Health & Welfare

Assessment of supplemental Bacillus probiotics in whiteleg shrimp juveniles

26 March 2018

By Jee Eun Han, DVM, Ph.D. , Ji Eun Kim, M.S. , Sung Hun Kim, Ph.D. , Jong-Su Eun, Ph.D. and Se Min Choi, Ph.D.

Feeding study sees improvement in growth performance, feed utilization, survival

Dietary supplementation of *Bacillus* probiotics improved growth, feed efficiency and survival (under high ammonia levels) of whiteleg shrimp juveniles. Photo by Darryl Jory.

Bacillus probiotics are spore-forming bacteria that are heat stable and are able to survive passage through the acidic stomach and make it to the gut, where they confer unique health benefits. In this study, we investigated the effects of supplementing three *Bacillus* species (*B. subtilis*, *B. pumilus*, and *B. licheniformis*) on growth performance, feed efficiency and survival (ammonium stress) in Pacific whiteleg shrimp juveniles. We thank all the staff at Nong Lam University, Ho Chi Minh City, Vietnam, for their technical assistance.

Experimental design

Whiteleg shrimp (*Litopenaeus vannamei*) postlarvae were cultured and acclimated in confined water tanks for one month to reach an average weight of 2.3 grams. From these, selected shrimp juveniles (n = 600) were randomly distributed into 500-liter circular tanks at a stocking density of 100 shrimp per tank. The experimental system included six tanks connected through a recycled water system with mechanical aeration and a bio-filter, and salinity was kept at 10 ppt.

Experimental diets were prepared by supplementing three powder forms of *Bacillus* spp. (1×10^9 CFU/g) with a combination of *B. subtilis, B. pumilus* and *B. licheniformis* at 0.2 percent, and a 40 percent fishmeal-based diet was formulated as a basal diet. Two dietary treatments were tested, and a control (without probiotics vs. with probiotics). The bacteria were originally isolated from seawater or intestinal microflora of shrimp and cultured, and their purity was routinely monitored. The tested *Bacillus* spp. were provided in an endospore form from the Bio-Resources Research Institute, CJ CheilJedang Corp. (Suwon, South Korea).

In the shrimp bioassay, shrimp were fed to satiation four times per day with the feeding rate estimated from 3 to 10 percent body weight. Unfed feed was collected, preserved in freezer, and used to calculate the real consumed feed from the distributed sum. Each diet was fed in three tanks.

Growth performance and feed utilization

The feeding trial lasted for eight weeks and the experimental animals reach a harvest weight of 15 to 20 grams. All shrimp in each experimental unit was weighed (initial and final weights). To evaluate growth performances and feed utilization of the shrimp, daily weight gain (DWG), specific growth rate (SGR) and feed conversion ratio (FCR) were calculated. Mortality in each tank was recorded daily during the entire experimental period in order to calculate survival rate. The following formulas were used

$DWG = [(W_{t2} - W_{t1})/(/T2 - T1)] \times 100$ (g/shrimp/day)

where:

Wt1 = average weight at the beginning of the experiment

Wt2 = average weight at the end of the experiment

$SGR = [(LnW2 - LnW1)/(T2 - T1)] \times 100$ (%/day)

where:

W2 = average weight at the end of the experiment

W1 = average weight at the beginning of the experiment

T2 – T1 = duration of the experiment

FCR = total feed intake/shrimp growth (W2 – W1)

Feed intake = total feed intake/total shrimp (g/shrimp/day)

Han, Bacillus, Table 1

	Control group	Probiotics group
Initial weight (g)	2.37	2.32
Final weight (g)	16.8	17.7
DWG (g/shrimp/day)	0.26	0.27
SGR (%/day)	3.47	3.62
Feed intake (g/shrimp/day)	0.22	0.24
FCR 1	2.30	2.06
FCR 2	2.14	1.97
Survival rate (%)	74.0	78.7

Table 1. Growth performances, feed utilization and survival rate of shrimp fed experimental diet for eight weeks.
DWG = daily weight gain
SGR = specific growth rates

