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# Dietary *Euterpe oleracea* Essential Oil, the Amazon Açaí, as Feed Additive to Amazonian Ornamental Fish, during Post-Larvae Growing Stage: A Preliminary Study

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**Abstract:** The present study aimed to evaluate the growth performance, batch uniformity, and survival rate of the Amazonian ornamental fish *Heros severus* and *Pterophyllum scalare* during the post-larvae growing stage, fed with diets containing levels of *Euterpe oleraceae* essential oil (EOO) during 30 days. In the first experiment, 160 *H. severus* post-larvae were distributed in 16 aquariums (1 L), and in the second, 200 *P. scalare* post-larvae were randomly distributed in 20 aquariums (1 L). The experiments were carried out in a completely randomized design, with four treatments for *H. severus* (0.0%, 0.50%, 1.00%, and 2.00% of dietary EOO) and five treatments for *P. scalare* (0.0%, 0.5%, 1.0%, 2.0%, and 4.0% of dietary EOO), both with four replications and dietary EOO being included in replacing soybean oil. A quadratic effect ($p < 0.01$) was observed for the final length, length gain, specific growth rate for length, final weight, weight gain, specific growth rate for weight, and batch uniformity for weight for both fish species. As for ornamental fish larviculture, survival rate and batch uniformity are the most important parameters to be considered. For *P. scalare* and *H. severus* post-larvae, the inclusion of dietary EOO was beneficial in up to 2.48% and 0.88%, respectively.

**Keywords:** Amazonian vegetable oils; larviculture; altricial post-larvae

## 1. Introduction

The use of plant products has gained importance in the aquaculture sector, especially due to the presence of bioactive molecules that improve growth parameters, stimulate anti-pathogen and antimicrobial organic properties as the expression of genes related to antioxidant defense systems and immunological response of fish and crustaceans [1,2]. Thus, using plants rich in bioactive compounds, such as phenolic compounds and carotenoids, can act as a feeding strategy for reducing oxidative stress in aquatic organisms [2–4]. In this context, essential oils (EOs), which are a mixture of hydrophobic liquids from some kinds of plants [5], must be evaluated as a feed additive for fish [6].

The *Euterpe oleracea*, also known as the Amazon açaí, is an endemic fruit of the Amazon region. In recent years, it has been given considerable attention due to its antioxidant, anti-inflammatory, and nutritional properties [7]. The high antioxidant capacity of this fruit is related to the presence of polyphenols and flavonoids, including a great concentration of

anthocyanins in their composition [7,8]. This special lipid is not only responsible for giving the fruit color but also for modulating lipid metabolism, minimizing the damage caused by oxidative stress. Studies involving the administration of dietary *Euterpe oleracea* essential oil (EOO) for juvenile *Oreochromis niloticus* demonstrated an improvement in the organic antioxidant responses and the consequent reduction in lipid peroxidation, as well as free radical formation [9] and the mitigation of ammonia-induced histopathological changes in *Litopenaeus vannamei* [10]. According to the literature, EOO is also important for possessing high contents of palmitic acid (C16:0) and oleic acid (C18:1n9) and for polyunsaturated fatty acids such as linoleic acid (C18:3n3) [11].

Hatchery and larviculture are important phases for commercial aquaculture, responsible for producing fingerlings for stocking and pond culture. However, larviculture can be one of the most critical stages of fish production due to the high mortality rate caused, mainly by the lack of basic knowledge about fish nutrition and food management at this life stage [12]. In this context, post-larvae feeding is one of the critical factors during larviculture [13]. Once the live food supply during the post-larvae production occurs only in the first ten days after opening the mouth [14], it is necessary to administer the inert diet, properly balanced to meet the nutritional requirements of the fish [15–17]. The inert diet can be supplemented with feed additives objecting to the least nutritional stress possible during the first dry feed of fish.

