EFFECT OF DIETARY FISH OIL ON SELECTED MARKERS OF AN INFLAMMATORY STATUS IN PIGS

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Abstract: The objective of the experiment was to test a hypothesis that the biologically active substances present in fish oil (eicosapentaenoic acid, EPA, and docosahexaenoic acid, DHA) are able to stabilize inflammatory status in an organism. Thirty two pigs (Large White x Landrace) at the age of eight weeks with the mean live weight of 25.5 kg were used as a model organism. The pigs were divided into two groups with 16 animals each; the experimental and control group was fed the basic feed mixture with 2.5% of fish oil (F) and 2.5% of palm oil (P), respectively. The F – and P – pigs were randomly divided into two groups 70th day of fattening, and eight F – and eight P – pigs were treated with E. coli lipopolysaccharide (LPS). After anesthetizing, pigs were sacrificed by bleeding, the blood and liver samples were taken, and expression of the liver genes coding for 7 selected cytokines and plasma concentration of adiponectin and three cytokines was determined. No significant effect (P > 0.05) of dietary intervention on feed intake, live weight and live weight gain was found. Fish oil tended to increase (P > 0.05) relative expression of all tested cytokine genes, the effect being significant (P < 0.05) in the case of IL–6 and TGF–β1 after LPS application. Fish oil also increased (P < 0.05) plasma concentration of TNFα in pigs treated with LPS. On the other hand, fish oil tended to decrease (P = 0.22) plasma adiponectin in comparison with palm oil. The present study did not confirm anti-inflammatory effect of fish oil.

Key Words: cardiovascular diseases, eicosapentaenoic acid, docosahexaenoic acid, cytokines, adiponectin

INTRODUCTION

Dietary docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), present in high quantities e.g. in fish oil, are able to modulate, among other things, chronic low-grade inflammation (Calder 2013), one of the hallmarks of atherosclerosis, which is a basis of cardiovascular diseases. EPA and DHA are endogenous ligands of the transcription factor peroxisome proliferator-activated receptor gamma (PPARγ). PPARγ ligation increases an amount of adiponectin, adipose tissue anti-inflammatory hormone (Siriwardhana et al. 2013). Anti-inflammatory effect of EPA/DHA is further mediated by a G - protein coupled receptor-sensor GPR120, whose activation leads to a repression of the macrophage induced inflammation (Flock et al. 2013). This repression is caused by inhibition of the signaling pathway of the transcription factor NF – κB (nuclear factor kappa B) (Calder 2012). Positive effects of EPA/DHA were mostly obtained by in vitro studies using higher-than-physiological EPA/DHA concentrations (Yates et al. 2014).

The objective of the present study was to use pigs as a model organism for testing a hypothesis that fish oil is able to stabilize inflammatory markers. Due to the fact that the length of the experiment was limited, it has not been possible to induce low-grade chronic inflammation status in experimental animals. Therefore a more robust intervention via lipopolysaccharide (LPS) intervention at the end of the experiment was necessary. The rationale behind the tested hypothesis was that fish oil (i.e. EPA and
DHA) increases plasma adiponectin and decreases nuclear fraction of the transcription factor NF – κB with a consequence of modulation of the pro- and anti-inflammatory cytokine plasma levels.

**MATERIAL AND METHODS**

**Animals, dietary interventions, analyzed tissues**

Thirty-two pigs of both sexes (16 males, 16 females; Large White x Landrace) (Bioprodukt Knapovec a.s., Ústí nad Orlicí, 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 m²) of four animals each.

The experiment was performed in compliance with the Czech National Council Act No. 246/1992 Coll. to protect animals against cruelty, the Amended 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 experimental group was fed the basic feed mixture with 2.5% of fish oil (F) and the control group was fed the basic feed mixture with 2.5% of palm oil (P) (amount which the animals are able to consume according to their weight). Both F and P diet contained in one kg 138 g of crude protein, 56 g of fat, 48 g of crude fibre and 758 g of nitrogen-free extractives. Metabolizable energy content was 13.6 MJ/kg.

Content of quantitatively and physiologically important fatty acids in the F and P diet was as follows (g/kg): 14:0 0.92 and 0.96; 16:0 6.79 and 14.78; 18:0 9.56 and 13.97; 18:2n-6 11.55 and 10.61; 18:3n-3 0.88 and 0.77; 20:5n-3 2.83 and 0.05; 22:5n-3 0.58 and 0.04; 22:6n-3 4.34 and 0.05, respectively. The animals had free access to the drinking water and were fed daily ad libitum. The fattening lasted for 70 days.

