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# The Effect of Dietary Intake of Omega-3 Polyunsaturated Fatty Acids on Cardiovascular Health: Revealing Potentials of Functional Food

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Additional information is available at the end of the chapter

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## Abstract

Functional food is a food containing components that show beneficial effects on one or more body functions and improve general condition and health or significantly affect lowering of disease risks. This chapter is aimed to examine the effect of dietary intake of omega-3 polyunsaturated fatty acids (n3-PUFA) on cardiovascular health. This chapter presents current knowledge on functional poultry products and the reasons to consume them, omega-3 enrichment of eggs and poultry meat, and the differences in profile of fatty acids in conventional and omega-3-enriched eggs. The second part of the chapter focuses on the metabolism of fatty acids and effectiveness of n-3 PUFA in the improvement of endothelial function, improvement of elasticity of the vascular wall and the anti-inflammatory effects in patients with chronic diseases, such as metabolic syndrome, diabetes mellitus and hypercholesterolemia, and overall effect on cardiovascular health and protection. To achieve long-term protective effects, the functional food should be consumed on daily basis. There are no specific constrains in taking functional food; even more, it can be recommended to athletes and cardiovascular patients. General population can also benefit from eating functional food enriched with n-3 PUFA due to their anti-inflammatory and vascular-protective effects.

**Keywords:** omega-3 fatty acids, enriched eggs and poultry, cardiovascular risk

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## 1. Introduction

Definitions of functional food differ in different parts of the world; however, they all have in common the reference toward food of natural origin that contains ingredients with beneficial

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effect on human health. In the United States, the definition of functional food says that functional food was “natural or processed food that contains known or unknown biologically active ingredients, which in certain, effective and non-toxic concentrations provide clinically proven and documented health benefits for prevention, treatment or healing of chronic diseases” [1]. This way of defining functional food is different from the definition in Europe, which does not mention effects of functional food in the treatment of diseases, but mainly refer to benefits in maintaining good health or reducing the risk of developing diseases. The European Commission document “Scientific Concepts of Functional Foods in Europe” states a working definition saying that food can be considered functional if it is satisfactorily shown that, in addition to appropriate nutritional effects, it has beneficial effects on one or more target functions of an organism, in a way that it is important for improving health condition and general well-being or reducing disease risks. Functional food has to be food (not in the form of pills or capsules) and it has to show its effects when consumed in normal daily amounts [2]. Regardless of different definitions [3], concluded that the main purpose of functional food had to be clear—it improves human health and well-being or general condition of the body.

Functional poultry products refer to meat and poultry eggs enriched with ingredients that have positive influence on human health. Poultry is particularly suitable for functional food products because of their ability to use the physiological and metabolic processes of their body to deposit beneficial ingredients from feed into products, that is, into meat and eggs. Meat and poultry eggs are enriched with functional ingredients (fatty acids, vitamins, and antioxidants) by feeding poultry feed supplemented with increased concentrations of those ingredients. The most common functional poultry products are meat and eggs with increased content of desirable omega-3 fatty acids, vitamin E, selenium, and carotenoids. Poultry meat is rich in protein and low in fat. As of its nutritional composition, it can be considered a dietary foodstuff. It is easily digested and especially recommended for consumption of the elderly and children. If considering all stated nutritional benefits, poultry meat enriched with functional ingredients can be considered as functional product. Chicken meat is available to wide population of consumers because of its price, which is more affordable if compared to red meat. High-quality nutritional composition of chicken meat is also one of the reasons for its frequent consumption, which is especially emphasized in recent years when consumers became more aware about the composition of foods and their effects on health.

Egg is a foodstuff that contains high-quality and easily digestible proteins, where amino acid composition is the most similar to proteins of the human body. Egg proteins are fully exploited in the human body and have greater biological value than meat proteins. Egg yolk contains essential fatty acids, vitamins, and minerals needed for proper functioning of human organism. Egg is considered a natural functional foodstuff because of its nutritional value. When compared to poultry meat, enrichment of eggs with functional ingredients is easier because of the high content of fat in egg yolk [4].

If consuming meat and eggs enriched with functional ingredients, consumers can affect the increase of the content of such functional ingredients in blood and tissue in a natural way, thus avoiding taking in some dietary supplements. The importance of functional ingredients for human health is elaborated further in the text.

## 2. Functional poultry products production

### 2.1. Metabolism of n-3 and n-6 polyunsaturated fatty acids

Fatty acids are constituent parts of fat and oil molecules. Polyunsaturated fatty acids (PUFAs) are divided into two groups of n-3 and n-6, depending on where the first double bond is found in the carbon chain, that is, where hydrogen atoms are missing. Linoleic fatty acid (LA) and arachidonic fatty acid (AA) are typical representatives of the n-6 group, and  $\alpha$ -linolenic fatty acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) represent the n-3 group. The metabolism and the role of PUFA n-6 and n-3 may differ in living organism. Fatty acids of the n-6 and n-3 groups (LA and ALA) cannot be synthesized in an organism and are therefore called essential fatty acids (EFAs).

Importance of designed products (enriched with n-3 PUFA) is found in the fact that LA and ALA (precursors with 18 carbon atoms) may extend in human organism and desaturate into arachidonic acid and DHA. Processes are catalyzed by elongase,  $\Delta^6$ - and  $\Delta^5$ -desaturase [5]. The limiting factor of metabolizing n-6 PUFA and n-3 PUFA is the enzyme  $\Delta^6$ -desaturase. Unfortunately, final conversion into docosapentaenoic acid (DPA) and docosahexaenoic acid is still not clear; however, the important role is attributed to  $\Delta^4$ -desaturase [6]. Infante and Huszagh stated that the biosynthesis of DHA took place in the mitochondria membranes, and biosynthesis of AA, EPA, and DPA occurred in the endoplasmic reticulum [7]. Supported by enzymes of cyclooxygenase (COX) and lipoxygenase (LX) within certain processes, EFA is converted into hormone-like substances called eicosanoids. Numerous studies confirmed that linoleic, linolenic, and oleic acids during biosynthesis compete for the same  $\Delta^6$ -desaturase. It was also found that linolenic acid acted as the inhibitor of n-6 PUFA metabolism. At the same time, 10 times more linoleic acids are required to inhibit metabolism of n-3 PUFA at the same level [6]. LA, ALA, and AA are essential fatty acids for poultry. The greatest importance in the composition of poultry feed should be given to those mentioned fatty acids because they are precursors to eicosapentaenoic acid and docosahexaenoic acid, both of which are also considered as essential for humans. Human organism requires daily intake of 290–390 mg of ALA and 100–200 mg of EPA and DHA. **Figure 1** depicts the metabolic pathways of fatty n-3 PUFA and n-6 PUFA.

