Review: Use of Highly unsaturated fatty acid (HUFA) in Ornamental Fish Feeds

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Abstract
Ornamental fish are well known because they can be seen every day in the home aquarium, ornamental pond, pet store, and public aquarium, while at the same time they are unknown because knowledge regarding their Nutritional requirements is limited. ornamental fish production, like all other segments of aquaculture, relies heavily on the successful spawning, hatching, and survival of larvae. Little is known about the nutrient requirements of most ornamental fish species. In many cultivars of fishes, such as ornamental fish, especially in those new ornamental fish species introduced to the aquaculture industry, the rate of fecundity is an important factor in the mass production of young fish and its final selection. Normally, improving the quality of feed used by breeders not only improves the quality of eggs and sperm, but also increases egg production. Gonadal development and fecundity are influenced by some of the nutrients present in the diet and are well documented in frequent spawning of broodstocks and short-term vitellogenic periods. The combination of lipids and fatty acids is one of the main determinants of the successful reproduction of ornamental fish and the survival of the offspring. The presence of fats in the diet as a source of energy and essential fatty acids, sterols and phospholipids are considered as carriers of fat-soluble vitamins and are of considerable importance.

Keywords: Fish feeding, EPA, DHA, Arachidonic acid (ARA), Aquarium fish.

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Introduction
Ornamental fish are well known because they can be seen every day in the home aquarium, ornamental pond, pet store, and public aquarium, while at the same time they are unknown because knowledge regarding their Nutritional requirements is limited. Aquarium keeping is amongst the most popular of hobbies with millions of enthusiast’s worldwide. They also come under the broader category of 'Pets'. Though the term 'Aquarium Pets' is the term used to denote these small pets kept at home; nowadays it is being replaced by the term 'ornamental Fish'. Variations in color, shapes, forms and species of these small (to medium size) fish are the major criteria for their popularity. Experienced aquarium keepers prefer to go in for rare species and Specific species varieties of ornamental fish. Certain types of these small ornamental fish require special environments and special diet for their health and survival. There are many of these small fish from tropical and subtropical regions, which require maintenance of the water temperature in the aquarium if kept in colder climates. Similarly, those small aquarium fish with origin of colder region (such as temperate regions) will get in to distress if the temperature rises above the optimum. However, today the possibility of keeping large fish in public aquariums is well provided.

Although most fish kept in aquariums are from freshwater, the acquisition of marine ornamental fish has greatly increased (Livengood and Chapman, 2000). ornamental fish production, like all other segments of aquaculture, relies heavily on the successful spawning, hatching, and survival of larvae. Little is known about the nutrient requirements of most ornamental fish species. There are no commercially available pelleted diets that are specifically designed to feed broodstock of ornamental species. Currently, the artificial diets available to commercial ornamental fish producers are primarily based on nutritional trials conducted on one or more of the major food fish species and do not always match ornamental producer needs for particle size, buoyancy, moisture content, palatability, and shelf life (Ohs et al., 2013).

