Effects of n-3 HUFA on Reproductive Performance of Fish Broodstock and Their Mechanisms of Action: Postprint

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Date: 2018-12-24T00:00:00+00:00

Abstract

n-3 highly unsaturated fatty acids (HUFA) exert significant influences on gonadal development, gamete quality (eggs and sperm), fecundity, fertilization rate, hatching rate, larval survival, and quality in fish broodstock. In fish, n-3 HUFA are synthesized through the action of a series of desaturases and elongases. Freshwater and salmonid fishes generally obtain linolenic acid from dietary sources for the endogenous synthesis of n-3 HUFA, whereas marine fishes require direct dietary intake of preformed n-3 HUFA. This review summarizes the biosynthetic pathways of n-3 HUFA in fish and their impacts on broodstock reproductive performance, while also addressing natural and alternative sources of n-3 HUFA, aiming to provide a reference for research on fatty acid nutrition and underlying mechanisms in fish broodstock.

Full Text

n-3 Highly Unsaturated Fatty Acids: Effects on Reproductive Performance of Fish Broodstock and Their Mechanisms of Action

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Abstract

n-3 highly unsaturated fatty acids (HUFA) exert critical influences on gonadal development, egg and sperm quality, fecundity, fertilization rate, hatching rate, and larval survival and quality in fish. Fish synthesize n-3 HUFA through a series of reactions catalyzed by desaturases and elongases. Freshwater and salmonid fish generally obtain n-3 HUFA by synthesizing them from dietary linolenic acid, whereas marine fish require direct dietary intake of n-3 HUFA. This review summarizes the biosynthetic pathways of n-3 HUFA in fish and their effects on broodstock reproductive performance, while also discussing natural and alternative sources of n-3 HUFA, aiming to provide a reference for research on fatty acid nutrition and mechanisms in fish broodstock.

Keywords: n-3 highly unsaturated fatty acid; fish; broodstock; reproductive performance; vegetable oil

Introduction

With the rapid development of aquaculture, producing large quantities of healthy, high-quality seedlings has become the primary objective of fish broodstock cultivation. During gonadal development, the gonads—particularly eggs—must accumulate substantial nutrients to support normal embryonic and early larval development [1-3]. Lipid nutrition, especially polyunsaturated fatty acids (PUFA), plays a crucial role in gonadal development and egg quality in fish broodstock [1-4]. Highly unsaturated fatty acids (HUFA) are defined as PUFA with carbon chain lengths 20 and three or more double bonds, such as eicosapentaenoic acid (C20:5n-3, EPA), docosahexaenoic acid (C22:6n-3, DHA), and arachidonic acid (C20:4n-6, AA). EPA and DHA are collectively referred to as n-3 HUFA [5].

n-3 HUFA are essential components of cell membrane phospholipids, provide energy, and serve as precursors for important bioactive metabolites. They play vital roles in promoting neural development, regulating metabolism in aquatic animals, and enhancing immunity [6-7]. Current research on HUFA in fish broodstock has primarily focused on reproductive performance, tissue nutrient composition, and endocrine regulation [8-10]. High dietary levels of n-3 HUFA promote oocyte growth and development, regulate androgen synthesis, and facilitate sperm maturation [9-10]. A stable supply of n-3 HUFA in feed is beneficial for broodstock cultivation. While fish oil represents the primary source of n-3 HUFA, declining global fish oil production has made the search for alternative lipid sources a research priority [11-12]. This article reviews the biosynthetic pathways of n-3 HUFA in fish, their effects on reproductive performance, and analyzes both sources and alternative lipid sources to provide a reference for aquaculture practitioners.

Biosynthetic Pathways of n-3 HUFA in Fish

The biosynthetic pathway of very long-chain polyunsaturated fatty acids (VLC-PUFA) in fish is illustrated in [Figure 1: see original paper]. Through the action of a series of desaturases and elongases, linolenic acid (LAN, C18:3n-3) and linoleic acid (LOA, C18:2n-6) are converted to n-3 HUFA and n-6 HUFA, respectively [7,13-14]. Two distinct pathways convert EPA to DHA. The first involves elongation of EPA to C22:5n-3, followed by Δ6 desaturation and peroxisomal -oxidation to form DHA [15]. The second pathway, initially discovered in the marine rabbitfish (*Siganus canaliculatus*), involves direct Δ4 desaturation of C22:5n-3 to DHA [16]. This Δ4 desaturase pathway has subsequently been identified in Senegal sole (*Solea senegalensis*), Mexican silverside (*Chirostoma estor*), and striped snakehead (*Channa striata*) [17-19].

The capacity for VLC-PUFA synthesis varies among fish species. Freshwater and salmonid fish generally have essential fatty acid requirements of C18:2n-6 and C18:3n-3, whereas most marine fish lack the ability to synthesize long-chain unsaturated fatty acids [5,15,20].

Effects of n-3 HUFA on Broodstock Reproductive Performance

Reproductive performance is evaluated through indicators including gonadal development, gamete quality, fecundity, fertilization rate, larval survival, and larval body composition [6-7]. Different fish species have varying requirements for dietary HUFA, with optimal levels promoting reproduction while both deficiency and excess can have detrimental effects.

