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# Larval Nutrition - a nutritional perspective

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## Introduction

Fish naturally contain high levels of HUFA i.e., docosahexaenoic acid (22:6n-3), eicosapentaaenoic acid (20:5n-3) in their body tissues and juvenile marine fish require 0.5 to 1% (n-3) HUFA as dry weight. 5-21% substitution of triacylglycerol fish oil, present either as a natural constituent of fish meal, or as added fish oil meets this requirement. Problems in altricial fish are 1. Marine fish larvae grow more rapidly than juveniles. 2. Natural diets of marine fish larvae are rich in phospholipids rather than triacylgcerol and, 3. The ratio of 22:6(n-3): 20:5 (n-3) in phospholipids naturally consumed is ca. 2:1 whereas this ratio in triacylglycerols in fish oil is less than or equal to 1:1. Thus the marine larval fish feeds based on conventional fish oils with ratios of 22:6 (n-3): 20:5(n3) less than or equal to 1:1 are sub-optimal, either by not providing 22:6(n-3) or by providing an excess of 20:5(n-3). Over emphasis of (n-3) HUFA has resulted in the neglect of arachidonic acid (20:4n-6) as a dietary essential fatty acid for marine fish and the role of monounsaturated fatty acids as major energy yielding nutrients in fish.

***Metabolic interrelationships, conversions and competitions.***

18:3 (n-3) ⟶ 20:5 (n-3)
Linolenate EPA

Either non-conversion, or very low conversion due to Δ-5 fatty acid desaturase activity.

20:5 (n-3) ⟶ 22:6 (n-3)
EPA DHA

Conversion at low rates not likely to meet the high demands of larval fish growth fully Problems: Visual impairment due to impaired rod function leading to decreased efficiency in capturing prey at low light intensities.

22:5 (n-3) ⟶ 22:6 (n-3) is not by direct Δ-4 desaturation but by a complex pathway where,

20:5 (n-3) is chain elongated to 22:5 (n-3) and then converted to 24:5 (n-3). 24:5 (n-3) is converted to 24:6 (n-3) by Δ-6 desaturase and 24:6 (n-3) is chain shortened through peroxisomal β-oxidation to 22:6 (n-3) or DHA.

Or

18:2 (n-3) ⟶ 18:4 (n-3)

by Δ-6 desaturase

and interestingly, 18:4 (n-3) and 24:5 (n-3) are substrates for the same enzyme.

Thus, 18:3 (n-3) competitively depresses conversion of 20:5 (n-3) ⟶ 22:6 (n-3)

High concentration of 22:6(n-3) exists in the neural tissues. The acyslases and transacylases that esterify fatty acids into phospholipids do not have absolute specificities for particular fatty acids. Therefore, fatty acid compositions of tissues are partly determined by the levels of fatty acids available from the diet. This is true in the case of PUFA where an excess of one dietary PUFA e.g., 20:5n-3, can lead to and elevation of that PUFA in tissue phospholipids at the expense of another PUFA present in much lower concentrations in the diet e.g., 22:6n-3. This effect has been established for phospholipids of fish brain.

*Artemia,* rotifers and copepods contain substantial amounts of 18:3(n-3) linolenic acid and probably linolenic acid competitively inhibits 20:5(n-3) ⟶ 22:6(n-3) conversion, even if the fish has the capacity to carry out this conversion. *Artemia* nauplii supplemented with fish oils preferentially catabolize 22:6(n-3) relative to 22:5 (n-3). Thus final ratio of 22:6 (n-3): 20:5(n-3) is invariably substantially less than the starting feed. Oils with a high ratio of DHA: EPA should be used in live feed enrichment protocols. Relative excess of 20:5(n-3) over 22:6(n-3) can be harmful in larval feeds. 20:5(n-3) competitively inhibits production of eicosanoids from arachidonic acid 20:4(n-6). Arachidonic acid is the major precursor of eicosanoids in fish and higher vertebrates, despite the surfeit of 20:5(n-3) over 20:4(n-6) in fishes. Current emphasis is on a desirable ratio of 20:5(n-3):20:4(n-6) in larval fish feeds.

General understanding is that marine fish lack Δ-5 desaturase activity. Hence they cannot convert 18:2 (n-3) to 20:4(n-6). Therefore, 20:4(n-6) has been an essential function of producing eicosanoids making it an essential fatty acid (EFA) in marine fish, which has to be provided in larval feeds. Supplementation of marine fish larval feeds with (n-3) HUFA fish oils has obscured the potential importance of 20:4 (n-6) in larval nutrition.

