COMBINED EFFECT OF LC-PUFA-RICH LIPIDS AND CAROTENOIDS IN ROTIFERS ENRICHMENT PRODUCTS FOR GREATER AMBERJACK LARVAE

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Introduction

The greater amberjack (*Seriola dumerili*) is a species with high potential for the aquaculture. However, the scarce knowledge on larval nutrition, including lipid and carotenoids requirements, results in inadequate larval feeds that lead to low larval survival and poor juvenile quality. The overall objective of the present study was to determine the combined effect of LC-PUFA-rich lipids and carotenoids in greater amberjack larval enrichment products evaluating their effects on survival, growth, welfare, and lipid composition compared to a commercial enrichment protocol.

Materials and methods

Newly hatched larvae of greater amberjack, at a total density of 5000 larvae per tank (mean total length 3.14± 0.08 mm), were randomly distributed in 12 experimental tanks of 100 l capacity. Rotifers enriched with enrichment commercial protocol (C) were compared with three experimental emulsions (E1; E1,10 and E3,10) added at a 6% concentration for 3h to the rotifer enrichment tanks. E1 was based on a polar lipid rich emulsion containing a marine natural lecithin LC60 (PhosphoTech Laboratories, France) with up to 60 % phospholipids (40% PC + 20% PE) rich in DHA, and a DHA/EPA ratio of 2.5/1. E3 was based on a mixture of different TAG sources (Incromega DHA500 TAG and cod liver oil) although a slight supplementation with soybean lecithin was performed to help emulsification and absorption of lipids. E1,10 and E3,10 consisting of these two lipid emulsions were combined with 10 ppm (mg l^{-1}) of Naturose, a commercial product containing 2% of astaxanthin monoester. All larval sampling at each age (1, 6, 10 and 14 dph) was carried out randomly from the experimental tanks. Total length, percentage of larvae with swim bladder inflated and eye diameter were determined. At the end of the trial (14 dph) percentage of survival was calculated. Whole body levels of glucose, lactate, sodium and potassium were determined in duplicates using standard spectrophotometric assays (Spinreact). Cortisol level was determined by radioimmunoassay using ELISA kits (Arbor Assay, Michigan, USA). Total lipids were extracted (Folch et al. 1957) and fatty acids from total and polar lipids were obtained by acid-catalysed transmethylation according to Christie (1989). The fatty acid methyl esters (FAME) were purified by thin-layer chromatography (TLC) (Christie, 2003), and quantified using a TRACE-GC Ultra gas chromatograph (Thermo Scientific). Carotenoids were obtained according to the method of Barua et al. (1993) and quantified by spectrophotometry.

Results and discussion

Larval growth and survival were significantly affected by dietary treatments. Fish total length (TL) in Control group was significantly lower at 14 dph than larvae from the experimental treatments. Larvae from treatments E3,10 displayed the highest size but not significantly different from treatment E1,10. The lowest survival was recorded in those larvae receiving the treatment E3,10, whereas E1,10 showed the best results in terms of larval survival and eye diameter.

Elevated and significantly higher ($P < 0.05$) whole body cortisol levels were observed in the non-carotenoid-supplemented larvae from treatment E1, at 14 dph, whereas cortisol of larvae from treatment E1,10 was the lowest one. Whole body lactate showed higher values in Control larvae treatment when compared to the other groups, whereas whole

body glucose, Na+ and K+ levels in larvae fed with different treatments were not significantly different.

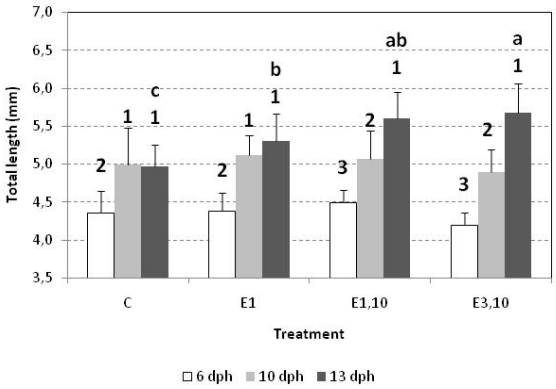

Fig. 1. Total length (mm) of greater amberjack larvae, fed with rotifers enriched with commercial (C) and experimental (E1, E1,10 and E3,10) emulsions at 6, 10 and 14 dph. Values are mean ± SD, n=3. Different numbers indicate significant differences among treatments at each age. Different letters indicate significant differences between treatments at each age.

