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Microbound diet (MBD) can replace the use of live feeds in larvae culture: A boon for fish larval nutrition

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Abstract

Successful aquaculture mainly depends on the provision of quality seed and feed. Initial stage of larval rearing is very delicate and critical, and due to improper supply of nutritional balanced feed, their survival gets affected. Live feeds are used during initial rearing, but they are sometimes not nutritionally balanced, and they require a separate set-up and expertise for their mass production. Microparticulate diets can be an ideal solution to overcome this constraint; hence, they can be used along with the live feeds for the culture of fish larvae. The different microparticulate diets, such as microbound (MBD), microcoated (MCD), and microencapsulated (MED) diets, can be used to partially or completely substitute the live feeds in fish larval rearing, but among the three, the production of MBD is comparatively easier. The quality MBD can be prepared as a dry type using a suitable binder matrix. The binders used in the MBD are: sodium alginate, carrageenan, zein, gelatin, etc. These binders reduce the leaching of nutrients, especially the water-soluble nutrients from it. MBD can be used along with live feeds in a hatchery's larval rearing of different fish species. Co-feeding of these live feeds and MBD can be the best solution in the larvae culture of fish. The MBD feed can be on-size or crumbled. The on-size MBD can be microextruder marumerization (MEM), and particle assisted rotational agglomeration (PARA). MEM is the most common type of MBD, and this idea has been adopted from the pharmaceutical industry. The cold extruded feed gets spheroid shape in the marumerizer, which helps in forming a smooth layer outside the MBD. Since these feed particles are not encapsulated, there is may be a chance of leaching water-soluble nutrients from the feed. It is most suitable for freshwater and marine fish larvae, but it is not suitable for the column-feeding fish larvae. The advantages and the disadvantages of use of MBD in larval rearing of fish with a view to replace the feeding of live feeds are briefly described in this article.

Keywords:
Microbound diet, larvae, live feeds, binders

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Introduction

Intensification of aquaculture needs a higher demand for fish seed supply; however, lower larval survival at the initial stage due to improper and unbalanced feed supply; nutrition is one of the major drawbacks for many years for the aqua-hatcheries around the globe. Live feeds have been used extensively in larval rearing of fish, but most of them are not nutritionally balanced, and they lack some essential nutrients like EPA (Eicosapentaenoic acid), DHA (Docosahexaenoic acid), vitamin C, taurine, etc. Also, their regular supply and chances of pathogenic infection poses serious issues in quality seed production. To overcome this problem, the use of a microparticulate diet in larvae culture can be an ideal solution. These diets can be used along with live feeds in larval rearing of fish. Although 100% replacement of live feeds may not be possible, co-feeding or mixed feeding can be followed. Live feeds, and microparticulate diets together can serve the nutritional requirement of larvae in the hatchery. Although manufacturing of microparticulate diet is much more complex and time-consuming than that of grow out fish feeds due to its extremely small particle sizes, the relatively undeveloped/underdeveloped digestive system of the larvae essentially required a nutritionally balanced diet with high palatability, water stability, and should have minimum nutrient leaching/loss in the culture environment. The size of larval feeds should be in the range of 2 to 7 μm. Some of the problems associated with the microparticulate diet are- high nutrient leaching, low water stability, low palatability, poor digestibility & retention of water-soluble nutrients. Mainly There is three different categories of microparticulate diets, viz., - microbound (MBD), micro coated (MCD) and microencapsulate (MED) diets.

Microbound diets (MBD)

The manufacturing process for MBD is the simplest and most used method of preparation of microparticulated diet. This is the most common particle type feed used in feeding the fish larvae. Dietary ingredients are bound together in a polymer matrix. These particles do not possess walls and, technically, should not be designated as microcapsules. This diet consists of a binding material such as agar, zein, gelatine, alginate, or carrageenan. Formulation of MBD particles typically requires activating or gelling a mixture of ingredients, followed by drying. The final dry mix is crushed and sieved to obtain the desired particle size. These particles do not possess walls, so there is a possibility of nutrient leaching, and they are also susceptible to direct bacterial attack. An outline of the procedure for MBD diet preparation is given below (Figure 1)

Preparation of an MBD diet bound with carrageenan: Powdered ingredients (10 g)

→ Water (35 ml) → Heat in a water bath at 80°C → Mix well with a high-speed homogenizer

→ K-Carrageenan (0.5 g) → Mix well with the homogenizer → Cool in a refrigerator at 4°C

→ Freeze-dry → Crush and sieve → K-Carrageenan microbinding diet (Kanazawa, 1986).

