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## **ω-6 and ω-3 Polyunsaturated Fatty Acids: Inflammation, Obesity and Foods of Animal Resources**

### **ABSTRACT**

Obesity, as defined by the World Health Organization (WHO), is excessive fat accumulation that can pose health risks and is a disorder of the energy homeostasis system. In typical westernized diets, ω-6 polyunsaturated fatty acids (PUFAs) vastly exceed the amount of ω-3 PUFAs, with ω-6/ω-3 ratios ranging from 10:1 to 25:1. ω-6 PUFAs, such as arachidonic acid (AA), have pro-inflammatory effects and increase obesity. On the other hand, ω-3 PUFAs, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have anti-inflammatory and anti-obesity effects. LA (linoleic acid) and ALA (alpha-linolenic acid) are synthesized in almost all higher plants, algae, and some fungi. However, in humans and animals, they are essential fatty acids (EFA) and must be consumed through diet or supplementation. Therefore, balancing LA/ALA ratios is essential for obesity prevention and human health. Monogastric animals such as pigs and chickens can produce meat and eggs fortified with ω-3 PUFAs by controlling dietary fatty acid (FA). Additionally, ruminant animals such as feeder cattle and lactating dairy cows can opt for feed supplementation with ω-3 PUFAs sources and rumen-protected microencapsulated fatty acids or pasture finishing. This method can produce ω-3 PUFAs and conjugated linoleic acid (CLA) fortified meat, milk, and cheese. A high ω-6/ω-3 ratio is associated with pro-inflammation and obesity, whereas a balanced ratio reduces inflammation and obesity. Additionally, probiotics containing lactic acid bacteria are necessary, which reduces inflammation and obesity by converting ω-6 PUFAs into functional metabolites such as HYA (10-hydroxy-cis-12-octadecenoic acid) and CLA.

### **KEYWORDS**

ω-3/ω-6 PUFAs, Inflammation, Obesity, *Lactobacillus*, Animal Food

## Introduction

In the late 1970s, Danish researchers Hans Olaf Bang and Jørn Dyerberg discovered that the Inuit had a very low incidence of cardiovascular disease (CVD) despite their diet of seal and whale blubber and oily fish (Dyerberg and Bang, 1979). Additionally, the Inuit were found to have nearly 2-fold longer bleeding times than Danes. The food consumed by the Inuit contains a high content of  $\omega$ -3 PUFAs, which replace  $\omega$ -6 PUFAs and reduce platelet aggregation.

In 1980, an epidemiological survey on the anti-arteriosclerotic effect of fish oil was conducted at Chiba University in Japan (Hirai, 1985). Fishermen with high fish intake were compared with rural residents with low fish intake, and the fishermen's EPA mean intake was 2,600 mg/day, approximately three times higher than rural residents' EPA mean intake. Fishermen had lower mortality rates from ischemic heart disease and CVD compared to rural residents. They also had lower platelet aggregation and blood viscosity, and triglyceride levels were about 40% lower.

The rural area population of the Nenets Autonomous Okrug (NAO) in Arkhangelsk city, Russia, eats lean reindeer meat and local cold-water white fish rich in  $\omega$ -3 PUFAs. Therefore, their serum lipid profiles were investigated and compared with those of the urban area population (Petrenya et al., 2012). Compared to the urban population, the NAO rural population has decreased total cholesterol, low-density lipoprotein cholesterol, very low-density lipoprotein cholesterol, and triglyceride. In contrast, high-density lipoprotein cholesterol has increased, showing positive health results. In Siberia and Chukotka, Chukchi residents who consumed a lot of sea fish and fish products had higher blood EPA and DHA levels than residents of the urban area of Novosibirsk city (Rezvukhin et al., 1996).

Fatty acid intake varies depending on human lifestyle (Fig. 1) (Simopoulos, 2016). As humans began to engage in agriculture in earnest, domestication of animals occurred, and many changes occurred in the fatty acid intake (LA and ALA) of humans and animals. During the Paleolithic and Neolithic periods, when food was mainly obtained through hunting,  $\omega$ -6 PUFAs were consumed at a lower rate than in modern times. Livestock that consumed grains had increased LA content in meat (Naughton et al., 2016). Since the Industrial Revolution Era, the intake rate of  $\omega$ -6 PUFAs has gradually accelerated. In the Late Paleolithic period, 8.84 (g/day) of LA and 12.6 (g/day) of ALA were consumed, and the  $\omega$ -6/ $\omega$ -3 ratio is estimated to be 0.79 (Simopoulos, 2016). Since 1960, consumption of vegetable oils such as rapeseed (canola), soybean, sunflower, and corn oils has increased 1000-fold in

the US, contributing to an increase in obesity, type 2 diabetes, nonalcoholic steatohepatitis (NASH), and other inflammatory disorders. Currently,  $\omega$ -6 PUFA-rich vegetable oils, such as soybean and canola, are widely used as cooking oils in homes and restaurants worldwide (Yamashima et al., 2020). In the Western diet, as the intake of high-fat diet (HFD) increases, the intake of  $\omega$ -3 PUFAs decreases, and the intake of  $\omega$ -6 PUFAs increases, resulting in a  $\omega$ -6/ $\omega$ -3 ratio of 10:1 to 25: 1 (Weiser et al., 2016).

High-ratio  $\omega$ -6/  $\omega$ -3 PUFAs are essential in increasing obesity through the hyperactivity of AA eicosanoid metabolites and cannabinoid system. Balancing the LA/ALA ratio is integral for obesity prevention and human health. Obesity is defined by the WHO (World Health Organization) as abnormal or excessive fat accumulation that can pose a risk to health (WHO, 2020). More than 2.1 billion people, or about 30% of the global population, are overweight or obese. The passive accumulation of excess weight does not cause obesity but is a disorder of the energy homeostasis system. Red blood cells from obese patients have high  $\omega$ -6 PUFAs and low  $\omega$ -3 PUFAs (Sot et al., 2022). Cardiovascular diseases (CVDs) affect one in four people in Europe and one in three in the United States (Dyńska et al., 2023). CVD is the most common global cause of death, with an average of 17.9 million people dying from this disease worldwide each year. In 2020, the number of deaths due to CVD exceeded 19 million, an increase of 18.7% compared to 2010, and approximately 23.3 million people are expected to die due to CVD by 2030. Diet is among the most critical risk factors in CVD prevention and treatment. Fish oil, rich in  $\omega$ -3 PUFAs, has immunomodulatory effects and can inhibit the production of proinflammatory cytokines.  $\omega$ -3 PUFAs (EPA and DHA) have anti-obesity and anti-inflammatory effects, and the European Food Safety Authority's average intake recommendation for human health is 250 to 500 mg/d (Avallone et al., 2019). However, most Western diets lack the recommended intake of  $\omega$ -3 PUFAs, so  $\omega$ -3 PUFAs supplementation is essential. This can be achieved by directly consuming fish, fish oils, krill oils, flaxseed oil, and leafy vegetables rich in  $\omega$ -3 PUFAs or meat, milk, and eggs fortified with  $\omega$ -3 PUFAs.

In the European Union (EU), foods containing 300 mg of ALA or 40 mg of combined EPA and DHA per 100 g and 100 kcal can be labeled as "Source of  $\omega$ -3 fatty acids", and foods containing 600 mg or 80 mg can be labeled as "High in  $\omega$ -3 fatty acids" (Commission Regulation EU, 116/2010). The primary source of  $\omega$ -3 PUFAs is fish oil, and the wholesale price averages 14 dollars per kg (Benvenga et al., 2022). The global market for  $\omega$ -3 PUFAs is

estimated to be worth 2.10 billion United States dollars (USD) in 2020, and with an annual growth rate of 7.8%, it is expected to reach 3.61 billion USD in 2028.

## **Sources of $\omega$ -3 and $\omega$ -6 PUFAs**

LA and ALA are essential fatty acids in animals and must be consumed through diet or supplementation (Bermúdez, 2009). Fish accumulate  $\omega$ -3 fatty acids derived from microalgae (phytoplankton) and are particularly rich in EPA and DHA. ALA is a  $\omega$ -3 fatty acid abundant in microalgae, fish, krill oils, flaxseed oils, green leafy vegetables, and animal fat, especially in grass-fed animals. LA is a  $\omega$ -6 fatty acid abundant in grains, meats, and the seeds of most plants (Table 1) (Amjad et al., 2017).

Microalgae are the earliest producers of very long-chain polyunsaturated fatty acids (PUFAs) in the marine food chain (Amjad et al., 2017).  $\omega$ -3 PUFAs synthesized in microalgae are biomagnified in the food web, reaching higher trophic levels. Therefore,  $\omega$ -3 PUFAs synthesized in microalgae accumulate in the tissues of marine fish such as salmon and herring. On the other hand, animals that consume cereal-based feed accumulate  $\omega$ -6 PUFAs in more significant proportions. However, animals that consume flaxseed oils and green leafy vegetables can also produce beef, pork, and chicken, accumulating  $\omega$ -3 PUFAs.  $\omega$ -3 PUFAs derived from external sources are incorporated into lipoproteins and membrane phospholipids in the body. DHA is highly accumulated in neuronal membranes (brain), retina photoreceptors (eyes), and muscles and is involved in the development and function of these organs (Fig. 2) (Bae et al., 2021).

## **Metabolism of $\omega$ -3 and $\omega$ -6 PUFAs**

### **Fatty acids synthesis**

The synthesis of fatty acids begins with acetyl-CoA in the cytoplasm, and two carbons are added to the fatty acid chain through malonyl-CoA (Fig. 3) (Qin et al., 2023). After eight cycles, the synthesized stearic acid (SA, C18:0) is transferred to the endoplasmic reticulum (ER) and begins the first unsaturation step. SA is desaturated by  $\Delta$ 9 desaturase to produce oleic acid (OA, C18:1 $\Delta$ 9), and OA is desaturated by  $\Delta$ 12 desaturase to produce  $\omega$ -6 linoleic acid (LA, C18:2 $\Delta$ 9,12). Afterward, LA produces  $\omega$ -3 alpha-linolenic acid (ALA, C18:3 $\Delta$ 9,12,15) by  $\Delta$ 15 desaturase.

Almost all higher plants, algae, and some fungi have  $\Delta 12$ -desaturases that convert OA to LA and  $\Delta 15$ -desaturases that convert LA to ALA (Ruiz-Lopez et al., 2015). However, animals and humans do not possess these desaturases, so LA and ALA are the essential FAs. The difference in the double bond between LA and ALA causes them to play different roles in inflammation and metabolism (D'Angelo et al., 2020). LA and ALA are synthesized into ARA (AA) and EPA by  $\Delta 6$  desaturase,  $\Delta 5$  desaturase,  $\Delta 4$  desaturase, and elongases in the endoplasmic reticulum of cells, and then into DHA in the peroxisome (Salem et al., 2015). LA and ALA are utilized competitively by these enzymes. When the intake of LA is high, the conversion rate of EPA and DHA from ALA decreases; when the intake of ALA is high, the conversion rate of ARA (AA) from LA decreases (Ailhaud et al., 2006). However, although humans and animals can synthesize both EPA and DHA, their conversion rates do not meet nutritional demands, so they must be supplied from external sources such as microalgae, fish, and flaxseed oils.

The key enzymes metabolizing LA and ALA are  $\Delta 6$  desaturases and  $\Delta 5$  desaturases, encoded by the fatty acid desaturases (FADS) 1/2 genes.  $\Delta 5$  desaturases are encoded by the *FADS1* gene and convert LA and ALA into long-chain PUFAs (such as ARA and EPA). *FADS1* rs174550 genotypes (TT and CC) strongly regulate plasma lipid PUFA composition. The *FADS1* haplotype has 43% and 24% lower plasma proportions of AA and DHA, respectively, compared to the homozygous haplotype. According to estimates from the Human Genome Diversity Project-Centre de référence du Polymorphisme Humain (HGDP-CEPH) panel, haplotype A has a frequency of 1% in African populations, 97% in native Americans, and 25–50% in Europeans and Asians (Ameur et al., 2012). People with the *FADS1* rs174550-TT genotype have increased LA phospholipids and inflammatory adipose tissue gene expression when consuming an LA-enriched diet compared to people with the *FADS1* rs174550-CC genotype. Humans of the *FADS1* rs174550-CC genotype play a protective role against LA-induced inflammation through the regulation of AA-derived eicosanoids biosynthesis (Vaitinen et al., 2022). Therefore, an approach that considers the variation of *FADS* should be applied to personalized dietary counseling.

### **Inflammatory response**

Polyunsaturated fatty acids (PUFAs), LA, and ALA exhibit pro-inflammation and anti-inflammation effects through metabolic and hydrolyzing processes in the body (Fig. 4) (D'Angelo S, 2020; Ishihara, 2019). LA and ALA are converted into AA, EPA, and DHA,

which are then converted into bioactive lipid mediators by cyclooxygenases (COXs), lipoxygenases (LOXs), and cytochrome P450 monooxygenases (CYPs) to perform biological actions.  $\omega$ -6 AA is released from membrane phospholipids by inflammatory stimuli and phospholipase A2 (PLA2). The released AA is converted into prostaglandins (PGs), leukotrienes (LTs), and lipoxins (LXs) by COXs and LOXs, showing pro-inflammation effects. On the other hand,  $\omega$ -3 EPA and DHA are converted into metabolites (3-series PGs, 5-series LTs) and anti-inflammatory mediators (resolvins, protectins, maresins, etc.) at inflammation sites and exhibit anti-inflammatory effects. Additionally,  $\omega$ -3 PUFAs (EPA, DHA) exhibit anti-inflammatory effects through substrate and receptor competition with  $\omega$ -6 AA.