2.1 Freshwater Fish Jaya-Ram et al. [21] investigated the effects of different dietary lipid sources (varying HUFA levels) on zebrafish (*Danio rerio*) broodstock. The highest fecundity and hatching rates occurred in the mixed oil group (fish oil:linseed oil = 1:1), demonstrating that both n-3 and n-6 fatty acids are important for zebrafish reproduction. Guo et al. [22] fed zebrafish broodstock diets containing fatty acids of different chain lengths and found that the n-3 HUFA group exhibited significantly higher DHA and EPA levels in gonads compared to control and medium-chain fatty acid groups. The n-3 HUFA group also showed significantly higher gonadosomatic index, absolute fecundity, and relative fecundity, indicating that dietary DHA and EPA enhance reproductive performance in zebrafish.

DHA and EPA can improve fertilization rates by enhancing egg quality. Luo et al. [23] found that high dietary DHA levels in Siberian sturgeon (*Acipenser baeri*) increased EPA and DHA content in eggs and improved fertilization rates. Different freshwater species have distinct HUFA requirements. Qi et al. [24] studied the effects of different dietary lipid sources on female Amur sturgeon (*Acipenser schrenckii*) after five months of feeding. An n-3 HUFA supplementation level of 2.5% resulted in higher broodstock maturity and larval survival rates. In rainbow trout (*Oncorhynchus mykiss*), dietary supplementation with 2% n-3 PUFA improved sperm quality and reproductive success [25]. Salinity also affects n-3 HUFA requirements. El-Sayed et al. [26] fed Nile tilapia (*Oreochromis niloticus*) broodstock different lipid sources (soybean oil, fish oil, or a mixture) at various salinities (0, 7, and 14). At 0 salinity, n-3 HUFA was not required, but at salinities of 7 and 14, n-3 HUFA was necessary for reproduction, possibly because high salinity inhibits enzymes involved in n-3 HUFA synthesis.

2.2 Marine Fish Zakeri et al. [4] fed yellowfin sea bream (*Acanthopagrus latus*) broodstock diets with different n-3 HUFA levels (6.67%, 4.26%, and 2.92%) for 132 days. Relative fecundity, oil globule buoyancy, hatching rate, and 3-day post-hatch larval survival were significantly higher in the high-level (6.67%) group, indicating that high dietary n-3 HUFA improves reproductive performance in this species. Callan et al. [27] fed flame angelfish (*Centropyge loriculus*) broodstock diets with three n-3 HUFA levels (1.8%, 2.9%, and 3.6%) for five months and found that high n-3 HUFA (3.6%) enhanced female reproductive performance.

However, different marine species have varying n-3 HUFA requirements, and excessive levels can reduce reproductive performance. Furuita et al. [28] fed Japanese flounder (*Paralichthys olivaceus*) broodstock diets containing 2.1%, 4.8%, and 6.2% n-3 HUFA for two months during the spawning season. While the high-HUFA group produced the most eggs, egg quality parameters including hatching rate and normal larval hatching rate were significantly higher in the low-HUFA group, suggesting that high dietary n-3 HUFA negatively affected egg quality. Fernández-Palacios et al. [29] reported similar results in gilthead sea bream (*Sparus aurata*), where a diet containing 1.6% n-3 HUFA yielded the best fecundity, hatching rate, and larval survival. As dietary n-3 HUFA levels increased, egg n-3 HUFA content increased, but excessive levels reduced fecundity and caused hypertrophy of the yolk sac in newly hatched larvae.

Li et al. [30] fed spotted knifejaw (*Plectorhynchus cinctus*) broodstock diets with five n-3 HUFA levels (1.12%, 2.40%, 3.70%, and 5.85%). Optimal egg buoyancy, egg production per kg female body weight, and 3-day post-hatch larval length and survival occurred at 2.4% and 3.7% n-3 HUFA, while both low (1.12%) and high (5.85%) levels negatively affected egg and larval quality.

2.3 Mechanism of Action During gonadal development in fish, various endogenous factors (sex hormones and their receptors) and exogenous factors (nutritional and environmental factors) act on the hypothalamic-pituitary-gonadal (HPG) axis to stimulate gonadal production of sex steroids, which bind to corresponding receptors to promote gonadal development, ovulation, and spermiation [31-33]. Follicles secrete estradiol (E2), which is transported via blood to the liver and binds to receptors to induce vitellogenin (Vg) synthesis. n-3 HUFA are important components of Vg, and Vg synthesis requires both n-3 HUFA and E2 stimulation. Dietary n-3 HUFA may alter sex steroid hormone levels, thereby regulating gonadal development [34]. Studies have shown that optimal dietary n-3 HUFA levels can increase sex steroid hormone levels in marine fish, promoting gametogenesis and maturation [35].

