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Health & Welfare

Brazil shrimp farm performs genetic selection for IMNV resistance, growth

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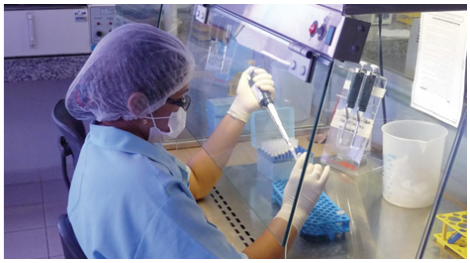
Mean survival rate increased from 3.2 percent to 55.3 percent



The main clinical sign of IMNV infection is the appearance of white, opaque areas in the tail muscles of affected shrimp. Broodstock are individually analyzed for IMNV and other pathogens prior to entering maturation to produce disease-free nauplii and postlarvae.

Since 2002, the epidemics due to infectious myonecrosis virus (IMNV) in the aquaculture of Pacific white shrimp (*Litopenaeus vannamei*) in northeastern Brazil have caused severe impacts on production and still represent a threat to sustainable culture in most affected areas.

Queiroz Galvão Alimentos S.A. (QGA) owns a 960-hectare shrimp farm, a hatchery with a monthly production capacity of 300 million postlarvae and a processing plant in Rio Grande do Norte, Brazil. In October 2004, QGA contracted technical assistance from the international team of Concepto Azul to implement a program for disease prevention and genetic breeding similar to those implemented against white spot syndrome virus (WSSV) and infectious hypodermal and hematopoietic necrosis virus (IHHNV) in other countries.

Due to severe mortalities caused by IMNV and other losses to IHHNV and the intracellular bacteria that cause necrotizing hepatopancreatitis (NHP), the program focused on selecting broodstock resistant to IMNV with simultaneous IHHNV- and NHP-free certification. It also examined several physiological stress factors and simultaneous pathogen-free certification.

QGA built a specific infrastructure for the program, including a molecular biology laboratory for polymerase chain reaction (PCR) analysis, individual spawning and larval culture units, an experimental pathology bio-secure unit and broodstock culture units at the hatchery and shrimp farm.

(https://link.chtbl.com/aquapod)

IMNV transmission

Pathogens such as viruses can be vertically and horizontally transmitted. Vertical transmission, mostly from infected females to their progeny, has been established for most of the viruses that impact shrimp production worldwide, including WSSV and IHHNV.

In the case of IMNV, a preliminary study performed in 2005 showed that IMNV could be detected by nested PCR testing in broodstock ovaries, hemolymph and muscle. Disinfected eggs, nauplii and postlarvae produced from these naturally infected brooders were also detected positive. These data indicated that vertical transmission is very likely.

Horizontal transmission can occur by cannibalism or by host vectors. IMNV virus was detected in farm samples of zooplankton (77 percent), crabs (80 percent) and oysters (40 percent). Quantitative real-time PCR analyses for quantification of IMNV in water samples from the farm inlet canal showed high concentrations from 1.0×10^5 to 3.0×10^7 viral RNA molecules/mL.

Production impacts

Considering the impracticality of sterilizing water and soils in the ponds of a semi-intensive system, total eradication of pathogens from the environment is almost impossible. Moreover, several abiotic stress factors that occur at the shrimp farm do affect production.

Eutrophic estuarine water can cause hypoxia and subsequent mortality. Due to adjacent river floods, low salinity values of 0 to 1 ppt and rapid drops in concentration cause higher susceptibility to pathogens. The mean survival rate in QGA's shrimp ponds in the winter of 2004 bottomed at 19 percent.

Postlarvae experimentally infected with IMNV at the biosecure facility.

Specific pathogen-free postlarvae without selection for resistance to pathogens and stress factors would get infected at the farm due to contamination in the semi-intensive system. As a consequence, it was necessary to stock postlarvae selected for resistance, growth and certified free of disease to reduce the risks of pathogen transmission and mortality.

Genetic breeding

The main criteria considered for the genetic selection program are resistance to IMNV and growth. The program relies on individual selection operations that permit a precise estimation of the genetic value of all broodstock candidates for each criterion.

Selected broodstock are used to establish pure lines through inbreeding, which increases homozygosity, in order to stabilize genes involved in resistance. For commercial production of nauplii and postlarvae, crosses are outbred between pure lines.

Production and selection of new generations are realized through directed crosses and artificial insemination. Nauplii are reared to the P.L.$_{10}$ stage in the individual larval culture unit. Each family is submitted to high-pressure selection for 60 days after experimental infection with IMNV.

During these 60 days of challenge, all families also undergo several physiological stress factors, such as a salinity stress from 34 to 1 ppt, hypoxia and a temperature drop from 29 to 22 degrees-C in order to combine viral challenge with stress conditions that naturally occur at shrimp farms.

Then families and individuals are selected for growth. Selected individuals are tagged with elastomer markers and transferred to tanks and ponds for growth evaluation and selection. After reaching optimal weight, selected families are transferred to hatchery quarantine.

Preselected broodstock are individually analyzed for detection of IMNV, IHHNV and NHP by loop-mediated isothermal amplification, nested PCR or real-time PCR. All virus carriers are discarded. Only disease-free broodstock are transferred to maturation in order to produce disease-free nauplii and

postlarvae.

Results: controlled conditions, farm

In controlled conditions of challenge with IMNV for 60 days, mean survival rate increased from 3.2 percent in the F1 generation to 55.3 percent in F7 – a significant increase in the resistance factor of 17.3 times (Fig. 1).

In 2007, a comparative study between selected and unselected postlarvae (P.L.) under farm production conditions was conducted. Forty ponds were stocked in April at a density of 30 P.L. per square meter. The F3 IMNV-resistant postlarvae reached a final survival rate of 57.0 percent, while only 24.4 percent of the unselected control P.L. survived.

The growth rate under intensive conditions with a stocking density of 100 P.L. per square meter was tested during the winter months of 2010 in outdoor grow-out tanks. The mean weekly growth rate reached 1.1 grams during 93 days with mean water temperature around 27 degrees-C.

Immune gene expression

Quantitative real-time PCR allows quantifying gene expression at the mRNA level. The authors considered hemocyanin an interesting immune gene due to its double function of oxygen transport and antimicrobial activity.

They compared the constitutive expression of hemocyanin in two generations of selected shrimp for their resistance to hypoxia (control, F1 and F2). The F2 shrimp showed a constitutive expression of hemocyanin 5.6 times higher than in the control. These results could partly explain the higher survival rates in the F2 and following generations at the farm.

The genes Dicer and Argonaute, which are involved in the gene-silencing machinery through RNA interference, have been studied as antiviral gene candidates. IMNV injection in shrimp caused an up regulation of these two genes. A comparative study established that resistant asymptomatic shrimp surviving an experimental infection with IMNV had a 10,000 times lower load of virus than symptomatic susceptible shrimp. Dicer and Argonaute mRNA quantification by real-time PCR showed an almost two-fold higher expression level in resistant asymptomatic shrimp.

Perspectives

The QGA program has been successful in selecting and producing postlarvae significantly more resistant to IMNV and adapted to culture conditions in the shrimp farms in northeastern Brazil. Considering the impacts of pathogens in most shrimp-producing countries and at present of IMNV in Asia, the use of these selected lines could be of great importance to reduce the impacts of pathogens on the sustainability of shrimp aquaculture.

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