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Tissue Fatty Acid Deposition, Plasma Lipid and Cytokine Profile in Pigs Fed a Diet with Fish Oil or Palm Oil

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ABSTRACT

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The present study tested a hypothesis concerning a favourable effect of dietary fish oil on the tissue polyunsaturated fatty acid (PUFA) deposition, and on plasma lipid and cytokine profile. Thirty-two pigs divided into two groups of 16 animals each were fed for 70 days a diet with 2.5% of fish oil (F) and palm oil (P), respectively. The content of PUFA n-3 in the liver, muscle (*m. quadriceps femoris*), and visceral adipose tissue (VAT) of the F- and P-pigs was 530 and 129, 84 and 19, 1365 and 191 mg/100 g of the fresh tissue, respectively (differences between dietary groups were significant at $P < 0.01$ in all tissues). Dietary fish oil in comparison with palm oil decreased ($P < 0.05$) total plasma cholesterol, but also desirable high-density lipoprotein cholesterol, and had no effect ($P > 0.05$) both on low-density lipoprotein cholesterol and triacylglycerols. Moreover, dietary fish oil increased ($P < 0.05$) expression of the genes coding for not only anti-inflammatory cytokines IL-10 ($P < 0.01$) and TGF-β1 ($P < 0.05$), but also for pro-inflammatory cytokines IL-6 ($P < 0.01$), IL-12, and TNF-α ($P < 0.05$) in the VAT, and increased ($P < 0.05$) the expression of the *IL6* gene in the liver. On the other hand, no significant differences ($P > 0.05$) between the F- and P-pigs in plasma levels of any tested cytokine were found out. It was concluded that an effect of dietary fish oil on tissue fatty acid deposition is undeniable, but its effects on plasma markers related to the risk of chronic degenerative diseases require further research.

Keywords: EPA; DHA; cholesterol; IL-10; TNF-α; cardiovascular diseases; feed ration; pig; muscle

Cardiovascular diseases (CVD) are currently a leading cause of mortality in economically developed countries. Eating habits significantly affect risk of CVD via food composition, especially from the viewpoint of lipid fractions of ingested foods. As far as lipids are concerned, the so-called Western-type eating habit is characterized by a relatively high intake of saturated fatty acids (SFA) and lower consumption of monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA). Within PUFA, intake of PUFA n-6 highly prevails over PUFA n-3, which further increases the risk of CVD (Bragt and Mensink 2012).

Pork is characterized by a relatively high SFA ratio. However, within a current trend of production of the so-called functional foods, it is possible

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to enrich pork with PUFA n-3. Possibilities of such an enrichment in foods in general were reviewed e.g. by Komprda (2012). Sobol et al. (2016) reported that loin and shoulder of pigs (with high intramuscular fat content) fed a diet enriched with the mixture of linseed, rapeseed, and fish oils meet the European Union recommendations for human nutrition for products considered as either PUFA n-3 sources or products with high PUFA n-3 content. Alvarez-Rodriguez et al. (2016) found out that total PUFA n-3 content (mainly α-linolenic acid, ALA) was greater in organic than in conventional pork, probably due to ALA content from dietary vegetable oils.

However, the conversion rate of dietary ALA (a precursor of the PUFA n-3 group) to higher functional metabolites eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) in animal tissues is very low (Komprda et al. 2013). Therefore it is more suitable to use directly dietary EPA + DHA, a rich source of which is fish oil. From the viewpoint of a healthy nutrition, palm oil (with its high content of SFA), which is currently present in many foods, stays on the opposite pole to fish oil (PUFA n-3).

Therefore, one of the objectives of the present study was to compare fish oil and palm oil as the components of the pig feed ration from the viewpoint of a deposition of dietary fatty acids in the tissues relevant to human consumption: liver, muscle, and adipose tissue.

Moreover, regarding the above-mentioned CVD, their hallmarks are, among others, dislipidemia and low-level chronic inflammation in an organism (especially in the vascular wall) (Calder 2013). Dislipidemia is characterized by a high plasma level of triacylglycerols (TAG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDLC), and low level of high-density lipoprotein cholesterol (HDLC). As far as pigs are concerned, Sopkova et al. (2017) evaluated an effect of combining a dietary source of PUFA n-3 (flaxseed) with probiotics on the lipid metabolism of piglets after weaning, and reported a decrease of HDLC, but no change in TAG, TC, and LDLC, respectively.

Low-level chronic inflammation manifests itself (among others) by an increased plasma level of pro-inflammatory cytokines (Bragt and Mensink 2012). The effect of dietary fish oil on cytokine production in pigs is only scarcely described in available literature. Liu et al. (2003) tested the fish oil (7%) effect on cytokine production in not-challenged and lipopolysaccharide-challenged crossbred pigs; in comparison with corn oil, fish oil only tended to reduce IL-1β and IL-2 production, respectively.