The number of fish farmers dedicated to producing Amazonian ornamental fish species is still very small [18,19]. Hence, studies on nutritional management during larviculture become mandatory for generating good quality ornamental fish, better adapted to captive conditions, and with greater market value [20]. Among the different species of ornamental Amazonian fish, the *Pterophyllum scalare* and *Heros severus* stand out for presenting exuberant coloration and being much appreciated by the aquarium market [4,21–23].

Thus, the objective of the present study was to evaluate the effect of the dietary EOO as a feed additive on growth performance, survival rate, and batch uniformity of the Amazonian ornamental fish *P. scalare* and *H. severus* post-larvae.

## 2. Materials and Methods

### *2.1. Fish and Culture Conditions*

The post-larvae were hatched in the Laboratory of Ornamental Fish, of the Federal University of Pará, in Brazil. Seven days after hatching, the post-larvae of *P. scalare* and *H. severus* were fed with 250 artemia nauplii post-larvae$^{-1}$ day$^{-1}$, three times a day, for 10 days. After this period, the food transition was carried out, according to [14]. From that, two trials with the same experimental design were carried out in parallel for 30 days. A total of 160 *H. severus* post-larvae (14.74 $\pm$ 0.61 mg and 43.00 $\pm$ 3.09 mm) and 200 *P. scalare* post-larvae (13.65 $\pm$ 0.60 mg and 30.69 $\pm$ 4.05 mm) were distributed in 16 and 20 translucent circular aquariums (1 L each), respectively. All aquariums were maintained with constant aeration at a photoperiod adjusted to 12 h Light: 12 h dark, using fluorescent lamps (G-Light®, 60 W, Feira de Santana, BA, Brazil). The aquariums were siphoned once a day to remove uneaten food and feces, then replenished with fresh water.

During the experimental period, water quality parameters such as temperature (26.86 $\pm$ 0.3 °C) and dissolved oxygen (6.98 $\pm$ 0.7 mg L$^{-1}$) were monitored daily, whereas the pH (6.94 $\pm$ 0.3) and total ammonium concentration (0.05 $\pm$ 0.03 mg L$^{-1}$) were monitored every two days. Temperature and dissolved oxygen were measured with a thermometer and a digital oximeter (Lutron DO -5510, São Paulo, SP, Brazil), and total ammonia and pH were measured with a multi-parameter device (HI 3512, Hanna Instruments, Barueri, SP, Brazil).

### *2.2. Experimental Design and Diets*

A completely randomized design with four treatments was used for *H. severus* (0.0%, 0.50%, 1.00%, and 2.00% of dietary EOO), and five treatments were used for *P. scalare* (0.0%, 0.5%, 1.0%, 2.0%, and 4.0% of dietary EOO), both treatments with four replicates

each. The oil extract was obtained from the Aromatech® industry and made from the fruit at the commercial maturity stage. Right after the obtention of the *E. oleraceae* pulp, a defatted processes was performed. The solvent removal was accomplished, and the resultant *E. oleraceae* oil was essentially free of water and solvents. The fatty acid profile was analyzed by reversed-phase high-performance liquid chromatography (HPLC) with a Waters 2695 Alliance system (Waters Corp., Milford, MA, USA) over 60 min (Pacheco-Palencia et al., 2007). The *E. oleraceae* oil extract fatty acid composition was lauric acid (C12:0; 2.9%), miristic acid (C14:0; 4.6%), palmitic acid (C16:0; 16.1%), estearic acid (C18:0; 3.2%), oleic acid (C18:1; 51.3%), linoleic acid (C18:2; 14.6%), araquidico acid (C20:0; 2.3%), and gondoic acid (20:1; 1.5%).

A basal diet was formulated to contain 42% crude protein and 4200 kcal $kg^{-1}$ gross energy (Table 1). The diets were then moistened with 25% water at 50 °C, manually mixed and pelleted in an electric meat grinder (G. PANIZ, MCR-22, São Paulo, SP, Brazil), dried in a forced air oven (QUIMIS, São Paulo, SP, Brazil) at 35 °C for 24 h, and stored in a refrigerator, at 4 °C. Before the feeding trial, all diets were crushed in a mill and manually sieved so that the granulometry of the pellets could reach 8 mm, a size that adequately fit into the post-larvae mouth. Both post-larvae fish species were fed to apparent satiation, a moment at which animals showed no interest in the diet, four times daily, at 08:00, 11:00, 14:00, and 17:00 h, for a period of 30 days for each assay.

