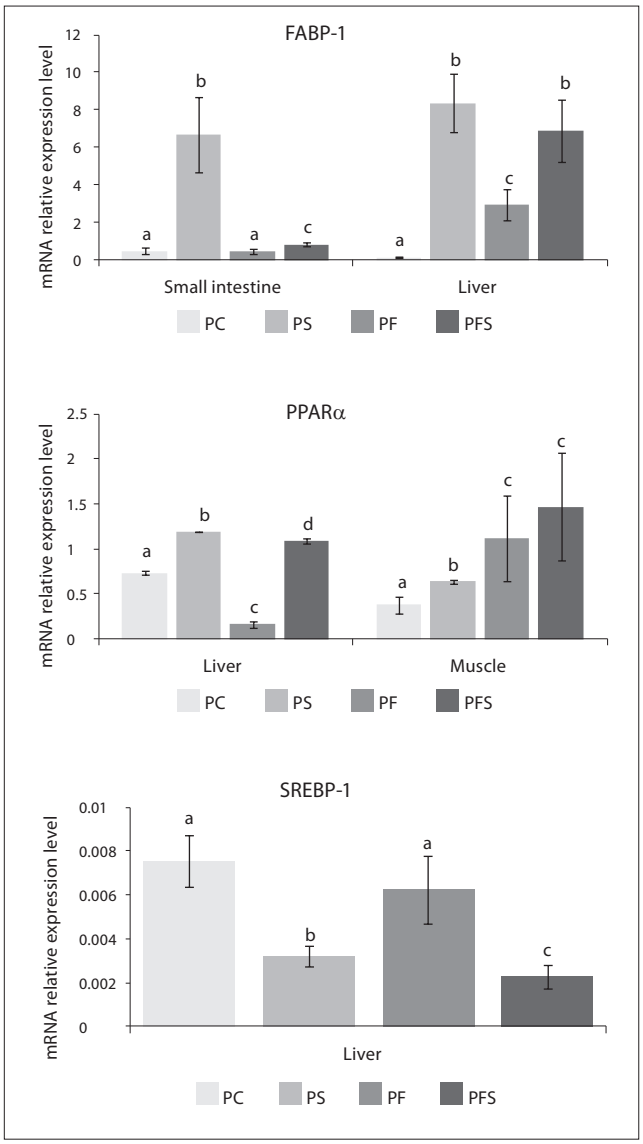

Figure 1. Relative mRNA abundance of selected genes in small intestine, liver and muscle of pigs.

a, b, c, d – means in a row with different letters are significantly different; $P \leq 0.05$. PC – piglets fed milk from control sows; PS – piglets fed milk from supplemented sows; PF – piglets fed formula milk; PFS – piglets fed formula milk and additionally received 1 ml/d fish oil.

Influence of piglets diet on mRNA abundance of genes. Fish oil in the diet of sows and formula milk increased levels of PPARα and FABP-1 mRNA in piglets' liver (PS and PFS), compared with the liver samples of piglets from PC group (fed by control sows) and piglets receiving formula milk without fish oil (PF). However, the PS and PFS piglets showed reduced mRNA expression of SREBP-1 in the liver. The highest level of PPARα and FABP-1 mRNA expression was in the liver of piglets from PS group, whereas PFS piglets showed the lowest expression of SREBP-1 in this tissue. In piglets receiving the formula milk addition of fish oil did not significantly affect mRNA expression of PPARα gene in muscle; however, the level of this gene was higher compared with the samples from piglets receiving sows' milk. In the small intestine, the FABP-1 mRNA expression increased in PS and PFS groups, compared with PC and PF, but between PS and PFS there were no significant differences.

DISCUSSION

Breast-feeding is widely regarded as the golden standard of infant nutrition, but very often and for different reasons, term infants must be fed formula milk. Human milk naturally contains n-3 LCPUFA (long chain polyunsaturated fatty acids), which are absent from many infant formulas. Nowadays, the supplementation of preterm infants' formulas with n-3 fatty acids is well accepted, while the benefits of adding n-3 fatty to formula for term infants remains controversial. For this reason, the objective of the presented research was to compare the effect of supplementation of the maternal diet with fish oil during pregnancy and lactation, as well as the influence of its addition to milk formula for term infants on mRNA expression genes involved in fatty acids metabolism, and fatty acids concentration in the small intestine, liver, and quadriceps femoris muscle of the offspring.

