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Artemia bioencapsulation delivers probiotics via digestive tracts of fish larvae

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Optimization of host animals' gut microbial flora leads to better growth, survival

When fed bioencapsulated *Artemia* enriched with *Bacillus*, larval-stage spiny sturgeons increased body weight and length.

The production of marine fish juveniles in commercial hatcheries largely depends on live preys such as rotifers and artemia as food sources. Optimization of the zootechnical, nutritional and microbial factors of live foods can reduce the commonly heavy mortalities of the fish larvae.

Although live preys are recognized as one of the pathways for the entry of pathogenic bacteria into fish larvae, they can also function as possible vectors for the delivery of nutrients, antimicrobial agents, vaccines and probiotics to larval fish. For example, research with varied fish species has shown that *Artemia urmiana* nauplii can be bioencapsulated with beneficial microorganisms.

Persian sturgeon larvae

In research, using *A. urmiana* nauplii in the process of bioencapsulation or enrichment by probiotic bacilii showed the artemia had a high potential to carry the produced suspension of bacteria. The high grazing rate of *A. urmiana* nauplii on bacteria illustrated that it can be used as a suitable vector to carry probiotic bacteria into the digestive tracts of cultured fish.

The probiotic *Bacillus* species were successfully incorporated into the *A. urmiana* nauplii at a level of 5.67×10^2 colony-forming units (CFU)/nauplii during a period of 10 hours. The concentration of bacteria in broth was 1×10^5 CFU/ml.

When larvae of Persian sturgeon (*Acipenser persicus*) were fed bioencapsulated *A. urmiana* nauplii at 30 percent of their body weight daily, a high concentration (6×10^7 CFU/larvae) of probiotic *Bacillus* was counted in the digestive tracts of the fish after 30 days of feeding.

This resulted in a survival rate of 89 percent, feed efficiency of 43.96 percent, total amylase of 1.8 international units (I.U.)/mg protein, protease of 0.37 I.U./mg protein and lipase of 1.66 I.U./mg protein. The related values for the control were 79 percent, 31.99 percent, 0.87 I.U./mg protein, 0.19 I.U./mg protein and 1.01 I.U./mg protein. The body weight of the sturgeon larvae increased from 901 mg for the control to 1,233 mg (Figure 1). Also, the survival time for Persian sturgeon larvae challenged with salinity stress increased significantly.

Treated artemia effectively carried probiotics to the digestive tracts of fish larvae.

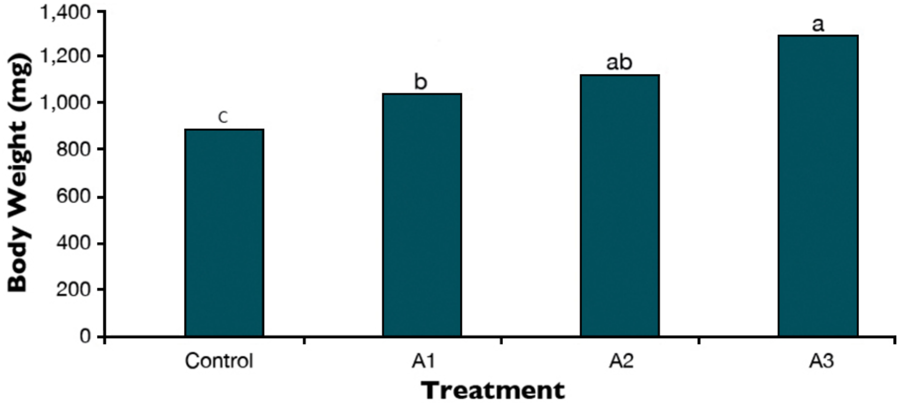

Fig. 1: Body weights of Persian sturgeon larvae.

Spiny sturgeon larvae

In another study, inistar-I *A. urmiana* nauplii were fed to larval-stage spiny sturgeons, *Acipenser nudiventris,* as a vector to carry probiotics to the larvae's digestive tracts. The *Artemia* were bioencapsulated at different levels of bacterial suspensions for 10 hours. The sturgeon larvae were fed 30 percent of their body weight with enriched *A. urmiana* nauplii six times a day.

The results indicated that the *A. urmiana* nauplii microenriched by the *Bacillus* significantly increased final body weights, final body lengths (Fig. 2) and specific growth rates in the experimental fish in comparison with the control. The study also showed that different concentrations of probiotics in the bioencapsulation broth of *A. urmiana* nauplii could have different effects on growth performance and feeding efficiency of the sturgeon larvae.

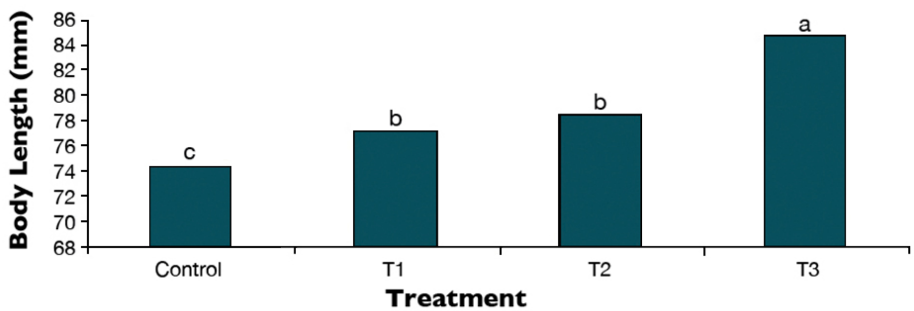

Fig. 2: Body lengths of spiny sturgeon larvae.

Beluga sturgeon larvae

Newly hatched *A. urmiana* nauplii were also employed for early feeding of larval beluga sturgeon, *Huso huso.* In this study, in which the *Artemia* nauplii carried the probiotic *Bacillus* to the digestive tracts of the larvae, results showed that the carcass proximate compositions of the larvae were significantly increased. The enriched artemia nauplii improved food intake, protein productive value, nitrogen retained efficiency, protein gain and energy retained as protein.

Silver carp larvae

Other research with larval silver carp, *Hypophthalmichthys molitrix*, showed that *A. urmiana* nauplii grazed the spores of profitable bacteria and had a high rate of inoculation with them. Spores of *Bacillus circulans* and *B. lichenifirmis* were used in the bioencapsulation suspension broth. Significant positive results for conversion efficiency ratio, condition factor, daily growth, specific growth rate and feed-conversion efficiency were obtained in the fish fed probiotics via bioencapsulation of *A. urmiana* nauplii.

Koi carp larvae

A. urmiana nauplii were also used as a vector to deliver the bakers yeast *Saccharomyces cerevisiae* into the digestive tracts of larval koi carp, *Cyprinus carpio carpio*. Fish treated with the yeast and probiotic *Bacillus* showed positive responses in such growth parameters as weight gain, feed-conversion ratio and specific growth. Significant positive correlations were observed between the concentration of probiotics in bacterial broth suspension of the artemia nauplii and weight gain ($r = 0.723$, $p < 0.05$), specific growth ($r = 0.669$, $p < 0.05$) and conversion efficiency ratio ($r = 0.776$, $p < 0.01$) of the carp larvae.

The bioencapsulation resulted in higher culture performance, with the best results in the trial in which fish larvae were fed artemia nauplii with 3×10^8 CFU yeast and *Bacillus*/L.

Perspectives

A. urmiana nauplii enriched with useful microorganisms can help in the optimization and development of gut microbial flora of host animals and lead to increased growth parameters and survival. Through such improvements, bioencapsulation can decrease the overall cost of aquaculture production and become a significant factor in the development of the industry.

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