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Short Communication

Replacement of *Artemia* spp. with zooplankton in *Penaeus vannamei* larviculture

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ABSTRACT. Supplying healthy and cost-effective postlarvae is critical to the success of the shrimp industry. Given the cost of *Artemia* in larvae culture, there is considerable interest in using alternative live feeds such as copepods. This study's objective was to evaluate five diets with different proportions of *Artemia* and copepods offered to *Penaeus vannamei* larvae. Biological factors such as growth, survival to postlarvae, and final weights were evaluated in conjunction with economic factors. The diets offered were: 1) 100% *Artemia* 2) 75% *Artemia* and 25% copepods 3) 50% *Artemia* and 50% copepods 4) 25% *Artemia* and 75% copepods and 5) 100% copepods. Concerning the biological response, there were no significant differences observed between the five treatments. The economic evaluation was based on a partial budget. It was concluded that there were no significant differences in survival or yields. However, the data collected in this experiment concluded that the treatment with 100% *Artemia* had higher annual net benefits due to the combined effect of survival rates and cost. These net benefits can vary in other repetitions, so it is recommended to experiment more times to observe differences in profits among diets.

Keywords: *Artemia*; *Penaeus vannamei;* copepods; larvae; partial budget; survival rate; aquaculture

High feed costs in the shrimp sector (larviculture) represent the main factors influencing the competitiveness and productivity of shrimp (Marroquín *et al*., 2012). Since the 1930s (Sorgeloos *et al*., 2017), larviculture has relied on *Artemia* spp. as a primary food source. Sanchez (2001) reported that import prices of *Artemia* from the USA ranged from USD 35 to 45 per kg in 2000, with current prices rising to around USD 100 per kg, leading producers to seek alternative larval feeding strategies to reduce production costs (Støttrup & Norsker, 1997; Tahim *et al*., 2014).

Finding a more affordable substitute that does not alter the final quality of postlarvae is necessary to maintain competitiveness, especially in crustaceans where diet quality affects their nutrient composition (D'Abramo & Sheen, 1993; Brett & Müller-Navarra, 1997). The alternatives for diets offered should take into consideration both nutritional and economic factor (Stoner & Zimmerman, 1988). These factors should not cause a significant change in the survival, development of the larvae' digestive system, and resistance to a stress test (Martín *et al*., 2006). Shrimp producers continue to use *Artemia* independent of cost due to its effectiveness (Puello *et al*., 2008).

Publications about replacement diets in shrimp farming have not focused on economic analysis and do not show the specific costs incurred in each diet and the benefits it generates (Støttrup & Norsker, 1997). This information would be useful for producers currently established in the market and investors interested in entering the larviculture industry, considering the cost-benefit of each of the diets provided to determine the best decisions (Martínez-Córdova *et al*., 2010).

This project aims to find a replacement in diets of shrimp larvae culture that is more profitable than *Artemia* spp. The overall objective of the study was to evaluate the biological and economic value of *Artemia* spp. replacement strategies using copepods in larviculture of Pacific white shrimp (*Penaeus vannamei*). The specific objectives of the study were to determine the performance of shrimp nauplii larvae according to the diet offered, characterize the annual cycle for each applied larval diet, determine costs that vary between each of the diets, determine the net bene fits of using

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different diets offered, and analyze the marginal rate return and residual value analysis to choose the optimal diet.

The experiment was conducted at Larvicultura del Pacífico S.A. (LARVIPAC), located in Honduras. The average temperature is 28°C, and the laboratory is located at 44 m above sea level. The experiment was done in 20-25 m^3 rectangular tanks (8.87×2×1.43 m) with an operating level of 22,000 L each. Controlled conditions were established for temperature, salinity, dissolved oxygen, and pH. Four 5 Hp blowers (Baldor Reliance Industrial Motor) supplied aeration for the 20 tanks.

Three variables were analyzed in the production stage: survival (final production), number of larvae per gram, and a stress test (survival rate after stress). Survival was determined by estimating the number of postlarvae (P-12) at the end of the production cycle, based on the initial density of 3.76 million nauplii 5 stocked into each tank. The stress test to determine the survival and number of viable animals per gram consisted of transferring 100 P-12 from fresh water of 21 of salinity for 15 min and returning them to 21 of salinity for another 15 min.