Corticosteroids exert anti-inflammatory effects by inhibiting the activity of phospholipase A2 (PLA2), which releases  $\omega$ -6 AA from cell membranes (Fig. 5) (Kousparou et al., 2023; Rice et al., 2017). Zileuton acts as a 5-lipoxygenase inhibitor, and Montelukast acts as a leukotriene receptor antagonist, showing anti-inflammatory effects. Nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin (acetylsalicylate), are COX irreversible inhibitors and exert anti-inflammatory effects by inhibiting the production of prostanoids (Bindu et al., 2020).

## **Obesity**

Excessive cellular AA (from dietary LA intake) can stimulate adipocyte differentiation through several interconnected pathways (Fig. 6) (Naughton et al., 2016). AA (20:4  $\omega$ -6) can be converted to prostacyclin via prostaglandin H2 (PGH2), but this reaction is reduced in the presence of cellular ALA. Prostacyclin stimulates the peroxisome proliferator-activated receptor (PPAR) family, leading to PPAR $\gamma$  activation and adipocyte differentiation. Prostacyclin can stimulate adipocyte differentiation through the CCAAT-enhancer binding protein family (CEBP $\beta$  and CEBP $\delta$ ). Additionally, AA can activate peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) by increasing cyclic adenosine monophosphate (cAMP) production and protein kinase A (PKA) activity (Petersen et al., 2003). The cAMP process is inhibited by a cyclooxygenase (COX) inhibitor (aspirin). ALA's metabolites, EPA (20:5  $\omega$ -3) and DHA (22:6  $\omega$ -3), inhibit the production of prostaglandin H2 (PGH2) and cAMP by AA (Massiera et al., 2003). Another mechanism by which LA causes obesity is the endocannabinoid (EC) pathway. The primary mediators of endocannabinoids include N-arachidonylethanolamine (AEA, anandamide) and 2-arachidonyl glycerol (2-AG) derived



from LA, which increase appetite and food intake by activating cannabinoid receptors (CB1 and 2). Conversion of the  $\omega$ -6 and  $\omega$ -3 PUFAs share the same biochemical pathway involving desaturation and elongation reaction processes. LA and ALA compete for metabolic enzymes. Therefore, balancing LA/ALA ratios is very important for obesity prevention and human health (Wang and Wang, 2023).

Current Dietary Guidelines for Americans (DGA) recommend replacing saturated fat (SFA) intake with polyunsaturated fatty acids (PUFAs) and monosaturated fatty acids (MUFAs). Additionally, modern Western diets are already rich in  $\omega$ -6 PUFAs, which increases the risk of chronic disease. Therefore, to resolve this problem, increasing intake of  $\omega$ -3 PUFAs rather than  $\omega$ -6 PUFAs is beneficial for obesity and related liver diseases caused by high-fat diets. For instance, in a study on the interventional effect of different dietary PUFAs on obesity, C57BL/6J mice were fed a high-fat diet (HF) for ten weeks to induce obesity. Then, the obese mice were divided into three groups and fed one of the following three diets for an additional ten weeks (Fig. 7) (Hao et al., 2023). The three groups consisted of the HF group, the HF+ $\omega$ -6 group (substituted half of SFA with  $\omega$ -6 PUFAs), and the HF+ $\omega$ -3 group (substituted half of SFA with  $\omega$ -3 PUFAs). In the HF+ $\omega$ -6 group, body weight and fat mass continued to increase like the HF group, but in the HF+ $\omega$ -3 group, body weight and fat mass decreased significantly, and the proportion of relative lean mass/body weight increased.

### **Approaches to enhance $\omega$ -3 PUFAs in animal food**

Fatty acids are major components of lipids, forming the lipid bilayer of cells and organelles (de Carvalho and Caramujo, 2018). Animal fatty acids originate from *de novo* or exogenous sources, and certain fatty acids are synthesized from glucose or through the metabolism of lipid precursors. Ruminant animals have a lower content of  $\omega$ -6 PUFAs compared to non-ruminant animals (Table 1). Different food sources have different types and contents of fatty acids, which are absorbed into the body and esterified and metabolized to form fatty acids or lipids (Kousparou et al., 2023). Different cells have different fatty acid compositions and different membrane fluidities and permeabilities. Membrane phospholipid fatty acids are mostly hydrocarbon chains of 12 to 24 carbon atoms. ALA sources mainly used in animal feed include linseed oil, rapeseed oil, microalgae, and fish oil (Irawan et al., 2022).

### **Mouse model**

In a mouse model, soybean oil, corn oil, and a mixture of flaxseed and canola oil were fed at LA/ALA ratios of 1:1, 7:1, and 44:1, respectively, for 14 weeks (Fig. 8) (Su et al., 2016; Watanabe and Tatsuno, 2020). And the levels of AA, EPA, and DHA in the liver, brain, and muscle were examined. When the LA/ALA ratio was 1:1, there was no difference in AA, EPA, and DHA levels in the liver, brain, and muscle. On the other hand, when the LA/ALA ratios were 7:1 and 44:1, AA levels in the liver and muscle increased, and EPA and DHA levels decreased. However, there was no significant difference in the levels of AA and DHA in the brain when the LA/ALA ratio was 1:1, 7:1, and 44:1.  $\Delta 6$  desaturase competitively uses LA and ALA to synthesize AA and EPA, and  $\Delta 4$  desaturase uses EPA to synthesize DHA. In human hepatoma cells, EPA and DHA formation is highest when the LA/ALA ratio is 1:1, with an EPA conversion rate of 17% and a DHA conversion rate of 0.7% (Harnack et al., 2009). The conversion of ALA to EPA and DHA in the body is minimal, with the conversion rate of EPA being 7.0-21% and that of DHA being 0.01-1% (Saini et al., 2021). Therefore, increasing ALA intake while reducing LA intake is an effective way to improve  $\omega$ -3 PUFA status.

Additionally, among  $\omega$ -3 PUFAs, DHA supplementation is the most effective way to increase  $\omega$ -3 DHA levels in the body (Harnack et al., 2009). The human brain is a lipid-rich organ that consumes up to 20% of daily energy intake despite making up only 2% of body weight. More than 50% of the brain's dry weight comprises lipids, 10%–20% of brain total lipids are  $\omega$ -3 PUFAs, and more than 90% of  $\omega$ -3 PUFAs are DHA (Weiser et al., 2016). DHA is a precursor of bioactive mediators that resolve the brain's inflammation of neurons, microglia, and endothelial cells. However, the brain's capacity to synthesize DHA is deficient, so it absorbs DHA from circulating lipid pools to maintain homeostatic levels. DHA transport from plasma to the brain is regulated by the blood-brain barrier (BBB) (Li et al., 2023). Endogenous synthesis of EPA and DHA from ALA is insufficient to meet the body's physiological needs and must be supplied from external sources such as microalgae, fish, and fish oils (Huang et al., 2020). In mice, a diet with a low LA/ALA ratio significantly increases the levels of ALA and DHA in erythrocytes, liver, epididymal fat, spleen, brain, heart, and gastrocnemius (Wang and Wang, 2023).

#### **Poultry (meat and eggs)**

According to the USDA (United States Department of Agriculture, 2011), the PUFA of regular egg yolk has an  $\omega$ -6/ $\omega$ -3 ratio of 19.9, while the PUFA of egg yolk from free-range

chickens has an  $\omega$ -6/ $\omega$ -3 ratio of 1.3 (Simopoulos, 1992). It is a common strategy to increase  $\omega$ -3 PUFAs in eggs by dietary supplementation of ALA, EPA, and DHA to laying hens. The total fatty acids of yolk lipids are saturated fatty acids (SFA) at 30–35% and monounsaturated fatty acids (MUFA) at 40–45%, which has an appropriate SFA/MUFA ratio, but PUFAs are very low at 20–25% (Nimalaratne and Wu, 2015). Eggs from laying hens fed standard feed mixtures contained a higher proportion of  $\omega$ -6 PUFAs and a lower proportion of  $\omega$ -3 PUFAs. The feeding mixtures of laying hens consisted of 5% soybean oil in the control group and 5% fish oil and linseed oil (1.5% + 3.5%) in the treatment group (Fig. 9) (Radanović et al., 2023). The egg yolk of the control group (5% soybean oil) contained 1553.51 mg/100g of  $\omega$ -6 PUFAs, 149.88 mg/100g of  $\omega$ -3 PUFAs, and 10.36 of  $\omega$ -6/ $\omega$ -3 ratio. In the treatment group (5% fish oil and linseed oil), 11,127.71 mg/100g of  $\omega$ -6 PUFAs and 598.59 mg/100g of  $\omega$ -3 PUFAs accumulated, and 1.8 of  $\omega$ -6/ $\omega$ -3 ratio. In this study, compared to the control group, ALA increased by 5.6 times, DHA increased by 2.4 times, and the  $\omega$ -6/ $\omega$ -3 ratio decreased by 5.5 times in the treatment group.  $\omega$ -3 PUFAs enriched eggs had improved atherogenic index, thrombogenic index, and hypo/hypercholesterolemic indexes of egg yolks compared to conventional eggs. They increased  $\omega$ -3 ALA content and egg health index of examinees' blood serum. Supplementation of broiler chicken feed with flaxseed oil as a  $\omega$ -3 source and the antioxidant quercetin, which limits lipoperoxidation, increased the deposition of  $\omega$ -3 PUFAs within breast muscles and decreased the  $\omega$ -6/ $\omega$ -3 ratio (Sierzant et al., 2022). These results provide a strategy to suppress the occurrence of unpleasant taste and smell while increasing the content of  $\omega$ -3 PUFAs in poultry meat.

#### **Pig (meat)**

Pigs are ideal for producing meat fortified with  $\omega$ -3 PUFAs by regulating dietary FA. In addition, pork is one of the most consumed meats, but it has a high level of saturated FA, so changes in the FA profile are necessary to improve its health properties.  $\omega$ -3 stearidonic acid (SDA; C18:4  $\omega$ -3) is in a more advanced position than  $\omega$ -3 ALA in the biosynthetic pathway. Therefore, SDA is converted to EPA and DHA more efficiently than ALA. *Echium plantagineum* is a good SDA source and grows abundantly in pastures. Recently, 60 crossbred gilts (Landrace  $\times$  Large White) were fed palm oil (control), soya oil, linseed oil, fish oil, and *Echium* oil, respectively, and the fatty acid composition and  $\omega$ -6/ $\omega$ -3 ratio of *M. longissimus thoracis* were investigated (Fig. 10) (van Wyngaard et al., 2023).  $\omega$ -3 PUFAs were highest in the following order: fish oil 3.19%, linseed oil 2.52%, *Echium* oil 2.37%,

soya oil 1.24%, and palm oil (control) 0.98%. The  $\omega$ -6/ $\omega$ -3 ratios were low in the following order: fish oil 4.05, *Echium* oil 4.93 and linseed oil 5.55, soya oil 12.43, and palm oil (control) 12.37. Dietary supplementation with fish oil, linseed oil, and *Echium* oil increased the  $\omega$ -3 PUFAs content and decreased the  $\omega$ -6/ $\omega$ -3 ratio in *M. longissimus dorsi* muscle. The accumulation of long chain n-3 PUFAs (EPA+DPA+DHA) was in the following order: fish oil with high DHA and EPA content, *Echium* oil with high SDA content, linseed oil with high ALA content, and soya oil and palm oil (control) with high LA content. *Echium* oil supplementation did not increase  $\omega$ -3 PUFA content to the same level as fish oil. However, when comparing *Echium* oil with linseed, soya, and palm oil, it improved long chain  $\omega$ -3 PUFAs content in muscle without any adverse effects on meat quality. Along with *Echium plantagineum*, seed oils of *Buglossoides arvensis* and *Ribes* sp. are also widely studied as sources of SDA to increase EPA and DHA status in animal foods. When Large White pigs were supplemented with 8% extruded flaxseed, which is a rich source of ALA, the level of  $\omega$ -3 PUFAs increased approximately 9-fold, and the  $\omega$ -6/ $\omega$ -3 ratio decreased significantly from 20 to 2.5 (Tognocchi et al., 2023). In the fat-rich back fat, bacon, and ham fatty parts, the accumulation level of  $\omega$ -3 PUFAs was higher than the threshold set by the EU to obtain the label “Source of  $\omega$ -3 fatty acids”. These results showed that diet supplementation with 8% extruded linseed improved the nutraceutical quality of pork meat. Perilla is a rich source of  $\omega$ -3 PUFAs, especially ALA, used in oil production. Perilla oil is composed of 90.60% total unsaturated fatty acids (TUFAs), 17.90% monounsaturated fatty acids (MUFAs), and 72.70% PUFAs, and in particular, 55–64% of PUFAs are ALA. Oil is extracted from perilla seeds using the screw pressing method and used commercially. This process produces perilla cake containing 9 to 10% fat as a by-product (Souphannavong et al., 2021). Supplementation of 2.5% perilla cake to the finishing crossbred pig diet increased intramuscular fat, marbling score, ALA, and decreased  $\omega$ 6/ $\omega$ 3 ratio in the *longissimus thoracic et lumborum* muscle. Supplementation of 2.5% perilla cake to the finishing crossbred pig diet changed the fatty acid composition required for healthy meat (Sringarm et al., 2022).