**Table 1.** Values of the percentage and chemical composition of the experimental diet.

| Ingredients | *Euterpe oleracea* Essential Oil Levels (%) | | | | |
|---|---|---|---|---|---|
| | **0.00** | **0.50** | **1.00** | **2.00** | **4.00** |
| Soybean Meal | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 |
| Fishmeal | 56.00 | 56.00 | 56.00 | 56.00 | 56.00 |
| Corn meal | 9.00 | 9.00 | 9.00 | 9.00 | 9.00 |
| Wheat meal | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 |
| Soybean oil | 5.60 | 5.10 | 4.60 | 3.60 | 1.60 |
| Acai oil | 0.00 | 0.50 | 1.00 | 2.00 | 4.00 |
| Dicalcium phosphate | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 |
| Premix vitamin/min [1] | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| DL methionine | 0.38 | 0.38 | 0.38 | 0.38 | 0.38 |
| BHT [2] | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Chemical Composition [3] | | | | | |
| Crude Protein (%) | 41.93 | 41.57 | 42.04 | 41.88 | 42.10 |
| Total Lipids (%) | 10.96 | 10.99 | 10.89 | 11.02 | 10.90 |
| Crude Fiber (%) | 1.56 | 1.61 | 1.54 | 1.58 | 1.52 |
| Crude Energy (kcal.$kg^{-1}$) | 4224.87 | 4198.23 | 4230.35 | 4212.91 | 4197.78 |

[1] Warranty levels per kilogram of product: Vit. A, 1,200,000 IU; Vit. D3, 200,000 IU; Vit. E, 12,000 mg; Vit. K3, 2400 mg; Vit. B1, 4800 mg; Vit. B2, 4800 mg; Vit. B6, 4000 mg; Vit. B12, 4800 mg; Ac. Folic, 1200 mg; Pantothenate Ca, 12,000 mg; Vit. C, 48,000 mg; Biotin, 48 mg; Choline, 65,000 mg; Niacin, 24,000 mg; Iron, 10,000 mg; Copper, 6.000 mg; Manganese, 4000 mg; Zinc, 6000 mg; Iodine, 20 mg; Cobalt, 2 mg; Selenium, 20 mg. [2] Buty hydroxy toluene (antioxidant). [3] The values of the chemical composition were performed in accordance with AOAC 2006.

### *2.3. Growth Performance, Batch Uniformity, and Survival Ration*

At the end of the feeding trial, all fish were counted, weighed on a precision scale (AG200 Gehaka® 0.0001 g, São Paulo, SP, Brazil). Growth performance was evaluated for the final weight, weight gain (final weight–initial weight), final standard length, standard length gain (final length–initial length), specific growth rate for standard length ($SGR_{(L)}$), and weight ($SGR_{(W)}$) being $SGR_{(L)}$ = ((ln final standard length–ln initial length)/number of experiment days) × 100 and ($SGR_{(W)}$) = ((ln final weight–ln initial weight)/number of experiment days) × 100 (Kestemont and Stalmans, 1992), batch uniformity for standard length (LU) and weight (WU) being LU = (number of fish with standard length varying ±20% from the average in each experimental unit/total number of fish per experimental unit) × 100 and WU = (number of fish with weight varying ±20% from the average in each

experimental unit/total number of fish per experimental unit) × 100, and survival rate (SR) SR = (final post-larvae number/initial post-larvae number) × 100.

