COMBINED EFFECT OF IMMUNE-STIMULANT ENRICHMENT PRODUCTS AND FEEDING FREQUENCY ON GREATER AMBERJACK LARVAL PERFORMANCE

M.V. Martín[1], J.A. Pérez[2], S. Jerez[1], E. Chaves-Pozo[3], A. Lorenzo[2], M. Arizcun[3], A. Bolaños[2], C. Rodríguez[2].

[1] Centro Oceanográfico de Canarias, Instituto Español de Oceanografía, Vía Espaldón, Dársena Pesquera PCL 8, 38180 Santa Cruz de Tenerife, Spain. virginia.martin@ ca.ieo.es
[2] Departamento Biología Animal, Edafología y Geología, Universidad de La Laguna, Santa Cruz de Tenerife, Spain
[3] Centro Oceanográfico de Murcia, Instituto Español de Oceanografía, Carretera de la Azohía s/n. 30860, Puerto de Mazarrón, Murcia, Spain

Introduction

A series of limitations constrain the production of greater amberjack (*Seriola dumerili*) juveniles in commercial hatcheries. Among those, the scarce knowledge on larval nutritional requirements and immune response to handling, results in inadequate larval feeding protocols with very low larval survivals. Since the stimulation of the larval immune system is a promising tool to increase survival rates at early stages of fish (Awad et al., 2013), *Echium* and black cumin oils were assayed in the present study using live preys as vectors that were previously enriched with a polar lipid rich emulsion containing a marine natural lecithin, carotenoids and arachidonic acid and supplied under different feeding frequency regimes. Therefore, the overall objective of the present study was to test the combined effect of enrichment products containing immune-stimulants (PUFA-rich lipids, carotenoids and *Echium* oil or black cumin oil, *Nigella sativa*) and the feeding frequency on *S. dumerili* larval performance, immune response, oxidative stress and digestive enzymes.

Materials and Methods

Newly hatched larvae of greater amberjack, at a total density of 8000 larvae per tank (total initial length 3.424±0.098mm), were randomly distributed in 24 experimental tanks of 100 l capacity. Rotifers were added to the larval rearing tanks twice (10:30h and 20:30h) or three times (10:30h, 15:30h and 20:30h) day^{-1}. The rotifer enrichment commercial protocol (S. presso®, Inve Aquaculture, Dendermonde, Belgium) (T1) was compared with three experimental emulsions (T2, T3 and T4) added at 6% (6g.100 l^{-1}) for 3h to the rotifer enrichment tanks. T2 consisted of a marine natural lecithin LC60 (PhosphoTech Laboratories, France) rich in DHA, and 1% supplementation of arachidonic acid (20:4n-6) combined with 10ppm (mg l^{-1}) of Naturose, a commercial product containing 2% of astaxanthin monoester. T3 and T4 consisted of this lipid emulsion combined with 20% *Echium* oil and 20% black cumin oil, respectively.

Larval sampling (7 and 12dph) was carried out randomly from the experimental tanks. Total length and percentage of survival was determined. Pooled samples of whole larvae collected in triplicate from each treatment and feeding frequency at 7 and 12dph were homogenized. The supernatants of larvae homogenates were used for evaluation of immune response (lysozyme, peroxidase, protease, anti-protease and bactericidal activity assays), digestive enzymes (amylase, lipase and alkaline proteases), antioxidant enzyme activities and lipid peroxidation.

Results and Discussion

Mean total length for all dietary treatments and feeding frequency was 4.631±0.409mm at 12dph (Figure 1). Regardless of dietary treatment and feeding frequency, larval total length remained unchanged at 7dph. However, dietary regime significantly affected larval growth at 12dph (P<0.05). T4 larvae were larger at 12dph than T3 fish but not significantly different (P>0.05) than T1 and T2 fish. No significant differences in total length, swim bladder inflation and survival were found between assayed feeding frequencies. Larval survival tended to increase in T2, T3 and T4 treatments.

The T4-larvae displayed lower levels of several activities in the humoral innate immune results. Thus, the T4-larvae presented significantly (P<0.05) lower peroxidase activity than T1-larvae at 7dph and the markedly lowest (P<0.05) activity of all groups at 12dph. The levels of bactericidal activity were also significantly (P<0.05) lower in both 7 and 12dph T4 larvae than in any other group. Protease activity decreased in T3 and T4 larvae compared to T1 fish at 7dph.

Regarding antioxidant enzymatic activities, despite a lack of significant differences due to the high variability of data, a general trend to increase glutathione transferase (GST) activity with age is evident for all dietary treatments. In addition, a trend for a lower superoxide dismutase (SOD) activity is also noticeable in T3 and T4 larvae at 12dph.

The digestive enzyme results clearly showed that alkaline proteases and lipase activities were significantly higher (P<0.05) in larvae receiving rotifers enriched with cumin oil (T4) whereas no marked differences were found for amylase activity among treatments.

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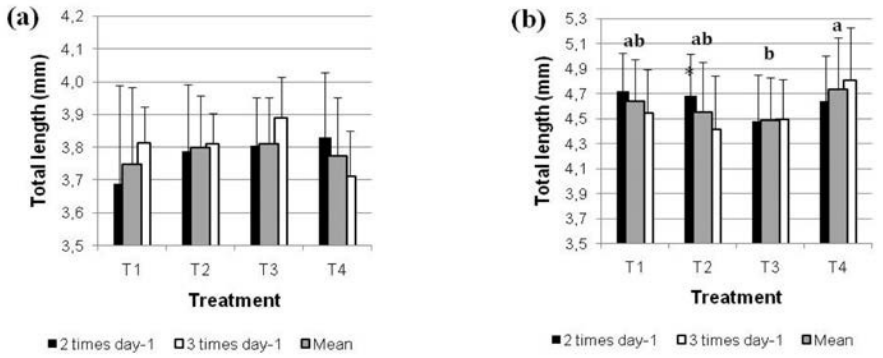

Figure 1. Total length (mm) of 7dph (a) and 12dph (b) greater amberjack larvae fed rotifers from T1 (Commercial enrichment), T2 (LC60/20:4n-6/10ppm carotenoids), T3 (LC60/20:4n-6/10ppm carotenoids+20% Echium oil) and T4 (LC60/20:4n-6/10ppm carotenoids+20% Black cumin oil). Values are mean±SD (n=3). Different letters indicate significant differences between treatments (ANOVA, $P<0.05$).

Conclusions

In summary, the results of the present study suggest the positive effect of experimental live prey enriching emulsions supplemented with immune modulators with particular reference to the combination including black cumin oil compared to commercial emulsions on *Seriola dumerili* larval performance, welfare and health status.

References

Awad, E., Austin, D., Lyndon, A.R., 2013. Effect of black cumin seed oil (*Nigella sativa*) and nettle extract (Quercetin) on enhancement of immunity in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquaculture, 388-391, 193-197.

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