## **Cow (meat and milk)**

As consumer interest in grass-fed animal foods increases, differences in beneficial bioactive compounds, such as  $\omega$ -3 PUFAs, micronutrients, and phytochemicals, between grass-fed and grain-fed animals are becoming more important. Over the past 30 years, research has shown that grass-based feeds can significantly improve beef's  $\omega$ -3 PUFAs composition and

antioxidant content, with various effects on overall taste. Grass-fed animals have improved levels of the  $\omega$ -3 PUFAs, conjugated linoleic acid (CLA), as well as antioxidants glutathione (GT) and superoxide dismutase (SOD) activity compared to grain-fed animals (Daley et al., 2010). In Red Angus steers, grass-finished beef contained more  $\omega$ -3 PUFAs and less  $\omega$ -6 PUFAs than conventional grain-finished beef (Fig. 11) (Krusinski et al., 2023). The experimental grass diet included diverse pasture (meadow fescue, clover, timothy grass, alfalfa, birds foot trefoil, chicory, orchard grass, and dandelion), and the experimental grain diet included a total mixed ration (TMR, orchard grass hay, corn, and pellets). The experimental grass diet contained 61.62% of  $\omega$ -3 PUFAs, 14.09% of  $\omega$ -6 PUFAs, and  $\omega$ -6/ $\omega$ -3 ratio of 0.24 in total fatty acids (TFAs). On the other hand, the experimental grain diet contained 4.29% of  $\omega$ -3 PUFAs, 56.12% of  $\omega$ -6 PUFAs, and a  $\omega$ -6/ $\omega$ -3 ratio of 15.12. Compared to the grain diet, the grass diet had more than 14 times higher  $\omega$ -3 PUFAs, while the  $\omega$ -6 PUFAs were 4 times lower and the  $\omega$ -6/ $\omega$ -3 ratio was 63 times lower. Grass-finished beef (ribeye) showed  $\omega$ -3 PUFAs 46.03mg/100g,  $\omega$ -6 PUFAs 67.07mg/100g and  $\omega$ -6/ $\omega$ -3 ratio 1.65, while grain-finished beef had  $\omega$ -3 PUFAs 10.13. mg/100g,  $\omega$ -6 PUFAs 100.32mg/100g and  $\omega$ -6/ $\omega$ -3 ratio 8.39. Compared to grain-finished beef,  $\omega$ -3 PUFAs in grass-finished beef increased by 4.5-fold, and  $\omega$ -6 PUFAs and  $\omega$ -6/ $\omega$ -3 ratio decreased by 1.5-fold and 5.1-fold, respectively. In particular, grass-finished beef decreased the sum of FAs while increasing ALA, EPA, and DPA. Grasses generally contain higher levels of PUFAs (especially  $\omega$ -3) when compared to grains. The higher concentration of  $\omega$ -3 PUFAs in grasses is due to the accumulation of FAs in the leaf tissue of fresh pasture, and  $\omega$ -3 PUFAs generally account for 50-75% of FA composition (Alothman et al., 2019). Additionally, pasture-finishing (i.e., grass-fed) bison reduced  $\omega$ -6/ $\omega$ -3 ratios (<3.2), improved metabolic health, and accumulated potential health-promoting compounds compared to pen-finished bison on concentrates (van Vliet et al., 2023). Grass-finished beef contains more  $\omega$ -3 PUFAs and a lower  $\omega$ -6/ $\omega$ -3 ratio than grain-finished beef. It contains more beneficial bioactive compounds such as micronutrients and phytochemicals, making it suitable for separating beef based on finishing feeds. It shows the possibility of production strategy.

To increase the content of  $\omega$ -3 PUFAs in cows' milk, linseed oil, fish oil, and microalgae are used as dietary supplements. Linseed oil is mostly composed of UFAs (unsaturated fatty acids), and contains 15.19% and 54.21% of LA and ALA, respectively. When this linseed oil enters the rumen, loss of  $\omega$ -3 PUFAs occurs due to an extensive biohydrogenation process. Therefore, dietary supplementation with rumen bypass microencapsulated linseed oil can

prevent the loss of  $\omega$ -3 PUFAs and increase the  $\omega$ -3 PUFAs content in milk. The milk of the control group fed total mixed ration (TMR) from Holstein dairy cows contained 9.24mg/100g of  $\omega$ -3 PUFAs, 63.40mg/100g of  $\omega$ -6 PUFAs, and  $\omega$ -6/ $\omega$ -3 ratio of 6.89. However, milk from the treatment group receiving dietary supplementation with 2% microencapsulated linseed oil contained 58.58mg/100g of  $\omega$ -3 PUFAs, 17.77mg/100g of  $\omega$ -6 PUFAs, and 3.48 of  $\omega$ -6/ $\omega$ -3 ratio. Dietary supplementation of 2% microencapsulated linseed oil increased the  $\omega$ -3 PUFAs content and lowered the  $\omega$ -6/ $\omega$ -3 ratio in the milk of dairy cows, and confirmed that rumen bypass microencapsulated fatty acids were safely transferred from the rumen to the small intestine (Fig. 12) (Kim et al., 2020).

#### **Goat (meat and milk)**

In a study to enhance the nutritional value of goat milk and raw cheese, the control diet was supplemented with 1.55%/DM (dry weight) palm oil and the two experimental diets were supplemented with 3.88%/DM flaxseed flakes and 2.64%/DM fish oil (Moya et al., 2023). The fatty acid profile of milk fed a control diet (palm oil 1.55%) showed 20.59mg/100g of  $\omega$ -3 PUFAs, 190.61mg/100g of  $\omega$ -6 PUFAs and 9.26 of  $\omega$ -6/ $\omega$ -3 ratio. The fatty acid profile of milk fed with 3.88%/DM flaked linseed diet showed 62.60mg/100g of  $\omega$ -3 PUFAs, 216.14mg/100g of  $\omega$ -6 PUFAs, and 3.45 of  $\omega$ -6/ $\omega$ -3 ratio. The fatty acid profile of milk fed with 2.64%/DM fish oil showed 45.27mg/100g of  $\omega$ -3 PUFAs, 208.24mg/100g of  $\omega$ -6 PUFAs and 4.59 of  $\omega$ -6/ $\omega$ -3 ratio. Compared to the control diet, the 3.88%/DM flaked linseed diet increased  $\omega$ -3 PUFAs by three times, and the  $\omega$ -6/ $\omega$ -3 ratio decreased by 2.7 times. These results were the same for fresh goat cheese. The flaked linseed-supplemented diet improved the nutritional value of milk due to a decrease in saturated fatty acids (SFAs) and an increase in polyunsaturated fatty acids (PUFAs) and conjugated linoleic acid (CLA). Dietary supplementation with flaked linseed or fish oil produced goats' milk and cheese with higher nutritional quality without affecting the sensory profile of the corresponding products obtained from animals fed routine diets.

According to the OECD/FAO report, the world's per capita consumption of milk and dairy products in 2015 was 111.3 kg and is expected to increase by about 12.5% by 2025 (OECD/FAO, 2017). Due to this, much research has focused on enriching  $\omega$ -3 PUFAs in milk, and its processed products (mostly from cows and sheep) consumed by humans (OECD/FAO, 2017).

Because  $\omega$ -6 and  $\omega$ -3 PUFAs are used competitively by desaturase and elongase enzymes that produce inflammatory and anti-inflammatory eicosanoids, a balanced intake of  $\omega$ -6 and  $\omega$ -3 PUFAs is necessary to suppress chronic diseases and maintain good health. The recommended value of EPA and DHA for the European adult population is 250 to 500 mg/d, and the recommended dietary ratio of  $\omega$ -6/ $\omega$ -3 is 1:1–2:1 (Avallone et al., 2019). However, the ratio of  $\omega$ -6/ $\omega$ -3 in typical Western diets is 15/1 to 16.7/1 (Simopoulos, 2006), and the amount of DHA contained in Western foods is approximately 100mg/day (Cardoso et al., 2016). In human hepatoma cells, EPA and DHA conversion rates are highest when  $\omega$ -6/ $\omega$ -3 is at a 1:1 ratio, resulting in 17% and 0.7% rates, respectively (Harnack et al., 2009). Therefore, balanced intake of  $\omega$ -6 and  $\omega$ -3 PUFAs and direct DHA supplementation are the most effective ways to increase body DHA levels.

### **$\omega$ -6 PUFAs and gut microbiota**

Gut microbes exist throughout the mammalian GI (gastrointestinal) tract and come into contact with all ingested dietary fats. Therefore, enzymes in gut microbes serve as a second liver to break down, transform, and detoxify dietary components that can have beneficial and detrimental impacts on host health (Brown et al., 2023). Just as the function of liver enzymes is to digest dietary and exogenous fats, gut microbial enzymes play a similar role in the gut. However, most functional by-products of gut microbial enzymes are still unknown. Dietary PUFAs may also be saturated by common gut microbial enzymes, limiting the number of double bonds and oxidative potential. Gut microbiomes containing *Bifidobacterium* and *Lactobacillus* species produce PUFA-derived intermediate metabolites, and PUFA-derived bacterial intermediate metabolites produced in the intestine exhibit anti-obesity and anti-inflammatory effects (Kishino et al., 2013). Gut microbes convert  $\omega$ -6 LA into HYA (10-hydroxy-cis-12-octadecenoic), reducing adipose inflammation and obesity caused by excessive dietary  $\omega$ -6 LA and AA cascade.

Additionally, HYA promotes the secretion of GLP-1 (Glucagon-Like Peptide-1) by activating G protein-coupled receptors GPR40 and GPR120. GLP-1 improves glucose homeostasis and suppresses appetite. HYA promotes intestinal peristalsis through EP3 (E-prostanoid 3 receptor) and inhibits lipid absorption (Fig. 13) (Miyamoto et al., 2019).

In *db/db* mice, EPA and DHA supplementation decreased the amount of LPS-containing *Enterobacteriaceae*. It increased the number of beneficial *Bifidobacterium*, *Lactobacillus*, and short-chain fatty acid (SCFA)-producing species (Zhuang et al., 2021). The genus

*Lactobacillus* is a facultatively anaerobic bacteria abundant in the small intestine, especially the ileum. This is consistent with the fact that fats are absorbed in the small intestine and that *Lactobacillus*-derived fatty acids HYA are abundant in the ileum.  $\omega$ -6 LA supplementation diet causes significant expansion of the intestinal *Lactobacillus* genus. Among the *Lactobacillus* genus, *Lactobacillus salivarius* has the high activity of a critical LA-metabolizing enzyme *Cla-hy* (conjugated linoleic acid-oleate hydratase), which converts LA into HYA, and LA induces the expression of *Cla-hy* gene. Additionally, *Lactobacillus plantarum* possesses conjugated linoleic acids oleate hydratase (CLA-HY), CLA short-chain dehydrogenase (CLA-DH), and CLA acetoacetate decarboxylase (CLA-DC) activities. These enzymes synthesize HYA and CLAs from LAs and have potential physiological activities such as anti-inflammatory, anti-cardiovascular disease, and anti-diabetes (Liu et al., 2021). These research results provide a central mechanism explaining the interrelationship between commensal bacteria and the host for maintaining energy homeostasis. They can be used to develop customized functional probiotics and foods to prevent metabolic disorders such as obesity, cardiovascular diseases, and type 2 diabetes.

## Conclusions

EPA and DHA are well known to prevent numerous metabolic disorders. Due to the increase in chronic diseases, consumer interest and demand for natural dietary sources of  $\omega$ -3 PUFAs are rapidly increasing.  $\omega$ -6 PUFAs and  $\omega$ -3 PUFAs have beneficial health effects and antagonistic (opposing) metabolic functions in the body.  $\omega$ -6 AA accounts for up to 25% of the fatty acids in phospholipids of muscles, brain, liver, platelets, and immune cells (Calder, 2007). Phospholipase A2 releases AA from the membrane into the cell cytosol (Korotkova and Lundberg, 2014). In “resting” conditions, eicosanoid production is low and the metabolites produced play a role in maintaining homeostasis by regulating physiological processes. However, AA is released in the presence of inflammatory stimuli and eicosanoid formation increases.  $\omega$ -3 EPA and DHA have various anti-inflammatory effects and reduce AA-derived eicosanoid production. It also competes with AA to form of cell membrane phospholipids and reduces the release of AA from membranes (Calder, 2015). EPA and DHA play various roles in maintaining body homeostasis and protecting against several chronic and metabolic diseases, including cardiovascular, inflammation, obesity, and diabetes.



$\omega$ -6 PUFAs greatly exceed the amount of  $\omega$ -3 PUFAs in most Western diets. Modern diet-related chronic diseases are rising due to the increasing unbalanced consumption of  $\omega$ -6 PUFAs rather than  $\omega$ -3 PUFAs. The essential fatty acids of LA and ALA compete with the same set of enzymes in the elongation and desaturation processes that are converted to longer PUFAs. When  $\omega$ -6 LA is present at a higher ratio than  $\omega$ -3 ALA, LA is converted to AA, so most cellular phospholipids become AA. In oxidative metabolism, AA produces many potent pro-inflammatory eicosanoids and contributes to the formation of thrombus and atheroma, allergic reaction and inflammatory disorders, and a hyperactive endo-cannabinoid system that affects appetite and food intake, increasing weight gain and obesity. On the other hand,  $\omega$ -3 PUFAs, such as EPA and DHA, have anti-inflammatory, anti-aggregatory, vasodilation, and bronchodilation effects, resolving inflammation and reducing the risk of cancer and cardiovascular diseases by changing the function of vascular and carcinogen biomarkers. Therefore, considering these antagonistic effects of  $\omega$ -3 and  $\omega$ -6 PUFAs, balancing the LA/ALA ratio is important in regulating body homeostasis.

Among plant sources, seed oils of *Salvia hispanica*, *Linum usitatissimum*, *Lepidium sativum*, *Echium plantagineum*, *Buglossoides arvensis*, and *Ribes sp.* are widely studied as sources of ALA and SDA. Additionally, oils from microalgae and *thraustochytrids* can directly supply EPA and DHA. Therefore, these plant sources are used for the commercial production of EPA and DHA (Saini et al., 2021).

