Health & Welfare

Brazil study: Beta-glucans improve survival of IMNV-infected white shrimp

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Outbreak forced production shift from intensive to semi-intensive

This shrimp collected from a grow-out pond exhibits severe signs of IMNV in the milky abdominal tissue.

In 2003, the infectious myonecrosis virus (IMNV) severely disrupted the production of farm-raised shrimp in Brazil after five years of continuous growth that had led to record production. The virus was first detected in September 2002 in farms located in the state of Piauí and rapidly spread to neighboring states within six months. Since then, production has recovered mainly by shifting from intensive to semi-intensive production methods.

Symptoms, treatment

Shrimp affected with IMNV typically show a loss of transparency in the abdominal tissue that often leads to a reddish color and persistent daily mortality after shrimp reach 7 g in body weight. Cumulative mortalities can approach 70 percent over a typical 120-day growout cycle and result in feed-conversion ratios well above 2 for 12- to 14-g shrimp.

To date, no single effective treatment against shrimp viral diseases exists. As shrimp are not able to respond to vaccination, enhancement of disease resistance has been attempted through stimulation of their immune systems. Several compounds have been reported to actively promote immune responses and increase survival in cultured penaeids after viral or bacterial challenge. These include peptidoglycans, glucans, lipopolysaccharides and sulfated fucoidans.

Brazil study

In a recent study funded by a grant from Financiadora de Estudos e Projetos, the authors' goal was to determine if a beta-1,3/1,6-glucan extracted from the cellular wall of the bakers yeast *Saccharomyces cerevisiae* could promote increases in the survival and growth of Pacific white shrimp *(Litopenaeus vannamei*) after an oral challenge with IMNV.

Shrimp growth and survival of juvenile *L. vannamei* orally challenged with IMNV were evaluated in 20 circular, 500-l polypropylene tanks stocked with 57 shrimp/tank (100 shrimp per square meter). Shrimp were fed for 70 days with lab-manufactured diets either deprived or containing a source of the beta-glucan.

The experimental design comprised one treatment and three control diets (Table 1): COM, a high-performance commercial diet fed to IMNV-free shrimp; REF, a basal diet manufactured in the lab without the beta-glucan and fed to IMNV-free shrimp; IREF, the basal diet fed to shrimp orally challenged with IMNV; and IBET, the basal diet with an inclusion of 1,000 mg/kg of a commercial beta-1,3/1,6-glucan.

Nunes, Experimental design for shrimp challenged, Table 1

Treatment	Oral IMNV Challenge	Beta-1, 3/1,6-glucan Supplementation
COM (35.0% crude protein)	No	–
REF	No*	No
(31.4% crude protein)		
IREF	Yes	No
IBET	Yes	1,000 mg/kg

Table 1. Experimental design for shrimp challenged with infectious myonecrosis virus.

The nearly isoenergetic and isoproteic diets were designed to result in the same nutritional composition except with regard to the inclusion of a beta-glucan source. The beta-glucan was included in the IBET diet at the expense of bentonite (Table 2). Over the growth cycle, shrimp were exposed to the feed for five hours daily. Meals were split equally between the two morning and evening feeding times.

Nunes, Composition and chemical proximate values of diets, Table 2

Ingredients	Feed (g/kg of diet) REF, IREF	Feed (g/kg of diet) IBET	Feed (g/kg of diet) COM
Wheat flour	350.0	350.0	–
Soybean meal	220.8	220.8	–
Broken rice	70.0	70.0	–
Fishmeal, anchovy	167.1	167.1	–
Fishmeal, offal and by-catch	100.0	100.0	–
Fish oil	30.0	30.0	–
Soy lecithin	20.0	20.0	–
Cholesterol	1.5	1.5	–
Attractant	5.0	5.0	–

Common salt	10.0	10.0	–
Vitamin-mineral premix	10.0	10.0	–
Synthetic binder	5.0	5.0	–
Bicalcium phosphate	12.0	12.0	–
Betonite	3.6	2.6	–
Beta-1,3/1,6-glucan	0	1.0	–
Chemical Composition			
Crude protein (%, dry matter)	31.4	31.3	36.6
Crude fat (%, dry matter)	10.1	10.0	8.7
Ash (%, dry matter)	10.0	10.3	10.6
Crude fiber (%, dry matter)	5.1	4.3	7.6
Gross energy (kcal/kg)	3,706	3,817	3,713

Table 2. Composition and chemical proximate values of diets used in the study.

