

**DIETARY LIPIDS, IMMUNE FUNCTION AND PATHOGENESIS
OF DISEASE IN FISH**

Santosh P. Lall and Joyce E. Milley
National Research Council Canada, Institute for Marine Biosciences,
1411 Oxford Street, Halifax, NS B3H 3Z1
Phone: 902-426-6272/Fax: 902-426-9413/e-mail: santosh.lall@nrc.ca

David A. Higgs
Department of Fisheries and Oceans, West Vancouver Laboratory,
4160 Marine Drive, West Vancouver, BC, V7V 1N6
Phone: 604-666-7924/Fax: 604-666-3497/e-mail: higgasd@dfo-mpo.gc.ca

Shannon K. Balfry
Faculty of Agricultural Sciences, University of British Columbia
2357 Main Mall, Vancouver, BC, Canada V6T 1Z4
Phone: 604-666-0034/Fax: 604-666-3497/e-mail: balfry@interchange.ubc.ca

EXTENDED ABSTRACT ONLY- DO NOT CITE

Lipids supply essential fatty acids (EFA) and energy in fish diets. Most fish cannot synthesize (*de novo*) polyunsaturated fatty acids (PUFA) and therefore they must be supplied in the diet for normal growth, reproduction and health. EFA include PUFA of the n-3 and n-6 series, e.g. α -linolenic acid, 18:3n-3 and linoleic acid, 18:2n-6. Generally, EFA requirements of freshwater fish can be met by the supply of 18:3n-3 and 18:2n-6 fatty acids in their diets. By contrast, the EFA requirement of marine fish can only be met by supplying the correct concentrations and ratios of the long-chain PUFAs, eicosapentaenoic acid (20:5 n-3; EPA) and docosahexaenoic acid (22:6n-3; DHA) with perhaps some arachidonic acid (20:4n-6; AA), a highly unsaturated member of the n-6 series. (NRC, 1993; Higgs and Dong, 2000). Freshwater fish are able to elongate and desaturate 18:3n-3 to 22:6n-3, whereas marine fish, which lack or have a very low activity of 5-desaturase, require the long-chain PUFAs, mainly from the n-3 series. This presentation will briefly review the status of knowledge on the relationship between EFA and immune functions with emphasis on eicosanoid production.

Fish tissues contain relatively higher concentrations of PUFA than are found in those from mammals. PUFAs are important components of all cell membranes,

which make fish tissue highly vulnerable to lipid peroxidation. Although the quantitative requirements and deficiency signs of EFA in several freshwater and marine fish have been documented (NRC, 1993; Higgs and Dong, 2000), the functional role of n-3 and n-6 PUFA in nonspecific and specific humoral and cellular immunity has not been studied extensively (Balfry and Higgs, 2001). Twenty-carbon PUFAs derived from EFA are precursors of two groups of eicosanoids that are comprised of prostaglandins and thromboxanes on the one hand, as well as leucotrienes and lipoxins on the other. These may have diverse pathophysiological actions that influence immune response and inflammatory processes especially if there is a preponderance of eicosanoids stemming from AA. Eicosanoids constitute a group of extracellular mediator molecules that are part of an organisms defense system. Further, they are synthesized from dihomo gamma linolenic acid (20:3, n-6), as well as AA and EPA by the action of two oxygenase enzymes, cyclooxygenase and lipoxygenase. Lipoxygenase yields a range of monohydroxy fatty acids while di- and tri-hydroxy fatty acids, such as leucotrienes (LT) and lipoxins (LX) are also formed via epoxy intermediates (Figure 1).