Monogastric animals, such as pigs and chickens, are the ideal for producing fortified meat and eggs with altered FA profiles by controlling dietary FA. Supplementing animal feed with oils high in  $\omega$ -3 fatty acids and antioxidants that limit lipoperoxidation has an antioxidant effect on feed lipids. It also produces  $\omega$ -3 PUFA enriched meat and has higher oxidative stability. These results serve as the basis for producing meats with desirable FA profiles and oxidative stability by using  $\omega$ -3 sources and antioxidant additives in feed mixtures (Fig. 14). Cattle management practices should be considered when making nutritional claims. Grass-finished beef produced in agroecological ways matches the preferences of consumers concerned about its impact on well-being and environmental impact. Grass-finished beef is an important alternative that contributes to food sustainability goals by providing higher amounts of potentially beneficial nutrients. Compared to grain-fed beef, grass-fed beef has higher  $\omega$ -3 PUFAs and conjugated linoleic acid (CLA) and lower  $\omega$ -6 PUFAs and cholesterol-raising saturated fatty acids (SFAs). This protects against several metabolic diseases, including obesity, diabetes, cancer, and cardiovascular disease (CVD). Therefore, beef's  $\omega$ -6/ $\omega$ -3 ratio,

total  $\omega$ -3 PUFAs, micronutrients, and phytochemicals are essential compounds in determining the finishing diet of cattle. Grass-finished beef, which contains high amounts of beneficial bioactive compounds, is beneficial to human health.

Supplementing the diets of lactating dairy cows with canola oil, linseed oil, and rumen-protected microencapsulated fatty acids can increase the concentration of  $\omega$ -3 PUFAs, CLA, and oleic acid, producing more nutritionally enhanced milk. Rumen-bypass microencapsulated  $\omega$ -3 fatty acids safely improve the pass rate of  $\omega$ -3 fatty acids from the rumen to the intestine and do not negatively influence ruminal fermentation in dairy cows. This rumen-protected microencapsulation approach of  $\omega$ -3 fatty acids sources represents a strategy for producing meat and milk enriched with  $\omega$ -3 fatty acids for ruminants. To produce meat, milk, and cheese high in  $\omega$ -3 PUFAs from ruminant livestock, including lactating dairy cows and goats, dietary supplementation with  $\omega$ -3 sources or pasture finishing and rumen-protected microencapsulated fatty acids can be selected. This choice benefits human health by improving animal metabolic health and nutritional compounds. Therefore, much research is needed in the future to link the impact of various production systems on the environment (soils, plants, animals) and human health.

A higher LA/ALA ratio than the high amount of fats included in the diet is the primary determinant of disease aggravation. A lower LA/ALA ratio has a prebiotic effect that suppresses abnormal expansion of *Proteobacteria* and *Escherichia-Shigella*. Additionally, the interaction between gut microbiota and host energy metabolism through dietary  $\omega$ -6 PUFA-derived bacterial intermediate metabolites provides a method to prevent metabolic disorders by targeting gut microbial metabolites.

A high  $\omega$ -6/ $\omega$ -3 ratio is associated with overweight and obesity, while a balanced ratio reduces obesity and weight gain. Therefore, the intake of  $\omega$ -3 PUFAs should be increased while reducing  $\omega$ -6 PUFAs in human diet and animal feed. Additionally, it is necessary to make every effort to increase the intake of probiotics, including lactic acid bacteria, which can convert  $\omega$ -6 fatty acids into functional metabolites.

521 **Author contributions**

522 HY Jeong: Investigation, data curation, software, formal analysis. YS Moon:  
523 Conceptualization, validation, writing - original draft. KK Cho: Conceptualization, validation,  
524 writing - review, editing & supervision .

525  
526 **Conflict of interest**

527 The authors declare no potential conflicts of interest.

528  
529 **Ethics Approval**

530 This article does not require IRB/IACUC approval because there are no human and animal  
531 participants.

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## References

- Ailhaud G, Massiera F, Weill P, Legrand P, Alessandri JM, Guesnet P. 2006. Temporal changes in dietary fats: role of n-6 polyunsaturated fatty acids in excessive adipose tissue development and relationship to obesity. *Prog Lipid Res* 45(3):203-36. doi: 10.1016/j.plipres.2006.01.003. Epub 2006 Feb 10. PMID: 16516300.
- Allothman M, Hogan SA, Hennessy D, Dillon P, Kilcawley KN, O'Donovan M, Tobin J, Fenelon MA, O'Callaghan TF. 2019. The "grass-fed" milk story: Understanding the impact of pasture feeding on the composition and quality of bovine milk. *Foods* 8: 350. doi: 10.3390/foods8080350. PMID: 31426489.
- Amjad Khan W, Chun-Mei H, Khan N, Iqbal A, Lyu SW, Shah F. 2017. Bioengineered plants can be a useful source of omega-3 fatty acids. *Biomed Res Int* 2017:7348919. doi: 10.1155/2017/7348919. Epub 2017 Feb 21. PMID: 28316988.
- Ameur A, Enroth S, Johansson A, Zaboli G, Igl W, Johansson AC, Rivas MA, Daly MJ, Schmitz G, Hicks AA, Meitinger T, Feuk L, van Duijn C, Oostra B, Pramstaller PP, Rudan I, Wright AF, Wilson JF, Campbell H, Gyllenstein U. 2012. Genetic adaptation of fatty-acid metabolism: a human-specific haplotype increasing the biosynthesis of long-chain omega-3 and omega-fatty acids. *Am J Hum Genet* 90(5):809-20. doi: 10.1016/j.ajhg.2012.03.014. Epub 2012 Apr 12. PMID: 22503634.
- Avallone R, Vitale G, Bertolotti M. 2019. Omega-3 fatty acids and neurodegenerative diseases: new evidence in clinical trials. *Int J Mol Sci* 20(17):4256. doi: 10.3390/ijms20174256. PMID: 31480294.
- Bae JH, Lim H, Lim S. 2023. The potential cardiometabolic effects of long-chain omega-3 polyunsaturated fatty acids: recent updates and controversies. *Adv Nutr* 14(4):612-628. doi: 10.1016/j.advnut.2023.03.014. Epub 2023 Apr 7. PMID: 37031750.
- Benvenga S, Famà F, Perdichizzi LG, Antonelli A, Brenta G, Vermiglio F, Moleti M. 2022. Fish and the thyroid: A janus bifrons relationship caused by pollutants and the omega-3

polyunsaturated fatty acids. *Front Endocrinol (Lausanne)* 13:891233. doi: 10.3389/fendo.2022.891233. eCollection 2022. PMID: 35712237.

Bermúdez Menéndez de la Granda M, Sinclair AJ. 2009. Fatty acids and obesity. *Curr Pharm Des* 15(36):4117-25. doi: 10.2174/138161209789909674. PMID: 20041813.

Bindu S, Mazumder S, Bandyopadhyay U. 2020. Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: A current perspective. *Biochem Pharmacol* 180:114147. doi: 10.1016/j.bcp.2020.114147. Epub 2020 Jul 10. PMID: 32653589.

Brown EM, Clardy J, Xavier RJ. 2023. Gut microbiome lipid metabolism and its impact on host physiology. *Cell Host Microbe* 31(2):173-186. doi: 10.1016/j.chom.2023.01.009. PMID: 36758518.

Calder PC. 2007. Dietary arachidonic acid: harmful, harmless or helpful? *Br J Nutr* 98(3):451-3. doi: 10.1017/S0007114507761779. PMID: 17705889.

Calder PC. 2015. Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance. *Biochim Biophys Acta* 1851(4):469-84. doi: 10.1016/j.bbalip.2014.08.010. Epub 2014 Aug 20. PMID: 25149823.

Cardoso C, Afonso C, Bandarra NM. Dietary DHA and health: cognitive function ageing. 2016. *Nutr Res Rev* 29(2):281-294. doi: 10.1017/S0954422416000184. Epub 2016 Nov 21. PMID: 27866493.

Daley CA, Abbott A, Doyle PS, Nader GA, Larson S. 2010. A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. *Nutr J* 9:10. doi: 10.1186/1475-2891-9-10. PMID: 20219103.

D'Angelo S, Motti ML, Meccariello R. 2020. omega-3 and omega-6 polyunsaturated fatty acids, obesity and cancer. *Nutrients* 12(9):2751. doi: 10.3390/nu12092751. PMID: 32927614.

- de Carvalho CCCR, Caramujo MJ. 2018. The various roles of fatty acids. *Molecules* 23(10):2583. doi: 10.3390/molecules23102583. PMID: 30304860.
- Dyerberg J and Bang HO. 1979. Lipid metabolism, atherogenesis, and haemostasis in Eskimos: The role of the prostaglandin-3 family. *Haemostasis* 8: 227-233.
- Dyńska D, Kowalcze K, Charuta A, Paziewska A. 2023. The ketogenic diet and cardiovascular diseases. *Nutrients* 15(15):3368. doi: 10.3390/nu15153368. PMID: 37571305.
- Hao L, Chen CY, Nie YH, Kaliannan K, Kang JX. 2023. Differential interventional effects of omega-6 and omega-3 polyunsaturated fatty Acids on high fat diet-induced obesity and hepatic pathology. *Int J Mol Sci* 24(24):17261. doi: 10.3390/ijms242417261. PMID: 38139090.
- Harnack K, Andersen G, Somoza V. 2009. Quantitation of alpha-linolenic acid elongation to eicosapentaenoic and docosahexaenoic acid as affected by the ratio of n6/n3 fatty acids. *Nutr Metab (Lond)* 6:8. doi: 10.1186/1743-7075-6-8. PMID: 19228394.
- Hirai A. 1985. An epidemiological study on the dietary ingestion of eicosapentaenoic acid (EPA) and platelet function in Japanese. *Intern Med* 74: 13-20.
- Huang S, Baurhoo B, Mustafa A. 2020. Effects of feeding extruded flaxseed on layer performance, total tract nutrient digestibility, and fatty acid concentrations of egg yolk, plasma and liver. *J Anim Physiol Anim Nutr (Berl)* 104(5):1365-1374. doi: 10.1111/jpn.13364. Epub 2020 May 5. PMID: 32372432.
- Irawan A, Ningsih N, Hafizuddin, Rusli RK, Suprayogi WPS, Akhirini N, Hadi RF, Setyono W, Jayanegara A. 2022. Supplementary n-3 fatty acids sources on performance and formation of omega-3 in egg of laying hens: a meta-analysis. *Poult Sci* 101(1):101566. doi: 10.1016/j.psj.2021.101566. Epub 2021 Oct 26. PMID: 34823172.

- Ishihara T, Yoshida M, Arita M. 2019. Omega-3 fatty acid-derived mediators that control inflammation and tissue homeostasis. *Int Immunol* 31(9):559-567. doi: 10.1093/intimm/dxz001. PMID: 30772915.
- Kawamura A, Nemoto K, Sugita M. 2022. Effect of 8-week intake of the n-3 fatty acid-rich perilla oil on the gut function and as a fuel source for female athletes: A randomised trial. *Br J Nutr* 129(6):1-11. doi: 10.1017/S0007114522001805. PMID: 3570519.
- Kim TB, Lee JS, Cho SY, Lee HG. 2020. *In Vitro* and *In Vivo* studies of rumen-protected microencapsulated supplement comprising linseed oil, vitamin E, rosemary extract, and hydrogenated palm oil on rumen fermentation, physiological profile, milk yield, and milk composition in dairy cows. *Animals (Basel)* 10(9):1631. doi: 10.3390/ani10091631. PMID: 32932849.
- Kishino S, Takeuchi M, Park SB, Hirata A, Kitamura N, Kunisawa J, Kiyono H, Iwamoto R, Isobe Y, Arita M, Arai H, Ueda K, Shima J, Takahashi S, Yokozeki K, Shimizu S, Ogawa J. 2103. Polyunsaturated fatty acid saturation by gut lactic acid bacteria affecting host lipid composition. *Proc Natl Acad Sci U S A* 110(44):17808-13. doi: 10.1073/pnas.1312937110. Epub 2013 Oct 14.
- Korotkova M, Lundberg IE. 2014. The skeletal muscle arachidonic acid cascade in health and inflammatory disease. *Nat Rev Rheumatol* 10(5):295-303. doi: 10.1038/nrrheum.2014.2. Epub 2014 Jan 28. PMID: 24468934.
- Kousparou C, Fyrilla M, Stephanou A, Patrikios I. 2023. DHA/EPA (omega-3) and LA/GLA (omega-6) as bioactive molecules in neurodegenerative diseases. *Int J Mol Sci* 24(13):10717. doi: 10.3390/ijms241310717. PMID: 37445890.
- Krusinski L, Maciel ICF, van Vliet S, Ahsin M, Lu G, Rowntree JE, Fenton JI. 2023. Measuring the phytochemical richness of meat: Effects of grass/grain finishing systems and grapeseed extract supplementation on the fatty acid and phytochemical content of beef. *Foods* 12(19):3547. doi: 10.3390/foods12193547. PMID: 37835200.

- Li J, Jian Y, Liu R, Zhao X, Mao J, Wei W, Jiang C, Zhang L, Wang Y, Zhou P. 2023. Choline and fish oil can improve memory of mice through increasing brain DHA level. *Foods*. 12(9):1799. doi: 10.3390/foods12091799. PMID: 37174337
- Liu XX, Xiong ZQ, Wang GQ, Wang LF, Xia YJ, Song X, Ai LZ. 2021. LysR family regulator LttR controls production of conjugated linoleic acid in *lactobacillus plantarum* by directly activating the *cla* operon. *Appl Environ Microbiol* 87(6):e02798-20. doi: 10.1128/AEM.02798-20. Print 2021 Feb 26. PMID: 33397697.
- Massiera F, Saint-Marc P, Seydoux J, Murata T, Kobayashi T, Narumiya S, Guesnet P, Amri EZ, Negrel R, Ailhaud G. 2003. Arachidonic acid and prostacyclin signaling promote adipose tissue development: a human health concern? *J Lipid Res* 44(2):271-9. doi: 10.1194/jlr.M200346-JLR200. Epub 2002 Nov 4. PMID: 12576509.
- Mierlita D, Santa A, Mierlita S, Daraban SV, Suteu M, Pop IM, Mintas OS, Macri AM. 2022. The effects of feeding milled rapeseed seeds with different forage:concentrate ratios in Jersey dairy cows on milk production, milk fatty acid composition, and milk antioxidant capacity. *Life (Basel)* 13(1):46. doi: 10.3390/life13010046. PMID: 36675995.
- Miyamoto J, Igarashi M, Watanabe K, Karaki SI, Mukouyama H, Kishino S, Li X, Ichimura A, Irie J, Sugimoto Y, Mizutani T, Sugawara T, Miki T, Ogawa J, Drucker DJ, Arita M, Itoh H, Kimura I. 2019. Gut microbiota confers host resistance to obesity by metabolizing dietary polyunsaturated fatty acids. *Nat Commun* 10(1):4007. doi: 10.1038/s41467-019-11978-0. PMID: 31488836.
- Moya F, Madrid J, Hernández F, Peñaranda I, Garrido MD, López MB. 2023. Influence of dietary lipid source supplementation on milk and fresh cheese from Murciano-Granadina goats. *Animals (Basel)* 13(23):3652. doi: 10.3390/ani13233652. PMID: 38067003.
- Naughton SS, Mathai ML, Hryciw DH, McAinch AJ. 2016. Linoleic acid and the pathogenesis of obesity. *Prostaglandins Other Lipid Mediat* 125:90-9. doi: 10.1016/j.prostaglandins.2016.06.003. Epub 2016 Jun 24. PMID: 27350414.