Lipids and fatty acids requirements
Lipids probably represent the most-studied nutrient group in fish nutrition, and some might say that this is in excess of their importance relative to that of other essential nutrients. However, part of the prominence of lipids, and fatty acids (FA) in particular, relates to the unique abundance of highly unsaturated fatty acid (HUFA) of the n-3 series that are found in fish. The importance of lipids for energy provision and how fish lipid synthesis, storage, and degradation are controlled by gene regulation are also discussed. The fatty acids are important sources of energy during early embryonic development (Tocher et al., 1985; Falk-Petersen et al., 1986, 1989; Rainuzzo, 1993; Sargent, 1995). They have an important structural function as components of the phospholipids in fish biomembranes. The ratio between saturated and unsaturated
fatty acids regulates the fluidity of cell and organelle membranes, and thereby their function (Bell et al., 1997; Sargent, 1995). Lipids represent the major energy contribution in aquaculture nutrition, and as such reach high inclusion levels in energy-dense feeds. As high-volume macro-ingredients, lipids have a significant impact on feed processing and quality. The proportion of different fatty acid classes according to chain length and degree of unsaturation clearly changes dietary lipid digestibility. Practical considerations of lipid raw material usage are raw material quality indices, such as oxidative status, and final feed quality indices (Gordon Bell and Koppe, 2011). Lipids, along with proteins and carbohydrates, comprise the major macronutrient classes that are required to provide both the essential nutrients for energy production and the building blocks for development of cells and tissues that allow growth and maintenance of homeostasis in all vertebrate organisms (National Research Council, 1993). However, the fatty acid composition of the lipids in fish eggs may vary with the species and even with different batches of the same species (Pickova et al., 1997). Defining the absolute requirement for an individual fatty acid is difficult, as the required amount provided by the diet is not the only deciding factor since the fish innate ability to modify a fatty acid, by either catabolism or anabolism, also impacts the requirement for that fatty acid. The intake level of one fatty acid can impact that of other fatty acid, with the most obvious example being that for Arachidonic acid (ARA, 20:4 n-6) and Eicosapentaenoic acid (EPA, 20:5 n-3), which are direct competitors at many levels of cellular function (National Research Council, 1993). The essential, HUFA, ARA, EPA and Docosahexaenoic acid (DHA; 20:6 n-3) have received special attention (Tocher, 2010). The levels of n-3 fatty acids will also affect the ratio of ARA to EPA and can influence eicosanoid production and fat metabolism in general, calling for a balanced supplementation of both n-3 fatty acids and ARA. ARA has similar biological importance as EPA and DHA and considered as the precursor of several eicosanoids which are produced by the ovarian tissues and play an important role in the ovulation process in viviparous fish (such as guppy) (Venkatesh et al., 1992). Finally, the n-3 fatty acids are susceptible to lipid oxidation, which may be a great challenge to the health of the organism (Fernández-Palacios et al., 2005). Therefore, the final level that EPA, or any other fatty acid, attains in fish depends not only on the dietary supply but also on the genetically determined ability to metabolize that fatty acid. For that reason, fatty acid requirements for individual fish species can vary significantly with species, season, temperature, and stage of development or reproductive cycle (National Research Council, 1993). The positive effect of n-3 fatty acids on spawning performance may be explained by their roles as structural components in biological membranes, where they increase fluidity (Fernández-Palacios et al., 2005). Finally, fish species, rearing conditions, and diet composition are the major inputs...
for the modeling and prediction of lipid digestibility.

