Assessment of Fatty Acid Allocation in Plasma and Tissues in Piglets, Using Feed Supplemented with Byproducts from Processed Olive Mill Wastewater

KONSTANTINOS GERASOPOULOS1,2, DIMITRIOS STAGOS1, ALEXANDROS KROUZEAS1, CHRISTINA KARAVELI1, CHRISTINA BARDA1, HELEN GKIKA1, DIMITRIOS MITSIOI1, KONSTANTINOS PETROTOS2, PANAGIOTIS GOLAΣ2 and DEMETRIOS KOURETAS1

1Department of Biochemistry and Biotechnology, University of Thessaly, Larissa, Greece;
2Department of Biosystem Engineering, Technical Education Institute of Thessaly, Larissa, Greece

Abstract. Background/Aim: A previous study revealed the improvement of redox status in blood and tissues of young piglets (ablationation period), that consumed feed containing polyphenolic additives from byproducts of processed olive mill wastewater (OMWW). The polyphenolic additives strengthened the antioxidant defense of the piglets. Herein we analyzed the fatty acid (FA) composition of these animals in various tissues. Materials and Methods: The steps followed during the analysis were: Preparation and isolation of byproducts containing polyphenolic compounds from OMWW processing, silage and piglet feed preparation, blood and tissue collection, fatty acid methyl esters synthesis and GC/MS analysis. Results: The young piglets, that consumed feed containing polyphenolic additives from byproducts of processed OMWW, were found to have a decreased ω6/ω3 ratio, compared to samples of the control group. For example, in the quadriceps tissue the control group has a ω6/ω3 ratio of 10.1, while in the polyphenolic group this ratio was decreased to 2.93. Regarding the ratio of UFA/SFA, no significant differences were observed. Finally, the polyphenolic group exhibited almost in all tissues lower values of the ratio of PUFA/MUFA than the control group.

Fatty acids (Fas), both free and as complex lipids, are playing key roles in the metabolism (e.g. for storage and transportation of energy), as essential components of all membranes and as gene modulators, usually, produced from triglycerides or phospholipids. Hydrolysis of triglycerides performed by lipases, results in releasing of energy. Through the action of lipases glycerol and fatty acids are produced. When not linked to other molecules, they are known as "free" fatty acids (FFA). These FAs are important energy sources, because when metabolized, they produce large quantities of ATP (nucleoside triphosphate) carrying chemical energy within cells for metabolism. The ratio phosphate/oxygen (P/O), depends on the ratio of H+ transported away from the mitochondrial matrix by 2e− passing NADH to O2 in the electron transport chain and the number of H+ traveling through ATP synthase, to synthesize a ATP (1, 2). A molecule of palmitic acid is produced after the complete oxidation at 106 ATP molecules. The fatty acids are cleaved to acetyl-CoA by oxidation. Also the beta-oxidation generates NADH. The beta-oxidation of fatty acids is the major route for energy release from lipids. The beta-oxidation enzymes are located in the mitochondria in animals and in the peroxisome in plants. At the end of beta-oxidation, generated acetyl-CoA enters in the citric acid cycle or the glycosylation cycle. Many cell types can use either glucose or fatty acids for this purpose. More particularly, in the heart and skeletal muscle fatty acids are preferred. Despite claims to the contrary, other than glucose and ketone bodies, fatty acids are used as energy source for brain cells, at least in some rodents (3, 4). Fats are decomposed in the digestive tract to glycerol and FAs by the enzymes secreted therein. FAs are the largest and most important part of fat. Full hydrolysis is performed at a rate of 30-45%, while a similar percentage of monoglyceride is hydrolyzed and the rest remain as lipids. By their absorption in the intestinal villi they are completely hydrolyzed, and both the glycerol and

Abbreviations: ATP, Adenosine triphosphate; FA, fatty acid; FAME, fatty acid methyl ester; GC-MS, gas chromatography-mass spectrometry; OMWW, olive mill waste waters; MDA, malondialdehyde; NADH, nicotinamide adenine dinucleotide; NIST, national institute of standards and technology; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TFA, trans fatty acid.

