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Prospects for the use of inert microdiets in shrimp hatcheries and nurseries

André Barreto, Wilson Pinto, Renata Serradeiro and Luis Conceição

The Pacific white shrimp (*Penaeus vannamei* Boone, 1931) has become one of the primary species produced in aquaculture. Although traditionally farmed in Asian and South American countries, production is expanding to European territory with a revamped approach, where quality is prioritized over quantity. The provision of high-quality adults relies on optimized husbandry practices during the early life-stages of shrimp, which still require to be enhanced. Shrimp hatchery production still depends on the costly live-prey production chain, generally based on *Artemia*, as the carnivorous stage mysis in traditional protocols requires a constant supply of the latter. The benefits of using live-prey are overshadowed by sub-optimal nutritional values and the potential to act as vectors for pathogenic agents, affecting the predictability of larval production. Therefore, the design of a high-quality inert microdiet that can completely, or partially in co-feeding regimes, replace live feeds, as well as improve PL nutritional status, would represent significant economic and ecological benefits for shrimp aquaculture. Although a great deal of work is still required, a similar path has been paved for some relevant cultured fish species with great success, which can inspire the shrimp industry. A better understanding of the nutritional requirements and digestive capacities at different larval stages will enable to progressively enhance inert diet formulations to better suit the needs of a developing shrimp organism. Nutritional studies on the first developmental stages of shrimp found in the literature are promising but scarce, with some reports of partial and/or total successful replacements of live feeds with inert diets.



Figure 1. One of Riasearch Lda RAS systems where shrimp larval trials are performed (left) and Pacific white shrimp post-larvae feeding on inert microdiets in one of the trial tanks (right).

Most microdiet formulation tests have been done on post-larvae and early juvenile shrimp and a wide range of ingredients has been contemplated. The rapid expansion of shrimp farming has generated increases in the demand for commercial feeds for the on-growing phase of production, that represents the bulk of

Shrimp's high tolerance to the inclusion of different ingredients in formulated diets opens prospects for an introduction of inert diets at earlier developmental stages and high live-prey replacement and the production of more eco-friendly diets for the post-larval/juvenile stage.

inert diet consumption. These microdiets commonly include fish and squid meals as the primary protein sources due to their balanced composition in essential amino acids, fatty acids, vitamins and minerals, but also due to their high palatability, which increases ingestion at early developmental stages. However, fishmeal supply has become limited and costly due to environmental concerns. Accordingly, the partial or total replacement of fish meal in aquaculture diets for more accessible and economical high-quality ingredients has been a prioritized field of study. Nonetheless, use of alternative ingredients at early life stages of shrimp should be regarded with caution. Soybean meal is one of the most widely available plant protein sources used in alternative aquafeed formulations, with demonstrated capacity to replace fishmeal in high proportions in practical juvenile shrimp diets.

In comparison with fishmeal, marine macro and microalgae have also been shown to produce similar or higher shrimp growth performances when incorporated as whole or broken biomasses in low dietary inclusion levels. Bioflocs, a by-product of biofloc technology systems, have also been successfully converted to meal form and used as an alternative protein source for *P. vannamei* diets. These consist of heterotrophic bacteria, phytoplankton, organic matter and grazers of the bacteria. Although research efforts have been focused

on testing the effectiveness of different protein sources, lipids and carbohydrates also represent the important parcels of shrimp diets indicating that even more changes to formulations are possible. These can minimize the protein requirements with no adverse effects if properly chosen and enable the reduction of production costs as they are the least expensive form of dietary energy.

Optimizing microdiets for shrimp post-larvae/juveniles

Recently, two experimental trials were performed in Portugal by SPAROS Lda - RIASEARCH Lda aiming at evaluating growth and survival of *P. vannamei* post-larvae/juveniles fed microdiets formulated with different ingredients. In the first trial, three different experimental premium microdiets were tested: 1) mix of plant-based and marine proteins; 2) marine and vegetable protein mix rich in plant-based phospholipids; and 3) macroalgae-rich and formulated to have high water-stability and high performance (Table 1).

TRIAL 1	Marine + Plant proteins	Marine + Plant proteins and Plant Phospholipids	Macroalgae
Main Ingredients	Krill meal, squid meal, fish protein hydrolysate, wheat gluten, pea protein	Krill meal, squid meal, fish protein hydrolysate, wheat gluten, pea protein, soy lecithin	Krill meal, squid meal, fish protein hydrolysate, wheat gluten, pea protein, macroalgae mix
Crude protein (g/100 g)	63	63	62
Total lipid (g/100 g)	17	17	16

Table 1. Main ingredients and crude protein and total lipid levels of the shrimp experimental microdiets tested on the first trial.

