

Live Feed Enrichment – A Possible Step Towards a Sustainable Blue Revolution

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Abstract: Enrichment is the process of increasing the quality or nutritional value of any substances, i.e., live feeds. The live feeds are essential for larval rearing of commercially critical aquatic organisms. Still, the significant constraint noticed in the live feed sector is that sometimes they lack vital nutrients such as EPA, DHA, etc. Enrichment with crucial nutrients can improve larval survivability during early larval stages, which is the major bottleneck observed during larvi culture techniques in aqua hatcheries. Simultaneously various live feed such as Rotifers, Cyclops, copepod, Cladocerans, and Artemia are widely used as a carrier for bio encapsulation process in aquaculture. Numerous substances, especially essential amino acids, fatty acids, attractants, vitamin-mineral premix, are widely used for bio encapsulation in live feed culture units. Live feed brings those substances into their body either by feeding or osmoregulation. The main idea behind this article is to demonstrate the proper encapsulation method for various live feeds in aqua hatcheries.

Keywords: Live feed, Bio enrichment, Larviculture, Nutrients, Essential fatty acids.

1. Introduction

Aquaculture is the farming of commercially important aquatic organisms such as fish, crustaceans, molluscs and aquatic plants etc., (FAO, 1990). It contributed 40% of the total production compared to wild fisheries production and it is expected to surpass the production of wild fisheries by 2020-2025 (Tacon 2003). Increase in the aquaculture production is mainly depends on better management practices starting with overall economic management, good water quality management, better feeding practices, more environment friendly feeds, genetically improved stocks and finally finish with overall health management (New and Wagner2000). World aquaculture has been expanding over the years, and declining trends of capture fishery in the last few years create huge potential for aquaculture to meet the protein requirement for the growing population in the future. Now a day's major constraint to be found in the aquaculture sector is larval nutrition. Larval nutrition is directly or indirectly affects on larval survivability and it varies from species to species i.e., finfish < 10%; mud crab < 1 %; shrimp 20-40%; molluscs < 20% (Imelda, 2003). Different types of live feed are used in aqua hatcheries such as Rotifers, Cyclops, copepod, Cladocerans, and Artemia. Generally live feed is being cultured in hatcheries to supply the demand of feed at initial life stages offish larvae.

Due to higher cost of commercially available encapsulated diet, live foods remain a permanent solution for larval nutrition in hatchery operations. Bio encapsulation or bio enrichment is one type of enrichment method by which we can improve the nutritional value of different live food organisms either by feeding or incorporating, within them with various kind of nutrients. In aquaculture, different practical and experimental enrichment diets are available in the form of microalgae, emulsion, liposomes and microencapsulated diets, etc. In case of mariculture sector, fish larvae generally require polyunsaturated fatty acids i.e., eicosapentanoic acid (EPA: 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), for their natural growth and survival. Apart from that, arachidonic acid (ARA; 20:4n-6) also play an important role in maintaining their physiological balance in a stressful condition such as it is required to maintain osmoregulation, cardiovascular functions, neural control, and reproduction (Imelda, 2003). The rotifer, *Brachionus plicatilis* and the anostracan *Artemia salina* are commonly used organisms in larvae culture in India. But the main problem with live feed is that they are generally poor in EPA and DHA, and in some cases, like Artemia, DHA is practically absent. For this purpose, they should be enriched with EPA and DHA before they are used for feeding the larvae. On the other hand, Phospholipids are potentially rich in DHA, so we can use this as a source of DHA in larvae culture techniques; otherwise, the marine fish larvae may develop neurological abnormalities. Fatty acid-enriched live feed improves the larval performance in the case of striped bass, palmetto bass, cod, red sea bream, yellowtail, and milkfish larvae culture techniques, etc. The optimum concentration of n3 (HUFA) in diet should be within the range of 2-4percent (1% EPA and 1% DHA) (Imelda, 2003).

2. Factors to be Considered Prior to Bio Enrichment

- Selection of the carrier or the live feed/ biofeed

Table 1
Commonly used carriers for bio enrichment process

S.no.	Carrier	Size
1	Micro algae	2-20 micron
2	Rotifers	5-200 micron
3	Artemia	200-400 micron
4	Moina	400-1500 micron
5	Daphnia	200-400 micron

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- b) Nutritional quality, digestibility and acceptability before and after Enrichment.
- c) Calculate the inclusion level or upto how much enriching media do we need to incorporate into the carrier organisms body.
- d) Economics feasibility of that enrichment process.
- e) Purity of the culture of the carrier organism.