In freshwater fish research, Guo et al. [22] proposed that n-3 HUFA may promote -oxidation of oleic acid in gonads, reducing its relative level and thereby alleviating its inhibitory effects on gonadal development, which benefits reproduction. In marine fish studies, Peng et al. [36] fed silver pomfret (*Pampus argenteus*) broodstock diets with four n-3 VLC-PUFA levels (5.18%, 4.01%, 3.02%, and 2.22%). During ovarian development, n-3 VLC-PUFA affected ovarian steroid hormone levels by altering plasma follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels and the expression of the aromatase 19A1 (Cyp19A1) gene. Higher dietary n-3 VLC-PUFA levels increased E2 secretion and Vg synthesis, stimulating ovarian development. Luo et al. [23] reported similar results in Siberian sturgeon, where high dietary DHA increased plasma E2 levels in females. Additionally, Da Silva et al. [9] demonstrated that high dietary DHA and EPA promoted oocyte growth and development in European eel (*Anguilla anguilla*).

Dietary n-3 HUFA affect reproductive performance by regulating E2 and testosterone (T) secretion. Li et al. [35] found that both low and high HUFA levels reduced plasma E2 and testosterone levels in spotted knifejaw, decreasing fecundity, fertilization rate, and larval survival. Regarding effects on male broodstock, Baeza et al. [10] showed that EPA and DHA regulate androgen synthesis during gonadal development in male European eel, particularly during final sperm maturation. DHA and EPA influence male gamete development by modulating androgen levels and ensuring proper sperm membrane composition [37]. Further research is needed to elucidate how n-3 HUFA affect sex steroid secretion and enhance reproductive performance in fish.

Sources and Alternative Lipid Sources for n-3 HUFA

Fish oil is the primary source of n-3 HUFA in aquafeeds. According to the 2012 annual report from the International Fishmeal and Fish Oil Organisation (IFFO), aquaculture accounts for 75% of global fish oil consumption, with salmonids using 62% and marine fish 19% [38]. Declining fish oil production has made the search for alternative lipid sources a critical research objective [12,39].

Freshwater and salmonid fish can synthesize EPA and DHA from C18 PUFA, making vegetable oils containing C18 PUFA viable alternatives. Vegetable oils are widely available and relatively inexpensive [7], including rapeseed oil, linseed oil, sunflower oil, palm oil, soybean oil, and olive oil. C18 PUFA can partially replace fish oil by promoting HUFA synthesis, primarily through upregulation of relevant enzyme transcription [20]. Most research has focused on juvenile fish, examining the effects of substituting fish oil with single or blended vegetable oils on tissue fatty acid composition and the expression of desaturases and elongases to enhance endogenous HUFA synthesis. However, few studies have investigated these effects in broodstock.

Afonso et al. [40] reported that a 50% replacement of fish oil with a blended vegetable oil (50% soybean oil, 25% rapeseed oil, and 25% linseed oil) in Senegal sole (*Solea senegalensis*) maintained flesh quality suitable for human consumption. Izquierdo et al. [41] fed gilthead sea bream broodstock diets with different linseed oil replacement levels (80-100% replacement) and found no reduction in female fecundity or egg production. Furthermore, offspring from broodstock fed 60% linseed oil diets showed higher growth rates and feed utilization at 4 months compared to those from 100% fish oil-fed broodstock when subsequently fed low-fish-oil diets. These results suggest that high dietary linseed oil in broodstock has long-term effects on offspring, and early nutritional strategies for marine fish broodstock can improve long-term progeny performance, though the molecular mechanisms remain unclear.

Marine microalgae such as *Schizochytrium*, *Nannochloris oculata*, and *Phaeodactylum tricornutum* are rich in EPA or DHA and have fatty acid profiles similar to fish oil, making them potential HUFA sources for fish feeds [42-43]. Studies on aquatic animal larvae have shown that dietary microalgae supplementation enhances growth, increases tissue DHA levels, and improves digestive capacity and immunity [42-43]. However, challenges including high protein content, presence of antibiotics and toxins, difficulty in breaking cell walls, and high costs associated with cultivation, harvesting, and processing limit their application in fish feeds. Future research should focus on developing improved algal strains through genetic engineering and optimizing cultivation and harvesting techniques to reduce costs [44].

Conclusion

n-3 HUFA significantly influence gonadal development, gamete quality, fecundity, fertilization rate, hatching rate, and larval survival in fish. However, research on the molecular mechanisms by which n-3 HUFA regulate broodstock reproductive performance remains limited. Declining fish oil production presents a major challenge for identifying suitable alternative lipid sources. Despite technical issues requiring resolution in cultivation and harvesting, marine microalgae represent a promising alternative to fish oil. Vegetable oils are widely available, and genetic engineering to develop high-oil-yielding plants offers another potential solution. Recent research has explored interactive effects of diet and hormones on broodstock to enhance reproductive performance. Additionally, given the long broodstock rearing period, short-term intensive n-3 HUFA supplementation could reduce fish oil usage while ensuring gonadal development and improving offspring quality. Future studies should investigate optimal n-3 HUFA requirements, feeding duration, and supplementation strategies to enhance broodstock reproductive performance.

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