### *2.4. Statistical Analysis*

For the verification of normality and homogeneity of variances, data were submitted to Lilliefors and Bartlett's tests, respectively. After assumptions were satisfied, a one-way analysis of variance (ANOVA) at 5% significance was performed. When differences were observed, a regression analysis between dietary EOO level and growth parameters was used. The values of each experimental unit were used to adjust the equations. The significance of the regression coefficients, the magnitude of the coefficients of determination, and the biological response of each variable were considered to choose the most suitable regression model. Statistical analyses were performed using SPSS (SPSS Inc., Chicago, IL, USA).

## 3. Results

The mean values of growth performance, batch uniformity, and survival ratio of *P. scalare* and *H. severus* fed diets with increasing values of dietary EOO replacing soybean oil are shown in Tables 2 and 3, respectively. There was no effect of the dietary EOO on the parameters of batch uniformity for length and survival rate for both fish species.

**Table 2.** Growth performance (mean ± standard deviation) of *P. scalare* post-larvae fed diets containing levels of *Euterpe oleracea* essential oil.

| Growth Performance | *Euterpe oleracea* Essential Oil Levels (%) | | | | | |
|---|---|---|---|---|---|---|
| | **0.00** | **0.50** | **1.00** | **2.00** | **4.00** | ***p*-Value** |
| FL (mm) | 12.85± 0.21 | 13.05± 0.05 | 13.47± 0.14 | 13.37 ± 0.14 | 12.81 ± 0.23 | 0.0001 |
| LG (mm) | 1.20± 0.21 | 1.40 ± 0.01 | 1.82± 0.14 | 1.72± 0.14 | 1.16 ± 0.23 | 0.0001 |
| $SGR_L$ (%) | 0.33 ± 0.06 | 0.38 ± 0.01 | 0.48± 0.03 | 0.4 ± 0.03 | 0.32 ± 0.06 | 0.0001 |
| FW (mg) | 65.24 ± 5.08 | 72.38 ± 1.97 | 72.05± 4.19 | 78.17 ± 4.11 | 63.26 ± 1.87 | 0.0001 |
| WG (mg) | 35.55 ± 5.08 | 42.69± 1.97 | 42.36± 4.19 | 48. 48± 4.11 | 33.57 ± 1.87 | 0.0001 |
| $SGR_W$ (%) | 2.61 ± 0.25 | 2.97± 0.09 | 2.95± 0.19 | 3.22± 0.18 | 2.52 ± 0.10 | 0.0001 |
| $BU_L$ (%) | 96. 15 ± 5.77 | 96.67± 5.00 | 96.15± 5.77 | 96.25± 3.75 | 97.50 ± 3.75 | 0.1384 |
| $BU_W$ (%) | 57.54 ± 10.58 | 72.99± 10.56 | 82.20± 8.65 | 88.89± 7.41 | 80.13 ± 1.13 | 0.0021 |
| SR (%) | 100.00 ± 0.00 | 100.00 ± 0.00 | 100.00 ± 0.00 | 90.00 ± 10.00 | 95.00 ± 5.00 | 0.1768 |

*p* value determined by analysis of variance (ANOVA). FL: final length; LG: length gain; $SGR_L$: specific growth rate for length; FW: final weight; WG: weight gain; $SGR_W$: specific growth rate for weight; $BU_L$: batch uniformity for length; $BU_W$: batch uniformity for weight; SR: survival rate.

**Table 3.** Growth performance (mean ± standard deviation) of *H. severus* post-larvae fed diets containing levels of *Euterpe oleracea* essential oil.