The second objective of the present study therefore was to evaluate the lipid and cytokine levels in plasma of pigs fed a diet enriched with fish oil and palm oil, respectively. An intention was to use such a level of these oils (2.5% of the feed ration), which would be relevant to humans. In this case, pigs were considered a useful model of human nutrition.

Therefore, the present experiment tested a hypothesis that pigs fed a diet with fish oil, in comparison with dietary palm oil, will deposit higher amounts of nutritionally favourable fatty acids in the food-processing relevant tissues, and will have a more favourable plasma lipid profile and a less inflammation-prone cytokine profile.

MATERIAL AND METHODS

Animals and dietary interventions. Thirty-two pigs of both sexes (16 males, 16 females – from technical reasons it was not possible to obtain a weight-and-age-homogeneous same-sex group; Large White × Landrace; Bioprodukt Knapovec a.s., Czech Republic) at the age of eight weeks with the mean live weight of 25.5 ± 1.15 kg were used. The pigs were housed in an experimental stable in floored indoor pens (10 m^2) of four animals each.

The experiment was performed in compliance with the Act No. 246/1992 Coll. of the Czech National Council on the protection of animals against cruelty, and with its amendment, the Act No. 162/1993 Coll., and was approved by the "Commission to protect animals against cruelty" of the Mendel University in Brno and of the Ministry of Agriculture of the Czech Republic.

The pigs were divided into two groups with 16 animals each (the same number of males and females was in each group): both the experimental and the control group were fed the basic feed mixture with 2.5% of fish oil (F) and 2.5% of palm oil (P), respectively. The P-diet was used as a control (instead of a control diet using either a basic feed mixture alone or a basic feed mixture with starch) in order to keep the diet not only isocaloric but also isolipidic. Both the F and the P diet contained in one kilogram: 138 g of crude protein (determined

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using a KD-310-A-1015 KjelROC Analyzer; Opsis AB, Sweden), 56 g of fat (quantified as hexane/2-propanol extract), 48 g of crude fibre (determined using an ANKOM 220 Fiber Analyzer; ANKOM Technology, USA), and 758 g of nitrogen-free extractives (calculated as a remainder to 100%). Metabolizable energy content (calculated from nutrient content) was 13.6 MJ/kg. Basic pelletized complete feed mixture for pig fattening (De Heus a.s., Czech Republic) was composed of wheat, barley, wheat bran, wheat middlings, dark distillery stillages, rapeseed expellers, vinas, sodium carbonate, animal fat, salt, premix of vitamins + minerals (the producer refused to communicate percentages of particular components due to the trade secret). The pelletized basic feed mixture was grinded and homogenized with an appropriate amount of particular oil.

The fatty acid content in fish oil and palm oil, and in the F and P diet, is presented in Table 1.

The animals had free access to drinking water and were fed daily *ad libitum*; the daily ration was divided into two parts and fed twice daily (at 7.00 and 14.00 h). By subtracting leftovers, the net feed consumption was measured twice daily; due to the *ad libitum* access to feed, significant differences in consumption between animals in a pen were not supposed. The animals were weighed in weekly intervals. The fattening lasted for 70 days.

Last day of fattening, all pigs were anesthetized by the intramuscular application of the TKX mixture (12.5 mg/ml of ketamine (Vétoquinol SA, France) + 12.5 mg/ml of xylazine (Bioveta, Czech Republic) + 12.5 mg/ml of tiletamine (Virbac, France) + 12.5 mg/ml of zolazepam (Virbac)) in the total volume of 0.2 ml/kg, and consequently sacrificed by bleeding.

Blood and tissue samples collection. Blood samples were collected (from the aorta) to the heparin-coated test tubes and subsequently centrifuged at 200 *g* for 10 min at 4°C to obtain blood plasma. Liver (200 g), muscle (*m. quadriceps femoris*; 200 g), and visceral adipose tissue (VAT) (pelvic; 200 g) samples were taken, aliquots (100 g of each tissue) were freeze-dried (Alpha 1-2 LDplus; Martin Christ Gefriertrocknungsanlagen GmbH, Germany; temperature programme: –45°C/27 h and –50°C/3 h) and stored at –20°C for subsequent fatty acid analyses. Total RNA was immediately isolated from another liver and VAT aliquots (50 and 50 mg, respectively).