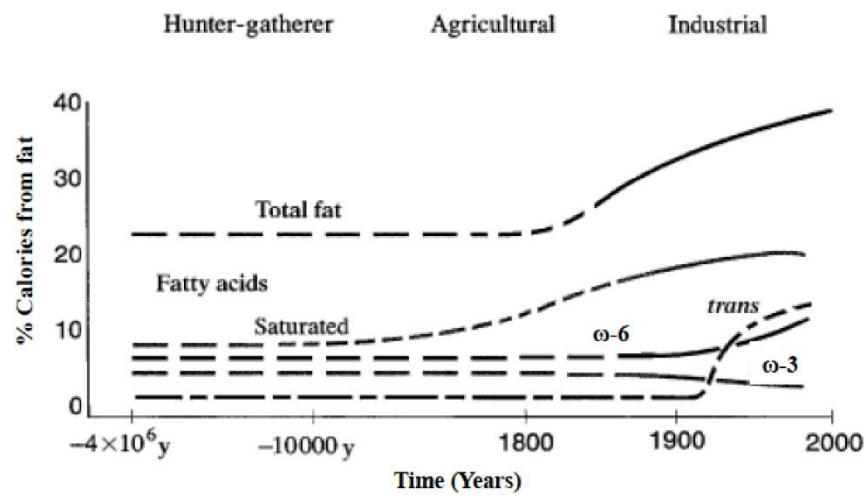
- Nimalaratne C, Wu J. 2015. Hen egg as an antioxidant food commodity: A review. *Nutrients* 7(10):8274-93. doi: 10.3390/nu7105394. PMID: 26404361.
- OECD/FAO. 2017. OECD-FAO Agricultural Outlook 2017–2026; OECD Publishing: Paris, France.
- Ortiz L.T., Velasco S., Treviño J., Jiménez B., Rebolé A. 2021. Changes in the nutrient composition of barley grain (*Hordeum vulgare* L.) and of morphological fractions of sprouts. *Scientifica (Cairo)* 2021:9968864. doi: 10.1155/2021/9968864.
- Ozturk I., Sagdic O., Hayta M., Yetim H. 2012. Alteration in  $\alpha$ -tocopherol, some minerals, and fatty acid contents of wheat through sprouting. *Chem Nat Compd* 47:876-879. doi: 10.1007/s10600-012-0092-9.
- Petersen RK, Jørgensen C, Rustan AC, Frøyland L, Muller-Decker K, Furstenberger G, Berge RK, Kristiansen K, Madsen L. 2003. Arachidonic acid-dependent inhibition of adipocyte differentiation requires PKA activity and is associated with sustained expression of cyclooxygenases. *J Lipid Res* 44(12):2320-30. doi: 10.1194/jlr.M300192-JLR200. Epub 2003 Aug 16. PMID: 12923227.
- Petrenya N, Brustad M, Cooper M, Dobrodeeva L, Bichkaeva F, Lutfalieva G, Odland JO. 2012. Serum apolipoproteins in relation to intakes of fish in population of Arkhangelsk County. *Nutr Metab (Lond)* 9(1):51. doi: 10.1186/1743-7075-9-51. PMID: 22681916.
- Qin J, Kurt E, LBassi T, Sa L, Xie D. 2023. Biotechnological production of omega-3 fatty acids: current status and future perspectives. *Front Microbiol* 14:1280296. doi: 10.3389/fmicb.2023.1280296. eCollection 2023. PMID: 38029217.
- Radanović A, Kralik G, Drenjančević I, Galović O, Košević M, Kralik Z. 2023. n-3 PUFA enriched eggs as a source of valuable bioactive substances. *Foods* 12(23):4202. doi: 10.3390/foods12234202. PMID: 38231614.

- Rezvukhin AI, Shalaurova Iu, Berezovskaia EV. 1996. Effect of actual diet on fatty acid composition of blood serum and indicators of immunity in Siberian and Chukotka populations. *Vopr Med Khim* 42(1):59-64. PMID: 8999660.
- Rice JB, White AG, Scarpati LM, Wan G, Nelson WW. 2017. Long-term systemic corticosteroid exposure: A systematic literature review. *Clin Ther* 39(11):2216-2229. doi: 10.1016/j.clinthera.2017.09.011. Epub 2017 Oct 19. PMID: 29055500.
- Ruiz-Lopez N, Usher S, Sayanova OV, Napier JA, Haslam RP. 2015. Modifying the lipid content and composition of plant seeds: engineering the production of LC-PUFA. *Appl Microbiol Biotechnol* 99(1):143-54. doi: 10.1007/s00253-014-6217-2. Epub 2014 Nov 25. PMID: 25417743.
- Saini RK, Keum YS. 2018. Omega-3 and omega-6 polyunsaturated fatty acids: Dietary sources, metabolism, and significance - A review. *Life Sci* 203:255-267. doi: 10.1016/j.lfs.2018.04.049. Epub 2018 Apr 30. PMID: 29715470.
- Saini RK, Prasad P, Sreedhar RV, Akhilender Naidu K, Shang X, Keum YS. 2021. Omega-3 polyunsaturated fatty acids (PUFAs): Emerging plant and microbial sources, oxidative stability, bioavailability, and health benefits-A review. *Antioxidants (Basel)* 10(10):1627. doi: 10.3390/antiox10101627. PMID: 34679761.
- Salem N Jr, Vandal M, Calon F. 2015. The benefit of docosahexaenoic acid for the adult brain in aging and dementia. *Prostaglandins Leukot Essent Fatty Acids* 92:15-22. doi: 10.1016/j.plefa.2014.10.003. Epub 2014 Oct 24. PMID: 25457546.
- Shingfield, K.J.; Bonnet, M.; Scollan, N.D. 2013. Recent developments in altering the fatty acid composition of ruminant-derived foods. *Animal* 7:132–162.
- Sierżant K, Korzeniowska M, Półbrat T, Rybarczyk A, Smoliński J. 2022. The use of an optimised concentration of quercetin limits peroxidation of lipids in the meat of broiler chickens fed a diet containing flaxseed oil rich in omega-3. *Animal* 16(8):100603. doi: 10.1016/j.animal.2022.100603. Epub 2022 Aug 9. PMID: 35961176.

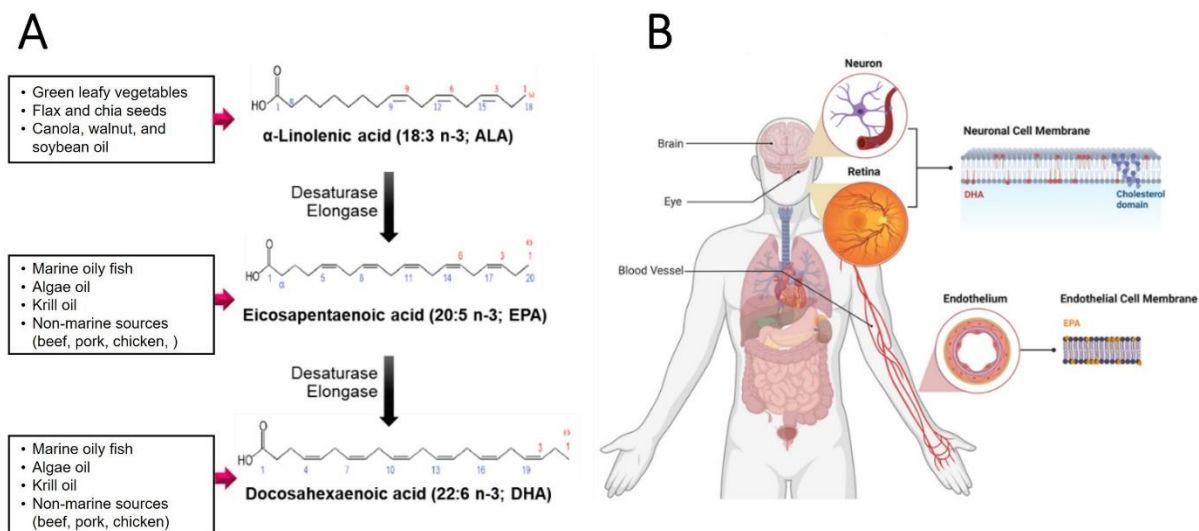
- Simopoulos AP. 2006. Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: nutritional implications for chronic diseases. *Biomed Pharmacother* 60(9):502-7. doi: 10.1016/j.biopha.2006.07.080. Epub 2006 Aug 28. PMID: 17045449.
- Simopoulos AP, Salem N Jr. 1992. Egg yolk as a source of long-chain polyunsaturated fatty acids in infant feeding. *Am J Clin Nutr* 55(2):411-4. doi: 10.1093/ajcn/55.2.411. PMID: 1734678.
- Simopoulos AP. 2016. An Increase in the omega-6/omega-3 fatty acid ratio increases the risk for obesity. *Nutrients* 8(3):128. doi: 10.3390/nu8030128. PMID: 26950145.
- Sot J, García-Arribas AB, Abad B, Arranz S, Portune K, Andrade F, Martín-Nieto A, Velasco O, Arana E, Tueros I, Ferreri C, Gaztambide S, Goñi FM, Castaño L, Alonso A. 2022. Erythrocyte membrane nanomechanical rigidity is decreased in obese patients. *Int J Mol Sci* 23(3):1920. doi: 10.3390/ijms23031920. PMID: 35163842.
- Souphannavong C, Arjin C, Sartsook A, Yosen T, Thongkham M, Seel-audom M, Mekchay S, Sringarm K. 2021. Nutritional values and nutrient digestibility of ground perilla cake (*Perilla frutescens*) in growing pig diets. *Vet. Integr. Sci.* 19:121–131.
- Sringarm K, Chaiwang N, Wattanakul W, Mahinchai P, Satsook A, Norkeaw R, Seel-Audom M, Moonmanee T, Mekchay S, Sommano SR, Ruksiriwanich W, Rachtanapun P, Jantanasakulwong K, Arjin C. 2022. Improvement of intramuscular fat in *longissimus* muscle of finishing Thai crossbred black pigs by perilla cake supplementation in a low-lysine diet. *Foods* 11(7):907. doi: 10.3390/foods11070907. PMID: 35406994.
- Su H, Zhou D, Pan YX, Wang X, Nakamura MT. 2016. Compensatory induction of *Fads1* gene expression in heterozygous *Fads2*-null mice and by diet with a high n-6/n-3 PUFA ratio. *J Lipid Res* 57(11):1995-2004. doi: 10.1194/jlr.M064956. Epub 2016 Sep 9. PMID: 27613800.
- Tognocchi M, Conte G, Mantino A, Foggi G, Casarosa L, Tinagli S, Turini L, Scicutella F, Mele M, Serra A. 2023. Linseed supplementation in the diet of fattening pigs: Effect on the

- fatty acid profile of different pork cuts. Meat Sci 204:109276. doi: 10.1016/j.meatsci.2023.109276. Epub 2023 Jul 4. PMID: 37421935.
- USDA. 2011. USDA National Nutrient Database for Standard Reference. <http://ndb.nal.usda.gov/>.accessed at June 2013.
- USDA. 2015. National Nutrient Database for Standard Reference. <http://ndb.nal.usda.gov/ndb/search>, accessed on 29th September 2017.
- Vaittinen M, Lankinen MA, Käkälä P, Ågren J, Wheelock CE, Laakso M, Schwab U, Pihlajamäki J. 2022. The FADS1 genotypes modify the effect of linoleic acid-enriched diet on adipose tissue inflammation via pro-inflammatory eicosanoid metabolism. Eur J Nutr 61(7):3707-3718. doi: 10.1007/s00394-022-02922-y. Epub 2022 Jun 14. PMID: 35701670.
- van Vliet S, Blair AD, Hite LM, Cloward J, Ward RE, Kruse C, van Wietmarschen HA, van Eekeren N, Kronberg SL, Provenza FD. 2023. Pasture-finishing of bison improves animal metabolic health and potential health-promoting compounds in meat. J Anim Sci Biotechnol 14(1):49. doi: 10.1186/s40104-023-00843-2. PMID: 3700410.
- van Wyngaard BE, Hugo A, Strydom PE, de Witt FH, Pohl CH, Kanengoni AT. 2023. A comparison of *Echium*, fish, palm, soya, and linseed oil supplementation on pork quality. Anim Biosci 36(9):1414-1425. doi: 10.5713/ab.22.0362. Epub 2023 May 2. PMID: 37170526.
- Wang Q, Wang X. 2023. The effects of a low linoleic acid/ $\alpha$ -linolenic acid ratio on lipid metabolism and endogenous fatty acid distribution in obese mice. Int J Mol Sci 24(15):12117. doi: 10.3390/ijms241512117. PMID: 37569494.
- Watanabe Y, Tatsuno I. 2020. Prevention of cardiovascular events with omega-3 polyunsaturated fatty acids and the mechanism involved. J Atheroscler Thromb 27(3):183-198. doi: 10.5551/jat.50658. Epub 2019 Oct 3. PMID: 31582621.