A good correlation among total carotenoid level in rotifers can be also observed in Fig. 2, with Control and E1 larvae displaying the lowest contents and a higher value being present in both E1,10 and E3,10. The high lipid contents of the commercial emulsion is not only evident in rotifers total lipids but in corresponding larvae. Although a good correlation was found among the lower lipid levels of the experimental larvae and the corresponding values in rotifers, an apparent trend for a higher incorporation of lipids in E1,10 and E3,10 larvae can be observed

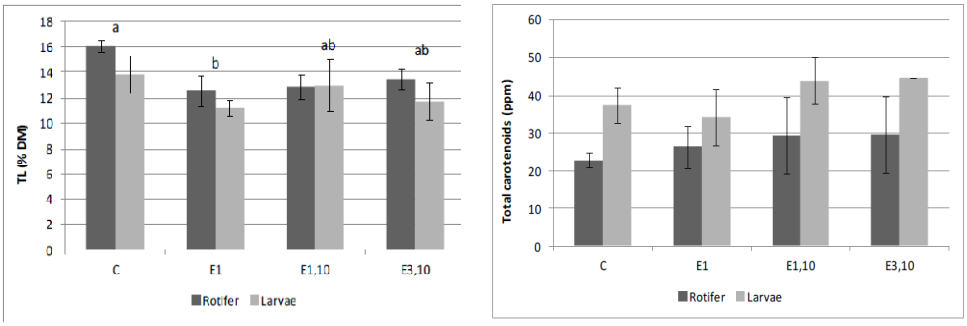

Fig. 2. Lipid content (% DM) and total carotenoids content (ppm) of rotifers and larvae enriched with the control (C) or one of three experimental emulsions (E1; E1,10; E3,10).

Conclusions

List of optimum levels and ratios of EFA and carotenoids in enrichment products for rotifers: DHA 14% TFA, DHA/EPA 2.3 and carotenoids levels 10 ppm.

References

Barua, A.B., Kostic, D., Olson, J.A., 1993. Journal of Chromatography 617B, 257–264.

Christie, W.W., 1982. Lipid Analysis. Oxford: Pergamon Press. 207pp

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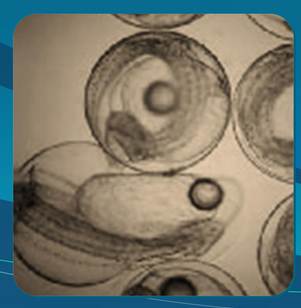


Combined effect of LC-PUFA-rich lipids and carotenoids in rotifers enrichment products for greater amberjack larvae

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Introduction

The scarce knowledge on larval greater amberjack (*Seriola dumerili*) nutrition, including lipid and carotenoids requirements, results in inadequate larval feeds that lead to low larval survival and poor juvenile quality. Most current enrichment protocols for live feed use triacylglycerols (TG) or fatty acid ethyl esters as dietary lipids, whereas oils rich in phospholipids (PL) have occasionally been used (Li et al., 2014) despite dietary PL seem to be a more efficient source of LC-PUFA (22:6n-3, DHA; 20:5n-3, EPA and 20:4n-6, AA) for larvae (Olsen et al., 2014). Carotenoids including astaxanthin, can inhibit LC-PUFA peroxidation (Guerin et al., 2003), and are found to be a determining factor for good egg quality in seriola (Watanabe and Vasallo-Agius, 2003). The present study was conducted to determine the combined effect of LC-PUFA-rich lipids and carotenoids in greater amberjack larval enrichment products evaluating their effects on growth, survival, welfare, and lipid composition compared to a commercial enrichment protocol.

Material & methods

Culture conditions

- 5000 larvae/tank (mean total length 3.14 ± 0.08 mm)
- 12 x 100 L conical tanks
- 4 treatments
- 13 dah larvae

Rotifer enrichment

- **Control group (C)**: commercial protocol
- **Experimental lipid emulsions:**
  - **E1** = based on a marine lecithin (PL-rich)
  - **E1,10** = E1 + 10 ppm Naturose®
  - **E3,10** = based on TG sources +10 ppm Naturose®
- Experimental emulsions supplemented with **AA (1.5 mg L^{-1})**
- **Concentration:** 60 mg L^{-1}
- **Enrichment period:** 3 h
- **Treatments:** Control; E1; E1,10; E3,10

Biochemical analysis

- Total lipid content and fatty acid profile (Christie, 1982)
- Carotenoids according to Barua et al. (1993)

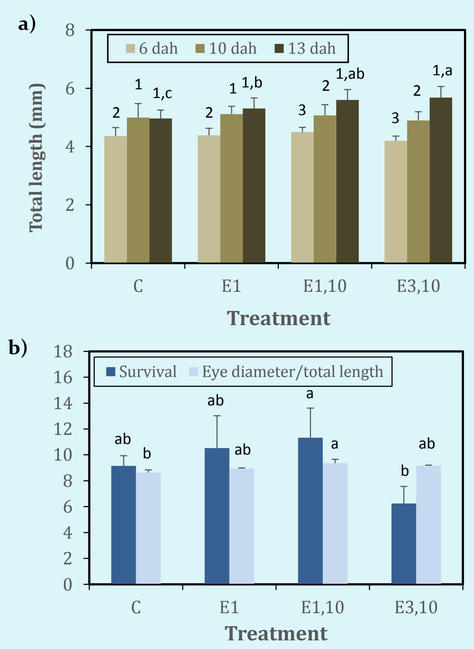
Statistical analysis

- ANOVA ($P < 0.05$)

