Improving Aquatic Animal Health

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Contributor: Murni Marlina Abd Karim

At the present time, no artificial larval diet is capable of entirely fulfilling the dietary requirements of several larval fish and crustacean species. Zooplankton live food is the basic foundation of fish larviculture, and successful rearing of fish larvae still heavily depends on an adequate supply of nutritious live food. Despite being important, the production protocols of copepods and cladocerans (*Moina*) are still underdeveloped in hatcheries. Rotifers and *Artemia* are the most commonly used live foods. However, these live foods are evidently lacking in crucial nutrient constituents. Hence, through nutrient enrichment, live food with the nutritional profile that meets the requirements of fish larvae can be produced. With the aim to maximize the effectiveness of production to optimize profitability, it is important to evaluate and improve culture techniques for the delivery of micro- and macro-nutrients as feed supplements to larvae in aquaculture systems. Bioencapsulation and enrichment are the evolving techniques in aquaculture that are commonly employed to enhance the nutritional quality of live food by integrating nutrients into them, which subsequently improves the growth, survival, and disease resistance of the consuming hosts.

live food enrichment nutrients nutritional quality aquatic health

1. Introduction

Larvae of many aquatic species either have complete dependence on zooplankton live food as a basal diet, or they have significantly better performance when started on live food[1]. Live food is commonly regarded as “living capsules of nutrition”, rich in proteins, vitamins, carbohydrates, minerals, amino acids, and fatty acids[2]. As a superior nutritional prey, some zooplankton contain high levels of digestive enzymes[3] and are capable of producing appetite-stimulating effects on larvae[4]. Live food organisms are able to swim freely in the water column, thereby being constantly accessible to finfish and crustacean larvae[5][6]. Their jerking movements are likely to stimulate larval feeding responses[7]. On the contrary, formulated feeds often accumulate on the water surface or some slowly sink to the bottom, whereby becoming less accessible to larvae[5]. Zooplankton such as rotifers and *Artemia* are by far the most commonly utilized live food in the cultivation of finfish and crustaceans[8].

Substitution of live food by formulated diets has been emphasized[9]. However, the sole application of a formulated diet may seem like a far-fetched idea due to low its digestibility and the deterioration of water quality[6][7]. Even though the use of live food in larval rearing has been reported to improve larval growth performance, survival, and disease resistance[1][10][11], the cultivation and management of live food for aquatic production is costly and unpredictable[12]. Multiple studies have demonstrated the success of total live food replacement or reduction in aquaculture[9][13]. It is important to understand the nutritional requirements of fish larvae in order to facilitate the optimization of diets and feeding protocols, which may subsequently enhance larval quality[7][14]. Consequently, several studies have emphasized developing practical methods to improve the nutritional status of live food with essential nutrients[15][16][17][18][19][20].

By taking advantage of primitive feeding characteristics, the manipulation of the nutritional status of zooplankton is achievable by pre-feeding them through the so-called “bioencapsulation” or “enrichment” protocols. Through enrichment techniques, essential nutrients lacking in zooplankton, prophylactics, and therapeutics can be delivered to fish larvae via zooplankton live food. The application of enriched live food is reflected in enhanced growth, survival, stress tolerance, and microbial diversity for a variety of aquatic species[19][20][21][22][23][24]. A very important aspect of live food enrichment is its reproducibility and predictability, which are crucial in commercial hatcheries. Hence, it is necessary to constantly produce high-quality live food on a large scale[15]. However, producing enriched live food with consistent levels of the important nutrients can be complex. This review aimed to emphasize the significance of live food and the implementation of different enrichment techniques to incorporate nutrients such as minerals, vitamins, microalgae, lipids, and probiotics to enhance the nutritional status of the live food and to subsequently boost the health of the aquatic animals.