| Growth Performance | *Euterpe oleracea* Essential Oil Levels (%) | | | | |
|---|---|---|---|---|---|
| | **0.00** | **0.50** | **1.00** | **2.00** | ***p*-Value** |
| FL (mm) | 20.29 ± 0.13 | 20.81 ± 0.15 | 20.33 ± 0.22 | 19.59 ± 0.26 | 0.0001 |
| LG (mm) | 5.55 ± 0.13 | 6.07 ± 0.15 | 5.59± 0.22 | 4.85 ± 0.26 | 0.0001 |
| $SGR_L$ (%) | 1.06 ± 0.02 | 1.15 ± 0.02 | 1.07 ±0.04 | 0.95 ± 0.04 | 0.0001 |
| FW (mg) | 146.36 ± 4.89 | 157.20 ± 3.42 | 144.48 ± 2.20 | 133.50 ± 3.89 | 0.0001 |
| WG (mg) | 103.36 ± 4.89 | 114.20 ± 3.42 | 101.48 ±2.20 | 90.50 ± 3.89 | 0.0001 |
| $SGR_W$ (%) | 4.08 ± 0.11 | 4.32 ± 0.07 | 4.04 ±0.05 | 3.77 ± 0.10 | 0.0001 |
| $BU_L$ (%) | 95.00 ± 5.00 | 100.00 ± 0.00 | 62.50 ± 13.75 | 95.00 ± 5.00 | 0.4262 |
| $BU_W$ (%) | 40.00 ± 6.67 | 70.00 ± 10.00 | 62.50 ± 13.75 | 23.81 ± 9.52 | 0.0027 |
| SR (%) | 100.00 ± 0.00 | 100.00 ± 0.00 | 100.00 ± 0.00 | 90.00 ± 10.00 | 0.1778 |

*p* value determined by analysis of variance (ANOVA). FL: final length; LG: length gain; $SGR_L$: specific growth rate for length; FW: final weight; WG: weight gain; $SGR_W$: specific growth rate for weight; $BU_L$: batch uniformity for length; $BU_W$: batch uniformity for weight; SR: survival rate.

A quadratic effect ($p < 0.01$) was observed in the parameters of final length, length gain, specific growth rate for length, final weight, weight gain, specific growth rate for weight, and on the batch uniformity for weight by the *P. scalare* post-larvae with the increasing levels of dietary EOO. The best level of replacement soybean oil by dietary AEO was 1.95% for the final length ($y = -0.1566x^2 + 0.6107x + 12.8590$; $R^2 = 0.70$), length gain ($y = -0.1568x^2 + 0.6116x + 1.2084$; $R^2 = 0.70$), and specific growth rate for length ($y = -0.0399x^2 + 0.1554x + 0.3289$; $R^2 = 0.70$). As for the final weight and weight gain, the best estimated level was 1.92% of dietary EOO, according to the equations $y = -3.1704x^2 + 12.1750x + 65.4300$ ($R^2 = 0.65$) and $y = -3.1704x^2 + 12.1750x + 35.7400$ ($R^2 = 0.65$), respectively. Moreover, for the specific growth rate for weight, the best-estimated value of dietary EOO was 1.91% ($y = -0.1505x^2 + 0.5757x + 2.6304$; $R^2 = 0.66$). The best-estimated value for the batch uniformity for weight was 2.48% of dietary EOO ($y = -5.3502x^2 + 26.5060x + 59.3280$; $R^2 = 0.64$) (Figure 1).