3. Techniques of Bio Enrichment

There are generally two methods are widely used for bio enrichment of livefeed such as - direct and indirect method.

A. Indirect Method

In this method a new type of yeast is designed by adding fish oil or cuttle fish liver oil as a supplement to the culture medium of baker's yeast and this new type of yeast is also known as co-yeast. As a result, this newly developed yeast contains a high amount of lipid and a significant amount of w-3 HUFA. Indirect method generally follows in copepod and Cladocerans enrichment unit.

B. Direct Method

Direct method was first developed by Watanabe et al., 1983. In this method specially a homogenate is prepared by using lipid emulsion, which usually rich in w-3HUFA and this emulsion is directly pour into the livefeed culture unit (Artemia, rotifer culture unit).

C. Preparation of Enrichment Media

For the preparation of enrichment media, firstly, 5gm of fish oil is taken in a container, and after that, fish oil is homogenized for 2-3 minutes with the help of a homogenizer or mixer or by vigorous shaking. After that, Proper emulsification is ensured by checking the emulsion under a microscope and the prepared emulsion may be stored under refrigeration until use. The enrichment media may be supplemented with water and as well fat-soluble vitamins like A, D, E and K prior to homogenization. Enrichment of Artemia nauplii and rotifers with w-3 HUFA is dictated by two factors-primarily, the amount of lipid content of the emulsion and secondly the type of lipid source used for making emulsion. The quantity of lipid depends on the population density of the carriers, their feeding activity, and the water temperature. After that, this emulsion can be used as a feed for Artemia and rotifer culture unit; when feeding is done, Artemia nauplii or rotifers are harvested using 60-micron mesh size plankton net and washed thoroughly with clean seawater or freshwater and fed to the larvae of finfish or shellfish in adequate numbers.

D. Bio Enrichment in Rotifers

Rotifers are commonly known as wheel animalcules and nearly 2200 species are widely distributed in fresh and marine water ecosystem. Out of 2200 species, 90% of rotifers are generally inhibited in freshwater habitats (Lavens and Sorgeloos, 1996). Their growth is generally occurred by plasma increase not by cell division. Rotifers could be used as a suitable live food organism for the early larval stages of marine fish culture unit and the filter feeding nature of these organisms are

main suitable criteria for selecting this species for bio enrichment.

Rotifers generally considered as an important live feed in aquaculture point of view because-

- They are generally planktonic in nature.
- Can tolerate to wide range of environmental conditions.
- High reproducibility (0.7-1.4 offspring/female/day) and small in size.
- Slow swimming nature.
- This species can be cultured at higher stocking densities (2000 animals/ml).

Enrichment of rotifers can be done various ways such as,

Techniques for n-3 HUFA enrichment: Done by using algae, formulated feed and oil emulsions. Generally, algae are used as a bio enrichment medium because they are usually rich in essential fatty acids *i.e.*, EPA (20:5n-3) and DHA (22:6n-3) compared to other bio enrichment medium. Rotifer can also be enriched by using selco diet (CS®), which is exceptionally high in EPA (5.4mg/g), DHA (4.4mg/g) and (n-3) HUFA (15.6 mg/g) respectively (Léger et al., 1989).

Techniques for vitamin C enrichment: It is done by using ascorbyl palmitate, as a source of vitamin C.

Techniques for protein enrichment: Protein enrichment is done by using Protein Selco® diet.

E. Bio Enrichment in Artemia

Among the live feeds used in the larviculture of fish and shellfish, Artemia nauplii constitute the most widely used food item. Annually, over 2000 metric tons of dry brine shrimp cysts are marketed worldwide. Artemia has that one unique property to from dormant embryos in stressful condition, which is also known as 'cysts'. Those cysts are available year-round in large quantities along the shorelines of hyper saline lakes, coastal lagoons and solar salt pans, where the salinity of the water is >200ppt. After harvesting and processing, cysts are generally stored in cans. Before going for feeding, the Artemia cysts should be incubated for 24hrs and after incubation it releases free-swimming nauplii that can be used as a food source to the larvae of a variety of marine as well as freshwater fishes, this unique property make them most convenient, least labour-intensive live food available for aquaculture. The main problem with that are nutritional deficiencies specially deficiency of eicosapentanoic acid (EPA; 20:5n-3) and docosahexanoic acid (DHA; 22:6n-3) (Watanabe et al., 1982). So, in terms of maintaining proper growth and health condition of fishes enrichment of Artemia should be done because enriched Artemia shows good growth and maximum survival performance compared to no enriched Artemia (Noori et al., 2005).