Fatty acid analysis. Total lipid extraction, sample derivatization, and fatty acid methyl ester separation were performed according to a procedure described in a paper of Komprda et al. (2013). The results were expressed as a percentage of the sum of all determined fatty acids and in mg/100 g of the fresh tissue.

Plasma lipids determination. TC, LDLC, HDLC, and TAG values were determined by the enzymatic-colorimetric method using an automated chemical analyzer BS-200 (Mindray, China) and commercial kits (Greiner Diagnostic GmbH, Germany).

Quantification of cytokine gene expressions. Total RNA isolation, reverse transcription, quantitative polymerase chain reaction (PCR), and calculation of the normalized relative quantity values were performed according to Komprda et al. (2016). The efficiency of the quantitative PCR for the target genes and the reference gene was determined using an external standard curve with serial dilutions of the cDNA plotted against cycle number to calculate the slope (Hellemans et al. 2007). *TBP1* was used as a reference gene based on the data of Nygard et al. (2007) reporting good expression stability and expression level for low abundant transcripts across different pig tissues. Specific primers for the pig genes used in quantitative PCR are characterized in Table 2.

Table 1. Fatty acid proportion in dietary oils and in the diets (% of the sum of all determined fatty acids)

Fatty acid	Oils		Diets	
	fish oil	palm oil	F	P
14:0	5.6	1.5	2.4	0.8
16:0	15.3	40.3	17.8	32.8
16:1	11.7	0.2	4.9	1.0
18:0	2.6	1.6	3.4	1.5
18:1	25.1	43.8	25.0	30.9
18:2n-6	3.5	11.3	30.2	30.2
18:3n-3	1.6	0.3	0.1	0.1
20:2n-6	0.7	0.1	0.3	0.1
20:3n-6	0.2	0.0	0.1	0.1
20:4n-6	0.7	0.1	0.5	0.3
20:5n-3	11.8	0.3	4.6	0.1
22:4n-6	0.3	0.1	0.2	0.1
22:5n-3	2.1	0.1	0.9	0.2
22:6n-3	18.5	0.1	7.1	0.1

F = diet with 2.5% of fish oil, P = diet with 2.5% of palm oil

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Table 2. Primers used for quantitative PCR (*TBP1* used as a reference gene)

Gene	Forward primer	Reverse primer	Reference
TNFα	CCCCCAGAAGGAAGAGTTTC	CGGGCTTATCTGAGGTTTGA	http://www.ncbi.nlm.nih.gov/pubmed/17894638
IL1β	GGGACTTGA AGAGAGAAGTGG	CTTTCCCTTGAT CCCTAAGGT	http://www.ncbi.nlm.nih.gov/pubmed/?term=pavlova+spi-1
IL4	TCGGCACATCTACAGACACC	CTTCTTGGCTTCATGCACAG	http://www.sciencedirect.com/science/article/pii/S0378113511005475
IL6	CACCGGTCT TGTGGAGTTTC	GTGGTGGCTTTG TCTGGATT	http://www.ncbi.nlm.nih.gov/pubmed/?term=pavlova+spi-1
IL10	TGAAGAGTGCCTTTAGCAAGCTC	CTCATCTTCATCGTCATGTAGGC	http://www.ncbi.nlm.nih.gov/pubmed/22817641
IL12 p40	CACTCCTGC TGCTTCACAAA	CGTCCGGAGTAA TTCTTTGC	http://www.ncbi.nlm.nih.gov/pubmed/?term=pavlova+spi-1
TGFβ1	TACGCCAAG GAGGTCACCC	CAGCTCTGCCCG AGAGAGC	http://www.ncbi.nlm.nih.gov/pubmed/14663153
TBP1	AACAGTTCAGTAGTTATGAGCCAGA	AGATGTTCTCAAACGCTTCG	http://www.ncbi.nlm.nih.gov/pubmed/?term=Nygard%2C+Jorgensen+et+al+BMC+Molecular+Biology+2007)

Determination of plasma cytokines. IL-1β, IL-4, IL-6, IL-10, IL-12, and TNFα concentrations in the pig blood plasma were measured by Milliplex® MAP Porcine Cytokine/Chemokine Magnetic Bead Panel kit (Millipore Corp., USA) according to the producer's recommendation.

Statistical evaluation. Normality of the data distribution was tested by Kolmogorov-Smirnov test. The differences between dietary interventions were evaluated by one-way ANOVA including the *post-hoc* Tukey's test and by the independent samples *t*-test (sets with a normal distribution, i.e. all data sets except gene expressions), and by the non-parametric Wilcoxon signed-rank test (data sets concerning relative expression of the liver and adipose tissue genes), respectively. For all evaluations, the STATISTICA Version 12 software package (StatSoft, USA) was used.