2. Enrichment with Fatty Acids

Highly unsaturated fatty acids (HUFA) with 20 or more carbon atoms are one of the major sources of metabolic energy during the embryonic and pre-feeding larval stages in fish. However, these energy sources rapidly declined during the endogenous feeding stage[25]. The n-3 series HUFA docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3), and the n-6 series HUFA arachidonic acid (ARA, 20:4n-6), play significant roles in fish larval development; thus, the deficiency of HUFA may impair fish growth, reproduction, and survival, causing pale or swollen liver, myocarditis, intestinal steatosis, lordosis, fin erosion, and shock syndrome[26]. HUFA are synthesized in very small concentrations from their precursors alpha-linolenic acid (ALA, 18:3n-3) and linoleic acid (LA, 18:2n-6) [27] due to the lack of delta-5 and delta-6 desaturases and elongases in marine fish larvae[28]. Therefore, HUFA must be incorporated through live foods such as copepods, rotifers and *Artemia* to meet the requirements for larval growth[27]. The requirements of HUFA in fish and crustaceans have been widely studied. The effects of dietary HUFA in the juveniles of golden pompano (*Trachinotus ovatus*[29], yellowtail (*Seriola dumerili*) [30] Asian seabass (*Lates calcarifer*)[31], and Pacific white shrimp (*Litopenaeus vannamei*)[32]are among the most recently published studies.

Enrichment of live food with commercial oil emulsion (Super Selco, DHA Selco, Selco S.presso) is a common practice[33][34][35][36][37][38][39][40]. Several studies have assessed the dietary fatty acid profiles of copepods and enriched *Artemia*[39][41][42] . The predominant fatty acids in copepods are DHA, EPA, and palmitic acid, while DHA, EPA, and oleic acid are the predominant fatty acids in *Artemia* enriched with Super Selco and DHA Selco[41]. Apart from the absolute amount of HUFA, the dietary DHA/EPA ratio is suggested to impact the normal growth and development of certain fish species[43][44]. The average DHA/EPA ratio for copepods ranged between 1.83 and 5.5 whereas the DHA/EPA ratio for *Artemia* enriched with DHA Selco ranged from 1.4 to 2.2[41][42]. The DHA/EPA ratio of *Artemia* enriched with Super Selco at 600 mg/L for 16 h was reported at 0.2 [39], whilst enrichment at 200 and 300 mg/L for 24 and 20 h, respectively, brought about 0.68 and 0.3 DHA/EPA ratio, respectively[38][41]. Altogether, *Artemia* enriched with DHA Selco recorded a higher DHA/EPA ratio than that of Super Selco. The instability of HUFA and the catabolism of these compounds by *Artemia* in addition to low DHA retention efficiency in *Artemia* during the first 24 h post enrichment might be the contributing factors to this variation[38][41][45]. Commercial emulsions are more stable and effective as the primary emulsions are mainly made from HUFA-rich fish oils and emulsified with egg yolk and seawater. However, these forms of enrichment formula are low in efficiency but are cheap alternatives in developing countries[7]. Higher DHA and EPA contents and DHA/EPA ratio were recorded in the freshwater cladoceran *Moina micrura* enriched with commercial emulsion (Maxepa MERCK, Delhi, India) in addition to gelatine, egg yolk, and Celin[19]. Modifications of dietary fatty acid compositions of rotifers and *Artemia* should be made in line with those of copepods.

Boosting of the nutritional status of rotifers[46][47], *Artemia*[48][49], copepods[50][51][52][53][54][55][56][57], and *Moina*[58] through algal enrichment techniques is a common practice to boost the quality of the otherwise nutrient-deficient feed. Microalgae is a rich source of HUFA and polyunsaturated fatty acids (PUFA) [59][60][61]. It is easier to control the essential fatty acid (EFA) composition of enrichment emulsions when microalgae-derived oil is used in comparison with purified fish oils [62] Due to the high cost and difficulty in producing, concentrating, and storing live microalgae, the development of different forms of microalgae as a replacement to live microalgae has become a major focus of research[63]. A cheaper microalga paste has been used in aquaculture practice as an alternative to live microalgae[64]. Rotifers fed on microalgal pastes (*Nannochloropsis oculata* and *Chlorella vulgaris*) at equal quantities were rich in palmitic acid, linoleic acid, and EPA after 48 h of exposure to the microalgal diet. However, the DHA content was only recorded at 6 mg/g dry weight (DW). Nevertheless, the DHA content was enough to improve the growth, development, and stress resistance of fish larvae[65]. This study underlined the importance of enriching rotifers fed to larvae with multiple microalgal species over monospecific diets.

