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ARTICLE

Enriched Artemia Nauplii with Commercial Probiotic in the Larviculture of Angelfish *Pterophyllum scalare* Lichtenstein (1823)

Natalino da Costa Sousa[1*] José Araújo da Silva[1] Emilly Monteiro Lopes[1] Arthur Felipe Lima dos Santos[1] Francisco Alex Lima Barros[1] Carlos Alberto Martins Cordeiro[1] Peterson Emmanuel Guimarães Paixão[2] Estela dos Santos Medeiros[3] João Carlos Nunes de Souza[3] Márcia Valéria Silva do Couto[2]

1. Federal University of Pará (UFPA), Campus Bragança, Alameda Leandro Ribeiro, Bragança, 68600-000, PA, Brazil
2. Tiradentes University (UNIT) - Av. Murilo Dantas, 300 - Farolândia, CEP 49032-490, Aracaju, Sergipe, Brazil
3. Faculdade Pio Décimo Av. Pres. Tancredo Neves, 5655 - Jabotiana, 49095-000, Aracaju – SE. Brazil

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ABSTRACT

This study evaluated the effect of enriched artemia nauplii with commercial probiotic for angelfish larvae determining growth perfomance, survival and modulation of the intestinal microbiota. Therefore, it experiment occurred in completely randomized design with five treatments (T1- 0.0, T2- 1.5, T3- 3.0, T4- 4.5 and T5- 6.0g of commercial probiotic) and four replaces during 20 days. After larvaculture, post larvae passed by biometric procedures to determine productive performance and then microbiological analysis. Occurred reduction of total heterotrophic bacteria while increased lactic acid bacteria in the intestinal tract from the post larvae for treatments T3, T4 and T5. The commercial probiotic also increased the survival and performance as final weight, weight gain and specific growth rate. For these reasons, the use of 3g of commercial probiotic promotes greater performance and intestinal modulation for angelfish larvae.

1. Introduction

Ornamental fish trade around the world has becomes a profitable activity moving approximately US 15 million [1,2]. Currently, it market look for several fish species with highest quality, different shape and colors [3,4]. Among the native fish species from Amazon, the angelfish *Pterophyllum scalare* has economic potential into national and international market due to this varied patterns and colors required by the ornamental market [4,5,6].

In freshwater ornamental fish, the larvaculture remains as the most problematic phase of production with highest mortality rate caused by management, inadequate nutrition and stocking density [4,7,8]. These factors can reduces its productive performance and health of the larvae [4,5,9].

The larval phase, live feed have an important paper to supply the nutritional requirement and its development [8]. Currently, the ornamental fish sector use widely artemia nauplii due to the size, protein and lipid profile as well as enzymes that aid its digestion process [10,11]. In the last de-

**Corresponding Author:*
Natalino da Costa Sousa,
Federal University of Pará (UFPA), Campus Bragança, Alameda Leandro Ribeiro, Bragança, 68600-000, PA, Brazil;
Email: natal.engpesca@gmail.com

cade, fish farmers has applied different strategies for larvaculture to improve the performance and health [8,12,13,14]. Among the new strategies, enriched artemia nauplii with probiotic showed nice results about the productive performance, survival, improvement of immunological system and intestinal modulation [9,15,16,17,18,19].

In front this, enriched diets for ornamental fish larvae becomes an efficient strategy in captivity rearing. Nonetheless, still missing scientific data about the use of probiotic for larval diet. Thus, this study evaluated the effect of enriched artemia nauplii with commercial probiotic for angelfish larvae *Pterophyllum scalare* determining performance, survival and intestinal modulation.

2. Material and Method

2.1 Experimental Design

This study used larvae of angelfish *Pterophyllum scalare* (1.10±0.01 mg and 4.70±0.25 mm) from natural reproduction, placed 10 fish per polyethylene tanks (total capacity for one liter). Therefore, it experiment occurred in completely randomized design with five treatments (C-0.0, T1- 1.5, T2- 3.0, T3- 4.5 and T4- 6.0 g of commercial probiotic) and four replaces during 20 days.

The feeding management have four daily frequencies (08, 11, 14 and 17 hours) providing one hundred artemia nauplii per larvae [5]. After the last daily feeding, it was carried out water exchange (30%). The water quality parameters were: temperature (28.6±0.35 ºC), dissolved oxygen (5.89±0.11 $mg.L^{-1}$) pH (6.56±0.42) total ammonia (0.18±0.04 $mg.L^{-1}$) and electric conductivity (168±28.32 $\mu S.cm^{-1}$).

2.2 Biological Culture and Enrichment Process

To obtain the artemia nauplii, it used cyst 1g into the tank (1 liter) with constant aeration during 24 hours [9]. After hatching time, artemia nauplii placed in becker (50mL) received commercial probiotic *Lactobacillus acidophilus* (1.1 x 10^8 $CFU.g^{-1}$ and *Enterococcus faecium* 7.7x 10^7 $CFU.g^{-1}$ during 40 minutes before feeding management [20].

2.3 Intestinal Microbiota

For determine bacterial amount in the intestine from post larvae, its intestinal tract (pools of five larvae) macerated with sterile saline solution 0.65% passed by serial dilution (1:10 factor). An aliquot (100 µL) from each of three dilutions (10^{-1}, 10^{-3} and 10^{-5}) was used to inoculate petri dishes containing either Triptone Soy Agar (TSA - incubated at 30 ºC for 24 hours) to obtain counts of total heterotrophic bacteria, or MRS Agar (incubated at 30 ºC for 48 hours) to obtain counts of lactic acid bacteria [19].

2.4 Growth Perfomance

At the end of experiment, all post larvae passed per biometric procedure to determine weight and length evaluating: Total length (TL), final weight (FW), weight gain (WG), specific growth rate (SGR), survival (S), uniformity (U) [21] and relative condition factor (Kr) [22].