RESULTS

Feed intake, live weight, daily weight gain. No significant differences ($P > 0.05$) between the F- and P-pigs were established either in feed intake (10.5 and 10.6 g/kg per day) or in daily weight gain (0.85 ± 0.05 kg/day and 0.86 ± 0.04 kg/day) or in the final live weight (83.64 ± 1.82 kg and 84.06 ± 3.35 kg, respectively; data not presented in tables or figures).

Fatty acid deposition in the tissues. Deposition of the tested fatty acids in the three tissues relevant from the viewpoint of human nutrition was quantified either as percentages from the sum of all analyzed fatty acids (Table 3) or as absolute amounts in mg/100 g of the fresh tissue (not presented in tables or figures).

As far as a comparison of the tissues is concerned, it follows from Table 3 that MUFA were deposited in the highest ($P < 0.05$) and PUFA in the lowest ($P < 0.05$) percentage in the muscle tissue. On the other hand, PUFA deposited preferentially in the liver: the highest ($P < 0.05$) PUFA percentage from all tested tissues was found in the liver not only of the F-pigs, but also of the pigs fed a diet with palm oil (38.5% of all fatty acids deposited in the P-livers were PUFA, contrary to e.g. only 22.3% in the VAT of the fish oil-fed pigs; $P < 0.05$; Table 3).

As far as a comparison of the dietary oils from the viewpoint of PUFA is concerned, the F- and P-pigs did not differ ($P > 0.05$) in deposition of PUFA n-6 in either of the tested tissues. On the

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other hand, sum of PUFA n-3 was deposited more ($P < 0.05$) in the F-pigs as compared to the P-counterparts in all tested tissues. The F-pigs also had a more favourable (lower; $P < 0.05$) PUFA n-6/PUFA n-3 ratio in all tested tissues (data not explicitly presented in Table 3).

When expressed in the absolute values, the contents of nutritionally desirable PUFA n-3 in the liver, muscle tissue, and VAT of the F- and P-pigs were 530 and 129, 84 and 19, and 1365 and 191 mg/100 g of the fresh tissue, respectively (differences between dietary groups were significant at $P < 0.01$ in all tissues).

Plasma lipids. One of the objectives of the present experiment was to use pigs as a model for evaluating the effect of dietary fish oil on plasma lipids as markers of the risk of CVD. Plasma levels of total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triacylglycerols are shown in Figure 1. Dietary fish oil in comparison with palm oil decreased ($P < 0.05$) TC. However, this decrease was apparently at the expense of the desirable HDL fraction, the level of which was also lower ($P < 0.05$) in plasma of the F-pigs. On the other hand, dietary fish oil had no effect ($P > 0.05$) either on LDL cholesterol or TAG.

Cytokines. Other hallmark of the CVD risk is a chronic low inflammation in an organism. Therefore the next objective of the present experiment was to test dietary fish oil as a means for amelioration of an inflammatory status in a model organism; pro- and anti-inflammatory cytokines were used as pertinent markers. Expression of the genes coding for selected cytokines in the liver and VAT, and plasma levels of these cytokines (except of TGF-β1, whose antibody was not available in the kit used for the analysis) in plasma are presented in Figures 2 and 3.

Table 3. Fatty acid proportion in the liver, muscle tissue (*m. quadriceps femoris*), and visceral adipose tissue of pigs fed 70 days a diet with 2.5 % of fish oil (F) and 2.5 % of palm oil (P), respectively (values are means ± standard error of the means, $n = 16$)

