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Aquafeeds

Global brine shrimp supply a potential bottleneck to aquaculture expansion, part 2

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A review of synthetic artemia results in commercial facilities

View of shrimp postlarvae after feeding on synthetic Artemia (note full guts and reddish coloration) during a test at an Indian shrimp hatchery. Photo by Chris Stock.

The first part of this article discussed the importance of artemia to the globally expanding aquaculture industry, the status of artemia resources and the possibility that its limited supplies could become a bottleneck to industry growth, and the possibility and advantages of replacing artemia with manufactured and novel synthetic products. In part two, we present and discuss results of some of the hatchery and pond trials carried out at commercial facilities, and some perspectives on the future of artemia replacement.

Commercial hatchery tests – Western Hemisphere

Two feeding protocols using 100 percent synthetic artemia (not from hatched cysts) were compared in an earlier test, assessing the dietary impact of the two protocols on survival and growth of larval shrimp in production systems under commercial conditions. This is our synthetic artemia, with over 10 years of proven commercial application in hatcheries and nurseries in several countries.

We tested two feeding protocols (treatments, each with three replicates) for *L. vannamei* using two types of rectangular 20-cubic-meter fiberglass tanks representing those typically used in shrimp larviculture. In one test protocol, flat bottom Asian-style tanks were used, with easily suspended liquid larval feeds with synthetic artemia. In the other protocol, parabolic American style tanks were used, with low-cost, dry diets and synthetic artemia.

Tanks were stocked at 160 shrimp nauplii/L, and the trial carried out for 21 days, between the shrimp larval stages of N_5 to PL_{15}. The microalgae *Thalassiosira pseudonana* was added and maintained at a density of 60,000 – 100,000 cells/mL from N_5 to PL_4. Water temperature was 30.8 to 31.8 degrees-C and dissolved oxygen 4.5 to 5.0 mg/L, and pH at 8.2 during the trial.

Table 1 shows growth and survival results of N_3 to PL_{15} in each treatment. Both treatments yielded adequate to superior results for a commercial hatchery using no artemia in its feeding protocol. Slightly better growth was observed when using dry diets, while under the conditions of these tests, somewhat better survival was achieved with the liquid feeds treatment.

Browdy, synthetic artemia, Table 1

TREATMENT	Stocking density	Survival (%)	Size (cm)
Protocol 1 (liquid diet tanks)	163a±6.76	163a±6.76	163a±6.76
Protocol 2 (dry diets tanks)	163a±6.76	163a±6.76	163a±6.76

Table 1. Results of commercial hatchery trials comparing two feeding protocols eliminating 100 percent of artemia from cysts.

This test was carried out at a Western Hemisphere hatchery that had removed the use of hatched artemia and had exclusively used synthetic artemia during the previous two years. The hatchery reported increased survival, sustained improvements in water quality and increased hatchery productivity, leading to lowered production costs and higher profitability.

We then carried out additional trials between 2014 and 2016 at commercial hatcheries in five countries in Latin America, where we evaluated our artificial or synthetic artemia at 100 percent replacement levels vs. controls fed conventional diets. Test conditions and results are summarized in Table 2, presenting results for survival percent and animal weight at PL_4 that were comparable or better than those of controls, demonstrating that 100 percent replacement is possible depending on the hatchery setup and equipment, operators and other conditions.

Management protocols have been developed at these pioneering hatcheries in Latin America, which enabled the complete replacement of artemia. Reported benefits include reduced vibrio counts and improved biosecurity while enhancing production consistency and efficiencies. The trials conducted with synthetic artemia used stocking densities from 85 to 300 nauplii/L. Positive results have been achieved in several countries, using different management and feeding protocols, with the best cases reporting survivals from 50 to 92 percent. Table 2 also shows that shrimp growth and larval development rates were similar to those obtained in normal cultures fed with artemia, ranging from 0.7 mg in 10 days for a PL_4, up to 5.5 mg for a PL_{13} in 20 days of culture.