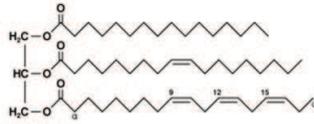
### 2.2. Poultry meat and eggs enriched with n-3 PUFA

The intake of plant sources, especially linseed oil, significantly increases the content of omega-3 fatty acids in the form of ALA; however, they fail to increase the content of long-chain omega-3 fatty acids in meat and eggs. The best sources of long-chain omega-3 PUFA, EPA, and DHA are oils of sea organisms and of fish. The use of these oils is limited because of poorer organoleptic properties of final products [8]. In order to avoid unpleasant odor or taste in meat and eggs, portions of fish oil, as well as of linseed oil in feeding mixtures, must be taken into account.

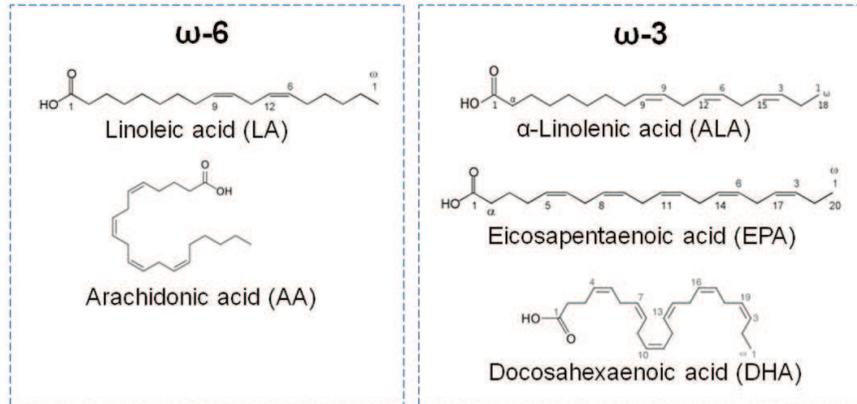
In their research into effects of linseed contained in laying hens' feeding mixtures in different portions (0, 5, 10, and 15%) on the content of ALA in egg yolks, the increase of the content of

**Dietary triglycerids/FFAs**

- a. Saturated FAs
- b. MUFAs
- c. PUFAs, including  $\omega$ -3 and  $\omega$ -6 EFAs:



Digestion



Cells and tissues

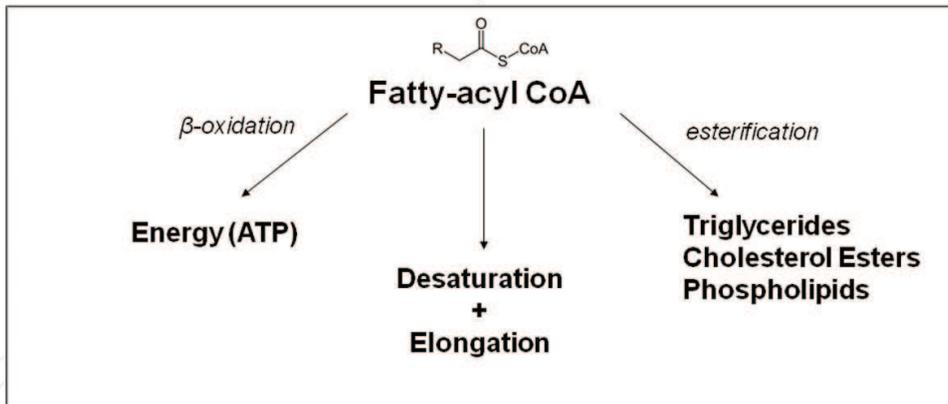


Figure 1. Fatty acids (FAs) source and metabolism.

ALA from 1.80% in the group without linseed to 7.07, 8.35, and 12.20% in the group with the highest content of linseed in feeding mixture [9] was determined. Valavan et al. recorded the increase of ALA content in egg yolks from 0.62% in the control group to 0.83, 0.93, and 1.00% in the experimental groups fed diets supplemented with linseed oil in the amounts of 1, 2, and 3% [10]. They also determined the increase in the content of EPA and DHA. Supplementation of linseed oil in the portion of 5 and 10% to laying hens' feed affected the increase of ALA portion from 0.37% to high 10.3% and 14.9% [11]. These results referring to the increase of ALA content in egg yolk correspond to the fact that linseed and linseed oil are rich in ALA.

Meluzzi et al. stated that the supplementation of 3% fish oil to laying hens' diet influenced the increase of EPA and DHA content in the egg of 19.53 and 143.70 mg/egg [12]. Gonzalez-Esquerria and Leeson pointed out that the supplementation of 6% fish oil to laying hens' diet affected the increase of EPA and DHA contents, as well as the content of total n-3 PUFA, which amounted to 246 mg on average [13]. In their paper about the production of Bio-omega-3 eggs, Imran et al. fed laying hens with mixtures supplemented with extruded linseed and determined that an increased content of extruded linseed in feeding mixtures affected the increase of DHA portion in eggs and reduced AA portion, as well as the ratio of total n-3 PUFA [14]. Apart from oils that can be purchased on the market, there are different commercial preparations, which can be used in poultry feeding in order to achieve the increased content of fatty acids in their products. Kralik et al. investigated the influence of Pronova Biocare EPAX 3000 (PBE), which is rich in fish oil, on the profile of fatty acids in chicken eggs [15]. The authors determined that the replacement of 3.33% of corn in chicken diet with the PBE oil resulted in the reduction of arachidonic fatty acid portion in egg yolks ( $C = 1.66\%$ ,  $E = 0.58\%$ ), and in the increase of EPA ( $C = 0.01\%$ ,  $E = 0.24\%$ ) and of DHA ( $C = 0.72\%$ , and  $E = 1.76\%$ ). Moreover, these authors reported that the n-6/n-3 PUFA ratio was lowered from 14.88 in the control to 7.25 in the experimental group.