- Weiser MJ, Butt CM, Mohajeri MH. 2016. Docosahexaenoic acid and cognition throughout the lifespan. *Nutrients* 8(2):99. doi: 10.3390/nu8020099. PMID: 26901223.
- World Health Organization. Obesity: preventing and managing the global epidemic, report of a WHO consultation. 2020. [cited 2020 Sep 7]. Available from: [https://www.who.int/nutrition/publications/obesity/WHO\\_TRS\\_894/en/](https://www.who.int/nutrition/publications/obesity/WHO_TRS_894/en/).
- Xu C, Zhang S, Sun B, Xie P, Liu X, Chang L, Lu F, Zhang S. 2021. Dietary supplementation with microalgae (*Schizochytrium* sp.) improves the antioxidant status, fatty acids profiles and volatile compounds of beef. *Animals (Basel)* 11(12):3517. doi: 10.3390/ani11123517. PMID: 34944292.
- Yamashima T, Ota T, Mizukoshi E, Nakamura H, Yamamoto Y, Kikuchi M, Yamashita T, Kaneko S. 2020. Intake of  $\omega$ -6 polyunsaturated fatty acid-rich vegetable oils and risk of lifestyle diseases. *Adv Nutr* 11(6):1489-1509. doi: 10.1093/advances/nmaa072. PMID: 32623461.
- Zhuang P, Li H, Jia W, Shou Q, Zhu Y, Mao L, Wang W, Wu F, Chen X, Wan X, Wu Y, Liu X, Li Y, Zhu F, He L, Chen J, Zhang Y, Jiao J. 2021. Eicosapentaenoic and docosahexaenoic acids attenuate hyperglycemia through the microbiome-gut-organs axis in *db/db* mice. *Microbiome* 9(1):185. doi: 10.1186/s40168-021-01126-6. PMID: 34507608.

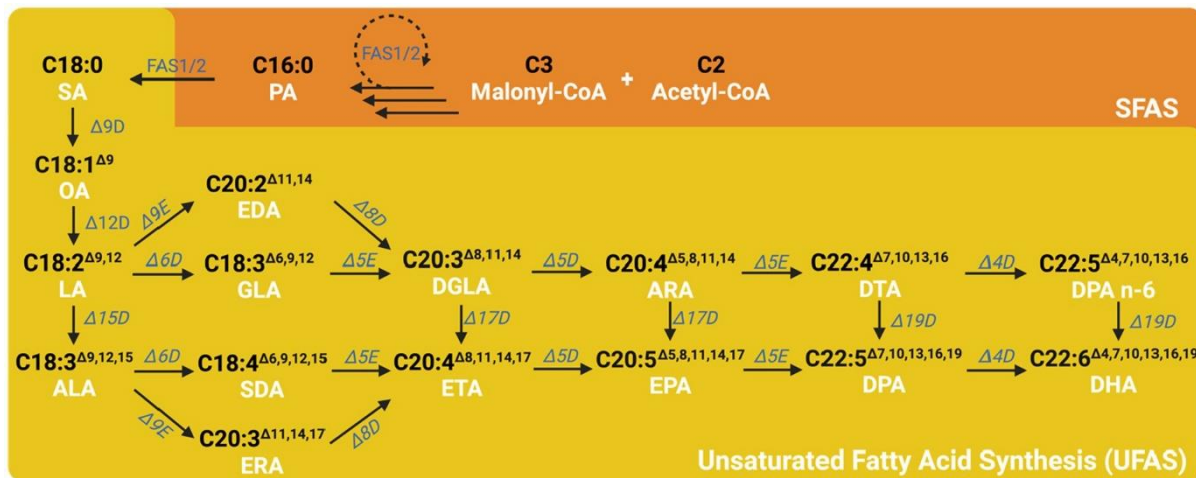


**Fig. 1. Hypothetical changes in total fat, saturated,  $\omega$ -6/ $\omega$ -3, and trans-fatty acid intake during human evolution.** As hunter-gatherer populations evolved into agricultural societies, the  $\omega$ -6/ $\omega$ -3 ratio changed from 1:1 to 20:1, adversely affecting health. Adapted from Simopoulos, (2016) with CC-BY 4.0.



**Fig. 2. Functions of  $\omega$ -3 PUFAs in the human body.** (A) Chemical structures and biosynthetic pathways of ALA, EPA, and DHA. The efficiency of ALA in being converted to EPA and DHA by enzymes in the body is very low, so EPA and DHA must be consumed from external sources. (B) Role of DHA in neurons of the brain, retina of the eyes, and heart endothelium.  $\omega$ -3 PUFAs are essential components of phospholipids in cell membrane membranes. Adapted from Bae et al., (2023) with CC-BY 4.0.

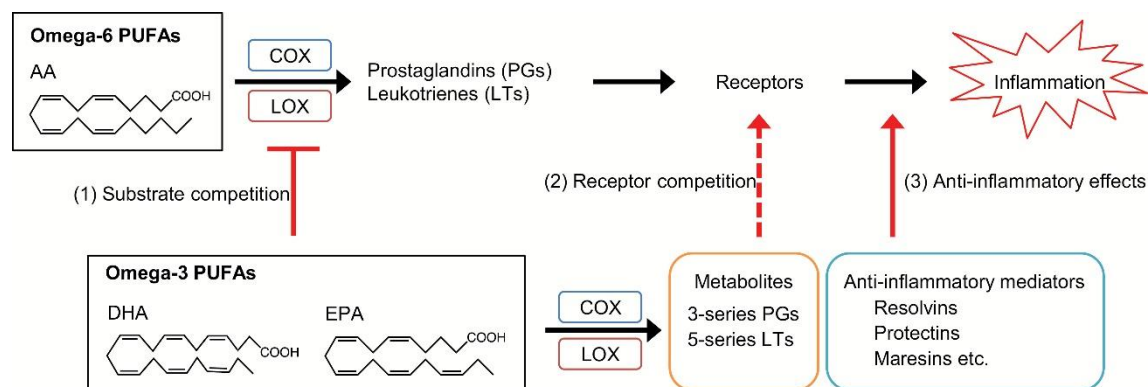
Abbreviations: ALA, Alpha-linolenic acid; DHA, Docosahexaenoic acid; EPA, Eicosapentaenoic acid; PUFAs, Polyunsaturated fatty acids.



**Fig. 3. Synthetic pathway of  $\omega$ -6 and  $\omega$ -3 PUFAs.** Saturated fatty acids are synthesized by fatty acid synthase (FAS), and desaturated fatty acids are synthesized by desaturases and elongases. Photosynthetic organisms with  $\Delta 12$  and  $\Delta 15$  desaturases can synthesize LA and ALA, but animals cannot because they lack these two enzymes. Therefore, LA and ALA are essential fatty acids (EFAs) in animals and must be supplied from plants. Adapted from Qin et al., (2023) with CC-BY 4.0.

Abbreviations: FAS, Fatty acid synthase; PA, Palmitic acid; SA, Stearic acid;  $\Delta 9D$ ,  $\Delta 9$  Desaturase; OA, Oleic acid;  $\Delta 12D$ ,  $\Delta 12$  Desaturase; LA, Linolenic acid;  $\Delta 15D$ ,  $\Delta 15$  Desaturase; ALA, Alpha-linolenic acid;  $\Delta 6D$ ,  $\Delta 6$  Desaturase;  $\Delta 9E$ ,  $\Delta 9$  Elongase; EDA, Eicosadienoic acid; GLA, Gamma-linolenic acid; SDA, Stearidonic acid; ERA, Eicosatrienoic acid;  $\Delta 8D$ ,  $\Delta 8$  Desaturase;  $\Delta 5E$ ,  $\Delta 5$  Elongase; DGLA, Dihomo- $\gamma$ -linolenic acid; ETA, Eicosatetraenoic acid;  $\Delta 5D$ ,  $\Delta 5$  desaturase; ARA, Arachidonic acid; EPA, Eicosapentaenoic acid;  $\Delta 17D$ ,  $\Delta 17$  Desaturase; DTA, Docosatetraenoic acid; DPA, Docosapentaenoic acid;  $\Delta 19D$ ,  $\Delta 19$  Desaturase;  $\Delta 4D$ ,  $\Delta 4$  Desaturase; DHA, Docosahexaenoic acid.

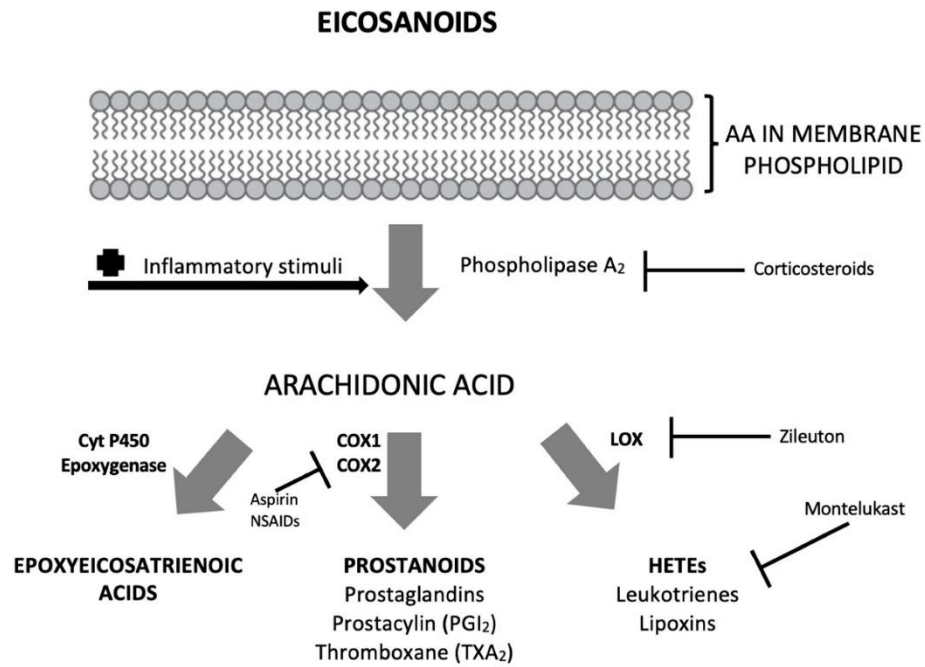




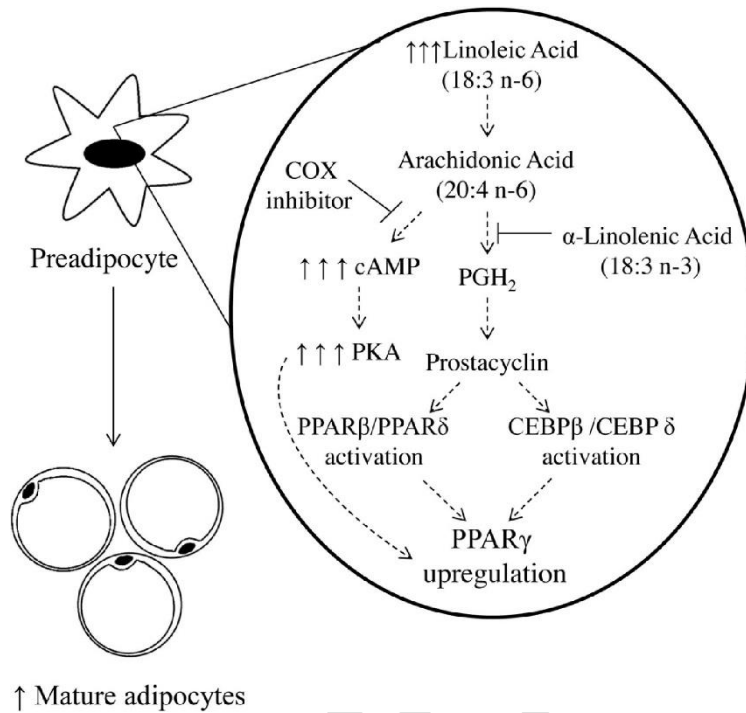
**Fig. 4. Pro-inflammatory effects of  $\omega$ -6 PUFAs and anti-inflammatory effects of  $\omega$ -3**

**PUFAs.**  $\omega$ -6 AA is released from membrane phospholipids in response to inflammatory stimuli and converted into PGs and LTs by COXs and LOXs.  $\omega$ -3 EPA and DHA are converted into anti-inflammatory bioactive mediators by COXs and LOXs.  $\omega$ -3 EPA and DHA exert their beneficial effects through substrate competition, receptor competition, and anti-inflammatory effects. Adapted from Ishihara et al., (2019) with CC-BY 3.0.