**Essential fatty acids**

Essential fatty acids are fatty acids many of fish and other animals must ingest because the body requires them for good health but cannot synthesize them. The term "essential fatty acid" refers to fatty acids required for biological processes but does not include the fats that only act as fuel (Goodhart and Shils, 1980). Fish, as is the case for all organisms, can synthesize *de novo* the saturated fatty acids 16:0 and 18:0 (Cook, 1996) by the action of fatty acids synthase in a cytosolic reaction utilizing Acetyl-CoA and nicotinamide adenine dinucleotide phosphate (NADPH) (Sargent *et al*., 2002). Fatty acids of the n-3 series and the n-3 HUFA found in gilthead sea bream eggs and cod (*Gadus morhua*) gonads increased when n-3 HUFA increased in the diet (Fernández-Palacios *et al*., 1995). The fatty acid composition of fish lipids varies according to the fatty acid profile of the dietary lipid. The fatty acid composition of fish also responds to temperature changes in an adaptive mechanism for maintenance of membrane homeoviscosity (cell membrane lipid) and physiological function over a range of temperatures. The dietary intake of essential fatty acids by brood stock must be adequate for ova formation and for embryonic development, with the latter requirement being more critical for reproductive success (March, 1993). The essential fatty acid requirements for different species of fish reflect different dietary and metabolic adaptations to different habitats and consider how such knowledge can be exploited to develop improved diets for fish, especially in their early stages of development (Sargent *et al*., 2002). The fatty acid composition of fish eggs is directly affected by the fatty acid content of the broodstock diets (Fernández-Palacios *et al*., 1995) or with the environmental conditions in which gametogenesis occurs (Dantagnan *et al*., 2007). The 20-carbon fatty acids ARA and EPA are eicosanoid precursors, producing PGs of the II and III series, respectively, and leukotrienes LTB4 and LTB5 (Ganga *et al*., 2005). The PGs have been shown to be involved in numerous reproductive processes, including the production of steroid hormones, gonadal development, and ovulation. The key enzymes for production of PGs and leukotrienes from fatty acids are the cyclooxygenases and lipoxygenases, respectively (Moore, 1985). The inhibitors of lipoxygenases reduce the maturation of Sea bass (*Dicentrarchus labrax*) oocytes induced by gonadotropin, suggesting that the leukotrienes may also be involved in reproduction in fish (Astoriano, 1999). The ARA derived prostaglandin PGE2 stimulates ovarian synthesis of E2 in mammals, and ARA stimulates synthesis of testosterone (T) and E2 in vitro in Goldfish (*Carassius auratus*) and Zebrafish (*Danio rerio*) ovarian follicles (Lister and Van Der Kraak, 2008). ARA, EPA, and DHA, compete with each other for the enzymes that regulate the synthesis of eicosanoids. The relationship between them in the diet (the EPA/DHA and the...
ARA/EPA ratios) will be decisive, for example, for the presence of PGs of the II and III series in the tissues. DHA is especially important in the neural tissue, the retina, and the optic nerve and is related to other structures of the sensory organs that develop during early larval stages (Benítez et al., 2007). These PGs have very different activities in the regulation of various physiological functions, including reproduction. Therefore, a deficiency or imbalance of ARA, EPA, and DHA in the broodstock diet can have large effects on reproduction. By supplementation with ARA, the ratio of ARA to the other fatty acids and the ARA/EPA ratio will increase. There is an optimal level of ARA in broodstock diets, where addition below or above this level gives reduced performance (Furuita et al., 2003). The optimal ratio of ARA to EPA is species-dependent in fish larvae (Sargent et al., 1999). In vitro, ARA, but not EPA or DHA, stimulates the release of testosterone in Goldfish (Carassius auratus) testicles through its conversion into prostaglandin PGE2, while EPA and DHA block the steroidogenic action of ARA and the PGE2 (Wade et al., 1994). Studies on broodstock nutrition in cod (Gadus morhua), as well as other fish species, generally focus on the effect of diet on fecundity, egg and larval viability and biochemical composition of the eggs (Lanes et al., 2012). Increasing these fatty acids may lead to improvement of fecundity and egg and larval quality in terms of survival, stress resistance, larval weight, and inflation of the swim bladder. Diets deficient in essential fatty acids also cause an increase in the number of fat droplets in fish eggs (Fernández-Palacios et al., 2005). On the other hand, very high levels of n-3 fatty acids caused a decrease in the total number of eggs produced and egg quality in Gilthead sea bream, Swordfish, (Xiphophorus helleri), and Pacific sole (Paralichthys olivaceus) (Ling et al., 2006). The requirement of n-3 fatty acids in sea bream broodstock varies between 1.5 and 2.5% in the diet (Fernández-Palacios et al., 2005). Being higher than that established for juveniles, ranging between 0.5 and 0.8% (Izquierdo, 1996). While the actions of ARA in vitro have been extensively studied, and the influence of fatty acids in general on realized fecundity and egg and larval quality has received much attention, there is relatively limited knowledge on the effects of ARA and other HUFAs on fish reproductive physiology in vivo (Norambuena et al., 2013; Xu et al., 2017).