Five treatments with four replicates tanks were used, in which the supply of copepods and *Artemia* spp. offered throughout the cycle varied. The amount of these two feeds was modified from the standard amount regularly used by the company per cycle (Table 1). Nauplii at stage N5 was stocked per tank in 10,000 L of water, with 2,000 L of water with algae periodically added to reach 22,000 L in each tank. Two days after stocking, animal quality in each tank was assessed with a microscope to standardize the animals' quality by determining the percentage of deformed animals, which cannot exceed 20%.

Daily water quality parameters (temperature, salinity, and dissolved oxygen) in the tanks were measured (Table 2) using a YSI Professional Series 2030 Pro meter. Also, algae counts were done every day. Treflan® (0.5 mL) was added in each tank to prevent fungal growth every 12 h from nauplii five to zoea three stages. Epicin®G2 (5 g) was added daily through two daily doses throughout the larval cycle. Additionally, 1,500 g of EDTA and 4,000 g of sodium bicarbonate were applied to the water reservoirs to control the presence of heavy metals and maintain alkalinity.

Diets in each treatment shared the same characteristics except for the amount of *Artemia* and copepods used. Diet with 0% *Artemia* was supplemented with a higher quantity of liquid supplements to compensate for the absence of *Artemia* and copepods in mysis 1, 2, and 3. The number of copepods and *Artemia* used was based on the expected production of two million larvae from the 3,760,000 stocked. The number of copepods in the diet with 100% inclusion was estimated at 8.1 kg per tank per cycle; for the *Artemia*, the estimation at 100% inclusion was 2.1 kg per tank per cycle.

Table 1. Amount of *Artemia* and copepods in larviculture diets used in LARVIPAC. Art: *Artemia*; Cop: copepods.

Treatment	Component (g)	
	Artemia	Copepod
100-0% Art-Cop	2,100	0
75-25% Art-Cop	1,575	2,025
50-50% Art-Cop	1,050	4,050
25-75% Art-Cop	525	6,075
0-100% Art-Cop	0	8,100

Table 2. Optimal water quality parameters for larval development.

Dissolved oxygen	Temperature	Salinity
≥ 3 mL L^{-1}	31-32°C	22-25

All diets were evaluated using a partial budget designed by the International Maize and Wheat Improvement Center (CIMMYT). Annual net benefits were determined, and marginal return rates on each diet were compared to the minimum acceptable rate set for farmers. The expected minimum return rate and residual analysis were determined to define which alternative among the five diets used is best for the producer (Harper *et al*., 2013). Annual net benefits (USD) for each treatment were calculated using Equation 1:

$$\text{Annual net benefit} = \text{annual gross benefit} - \text{annual costs that vary} \quad (1)$$

Gross benefit was obtained by multiplying the average annual production of larvae of each treatment (larvae m^{-3} yr^{-1}), by its price in USD. Yearly production is estimated using the available number of cycles per year per treatment.

The selling price in Honduras, used to develop the partial budget, was USD 3,000 per million postlarvae (USD 3/thousand). Postlarvae quality is a determining factor in establishing the prestige factor of a laboratory to compensate for mortality. The marginal rate of return was obtained by dividing marginal annual net benefit by the increase in costs that vary due to the change of one diet to another (Eq. 2).

$$\text{Marginal rate of return} = \frac{\Delta\ \text{annual net benefit}}{\Delta\ \text{annual costs that vary}} \times 100 \quad (2)$$

The farmer must establish a minimum acceptable rate for which he would be willing to change the current diet for a different one for shrimp larvae production, setting a minimum percentage between 50 and 100%.

According to CIMMYT, the marginal rate of return must be higher than the prescribed minimum acceptable rate of return, considered an alternative, along with a residual analysis to determine the optimal diet for the fish farmer.