Correspondence to: Demetrios Kouretas, Department of Biochemistry and Biotechnology, University of Thessaly, Ploutonos 26 & Aiolou, Larissa 41221, Greece. Tel: +30 2410565277, Fax: +30 2410565293, e-mail: dkouret@uth.gr

Key Words: Olive mill wastewater, antioxidant feed, fatty acid, piglet, ω6/ω3 ratio.
the FAs are used in the villi for reconstruction of body fat while others, especially the FAs of carbon atoms less than 12 (C12: 0 and below) move directly to the liver where metabolized without being used to form lipids (5). Out of the FAs in food technology those with a straight aliphatic chain, an even number of carbon atoms and a single carboxyl group are the most interesting.

Lipid oxidation is influenced by many factors: the medium, oxygen concentration, temperature, light, degree of unsaturation, and metal ions among others. In the presence of oxygen, oxidation cannot be entirely prevented nor can it be reversed, but it can be inhibited, delaying the buildup of oxidized products to unacceptable levels. Anti-oxidants can interact with several steps of free-radical or photo oxidation. Their performance is medium and concentration – dependent and requires care as they can also act as prooxidants under certain conditions (6). The most widely used antioxidants are free radical scavengers that remove reactive radicals formed in the initiation and propagation steps of auto-oxidation. Many fatty foods are susceptible to auto-oxidation, involving the oxidation of lipid molecules to produce malodorous ketones, alcohols and acids that adversely affect the texture, flavour and taste of food.

The polyphenols that have been identified in OMWW include hydroxytyrosol and tyrosol as their major components, as well as p-coumaric acid, homovanillic acid, caffeic acid, protocatechuic acid, 3,4-dihydroxymandelic acid, vanillic acid and ferulic acid (7). It should be noted that polyphenols in OMWW contain about 50% of the total polyphenols found in olive fruit (8). Despite the toxic effects exhibited at high concentrations, these polyphenols also possess antioxidant activity (7). The findings of the study (9) suggest that feed containing OMWW byproducts could be used for enhancing the redox status of piglets by reducing the oxidative damage of biological molecules (i.e. protein oxidation, lipid peroxidation) and increasing antioxidant mechanisms. Furthermore, in the present study, by means of gas chromatography-mass spectrometry, the FAs allocation in pigs (plasma and in nine different tissues; brain, cardiac, kidney, liver, lung, pancreas, stomach, spleen and quadriceps muscle) was assessed, at the different tissues; brain, cardiac, kidney, liver, lung, pancreas, stomach, spleen and quadriceps muscle) was assessed, at the

Materials and Methods

Preparation and isolation of byproducts containing polyphenolic compounds from OMWW processing. The byproducts isolation containing polyphenolic compounds from OMWW processing was based on a patented OMWW polyphenol powder production scheme that has been previously described (9).

Silage and piglet feed preparation. OMWW retentate was used for making silage corn. For this purpose, corn was mixed with OMWW retentate at a ratio of 24:1. Thus, silage corn was made that contained 56% solids, 4% OMWW retentate and 40% liquid. Then, standard commercial formulation of lactic bacteria (11CFT Pioneer, Buxtehude, Germany,) was used for the lactic fermentation of corn. The lactic bacteria had been dissolved in water (10% w/v) by stirring and warmed at 40°C in order to be activated prior to mixing with corn. After activation, lactic bacteria were mixed with corn (1 g of bacteria with 100 kg of corn). For producing the silage, the mixture of lactic bacteria and corn was placed in special airtight-seal plastic bags and was fermented for 3-4 weeks. To prevent the bags from rupturing due to the inflation caused by the carbon dioxide production during fermentation, the material was repackaged in new plastic bags every two to three days. Finally, the resulting silages were mixed with other ingredients to make the final piglet feed (Table I).

Blood and tissue collection. At 50 days post birth (i.e. after piglets had been fed with ration for 30 days), 7 blood samples were obtained from each group. For blood collection, piglets were restrained manually and 4 ml of blood was collected from the anterior vena cava and placed in ethylenediamine tetraacetic acid Vacuette® K3 EDTA tubes (VWR, Radnor, PA, USA). Blood samples were centrifuged immediately at 1,370 × g for 10 min at 4°C and the plasma was collected and then stored at −80°C until gas chromatography (GC) analysis.