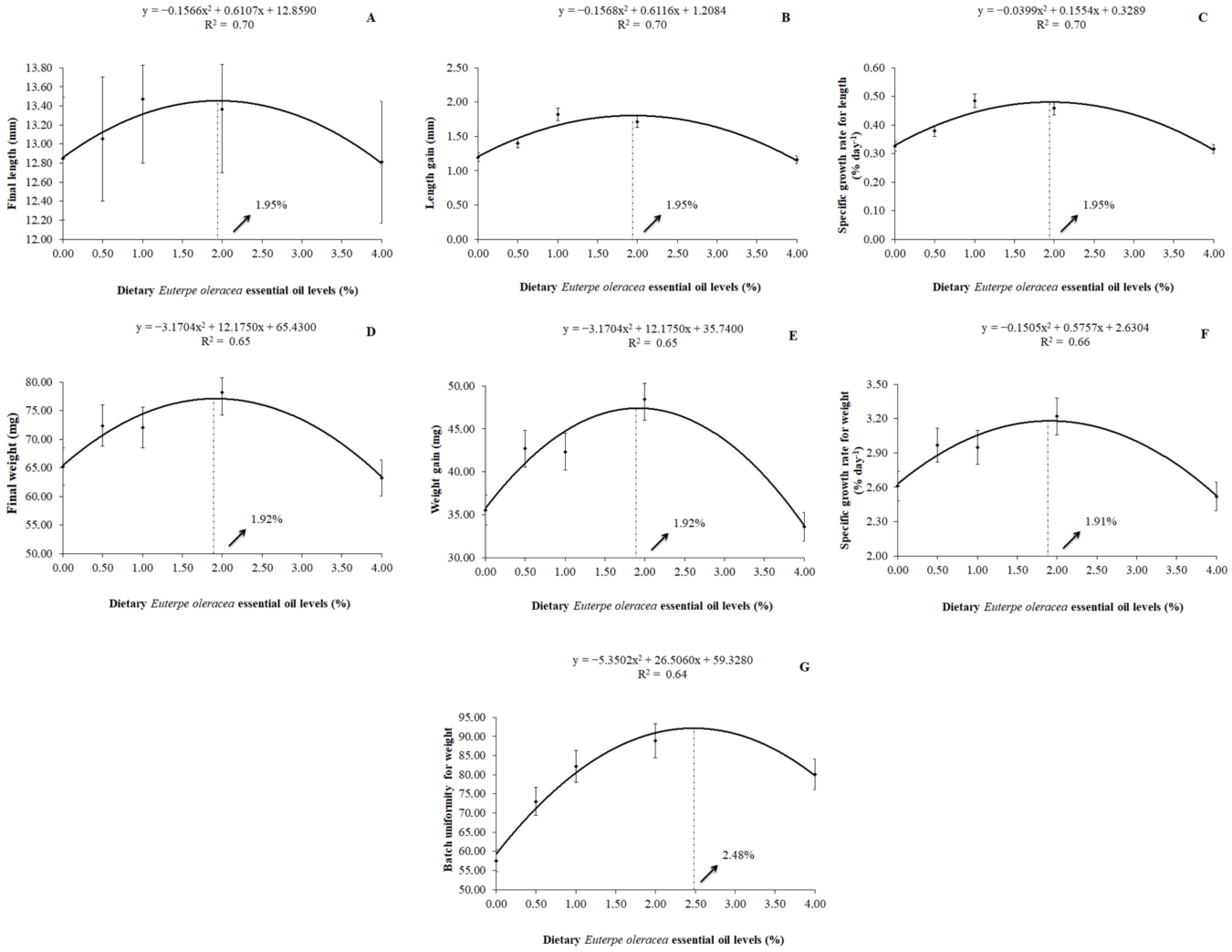



**Figure 1.** Final length (**A**), length gain (**B**), specific growth rate for length (**C**), final weight (**D**), weight gain (**E**), specific growth rate for weight (**F**), and batch uniformity for weight (**G**) of *P. scalare* post-larvae fed diets containing levels of dietary *Euterpe oleracea* essential oil.

It was also observed that a quadratic effect ($p < 0.01$) of dietary EOO on the final length, length gain, specific growth rate for length, final weight, weight gain, specific growth rate for weight, and on the batch uniformity for weight by the *H. severus* post-larvae fed with levels of dietary EOO. The best replacement level of soybean oil by dietary EOO was 0.62% for the final length ($y = -0.5337x^2 + 0.6581x + 20.3760$; $R^2 = 0.74$) and length

gain ($y = -0.5337x^2 + 0.6581x + 5.6360$; $R^2 = 0.74$). For the specific growth rate for length, the best-estimated value was 0.53% of dietary EOO ($y = -0.0882x^2 + 0.1083x + 1.0790$; $R^2 = 0.74$). For the final weight and weight gain, the best-estimated value was 0.53% of dietary EOO, according to the equations $y = -8.4137x^2 + 8.8486x + 148.6800$ ($R^2 = 0.64$) and $y = -8.4137x^2 + 8.8486x + 105.6800$ ($R^2 = 0.65$), respectively. As for the specific growth rate for weight, the best-estimated value was 0.52% of dietary EOO ($y = -0.1961x^2 + 0.2052x + 4.1315$; $R^2 = 0.67$) and, for the batch uniformity for weight, the better-estimated value was 0.88% of dietary EOO ($y = -36.2240x^2 + 63.8500x + 40.1270$; $R^2 = 0.74$) (Figure 2).