Benefits of MBD:

This is one of the most preferred artificial diets which are suitable for delivering high- molecular-weight proteins, carbohydrates, and water-insoluble nutrients. The benefits of use of MBDs in larval rearing of fish are-

- Inexpensive to produce
- Easy to produce/Ease of preparation
- It can be produced within a narrow size range
- Binders can be nutritionally inert.
- Possibly more attractive to larvae because of the absence of capsule wall, allowing leaching of nutrients that may promote ingestion
- Possibly more digestible because no capsule wall to break down before digestion of dietary ingredients
- MBD particles allow short to medium-term feed storage (Southgate and Partridge, 1998).

Types of MBD:

Microbound diets can be characterized as crumbled and on-size feeds (or shaped particles) based on the manufacturing processes.

Crumbled feeds:

Crumbled feeds are produced by first making a pellet, flake, or a cake, crumbling, and then sieving to the appropriate size range. All the ingredients are ground, mixed with a binder, activated by temperature or chemicals, dried (drum drying or spray drying), ground, and sieved to the required size. The final product is usually uneven and rough-edged particles. (Kolkovski, 2001; Kolkovski *et al.*, 2009; Langdon and Barrows, 2011). This constitutes waste in crumbled feed production. Moreover, the resulting increase in surface area-to-volume ratios will adversely affect the leaching rates of water-soluble nutrients.

Types of crumbled feeds

1. **Steam-pelleted:** Used effectively for many years in the salmonid and catfish industries, not effective for producing larval feeds.
2. **Extruded crumbles:** To prevent leaching of water-soluble nutrients
3. **Crumbled flake**: Flake feeds are the most common feeds fed to aquarium fish. Effective binders used for production includes agar, gelatin, carrageenan, and alginates. The most common production method is the double-drum drier. This machine consists of two parallel drums rotating in opposite directions. A feed slurry coats the drums, and as the drums rotate, the slurry is spread to a uniform thickness. Steam is used as a heat source for the drum, which quickly dries the feed. The thickness of the flake can be adjusted by altering the distance between the drums. Drying conditions may influence the product's nutritional value; therefore, the feed is exposed to the heat for only a short time (~30 sec). Flake feeds come off the drum drier as sheets, which must be ground, and sometimes sifted to produce appropriately sized thin flakes. This process results in a particle with a high surface-area-to-volume ratio. Because of this high ratio, particles float on the surface for a long time before becoming saturated with water and sinking.
4. **Crumbled cake:** Many binders can be used to produce crumbled cake feeds. Each binding system is activated differently. For example, zein is a protein found in corn and is soluble in alcohol but not water. Zein was used effectively in preparing diets for prawn larvae. Egg albumin can also form a matrix but must be activated by heat. The cake

can be dried by freeze-drying, oven-drying, or drum-drying. Nutritional quality is affected by the type of drying. Crumbled cake feeds are currently produced commercially and can be very effective larval feeds.

On-size feeds/Shaped particles

Shaped particles are prepared in the desired particle sizes, and thus it does not require crumbling of feed to achieve the appropriate size. It eliminates under or oversized particles, therefore reducing the waste production. It also increases particle stability and has a suitable range. In addition to eliminating a production step, it also creates different physical shapes of on-size. There are three types of shaped particles, namely, (a) Microextruded marumerized (MEM) particles, (b) Particle-assisted rotational agglomerated (PARA) particles, and (c) Spray beads. The first two techniques have gained attention in the past few years, with commercially available diets produced using these methods.

Microextruded marumerization (MEM)

Applicable to fish feeds and has been used in the pharmaceutical industry for many years. This is a two-step process of cold extrusion followed by marumerization (spheronization). These cold extruders can produce noodles down to 1000 μm in diameter. Commercial manufacturing systems are currently being used to make specialty, ornamental, and salmonid feed.

Cold extrusion

Extremely finely ground ingredients are required to produce all types of larval feeds, but it is particularly critical with the MEM process to prevent die blockage. It is generally recommended to have all ingredients smaller than 20% of the die opening. The best grinding method may include hammer mills, ball mills, pin mills, and homogenizers, but it depends on the ingredients' moisture, starch, and fat content. For high fat, low moisture ingredients, the air-swept pulveriser has been proven to be very effective on a large scale but costly in terms of energy demands.

Types of extruders capable of producing a small extrudate

1. Axial discharge extruder designs with a flat plate/screen (traditional), the high pressure generated from making such small extrudate results in damage to the die.
2. Radial discharge extruders increase die surface area since the die holes wrap around the screws, resulting in less pressure on the screen.
3. Twin-dome extruders also reduce pressure at the die screen and produce these tiny particles.