The possibility of altering the fatty acid composition of chicken meat is an objective in many studies. Kralik et al. emphasized more favorable ratio of total n-6/n-3 PUFA in thigh muscle lipids of chickens fed diets supplemented with linseed oil, in comparison to the control group that consumed diets with sunflower oil (2.75 and 12.23, respectively) [16]. Since poultry diet is based on corn rich in saturated fatty acids (SFAs), which are then through feed deposited into muscle tissue, feeding mixtures for chickens should be supplemented with linseed or rapeseed or their oils or with fish oil if wanting to enrich their meat with desirable n-3 fatty acids [16–18]. These authors agreed that dietary supplementation of plant oils (linseed and rapeseed oils) instead of sunflower oil affected the increase of n-3 PUFA, and the reduction of n-6 PUFA in poultry meat.

Fish oil or seafood oil are an excellent source of n-3 PUFA fatty acid, such as EPA and DHA. Mirghelenj et al. reported that the increase in the portion of fish oil contained in broiler feed influenced the increase of EPA and DHA fatty acids content in thigh and breast muscles [19]. The content of EPA in breast muscle increased from 0.014 mg/g in the group K to 0.090 mg/g in the group P4, and the content of DHA was raised from 0.046 mg/g in the group K to 0.338 mg/g in the group P4. The authors also reported the increase of EPA in thigh meat, from 0.028 mg/g in the control to 0.232 mg/g in the group P4, while the content of DHA was the lowest in the control (0.0085 mg/g), and the highest in the group P5 (0.578 mg/g). Unlike plant oils used in feeding mixtures, fish oil can negatively affect the organoleptic properties of meat [19, 20].

### **2.3. Specifics of fatty acid profile in conventional and n-3 PUFA-enriched products**

Composition of fatty acids in eggs is influenced by many factors, such as genetic background and age of laying hens, housing system, and composition of feed [11, 21–23]. Simopoulos

reported that eggs produced outdoors in the Peloponnese contained as much as 20 times more n-3 fatty acids than conventional eggs [24]. Huyghebaert et al. confirmed that feed composition significantly affected the profile of fatty acids in egg yolk [25]. The content of DHA in egg yolk is in positive correlation with the content of ALA, EPA, and DHA contained in feed, but in negative correlation with LA. Bavelaar and Beynen pointed out that EPA contained in egg yolk could be modified through laying hens' feed containing EPA, while DHA in yolk might be increased if the feed was rich in ALA or DHA [26]. **Table 1** overviews the results of the authors' own research referring to enrichment of egg yolk with n-3 PUFA.

Fatty acid	Conventional eggs	n-3 PUFA eggs	
	SFO*	SO**	MO***
$\Sigma$ SFA <sup>1</sup>	35.34 ± 1.77	34.72 ± 1.13	31.33 ± 0.64
$\Sigma$ MUFA <sup>2</sup>	41.21 ± 2.03	41.82 ± 2.04	41.86 ± 1.08
$\Sigma$ n-6 PUFA <sup>3</sup>	21.74 ± 1.44	20.85 ± 1.91	19.59 ± 0.83
ALA (C18:3n-3)	0.89 ± 0.18	1.17 ± 0.15	4.73 ± 0.21
ETA (C20:3n3)	0.01 ± 0.005	0.02 ± 0.01	0.03 ± 0.21
EPA (C20:5n-3)	0.01 ± 0.004	0.06 ± 0.02	0.20 ± 0.03
DHA (C22:6n-3)	0.68 ± 0.22	1.35 ± 0.22	2.37 ± 0.18
$\Sigma$ n-3 PUFA	1.68 ± 0.39	2.60 ± 0.18	7.32 ± 0.23
$\Sigma$ n-6 PUFA/ $\Sigma$ n-3 PUFA	12.94	8.02	2.67

\*SFO sunflower oil.  
\*\*SO soybean oil, fish oil.  
\*\*\*MO mixed oil (sunflower oil, soybean oil, linseed oil, fish oil).  
<sup>1</sup> $\Sigma$ SFA: C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C21:0 + C23:0.  
<sup>2</sup> $\Sigma$ MUFA: C14:1 + C16:1 + C18:1n9t + C18:1n9c + C20:1n9 + C22:1n9.  
<sup>3</sup> $\Sigma$ n-6 PUFA: C18:2n6 + C18:3n6 + C20:2n6 + C20:3n6 + C20:4n6 + C22:2n6.

**Table 1.** Profile of fatty acids in yolk lipids of conventional and n-3 PUFA eggs (% of total fatty acids).

Eggs enriched with n-3 PUFA contain 5.3 times more ALA, 20 times more EPA, and 3.5 times more DHA compared to conventional eggs. The sum of n-3 PUFA in enriched eggs is 4.4 times higher than in conventional eggs. Samman et al. analyzed the profile of fatty acids in conventional table eggs bought in a store and omega-3 eggs [27]. They determined that the ratio of n-6/n-3 PUFA in conventional eggs was 11.03, and in eggs enriched with n-3 PUFA only 2.17. Our researches proved that lipids of omega-3 eggs contain less percentage of SFA and n-6 PUFA, and a higher percentage of ALA, EPA, and DHA than conventional eggs. The n-6/n-3 PUFA ratio in conventional eggs was 12.94 and 8.02, respectively, and in omega-3 eggs it was only 2.67. Many health organizations recommend that the n-6/n-3 PUFA ratio shall range between 3:1 and 10:1. In the USA, it is determined as 15:1, and in Japan that ratio is only 1:1 to 3:1. In Croatia, such ratio is quite wide, from 11:1 to 35:1.

