THE EFFECT OF FISH AND PALM OIL ADDITION ON FATTY ACIDS CONTENT OF PIG TISSUES

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Abstract: Thirty-two piglets at the age of eight weeks were divided into two groups for 60 days of fattening. The experimental group (FO) was fed the basic feed mixture with addition of fish oil (2.5%) and the control group (PO) was fed basic feed mixture with addition of palm oil (2.5%). Fish oil is characterized by high proportion of n–3 polyunsaturated fatty acids. Palm oil has high level of palmitic, oleic and linoleic acids. At the end of fattening period, fatty acids content in the liver, muscle and adipose tissues were determined. FO-diet caused increase of PUFA n–3 in all observed tissues (P < 0.01). PO-diet had effect only in adipose tissue, where significant increase of palmitic, stearic and linoleic acids was found (P < 0.05).

Key Words: fatty acids, EPA, DHA, pig, fish oil, palm oil, liver, adipose tissue, muscle

INTRODUCTION
Component of total lipids in the human nutrition is important, because it depends on representation of fatty acids. Its chemical composition determines their physiological effects. Fatty acids are divided to groups according to number of double bounds in a molecule (saturated fatty acids – SFA, no double bound; monounsaturated fatty acids – MUFA, 1 double bound; polyunsaturated fatty acids – PUFA, 2 and more double bounds).

From the point of view of human nutrition are important PUFA n–3 and n–6 groups, which are characterized by different physiological effects. Representative of PUFA n–3 is essential α-linolenic acid (LNA; 18:3n–3) and their important metabolites eicosapentaenoic acid (EPA; 20:5 n–3) and docosahexanoic acid (DHA; 22:6n–3). The family of PUFA n–6 contains essential fatty acid linoleic acid (LA; 18:2n–6) and it`s important metabolite arachidonic acid (AA; 20:4n–6). The final metabolites of AA and EPA and DHA, respectively, are eicosanoids, which play important role in vasodilatation, resistance, evolution, inflammatory response and cholesterol homeostasis (Mourek 2007).

Consumption of long-chain polyunsaturated fatty acids of the n–3 group (EPA, DHA) is a possible dietary strategy to decrease risk of diseases with a chronic inflammation (Givens and Gibbs 2008). In human nutrition, ratio of PUFA n–6/n–3 under 5 : 1 or lower is recommended (Kouba and Mourot 2011). One of the possibilities to increase PUFA n–6/n–3 ratio is consumption of fish oil due to its amount of EPA and DHA.

Nowadays it is discussed using of palm oil, which has higher content of saturated fatty acids and very low content of PUFA n–3. Based on their different percentages of fatty acids we used these oils on our project for dietary intervention on animal model organism close to human – pig.

The aim of the research was to evaluate in a model organism an effect of fatty acids in animal diet on their deposition in physiologically important tissues, which could be applied to human nutrition.
MATERIAL AND METHODS
The experiment was carried out on 32 piglets (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. The pigs were housed in an experimental stable in floored indoor pens 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.

Average ambient temperature and relative humidity were 19 ± 3°C and 55 ± 10%, respectively. The animals were allocated into two groups based on individual live body weight and sex. The experimental group (FO) was fed the basic feed mixture with addition of fish oil (2.5%) and the control group (PO) was fed basic feed mixture with addition of palm oil (2.5%). During the course of the experiment (60 days) the pigs were fed ad semi-libitum.

At the end of the experiment, the pigs were anesthetized by the intramuscular application of the TKX (12.5 mg/ml ketamine, 12.5 mg/ml xylazine, 12.5 mg/ml tiletamin, 12.5 mg/ml zolazepam) and sacrificed by bleeding. Aliquots of the liver, adipose tissue and muscle (m. quadriceps femoris; 25 g, 25 g, and 10 g, respectively) were taken and stored at -20 ºC for fatty acids analysis.