Navas et. al. (1993) and Thrush (1993) fed a diet of fish meal + fish oil + vegetable oils rich in 20:5(n-3) and 22:6(n-3) to broodstock of sea bass (*Dicentrachus labrax*) and reported a production of lower quality eggs with lower survivability and hatchability. The diet contained 0.6%, 20:4(n-6) of the total fatty acids with a ratio of 20:5(n-3) : 20:4(n-3) in the range of 15:1. Animals receiving trash fish (*Boops boops*) produced better quality eggs in comparison. Trash fish diets contained 4.6%, 20:4(n-6) and the ratio of 20:5(n-3):20:4(n-6) was 1.5 : 1. Following up this work, Bruce (1997) found that phosphatidylcholine was the major egg lipid in sea bass and other marine fish eggs. And oil based diets had ratios of 20:5(n-3): 20:4(n-6) of 17.3:1. Trash fish diets had the ratio of 3.4:1. Phosphatidylinositol had the highest% of 20:4(n-6) and is the most likely source of 20:4 (n-6) for ecosanoid production. This is important in the context of broodstock diets because prostaglandins ($PGF2_{\alpha}$) is produced from 20:4(n-6) and has roles in natural shedding of eggs, synchronizing ovulation and spawning and avoids over ripening of eggs.

However, other than *Boops boops* no other natural feed source is found to contain 20:5(n-3):20:4(n-6) in the ration 1.5:1. Does this putatively high requirement of 20:4(n-6) in sea bass apply to other marine fishes?

Knowledge of the (n-3) PUFA requirements of different species of marine fish is still elementary. It is not sufficient to consider gross PUFA requirements, instead the ratio of 22:6 (n-3):20:5(n-3):20:4(n-6) has to be defined.

**Requirements**

Marine fish can neither biosynthesize 22:6(n-3) *de novo* nor from shorter chain precursors such as 18:3(n-3), therefore 22:6(n-3) and 20:5(n-3) are essential dietary constituents for marine fish. 22:6(n-3) is present in very high concentrations in neural and visual cell membranes and synaptosomal membranes, in fish as in mammals. An insufficiency of 22:6(n-3) in marine larval fish diet is likely to impair neural and visual development with significant if not serious consequences for a whole range of physiological and behavioural processes including those dependent on neuroendocrines. Abnormal pigmentation in cultured marine flatfishes is related to HUFA deficiencies.

Detailed studies examining the appropriate ratios of fatty acids, mainly 22:6(n-3), 20:5(n-3) and 20:4(n-6) have revealed that given a sufficiency of 22:6(n-3), excess of 20:5(n-3) is not deleterious, where as 20:4(n-6) is, because of a generalized biochemically-induced stress in the fish through excess eicosanoid production. In commercially available fish oils, 20:4(n-6) are found to be consistently at low levels (< 1% of the total fatty acids). Neither is an excess of dietary 22:5(n-6) a practical problem. But the major limiting fatty acid in commercial fish oils would be 22:6(n-3). The availability of the fatty acid through enriched *artemia* is also problematic because brine shrimp nauplii retro-converts 22:6(n-6) to 20:5(n-3). Thus, oils particularly rich in 22:6(n-3) are essential for the supplementation process and other than commercial (n-3) HUFA concentrates Tuna orbital oil (TOO) is the only identified natural oil, which has the levels and ratios of 22:6(n-3), 22:5(n-3) and 20:4(n-3) that lead to satisfactory, though not optimal survival growth and metamorphosis of turbot larvae which may not be applicable to all marine fishes. There is evidence that seabass larvae require more of 20:4(n-6) and TOO with ca. 2% proved to be satisfactory. Commercial fish oils with less than 1% arachidonic acid has to be blended with oils rich in arachidonate to achieve this objective.

**Presentation**

Nearly all mariculture production systems rely heavily on live feeds viz., rotifers, *atremia* nauplii and copepods of *Tisbe, Acartia, Eurytemora. Artemia* and *Brachionus plicatilis* are naturally deficient in 20:5(n-3) and no known strains of *artemia* contain significant levels of 22:6(n-3) making (n-3) HUFA enrichment necessary. Procedures for enrichment with emulsions of marine fish oils are well developed. Commercial products are readily available to achieve this objective. However, up gradation of current procedures in the light of recent knowledge of PUFA requirements is essential. Current problems in enrichment of live feed are – 1. 22:6(n-3) content is very small in triacylglycerol micelles generated in enrichment procedures and are prone to autooxidation, especially under vigorous aeration. 2. Natural antioxidants such as á-tocopheryl acetate and scorbyl palmitate are not effective especially until hydrolysed in the intestinal tract and absorbed. Ethoxyquin and Butylated hydroxy anisole minimizes peroxidation. However, the level of these in enrichment emulsions is an area where there is no information.