Reference	Diet	ALA % of total FA	EPA	DHA
Eggs				
Škrtić et al. [28]	Sunflower oil 6%	0.97	0.01	1.72
	Sunflower oil 4% + fish oil 2%	1.00	0.24	1.76
Kralik et al. [29]	Soybean oil 5%	1.17	–	0.16
	Rapeseed oil 1.5% + fish oil 3.5%	2.31	0.22	2.64
	Rapeseed oil 3.5% + fish oil 1.5%	1.21	0.10	2.23
Kralik et al. [30]	Linseed oil 1.5% + fish oil 3.5%	3.25	0.25	2.99
	Linseed oil 2.5% + fish oil 2.5%	4.33	0.20	2.35
	Linseed oil 3.5% + fish oil 1.5%	5.18	0.18	2.90
Gül et al. [31]	Soybean oil 2%	0.93	0.04	0.93
	Rapeseed oil 2%	0.51	0.68	0.71
	Rapeseed oil 4%	0.03	0.02	0.02
	Rapeseed oil 6%	0.84	1.15	1.55
Broiler breast meat				
Kralik et al. [17]	Sunflower oil 2.5%+ fish oil 2.5%	3.16	0.79	5.62
	Soybean oil 2.5% + fish oil 2.5%	2.37	0.93	6.44
	Rapeseed oil 2.5% + fish oil 2.5%	2.36	1.32	8.95
	Linseed oil 2.5% + fish oil 2.5%	6.25	1.18	5.66
Salamatdoustnobar [32]	Control	0.72	0.75	0.87
	Rapeseed oil 2%	0.37	1.18	2.03
	Rapeseed oil 4%	0.61	0.62	0.75
Galović et al. [33]	Sunflower oil 5%	1.44	0.12	0.96
	Soybean oil 5%	2.63	0.23	0.89
	Rapeseed oil 5%	2.89	0.22	0.90
	Linseed oil 5%	7.71	0.89	1.85
Gajčević [34]	Linseed oil 6%	7.09	0.77	0.90
	Linseed oil 6% + 0.3% Se	8.51	0.73	0.93
	Linseed oil 6% + 0.5% Se	6.78	0.51	0.84

**Table 2.** Supplementation of oils to laying hens' diet and its effect on enrichment of eggs and breast meat with n-3 PUFA.

The data presented in **Table 2** include the efficiency of enriching yolk lipids and broiler breast meat with n-3 PUFA, as reported by various authors. Most authors used feeding treatment with sunflower or soybean oil in the control groups, and for the purpose of enriching eggs with n-3 PUFA, those authors used rapeseed, linseed, and fish oils, as well as their combinations.

Results of their research showed that the most efficient enrichment of eggs with n-3 PUFA was achieved by supplementing fish oil to laying hens' diet, as well as by combining fish, rapeseed, and soybean oils. Combination of sunflower oil and fish oil was less effective in the deposition of n-3 PUFA in yolk lipids, if compared to treatments with a combination of fish oil and other plant oils. Enrichment of broiler breast meat with the n-3 PUFA was also more successful when supplementing fish oil to diets. The best deposition of EPA and DHA in breast meat (1.32 and 8.95%, respectively) was achieved in feeding treatment with 2.5% fish oil and 2.5% rapeseed oil, thus achieving 1.67 times more EPA and 1.59 times more DHA than in feeding treatment with a combination of fish oil and sunflower oil [17].

When enriching products with EPA and DHA by using particular plant oils: sunflower, soybean, rapeseed, and linseed oils supplemented in the amount of 5% in feed, and the best efficiency was proven with linseed oil, which achieved 0.89% EPA and 1.85% DHA in muscle lipids, being 7.41 and 1.92 times more than in the control group with sunflower oil [33]. Selenium supplemented to broiler feed in the amount of 3 and 6% did not have influence on enriching breast meat with n-3 PUFA.

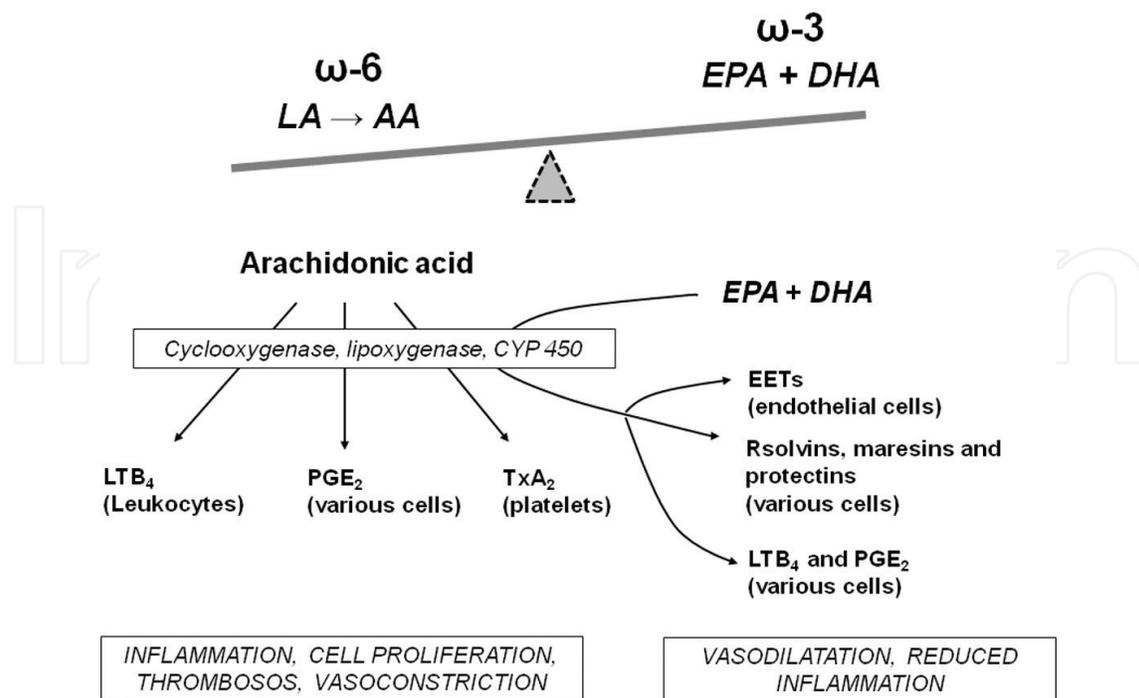
Soybean and rapeseed oils are rich in monounsaturated fatty acids (MUFA >65%). Linseed oil is rich in n-6 polyunsaturated fatty acids (n-6 PUFA >37%) and n-3 polyunsaturated fatty acids (n-3 PUFA >28%  $\alpha$ -linolenic acid). Fish oil is rich mostly in saturated fatty acids (39.7%) and n-3 PUFA (>31%). Our research confirmed that it was more efficient to use a combination of soybean, linseed, rapeseed, and fish oils as supplement to laying hens' diet than pure soybean oil. Results of our own research as well as of the abovementioned authors confirmed that the modification of poultry diets could influence the deposition of desirable n-3 PUFA in lipids of egg yolks and broiler meat.