A previous study investigated the fatty acid composition of rotifers enriched with a mixture of DHA-enriched *C. vulgaris* (Super fresh Chlorella V12, SV, Chlorella Industry, Tokyo, Japan) and DHA emulsion (Bio Chromis, Chlorella Industry, Tokyo, Japan) for 12 h[43]. The DHA content in enriched rotifers increased from 0.1 to 15.4 % and the DHA/EPA ratio was highest in the treatment. DHA was found to be dominant in rotifers enriched with DHA- and arachidonic acid (AA)-rich oils extracted from the dinoflagellate *Crythecodinium* sp. and the fungus *Mortierella alpina*, respectively, in addition to EPA-rich marine oil[66]. Rotifers have a better retention rate of EPA compared to DHA, regardless of the ratio in their enrichment [44]. Enrichment of rotifers can be achieved either through short-term enrichment (alteration of the lipid content of the rotifers just before larval feeding) and long-term enrichment (feeding of rotifers on a complete diet) [67][68]. Enriched DHA was stable in rotifers at 10 °C for at least 24 h post-enrichment under starving conditions, whereas a higher temperature of 20 °C significantly decreased the DHA level during starvation [69] Rotifers emptied their gut at a reduced rate as culture temperature decreased from 26 °C to 4 °C. Moreover, microalgae are often added to the enrichment formula to promote “green water” to maintain the nutritional quality of zooplankton[70][71]. The larvae of rainbow trout (*Oncorhynchus mykiss*) [72]. Russian sturgeon (*Acipenser gueldenstaedtii*)[73], Atlantic sturgeon (*Acipenser oxyrinchus*)[74], caspian kutum (*Rutilus frisii kutum*)[75], yellowtail flounder (*Limanda ferruginea*)[66], gilthead seabream (*Sparus aurata*) and greater amberjack (*S. dumerili*), whitefish (*R. kutum*) fry [76], and juvenile milkfish (*Chanos chanos*) [77]have been reared with live food enriched with essential fatty acids.

The high contents of EPA, DHA, and some digestive enzymes in copepods are among the important properties that make them a superior live food to *Artemia* and rotifers[6]. Therefore, it is recommended to enrich zooplankton in order to meet copepod HUFA levels. The enrichment emulsions are commonly prepared using commercial emulsions such as DHA Selco and Super Selco. To meet the copepod DHA/EPA ratio, it is recommended that *Artemia* and rotifers be enriched with DHA Selco. Even though studies on HUFA enrichment in *Moina* are fairly limited, a study has successfully enriched *Moina* with Maxepa. Additionally, HUFA enrichment can be performed using microalgae, either live or pastes. It is recommended that microalgae pastes be used as a cheaper alternative to live microalgae, and the application of multiple microalgal species over monospecific diets would be very beneficial. Moreover, a combination of commercial emulsions and microalgae in an enrichment mixture would be advantageous in terms of enhancing the DHA/EPA ratio.

3. Enrichment with Vitamins

3.1. Vitamin C

Vitamin C (VC) plays a vital role in the growth, immune response[78], hematology and histology[79], antioxidant and enzyme activities[80], reproduction [81], wound healing[82], and response to stressors[83]of fish and crustaceans. The addition of VC in aquaculture practices has been proven to enhance the growth performance, antioxidant defense system, and production of many aquatic animals including freshwater prawn (*Macrobrachium malcolmsonii*) [80] and kuruma shrimp (*Marsupenaeus japonicus* Bate)[84]. Enrichment of *Artemia* with of ascorbyl-6-palmitate for 24 h was observed to significantly reduce the mortality rate of seabream larvae[85]. However, it is necessary to note that a high dose of vitamin supplementation may cause lipid peroxidation in fish tissues under oxidative stress conditions[86]. The dietary requirement of VC may decrease with increased size[87]. Lack of VC can lead to structural deformities and internal hemorrhaging[88]. Some aquatic animals including the majority of crustacean and fish species are unable to synthesize VC because of the absence of the enzyme L-gluconolactone oxidase, which is essential for the last step of VC biosynthesis[89]. Hence, they depend on feed for a constant supply of VC.