Lecithin can be used to considerable advantage in enriching the nauplii with 22:6(n-3) rich fish oils, because lecithin acts as a natural emulsifying agent and a natural protectant against autooxidation. Thus the ideal enrichment mixture tested is a combination of 90% 22:6(n-3) rich fish oil + 10% lecithin from fish eggs. Lecithin derived from fish eggs is superior to soy lecithin because

fish egg lecithin contains readily assimilable 22:6(n-3) and 20:5(n-3) in the ratio of 2:1. Soy lecithin contains only 18:2(n-6) linoleic acid. Commercial availability of (n-3) PUFA rich phospholipids is limited. This limitation has to be overcome by exploring fishery products other than fish roe and milt.

Alternatives to fish oil fractions rich in 22:6(n-3) are (1) a heterotrophic dinoflagellate *Crytothecodenium cohnii* which is mass produced commercially to produce triacyl glycerol rich in 22:6(n-3) - commercial product by MARTEK®; frozen thawed cells are used to supplement *artemia*. (3) Spray dried *Schizochytrium* spp. rich in PUFA is a single celled heterotrophic marine protist of the group labyrinthulomycota - commercial product KELCO®. (4) Copepods cultures have to be developed because they have a preponderance of phospholipids rather than triacylglycerols in their body. Levels and ratios of 22:6(n-3): 20:5(n-3): 20:4(n-6) more closely resemble larval natural diets and the probability of natural protection of PUFA by natural antioxidants and delivery to larvae is always advantageous. Copepods enriched with freeze thawed cells of *C. cohni* or *Schizotricodinium* spp. is another possibility ensuring the appropriate HUFA ratio delivery to larval marine fishes, which is not popular.

**Sources**

Traditional commercial fish oils especially byproducts of industrial pelagic fisheries are the richest sources of fats and fatty acids. Basically fish oils are rich in 20:5(n-3) and the ratio of 22:6(n-3):20:5(n-3) is found to be < 1:2. MAXEPA™ type oils available commercially contain 12% 22:6(n-3) and 18% 20:5(n-3) which are sourced from southern hemisphere low latitude fisheries, mainly, plichards, anchovies, sardines and menhaden. Northern hemisphere high altitude fisheries yield oils with decreased (n-3) PUFA mainly from capelin, sand eels, herring, sprat and mackerel. 20:5(n-3):22:6(n-3) ratios do not differ from the former with an increased % of 20:1 (n-9) and 22:1(n-11) serving as metabolic source of energy. Cod liver oil has a higher PUFA (n-3) content and a lower% of 20:1(n-9) and 22:1(n-11).

Commercially fish oils are enriched with 22:6(n-3) and 20:5(n-3) by fractional distillation, solvent extraction or by urea adduction or by a combination of all these methods. (n-3) PUFA's are available as ethyl ester, free fatty acids and rarely as triacylglycerols among which ethyl esters are already used to enrich *artemia*. Commercial fish oils can meet enrichment requirements because saturated and monounsaturated fatty acids in fish oils are as important as energy yielding molecules and (n-3) PUFA are useful for structural purposes. Eventhough (n-3) PUFA can be catabolized for energy, they are more difficult to catabolize than saturated or monounsaturated fatty acids. Thus over enrichment with PUFA could conceivably result in an insufficient energy content in the diet.

The only 22:6(n-3) rich natural fish oil known so far is tuna orbital oil (TOO), which contains 30% 22:6(n-3), 7% 20:5(n-3) and 2% 20:4(n-6). It has been proven that blending of 90% TOO with 10% lecithin from fish roe produces the most ideal enrichment emulsion known to date. However, maintenance of the levels of DHA: EPA: AA in *artemia* till the larval fish feeds on it has not been successful because all these fatty acids especially DHA is metabolized by *artemia* after bioencapsulation leading to lowering of its content in the enriched organism. Surprisingly, a strain of *artemia* from China designated as *Artemia sinica* is found to retain the levels of DHA up to 24 h post-enrichment.

The future direction of PUFA nutrition in mariculture is to blend the range of products available to us to achieve either economical larval survival or brood stock maturation and spawning. The clues have naturally come from the nutrient profiles of mature fish eggs.