### 3. Health consequences of n3- and n6-PUFA consumption

#### 3.1. The fate of PUFA in human organism

Linoleic acid and alpha linolenic acid belong to the n-6 (omega-6) and n-3 (omega-3) series of polyunsaturated fatty acids, respectively. They are defined "essential" fatty acids since they are not synthesized in the human body and are mostly obtained from the diet. The best food sources of ALA and LA are the most vegetable oils, cereals and walnuts, fish meat, and fish oil. The adequate intake (AI) determined by the Food and Drug Administration (FDA) is for  $\alpha$ -linolenic acid 1.6 g/day for men and 1.1 g/day for women, while the acceptable macronutrient distribution range (AMDR) is 0.6–1.2% of total energy [35]. The FDA has recommended that adults can safely consume a total of 3 g/day of combined DHA and EPA, with no more than 2 g/day coming from dietary supplements [36]. Linoleic acid (18:2, n-6), the shortest-chained omega-6 fatty acid, is one of many essential fatty acids. Mammalian cells lack the enzyme omega-3 desaturase and therefore cannot convert omega-6 fatty acids to omega-3 fatty acids. This outlines the importance of the proportion of omega-3 to omega-6 fatty acids in a diet [35, 36]. Omega-6 fatty acids are precursors to endocannabinoids, lipoxins, and specific eicosanoids [6, 7].

Arachidonic acid and EPA are precursors of different classes of pro-inflammatory or anti-inflammatory eicosanoids, respectively (**Figure 2**). AA is found in small amounts in animal food sources (e.g., eggs and meats) and can also be formed by desaturation plus elongation reactions from its precursor, LA. The long-chain n-3 PUFA, DHA and EPA, can be formed in very limited amounts in the human body, or can be consumed preformed in the diet from sources rich in DHA/EPA such as fish meat or fish oils or enriched or fortified functional foods. Dietary n-3 PUFA may counteract the inflammatory effects of AA's eicosanoids in three ways: by counteracting the effects of their AA-derived counterparts via n-3 PUFA derivatives; by displacement, since dietary n-3 PUFA decreases tissue concentrations of AA, thus less of AA-derived eicosanoids is synthesized; and by competitive inhibition with AA for the access to the cyclooxygenase and lipoxygenase enzymes [37]. AA replacement by EPA or DHA n-3 PUFA results in reduced/inhibited production of pro-inflammatory mediators such as prostaglandins, leukotrienes, and lipoxins. EPA and DHA compete with AA for the conversion by cytochrome P450 (CYP) enzymes, resulting in the formation of alternative, physiologically active, metabolites. Renal and hepatic microsomes, as well as various CYP isoforms, displayed equal or elevated activities when metabolizing EPA or DHA instead of AA. CYP2C/2J isoforms converting AA to epoxyeicosatrienoic acids (EETs) preferentially epoxidized the -3 double bond and thereby produced 17,18-epoxyeicosatetraenoic (17,18-EEQ) and 19,20-epoxydocosapentaenoic acid (19,20-EDP) from EPA and DHA. Those -3 epoxides are highly active as antiarrhythmic agents. Moreover, rats given dietary EPA/DHA supplementation exhibited substantial replacement of AA by EPA and DHA in membrane phospholipids in plasma, heart, kidney, liver, lung, and pancreas, with less pronounced changes in the brain [38]. The metabolic pathways competition of n3-PUFA and n6-PUFA is schematically represented in **Figure 2**.



**Figure 2.** n3-PUFA and n6-PUFA metabolic pathways competition.

n-3 fatty acids, ALA, EPA, and DHA are especially important for good condition of heart and blood vessels, as well as for the prevention of diabetes and certain types of cancer [39]. Connor states that n-3 PUFA prevents heart diseases by preventing the occurrence of arrhythmia [40]. They have anti-inflammatory and hypolipidemic properties, they act antithrombotic, and slow down the development of atherosclerosis. Moreover, they also have a beneficial effect on digestion, improve the immune system, and reduce occurrence of allergic diseases [41]. DHA is an essential element in phospholipids of cell membranes, particularly in brain and eye retina. It is necessary for proper development and function of these organs, especially in fetuses and infants [42]. The anti-inflammatory effects of n-3 fatty acids through reduced production of pro-inflammatory mediators include reduced/inhibited leukocyte chemotaxis, adhesion molecule expression, and leukocyte-endothelial interactions. In addition, among the products of omega-3 fatty acid metabolism are the resolvins, maresins, and protectins [43, 44] which have an indispensable role in the contraction of inflammation [45].

Both n-3 and n-6 PUFA are essential in the diet; however, their ratio affects the ratio of produced pro-inflammatory and anti-inflammatory metabolites. Healthy ratios of n-6:n-3, according to some authors, is the ratio of n6 to n3 of 1:1 to 1:4 (an individual needs more omega-3 than omega-6) [46]. Typical Western diets provide ratios of n6:n3 PUFA between 10:1 and 30:1 [47].

In human, after digestion in the small intestine and transport to the blood, the n-6 and n-3 PUFAs are assimilated within tissues themselves through the body. They can be used in energy metabolism by beta-oxidation to form ATP-EFAs and can also undergo esterification into cellular lipids including triglyceride, cholesterol ester, and phospholipid or can be stored in the form of triglycerides and released later by enzymatic/hydrolytic processes. EFAs can also be temporarily stored as cholesterol ester and also released to be utilized in energy metabolism. Both n-6 and n-3 PUFAs in the form of phospholipids are particularly important as they maintain both the structural integrity and the critical functioning of cellular membranes throughout the body. In addition, LA and ALA are activated to high-energy forms known as fatty-acyl CoA which provides the conversion of these dietary PUFAs into their longer-chain and more polyunsaturated products as derived by a series of desaturation plus elongation reactions which are particularly active in the liver and to a lesser extent in other tissues [48].