Brown and Hohmann[90] reported a significant effect of the algal growth phase on the percentage of ascorbic acid in the culture of *Isochrysis* sp. However, the results need to be contextualized with the standard hatchery practice for algal production, the balance of other nutrients in the microalga, and the dietary requirements of the aquatic animal[90]. The enrichment of *A. franciscana* with the microalgae *Isochrysis galbana* for 72 h had a favorable impact on the amount of ascorbic acid in *Artemia* depending on the ascorbic acid content of *I. galbana*[45]. Moreover, the enrichment of rotifers with ascorbyl palmitate improved the assimilation of ascorbic acid when the percentage of ascorbyl palmitate incorporation in the enrichment media increased, thus suggesting that the ascorbic acid levels in *Artemia* nauplii can be manipulated via bioencapsulation of different ascorbyl palmitate concentrations [91] The positive effects of feeding VC-enriched live food were reported on the larvae of milkfish (*C. chanos*) [92]and climbing perch (*Anabas testudineus*)[19], Senegalese sole (*Solea senegalensis*)[93], and Patagonian red octopus paralarvae (*Enteroctopus megalocyathus*)[94]. Generally, boosting of ascorbic acid content in zooplankton live food through the algal enrichment technique at a commercial hatchery yielded a lower ascorbic acid concentration than in the laboratory, probably due to differences in the culture conditions[91]. Different species and enrichment procedures resulted in different ascorbic acid levels in zooplankton[6], but the enrichment of microalgae with VC had been reported to increase the concentration of VC in *Artemia* [93], rotifers[95] and copepods[96]. However, it is crucial to have prior knowledge on the natural content of vitamins in microalgae before they are subjected to any enrichment procedure. There may be variations due to species and culture conditions with regards to light and nutrient conditions; protocols for harvesting, processing, and storage; extraction; and analysis[97]. The compositional data of vitamin contents in microalgae need to be compared to the dietary needs of the consuming aquatic animals. Unfortunately, the dietary requirements for larval or juvenile animals that feed on zooplankton are poorly understood[97]. Moreover, the concentrations of vitamins in the intermediary zooplankton and the transfer efficiency in the food chain are the area that requires further research [97][98].

The pre-enrichment of microalgae with VC would be beneficial to a large group of filter-feeding zooplankton that utilize microalgae as a major source of food. Therefore, enrichment of zooplankton such as *Artemia*, rotifers, and copepods with microalgae to boost their VC levels is a practical approach. The levels of VC in zooplankton can be manipulated by enriching them with ascorbyl palmitate at different concentrations. However, when utilizing microalgae in an enrichment procedure, several factors including culture conditions, harvesting, processing, and storage protocols, as well as extraction and analysis must be taken into account, as these may greatly impact the VC levels in zooplankton live food.

3.2. Vitamin A

Vitamin A (VA) is a vital nutrient for fish as the compound cannot be synthesized *de novo*. Many VA or retinoid forms are available as dietary supplements including retinol (the alcohol form of VA), retinal (the aldehyde form), retinoic acid (the acid form), and retinyl acetate and retinyl palmitate (the ester form)[99]. VA hypervitaminosis can cause skeletal malformations in different vertebral regions, as well as cephalic malformations in jaw and fin complexes in other marine fishes, including the larvae of gilthead sea bream (*S. aurata*) [100], red sea bream (*Chrysophrys major*)[101], European sea bass (*Dicentrarchus labrax*) [102] Japanese flounder (*Paralichthys olivaceus*)[103][104], striped trumpeter (*Latris lineata*) and post larvae [99], and summer flounder (*Paralichthys dendatus*) [105]. The positive effect of dietary VA were reported on the juveniles of Nile tilapia (*Oreochromis niloticus*)[106] and spotted grouper (*Epinephelus coioides*)[107], on-growing gibel carp (*Carassius auratus gibelio*) [108], rainbow trout (*O. mykiss*) fry[109], and on Japanese flounder (*P. olivaceus*) larvae[110].