Residual analysis indicates the difference in net benefit and the cost of additional investment (CIMMYT, 1998). This analysis supports conclusions with the marginal analysis. The residual is obtained by subtracting the product of the minimum rate of return and costs that vary from the net benefit (Eq. 3). Once the difference is obtained, the analysis continues comparing the residuals obtained in each treatment.

Residual = annual net benefit Ti - (minimum acceptable rate × annual costs that vary Ti) (3)

where, minimum acceptable rate: 100%; Ti: treatment evaluated.

A completely randomized block design was used to group the experimental units into homogeneous groups (blocks). Each block included all treatments, and four repetitions were made simultaneously. An analysis of variance (ANOVA) was used to compare various groups in a quantitative variable with the "Statistical Analysis System" (SAS 9.4) program, and Duncan's multiple range test and LSMEANS ($P \leq 0.05$) were used to detect statistical differences in means. The larvae obtained at harvest time were used to measure the following technical variables: 1) survival, 2) postlarvae per gram, 3) survival after a stress test.

After harvest, survival for all treatments showed no significant difference, which indicates that providing diets with reduced *Artemia* content, which has been the basis of larviculture shrimp feed, does not affect survival at the time of harvest, a possibility for cost reduction. Survival rates differ from the study by Amaya (1991), who concluded that postlarvae fed *Artemia* had a higher survival rate than fed copepods. Martín *et al*. (2006) determined that survival above 80% was possible with the use of zooplankton, specifically *Moina micrura*.

The average postlarvae per gram per treatment required to achieve the weight of one gram are shown in Table 3, and the stress test showed no differences between the treatments offered (Table 4). Results in this study differ from those of Martin *et al*. (2006), where complete *Artemia* replacement had a significant effect on growth. *Artemia* has a large energy reserve, highlighting its polyunsaturated fatty acids content (Villamar-Ochoa, 2000), although this could not cause differences in the size and weight of this food treated larvae.

Table 3. Survival of postlarvae (mean ± standard deviation) in LARVIPAC.

Treatment	Harvest survival (%)	Postlarvae per gram
Artemia (100%)	68	211 ± 52.7
Artemia (75%)	61	271 ± 37.5
Artemia (50%)	56	265 ± 43.5
Artemia (25%)	59	250 ± 53.5
Artemia (0%)	59	271 ± 40.1
P	0.34	0.34
CV %	14.13	18.09

Table 4. Stress test survival in LARVIPAC.

Treatment	Survival (%)
Artemia (100%)	100
Artemia (75%)	99.3
Artemia (50%)	100
Artemia (25%)	100
Artemia (0%)	99.3
P	0.3
CV %	4.62

Table 5. Breakdown of production cycle gross benefit of *Penaeus vannamei* diets in LARVIPAC.

Treatment	Average yield (P m^{-3})	Gross benefit (USD)
Artemia (100%)	2,356,500	6,363
Artemia (75%)	2,110,500	5,698
Artemia (50%)	1,975,750	5,335
Artemia (25%)	2,081,750	5,621
Artemia (0%)	2,055,250	5549

As per the stress test, Ogle *et al*. (1992) suggest that survival to different salinity changes is associated with age and time of exposure. Stress test results differ from the studies by Amaya (1991), Rees *et al*. (1994), and Sorgeloos *et al*. (2017), who concluded that larvae fed *Artemia* showed higher survival to this stress because of the energy reserves that it provided to the animal. The differences reported by Rees *et al*. (1994) and Sorgeloos *et al*. (2017) are probable because their experiments used *Artemia* with different polyunsaturated fatty acid enrichment while in this study the *Artemia* for all treatments used was the same. However, the stress data are consistent with the study by Martin *et al*. (2006), where P-9 shrimp fed *Artemia* did not outperform that fed zooplankton.