The hatcheries that were most successful replacing artemia used the strategy of splitting of daily rations into larger numbers of feedings. The traditional feeding of live artemia two, four or six times per day has been replaced with a strategy feeding dry and liquid manufactured feeds up to 12 times per day, providing access to the developing larvae to fresh feeds on a semi-continuous and frequent basis. These successful results achieved by progressive hatchery managers who have adopted artemia replacement are being shared among producers, and more hatcheries are gradually increasing the replacement rates over time using liquid synthetic artemia.

Another important application for synthetic artemia, in addition to its use in first- and second-phase larval culture systems, is its growing commercial use for the transport of PLs from hatcheries to nursery systems or grow-out ponds in Latin America. The product is easily maintained at the farm to reduce cannibalism during acclimation prior to stocking in the culture ponds. In tanks, tubs or plastic bags, water quality is one of the primary concerns of every shrimp producer, particularly during long

Growing demand for brine shrimp – a major feed for the larval stages of various shrimp and fish species – could make its global supply and availability a potential bottleneck for the growth of the aquaculture industry in the near future.

transports. The stability of synthetic artemia provides an effective and economical option for reducing handling stress and cannibalism while maintaining needed water quality.

Browdy, synthetic artemia, Table 2

PARAMETERS	Country 1 (2014)	Country 2 (2015)	Country 1 (2015)	Country 3 (2016)	Country 4 (2016)	Country 5 (2016)
Artemia used	100% replacement	100% replacement	100% replacement	100% replacement	100% replacement	100% replacement
Nauplii/L	185	200	85	300	190	117
Feed (kg/million)	2.75	1.66	4.5	4	2.02	1.68

PARAMETERS	Country 1 (2014)	Country 2 (2015)	Country 1 (2015)	Country 3 (2016)	Country 4 (2016)	Country 5 (2016)
Culture cycle	10 days Z1-PL4	11 Days Z1-PL4	12 Days Z1-PL4	15 Days Z1-PL8	20 Days Z1-PL13	12 Days Z1-PL6
Survival (%)	85	69	70	50	70	92
Weight at PL4 (mg)	0.87	0.72	–	1	5.5	1.1-1.2

Table 2. Results of trials with 100 percent replacement of hatched artemia with a commercial synthetic artemia product, in five major shrimp farming countries in Latin America. The hatchery in Country 3 had replaced artemia for two years, Country 4 had an 8-month protocol, and for Country 5 it was a first-cycle protocol.

Commercial hatchery tests in India

In recent months, several artemia replacement trials were carried out at six commercial shrimp hatcheries in Andhra Pradesh, India. Three larval shrimp rearing tanks were used at each hatchery, and the amount of artemia fed was reduced by 50 percent and substituted with synthetic artemia. At each hatchery, an additional three tanks were used as controls stocked as a group or pairwise from the same batches of nauplii. Survival rates were generally typical for Indian hatcheries and varied between facilities and according to the season when the tests were run. Artemia was successfully replaced with no real changes in survival rates, as indicated by the standard deviation bars (Fig. 1). Performance results at the hatchery with the best culture conditions (based on high survivals during the cooler season) for tanks where artemia was replaced equaled or exceeded the results achieved for the artemia-fed tanks in survival, growth and measures of PL quality (Table 3).

Fig. 1. Average survival values to harvest for hatchery tanks fed 50 percent synthetic liquid Artemia replacement vs. 100 percent artemia controls (three tanks per treatment) during trials carried out at six different shrimp hatcheries in India.

Browdy, synthetic artemia, Table 3

Synthetic artemia average (%)	*Synthetic artemia std. dev. (%)*	*Artemia cysts average (%)*	*Artemia cysts std. dev. (%)*
74	*5*	*69*	*4*
46	*3*	*44*	*2*
53	*3*	*55*	*3*
38	*2*	*36*	*3*
42	*2*	*49*	*3*
52	*4*	*51*	*5*

Table 3. Performance results at the hatchery with the best culture conditions for tanks where artemia was replaced equaled or exceeded the results achieved for the artemia fed tanks in survival, growth and measures of PL quality.