It was reported that VA deficiency in *Artemia* caused incomplete migration of the eye during Atlantic halibut (*Hippoglossus hippoglossus* L.) larvae metamorphosis[111]. Another study reported a higher concentration of VA in Atlantic halibut when fed with marine copepod than those fed with *Artemia*[112], probably because *Artemia* contains carotenoids in the form of cryptoxanthin or canthaxanthin, while the source of carotenoids in copepods is lutein and astaxanthin[61]. Thus, it is appropriate to enrich *Artemia* with copepod-type carotenoids. Furthermore, it was reported that the vitamin content in rotifers was below the levels found in copepods, but generally still within the range required by fish larvae[113]. Considering the limited amount of literature on fish larval nutrient requirements, the nutrient levels of copepod are considered as a target for the enrichment[114]. Moreover, VA was not detected in rotifers fed with basic diet containing Baker’s yeast, suggesting a need for further enrichment with pure VA [113] It is reported that the total VA accumulation in rotifers was independent to the dose[17][114], but dependent to the added doses in the case of *Artemia*[115]. However, another study demonstrated otherwise[100]. Studies have demonstrated the possibility of zooplankton enrichment using liposomes[115] and commercial emulsion[100][116], in which retinyl palmitate was the dominant form of the retinoids in the emulsions and in the enriched zooplankton. However, Monroig et al.[115] [115] reported a noteworthy result of the poor efficiency of the commercial emulsion in *Artemia*, despite it containing retinyl palmitate, probably due to a partial degradation during the enrichment process influenced by different abiotic conditions. Moreover, the emulsion quality and properties; differences in the strains and batches of the zooplankton used; or the stages of development, metabolic capability, and filtration rates may contribute to the enrichment efficiency of the emulsions[17]. Therefore, the application of vesicles such as liposomes to bioencapsulate VA in live food is a promising approach. Liposomes as a retinyl palmitate carrier to zooplankton provide extra protection for VA from oxidation, and thus a higher amount of retinyl palmitate can be bioencapsulated in the zooplankton to be fed to aquatic animals[115].

Given the limited number of studies on fish larval nutrient requirements, the VA levels of copepods are considered as a target for enrichment. *Artemia* can be enriched with VA in the form of lutein and astaxanthin to meet the copepod VA levels. Even though the vitamin content in rotifers was below the levels found in copepods, it is still within the range required by fish larvae. Several studies reported that zooplankton such as *Artemia* can be enriched with liposomes and commercial emulsion. However, the partial degradation of VA during the enrichment process must be taken into account when employing commercial emulsions for zooplankton enrichment. Various studies on the applications of vitamin C and A in live food have been compiled in Table 1.

Table 1. Enrichment of live food with vitamins.

Vitamin	Live Feed	Biological Model	Method	Effects	References
Vitamin C	*M. micrura*	Climbing perch (*Anabes testudineus*)	Co-enrichment with HUFA for 24 h	Growth promoter	[19]
	Artemia nauplii	Senegalese sole (*S. senegalensis*)	Co-enrichment with dietary Fe for 24, 29, and 33 h	Growth promoter	[93]
		Patagonian red octopus (*E. megalocyathus*)	Co-enrichment with algae for 2 h	Growth promoter and survival improvement (not significant)	[94]

		-	Enrichment with unilamellar liposomes composed of soybean phosphatidylcholine and loaded with sodium ascorbate	Low vitamin C content in the nauplii	[115]
	Moina, Daphnia, Cyclops, and *Diaptomus*	Rohu (*Labeo rohita*)	Enrichment with 20% ascorbyl palmitate for 12 h	Growth promoter and survival improvement	[117]
	Artemia nauplii and rotifer *Brachionus plicatilis*	Milkfish (*C. chanos*)	Co-enrichment with HUFA for 24 h	Growth promoter, survival improvement, and lower incidence of opercular deformity	[92]
	Rotifer *Brachionus* sp.	-	Co-enrichment with thiamine, vitamins A and E, and iodine	Enrichment with 4.6% Stay C in the diet could give copepod levels of vitamin C	[114]
Vitamin A	*Artemia* nauplii	Striped trumpeter (*L. lineata*)	Enrichment emulsions contain retinyl palmitate together with lipids, and vitamin E and C (24 h)	Growth and survival were not significantly affected by increasing dietary doses of retinyl palmitate	[118]
	Artemia metanauplii	Senegalese sole (*S. senegalensis*)	Addition of retinyl palmitate to a	Survival improvement	[119]

		commercial enrichment emulsion		
Rotifer *B. plicatilis*	Striped trumpeter (*L. lineata*)	Enrichment with retinyl palmitate for 2 h	Growth and survival were not significantly affected by increasing dietary doses of retinyl palmitate Retinyl palmitate enrichment in rotifers did not affect the type or severity of jaw malformations	[99]
Rotifer	Atlantic cod (*Gadus morhua*)	Co-enrichment with oil mixtures for 2 h along with the addition of fish meal, Selplex, and iodine	Alteration of the skeletal metabolism during larval development	[120]

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