The small intestine is the first place exposed to the fatty acids from milk or formula. Because the small intestine is the main place where fatty acids are digested and absorbed, the signaling pathways for fatty acids absorption and metabolism in the intestinal epithelial cells during the first month of life are thought to be important for development. Several studies have shown that genes involved in fatty acids absorption are highly expressed in the small intestine during the suckling-weaning period [11]. A number of experimental approaches have demonstrated that fatty acid binding proteins of both liver-type (FABP-1) and intestine-type (FABP-2), which mediate fatty acids absorption from lumen to enterocyte, are inducted by dietary fat [12]. Moreover, structural studies clearly demonstrated that most FABPs bind long-chain fatty acids (C16-C22) with high affinity, but FABP-1 also binds other acyl ligands [13].

The presented experiment demonstrates that piglets fed by sows supplemented with fish oil (PS) compared with piglets fed supplemented formula milk (PFS) had higher mRNA FABP-1 gene level in the small intestine. These findings are not convergent with the results of n-3 LC PUFA concentration in the small intestine. Despite similar concentration of EPA and DHA in plasma, the content of EPA was higher in PFS group of piglets. The inverse relation between the content of DHA and gene expression can probably be explained by the above-mentioned binding characteristics of FABP-1. FABP-1, apart from LC-PUFA, can also bind hydrophobic ligands, including acyl coenzyme A, lysophospholipids, heme and bile salts. Therefore, the high expression of this gene does not exactly reflect the amount of LC-PUFA in enterocytes. In the small intestine, similar levels of FABP gene expression in PC and PF group of piglets were found, as well as similar DHA and EPA concentrations. Many studies have reported that EPA and DHA status of breast-fed infants is higher than that of formula-fed infants when formulas do not contain LCPUFA [14]. However, the presented study undermines the suggestion of these authors, that mother's milk and formula cannot be considered as equivalents in terms of the true availability of PUFA.

These results suggest that the feeding of LC-PUFA-free formula up-regulates the accretion of n-3 fatty acids from their precursors. An additional explanation can be provided by the fact that formula does not contain immunoglobulin and other biologically-active compounds. It is speculated that altered and/or increased demands of the immune system of offspring fed formula may cause increased demands for n-3

fatty acids which are the precursors to eicosanoids. Although their specific effects on conversion are not known, it is likely that greater demand would induce the turnover of n-3 fatty acids via FABP, which is involved in the intracellular fatty acid export process by regulation of substrate and/or product concentrations in the cytosolic compartment [15].

The liver plays a central role in lipid metabolism of the whole body and responds rapidly to changes in dietary fat composition. Polyunsaturated fatty acids from n-3 family play a key role in hepatic fatty acid oxidation and inhibit fatty acid synthesis and VLDL secretion, in part, by regulating gene expression. The results of many studies have shown that fish oil decreases the mature form of sterol regulatory element-binding proteins (SREBPs), which up-regulate the expression of genes promoting fat synthesis, and increases the expression of peroxisome proliferator-activator receptor (PPARα) stimulating fatty acid oxidation in the liver [16].

The results obtained in the presented study provide evidence that fish oil supplementation, irrespective of the type of diet, increases the levels of mRNA of FABP-1 and PPAR-α genes, and decreases SREBP-1 expression. Also, the concentration of EPA and DHA in piglets' liver reflects the fatty acids composition of the consumed diets, because the content of these fatty acids in the liver of PS and PFS piglets was several times higher compared with PC and PF piglets. On the other hand, comparison of n-3 fatty acids concentration in the liver of PS vs. PFS piglets showed the highest content of DHA in the liver samples of PS piglets, but the highest EPA content was found in the small intestine of the PFS group of piglets. These results may be related to the gene expression in this group of piglets. In the liver, no significant differences between PS and PFS groups of piglets were observed regarding the FABP-1 gene expression level, which suggests that lipid absorption in the liver was similar, irrespective of the type of diet, whereas mRNA levels of SREBP-1 was higher in PS group, compared with the formula milk group. SREBP-1 mainly activates the transcription of fatty acid synthesis-related genes, such as fatty acid synthase (FAS), stearoyl-CoA desaturase (SCD) and elongase enzymes (ELOVL) [16]. The synthesis of DHA from EPA requires a second pass of the Δ6 desaturase after it is elongated to 24:5n-3 [17]. Thus, it is possible that in piglets fed milk from supplemented sows, a part of the absorbed EPA was converted to DHA, increasing its amount in the liver, and thereby decreasing EPA content.