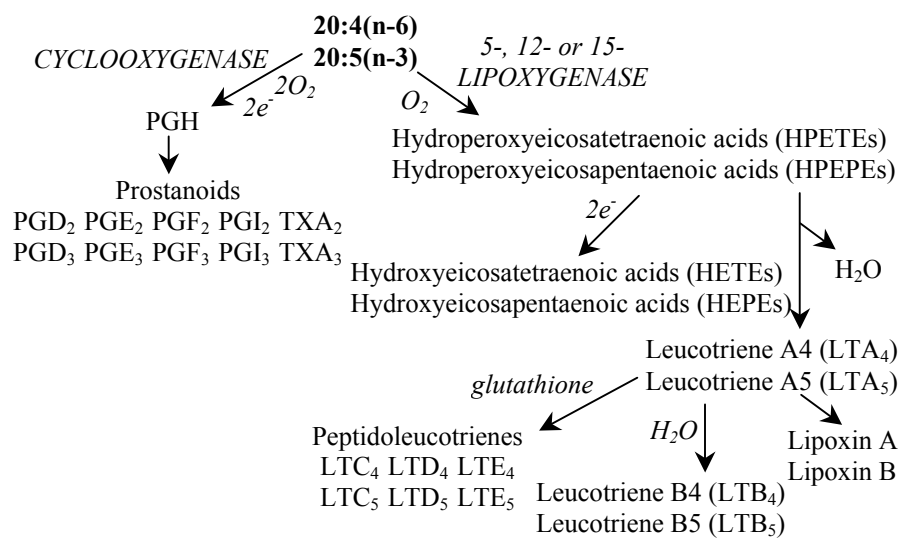


Figure 1. Pathways of conversion of 20:4(n-6) and 20:5(n-3) to eicosanoids.

The major source of n-3 EFA in fish diets is marine fish oil (MFO) and the partial substitution of MFO with vegetable and animal lipid sources in fish diets

affects the tissue and cellular lipid composition. Diets containing high levels of n-3 and n-6 fatty acids from fish and vegetable oils modify the fatty acid composition of cell phospholipids of Atlantic salmon (Bell *et al.* 1993) and halibut leucocytes (Table 1). Total n-3 and n-9 fatty acid concentrations increase significantly in liver, heart and muscle of halibut and Atlantic salmon fed flaxseed and canola oil, respectively. Recently we have separated major eicosanoids in several tissues of Atlantic salmon, haddock and halibut by a newly developed HPLC method using a reversed-phase C₁₈ column, a linear gradient mobile phase and diode-array detector. Their identities have been confirmed by LC-MS with negative-ion electrospray ionization. LTB₅ and LTB₄ were found to be the most abundant leucotrienes generated by these fish followed by prostaglandins and hydroxy fatty acids respectively. 11-HETE and 12-HETE were the most common hydroxy fatty acids in the foregoing fish species. The changes in fatty acid composition of phospholipids affected the synthesis of eicosanoid precursors in halibut leucocytes. When the dietary intake of n-6 fatty acids increased, a higher level of AA-derived eicosanoids was observed.

Studies conducted on the effect of n-3 and n-6 fatty acids on the immune responses in fish are preliminary and often inconclusive. Essential fatty acid deficiency in rainbow trout reduces the *in vitro* killing of bacteria by macrophages and reduces antibody production (Kiron *et al.*, 1995). The increased activity of head kidney macrophages has been associated with higher levels of dietary n-3 fatty acids in catfish (Sheldon and Blazer, 1991). The impacts that dietary fatty acids have on the immune responses are complex and depend on several factors that influence eicosanoid production including competition between n-3 and n-6 fatty acid during metabolism for chain elongation and desaturation, the cell types involved, and the sources of fatty acids in the diet. Although the nature of dietary lipids and the concentration of essential fatty acids have direct effects on eicosanoid metabolism, the regulation of the immune system by their direct effects on cells such as macrophages and lymphocytes or their indirect effects via cytokines requires further studies. Recent advances in our understanding of PUFA metabolism and immunology provide an opportunity to further investigate the role of n-3 and n-6 PUFAs in defense mechanisms of fish and to prevent diseases.

Table 1. Fatty acid composition of head kidney leucocytes from halibut fed diets containing anchovy oil, vegetable oils and poultry fat.