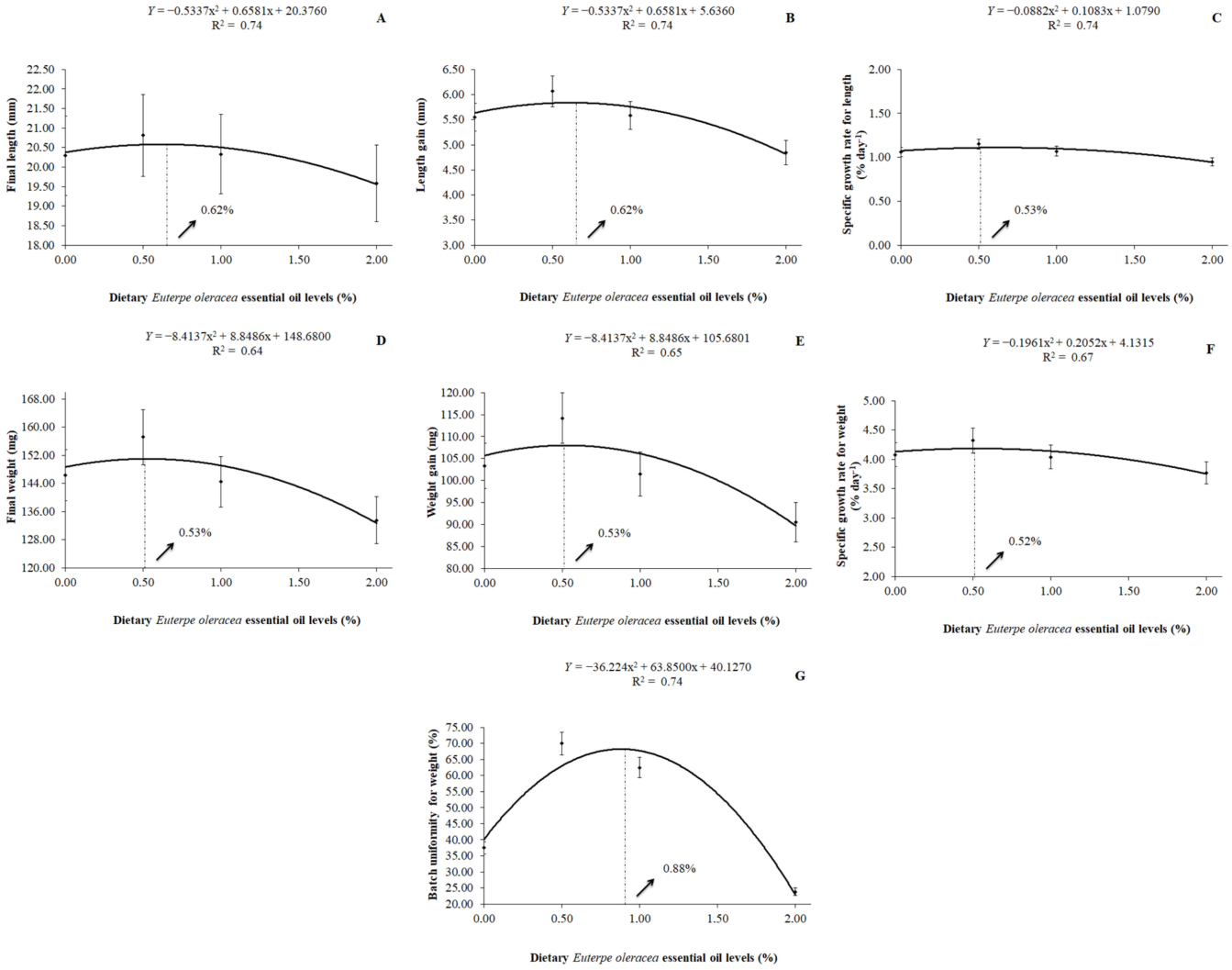



**Figure 2.** Final length (**A**), length gain (**B**), specific growth rate for length (**C**), final weight (**D**), weight gain (**E**), specific growth rate for weight (**F**), and batch uniformity for weight (**G**) of *H. severus* post-larvae fed diets containing levels of dietary *Euterpe oleracea* essential oil.