Fatty acid	Fatty acid content (% of the sum of all determined fatty acids)					
	liver		muscle (*m. quadriceps femoris*)		visceral (pelvic) adipose tissue	
	F	P	F	P	F	P
14:0	$0.5^{A} \pm 0.0$	$0.5^{A} \pm 0.1$	$1.4^{B} \pm 0.0$	$1.3^{B} \pm 0.0$	$1.8^{C} \pm 0.0$	$1.4^{B} \pm 0.0$
16:0	$15.3^{A} \pm 0.6$	$17.4^{B} \pm 0.5$	$26.0^{CD} \pm 0.4$	$25.6^{C} \pm 0.3$	$27.5^{DE} \pm 0.4$	$25.8^{E} \pm 0.4$
17:0	$2.7^{A} \pm 1.8$	$0.5^{A} \pm 0.0$	$0.4^{A} \pm 0.0$	$0.3^{A} \pm 0.0$	$0.4^{A} \pm 0.0$	$0.5^{A} \pm 0.0$
18:0	$21.6^{C} \pm 0.1$	$21.1^{C} \pm 1.2$	$6.1^{A} \pm 0.7$	$7.5^{A} \pm 0.6$	$9.1^{AB} \pm 0.6$	$12.0^{B} \pm 0.8$
∑SFA	$40.1^{C} \pm 2.2$	$39.7^{C} \pm 0.9$	$33.8^{A} \pm 0.8$	$34.7^{AB} \pm 0.6$	$38.7^{BC} \pm 0.5$	$42.4^{C} \pm 0.5$
16:1	$1.0^{A} \pm 0.1$	$0.9^{A} \pm 0.1$	$3.0^{D} \pm 0.0$	$2.6^{C} \pm 0.1$	$2.8^{CD} \pm 0.1$	$1.6^{B} \pm 0.1$
18:1	$16.5^{A} \pm 0.5$	$20.2^{B} \pm 0.8$	$43.0^{D} \pm 0.5$	$45.9^{E} \pm 0.6$	$36.1^{C} \pm 0.8$	$36.6^{C} \pm 0.1$
∑MUFA	$17.6^{A} \pm 0.6$	$21.2^{B} \pm 0.8$	$46.1^{D} \pm 0.6$	$48.4^{D} \pm 0.6$	$38.9^{C} \pm 0.8$	$38.3^{C} \pm 0.5$
18:2n-6	$15.2^{A} \pm 0.2$	$16.5^{A} \pm 0.3$	$16.9^{A} \pm 3.6$	$12.9^{A} \pm 0.3$	$15.6^{A} \pm 0.5$	$16.3^{A} \pm 0.4$
18:3n-6	$2.9^{A} \pm 2.2$	$0.6^{A} \pm 0.0$	$1.0^{A} \pm 0.0$	$0.8^{A} \pm 0.02$	$1.3^{A} \pm 0.1$	$1.1^{A} \pm 0.0$
20:2n-6	$1.8^{A} \pm 1.4$	$0.7^{A} \pm 0.0$	$0.5^{A} \pm 0.0$	$0.6^{A} \pm 0.0$	$0.5^{A} \pm 0.0$	$0.5^{A} \pm 0.0$
20:3n-6	$2.2^{A} \pm 1.5$	$0.6^{A} \pm 0.0$	$0.2^{A} \pm 0.0$	$0.2^{A} \pm 0.0$	$0.1^{A} \pm 0.0$	$0.1^{A} \pm 0.0$
20:4n-6	$5.3^{B} \pm 0.1$	$13.7^{C} \pm 0.66$	$0.7^{A} \pm 0.1$	$1.3^{A} \pm 0.1$	$0.3^{A} \pm 0.0$	$0.3^{A} \pm 0.0$
22:4n-6	$1.6^{A} \pm 1.2$	$1.0^{A} \pm 0.0$	$0.2^{A} \pm 0.0$	$0.3^{A} \pm 0.0$	$0.1^{A} \pm 0.0$	$0.1^{A} \pm 0.0$
PUFA n-6	$29.0^{BC} \pm 6.3$	$33.0^{C} \pm 0.7$	$19.5^{AB} \pm 3.6$	$16.1^{A} \pm 0.4$	$17.9^{AB} \pm 0.5$	$18.4^{AB} \pm 0.4$
18:3n-3	$0.9^{A} \pm 0.6$	$0.3^{A} \pm 0.0$	$0.1^{A} \pm 0.0$	$0.1^{A} \pm 0.0$	$0.1^{A} \pm 0.0$	$0.2^{A} \pm 0.0$
20:5n-3	$10.0^{B} \pm 0.2$	$0.9^{A} \pm 0.1$	$1.1^{A} \pm 0.1$	$0.2^{A} \pm 0.0$	$1.0^{A} \pm 0.0$	$0.1^{A} \pm 0.0$
22:5n-3	$3.3^{D} \pm 0.13$	$2.2^{C} \pm 0.1$	$1.1^{B} \pm 0.0$	$0.4^{A} \pm 0.0$	$1.1^{B} \pm 0.0$	$0.2^{A} \pm 0.0$
22:6n-3	$7.4^{D} \pm 0.2$	$2.1^{BC} \pm 0.1$	$1.8^{B} \pm 0.1$	$0.3^{A} \pm 0.0$	$2.2^{C} \pm 0.1$	$0.2^{A} \pm 0.0$
PUFA n-3	$21.7^{D} \pm 0.7$	$5.5^{C} \pm 0.2$	$4.0^{BC} \pm 0.2$	$0.9^{A} \pm 0.1$	$4.4^{B} \pm 0.2$	$0.6^{A} \pm 0.1$
∑PUFA	$50.7^{B} \pm 6.9$	$38.5^{B} \pm 0.9$	$23.5^{A} \pm 3.54$	$17.0^{A} \pm 0.4$	$22.3^{A} \pm 0.7$	$19.0^{A} \pm 0.4$