For tissue collection, the piglets were sacrificed in a fully automated slaughter complex (Slaughterhouses of Larissa S.A., Girtoni, Greece). All relevant procedures (e.g. CO₂ stunning, slaughter, bleeding, skin removal, gutting, viscera separation and washing) were executed by special machines and specialized staff. Tissues were quickly removed and snap-frozen in liquid nitrogen. In preparation for tissue biochemical analysis, mortar and pestle were used for crushing and grinding the samples with the assistance of liquid nitrogen. One part of tissue powder was then homogenized with two parts (weight/volume) of 0.01 M phosphate buffered saline pH 7.4 (138 mM NaCl, 2.7 mM KCl, and 1 mM EDTA) and a cocktail of protease inhibitor tablet (complete mini inhibitor protease cocktail tablet; Roche, Munich, Germany) was added. The homogenate was vigorously vortexed and a brief sonication treatment on ice was applied. The homogenate was then centrifuged at 12,000 × g for 30 min at 4°C and the supernatant was collected. Tissues were then stored at ~80°C until GC analysis.

Fatty acid methyl esters (FAME) synthesis. The method of FAME synthesis (10) was applied as follows: 0.5 ml of sample was homogenized in PBS with addition of proteases inhibitors, by means of a special microspatula placed in heat resistance glass tubing length 16 cm, diameter 1.6 cm that had a teflon screw cap for hermetic seal. Then, 1 ml methanolic solution of tridecanoid acid

Table I. Final piglet feed. Ingredients and nutrient composition of experimental diets.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Composition (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>46.5*</td>
</tr>
<tr>
<td>Soybean</td>
<td>21.0</td>
</tr>
<tr>
<td>Milk powder</td>
<td>20.0</td>
</tr>
<tr>
<td>Pig grower concentrate</td>
<td>10.0</td>
</tr>
<tr>
<td>Balancer (piglet corn)</td>
<td>2.5</td>
</tr>
</tbody>
</table>

*Corn contained 60% solids and 40% liquid in control feed; 56% solids, 4% OMWW retentate and 40% liquid in feed supplemented with OMWW polyphenols.
(C13:0) was added at a concentration of 600 μg/ml, as an internal standard. Subsequently, 10 N 0.4 ml KOH (potassium hydroxide) and 2.7 ml of pure methanol were added. For proper hydrolysis of samples, the tubes by careful secure-screwing were placed in a water bath at 55°C for a period of 1.5 and every 20 min vigorous stirring followed. Cooling with tap water was followed for 15 min. For the correct composition of fatty acid methyl esters, 0.3 ml 24 N H₂SO₄ were added, hermetic screw and the tubes were placed in a water bath at 55°C for a period of 1.5 h, followed by vigorous stirring every 20 min. Cooling with tap water was followed for 15 min. Finally, 3 ml hexane were added as solvent and the samples were stirred at vortex for 3 min. Then, placed in the centrifuge 6.000 × g, 15 min at room temperature and the supernatant (the hexane layer containing the FAME), placed in GC vials of 2 ml and stored at -20°C until GC/mass spectrometry (MS) analysis.

**GC-MS analysis.** The fatty acids profile assessment in plasma and nine different tissues (brain, heart, kidney, liver, lung, quadriceps, pancreas, spleen and stomach) of young pigs (50 days old), was determined using the methodology FAME final optimal. A paired calculation was carried out, using the following assay protocol; The assay of concentrations was made using the peak areas after correction with the internal standard and using standard 37 fatty acids supplied by SUPELCO (Sigma-Aldrich, Munich, Germany) known as 37 Component FAME Mix Standard (this certified reference material may be used to identify and quantify key fatty acid methyl esters). To identify the various esters of lipid oxides, the ability of the MS to perform recognition/identification of peaks in conjunction with the number of spectral data available from the NIST (National Institute of Standards and Technology) chromatograph was used. The GC-MS Agilent 7890A GC chromatography apparatus (Agilent, Frankfurt, Germany) was used. GC columns were located inside a temperature-controlled oven. Generally, one end of the column is attached to the inlet, while the other end is attached to the detector. Columns vary in length, diameter, and internal coating. Each column is designed for use with different compounds. The purpose of the column and the oven is to separate the injected sample into individual compounds as it travels through the column. To aid this process, the GC oven can be programmed to speed the sample flow through the column. The silica column Agilent J&W 112-88A7: 804.11246 HP-88 250˚C: 100 m × 250 μm × 0.25 μm (Agilent,) was used. The gas carrying the substances to be separated should be inert and not react with the stationary phase or the analytes. The gas is driven to the column from the high pressure cylinder through the flow regulator. The entire sample is introduced to the importer of the gas chromatograph directly or with an auto sampler, entrained by the carrier gas, along the column in which the separation of the analytes occurs. Fractions of components are led to a computer. Helium gas with 45.2 ml/min flow was used.