Environmental influences Salinity

The difference between fatty acid compositions of marine and freshwater fish has been noted by several researchers. Essential fatty acid requirements vary qualitatively and quantitatively with both species (marine and freshwater fish) and during ontogeny of fish, with early developmental stages and broodstock being critical periods (Tocher, 2010). Although these fish lipids are higher in ω-3(omega 3) fatty acids, it is clear that freshwater fish have higher levels of ω-6 (omega 6) fatty acids than marine species. The average ω-6/ω-3 ratios are 0.37 and
0.16 for freshwater and marine fish, respectively. Fish in general contain more ω 3 than ω 6 polyunsaturated fatty acids (PUFA) and should have a higher dietary requirement for ω 3 PUFA; thus the dietary EFA requirement of marine fish for ω 3 PUFA may be higher than that of freshwater fish. The same type of difference in the ω 6/ω 3 ratio between freshwater and seawater is seen when some species of fish migrate from oceans to streams or vice versa (National Research Council, 1993). Environment and/or trophic level are major factors, with freshwater/diadromous species generally requiring C18 PUFA whereas marine fish have a strict requirement for long-chain PUFA, Eicosapentaenoic, Docosahexaenoic and Arachidonic acids (Tocher, 2010). The PUFA ratio of Sweet smelt (Plecoglossus altivelis) changes drastically in only one month as they migrate from the sea to a freshwater river. A similar but reverse change occurs in the Masu Salmon (Oncorhynchus masu) as they migrate from freshwater to seawater. Even within the same species of fish, the salinity of the water seems to cause a dramatic change in the fatty acid pattern (National Research Council, 1973). The difference between marine and freshwater fish may be due simply to differences in the fatty acid content in the diet or it may be related to a specific requirement in fish related to physiological adaptations to the environments (National Research Council, 1993).

Temperature

There are several other factors besides the salinity of the water which affect the fatty acid composition. The fatty acids from a number of marine animals from temperate and arctic waters show some significant differences in the general pattern. The intake level of one Fatty Acid can impact that of other Fatty Acid. The ω 6/ω 3 ratio decreases with a decrease in temperature. With the most obvious example being that for arachidonic acid (ARA, 20:4n-6) and Eicosapentaenoic acid (EPA, 20:5n-3), which are direct competitors at many levels of cellular function. Therefore, the final level that EPA, or any other Fatty Acid, attains in fish depends not only on the dietary supply but also on the genetically determined ability to metabolize that Fatty Acid. For that reason, Fatty Acid requirements for individual fish species can vary significantly with species, season, temperature, and stage of development or reproductive cycle (National Research Council, 1993). If the trends in fatty acid composition can be taken as clues to the Essential Fatty Acids requirements of fish, the ω 3 requirement would be greater for fish raised at lower temperatures. Fish raised in warmer waters, such as Common carp, Goldfish, Channel catfish, and Tilapia may do better with a mixture of ω 6 and ω 3 fatty acids (Ackman, 1967).

Factors affecting long-chain fatty acid synthesis and requirements

The ability to perform the series of desaturation and elongation steps required to synthesize DHA from 18:3n-3 varies
between fish species and is partly dependent on whether the species is freshwater, diadromous, or marine (Tocher, 2003). It is generally accepted that freshwater fish can convert C18 PUFA of both the n-6 and n-3 series to their longer chain PUFA, and the elongation and desaturation steps appear to be qualitatively similar to those in mammals (Buzzi et al., 1996).