It also cannot be excluded that the higher EPA concentration found in the liver of PFS piglets results from the presence of short chain fatty acids in formula milk, as the preferential transport and oxidation could spare dietary PUFA from oxidation for energy, and hence, increase EPA bio-availability. Moreover, ingestion of dietary n-3 fatty acids in the form of triglycerides from fish oil, rather than as milk triglyceride and phospholipid, probably effectively improves absorption of EPA first in piglets' small intestine and next in the liver [18]. It is also worth noting that in piglets receiving milk from control sows, the level of PPAR-α mRNA was higher in comparison to the expression detected in formula fed piglets without fish oil. It has been reported that n-3 LC-PUFA and their metabolites can bind with PPARα with greater affinity than their parent fatty acids [19]. The results of the presented study are consistent with these observations, indicating that milk naturally containing small amounts LC-PUFA can induce the expression of genes more efficiently in comparison to formula milk, which generally contains only the precursors of LC-PUFA.

Postnatal growth of skeletal muscle in mammals occurs rapidly and continues until adulthood. The high rate of neonatal muscle growth is due to accelerated rates of protein synthesis, accompanied by rapid accumulation of energy. One of the debated issues that relates to the growth of infants is whether n-3 LCPUFA could be added to formula milk without a source of AA, because AA are never absent from human milk. This question arose from the early observations of growth deficit in preterm infants receiving formulas supplemented only in n-3 fatty acids, when compared with control formulas [20]. It was hypothesized that the depletion of plasma AA caused by dietary n-3 fatty acids supplementation may be a factor that contributes to the growth deficit. A randomized trial performed by Carlson et al. indicated an association between plasma AA and weight and length of the offspring [21]. In the presented study no significant differences in liver AA concentration were found between piglets from PC and PF groups, and only a slight increase in the AA content was noted in PS group of piglets in comparison with PFS piglets. Moreover, in the current experiment the formula feeding caused an increase in the expression of PPARα gene compared with piglets fed sows milk. After birth, lipid oxidation is the predominant metabolic activity of skeletal muscle [22], and the majority of the energy requirement of skeletal muscle is obtained from fatty acid oxidation. Thus, the presented results indicate that formula milk, with or without fish oil, influences muscle metabolism and development by elevating the process of β-oxidation and AA fatty acids concentration. In the opinion of many authors, the decrease of plasma and liver AA content in formula milk fed piglets can be associated with a high amount of LA in the formula [23, 24].

The presented study does not support the hypothesis that an increased amount of substrate (LA) suppresses AA desaturation in the liver, or competes with AA for acylation to the 2-position of phospholipids, because in piglets from PFS group the concentration of AA in plasma and the small intestine was higher than in PS group of piglets. It is possible that the lower AA content observed in the studies by other authors resulted from inappropriate conditions used in the experiments in which optimal conversion of LA to AA was not promoted.

Although human milk is the first choice for the newborn infants, milk substitutes play an indispensable role in infant nutrition when breastfeeding is not possible, desirable or sufficient. Nowadays, research to improve the quality of formula milk is focused mainly on achieving the functional effects that are observed in breastfed infants. The present study provides evidence that supplementation of the maternal diet with fish oil during pregnancy and lactation results in a more efficient induction of genes involved in fatty acids metabolism in the offspring than the supplementation of formula milk. However, the differences in gene expression and in n-3 LP-PUFA concentration in tissues between PS and PFS groups of piglets were small, it is therefore suggested that supplementation of formula with fish oil for term infants is as good way to enhance the development of infants. In addition, the results presented show that formula milk with n-3 LCPUFA increases the level of PPARα gene expression, and does not completely inhibit AA synthesis; therefore, it should not affect growth and in this respect it is safe for infants.

Acknowledgment

This research was supported by a Grant No. NN308563640, financed by the National Centre of Science in Poland.