**Statistical analysis.** Data were analyzed by one-way ANOVA followed by Dunnett’s test for pairwise comparisons. The level of statistical significance was set at p<0.05. All results are expressed as mean±SEM. Data were analysed using SPSS, version 13.0 (SPSS Inc., Chicago, IL, USA).

**Results**

**Assessment of lipid oxides in plasma.** Seven FAs were detected in the plasma in both groups. According to the retention time these were: palmitic, palmitoleic, stearic, oleic (ω-9), linoleic (ω-6), linolenic (ω-3) and arachidonic acid (ω-6) (Figure 1A). Even though, there were no statistically significant differences between the polyphenolic and control group, the ω6/ω3 ratio in the polyphenolic group was 8.71 and in the control group was 9.12 (Table II). The FAs allocation in the control group was: SFA 34.5%, MUFA 26.4% and PUFA 39.1%, while in the polyphenolic group it was: SFA 32.3%, MUFA 29.1% and PUFA 38.6%.

**Assessment of lipid oxides in tissues**

**Brain.** Seven FAs were detected in the control group: palmitic, palmitoelaidic, stearic, oleic (ω-9), linoleic (ω-6), linolenic (ω-3) and arachidonic acid (ω-6). In the polyphenolic group two more FAs were detected: palmitoleic and eicosepentaenoic (timnodonic) acid (ω-3), a PUFA that acts as a precursor for prostaglandin-3 (that inhibits platelet aggregation), thromboxane-3 and leukotriene-5 eicosanoids. In most brain FAs, there were statistically significant differences between the polyphenolic and control group (Figure 1B). Even if the ω6/ω3 ratio in the control group is as low as 2.60, in the polyphenolic group it is getting lower and reaches 1.28 (Table II). The FAs allocation in the control group was: SFA 37.8%, MUFA 36.8% and PUFA 25.4%, while in polyphenolic group it was: SFA 40.9%, MUFA 25.4% and PUFA 33.7%.

**Heart.** Sixteen FAs were detected in the control group: myristic, pentadecanoic, palmitic, palmitoleic, margaric, palmitoelaidic (ω-9), stearic, oleic (ω-9), linoleic (ω-6), linolenic (ω-3), eicosenoic (gondoic) (ω-9), dihomo-γ-linolenic (ω-6), arachidonic (ω-6), eicosepentaenoic (timnodonic) (ω-3), tetracosenoic (nervonic) (ω-9) and ducosahexuenoic acid (cervonic) (ω-3). In the polyphenolic group one more FA was detected and this was the arachidic acid in a low percentage (Figure 2A). In six (6) heart FAs, there were statistically significant differences between the polyphenolic and control group, especially in the ω-3FAs. The ω6/ω3 ratio in the control group was 9.53 and in the polyphenolic group drastically dropped to 2.24 (Table II). The FAs allocation in the control group was: SFA 39.4%, MUFA 36.8% and PUFA 25.4%, while in polyphenolic group it was: SFA 40.9%, MUFA 25.4% and PUFA 33.7%.

**Kidney.** Seventeen FAs were detected in the control group: pentadecanoic, palmitic, palmitoleic, margaric, palmitoelaidic (ω-9), stearic, oleic (ω-9), linoleic (ω-6), linoleic (ω-6), arachidic, linolenic (ω-3), eicosenoic (gondoic) (ω-9), dihomo-γ-linolenic (ω-6), arachidonic (ω-6), eicosepentaenoic (timnodonic) (ω-3), tetracosenoic (nervonic) (ω-9) and ducosahexuenoic acid (cervonic) (ω-3). In the polyphenolic group one more FA was detected and this was the myristic acid (Figure 2B). In ten FAs, there were statistically significant differences between the polyphenolic and control group, especially in the ω-3FAs. The ω6/ω3...
ratio in the control group was 4.79 and in the polyphenolic group was decreased to 2.25 (Table II). The FAs allocation in the control group was: SFA 38.3%, MUFA 26.8% and PUFA 34.9%, while in polyphenolic group it was: SFA 38.4%, MUFA 33.2% and PUFA 28.4%.