While conversion of 18:3n-3 to EPA is appreciable in freshwater fish and anadromous salmonids, the final steps in the synthesis of DHA are relatively inefficient and DHA production is negligible (Sargent et al., 2002; Tocher et al., 2003). It is a general rule that larval and juvenile fish, as well as other animals, tend to have a higher requirement for n-3 Long-chain PUFA than later life stages (Sargent et al., 2002). In tropical freshwater fish such as the tilapia (Oreochromis niloticus), conversion of 18:3n-3 to DHA might be expected to be greater than that in salmonids, given the low levels of n-3 Long-chain PUFA in tropical ecosystems. However, while the pathways for DHA synthesis has been elucidated in tilapia (Olsen et al., 1990). Their capacity for DHA synthesis is low, perhaps due to their reduced requirement for Long-chain PUFA compared to Coldwater species (De Silva and Anderson, 1995). When tilapia was fed diets rich in 18:3n-3, increased concentrations of longer chain products were observed with concomitant decreases in n-6 PUFA in muscle lipids (Karapanagiotidis et al., 2007). Observed a decrease in egg hatchability for Striped Trumpeters (Latis lineata) fed diets with increased levels of DHA and EPA (Morehead et al., 2001). Decreases in egg hatchability have also been observed for Nile Tilapia when broodfish were fed diets with high levels of DHA, EPA, and ARA (Wing-Keong and Wang 2011). Observed a positive correlation between the DHA level in wild Common Snook (Centropomus undecimalis) eggs and hatching percentage and larval survival (Yanes-Roca et al., 2009). While the biosynthetic activity of the Long-chain PUFA synthesis pathway has been studied and characterized in a number of freshwater and anadromous species, the conversion activity along the pathway is generally much lower, or even absent, in most marine fish studied to date (Sargent et al., 1995; Tocher 2003). Since the marine ecosystem generally has an abundance of EPA and DHA across the trophic layers, there is probably little requirement for a synthetic pathway to synthesize n-3 Long-chain PUFA (Bell et al., 2006). Marine fish generally have an absolute requirement for DHA, and probably EPA, to be supplied by the diet. These requirements for different groups of fish species residing in different ecosystems and different trophic levels are very generalized, and the absolute Essential Fatty Acids requirements not only are dependent on species, but also must consider the developmental stage (larvae, juvenile, adult, or broodstock), the season and temperature of the culture, and the dietary lipid content and relative Fatty Acids compositions (Zheng et al., 2005). For any given species, the Essential Fatty
Acids requirement will be influenced by a range of factors including life stage, growth rate, fish age and weight, dietary lipid level, feed raw materials and their Fatty Acid digestibility and retention efficiency, trophic level, and temperature of culture (Glencross et al., 2007). For the freshwater-brackish and anadromous species including Koi (Cyprinus carpio), Grass carps (Ctenopharyngodon idella), Channel catfish (Ictalurus punctatus), Milkfish (Chanos chanos), Chum (Oncorhynchus keta), and Atlantic salmon (Salmo salar), Arctic char (Salvelinus alpinus), Rainbow trout (Oncorhynchus mykiss), and Eel (Anguilla sp.), the α-Linolenic Acid (18:3 n-3) requirements is in the range of 0.3% –2.0% of dry diet. The literature evidence suggests that tilapias (Tilapia zilli and Oreochromis niloticus) have a requirement for Linoleic Acid (18:2 n-6) in the range 0.5% –1.0% of dry diet, which is similar to the range for Linoleic Acid in the other species where a α-Linolenic Acid requirement is also quoted. It is likely that for all these species both Linoleic Acid and α-Linolenic Acid are required, but the requirement levels in some species are at a low level that is difficult to quantify accurately. For all of the above species, their requirements for α-Linolenic Acid and Linoleic Acid can be overcome by supplying n-3 Long-chain PUFA in the range of 0.2%–1.0% of dry diet (Glencross, 2009; Tocher, 2010; Turchini et al., 2009). In a scientific review it became clear: in the broodstock Blue gourami (Trichopodus trichopterus) as a model of asynchronous multi-batch spawning fish, needs to receive at least 1% ARA in their maturation diet to improve reproductive performance. The best efficiency was achieved in 2% ARA, based on fecundity, yolk sac diameter and hatching rate (Masoudi Asil et al., 2017). For the marine species, including Gilthead (Sparus aurata) and red Seabream (Pagrus major), Turbot (Psetta maxima), Japanese flounder (Paralichthys olivaceus), Red drum (Sciaenops ocellatus), Yellowtail flounder (Pleuronectes ferrugineus), Amberjack (Seriola sp.), and Groupers (Epinephelus sp.), the requirements for n-3 Long-chain PUFA are in the range of 0.4% –3.7% of dry diet (Tocher, 2010). In marine fish a requirement for Arachidonic acid was not considered for many years despite the fact that Arachidonic acid, or its metabolic products, has a number of important physiological roles including improved growth and survival rates, especially in early life stages; stress resistance; egg and larval quality; and immune function (Bell and Sargent, 2003). The essentiality of Arachidonic acid was proven in turbot juveniles, and a requirement was established for 0.3% of dry diet (Bell et al., 1995). It is likely that Arachidonic acid is also essential for other marine species. While this may not be a problem when feeding Fish Oil, it may become so if diets with higher Vegetable Oil inclusion are fed to marine species that have no capacity to synthesize Arachidonic acid from Linoleic Acid.

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