Fatty Acid	Diets				
	anchovy	flaxseed	canola	sunflower	poultry
14:0	2.0±0.5	1.6±0.2	1.4±0.1	1.3±0	1.2±0.2
16:0	12.8±0.9	13.4±0.7	12.2±0.3	12.2±1.0	14.9±0.5
18:0	7.3±0.7	9.8±0.4	7.4±0.3	8.3±0.7	8.9±0.1
Total saturates¹	23.8	26.6	23.1	24.6	27.6
16:1	3.4±0.6	2.7±0.2	2.7±0.1	2.3±0.1	3.4±0.3
18:1 n-9	9.1±0.3	11.6±0.9	14.2±0.8	10.3±0.7	14.3±0.3
18:1 n-7	3.2±0.5	2.6±0.2	2.9±0.2	2.6±0.2	3.4±0.2
20:1	1.1±0.3	1.3±0.2	1.6±0.1	1.2±0.2	1.5±0.1
22:1	0.9±0.7	0.4±0.1	0.3±0.04	0.3±0.04	0.2±0
24:1	0.8±0.1	0.8±0.1	0.9±0.1	1.0±0.01	1.6±0.04
Total monoenes¹	20.2	21.1	24.0	18.9	25.5
18:2 n-6	1.4±0.2	7.0±0.9	6.7±0.2	18.4±1.7	7.4±0.4
18:3 n-6	0.1±0.08	0.04±0.04	0.05±0.01	0.01±0.02	-
20:2 n-6	0.2±0.1	0.5±0.04	0.7±0.02	1.9±0.23	0.7±0.07
20:3 n-6	0.1±0.07	0.04±0.04	0.1±0.01	0.05±0.01	0.2±0.01
20:4 n-6	2.8±0.9	1.7±0.03	2.1±0.1	1.6±0.2	2.7±0.2
22:2 n-6	1.8±1.7	1.5±0.5	1.5±0.2	1.5±0.5	0.7±0.1
22:5 n-6	0.4±0.1	0.2±0.02	0.3±0.03	0.2±0.03	0.3±0
Total n-6¹	6.8	11.0	11.4	23.6	12.0
18:3 n-3	0.3±0.04	5.9±1.1	1.3±0.06	0.4±0.1	0.4±0.04
18:4 n-3	0.4±0.05	0.14±0.06	0.2±0	0.1±0.01	0.2±0.02
20:3 n-3	0.2±0.2	1.8±0.3	0.4±0.02	0.1±0.05	0.1±0.01
20:4 n-3	0.3±0.1	0.1±0.02	0.1±0.01	0.1±0.01	0.2±0.01
20:5 n-3	9.9±2.5	6.0±0.6	7.6±0.4	5.4±0.4	7.7±0.2
22:4 n-3	1.3±1.5	1.1±0.4	1.1±0.1	0.9±0.4	0.4±0.1
22:5 n-3	2.0±0.6	1.2±0.1	1.4±0.1	1.2±0.1	1.7±0.1
22:6 n-3	15.8±4.1	9.1±1.3	12.9±1.2	10.1±1.8	12.6±0.3
Total n-3¹	30.1	25.4	25.0	18.4	23.3
Total PUFA¹	52.4	49.5	50.2	53.6	44.0
n-3/n-6	4.4	2.3	2.2	0.8	1.9

¹Includes all the fatty acids identified in the respective group

References

- NRC (National Research Council), 1993. Nutrient requirements of fish. National Academy of Sciences, Washington, DC, 114 p.
- Balfry, S.K. and D.A. Higgs. 2001. Influence of dietary lipid composition on the immune system and disease resistance of finfish. In: Nutrition and Fish Health (eds. C. Lim and C.D. Webster), pp. 213-234, The Haworth Press Inc., New York.
- Higgs, D.A. and F. M. Dong. 2000. Lipids and fatty acids. In: Encyclopedia of Aquaculture (ed. R.R. Stickney), pp. 476-496, John Wiley & Sons, Inc., New York.
- Kiron, V., H. Fukuda, H., T. Takeuchi, T. Watanabe. 1995. Essential fatty acid nutrition and defence mechanisms in rainbow trout *Oncorhynchus mykiss*. Comp. Biochem. Physiol. 111A: 361-367.
- Bell, J.G., D.R. Dick, A.H. McVicar, J.R., Sargent and K.D. Thompson. 1993. Dietary sunflower, linseed and fish oils affect phospholipid fatty acid composition, development of cardiac lesions, phospholipase activity and eicosanoid production in Atlantic salmon (*Salmo salar*). Prostaglandins, Leukotrienes and Eicosanoid Fatty Acids 49: 665-673.
- Sheldon, W.M., Jr. and V.S. Blazer. 1991. Influence of dietary lipid and temperature on bactericidal activity of channel catfish macrophages. J. Aquat. Anim. Health 3: 87-93.

Acknowledgements

The review contains research information from a project (AP 7) supported by AquaNet, a NSERC's Network of Centre of Excellence in Aquaculture. The support of the National Research Council, Department of Fisheries and Oceans and AquaNet is gratefully acknowledged.