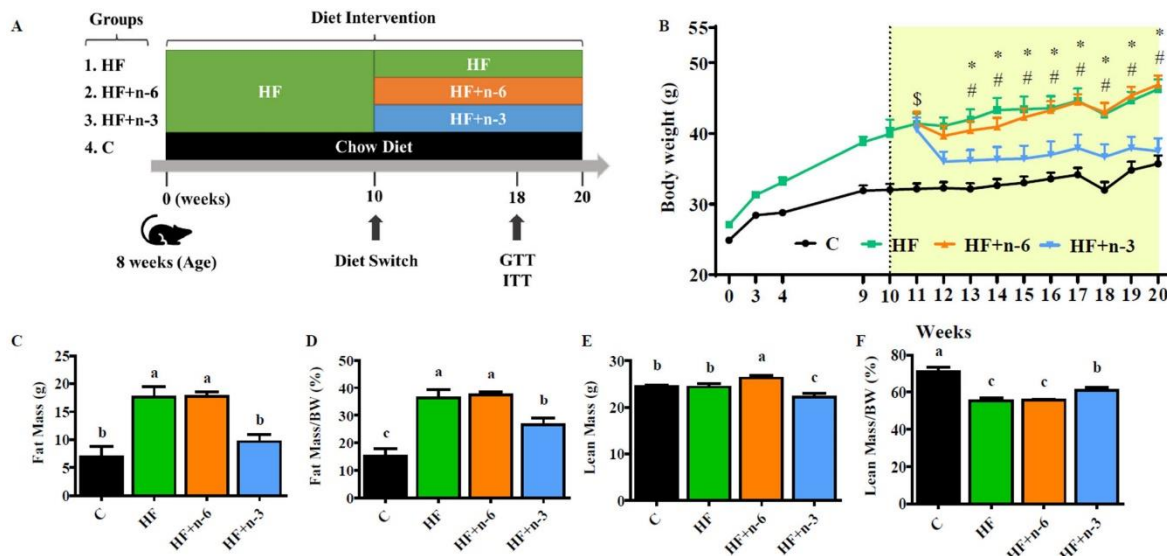
Abbreviations: AA: Arachidonic acid COXs: Cyclooxygenases DHA: Docosahexaenoic acid EPA: Eicosapentaenoic acid LOXs: Lipoxygenases LTs: Leukotrienes PGs: Prostaglandins PUFAs: Polyunsaturated fatty acids.



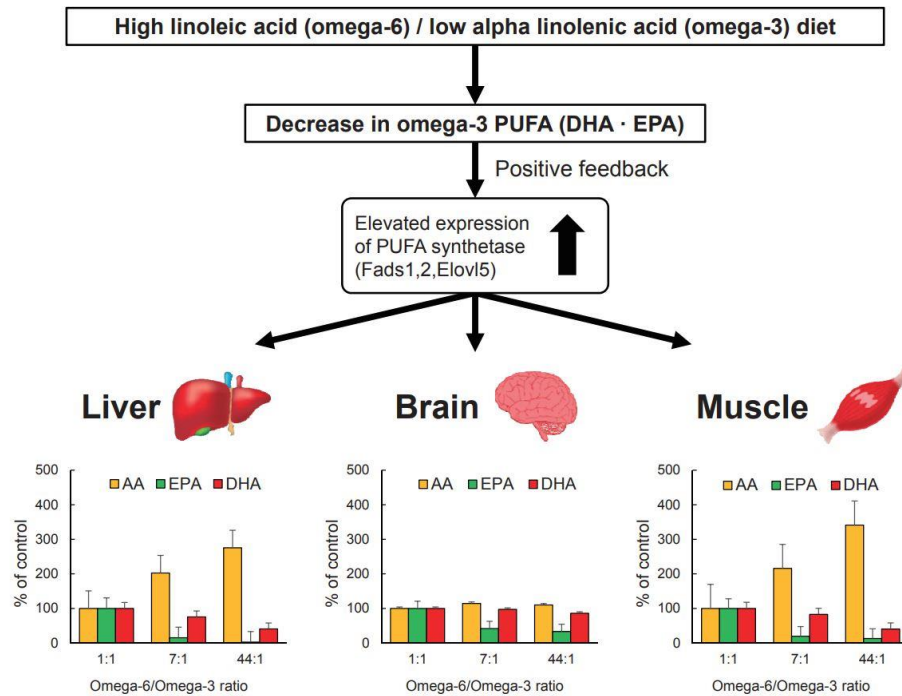
**Fig. 5. Inhibition of  $\omega$ -6 AA inflammatory responses by corticosteroids and nonsteroidal anti-inflammatory drugs (NSAIDs).** Synthetic corticosteroids exhibit anti-inflammatory effects by inhibiting cytosolic phospholipase A<sub>2</sub> (PLA<sub>2</sub>) activity and NSAIDs, including aspirin, by inhibiting cyclooxygenase-1 and 2 (COX-1 and 2) activity. Zileuton acts as a 5-lipoxygenase (LOX) inhibitor, and montelukast acts as a leukotriene receptor antagonist to exhibit anti-inflammatory effects. Adapted from Kousparou et al., (2023) with CC-BY 4.0.



**Fig. 6. Adipocyte differentiation and  $\omega$ -6 arachidonic acid (AA).** AA converted from dietary linoleic acid (LA) can promote adipogenesis and weight gain through the prostacyclin pathway. AA is converted to prostacyclin via prostaglandin H2 (PGH<sub>2</sub>). This process is inhibited by  $\omega$ -3  $\alpha$ -linolenic acid (ALA). Prostacyclin induces adipocyte differentiation by activating the peroxisome proliferator-activated receptor (PPAR) family, CCAAT-enhancer binding protein family (CEBP $\beta$  and CEBP $\delta$ ), and PPAR $\gamma$ . In addition,  $\omega$ -6 AA induces adipocyte differentiation by increasing cAMP (cyclic adenosine monophosphate) and PKA (protein kinase A). Dashed lines represent adipocyte differentiation pathways of  $\omega$ -6 AA, and blunt arrows represent adipogenesis inhibitory factors adipogenesis. Adapted from Naughton et al., (2016) with CC-BY 4.0.

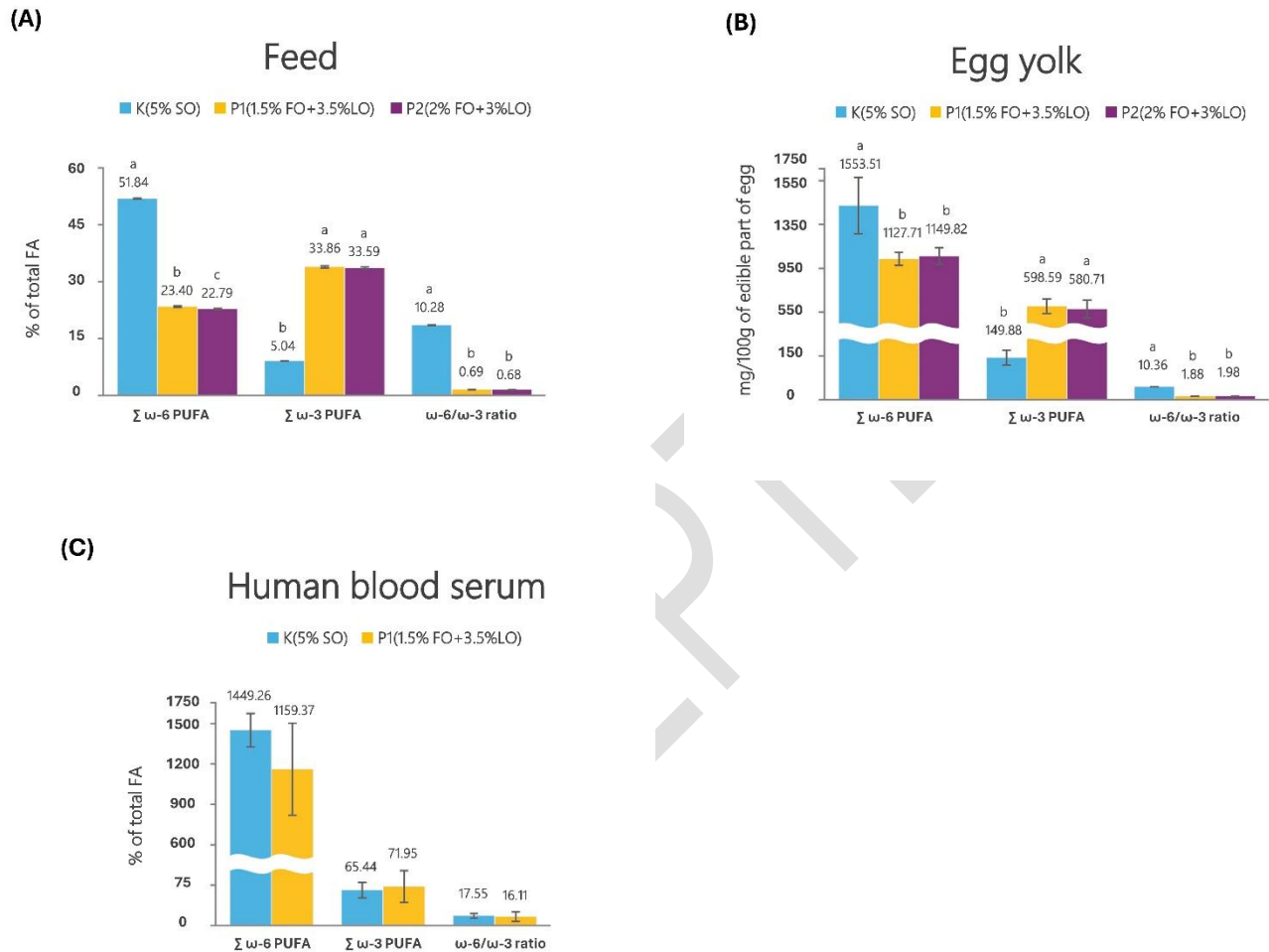


**Fig. 7. Effects of  $\omega$ -6 and  $\omega$ -3 PUFAs on obesity.** C57BL/6J mice were fed a high-fat diet for 10 weeks. In a study conducted on C57BL/6J mice, the mice were initially fed a high-fat diet for 10 weeks. Subsequently, they were divided into three groups and fed HF, HF+ $\omega$ -6 and HF+ $\omega$ -3 diets for another 10 weeks. The control groups were fed chow diets (A). Body weight in the HF+n-3 diets group decreased to the level of the control groups (chow diets), and in the HF+ $\omega$ -6 diets group, it was the same as that of the HF group (B). Total fat mass (C) and relative fat mass to body weight (D) also decreased in the HF+ $\omega$ -3 diet group. Total lean mass (E) decreased in the HF+ $\omega$ -3 diet group, but relative lean mass to body weight (F) increased in the HF+ $\omega$ -3 diet group. Data shown are the mean or mean  $\pm$  S.E.M (n = 6-7/group). \*  $p < 0.05$  HF+n-3 compared to HF #  $p < 0.05$  HF+n-3 compared to HF+n-6, \$  $p < 0.05$  All high-fat diet feed groups compared to C, the different letter indicates significant difference. Adapted from Hao et al., (2023) with CC-BY 4.0.



**Fig. 8.  $\omega$ -6 LA/ $\omega$ -3 ALA ratio and AA, EPA and DHA contents in liver, brain and muscle.**

In mouse model study, AA, EPA and DHA contents in liver, brain and muscle were compared when the  $\omega$ -6 LA/ $\omega$ -3 ALA ratio increased. When the  $\omega$ -6 LA/ $\omega$ -3 ALA ratio was 1:1, the contents of AA, EPA and DHA in liver, brain and muscle showed no difference. When the  $\omega$ -6 LA/ $\omega$ -3 ALA ratio increased to 7:1 and 14:1, the  $\omega$ -6 AA content in the liver and muscle increased, and the contents of  $\omega$ -3 EPA and DHA decreased. However, the  $\omega$ -6 AA content in the brain did not increase, and the content of DHA remained constant. These results suggest the brain has an *in vivo* autonomic regulation to maintain DHA content. Adapted from Hao et al., (2023) with CC-BY 4.0.

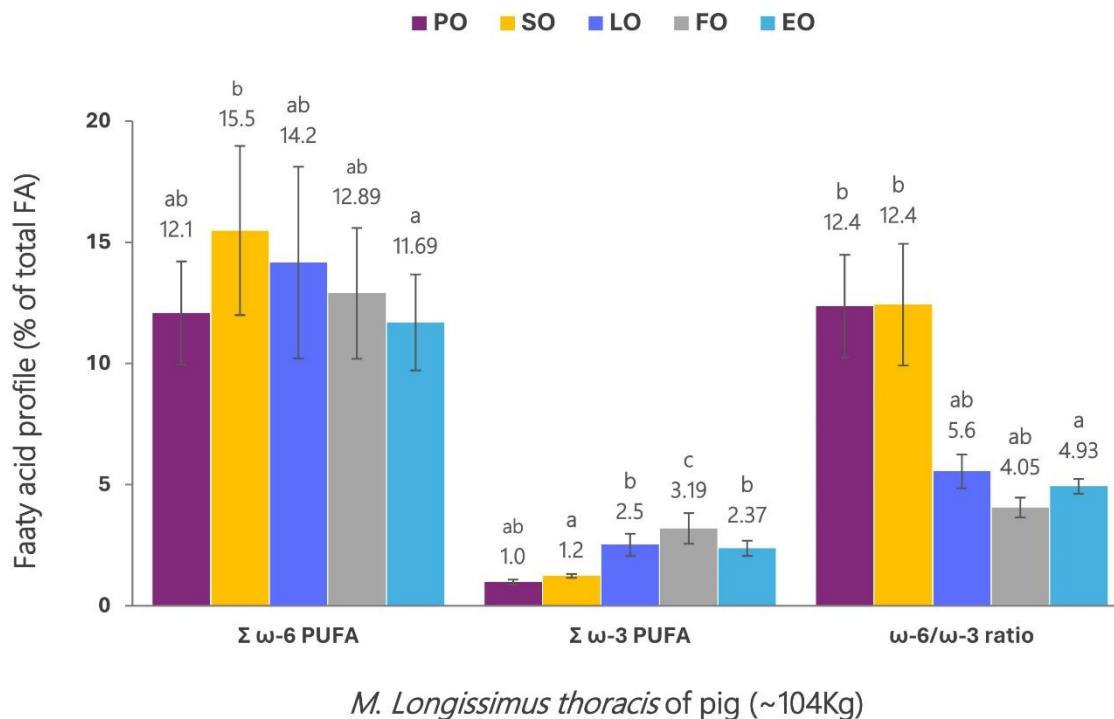


**Fig. 9. Effects of  $\omega$ -3 PUFA in laying hens' feeding mixtures on egg yolks and human blood serum.** (A) Fatty acid profile in laying hens' feeding mixtures (% of total FA), (B) egg yolks (mg FA/100g of edible part of egg), (C) examinees' blood serum (% of total FA). Supplementation of  $\omega$ -3 PUFA in laying hens' feeding mixtures increased  $\omega$ -3 PUFAs in egg yolks. In clinical trials, people who consumed eggs rich in  $\omega$ -3 PUFAs had decreased  $\omega$ -6 PUFAs and increased  $\omega$ -3 PUFAs in their serum. Superscript <sup>a, b, c</sup> differ significantly at  $p < 0.05$ . Adapted from Radanović et al., (2023) with CC-BY 4.0.