The partial budget used the annual gross benefit generated by the sale of the average production of larvae of each treatment, represented as live larvae at the end of the production cycle (Table 5). The live larvae are sold at a retail price of USD 3/thousand adjusted to the standard rate (10%) of additional larvae that the laboratory provides to the buyer. The estimated number of production cycles for each treatment was approximately 12. Treatments differed on costs that vary due to the singular percentages of *Artemia* and copepods used in each. The costs that vary per cycle, in descending order of percentage of *Artemia* (100, 75, 50, 25, and 0) and increasing percentage of copepods, are approximately 177, 159, 141, 122, and 111 USD per 22 m^3. The annual costs that vary, in descending order of percentage of *Artemia* (100, 75, 50, 25, 0) and increasing percentage of copepods, with the aforementioned costs per cycle and the 12 cycles per year, amount to 2,158; 1,935; 1,712; 1,489; and 1,351 USD per 22 m^3, respectively. The data in the following tables may vary due to rounding. The annual net benefit, the difference between the annual gross benefit and the annual costs that vary, is higher using only *Artemia* compared to the other treatments, although with higher costs that vary (Table 6).

The marginal rate of return is part of the economic analysis and shows how many dollars would be obtained in return by switching from one diet to another for every additional dollar invested. Data obtained from the experiment determined that the treatment with 100% *Artemia* had the highest marginal rate of return, followed by treatment 75:25% *Artemia*: copepods and treatment 25:75% *Artemia*:copepods. Treatment 50:50% *Artemia*:copepods is dominated since it has the lowest net benefits and higher costs that vary and lower net benefits, as shown in Figure 1. The minimum rate of return used was 100% on the investment. This rate was taken considering the cost of capital and risk of using alternatives to *Artemia*, this being the current paradigm of the shrimp industry. A graph is presented indicating the relationship of annual net benefits and annual costs that vary (Fig. 1) to establish dominance among treatments. According to this experiment, the farmer would get the highest return on his investment with 100% *Artemia* compared to other treatments for its expected higher net benefit compared to the low additional cost incurred compared to other diets.

Table 6. Annual net benefits (USD/22 m^3) for *Penaeus vannamei* in LARVIPAC.

Treatment	Annual gross benefit	Annual costs that vary	Annual net benefits
Artemia (100%)	77,411	2,158	75,253
Artemia (75%)	69,330	1,935	67,395
Artemia (50%)	64,903	1,712	63,191
Artemia (25%)	68,385	1,489	66,896
Artemia (0%)	67,515	1,351	66,164

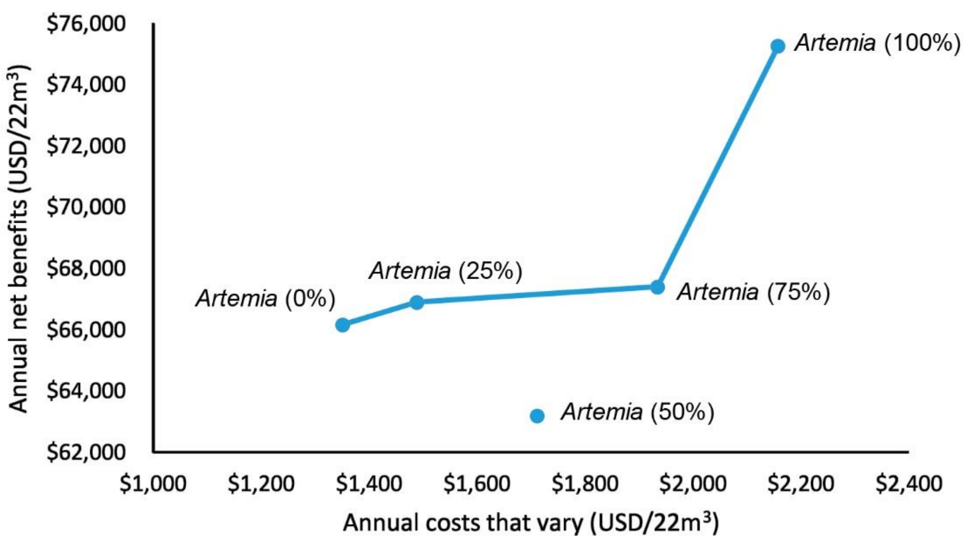

Figure 1. Annual net benefits and annual costs vary for different diets for *Penaeus vannamei* shrimp larvae (USD/22m^3), LARVIPAC.

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