Tissue lipids and fatty acids determination
Total lipids were extracted by hexane/isopropanol solvent according to Komprda et al. (2015). Derivatization of the samples were performed according to Komprda et al. (2013). Methyl esters of fatty acids (FAME) were separated using an Fisons GC 8000 series chromatograph with capillary column DB-23 (60 m x 0.25 nm x 0.25 µm; Agilent Technologies, J&W Scientific, USA). The injector was heated to 250 °C and detector (FID) to 260 °C. The temperature program was 140 °C/1 min, gradient 5ºC/min to 200 °C held for 1 min, gradient 3 °C/min to 240 °C and held for 15 min. The carrier gas was nitrogen, flow rate of 1.5 mL/min, the pressure was 200 kPa and split ratio was 20 : 1. FAME identification was performed by using GLC–455 reference standards (Nu–Chek–Prep, USA). The content of fatty acids (FA) in fish and palm oil, respectively, was expressed as percentage of the sum of determined fatty acids (Table 1). FA content in the diet and in the analyzed tissues was expressed in mg/100 g of the diet or the analyzed tissues.

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>14:0</th>
<th>16:0</th>
<th>16:1</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2n6</th>
<th>18:3n3</th>
<th>20:2n6</th>
<th>20:3n6</th>
<th>20:4n6</th>
<th>20:5n3</th>
<th>22:4n3</th>
<th>22:5n3</th>
<th>22:6n3</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>5.6</td>
<td>15.3</td>
<td>11.7</td>
<td>2.6</td>
<td>25.1</td>
<td>3.5</td>
<td>1.6</td>
<td>0.7</td>
<td>0.2</td>
<td>0.7</td>
<td>11.8</td>
<td>0.3</td>
<td>2.1</td>
<td>18.5</td>
</tr>
<tr>
<td>P</td>
<td>1.5</td>
<td>40.3</td>
<td>0.2</td>
<td>1.6</td>
<td>43.8</td>
<td>11.3</td>
<td>0.3</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
</tr>
</tbody>
</table>

Legend: F - fish oil; P - palm oil

The differences in absolute amount of fatty acids in the analyzed tissues and pig’s feed (FO, PO) were evaluated by one-way analysis of the variance ratio test including post–hoc Tukey’s test using STATISTICA 12 package (StatSoft, USA).

RESULTS AND DISCUSSION
The experiment was focused on the effect of dietary fish oil and palm oil, respectively on deposition of fatty acids in the tested tissues. The composition of fatty acids in the diets with added palm oil and fish oil is shown in table 2.
Table 2 The composition of fatty acids in pig diets (mg/100 g of fresh matter ± mean error)

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>FO</th>
<th>PO</th>
</tr>
</thead>
<tbody>
<tr>
<td>C 14:00</td>
<td>92±3.8</td>
<td>34±2.2</td>
</tr>
<tr>
<td>C 16:00</td>
<td>679±1.9</td>
<td>1472±26.9</td>
</tr>
<tr>
<td>C 16:1</td>
<td>186±5.7</td>
<td>43±6.7</td>
</tr>
<tr>
<td>C 18:00</td>
<td>130±49.8</td>
<td>65±6.7</td>
</tr>
<tr>
<td>C 18:1</td>
<td>957±65.1</td>
<td>1389±6.7</td>
</tr>
<tr>
<td>C 18:2n6</td>
<td>1156±0.5</td>
<td>1356±4.5</td>
</tr>
<tr>
<td>C 18:3n3</td>
<td>88±9.3</td>
<td>76±6.1</td>
</tr>
<tr>
<td>C 20:2n6</td>
<td>11±0.1</td>
<td>4±0.2</td>
</tr>
<tr>
<td>C 20:3n6</td>
<td>4±0.5</td>
<td>2±1.2</td>
</tr>
<tr>
<td>C 20:4n6</td>
<td>17±1.9</td>
<td>11±2.2</td>
</tr>
<tr>
<td>C 20:5n3</td>
<td>176±3.8</td>
<td>4±0.0</td>
</tr>
<tr>
<td>C 22:4n3</td>
<td>8±0.2</td>
<td>4±0.0</td>
</tr>
<tr>
<td>C 22:5n3</td>
<td>36±1.9</td>
<td>9±0.0</td>
</tr>
<tr>
<td>C 22:6n3</td>
<td>270±1.9</td>
<td>4±0.0</td>
</tr>
</tbody>
</table>

Legend: FO – basic feed mixture with addition 2.5% fish oil; PO – basic feed mixture with addition 2.5% palm oil; A, B – means with different superscripts differ at P < 0.01; one way ANOVA with post hoc Tukey’s test; n = 4.

