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Stand-alone live food diets, an alternative to culture and enrichment diets for rotifers

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ABSTRACT

In order to verify the hypothesis that a formulated live food diet could completely substitute any enrichment step, an existing rotifer culture diet was transformed to slowly release the enrichment components such as protein, fat, vitamins and minerals in non-suffocating concentrations. Nutrients being present at metabolizing concentrations allowed not only gut filling but also gradually enriched the tissue of the rotifers without consuming excessive oxygen or compromising health nor reproduction of the rotifers.

Similar growth and reproduction figures were registered with the traditional culture diet as with the stand-alone diet in a lapse of 9 weeks corresponding with 15 rotifer cycles and average FCR's of 1.0. The nutritional profile of the rotifers was similar to that of rotifers undergoing a classic enrichment for all major nutrients (protein, HUFA, vitamins) except for some slow enriching nutrients (i.e. taurine, Zn, Se) that could be incorporated successfully thanks to the long term enrichment approach.

Besides the nutritional advantage, benefits were noticed on the practical use of the diet (i.e. direct available enriched live food at any moment); the reduced amount of losses and handling of rotifers and the stability in the process thanks to a higher degree of standardization of the operations. These optimizations lead to less damage of the live food resulting in stronger and better quality rotifers.

1. Introduction

Although formulated starter feeds slowly substitute live feed in commercial marine hatchery applications (Cahu and Zambonino Infante, 2001; Curnow et al., 2006; Engrola et al., 2009; Kaushik et al., 2011), it is generally restricted to larger preys as *Artemia* and copepods. Rotifers, more particularly mass cultured *Brachionus*, is still a popular and important food source contributing to the successful larval fish production among commercial marine species. Shortly after the discovery in the early eighties when it was demonstrated that enriched live food substantially increased larval rearing success, the practice of "boosting" or "enrichment" was readily accepted and applied at the academic and commercial level on *Artemia* and rotifers (Lavens and Sorgeloos, 1996; Léger et al., 1986).

Numerous publications report on this successful bio-encapsulation of mostly single active ingredients to fish larvae (HUFA, vitamins, amino acids and minerals) showing a direct correlation between the bio-accumulating agent and its concentration in the rotifer. Only few reports, however, pay attention to possible disturbance (Negm et al., 2013) in the overall nutrition or energy balance caused by bio-encapsulation (i.e. modified protein/fat balance, protein/amino acid balance, mineral and/or vitamin deficiency,…). Also, the physiological impact of bio-encapsulation which most likely drives stress-induced hormonal functions (Gallardo et al., 1999) and enzyme activity is unknown as well as its impact further in the food chain. Physical consequences of the enrichment, however, are obvious and often reported in hatcheries. Losses in quality due to decreased vitality, damage, mortality, clustering, etc.…are only a few examples of probable overlooked signs of the impact and possible causes of emerging events in the later hatchery phase of the larval fish rearing. On a commercial scale, live food husbandry in all its aspects of culture and enrichment, is indeed often treated in a "Machiavellian way" considering prey numbers the first prerequisite over the general quality of the presented live food to larval fish. Unfortunately, the "quality of the meal" is too often regarded as acceptable whenever the boosted nutrients fill the gut (app. 10–15% of the live food) of the often manhandled "living" pill.

It is clear that from a holistic nutritional consideration, the enrichment practice merits a more indebt investigation and that one should evolve from simply vectoring individual nutrients to complete formulations with preferably, stable compositions of the entire live food capsule (Dhert et al., 2001). It is only in this way that limiting nutrient analyses can be performed in a rational way, gradually tending to improve larval quality and survival.

Also to the benefit of commercial applications, under the impulse of time, financial and ecological constraints, the classic concept of

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boosting/enrichment needs to be improved in general terms of effectiveness. This optimization in production will likely be found in a reduction of labor expenses for live food preparation which is in principal not belonging to the core business of a hatchery.

Analyzing enrichment as a biological process, it could be seen as force-feeding, a form of "gorging" in aquaculture. This practice is counter biological and not encountered in nature where wild filter feeders (ciliates, rotifers, copepods, larval stages of mollusks and shrimp…) are swimmingly suitable for wild fish larvae. Furthermore, one can wonder if the readily availability of clean and nutritious preys in nature does not hide some keys to further success; and enrichment is a step too far in counter-productive actions to produce healthy and microbiologically acceptable live food.

In a similar way as in nature, where zooplankton is accumulating all its nutrients from suspended organic material under the form of phytoplankton and "marine snow" (Keppel, 1993), it should be possible to enhance the dietary composition of rotifers during their mass cultivation by improving the feeding and culture conditions in such a way that a continuous enrichment forms an integral part of the culture process.

In order to verify the hypothesis that a well formulated live food diet could avoid the use of an enrichment step, we analyzed rotifers produced on a stand-alone diet that slowly released the enrichment components in non-suffocating concentrations during rotifer culture and investigated its impact on the nutritional status and general health condition of the rotifers.