$^{A–E}$means with different superscripts in rows differ at $P < 0.05$ (one-way ANOVA with *post-hoc* Tukey's test)

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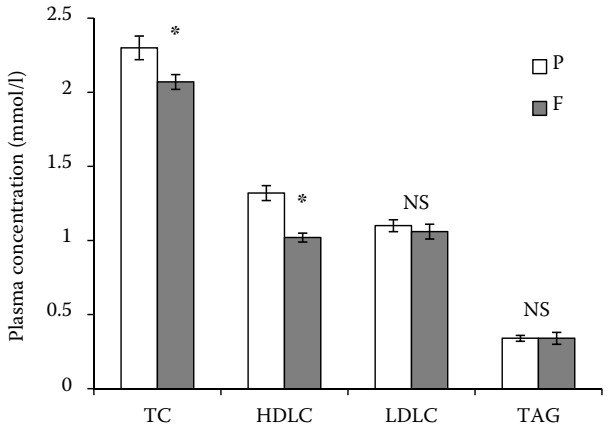

Figure 1. Concentration of total cholesterol (TC), high-density lipoprotein cholesterol (HDLC), low-density lipoprotein cholesterol (LDLC), and triacylglycerols (TAG) in plasma of pigs fed 70 days a diet with 2.5% of fish oil (F) and 2.5% of palm oil (P), respectively. Values are means ± SEM; $n = 16$

*$P < 0.05$, NS = not significant (independent samples t-test)

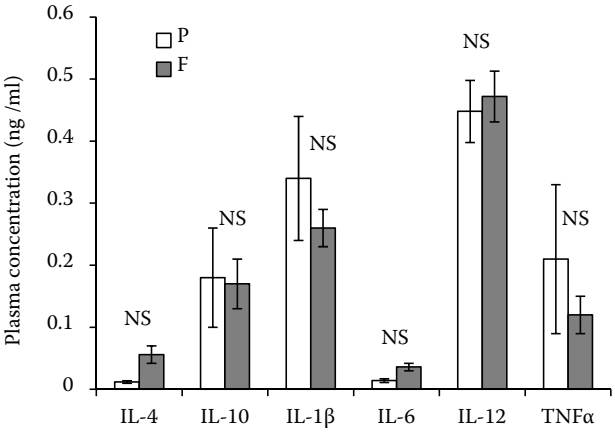

Figure 3. Concentration of selected cytokines in the plasma of pigs fed 70 days a diet with 2.5% of fish oil (F) and 2.5% of palm oil (P), respectively. Values are means ± SEM; $n = 8$

NS = not significant (independent samples t-test)

The most interesting finding following from Figure 2 is a tendency of fish oil to increase expression of the genes coding for both anti- and pro-inflammatory cytokines. Due to a high variability between pigs, only difference in the *IL6* gene was significant ($P < 0.05$) in the liver. However, in the VAT, dietary fish oil significantly increased expression of the genes coding for anti-inflammatory cytokines IL-10 ($P < 0.01$) and TGF-β1 ($P < 0.05$), but also for pro-inflammatory cytokines IL-6 ($P < 0.01$), IL-12, and TNF-α ($P < 0.05$).

The plasma level of respective cytokines (Figure 3) did not correspond with the results of the genomic analysis (Figure 2). Due to the above-mentioned high variability between pigs within both dietary groups, no significant differences in plasma cytokines between the F- and P-pigs were found out ($P > 0.05$). However, some interesting tendencies are worthy to mention. Dietary fish oil tended to increase plasma level of anti-inflammatory cytokine IL-4 ($P = 0.07$) on the one hand and to decrease plasma level of pro-inflammatory cytokines IL-1β ($P = 0.12$) and TNF-α ($P = 0.16$) on the other.

DISCUSSION

Weight, weight gain. Functional components of fish oil, EPA and DHA, can probably contribute to weight loss in obese animals or humans (Howe et al. 2014). However, this is likely not the case