Abbreviations: FA, fatty acid; FO, fish oil; LO, linseed oil; SO, soybean oil; PUFA,

polyunsaturated fatty acids.

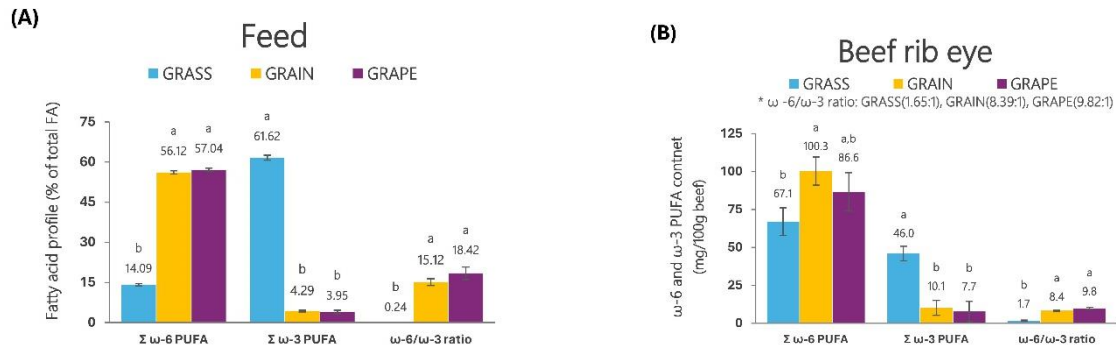
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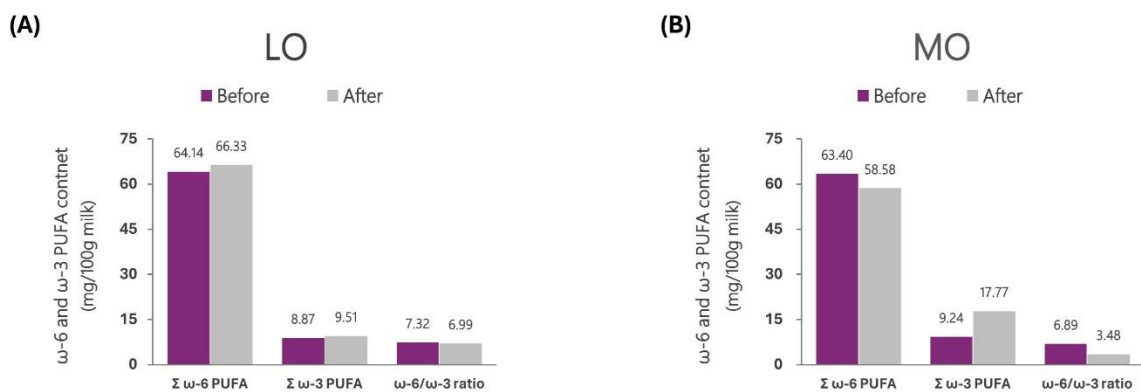
**Fig. 10. Effects of supplementation of five dietary oils (palm oil, soybean oil, flaxseed oil, fish oil, and echium oil) on the  $\omega$ -6 and  $\omega$ -3 PUFAs contents in *M. longissimus thoracis* in pigs.** Five dietary oils were fed from an average live weight of  $\pm 30$  kg to an average live weight of approximately 104 kg. The  $\omega$ -6 PUFA content of *M. longissimus thoracis* was highest in 1% soya oil and lowest in 1% *Echium* oil. The  $\omega$ -3 PUFA content was highest in 1% fish oil and lowest in 1% palm oil. The  $\omega$ -6/ $\omega$ -3 ratio was lowest in 1% fish oil and 1% *Echium* oil. Therefore, it indicated that inexpensive *Echium oil* could replace expensive fish oil. <sup>a-c</sup> Means with different superscripts differ significantly ( $p < 0.05$ ). Adapted from van Wyngaard BE et al., (2023) with CC-BY 4.0.

Abbreviations: EO, *Echium* oil; FO, Fish oil; LO, Linseed oil; PO, Palm oil; SO, Soya oil.



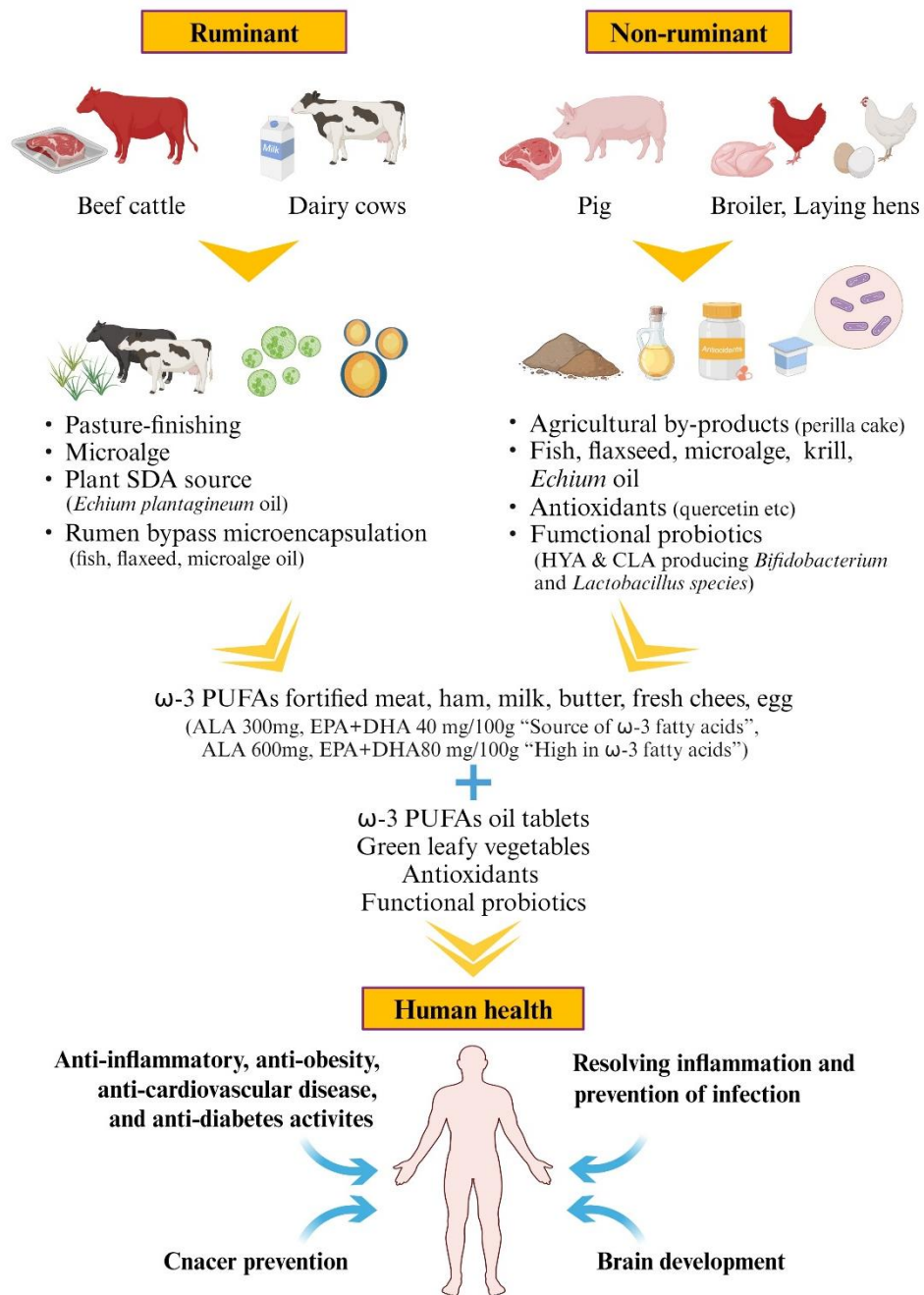


**Fig. 11. Effect of grass/grain finishing systems on  $\omega$ -6 and  $\omega$ -3 PUFAs content of beef rib eye in Red Angus steers. (A)  $\omega$ -6 and  $\omega$ -3 PUFAs content of experimental feed. (B)  $\omega$ -6 and  $\omega$ -3 PUFAs content of beef rib eye.** Grass feeds composed of diverse pastures are high in  $\omega$ -3 PUFAs and low in  $\omega$ -6 PUFAs, while GRAIN and GRAPE feeds containing 74% corn are low in  $\omega$ -3 PUFAs and high in  $\omega$ -6 PUFAs. Beef rib eye from grass finishing systems is high in  $\omega$ -3 PUFAs and low in  $\omega$ -6 PUFAs, while beef rib eye from grain finishing systems is low in  $\omega$ -3 PUFAs and high in  $\omega$ -6 PUFAs. GRASS, diverse pasture; GRAIN, total mixed ration (TMR); GRAPE, TMR+5% DM grape seed extract. Different letters denote statistical significance at  $p < 0.05$ . Adapted from Krusinski et al., (2023) with CC-BY 4.0.



**Fig. 12. Effects of 0.7% linseed oil (A, LO) and 2% rumen-protected microencapsulated supplement (B, MO) on milk  $\omega$ -3 PUFAs content in Holstein dairy cows. 2% MO supplementation in cows' diets inhibited the biohydrogenation of  $\omega$ -3 fatty acids in rumen, increased the content of omega-3 PUFAs in milk, and decreased the  $\omega$ -6/ $\omega$ -3 ratio. Adapted from Kim et al., (2020) with CC-BY 4.0.**





**Fig. 14. Strategies to enhance intake of  $\omega$ -3 PUFAs to improve human health.** Ruminants, including beef cattle and dairy cows, can produce  $\omega$ -3 PUFAs fortified meat and milk using pasture-finishing and rumen bypass microencapsulated oil. Non-ruminants, including pigs, broilers, and laying hens, can be used to produce  $\omega$ -3 PUFAs fortified meat and eggs using agricultural by-products, oils high in  $\omega$ -3 content, antioxidants, and functional probiotics. Humans can expect anti-inflammatory, anti-obesity, cancer prevention and brain development effects by consuming  $\omega$ -3 PUFAs fortified functional foods (Created with [www.Biorender.com](http://www.Biorender.com)).

Abbreviations: ALA, Alpha-Linolenic acid; SDA,  $\omega$ -3 stearidonic acid (C18:4n-3); DHA, Docosahexaenoic acid; EPA, Eicosapentaenoic acid; HYA, 10-hydroxy-cis-12-octadecenoic; CLA, Conjugated linoleic acid; PUFAs, Polyunsaturated fatty acids.

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Table 1. Contents of  $\omega$ -3 and  $\omega$ -6 PUFAs in selected plant and animal-based food

Dietary sources		ALA (18:3, $\omega$ -3)	EPA (20:5, $\omega$ -3)	DHA (22:6, $\omega$ -3)	LA (18:2, $\omega$ -6)	AA (20:4, $\omega$ -6)	$\omega$ -6/ $\omega$ -3 ratio	Units	References
Microalgae	<i>Schizochytrium</i> sp.	0.97	1.90	202.62	0.15	0.59	0.0:1	mg / g DP	(Xu et al., 2021)
Fishes	Salmon (raw)	-	1.01	0.94	-	-	0.0:1	g / 100g	(USDA, 2015)
	Herring (raw)	-	0.97	1.18	-	-	0.0:1	g / 100g	(USDA, 2015)
Plant oils	Flaxseed	53.4	-	-	14.3	-	0.3:1	g / 100g	(USDA, 2015)
	Perilla	62.6	-	-	15.4	-	0.3:1	g / 100g	(Kawamura et al., 2022)
	Soybean	6.79	-	-	50.42	-	7.4:1	g / 100g	(USDA, 2015)
	Corn	1.16	-	-	53.23	-	45.9:1	g / 100g	(USDA, 2015)
Grains	Wheat	4.04	-	-	59.09	-	14.6:1	g/kg total FA	(Ozturk et al., 2012)
	Barley	4.52	-	-	56.65	-	12.5:1	g/kg total FA	(Ortiz et al., 2021)
Pastures	Grass hay	61.62	-	-	14.09	-	0.2:1	g/kg total FA	(Krusinski et al., 2023)
	Grass silage	54.27	-	-	12.24	-	0.2:1	g/kg total FA	(Mierlita et al., 2022)
	Alfalfa hay	17.95	-	-	8.25	-	0.5:1	g/kg total FA	(Mierlita et al., 2022)
Animals products	Beef (lean, sirloin)	4	0	0	33	5	9.5:1	g/kg total FA	(USDA ,2011)
	Pork (lean, trimmed)	4	0	0	95	10	26.3:1	g/kg total FA	(USDA, 2011)

Chicken (light meat)	7	3	10	179	26	93:1	g/kg total FA	(USDA, 2011)
Milk (whole)	15	0	0	23	0	15:1	g/kg total FA	(USDA, 2011)
Egg (yolks)	4	0	4	134	17	18.9:1	g/kg total FA	(USDA, 2011)

AA, Arachidonic acid; ALA, Alpha-Linolenic acid; DHA, Docosahexaenoic acid; DW, dried powder; EPA, Eicosapentaenoic acid; FA, fatty acids; LA, Linoleic acid; PUFAs, Polyunsaturated fatty acids.