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Use of Tracer Techniques for Production of Quality Fish Juveniles

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Abstract

The production of good quality marine fish fry for farming has improved in recent years due to scientific and technical advances. However, the mass production of good quality juveniles of many species is still unavailable due to the limited knowledge on nutritional requirements. The understanding of larval nutritional physiology is essential to adapt the larva on inert microdiets by replacing the live food. The knowledge of digestion, absorption, and nutrient assimilation in marine fish larvae are seriously limited by several factors viz. small size larva (~2–3 mm) at the start of exogenous feeding, mouth gape is also small and requiring small feed-particle sizes (~50–150 µm), which creates difficulties for microdiet production technology.

Keywords: Production; Quality; Requirements

Introduction

The production of good quality marine fish fry for farming has improved in recent years due to scientific and technical advances. However, the mass production of good quality juveniles of many species is still unavailable due to the limited knowledge on nutritional requirements [1]. The understanding of larval nutritional physiology is essential to adapt the larva on inert microdiets by replacing the live food [2]. The knowledge of digestion, absorption, and nutrient assimilation in marine fish larvae are seriously limited by several factors viz. small size larva (~2–3 mm) at the start of exogenous feeding, mouth gape is also small and requiring small feed-particle sizes (~50–150 µm), which creates difficulties for microdiet production technology [3]. The acceptance of inert microdiets by larvae of most commercial marine species is limited resulting in low or variable ingestion rates in feeding experiments. Moreover, the use of live food in nutritional studies with fish larvae creates major limitations because it is difficult to manipulate the nutritional composition of live prey, with the exception of some lipid components [4]. Therefore, knowledge on nutritional requirements of marine fish larvae is still limited and often qualitative rather than quantitative. The marine fish larvas have generally a poorer capacity to digest and absorb the complex form of nutrients [5]. However, small size larva have much higher growth rates [6], typically 10 to 30%/day and up to 100%/day. Due higher growth rates, larva require the high amount of amino acids (AAs), highly unsaturated fatty acids (HUFAs), phospholipids (PLs) and other nutrients,

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although the exact requirements are poorly understand. Moreover, quantification of feed intake and diet digestibility is a major difficulty in larval nutrition studies. To overcome some of these difficulties, tracer studies have been used intensively in recent years [7]. In tracer techniques, the tracer molecule are used to quantify in vivo feed intake, digestion, absorption and utilization of nutrients in fish larvae. Isotopic tracers have one or more of the naturally occurring atoms in the tracee molecule replaced in a specific position(s) by an isotope of an atom with a less common abundance. Both stable (e.g., containing ^{13}C and ^{15}N,) or radioactive tracer molecules (e.g., containing ^{14}C, ^{35}S and ^{3}H) are available.

Methods used in Delivery of Tracers to Larval Fish

Uptake of Tracers from Water

This method most commonly used for the uptake of amino acids (AAs) from incubated water, to measure the rates of oxidation, protein synthesis and turnover in different species of fish larvae and fry [8]. The fish larva can easily absorb dissolved AAs and some other molecules across the gills, digestive tract and skin [9]. A suitable incubation time is necessary to allow tracer uptake.

Microdiet Labeling

Microdiets containing radiolabeled tracers have been employed in a number of larval studies. In this method the tracer molecules are simply mixed with the other diet ingredients, taking care that the tracer is uniformly distributed in the diet. Hadas, et al. [10] used radiolabeled diets to study the effect of dietary PLs on absorption of dietary fatty acids (FAs). Radiolabeled microdiets have also been used to assess how feed intake and lipid absorption are affected by dietary lipid level and composition, [11] or to study the effect of dietary exogenous digestive enzymes on feed intake and diet digestibility [12]. The advantage of using the microdiets in the larval nutritional studies is controlling its nutritional composition and the exact form in which the label will be delivered to the larvae, which is not the case with live diets. There are some problems in this methodology is its normally low efficiency, large amount of label being lost during the microdiet production process and the production of labelled microdiets can be a very expensive process.

Labelling of Live Food

Labelling of live diets has been typically used to quantify larval feed intake and/or to characterize digestion, absorption, metabolism or retention of dietary nutrients such as AAs and FAs. Both radioactive and stable isotopes have been used to label live prey, either rotifers or *Artemia* [11]. The Rotifers and Artemia can be labelled by feeding them on [^{14}C]-labelled algae that are in turn labelled by the inclusion of NaH[^{14}C]O_3 in the algal growth media [13]. Alternatively, *Artemia* metanauplii can also be labelled through the use of liposomes containing a radiolabeled triacylglycerol (TAG) or a free Fatty acid (FFA), which are added to the enrichment media, resulting in a large proportion of the ^{14}C-FA being incorporated into the structural polar lipid fraction of the *Artemia* [11], but this approach used to label the live prey will thus affect differently their composition and determine the type of studies that can be conducted.

Tube Feeding

Tube feeding has been used to obtaining quantitative data on the digestibility, absorption, and metabolic handling of water soluble and particularly of low molecular weight dietary ingredients in fish larvae. Tube feeding allows the experimenter to deliver the studied nutrients directly into the larval gut. There are many studies used protein, peptides and FAAs, but nutrients like FAs and lipid classes have also been investigated. The set-up comprises of a dissecting microscope with a camera and micromanipulator, to which a Nano litre injector is attached. A plastic or handmade polished glass capillary tube that is adapted to the mouth and oesophagus diameter of the larvae is fastened to the Nano litre injector. With appropriate operator training, the total handling time per larva is less than 1 min.