Liver. Seventeen FAs were detected, both in the control and polyphenolic groups: myristic, pentadecanoic, palmitic, palmitoleic, margaric, palmitoelaidic (ω-9), stearic, oleic (ω-9), linoleic (ω-6), arachidic, linolenic (ω-3), eicosenoic (goedoic) (ω-9), dihomo-γ-linolenic (ω-6), arachidonic (ω-6), eicosenoic (timnodonic) (ω-3), tetracosenoic (nervonic) (ω-9) and docosahexaenoic acid (cervonic) (ω-3). (Figure 3A). The ω6/ω3 ratio in the control group was 2.79 and in the polyphenolic group it dropped to 2.72 (Table II). The FAs allocation in the control group was: SFA 41.8%, MUFA 23.7% and PUFA 34.5%, while in polyphenolic group it was: SFA 37.6%, MUFA 33.3% and PUFA 29.1%.

Lung. Fourteen FAs were detected in the control group: myristic, pentadecanoic, palmitic, palmitoleic, margaric, palmitoelaidic (ω-9), stearic, oleic (ω-9), linoleic (ω-6), arachidic, dihomo-γ-linolenic (ω-6), arachidonic (ω-6), eicosenoic (timnodonic) (ω-3), and docosahexaenoic acid (cervonic) (ω-3). In the polyphenolic group one more FA was detected and this was the tetracosenoic (nervonic) (ω-9) acid (Figure 3B). The ω6/ω3 ratio in the control group was 3.14 and in the polyphenolic group was 3.00 (Table II). The FAs allocation in the control group was: SFA 50.9%, MUFA 27.9% and PUFA 21.2%, while in polyphenolic group it was: SFA 50.6%, MUFA 29.4% and PUFA 20.0%.
Quadriceps. Seventeen FAs were detected, both in the control and polyphenolic groups: myristic, pentadecanoic, palmitic, palmitoleic, margaric, palmitoelaidic (ω-9), stearic, oleic (ω-9), linoleic (ω-6), arachidic, linolenic (ω-3), eicosenoic (gondoic) (ω-9), dihomo-γ-linolenic (ω-6), arachidonic (ω-6), eicosepentaenoic (timnodonic) (ω-3), tetracosenoic (nervonic) (ω-9) and ducosahexuenoic acid (cervonic) (ω-3) (Figure 4A). The ω6/ω3 ratio in the control group was 10.15 and in the polyphenolic group drastically dropped to 2.91 (Table II). The FAs allocation in the control group was: SFA 26.3%, MUFA 50.4% and PUFA 23.3%, while in the polyphenolic group it was: SFA 34.3%, MUFA 42.7% and PUFA 23.0%.

Pancreas. Seventeen FAs were detected, both in the control and polyphenolic groups: myristic, pentadecanoic, palmitic, palmitoleic, margaric, palmitoelaidic (ω-9), stearic, oleic (ω-9), linoleic (ω-6), arachidic, linolenic (ω-3), eicosenoic (gondoic) (ω-9), dihomo-γ-linolenic (ω-6), arachidonic (ω-6), eicosepentaenoic (timnodonic) (ω-3), tetracosenoic (nervonic) (ω-9) and ducosahexuenoic acid (cervonic) (ω-3) (Figure 4B). The ω6/ω3 ratio in the control group was 10.51 and in the polyphenolic group drastically dropped to 2.17 (Table II). The FAs allocation in the control group was: SFA 40.4%, MUFA 42.0% and PUFA 23.3%, while in the polyphenolic group it was: SFA 44.3%, MUFA 29.6% and PUFA 26.1%.