An additional, controlled trial, with the kind support of a customer – Blue Star Marine Hatchery in Ramatheertham-Nellore, India and with the efficient backing of Dr. Ravikumar – was recently conducted to prove that our synthetic artemia could replace hatched artemia and improve PL quality and survival. The control treatment included several commercial shrimp larval feeds and a commercial probiotic. The experimental treatments included the same commercial feeds and probiotic as in the control group, and our synthetic artemia product.

The rectangular, 10-ton larviculture tanks used in the trial had flat bottoms and were filled with dechlorinated water (EDTA was added). Each tank was stocked with two million nauplii (N_5) from the hatchery's broodstock (sourced from a commercial U.S. supplier). Tanks had grid aeration, and water was exchanged at 40 percent daily from PL_6 until harvest. Additions included a commercial probiotic at 20 grams daily, sugar at 250 grams starting at the M_3 stage, and EDTA. NH_4-N, alkalinity and pH were monitored, and the larval shrimp were fed six times per day.

The PL fed with our synthetic artemia scored 100 percent survival in stress tests, and microscopic observations of the animals showed muscle-gut ratios of 4:1 and high numbers of fat globules. We concluded that artemia replacement with our synthetic artemia liquid diet demonstrated similar performance when compared with hatched artemia and was cost-effective.

Practical prevention strategy in hatcheries

Unusual mortalities during larviculture, likely due to bacterial and other pathogens, are usually experienced by any shrimp hatcheries experience. These mortalities typically involve an increase in the concentrations of *Vibrio sp.* (*V. parahaemolyticus*, *V. vulnificus, V. alginolyticus*), in the culture tanks and that affect various shrimp larval stages. Recently, particularly virulent strains of *Vibrio sp.* have emerged that cause a reduction in appetite, a reduction in overall activity, and progressive atrophy of the hepatopancreas. A small percentage (2 to 4 percent) of the population in a tank is initially affected, with exponential contagion of the rest and total mortalities in 12 to 14 hours. Synthetic artemia can be an important component in a practical prevention strategy, as shown in the data in Table 4 for bacterial counts for a commercial hatchery in the Western Hemisphere that tested using artemia nauplii vs. synthetic artemia.

Browdy, synthetic artemia, Table 4

	Total bacteria (CFU)		Vibrio alginolyticus (CFU)		Vibrio parahaemolyticus (CFU)	
Diet	Culture water	Larvae	Culture water	Larvae	Culture water	Larvae
Synthetic artemia	2 X 10(5th)	1 X 10(5th)	5 X 10(4th)	1.8 X 10(3rd)	2 X 10(3rd)	4 X 10(2nd)
Artemia nauplii	2.8 X 10(5th)	1.5 X 10(5th)	2.1 X 10(5th)	3.2 X 10(3rd)	4.2 X 10(3rd)	2.6 X 10(3rd)

Table 4. Comparison of bacterial counts (in colony forming units, CFU) in culture water and larvae for larval tanks fed synthetic artemia or artemia nauplii. The consumption of synthetic artemia was 3.8 kg. per mm PL; and 0.22 kg. per mm PL.

Some of the practical measures undertaken that have reportedly helped, according to one major hatchery in the Western Hemisphere, include:

- Establishment of microbial health indices: identification of the levels, areas and foci of bacterial contamination in hatchery systems through periodic, ongoing evaluations of animal behavior and bacterial analyses.
- Bacteriological control: reduction of *Vibrio sp.* loads in the environment and larvae; determine loads in algae and reservoir water; and evaluate efficiency of all treatments, including probiotics, chlorination and addition of organic acids.
- Control of larvae quality: rate of development through larval stages, growth rate, water quality management, stress tests, evaluations of size variation and bacterial loads.
- Rigorous control of algae quality: externally procure certified microalgae, and use it during its optimum growth phase.
- Replace artemia nauplii with synthetic artemia: providing a very useful tool in larval production, beneficial for reducing bacterial loads in the system without affecting larval quality during the larviculture phase.