2. Materials and methods

2.1. Rotifer rearing

L-type rotifers, *Brachionus plicatilis Cayman* (lorica length: 192 ± 28 μ) were reared in 1000 l cylindro-conical tanks in 3 replicates in 30 ppt seawater. Prior to stocking the seawater was chlorinated for 24 h (15 mg·l^{-1}) and neutralized with sodium thiosulphate (15 mg·l^{-1}). The 3 replicates of each treatment were stocked at a density of 500 rotifers per ml. A fixed feeding regime of 0.4 g·million^{-1} of rotifers was used for the culture diets. The experiment was conducted over several culture cycles to ensure that the diet did not compromise health or reproduction of the rotifers. Diets were obtained from Skretting (France) and consisted of Ori-Culture (protein 54% protein, 14% fat) and Ori-Green (43% protein, 30% fat) for the traditional culture and enrichment diet. Ori-One (56% protein, 18% fat) was used as the stand-alone diet.

The culture diets were fed manually 8 times a day at 3 h interval. Temperature was kept constant at 27 °C using submerged heaters. Harvesting of the rotifers was performed at the beginning of day 4. For the traditional culture/enrichment experiment rotifers were enriched during 3 h in 450 l tanks at a starting density of 3000 individuals·ml^{-1} and a single enrichment dose of 0.17 g·mill^{-1}.

The rotifer populations (L-type) originating from the Ori-One (end of first cycle) and the Ori-Green enrichment were sieved in different size classes to separate the new-born rotifers (neonates) from the adults and were analyzed on HUFA and protein composition.

S-type rotifers were reared in 2000 l tanks following the same procedure as for L-type rotifers. Starting density was 1000 rotifers per ml. The feeding regime was adjusted according to the growth of the rotifers and was 0.5, 0.4, 0.25 and 0.2 g·million^{-1} of rotifers on days 0, 1, 2, and 3 respectively.

2.2. Chemical analyses

Protein content and fatty acids were measured by Kjeldahl and FAME analysis (Lepage and Roy, 1984), respectively.

The determination of amino acids was obtained after the sample material was hydrolyzed with hydrochloric acid for 24 h. This solution was analyzed on a cat-ion exchange column using a step-gradient with after column derivatization with ninhidrin. The amino acid derivative was measured colorimetric at 440 nm and 570 nm (NEN-EN-ISO, 13903, 1985).

The mineral sample was ashed at 500 °C and dissolved in diluted hydrochloric acid. After this the solution was measured by Atomic Absorption Spectrometry.

Vitamins were quantified by HPLC with fluorescence detection.

2.3. Calculations

Food conversion rate (FCR) is expressed as the amount of diet (in g) needed to produce one million of rotifers.

2.4. Rotifer quality and physical parameters

The losses of rotifers due to rafting and sticking were quantified indirectly as losses during harvest (Table 2). Lethal injuries to rotifers incurred during harvest were also taken up as losses during harvest. Non-lethal injuries were documented by microscopy but were not quantified. Water quality measurements, such as temperature, dissolved oxygen and pH were measured on a daily basis.

2.5. Statistical analysis

All data on growth of rotifers were statistically treated using one-way ANOVA. Significant differences among means ($P < 0.05$) were tested by Duncan's multiple range test. Samples of rotifers for nutritional analyses were taken from each of the 3 replicated tanks and pooled for nutritional analyses.

3. Results

The growth performance of the L-type rotifers on the traditional culture diet was not significantly different from the stand-alone diet (Fig. 1). Similar growth and reproduction figures were registered with Ori-Culture as with the stand-alone diet in a lapse of 9 weeks corresponding with 15 rotifer cycles (average FCR 0.9–1.0, egg ratio 25–40%). When the same diet was offered to S-type rotifers, the rotifers grew from 1000 individuals per ml to 4500 rotifers per ml in 4 days (FCR 0.7, egg ratio 20–35%).

The enrichment kinetics for the L-type rotifers on the stand-alone diet showed saturation in lipid enrichment after 4 days of culture corresponding with the first culture cycle (Fig. 2). The n-3 HUFA culminated at 30 mg·g^{-1} DW at the end of the first cycle which was comparable to the enrichment value obtained with a traditional enrichment with Ori-Green. Rotifers harvested before the end of the first cycle reached 75%, 85% and 90% of the plateau value on days 1, 2 and 3 respectively.

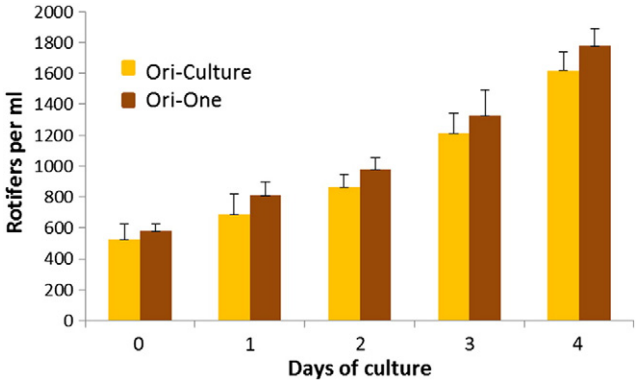

Fig. 1. Growth of L-type rotifers in three replicates fed on Ori-Culture and Ori-One in a 4-day rotifer culture cycle.