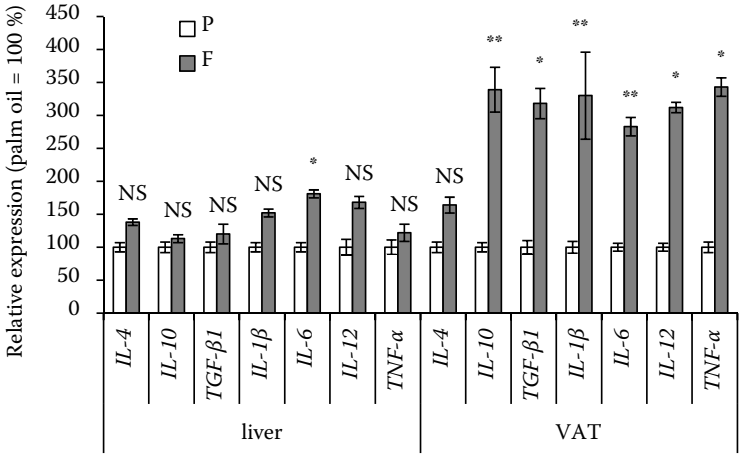

Figure 2. Expression of the genes coding for selected pro- and anti-inflammatory cytokines in the liver and visceral adipose tissue (VAT) of pigs fed 70 days a diet supplemented with 2.5% of fish oil (F) relative to expression of these genes in the control pigs fed a diet with 2.5% of palm oil (P). Values are means ± SEM; $n = 8$

*$P < 0.05$, **$P < 0.01$, NS = not significant (Wilcoxon signed-rank test)

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as far as normal-weight, non-obese animals are concerned. Chen et al. (2012) reported no significant effect of DHA oil in comparison with beef tallow on the body weight gain in weaned pigs, which agrees with the findings of the present experiment. Similarly, dietary intervention had no significant effect on either daily weight gain or the final live weight of rats fed a diet with fish oil and palm oil, respectively, in our previous experiment (Komprda et al. 2014).

Fatty acid deposition in the tissues. As follows from Table 3, a relatively high proportion of the sum of total PUFA in all tested tissues was found out in the present experiment not only in the F-, but also in the P-pigs. This is likely a reflection of a high PUFA proportion both in the F-diet (44.0%) and in the P-diet (31.3%) (the data follow from individual fatty acid contents presented in Table 1); however, more than 30% of PUFA in both diets represented linolenic acid (n-6).

In an effort to enrich animal products (including pork) with desirable long-chain (LC)-PUFA n-3, supplementation of a diet with fish products (EPA + DHA) is more efficient in comparison with linseed (ALA); however, organoleptic properties may be compromised (Moghadasian 2008). Unfortunately it was not possible to address this question in the present study; we were not able to perform sensory analysis, because the pigs were sacrificed after previous anesthesia by a mixture of ketamine, xylazine, tiletamine, and zolazepam.

Regarding PUFA deposition, it is interesting that Wojtasik et al. (2012), when fattening pigs with a diet containing 2.5% of linseed oil + 1% of fish oil, reported higher PUFA/SFA ratio in a subcutaneous fat than in a muscle tissue (*m. longissimus dorsi*), which is contrary to our finding: the PUFA/SFA ratio was 0.58 in the VAT and 0.70 in the muscle tissue (*m. quadriceps femoris*) in the present experiment.

PUFA n-6/n-3 ratio established in the present experiment (5.6 in the muscle tissue, 4.2 in the VAT) is within the range of the results of the recent similar experiments in pigs fed diets containing fish oil: 6.39 in the outer shoulder fat layer (Hallenstvedt et al. 2012), and 3.57 and 3.11 in the muscle (*m. triceps brachii*) and backfat, respectively (Lisiak et al. 2013), or 3.51 and 3.99 in the muscle tissue (*m. longissimus dorsi*) and subcutaneous fat, respectively (Wojtasik et al. 2012). However, in the two last-mentioned experiments reporting lower PUFA n-6/n-3 ratios (Wojtasik et al. 2012; Lisiak et al. 2013), in addition to fish oil the authors used also linseed oil, which has a very high content of α-linolenic acid; though a conversion efficiency of dietary ALA to the tissue LC-PUFA n-3 is very low (Komprda et al. 2013), ALA itself is efficiently transported from a diet to the animal (pig) tissues (Skiba et al. 2015) and therefore substantially decreases the total PUFA n-6/n-3 ratio.

Nevertheless, from the viewpoint of human nutrition, the most valuable components of meat of pigs fed diets enriched with fish oil are LC-PUFA n-3, especially EPA + DHA. The EPA + DHA content found out in the muscle tissue of pigs fed a diet with 2.5% of fish oil in the present experiment (1.1 + 1.8 = 2.9% of the sum of all determined fatty acids; Table 3) is similar to the values reached using a diet with both a half (1.2%) and a double (5.0%) of fish oil content as reported by Haak et al. (2008) (EPA + DHA content was 2.4% of the sum of fatty acids in *m. longissimus thoracis*) and by Skiba et al. (2015) (EPA + DHA content was 2.4% after re-calculation: the authors reported kilograms of total fatty acids and grams of EPA and DHA in the whole body of pigs), respectively.