1) Process involved in artemia enrichment

30gm of gelatin was taken in 800ml boiled deionised water and left the solution for few minutes at 40°C (Solution-A). On the other hand 160 ml cod liver oil was mixed with ascorbic acid (16g) and 4 raw egg yolks (Solution-B), then the full solution (Solution-A+Solution-B) is homogenized using a blender and stored in a refrigerator for 1 week. Emulsion dose

is applied 0.5 ml/lit of water, at 12 hrs interval (Treece, 2000).

F. Bio Enrichment in Copepod

Copepods are the common zooplankton, which are generally found in fresh and brackish water habitats. They have great ecological importance in the food chain and side by side, it serves as an important diet for many aquatic organisms, including finfish and shellfish (Kahan & Azoury, 1981). Among the live feeds used in aquaculture, Copepods are considered as best live feed in terms of their high dietary profiles that meet larval fish requirements (Hamre et al., 2008) and also has right quantity of digestive enzymes (Conceição et al., 2010). Proximate composition of copepod shows good amount of essential fatty acids (DHA, EPA, ARA), which promote excellent growth and survival to fish larvae (Shields, 2001). Earlier studies also reported that copepods contain high percentage of carotenoids, proteins, minerals and vitamins (Mollan, et al., 2008; Karlsen et al., 2015). In general, copepods have high protein content (44%–52%) and an excellent amino acid profile, except for methionine and histidine (Kandathil Radhakrishnan et al., 2019). Copepods cannot be enrich with traditional method, for that the alteration of nutritional composition in copepods has to done through manipulation of the dietary nutrition throughout culture period (Kandathil Radhakrishnan et al., 2019).

G. Bio Enrichment in Cladocerans

Cladocerans are commonly known as ‘water fleas’. Two genera, in particular, *Daphnia* and *Moina*, are widely used as live food, particularly in pond cultures (Qin & Culver, 1996). But in case of aquaculture point of view, *Moina* used to be the most common live food organism for fish larvae. For *moina* enrichment rice bran is used because it contains various nutrients such as protein (12%–13%), lipids (16%–20%), linoleic acid (6.35%–6.85%), α linolenic acid (0.2%–0.27%), vitamin B and minerals (dominated by calcium and iron; 6%–9%) (Faria et al., 2012).

4. Nutritional Composition in Live Feed

Table 2
Fatty acid profile (%) of live feeds according to former studies

Fatty acids	Rotifers ^a		Artemia ^a		Moina ^b	
	UE	E	UE	E	UE	E
C14:0	0.98	1.34	0.47	0.52	4.25	1.79
C16:0	17.00	18.3	10.5	10.6	10.53	20.56
C16:1	1.38	1.90	1.46	1.56	21.67	1.31
C18:1	5.61	5.00	6.57	6.23	9.1	14.49
C18:1n-9	7.92	10.6	18.9	17.7	11.83	8.26
C18:2n-6	22.2	20.7	5.29	7.90	2.35	5.02
C18:3n-3	6.28	5.44	31.4	25.9	20.19	3.39
C20:4n-6	2.38	1.07	0.48	0.99	2.66	4.61
C20:5n-3	3.53	3.36	2.19	4.21	3.04	8.20
C22:6n-3	5.05	11.00	0.39	4.63	1.31	10.36
C22:5	0.28	0.68	0.01	0.28	-	-

N/B: UE-Un-enriched; E- enriched

^aRocha et al., 2017

^bSingh et al., 2019

5. Conclusion

Live feeds are generally considered as a living nutritious capsule in aquaculture industry, even though their nutritional composition does not naturally meet the requirements of the target species. Generally, the nutritional quality of live feed organisms reflects the culture conditions and quality of the feed when cultured. Bio enrichment is very important because when we enrich the live feed with HUFA, PUFA, vitamin and minerals, their nutritional composition will change and helps in improving the growth and survival of fish during early stages of life. Currently, *Artemia* nauplii are considered to be one of the best live feeds in aquaculture industry, but considering the high cost and low availability of *Artemia*, other economically viable live feeds should be developed by isolating and culturing locally available species. Other common live food organisms that have introduced in hatcheries include the rotifer, copepods and freshwater Cladocerans *Daphnia* sp., *Moina* sp., and fairy shrimp which exhibits high reproduction rate, short generation time and can grow and live in high stocking densities.

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