## 4. Discussion

For the ornamental fish industry, the survival rate and the uniformity of the batch throughout the larviculture phase are extremely important parameters. As ornamental fish are sold individually, small losses during cultivation can considerably reduce the sector's profitability due to the high market value of the ornamental fish unit. Furthermore, an irregular batch can cause production scheduling problems, increase competition between individuals, and generates a stressor factor that can inhibit food consumption [24]. Thus, according to our results, the dietary EOO as a feed additive was beneficial in up to 2.48% and 0.88% for both species studied, *P. scalare* and *H. severus* post-larvae, respectively, as

both values resulted in a greater batch uniformity for weight. However, it is important to highlight that although an irregular batch for weight was observed, the post-larvae batch uniformity for length and survival rate was not influenced by the dietary EOO.

Fish post-larvae present high metabolic rates with a gradual increase production of reactive oxygen species [25]. Additionally, both species present an altricial post-larvae type which corresponds to digestive and immune systems still in development and, therefore, lacking digestive enzymes and antioxidant molecules. In this context, it is possible that the growth performance results observed in the present study are a response to the antioxidant characteristic of dietary EOO well determined by [26] on the typical oxidative stress of post-larvae metabolism, as observed by [27]. Previous works have reported a positive role of dietary EOO in modulating the production of reactive oxygen species and in the expression of antioxidant genes in the liver of rats [28]. Furthermore, according to [29], the antioxidant activity of dietary EOO can effectively protect *Caenorhabditis elegans* against oxidative stress and reduce the levels of endogenous reactive oxygen species. The authors also found that EOO performed its antioxidant activity not only by scavenging the radicals but also by modulating the expression of stress-response genes. Similar results were also observed by [9] in *O. niloticus* juveniles with fed diets containing different levels of inclusion of dietary EOO in place of soybean oil, which concluded that the addition of dietary EOO stimulated the antioxidant responses of the fish. However, as opposed to our observations, no effect of dietary EOO on the growth performance of *O. niloticus* was observed. Nevertheless, according to these authors, the exact mechanism for the absence of effects remains unknown but appears linked to a dose-dependent effect.

The fish growth performance improvement observed in the present study could also be in response to the high content of palmitic acid (C16:0) in EOO composition [8,11]. According to [30], in mammals, of the 20–30 g daily intake of palmitic acid, 20–30% is beta-oxidized [31], and about 60–70% is incorporated into phospholipids [32], both enzymatic pathways highly activated in post-larvae metabolism. However, it is important to highlight that these observations were made only in juvenile fish, leaving doubt about the dietary palmitic acid effect of fish with different physiological conditions and higher metabolic rates.

Another important result observed was the difference between the effect of the dietary EOO on the growth performance of each fish species. While *H. severus* post-larvae required an average of 0.52% in dietary EOO to achieve its highest growth rate, *P. scalare* post-larvae required more than triple to achieve the same result. Although *H. severus* nutrient requirement is similar to species of the same family (Cichlidae) as *P. scalare* and *O. niloticus* [33–36], it is possible that *H. severus* post-larvae are more responsive to dietary EOO than *P. scalare*, which suggest specific studies on the lipid source requirement for both fish species. As for ornamental fish larviculture, survival rate and batch uniformity are the most important parameters to be evaluated. According to the present study, for *P. scalare* post-larvae and *H. severus* post-larvae, the inclusion of dietary EOO in replacing soybean oil was beneficial in up to 2.48% and 0.88%, respectively.

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