Marumerization/Spheronization

Once the wet mash is extruded into noodles, the second step begins. The noodles are placed into a spheronizer (originally termed a marumerizer), consisting of a cylindrical chamber with a high-speed rotating plate at the bottom. Plates are grooved and available with different grooves to break the noodles, reshaping and compacting the particles. The depth of the groove affects the amount of energy that is transferred to the feed during marumerization. A firm noodle will require a deeply grooved plate and a soft noodle will be processed most effectively with a shallowly grooved plate. The spheronizer is equipped with a variable speed motor to deliver a range of energies to the extrudates.

The marumerizer imparts two effects to the feed. (a) The first effect is to reshape the particle into a spheroid shape. Noodles are broken into particles with lengths equivalent to the diameter of the noodle. (b) The second effect is to impart surface densification to the particle.

Many types of binders can be used with MEM particles if they are moisture and pressure activated. No heat is added to the process, but some are generated at the extrusion screen due to friction. Binding systems based on gums have been used, but protein hydrolysates are also effective. Particles produced by MEM can be characterized as smooth and spheroid, with a high density. The smooth shape may decrease nutrient leaching by reducing the surface area-to-volume ratio. The high density of the feed results in a faster-sinking particle, which is a negative property for fish species

Fig: A: Microextruder marumerization

B: MBD produced by marumerization

that feed in the water column.

Particle-Assisted Rotational Agglomeration (PARA):

This is a single-step process capable of producing particles from 50–500 µm that are lower in density than particles produced by the MEM. This method differs from MEM because only a cylindrical shaper is used, and the process does not involve an extruder. Compared to MEM, the lower capital expenditure required and lower operating costs. The ingredients are mixed, and slightly higher moisture levels are required. Feed formulation and moisture content are essential in this method. The wet mixture of ingredients is then added to the spheronizer with a charge of 3.0-mm inert particles (75% weight of beads/weight of wet mesh). The rotation of the spheronizer imparts energy to the inert particles, which in turn transfer energy to the mash, producing roughly spheroid particles in a wide size range. Particles and inert beads are discharged from the spheronizer and sieved through a 2.0-mm screen to remove the beads.

The PARA process produces a wide range of particle sizes and is less uniform in shape but manipulating moisture levels and spheronization time can alter the particle size distribution. The PARA process depends on adequate binding capacity from the diet formulation than the MEM method since high pressure is not used to agglomerate the particles. The same binders used with MEM particles are effective in PARA particles, with minor modifications. The PARA process is a low-pressure agglomeration method that results in a low-density feed particle. The low-density results in a slower sinking rate of the particles than MEM particles, which can be beneficial for slow-feeding larvae of some species, such as halibut.

Spray Beadlets

These are particles produced by spraying a slurry of material into a liquid that assists in forming particles. Binders such as alginate and gelatin can be used

Table 1: Binders used in the preparation of MBD diets for altricial fish larvae (Langdon, 2003)

Binder type	Species	Reference
Alginate	*Dicentrarchus labrax* *Lates calcarifer* *Stizostedion vitreum* *Brachydanio rerio*	Partridge and Southgate, (1999); Guthrie *et al.* (2000) Onal and Langdon (2000)
Agar	*Lates calcarifer*	Partridge and Southgate (1999)
Carboxymethyl cellulose	*Stizostedion vitreum*	Guthrie *et al.* (2000)
Carrageenan	*Plecoglossus altivelis* *Acipenser transmontanus* *Lates calcarifer* *Gadus morhua* *Stizostedion vitreum*	Kanazawa *et al.* (1982) Gawlicka *et al.* (1996) Partridge and Southgate(1999); Baskerville-Bridges & Kling (2000) Guthrie *et a.* (2000)
Fish meal	*Dicentrarchus labrax*	Cahu *et al* (1999)
Gelatin	*Pleuronectes platessa* *Solea solea* *Sparus aurata* *Lates calcarifer*	Kolkovski and Tandle, (2000); Partridge and Southgate, (1999)
Starch	*Gadus morhua*, *Pleuronectes platessa* *Stizostedion vitreum* *Hippoglossus hippoglossus*	Guthrie *et al.* (2000) Hamre *et al.* (2001) Naess *et al.* (2001)
Zein	*Pagrus major* *Plecoglossus altivelis* *Pagrus major* *Paralichthys olivaceus* *Dicentrarchus labrax* *Lates calcarifer* *Stizostedion vitreum*	Kanazawa *et al.* (1982) ; Kanazawa *et al.* (1989); Partridge and Southgate (1999); Guthrie *et al.*(2000)

effectively. This process has a wide range of sizes, so particle sieving is often necessary to obtain appropriate size categories. Shaped microbound particles can also be formed by spraying a mixture of diet and binder into a gelling solution or drying chamber where particles are formed on contact; for example, alginate- and CMC-bound particles can be prepared by spraying a mixture of dietary components and binder into a solution of calcium salt the spray method results in lower-density particles with a slower sinking rate than MEM particles

A disadvantage of spraying dietary mixtures into an aqueous medium is that leaching of water-soluble dietary ingredients will likely occur, changing the dietary composition of the particles. The problem can be addressed by spraying a mixture of binder and diet into heated air to evaporate the solvent and deposit the binder material around the dietary particles

Binders used in MBD:

Binding agents used to make microbound particles include hydrocolloids, such as alginate, carrageenan, carboxymethyl cellulose, starches, chitosan, gelatine, fish proteins, and zein. Some of these binders, such as wheat gluten or pre-gelatinized starches, can have nutritional value for the animals. In contrast, others are inert raw materials used purely as gelling agents or thickeners in the diet (Melcion, 2001).