Spleen. Seventeen FAs were detected, both in the control and polyphenolic groups: myristic, pentadecanoic, palmitic, palmitoleic, margaric, palmitoelaidic (ω-9), stearic, oleic (ω-9), linoleic (ω-6), arachidic, linolenic (ω-3), eicosenoic (gondoic) (ω-9), dihomo-γ-linolenic (ω-6), arachidonic (ω-6), eicosapentaenoic (timnodonic) (ω-3), tetracosenoic (nervonic) (ω-9) and ducosahexuenoic acid (cervonic) (ω-3) (Figure 5A). The ω6/ω3 ratio in the control group was 2.65 and in the polyphenolic group was 2.17 (Table II). The FAs allocation in the control group was: SFA 48.3%, MUFA 21.0% and PUFA 30.7%, while in the polyphenolic group it was: SFA 44.3%, MUFA 29.6% and PUFA 26.1%.

Stomach. Seventeen FAs were detected, both in the control and polyphenolic groups: myristic, pentadecanoic, palmitic, palmitoleic, margaric, palmitoelaidic (ω-9), stearic, oleic (ω-9), linoleic (ω-6), arachidic, linolenic (ω-3), eicosenoic (gondoic) (ω-9), dihomo-γ-linolenic (ω-6), arachidonic (ω-6), eicosapentaenoic (timnodonic) (ω-3) and tetracosenoic (nervonic) (ω-9) (Figure 5B). The ω6/ω3 ratio in the control group was 7.45 and in the polyphenolic group drastically dropped to 2.54 (Table II). The FAs allocation in the control group was: SFA 39.2%, MUFA 34.5% and PUFA 26.3%, while in the polyphenolic group it was: SFA 38.7%, MUFA 34.0% and PUFA 27.3%.

Discussion

Industrialized societies are characterized by an increase in energy intake and decrease in energy expenditure, an increase in SFA, ω-6 FAs and TFAs and a decrease in ω-3 FAs intake (11). The beneficial health effects of ω-3 fatty acids, eicosapentaenoic acid
(EPA) and docosahexaenoic acid (DHA) were first described in Greenland Eskimos who consumed a seafood diet and had low rates of coronary heart disease, asthma, type 1 diabetes mellitus, and multiple sclerosis. Since that observation, the beneficial health effects of ω-3 FAs have been extended to include benefits related to cancer, inflammatory bowel disease, rheumatoid arthritis, and psoriasis (12).

In recent years it has become apparent that the oxidation of lipids, or lipid peroxidation, is a crucial step in the pathogenesis of several disease states in adult and infant patients. Lipid peroxidation is a process that occurs naturally in small amounts in the body, mainly due to the effect of several reactive oxygen species (e.g. hydroxyl radical, hydrogen peroxide etc.). It can also be caused by the activity

Figure 2. Fatty acids allocation, in heart (A) and kidney tissue (B). *Significantly different from values of control group at the same sampling p<0.05. Results are presented as mean±SEM.
of phagocytes. These reactive oxygen species readily attack the polyunsaturated fatty acids of the fatty acid membrane, initiating a self-propagating chain reaction (13).

According to the Joint FAO/WHO (Food and Agriculture Organization/World Health Organisation) expert consultation on fats and FAs in nutrition, November 10-14, 2008, WHO HQ, Geneva, there exists convincing evidence that: replacing SFA (C12:0–C16:0) with PUFA decreases LDL cholesterol concentration and the total/HDL cholesterol ratio. A similar but smaller effect is achieved by replacing these SFA with

Figure 3. Fatty acids allocation, (A) in liver tissue and (B) in lung tissue. *Significantly different from values of control group at the same sampling time p<0.05. Results are presented as mean±SEM.
MUFA. Replacing SFA (C12:0–C16:0) with trans-fatty acids (TFA) decreases HDL cholesterol and increases the total /HDL cholesterol ratio. Based on coronary heart disease (CHD) morbidity and mortality data from epidemiological studies and controlled clinical trials (using CHD events and death), it was also agreed that: there exists convincing evidence that replacing SFA with PUFA decreases the risk of CHD. Therefore, it is recommended that SFA should be replaced with PUFA (ω-3 and ω-6) in the diet and the total intake of SFA does not exceed 10% E (energy percentage). Based on both the scientific evidence and conceptual limitations, there is no compelling scientific rationale for the recommendation of a specific ratio of ω-6 to ω-3 FAs or LA (linoleic acid) to ALA (α linolenic acid). The ω-3 and ω-6