References: Christie, 1982. Lipid analysis. Pergamon Press, Oxford, pp. 17–23, 51–61; Guerin et al., 2003. Trends in Biotechnology 21(5): 210-216; Li et al., 2014. Aquaculture Nutrition 21: 85-97; Olsen et al., 2014. Aquaculture 428-429: 203-214; Watanabe and Vasallo-Agius, 2003. Aquaculture 227: 35-61

Results

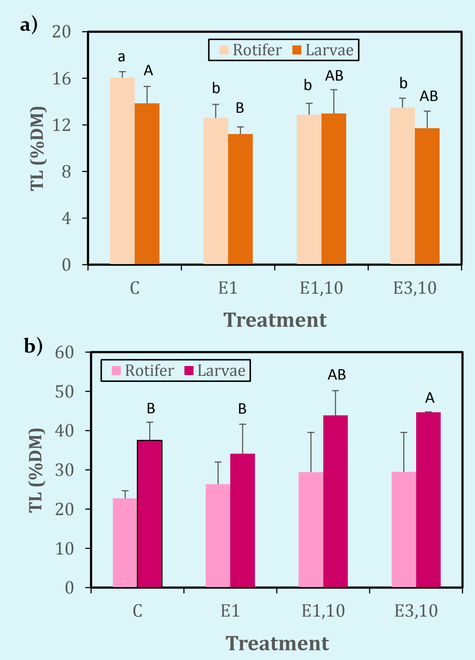
Figure 1. Greater amberjack larval performance. a) growth; b) survival and ratio eye diameter/total length



Different numbers denote significant differences among ages for a particular dietary treatment. Different letters indicate significant differences among dietary treatments for each larval age. C, commercial booster; E1, PL-rich lipid emulsion; E1, 10, E1 + 10 ppm Naturose; E3, 10, blend of oils rich in TG + 10 ppm Naturose.

- **The worst results in terms of larval growth performance were obtained with the commercial treatment.**

Figure 2. Biochemical composition of rotifers and greater amberjack larvae. a) total lipid (% dry matter); b) carotenoids (μg g^{-1})



Significant differences among dietary treatments are shown with lowercase letters for rotifers and uppercase letters for larvae. C, commercial booster; E1, PL-rich lipid emulsion; E1, 10, E1 + 10 ppm Naturose; E3, 10, blend of oils rich in TG + 10 ppm Naturose.

- **Lower values of TPL in control rotifers (treatment C), mainly due to lower PC levels.**

Table 1. Main fatty acid composition of TL and PL from 13 dah larvae fed rotifers enriched with the commercial (C) or one of the experimental lipid emulsions.

	TOTAL LIPID				POLAR LIPID			
	C	E1	E1,10	E3,10	C	E1	E1,10	E3,10
TG (% TL)	21.5±0.9a	15.0±1.1b	15.4±2.1b	10.9±1.4c				
TPL (% TL)	51.9±1.7b	57.4±0.9a	56.0±1.5a	58.0±1.2a				
Fatty acids (%)								
18:1n-9	12.0±1.6	10.4±0.3	12.2±1.7	11.8±0.9	10.4±1.3ab	10.0±0.3b	10.8±0.6ab	11.3±0.3a
18:2n-6	5.5±0.5a	3.0±0.2b	3.4±0.5b	3.7±0.4b	4.3±0.6a	2.8±0.2b	3.2±0.5b	3.3±0.5b
18:3n-3	1.4±0.2	1.1±0.3	1.7±0.9	1.9±0.8	1.3±0.4	1.1±0.4	1.7±0.8	1.8±0.8
20:4n-6	3.0±0.6b	6.9±0.2a	6.4±0.6a	6.3±0.4a	3.2±0.4b	6.9±0.3a	6.5±0.4a	6.1±0.3a
20:5n-3	4.9±0.8b	4.9±0.5b	5.7±1.4b	8.7±0.7a	4.6±0.9b	4.8±0.7b	5.6±1.4b	8.0±0.9a
22:6n-3	26.0±1.9a	24.8±1.3a	22.0±2.5a	17.8±1.4b	27.5±3.1a	25.3±1.3a	23.2±2.0a	18.1±1.1b
∑ n-3 HUFA	34.6±2.7a	31.7±0.8ab	29.7±1.8bc	29.5±0.7c	35.2±2.8a	32.2±0.5a	30.9±0.5b	29.0±0.5c

Conclusions

- List of optimum levels and ratios of EFA and carotenoids in enrichment products for rotifers: DHA 14% TFA, DHA/EPA 2.3 and carotenoids levels 10 ppm.

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