REFERENCES

1. Alessandri JM, Guesnct P, Vancasscl S. Polyunsaturated fatty acids in the central nervous system: evolution of concepts and nutritional implications throughout life. Reprod Nutr Dev. 2004; 44: 509-538.
2. Das UN. Essential fatty acids- a review. Curr Pharm Biotech. 2006; 7: 467-482.
3. Bourre MJ. Diet, brain lipids and brain function. Polyunsaturated fatty acids, mainly omega-3 fatty acids. Neurochem Mol Neurobiol. 2011; 4: 409-44.
4. Dunstan JA, Simmer K, Dixon G, Prescott SI. Cognitive assessment 2(1/2) years alter fish oil supplementation in pregnancy: a randomized controlled trial. Arch Dis Child Fetal Neonatal Ed. 2008; 93: 45-50.
5. Brenna JT, Salem N, Sinclair A, Cunnane S. Alpha-linolenic acid supplementation and conversion to n-3 long-chain polyunsaturated fatty acids in humans. Prostaglandins Leukot Essent Fatty Acids 2009; 80: 85-91.
6. Givens ID, Gibbs RA. Current intakes of EPA and DHA in european populations and the potential of animal-derived foods to increase them. Proc Nutr So. 2008; 67: 273-280.
7. Martin CR, Dasilva DA, Cluette-Brown JE, Dimonda C, Hamill A, Bhutta AQ, et al. Decreased postnatal docosahexaenoic and arachidonic acid blood levels in premature infants are associated with neonatal morbidities. J Pediatr. 2011; 159(5):743-749.
8. Amusquivar E, Sanchez M, Hyde MJ, Laws J, Clarke L, Herrera E. Influence of fatty acids profile of total parenteral nutrition emulsion on the fatty acid composition of different tissues of piglets. Lipids. 2008; 43: 713-722.
9. Heuvel JP van den. Nutrigenomics and nutrigenetics of ω3 polyunsaturated fatty acids. Prog Mol Biol Transl Sci. 2012; 108: 75-112.
10. Folch J, Lees M, Stanley GHS. A simple method for total lipid extraction and purification. J Biol Chem. 1957; 226: 497-509.
11. Koldovský O, Dobiásová M, Hahn P, Kolínská J, Kraml J, Pácha J. Development of gastrointestinal functions. Physiol Res. 1995; 44: 341-348.
12. Hsu KT, Storch J. Fatty acid transfer from liver and intestinal fatty acid binding-proteins to membranes occurs by different mechanisms. J Biol Chem. 1996; 271: 13317-13323.
13. Hubbell T, Behnke WD, Woodford JK, Schroeder F. Recombinant liver fatty acid binding protein interacts with fatty acyl-coenzyme A. Biochem. 1994; 33: 3327-3334.
14. Schwartz J, Drossard C, Dube K, Kannenberg F, Kunz C, Kalhoff H et al. Dietary intake and plasma concentrations of PUFA and LC-PUFA in breastfed and formula fed infants under real-life conditions. Eur J Nutr. 2010; 49: 189-195.
15. Storch J, Thumser AE. The fatty acid transport function of fatty acid-binding proteins. Biochim Biophys Acta. 2000; 1486: 28-44.
16. Yen CF, Jiang YN, Shen TF, Wong IM, Chen CC, Chen KC, et al. Cloning and expression of the genes associated with lipid metabolism in Tsaiya ducks. Poult Sci. 2005; 84(1): 67-74.
17. Blank C, Neumann MA, Makrides M, Gibson RA. Optimizing DHA levels in piglets by lowering the linoleic acid to α-linolenic acid ratio. J Lip Res. 2002; 43: 1537-1544.
18. Amate L, Gil A, Ramirez M. Feeding infant piglets formula with long-chain polyunsaturated fatty acids as triacylglycerols or phospholipids influences the distribution of these fatty acids in plasma lipoprotein fractions. J Nutr. 2001; 4: 1250-1255.
19. Krey G, Braissant O, L'Horset F, Kalkhoven E, Perroud M, Parker MG, et al. Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay. Mol Endocrinol. 1997; 11(6): 779-791.
20. Ryan AS, Montalto MB, Groh-Wargo S, Mimouni F, Sentipal-Walerius J, Doyle J, Siegman JS, Thomas AJ. Effect of DHA-containing formula on growth of preterm infants to 59 weeks postmenstrual age. Am J Human Biol. 1999; 11: 457-467.
21. Carlson SE, Werkman SH, Peeples JM, Cooke RJ, Tolley EA. Arachidonic acid status correlates with first year growth in preterm infants. Proc Natl Acad Sci. 1993; 90: 1073-1077.
22. Dagenais GR, Tancredi RG, Zierler KL. Free fatty acid oxidation by forearm muscle at rest, and evidence for an intramuscular lipid pool in the human forearm. J Clin Invest. 1976; 58: 421-431
23. Innis SM, Nelson CM, Rioux FM, King DJ. Development of visual acuity in relation to plasma and erythrocyte ω-6 and ω-3 fatty acids in healthy term gestation infants. Am J Clin Nutr. 1994; 60: 347-352.
24. Farquharson J, Jamieson EC, Logan RW, Patrick WJA, Howatson AG, Cockburn F. Age-and dietary-related distributions of hepatic arachidonic and docosahexaenoic acid in early infancy. Pediatr Res. 1995; 38: 361-365.