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## Microalgae - a reliable renewable feed stock for future fuel

*Syamlal*

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Microalgae are microscopic unicellular plants usually found in aqueous environments. The size ranges from a few microns to hundreds of microns. They exists individually or in colonies. Microalgae, capable of performing photosynthesis, are important for life on earth; they produce approximately half of the atmospheric oxygen and use simultaneously the greenhouse gas carbon dioxide to grow photoautotrophically

The biodiversity of microalgae is enormous and they represent an almost untapped resource. It has been estimated that about 200,000-800,000 species exist of which about 35,000 species are described. Over 15,000 novel compounds originating from algal biomass have been chemically determined. Most of these microalgae species produce unique products like carotenoids, antioxidants, fatty acids, enzymes, polymers, peptides, toxins and sterols.

The chemical composition of microalgae is not an intrinsic constant factor but varies over a wide range, both depending on species and on cultivation conditions. It is possible to accumulate the desired products in microalgae to a large extent by changing environmental factors like temperature, illumination, pH, CO2 supply, salt and nutrients.

In addition, because the cells grow in aqueous suspension, they have more efficient access to water, CO2, and other nutrients.

While fish oil has become famous for its omega-3 fatty acid content, fish don't actually produce omega-3s, instead accumulating their omega-3 reserves by consuming microalgae.

Microalgae are sunlight-driven cell factories that convert carbon dioxide to potential biofuels, foods, feeds and high-value bioactives

**Why microalgae**

The idea of using microalgae as a source of fuel is not new, but it is now being taken seriously because of the escalating price of petroleum and, more significantly, the emerging concern about global warming that is associated with burning fossil fuels. The fear of depleting oil reserves also contribute to the rush for renewable fuels.

There are several aspects of algal biofuel production that have combined to capture the interest of researchers and entrepreneurs around the world.

These include:

1) High per-acre productivity compared to typical terrestrial oil seed crops,

2) Non-food based feedstock resources,

3) Use of otherwise non-productive, non-arable land,

4) Utilization of a wide variety of water sources (fresh, brackish, saline, and wastewater),

5) Production of both biofuels and valuable co-products.

6) Nutrients from the wastewater and CO2 from the flue gas can be utilized.

Microalgae appear to be the only source of renewable biodiesel that is capable of meeting the global demand for transport fuels.

Microalgae can provide several different types of renewable biofuels. These include methane produced by anaerobic digestion of the algal biomass biodiesel derived from microalgal oil; and photobiologically produced biohydrogen. Out of these biodiesel from algae oil is the major energy product. Biodiesel is produced by transestrification of algal oil (lipid).

**Oil content of some microalgae**

| **Microalga Oil content** | **(% dry wt)** |
|---|---|
| *Botryococcus braunii* | 25–75 |
| *Chlorella* sp. | 28–32 |
| *Crypthecodinium cohn*ii | 20 |
| *Cylindrotheca* sp. | 16–37 |
| *Dunaliella primolecta* | 23 |
| *Isochrysis* sp. | 25–33 |
| *Monallanthus salina* | 20 |
| *Nannochloris* sp. | 0–35 |
| *Nannochloropsis* sp. | 31–68 |
| *Neochloris oleoabundans* | 35–54 |
| *Nitzschia* sp. | 45–47 |
| *Phaeodactylum tricornutum* | 20–30 |
| *Schizochytrium* sp. | 50–77 |
| *Tetraselmis sueica* | 15–23 |

Ideally, microalgal biodiesel would be carbon neutral, as all the power needed for producing and processing the algae would come from biodiesel itself and from methane produced by anaerobic digestion of biomass residue left behind after the oils has been extracted. Although microalgal biodiesel can be carbon neutral, it will not result in any net reduction in carbon dioxide that is accumulating as a consequence of burning of fossil fuels.

**Farming algae for fuel**

Culture requirements

Light
Temperature
Mixing
Gas exchange
Nutrients

Culture Systems

Open pond
Closed photobioreactors
Fermenters

Biomass harvest
Downstream processing
Economic analysis
Energy balance
Co-products

**Critical issues of algae fuel industry**

Production systems
Trained personnel
Algal strain selection
Input costs
Develop high lipid strains
Component separation
Light management
Extraction
Contamination
Nutrient delivery
Temperature management
Intellectual property
Monitoring systems
Mixing

**Low cost microalgae biomass production system developed at M/s Energymicrolgae.**

The system is able to produce the biomass @Rs. 4/ Kg DW in a commercial unit. The system powered by ACCaS technology allows the algae farm to be located any remote place where there is no CO2 source.