### **3.2. Metabolic effects of n-3 PUFA consumption on triacylglycerol and very low-density lipoprotein**

It is known that n-3 PUFA fatty acids reduce triacylglycerol (TG) synthesis in the liver and increase very low-density lipoprotein (VLDL) clearance in the peripheral circulation. This occurs because n-3 PUFA inhibit diacylglycerol acyl transferase (DGAT), and phosphatidic acid phosphohydrolase (PA), two crucial enzymes involved in hepatic TG biosynthesis which results in decreased hepatic VLDL secretion. Furthermore, the availability of FAs for TG synthesis is decreased because of increased peroxisomal beta-oxidation of FA. Finally, due to the action of lipoprotein lipase, there is an increased plasma lipolytic activity in the peripheral circulation [49].

### 3.3. Anti-inflammatory effects of n-3 PUFA in chronic cardiometabolic diseases

n-3 PUFAs have numerous positive effects on immune response [50]. They are components of the plasma membrane and as such are important for cell permeability, fluidity, and flexibility [51]. Increased intake of fish oil or n-3 PUFA supplementations due to its anti-inflammatory function has beneficial effects on cardiovascular disease (CVD), metabolic syndrome, diabetes mellitus, and other diseases [52, 53].

### 3.4. Anti-inflammatory effect of n-3 PUFA in adiposity and glucose metabolism

Adipocytes play an important endocrine role regulating metabolism, and immune response by secreting adipokines [54]. Adipocyte in healthy subjects maintains the balance between pro- and anti-inflammatory adipokines, but in obesity secretion of inflammatory adipokines is shifted to pro-inflammatory cytokines [55], which may contribute to the pathogenesis of metabolic disorders. Accumulation of triacylglyceride in adipocytes results in adipocyte hypertrophy and dysregulation in secreting bioactive components. Obese patients are a high-risk population for developing diabetes mellitus and cardiovascular complications because they become insulin insensitive, have higher blood pressure, and heart rate (HR). It has been shown that obesity-related metabolic disorders originate from a low-grade inflammation [56].

Macrophage, lymphocyte, adipose stem cells, and preadipocytes that reside in adipose tissue also contribute to increased secretion of pro-inflammatory cytokines such as monocyte chemoattractant protein (MCP)-1, IL-8, IL-6, IL-1, and tumor necrosis factor alpha (TNF- $\alpha$ ) [57].

Anti-inflammatory effects of n-3 PUFAs have a protective effect and decrease the pro-inflammatory action of adiponectin [58, 59], as a result of the activation of AMP-activated protein kinase [60], which further regulates carbohydrate metabolism [61], and reduce the risk of developing cardiovascular diseases [62]. Although mechanisms involved in anti-inflammatory effect of n-3 PUFA are poorly understood, G protein-coupled receptor 120 (GPR120) is highly expressed on adipocytes and pro-inflammatory macrophage serves as an n-3 PUFA receptor. In mice fed with high-fat diet, supplemented with n-3 PUFA, inflammation was decreased (lower levels of TNF- $\alpha$  and IL-6) and systemic insulin sensitivity was enhanced, while in GPR120 knockout mice these effects were not observed [63]. They also showed that  $\beta$ -arrestin2 and GPR120 signaling induce the inhibition of TAB-mediated activation of transforming growth factor- $\beta$  activated kinase 1 (TAK1) and inhibit toll-like receptor2/3/4 (TLR) and TNF- $\alpha$  pro-inflammatory-signaling pathway.

Increased intake of n-3 PUFA increases DHA and EPA in immune cells of experimental animals and human subjects [64, 65]. Since immune cells integrate more n-3 PUFAs, there is a decrease in AA content, and therefore a drop of pro-inflammatory eicosanoids secretion [50, 66]. Recent *in vitro* and *in vivo* studies show that the anti-inflammatory effects of n-3 PUFA are mediated through the inhibition of NF- $\kappa$ B-signaling pathway and decreased macrophage TNF- $\alpha$  transcription [67, 68]. Macrophages stimulated with LPS in n-3 PUFA-

enriched medium significantly decreased serine 32 phosphorylation [69]. Without proper phosphorylation, NF- $\kappa$ B remains in the cytoplasm, inactively coupled with I $\kappa$ B, and in these conditions, the inflammatory response is downregulated or missing [70]. The fatty acid can also act as a ligand for peroxisome proliferator-activated receptors—PPAR $\alpha$  and PPAR $\gamma$  [71]. Specifically, 8(S)-HETE and 15d-J2-PUFA metabolites are PPARs potent selective activators. PPAR receptors are a group of transcription factors regulating energy homeostasis [72] and inflammation and immunity directly inhibiting NF- $\kappa$ B and its downstream effects [73]. All together, these studies prove n-3 PUFA to be a potent anti-inflammatory compounds.

Besides the anti-inflammatory effect of the n-3 PUFAs, study of Mori et al. showed that the incorporation of fish into a low-fat, energy-restricted diet has decreased triglyceride level, insulin-glucose metabolism [74], and thereafter reducing the risk of developing metabolic disorders. In addition, n-3 PUFA improves glucose tolerance and insulin sensitivity in mice models of type-2 diabetes and metabolic syndrome [75]. Rats fed with high-fat diet supplemented with n-3 PUFAs showed increased insulin receptor (IR) density and increased IR and IRS1 phosphorylation, phosphatidylinositol (PI) 3'-kinase activity, and GLUT-4 content in muscles, but rats show no beneficial effect on hyperglycemia and hyperinsulinemia, indicating important role of liver in glucose metabolism [76]. Documented data seen in animal models were not always translated to human subjects [77]. Some studies show beneficial effects of n-3 PUFA on glucose metabolism, and others not. Mostad et al. showed that n-3 PUFA supplementation in type-2 diabetic and obese patients did not improve insulin sensitivity, although those patients had improved lipid metabolism [78]. On the contrary, Albert et al. showed that higher n-3 PUFA concentrations were associated with improved insulin sensitivity, lower free fatty acid, and C-reactive protein (CRP) level in a group of middle-aged overweight men [79]. Another study in women patients with type-2 diabetes showed that in 2 months of n-3 PUFA supplementation, they had decreased adiposity, significantly lower plasma triacylglycerol, but without changes in insulin sensitivity [80]. These opposing results in human insulin sensitivity may be explained by different phenotypes, sex, age, adiposity, and environmental factors of patients, but also by different n-3 PUFA dosage in studies [81].