On the other hand, Wojtasik et al. (2012) and Lisiak et al. (2013) found out a lower EPA + DHA content (1.6% in *m. triceps brachii* and 0.8% in *m. longissimus dorsi*, respectively) in pigs fed a diet with 3.5% of a mixture of fish oil and linseed oil.

Plasma lipids. The effect of fish oil on total plasma cholesterol found out in the present experiment (significant decrease in comparison with the palm oil control; Figure 1) agrees with the results of our previous experiment in rats (Komprda et al. 2014). However, a response of pigs (present experiment) and rats (Komprda et al. 2014) on dietary fish oil in comparison with palm oil was totally different as far as plasma TAG are concerned: no difference (Figure 1) and a substantial (twofold) decrease, respectively. An explanation is likely based on the differences between rats and pigs in a response to PUFA n-3 as natural ligands of various isoforms of peroxisome proliferator-activated receptors (PPAR); rats and pigs are labelled in this context as proliferating and non-proliferating species, respectively (Komprda 2012). PPARα activation by the active components of fish oil in the proliferating rats is presumably stronger in comparison with non-proliferating pigs, which leads to a stronger inhibition of the sterol response

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element-binding protein 1 (SREBP-1) signaling pathway that stimulates fatty acid β-oxidation and simultaneously inhibits fatty acid synthesis with the result of a decreased serum TAG (Jump 2008).

Unexpected results were found out in the present experiment regarding the plasma HDL-cholesterol fraction (Figure 1): significantly ($P < 0.05$) lower values in the F-pigs in comparison with the P-controls. This finding can be (rather counter-intuitively) interpreted as an ability of saturated fats to increase this favourable cholesterol fraction, which is, however, in agreement with the results of Puccinelli et al. (2015), who reported nearly four-times higher HDLC (2.28 vs 0.62 mmol/l) in plasma of pigs fed intermittently a diet with 20% of lard in comparison with control.

Cytokines. The findings of the present experiment regarding the expression of cytokine genes (a tendency to increase and significant increase in the liver and VAT, respectively, of some genes coding for pro-inflammatory cytokines; Figure 2) are difficult to explain because they do not correspond with the presumptive effect of EPA/DHA as endogenous ligands of PPARγ, the ligation of which contributes to the inhibition of the signaling pathway of nuclear factor kappa B (NF-κB) (Komprda 2012); NF-κB is a transcription factor of (among others) the genes coding for the pro-inflammatory cytokines (Calder 2013).

Nevertheless, ligand induced activation of PPARγ did not ameliorate, but enhanced the pro-inflammatory cytokine production in weaned pigs in an experiment of Liu et al. (2009); however, this finding can only partly explain the above-mentioned results of the present experiment (Figure 2), because it does not concern gene expressions, but circulating proteins, and it was obtained in lipopolysaccharide (LPS)-challenged animals. Nevertheless, similarly to our results, Cormier et al. (2016) reported slightly over-expressed *TNFα* and *IL6* genes in human subjects supplemented for six weeks with 5 g/day of fish oil.

As far as plasma cytokines are concerned, an insignificant effect of fish oil found out in the present experiment (Figure 3) was likely a consequence of the fact that there was no apparent inflammation in an organism of pigs and concentrations of circulating cytokines were therefore very low (only tenths of pg/ml). On the other hand, Upadhaya et al. (2015) reported significantly reduced serum TNF-α in the LPS-challenged pigs supplemented with PUFA n-3 (from 50 to 44 pg/ml) – compare with only a tendency ($P > 0.05$) of fish oil to decrease plasma level of TNF-α in the present experiment (from 0.21 to 0.12 pg/ml) (Figure 3).

CONCLUSION

The hypothesis tested in the present experiment was confirmed only partially. On the one hand, fish oil fed at an amount of 2.5% of the feeding ration conspicuously increased the content of nutritionally valuable PUFA n-3 in all tested pig tissues (liver, muscle, fat). On the other hand, dietary fish oil decreased not only total plasma cholesterol, but also its favourable HDL fraction, and had no effect on plasma triacylglycerols, important risk factor of CVD. Moreover, the hypothesis concerning a favourable effect of fish oil on the inflammatory status of a model organism was also not confirmed: fish oil increased expression not only of some genes coding for anti-inflammatory cytokines, but also of some genes coding for pro-inflammatory cytokines, and affected plasma cytokine profile only insignificantly.

In conclusion, as far as dietary fish oil is concerned, its effect on tissue fatty acid deposition is undeniable, but its effect on plasma markers related to the risk of chronic degenerative diseases requires further research.

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