Results from commercial grow-out ponds

To test if PLs produced with synthetic artemia have the same quality as PLs produced with real, live artemia, trials were conducted to compare pond results at a Latin American shrimp farm using PLs produced with hatched artemia cysts and with synthetic artemia.

For the test, two hatcheries produced 6 million PLs for the test, 3 million PLs with a larviculture protocol using synthetic artemia and 3 million PLs following the larviculture protocol using artemia hatched from cysts. The PLs were kept in nursery tanks for 7 to 12 days before being stocked in the grow-out ponds. A total of 72 hectares of ponds were stocked at a large commercial shrimp farm with animals from the nursery tanks: seven ponds (36 ha) of the area were stocked with the PLs produced with synthetic artemia and six ponds (36 ha) with the PLs produced with live artemia from cysts. The results of the test in commercial ponds, showing equal pond performance from PLs produced using artificial artemia vs. PLs fed hatched artemia, are presented in Fig. 2.

Fig. 2. Statistical analysis of the commercial pond results demonstrates equal performance from PLs produced using synthetic artemia vs. hatched artemia.

Looking at the future

Shrimp larvae do not need artemia as they evolved without it in their natural habitats, so the key is to effectively provide its nutrients in a replacement product. In the industry's early years, many shrimp producers evaluated aquafeed quality based exclusively on their fishmeal and crude protein content. Nowadays, many shrimp feeds are formulated using nutritional strategies to meet specific nutrient requirements for amino acids, fatty acids, various micronutrients and others. Aquacultured animals require specific nutrients in their feeds, and the same strategy can and is being applied during the larval phase with synthetic artemia.

When the projected global growth of aquaculture production is considered, continued demand increases for artemia cysts could create a supply, availability and price bottleneck. In particular, when environmental conditions deteriorate in places like the Great Salt Lake, which currently provide the majority of the high-quality cysts.

The shrimp farming sector is one of the most important aquaculture industries in the world, and as such, it cannot completely depend on wild production for such a critical resource as artemia, with its historical production variability in production and price increases. The expected growth of the aquaculture industry means increasing demand for artemia, so where will this additional supply come from? Artemia culture on land could be an alternative, but to date, large-scale culture efforts have not been successful.

The development of improved, advanced hatchery diets designed to reduce dependency on live feeds remains an ongoing process in the evolution of the aquaculture industry. Numerous hatcheries have successfully decreased their overall reliance on artemia, but it often remains a key element of most larval feeding regimes. However, some pioneering and innovative shrimp hatcheries have been able to completely eliminate artemia from their larval protocols while concurrently improving overall results.

Artemia has unquestionably being a key resource to support the global hatchery business and the aquaculture industry. But the industry should accept its limitations and continues working with partners in academia and industry to gradually replace it, to reduce the risks and costs of its disproportionate reliance on this resource. The continued, cost-effective supply of enough artemia cysts is a potential bottleneck to the growth of the aquaculture industry, therefore, the development of cost-effective and practical alternatives to artemia will support aquaculture's increasing role as a responsible provider of food for the world's growing human population.

A considerable increase in the supply of hatchery-reared shrimp postlarvae – and a concomitant demand for additional artemia – will be required to significantly expand global production of farmed shrimp.

The complete replacement of artemia is already possible using an available, complete, synthetic artemia product with a consistent nutritional profile. It is pathogen-free and has no biosecurity issues, and has constant availability and quality with no storage or hatch-out concerns. This synthetic artemia can be a delivery means for higher levels of nutrients, probiotics, immunostimulants and enzymes to enhance digestion and improve water quality and animal health.

Can all hatcheries replace 100 percent of the artemia cysts they use? The answer today is no, because it completely depends on the specific hatchery, its resources, infrastructure and technical personnel capabilities. But we believe a starting goal of a 50 percent replacement level could certainly be achieved by many shrimp hatcheries, and increasingly higher levels of replacement can be reached with additional experience and training.

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