2.5 Statistical Analysis

Data was tested for normality (Shapiro-Wilk) and homoscedasticity (Levene's). Subsequent analysis of variance tests were performed applying post hoc Tukey tests for separation of means ($P<0.05$) [23]. Microbiological counts showed non-parametric characteristics and were therefore log transformed [logX10 (x+1)] and arc sen square root (x).

3. Result

At the end of experiment, occurred reduction ($p<0,05$) of total heterotrophic bacteria (T2= 3.41±0.061; T3= 3.43±0.078 and T4= 3.36±0.04 Log $CFU.g^{-1}$), while increased ($p<0,05$) lactic acid bacteria in the intestinal tract from the post larvae for treatments T2 (3.81±0.06 Log $CFU.g^{-1}$), T3 (3.95±0.08 Log $CFU.g^{-1}$) and T4 (3.95±0.12 Log $CFU.g^{-1}$), compared to control treatments (Figure 1).

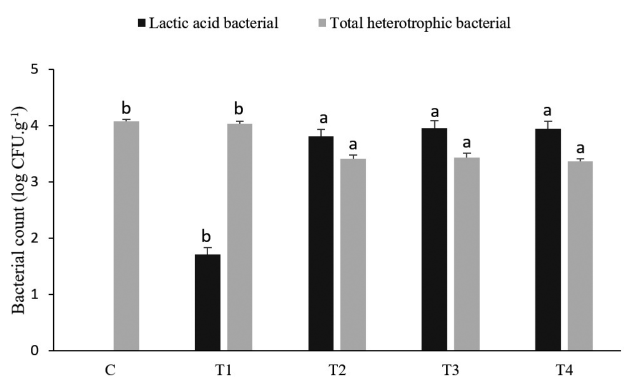

Figure 1. Heterotrophic and lactic acid bacterial counting from intestinal tract of angelfish larvae *Pterophyllum scalare*

Note: Different letters mean statistical difference ($p<0.05$) among the treatments.

After 20 days, With regard to the productive performance, larvae fed with the enriched artemia nauplii with T2, T3 and T4 treatment, increased ($p<0,05$) final weight (FW), weight gain (WG) and specific growth rate (SGRw) for when compared to the control (table 1). Furthermore, larvae mortality significantly increased ($p<0,05$) in the control (72,0±8,5) (table 1).

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Table 1. Productive performance and survival from angelfish larvae *Pterophyllum scalare* fed with Artemia sp. without enrichment (C) and enriched with commercial probiotic (T1: 1.5 g; T2: 3.0g; T3: 4.5g and T4: 6.0g) during larviculture

Treatments	TL (mm)	FW (mg)	WG (mg)	SGR_W (%)	SGR_L (%)	UNI (%)	Kr	S (%)
C	12,48±0,35 a	38±1,1 b	35±1,1 b	23,2±0,17 b	5,74±0,03 a	66.0±16,2 a	0,99±0,02 a	72,0±8,5 b
T1	12,47±0,32 a	39±1,2 b	35±1,2 b	23,4±0,18 b	5,71±0,16 a	75,8±12,4 a	0,99±0,02 a	84,5±12,5 a
T2	12,83±0,24 a	42±1,3 a	39±1,8 a	24,8±0,32 a	5,90±0,20 a	78,1±15,0 a	1,00±0,01 a	92,0±9,4 a
T3	12,86±0,48 a	41±1,7 a	40±2,1 a	25,0±0,47 a	5,77±0,23 a	76,2±14,8 a	0,99±0,01 a	95,5±8,8 a
T4	12,71±0,18 a	42±1,9 a	39±1,6 a	25,2±0,53 a	5,83±0,23 a	75,0±12,8 a	0,99±0,02 a	94,0±8,2 a

Note: Mean values ± standard deviation from productive performance, different letters in column mean statistical difference ($p<0.05$).

4. Discussion

The use of bacteria with probiotic potential has showed positive results to the ornamental aquaculture [9,17,24,25]. Nonetheless, still missing reports about their use for angelfish larvae on productive performance, intestinal modulation and survival.

Colonize the intestinal tract stands out as the main aspect to determine its probiotic potential [19,26]. For this study, the artemia nauplii works as transporter of probiotic bacterium to the host. Its intestinal tract reduced the heterotrophic bacteria amount with the increases of probiotic bacterium due to the competition for space, nutrients and releasing of bactericides [15,19,27].

[17] reported it modulator effect in the intestine for angelfish adult, as also to *Carassius auratus* [24] and *Xiphophorus helleri* [25]. In addition, probiotic act as immune stimulant forming barriers against pathogenic bacteria and increasing its defense cell numbers [16,19,25,28]. Thus, it increases above 80% of survival could be related to the effects of probiotic bacterium included in the exogenous diet during larvaculture.

The larval performance improved in this study with the enrichment of artemia nauplii promoting greater weight gain, specific growth rate and survival. It benefits would be related to the intestinal modulation that provides increased intestinal villi and better nutrient absorption [9,19]. According to [29], they observed similar result with increased performance for angelfish larvae Pterophyllum scalare using a different commercial probiotic.

For these reasons, enrichment of artemia nauplii becomes an efficient strategy to ornamental fish farming considering the probiotic amount to reach its benefits results. However, its microorganisms could influenced by environmental factors, age, development phase and physiological aspects [19,30,31,32].

5. Conclusion

The enriched artemia nauplii with commercial probiotic (3g/L) can modulates the intestinal tract, increases the performance and survival. Thus, it enriched diet can be used as a new strategy for larvaculture of the angelfish larvae *Pterophyllum scalare*.

Conflicts of interest

The authors have no conflicts of interest to declare.

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