**Lipopolysaccharide (LPS) treatment**

Last (i.e. 70th) day of fattening the F– and P – pigs, respectively, were randomly divided into two groups of eight animals each. E. coli LPS at an amount of 25 μg/kg of live weight (W) was applied i.v. (vena auricularis) to eight F – and eight P – pigs. Three hours after the LPS application (time sufficient for measuring the effect before dead of animals could occur), all pigs were anesthetized by the intramuscular application of the TKX (12.5 mg/ml ketamine, 12.5 mg/ml xylazine, 12.5 mg/ml tiletamine, 12.5 mg/ml zolazepam) mixture and sacrificed by bleeding.

**Blood and liver sample collection**

Blood samples were collected (from the aorta) to the heparin-coated test tubes and the liver samples (300 g) were taken. Blood samples were centrifuged at 200 g for 10 min at 4 °C to obtain plasma. Aliquots of the liver samples (100 g) were freeze-dried and stored at -20 °C for subsequent fatty acid analyses. Total RNA was immediately isolated from another liver aliquots (50 mg).

**Fatty acid analysis**

Fatty acids in the liver samples, and in the diet were determined (after total lipid extraction by the hexane/2-propanol mixture) using the procedure described in our previous study (Komprda et al. 2016a).

**Quantification of cytokine genes expression**

Total RNA isolation, reverse transcription and quantitative PCR was performed according to Komprda et al. (2016a).

**Determination of plasma cytokines and plasma adiponectin**

IL – 1β, IL – 10 and TNFα concentration in the pig plasma was measured by Milliplex® MAP Porcine Cytokine/Chemokine Magnetic Bead Panel kit (Millipore Corporation, Billerica, MA, USA) according to the producer’s recommendation.

Plasma adiponectin was determined by Porcine Adiponectin ELISA (BioVendor, Brno Czech Republic) 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 the 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 12 package (StatSoft, Tulsa, OK, USA) was used.

RESULTS AND DISCUSSION

Feed intake, live weight, daily weight gain

Feed intake of pigs fed the F– and P – diet was 880 and 890 g/day; corresponding values expressed per kg of live weight (W) were 10.5 and 10.6 g · kg/W/d, respectively. Daily weight gain and the final live weight of the F– and P – pigs was 0.85 ± 0.05 kg/day and 0.86 ± 0.04 kg/day, and 83.64 ± 1.82 kg and 84.06 ± 3.35 kg, respectively. No significant effect of the type of dietary oil on any of the above-mentioned traits was established (P > 0.05).

Expression of the cytokine genes

Relative expression of the liver genes coding for selected pro- and anti-inflammatory cytokines is presented in Figure 1.

Plasma cytokine and adiponectin levels

Plasma concentration of selected pro-inflammatory (IL – 1β; TNFα) and anti-inflammatory (IL – 10) cytokines is shown in Figure 2. An increase of TNFα, one of the acute phase proteins in the plasma of the F – pigs after LPS application is contrary to the hypothesis of a fish oil anti-inflammatory effect. In corresponding human studies, level of pro-inflammatory cytokines (IL – 6, TNFα) was low and not affected by EPA and DHA in obese patients in an experiment of Labonté et al. (2013); fish oil had no effect on TNFα and IL – 6 in overweight subjects (Bragt and Mensink 2012). No effect of EPA and DHA on IL – 1β, IL – 6 and TNFα in healthy volunteers reported Skulas–Ray et al. (2011).
Regarding adiponectin, fish oil tended ($P = 0.22$) to decrease plasma adiponectin in comparison with palm oil. Again, this finding is contrary to the tested hypothesis, and is also contrary to our previous finding in rats (Komprda et al. 2016b). On the other hand, as compared to the status before the LPS application, fish oil tended ($P = 0.24$) to increase plasma adiponectin after the LPS application, contrary to palm oil (Figure 3).

**Figure 3** Adiponectin level in the plasma of pigs fed a diet supplemented with either 2.5% of fish oil ($F$) or 2.5% of palm oil ($P$); either mean values irrespective of the lipopolysaccharide (LPS) application ($F; P; n = 16$) or the values measured before ($F-; P-$; $n = 8$) and after ($F+; P+; n = 8$) the LPS application, respectively, are presented; $NS$ – not significant

**CONCLUSION**

The tested hypothesis was the influence of fish oil, respectively biologically active substances EPA and DHA, on stabilization of inflammatory status in organism. In our study this hypothesis wasn’t proved.
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REFERENCES