FCR 1 = total feed intake per tank/total shrimp growth per tank (total W2 – total W1)
FCR 2 = feed per shrimp/(average final weight – average initial weight)

Ammonia challenge test

At the end of the feeding trial, 15 shrimp in each tank were maintained in the tank and then the ammonia concentration in the tanks was increased to 40 to 50 mg/L at pH 8.0 (lethal dose for 50 percent of animals, LD_{50}) using ammonium chloride (NH_4Cl), to evaluate stress resistance of shrimp. Mortality was observed until the shrimp all died, and results are shown in Fig. 1.

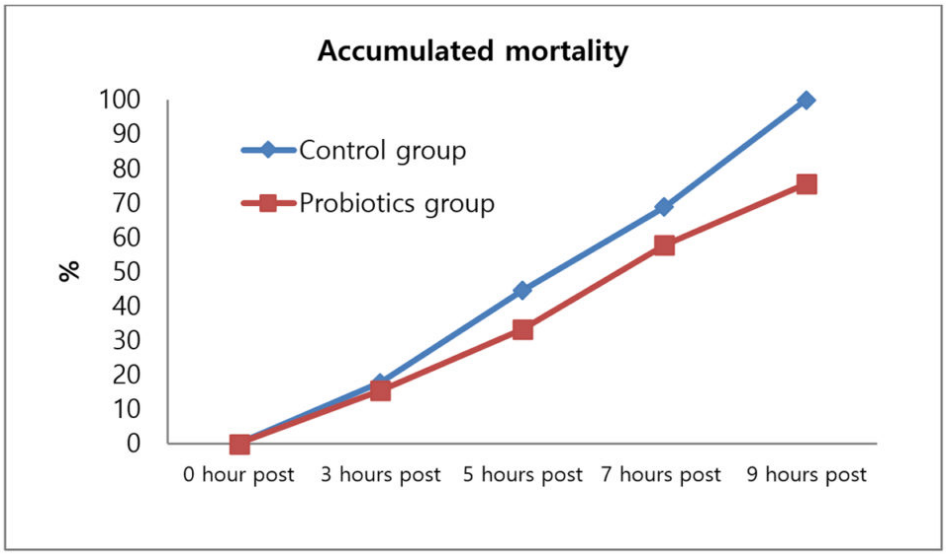

Fig. 1: Accumulated mortality rate of shrimp exposed to high ammonia concentration in hours post challenge.

Conclusions

During the study, all experimental diets were readily accepted by the Pacific whiteleg shrimp at the start of the feeding trial, and they consumed those aggressively during the eight weeks of the feeding trial. Results showed that when the *Bacillus* species were supplemented in an appropriate concentration into feeds, the growth and feed efficiency of whiteleg shrimp could be improved; and that the survival rate of shrimp could be greatly improved under both normal and stressed (high ammonia levels) conditions. Further studies are needed to evaluate the effectiveness of the *Bacillus* probiotics on shrimp in field trials.

Authors

JEE EUN HAN, DVM, PH.D.

Biotechnology Research Institute
CJ CheilJedang, Suwon
Republic of Korea

hanje1223@gmail.com (mailto:hanje1223@gmail.com)

JI EUN KIM, M.S.

Biotechnology Research Institute
CJ CheilJedang, Suwon
Republic of Korea

jieun.kim7@cj.net (mailto:jieun.kim7@cj.net)

SUNG HUN KIM, PH.D.

Biotechnology Research Institute
CJ CheilJedang, Suwon
Republic of Korea

sunghun.kim@cj.net (mailto:sunghun.kim@cj.net)

JONG-SU EUN, PH.D.

Biotechnology Research Institute
CJ CheilJedang, Suwon
Republic of Korea

jongsu.eun@cj.net (mailto:jongsu.eun@cj.net)

SE MIN CHOI, PH.D.

Biotechnology Research Institute
CJ CheilJedang, Suwon
Republic of Korea

sm.choi@cj.net (mailto:sm.choi@cj.net)