The PO group had twice higher intake of palmitic acid (P < 0.01) oleic acid and linoleic acid than the FO group. Addition of fish oil to basic feed mixture caused significant increase (P < 0.01) of content of polyunsaturated fatty acids n–3 (eicosapentaenoic acid–EPA, docosatetraenoic acid–DTA, docosapentaenoic acid–DPA, docosahexaenoic acid–DHA) compared with addition of palm oil.

The composition of fatty acids in liver, muscle and adipose tissues of animals with different addition of oils are shown in Figures 1–5.

Figure 1 Fatty acid content in the liver of pigs (mg/100 g of liver)

Legend: FO – basic feed mixture with addition of 2.5% fish oil; PO – basic feed mixture with addition of 2.5% palm oil; ** – differences significant at P < 0.01; one way ANOVA with post hoc Tukey’s test; n = 32; NS – not significant (P > 0.05).

The differences between the dietary groups in saturated FA and monounsaturated fatty acids, respectively were not significant (P > 0.05). Ayleso et al. (2012) also did not report increase of saturated fatty acids in the liver tissue due to addition of palm oil to the feeding mixture. Significant differences were found in the present experiment between amounts of n-3 polyunsaturated fatty acids. The increase of EPA, DPA and DHA was significant (P < 0.01) in the liver tissue of FO group in comparison with the PO group.
The type of the added dietary oil did not influence content of saturated and monounsaturated fatty acids either in the liver (Figure 1) or muscle (Figure 2).

Addition of fish oil significantly ($P < 0.01$) increased amount of arachidonic, eicosapentaenoic, docosatetraenoic, docosapentaenoic and docosahexaenoic acids in comparison with PO group.

Addition of palm oil to the animal diet increased significantly ($P < 0.01$), stearic ($P < 0.05$), linoleic ($P < 0.05$) and eicosadienoic ($P < 0.01$) acids in adipose tissue of the PO group.
The FO group had higher amounts of EPA, DTA and DHA (P < 0.01) than the PO group. In pigs, the composition of fatty acids stored in adipose tissues largely reflects that of ingested lipids (Kouba and Mourot 2011).

The addition of palm oil increased level of linoleic acid (PUFA n–6; table 1) in the feed mixture. On the other hand, fish oil had higher amount of α-linolenic acid (LNA), EPA, DTA and DHA (PUFA n–3; table 1). The ratio of PUFA n–6 and n–3 is important for nutritional point of view on the grounds of production of eicosanoids and other biochemically active molecules, which has affect on cholesterol homeostasis, blood vessels, arthritis and inflammatory responses. The ratio of PUFA n–6 and PUFA n–3 in the tested tissues of is shown in Figure 6.

All tissues had significantly lower ratio (P < 0.01) of PUFA n-6/PUFA n–3 in the FO group compared with the PO group. Liver tissue had recommended ratio (5 : 1) in the PO group, but even lower ratio in the FO group. Harnack et al. (2009) suggested the LA/LNA ratio 1 : 1 positive for formation their metabolites. Determining content of PUFA in the liver of model animals, including the ratio of n–6/n–3 is significant for evaluation of cholesterol metabolism with the consequence of estimated risk of cardiovascular diseases (Komprda et al., 2015). The effect of fish oil to decrease ratio of n–6/n–3 in animal tissues was also confirmed by Feillet-Coudray et al. (2013).

CONCLUSION
The addition of fish oil affected the deposition of fatty acids in tested tissues. Deposition of EPA, DPA, DHA significantly increased (P < 0.01) in the liver, muscle and adipose tissues under the fish oil diet rich in PUFA n–3 compared with the control group (PO). Increased amount of PUFA n–3 in the diet
(FO) decreased the ratio of PUFA n–6/n–3 to a recommended value, which is associated with reduced risk of cardiovascular diseases. On the other hand, palm oil added to the feeding mixture increased (P < 0.01) amount of arachidonic acid in the liver and muscle tissues with no effect on saturated fatty acids, especially palmitic acid. Fatty acids content in adipose tissue mirrors dietary intake of these fatty acids.

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REFERENCES