n-3 PUFA effects on human and animals are dose and tissue dependent [82]. n-3 PUFA incorporates in the plasma membrane, and it binds to receptors as a ligand and modulates gene expression for immune and metabolic function, and by these decrease risk for cardiovascular disease. n-3 PUFA-enriched membranes have changed membrane fluidity and biophysics of lipid rafts affecting protein function and signaling events [53]. For example, n-3 PUFAs modulate the function of Na<sup>+</sup> and L-type Ca<sup>2+</sup> membrane ion channels, to prevent arrhythmias [83]. Altered channel function by n-3 PUFA leads to reduced myocyte excitability and cytosolic calcium fluctuation of ischemic myocardium myocyte which becomes susceptible to partial depolarization (resting inactivation) and prevents arrhythmia, while membrane potential of myocytes in the nonischemic myocardium is not drastically affected [84]. Beneficial and protective effects of n-3 PUFA are supported by many scientific studies, without notable side effect [53].

### **3.5. Effectiveness of n-3 polyunsaturated fatty acids in the improvement of endothelial function and improvement of elasticity of the vascular wall**

A large empirical data indicate that the consumption of n-3 PUFA has beneficial effect on the risk and progression of cardiovascular diseases acting via multiple pathways and molecular mechanisms [53, 85, 86]. Since atherosclerosis is one of the main features of CVDs characterized by morphological and functional changes in blood vessel wall and its endothelium, attention has been given to numerous studies to investigate whether n-3 PUFA may prevent or delay atherosclerosis progression acting on the initial steps in its pathogenesis—endothelium and vascular wall function.

Endothelium plays a critical role in maintaining vascular tone and the term “endothelial function” is commonly used to describe its ability to release vasoactive substances, thereby regulating the blood flow [87]. Classically, endothelial dysfunction (ED) refers to reduced production and/or bioavailability of the main vasodilator nitric oxide (NO) and/or an imbalance in the relative contribution of other endothelium-derived relaxing (e.g. cyclooxygenase-1 and -2 (COX-1,2) or CYP450-epoxygenase-derived metabolites) and contracting (e.g. COX-1,-2 or CYP450-hydroxylase-derived metabolites) metabolites of AA, resulting in impaired vascular relaxation mechanisms [88]. It is considered that increased oxidative stress level is one of the main causes for ED and the development in various pathological states associated with vascular diseases such as hypertension, diabetes mellitus, hypercholesterolemia, smoking, and aging. A number of studies have shown that there is a cross-talk between the enzymes producing the vasoactive metabolites (NOS, COX-1,-2, CYP450) and reactive oxygen species (ROS), in which ROS may affect the bioavailability of NO and/or affecting other enzymes to shift their production from vasodilators to vasoconstrictors [89]. ED becomes an accepted prognostic value for future cardiovascular events in both populations at low and high cardiovascular risk and its noninvasive assessment by flow-mediated dilation (FMD) of brachial artery (gold standard) is being routinely used not only in research but in clinical practice, as well [90].

As elaborated previously, the mechanism by which n-3 PUFA may influence endothelial function is its ability to incorporate into membrane phospholipids in which signaling molecules and receptors for endothelial cell function are located [91]. Some of the possible pathways activated in this way result in increased NO production and reduced synthesis of pro-inflammatory mediators [92]. Enhanced eNOS activity/expression by n-3 PUFA administration was demonstrated in several endothelial cell cultures or experimental animal studies [93–95]. In addition, n-3 PUFAs increase NO production by directly stimulating eNOS gene and protein expression, which was reported by several studies in healthy and disease animals including atherosclerosis, diabetes mellitus, and menopause [96–102].

Taken together, these results strongly suggested that n-3 PUFA increases the bioavailability of NO acting via different molecular mechanisms. Despite that high doses of n-3 PUFA have been considered as to have a pro-oxidant effect, several studies on cell cultures and isolated blood vessels have shown that n-3 PUFA may reduce the oxidative stress level by attenuating ROS production via its direct effect on ROS formation, or reducing peroxynitrite produc-

tion [97, 102, 103]. Both *in vitro* and *in vivo* experiments have demonstrated that n-3 PUFAs reduce the concentration of soluble cell adhesion molecules (sCAMs) VCAM-1 and E-selectin, as well as IL-6 and C reactive protein level resulting in the attenuation of cellular and systemic inflammation [104, 105]. It is important to emphasize that relatively high dose of n-3 PUFAs is needed to achieve this anti-inflammatory effect. Interestingly, high doses of n-3 PUFA significantly reduce triglycerides level, which indirectly also contributes to improved endothelial function in these conditions [86]. Taken together, these data suggest that n-3 PUFA has the potential to improve endothelial function by acting on the bioavailability of NO by various mechanisms, reducing oxidative stress and inflammation and thereby reducing pathological activation of the endothelium. The results of a number of functional vascular studies have been summarized in several recent meta-analyses; however, the conclusions of these meta-analyses have been a bit inconsistent. There are a few studies, both in animals and in humans which aimed to distinct the effect of n-3 PUFA on endothelium-independent vasodilation, as well, and whose results suggest that the effect of n-3 PUFA on endothelium-independent vasodilation (contribution of vascular smooth muscle cells) is negligible, as demonstrated in the meta-analysis by Wang et al. [106]. One of the main shortcomings of these functional studies was the lack of basal measurement of n-3 PUFA in the studied population. Another lack of mentioned studies is significant heterogeneity in the number of participants, inclusion criteria such as age of participants or whether participants were healthy or disease, markers of endothelial function that were measured, dose and duration of n-3 PUFA supplementation, forms of n-3 PUFA that were administered (EPA, DHA, or ALA) alone or in combination and concomitant therapy that was used. Because of the abovementioned structure heterogeneity of functional studies, conclusions that indicate that n-3 PUFA improves endothelial function are still adopted with great caution. Meta-analysis of Wang et al. from 2012 identified totally 16 eligible studies which investigated the effect of n-3 PUFA supplementation on endothelial function measured by FMD and involving 901 participants, which reported that n-3 PUFA supplementation significantly increased FMD by 2.30% at a dose range from 0.45 to 4.5 g/day during a median of 56 days. Furthermore, results of this meta-analysis suggested that the effect on n-3 PUFA on endothelial function can be modified by the health status of the participants or by the dose of n-3 PUFA supplementation [106]. A review on human intervention studies by Egert and Stehle reported that n-3 PUFA supplementation improved endothelial function in overweight DM type 2 patients with dyslipidemia; however, conflicting results were observed in CVD patients. The authors concluded that reasons for these discrepancies between studies lie in the heterogeneity in the participants' health status and age, as well as in dose, duration, and the type of n-3 PUFA supplementation [107]. A third large meta-analysis of randomized controlled trials on the fish oil supplementation on endothelial vascular function published in 2012 included 16 studies with 1385 participants involved and reported that fish oil supplementation significantly improved FMD. Furthermore, endothelial function was significantly improved particularly in normoglycemic subjects and participants with lower diastolic blood pressure [108]. But contradictory, sensitivity analysis including only double-blind, placebo-controlled studies indicated that fish oil supplementation has no significant effect on endothelial function. All together, these studies provide many indices that n-3 PUFA supplementation has beneficial effect and improves endothelial function, but large-scale and high-quality clinical trials are needed to evaluate this effect to get a definite conclusion.