Estimation of Digestion and Absorption Efficiencies

Hot Chase

In hot chase approach the tracer is fed to a larva as a single meal and larvae are allowed to ingest for a period that is shorter than the gut transit time or as a bolus of a radiolabeled nutrient in solution administered directly into the digestive tract by tube feeding. The hot chase approach used to investigate, total digestibility, gut absorption rates and also used to investigate the catabolism and retention (assimilation) of nutrients. In this approach the tracer content analyse by scintillation counting in the larvae and in the water after the digestive process is finished, *i.e.* when the gut is empty of visible contents, and based on this calculate the assimilation [14].

Cold Chase

In cold chase approach the larvae fed with radio or stable isotopes labelled diets until gut fullness (but before start of diet evacuation), after which this hot diet is replaced by

non-labelled (i.e., cold) diet. Larvae are sampled at the end of the labelled diet feeding period and periodic sampling continues until complete evacuation of the labelled diet [15]. The major problems of cold chase approach are, it is technically difficult to remove remnants of the labelled diet still in the water and replace it by unlabelled diet without causing significant stress to the larvae, which will have a full gut at the time. Moreover, catabolism cannot be measured as in the hot chase approach. The most common application of the cold chase approach has been to study FA absorption into larval tissues [11].

Study of Post-Absorptive Nutrient Utilization

Nutrient Catabolism

In nutrient catabolism studies the tracer may be delivered though the live food [16], water [17], microdiets [18] and by tube feeding. In this methods the ^{14}C labelled AAs or FAs utilize to estimate their respective oxidation rates. The metabolically produced $^{14}CO_2$ trapped through aeration and manipulation of pH of the incubation water has been adapted for marine fish larvae. The digestion and absorption of protein and AAs are analyse by $^{14}CO2$-metabolic trap, allowing the simultaneous quantification of the utilization of absorbed AAs as energy substrates.

Retention of Amino Acids

Conceição, et al. [19] proposed a method, combining high resolution 13C-NMR spectroscopy and the use of 13C-labeled live to study the qualitative AA requirements of fish larvae through the simultaneous estimation of the relative bioavailability of individual AAs. This method provides the most reliable measurement of qualitative IAA requirements when compared to the use of the IAA profile of fish carcass [20]. Relative bioavailability is a combined measure of absorption efficiency and rate of catabolism for each AA when compared with the other AAs studied. For instance, threonine had a relative bioavailability of 0.64, meaning that it is retained less efficiently by larvae, when compared to other IAAs. GC-IRMS is much more sensitive than the combination of 13C-NMR spectroscopy and HPLC Estimates of relative bioavailability of individual AAs can be used to correct the IAA profile of the larval protein, so that the ideal dietary IAA profile for a given species can be determined [19,20]. This approach is an alternative to dose response curves to study AA requirements in fish larvae.

Quantification of Feed Intake

The precise quantification of feed intake is intimately associated with the gut transit time, digestion and absorption efficiency towards dietary nutrients which is essential in any nutritional study, therefore controlling how much of the dietary constituents will actually be assimilated [11].

Now days there are several methods used for the feed intake quantification such as direct counting of colour live feed in the gut, by using the inert marker in the feed and using radio-isotops. The first approach is the simplest among others, in this method direct counting of rotifers or Artemia in the gut, preceded by colour labelling through the use of microalgae (inducing an intense green colour) [21], blackdrawing ink [22]or methylene blue [23]. However, this method is highly laborious and inaccurate due to most of the zooplankton organisms are easily broken in the larval digestive tract. In the second approach the inert markers (non-metabolised by the larvae) have been used, such as the measurement of ascorbic acid 2-sulfate accumulating in larvae after feeding Artemia decapsulated cysts [24], yttrium oxide [25] and auto-fluorescence of pigments associated with an alginate-based microparticulate diet [26]. But now days most of studies commonly employ radioisotopes for the labelling of larval diets, given its easiness and high accuracy in quantification by scintillation counting, and that the tracee nutrient can be further traced for digestion/absorption and catabolism determinations. The quantification of feed intake has been performed using either radiolabeled microdiets [11] or live prey [15].

Determination of Protein Synthesis and Turnover

The traditional injection system of tracers for determination of protein synthesis commonly used in larger size fish, but in small size fish such as larvae the “bath immersion” method has been used [27]. In immersion method, know number of larvae are kept in a small volume of water that contain a radioactive AA, most commonly L-[2,6–3H]phenylalanine, after which larval samples are taken at regular time intervals. Collected samples are homogenized in 0.5 M perchloric acid and centrifuged. After centrifugation, separation of FAA is performed from the precipitated protein. Specific radioactivity of protein-bound phenylalanine and free pool phenylalanine can be determined by dividing liquid scintillation counts by total phenylalanine contents [27], after liquid scintillation counting of both in the solubilised protein and in the FAA pools [27,8].

The estimation of rate of protein synthesis is based on the change in the different tracer (e.g., L-[2,6–3H] phenylalanine) and tracee (e.g., L-phenylalanine) pools between two time points. There are some major constraint in studies measuring protein synthesis using the uptake of AAs from water is that larval uptake of

phenylalanine from the incubation water, tends to be relatively slow, and an incubation time of several hours (4–8 h) is normally required. Other methods such as phenylalanine flux method [8] and flooding dose equation method [28] used to measure the protein synthesis and turnover.

Now days the tracer methodologies to implement in larval nutritional studies is currently available, that may be improve our understanding of the nutritional physiology of larvae (mostly marine fish larvae) and their nutritional requirements. The nutrient are labelled with either a radioactive isotope (e.g., 14C, 35S, 3H) or a stable isotope (e.g., 13C, 15N), that has been fed to a larva as a meal or as a bolus administered directly into the digestive tract. The relative digestion/absorption capacity for different AAs, protein and protein hydrolysates, FAs, and lipid classes, as well as their relative utilization for energy production, can be assessed by this methodology.

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