One binder may not be optimal for all species. Partridge and Southgate (1999) reported that alginate- and zein-bound particles were poorly digested by barramundi (*Lates calcarifer*) larvae, and they recommended gelatine or carrageenan as binders instead. Carrageenan was not considered suitable as a binder for larvae of white sturgeon due to poor digestibility (Gawlicka *et al.*, 1996) and first-feeding walleye (*Stizostedion vitreum*) (Guthrie *et al,*. 2000). Genodepa *et al.* (2007) used 14C-labeled MBD particles to determine the ingestion rate of diet particles prepared with either zein, gelatine, agar, alginate, or carrageen as the binding agent. From a nutritional perspective, for mud crab larvae, a protein binder (e.g., zein or gelatine) may be more appropriate than a polysaccharide binder (e.g., agar, alginate, or carrageen).

Considerations in the development of formulated larval feeds

The following factors are to be considered while the development of a suitable larval diet:

1. Microdiet characteristics
2. Nutrient leaching
3. Particle size
4. Buoyancy/ Sinking rate
5. Larval characteristics
6. Ingestion rate of the diet
7. Larval development
8. Ontogeny of digestive systems and digestive enzymes
9. Digestibility
10. Nutritional requirements
11. Microdiet formulation and nutrition experiments
12. Weaning and co-feeding methods

Microbound diets in advancing larval rearing:

Despite poor retention of water-soluble nutrients by microbound particles, researchers have reported success in weaning larvae with microbound diets after as initial period of feeding on live feeds or a combination of live feeds and microbound diets (Kolkovski *et al.*, 1997; Cahu *et al.*, 1999; Lazo *et al.*, 2000; Hamre *et al.*, 2001). In contrast to marine fish, larvae of several freshwater fish species have been

Table 2. Formulated microbound diets previously tested as live feed replacements for various crustacean species

Species	Survival	Reference
P. monodon	85%	Galgani and Aquacop (1988)
P. monodon	52% Nauplii to Mysis	Paibulkichakul *et al.* (1998)
P. japonicus	90% to PL1	Kanazawa *et al.* (1982)
P. japonicas	80% to PL	Kanazawa *et al.* (1986)
P. japonicus	75% to PL1	Kanazawa (1990b)
P. indicus	62% to M1	Galgani and Aquacop (1988)
P. indicus	100% PL20–PL50	Immanuel *et al.* (2003)
M. rosenbergii	77.3% from Z5 to PL1	Kovalenko *et al.* (2002)
Homarus Gammarus	Non beyond stage III	Kurmaly *et al.* (1990)
Scylla serrata		
(MBD+Artemia)	66% from Z111–ZIV	Holme *et al.* (2006b)
S. serrata	90% from ML to C1	Genodepa *et al.* (2004a)

successfully reared entirely on microbound diets such as larvae of goldfish, *Carassius auratus*; (Szlaminska *et al.*, 1993), pike perch (*Sander lucioperca* (Ostaszewska *et al.*, 2005), and zebrafish *Danio rerio*; (Carvalho *et al.*, 2004). Several hatcheries and laboratories around the world have reported successful use of MBD (Liao *et al.*, 1988; Kanazawa, 1990b); however, as these particles show relatively poor stability in water, there are potential problems relating to water quality and bacterial proliferation, as well as nutrient deficiency resulting from leaching (Amjad *et al.*, 1992).

Conclusion

In practice, high concentrations of water-soluble nutrients added to microbound diets is likely to compensate the rapid leaching rates and also the due care must be taken for the faster consumption of the supplied feed/nutrients by supplementing some suitable attractants to improve the acceptability and palatability of MBD. Larvae may also acquire leached nutrients by drinking the culture medium. Frequent exchanges of culture medium can remove the leached nutrients and minimise the potential for the problem of developing high pathological bacterial concentrations. The economic cost of wasted leached nutrients may be acceptable given the small amounts of feed required for larval production; however, the costs of poor larval performance due to inadequate delivery of essential nutrients may pose a serious problem for commercial fish hatcheries to produce the adequate quantity of quality seeds.

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