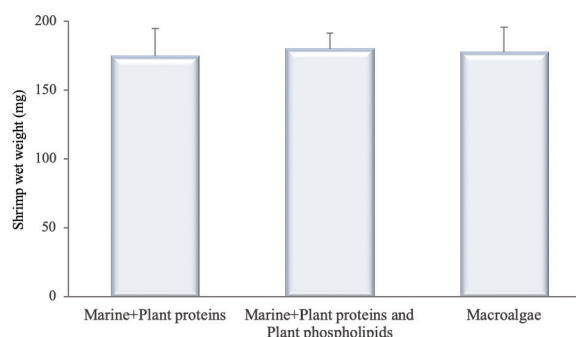


Figure 2. Final body wet weight of Pacific white shrimp reared for 21 days under three dietary treatments. 1) experimental diet rich in marine and plant-based proteins; 2) experimental diet rich in marine and plant-based proteins and plant-based phospholipids; 3) experimental diet rich in macroalgae and formulated to have high water-stability and high performance (n=3 experimental units).

TRIAL 2	Squid meal + Krill meal	Krill meal	Fish meal
Main Ingredients	Squid meal, krill meal, fish protein hydrolysate, wheat gluten, pea protein	Krill meal, squid meal, fish meal, fish protein hydrolysate, wheat gluten	Fish meal, squid meal, fish protein hydrolysate, wheat gluten
Crude protein (g/100 g)	62	66	66
Total lipid (g/100 g)	17	13	13

Table 2. Main ingredients and crude protein and total lipid levels of the microdiets tested in the second trial.

Pacific white shrimp post-larvae (2 mg wet weight) were reared for 21 days in 40L tanks part of a RAS system (Fig. 1) at a density of 5000 shrimp/m³. Shrimp were co-fed with *Artemia* nauplii and microdiets the first three days, with shrimp promptly exhibiting inert diet acceptance (Fig. 1).

The different experimental microdiets had very good performances, with no significant effects on growth and survival between them. At the end of the trial, shrimp from all treatments achieved final wet weight values around 180 mg (Fig. 2), feed conversion ratios (FCR) values around 0.67, relative growth rates (RGR) around 23 % day⁻¹ and survival around 97 %.

On a second trial, a premium commercial weaning microdiet designed for flatfish, rich in squid and krill meals (SPAROS, Portugal) was tested together with two experimental microdiets: 1) one rich in krill meal, and another with a marine protein mix rich in fishmeal followed by the microdiet rich in krill meal in the second half of the trial (Table 2).

Pacific white shrimp juveniles (0,328 g wet weight) were reared for 29 days in 40L tanks part of a RAS

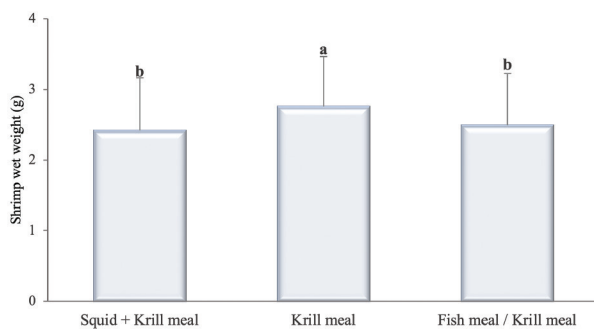


Figure 3. Final body wet weight achieved by Pacific white shrimp reared for 29 days under three dietary treatments. 1) premium commercial weaning microdiet diet for flatfish, rich in squid and krill meals; 2) experimental microdiet rich in krill meal; 3) experimental microdiet rich in fishmeal and experimental microdiet rich in krill meal the second half of the trial (n=4 experimental units). Different subscript letters indicate significant statistical differences between treatments ($p>0.05$).

system at a density of 2500 shrimp/m³. It was possible to observe a diet impact on shrimp weight (Fig. 3). Shrimp fed the microdiet rich in krill meal had a higher weight than those from the remaining treatments.

However, no significant differences were observed on the remaining growth parameters analysed, with all treatments achieving FCR values around 1.12 and RGR around 7.3 % day⁻¹. As for survival, shrimp fed both experimental microdiets had a significantly lower survival than those fed the commercial flatfish diet rich in squid and krill meals ($94 \pm 2\%$ and $97 \pm 0.8\%$, respectively).

The inclusion of krill meal as the main protein source instead of fishmeal marginally enhanced shrimp growth. Nevertheless, results from both trials suggest that the *P. vannamei* has a high tolerance to changes in diet formulations and good growth can be achieved utilizing a wide range of ingredients in the microdiets. Additionally, results suggest the importance of designing species-specific microdiets, as utilizing a diet for flatfish did not produce identical growth results as a diet designed for *P. vannamei*.

Future prospects

Awareness has increased to the importance of early nutrition as a critical component for larval production, as sub-optimal nutrition during early developmental stages has long lasting effects on quality criteria. Although further research needs to be conducted to better understand the nutritional requirements of shrimp during the early developmental stages, its high tolerance to the inclusion of different ingredients in formulated diets opens prospects for an introduction of inert diets at earlier developmental stages and high live-prey replacement and the production of more eco-friendly diets for the post-larval/juvenile stage, contributing to tackle the ecological challenges faced by shrimp farming.

More information:

André Barreto
Riasearch Lda., Portugal
E: andrebarreto@riasearch.pt