Figure 4. Fatty acids allocation, (A) in quadriceps tissue and (B) in pancreas tissue. *Significantly different from values of control group at the same sampling time *p＜0.05. Results are presented as mean±SEM.
FAs are important components of cell membranes and a precursor of many substances in the body, such as substances involved in regulating blood pressure and inflammatory response of the body. There is evidence that ω-3 FAs help protect against heart disease and are known for their anti-inflammatory effects, which play an important role in the above, but also in many other diseases (14). In the body the ALA and LA compete for their metabolism by the delta-6-desaturase enzyme. The above fact is considered important to health, since high uptake LA could reduce the amount of enzyme available for the metabolism of ALA, thereby increasing the risk for cardiovascular diseases. The foregoing theory was based on data showing that the last 150 years, the ω-6 intakes were increased, whereas the intake of ω-3

![Figure 5. Fatty acids allocation, (A) in spleen tissue and (B) in stomach tissue. *Significantly different from values of control group at the same sampling time p<0.05. Results are presented as mean±SEM.](image-url)
decreased alongside increasing heart disease. Consequently, an idea of having an "ideal" ratio of \( \omega-6 \) to \( \omega-3 \) FAs was developed (15).

Administration of feed supplemented with OMWW retentate reduced lipid peroxidation as demonstrated by reduction in TBARS levels in plasma and all tissues (1). Inhibition of lipid peroxidation is important, since it has been reported to affect pig productivity and health (16). Moreover, lipid peroxidation is one of the primary causes leading to meat’s quality deterioration, while it may also result in production of toxic compounds (17). It is also believed that lipid peroxidation and protein oxidation, especially when occurring in meat products, are interrelated processes at which the former probably takes place faster and subsequently induces the latter (18). Due to the increased amounts of \( \omega-6 \) FAs in the Western diet, the eicosanoid metabolic products from AA (arachidonic acid), specifically prostaglandins, thromboxanes, leukotrienes and lipoxins, are formed in larger quantities than those formed from \( \omega-3 \) FAs, specifically EPA (19). Furthermore, in the present study, the GS analysis in plasma and all tissues showed that the \( \omega-6/\omega-3 \) ratio is getting lower in the polyphenolic group compared to the control group. The quadriceps tissue in the polyphenolic group has a low ratio 2.91 compared with 10.2 of the control group and that is because in the polyphenolic group the presence of EPA is more than five times higher compared to that of the control group. The EPA acts as a precursor for the biosynthesis of eicosanoid groups: prostaglandin-3 (which inhibits platelet aggregation), thromboxane-3 (antithrombotic and vasodilator activity) and leukotriene-5 (cellular communication). In the brain tissue although the ratio \( \omega-6/\omega-3 \) in the control group is low 2.60, in the polyphenolic group further descends and reaches 1.28. Regarding the ratio of UFA/SFA, no significant differences are observed. The polyphenolic group almost entirely shows lower values than the control group in the ratio of PUFA/MUFA. Oleic (18:1 \( \omega-9 \)), linoleic (18:2 \( \omega-6 \)), and palmitic (C16:0) acids accounted for the greatest proportion of the total peak area of monounsaturated, polyunsaturated, and saturated fatty acids, respectively. Interestingly, it has been reported that \( \omega-3 \) fatty acids exerted a significant influence on subcutaneous and intramuscular fat and on meat quality (colour and tenderness) (20). Moreover, the reduction of the dietary \( \omega6/\omega3 \) resulted in nutritionally-enriched meat with higher content of desirable PUFA, especially AA, EPA and DPA (20).

This finding is important, since as mentioned above piglets’ weaning is a stressful event that may cause oxidative stress and subsequently manifestation of pathological conditions (21–23). The use of byproducts from OMWW processing for making animal feeds is also interesting, since OMWW cause serious environmental problems. It is of particular interest that polyphenols contained in the feed of pigs, from byproducts from OMWW, are beneficial for lipid ratio in the meat of pigs (decrease of \( \omega-6/\omega-3 \) ratio). Study of the specific mechanism is one of our aims in the near future.

Conflicts of Interest

The Authors declare that there are no conflicts of interest.

Acknowledgements

The work was funded by the ‘Biotechnology-Nutrition & Environment’ and ‘Molecular Biology and Genetics Applications’ MSc programmes in the Department of Biochemistry & Biotechnology at the University of Thessaly.

References


Received December 4, 2015
Revised January 20, 2016
Accepted February 2, 2016