IMNV challenge

Shrimp suspected of IMNV infection were collected in the geographic area where the first case of the disease was reported in Brazil. Sampled shrimp were 12 to 14 grams in body weight and exhibited severe gross signs of IMNV infection. Additionally, shrimp in the growout pond surveyed had attained a low 32 percent final survival at harvest.

Soon after capture, shrimp pleopods from 50 animals were fixed in a 95 percent ethanol solution for polymerase chain reaction (PCR) and real-time PCR analyses. For assurance, the size of the pleopod sample exceeded the amount required for analysis. The shrimp samples were found negative for Taura syndrome virus, white spot syndrome virus and infectious hypodermal and hematopoietic necrosis virus , but positive for IMNV.

In the lab, IMNV-free shrimp were fed roughly ground IMNV-contaminated tissue. Challenge occurred over three consecutive days, when shrimp had reached 4.9 to 6.9 grams in body weight or 29 days after continuous exposure to the experimental diets. Feeding rates over the challenge period varied 4.0 to 5.2 percent of estimated stocked shrimp biomass. During the viral challenge period, shrimp in treatments COM and REF were fed their regular diets.

Results

Shrimp survival started to decrease progressively after the oral challenge (Fig. 1). Three days after exposure to IMNV extract, shrimp mortality in the IREF treatment was already statistically different from all other treatments ($P < 0.05$). This trend prevailed until shrimp harvest. Conversely, cumulative shrimp mortality did not vary significantly among the COM, REF and IBET treatments ($P > 0.05$).

Fig. 1: Cumulative mortality of juvenile shrimp after IMNV challenge. The zero week refers to three days after the viral challenge. Data points that do not share the same letter are statistically different ($P < 0.05$).

Mortality of 30 percent and greater was observed three weeks after the viral challenge, regardless of the feed treatment. However, in the IREF treatment, shrimp mortality topped 67 percent.

At harvest, the highest shrimp survival rate (69.5 ± 12.7 percent) was observed in the COM treatment (Fig. 2), which did not differ statistically from the REF group. In contrast, the poorest survival (23.20 ± 5.76 percent) was reported for shrimp in the IREF treatment. Shrimp survival for IBET (48.10 ± 8.53 percent) was significantly higher than in IREF.

Fig. 2: Mean survival and final body weight of juvenile shrimp after IMNV challenge.

Shrimp grew continuously over the rearing period. Weekly growth rates varied from 0.56 grams for the first 14 days of rearing to 0.77 grams two weeks prior to harvest. At harvest, the highest shrimp body weight (11.20 ± 0.58 grams per shrimp) corresponded to the lowest survival rate (IMNV-Ref; Fig. 2).

Shrimp body weight in the IREF treatment was significantly higher than in the other treatments ($P < 0.05$). The final weights for COM and REF shrimp were not statistically different, while shrimp final weight for IBET was statistically lower than for the REF group ($P < 0.05$).

Observations

In the study, the IREF and IBET animals were found very susceptible to IMNV. In the former, average mortality as high as 76.8 percent was observed at harvest, when animals were close to 8 grams in body weight. This observation agrees with reports from shrimp farmers. In infected areas, clear signs of IMNV could be found in shrimp of all sizes, but the highest mortality rates were most often observed when animals were 6 to 8 grams in body weight.

The study results suggested that continuous exposure to a diet supplemented with beta-1,3/1,6-glucan enhanced *L. vannamei* survival after oral challenge with IMNV. However, the effects of the beta-glucan on shrimp growth performance were unclear, probably due to the significant mortalities observed.

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