Beside impairment of endothelial function, CVDs and atherosclerosis are closely linked to increased arterial wall stiffness which represents progressive deterioration in vessel elasticity [109]. Arterial wall stiffness is characterized by morphological changes in blood vessel wall structure and in mechanical properties of vascular wall, which result in changed functional possibilities of such blood vessels. The arterial stiffness, in addition to the changes in the anatomical structure of the blood vessel wall, is closely related to impaired endothelial function in promoting atherosclerosis development [86]. Therefore, the assessment of arterial stiffness became an accepted predictive factor for future cardiovascular events and mortality in patients with CVDs.

One of the most straightforward and reliable methods for large artery stiffness assessment is noninvasive measurement of pulse wave velocity (PWV), and the most commonly used methods are carotid-femoral PWV and brachial-ankle PWV. PWV presents the speed at what the so-called pulse wave (arterial pulsation produced by the ejection of blood from the heart) propagates from heart to the periphery. Higher PWV is associated with the greater blood vessel wall rigidity that is interpreted as increased arterial wall stiffness [110]. n-3 PUFA administration may influence arterial stiffness acting via passive mechanisms involving mechanical and elastic arterial wall properties, just as via active mechanisms involving cellular and molecular functions of endothelium, VMS, and extracellular matrix of blood vessel wall [86, 109]. It is well known that chronically increased blood pressure levels also increase arterial stiffness by remodeling of the artery wall itself. A large body of evidence indicates that n-3 PUFAs are able to decrease blood pressure level, and therefore act to reduce arterial stiffness as well [111–113]. A second possible link between n-3 PUFA and arterial stiffness is blood triglyceride levels, which are decreased by n-3 PUFA supplementation. Since abnormalities in lipid metabolism are considered to be one of the fundamental determinants for the atherosclerosis developments, its effect on stiffening of the arteries should be taken into account as well [114, 115]. It is considered that n-3 PUFA may act on arterial stiffness by reducing heart rate, since numerous studies in both animal model and humans reported that an increased heart rate is associated with an increased risk for CV events, and is independently associated with the progression of arterial stiffness. It has been speculated that n-3 PUFA may lower HR acting directly on cardiac electrophysiology, or through a modulation of vagal and sympathetic balance [53, 116, 117]. Therefore, beneficial effect of n-3 PUFA on arterial stiffness is multifactorial affecting both passive and active mechanisms relating to the structure and function of the arterial wall, which are very often changed and/or damaged by some major cardiovascular risk factors (such as hypertension, obesity, smoking, menopause, hyperlipidemia, etc.).

Recently, numerous studies tried to investigate the effect of n-3 PUFA supplementation on arterial stiffness in a variety of conditions associated with increased cardiovascular risk in both experimental animals and humans. Regarding results of studies in experimental animals, they have described beneficial effect of n-3 PUFA in animals with insulin resistance, hypertension, and in ovariectomized animals which presented an experimental mode for menopause [118–122]. In the meta-analysis from 2011 on the n-3 PUFA interventions to arterial stiffness which included nine studies (one on the acute effects of n-3 PUFA in healthy volunteers and others on the chronic supplementation in patients with various CVDs), all

but one study reported improvement in PWV or capacitive arterial compliance compared to the controls. Furthermore, combined supplementation of EPA and DHA had greater effect on arterial stiffness improvement than that of EPA alone, while one study reported that DHA supplementation alone had no significant effect on arterial stiffness. Just as studies on the effect on n-3 PUFA on endothelial function, the disadvantage of the above mentioned experiments is heterogeneity in the sample population and supplementation dose sizes. Yet, the authors of this meta-analysis pointed out that if the different doses of n-3 PUFA supplementation acted to improve arterial stiffness in diverse populations these findings could be potentially translated to general population [109].

In conclusion, there is a growing evidence that n-3 PUFA supplementation by targeting to endothelial function and vascular wall stiffness may have beneficial effect in preventing the development and progression of atherosclerosis and incidents related to CVDs. So far, we can concisely presume that this benevolent effect of n-3 PUFA on vascular health is a sum of their actions on vasodilator mediators' bioavailability, antioxidant and anti-inflammatory capacity, modulation of lipid profile, and structural arterial remodeling. Still, stronger evidence from large clinical trials with more homogeneous experimental populations and supplementation dose is needed before n-3 PUFAs can find their place in the clinical prevention and treatment of CVDs.

#### 4. Conclusions

The production of functional food enriched with n-3 PUFA (i.e. eggs and poultry meat) is a well-established process and the food is available at the market. Despite inconsistency in designs of the analyzed studies, up to date numerous accumulated data demonstrate beneficial effects of n-3 PUFA for human health, particularly in relation to cardiovascular and metabolic conditions. To achieve long-term protective effects, the functional food enriched with omega-3 fatty acids should be consumed on daily basis. There are no specific constrains in taking functional food; even more, it can be recommended to athletes and cardiovascular patients. General population can also benefit from eating functional food enriched with n-3 PUFA due to their anti-inflammatory and vascular-protective effects.

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