Yeast parietal fractions enhance the immune response of shrimp towards AHPND infections

By Dang Thi Hoang Oanh and Philippe Tacon

Four years ago, the early mortality syndrome (EMS) or acute hepatopancreatic necrosis disease (AHPND) appeared in shrimp farms in Asia. The disease has devastated the shrimp industry in China, Vietnam, Malaysia and Thailand. This disease is caused by a bacterium *Vibrio parahaemolyticus* which is potentially modified by a bacteriophage agent, causing a toxin secretion (TCP/VIE/3304 report, 2013). In order to prevent this disease, one of the measures taken by the industry is to design specific feeds for the shrimp.

Parietal fractions are isolated from yeasts and contain mannan oligosaccharides, beta glucans, chitin and components that are potential immune-stimulants (Ringo et al., 2012), and they have been shown to prevent bacterial infections. The expertise of Phileo Lesaffre Animal Care business has specifically designed parietal fractions with known and consistent concentrations of these components using a specific strain and optimised production processes including drying.

The aim of this study was two-fold: first to determine the optimum dosages for the parietal fraction Safmannan® to increase the survival of shrimp against the AHPND, and second to test if a short immune-stimulation of two weeks was sufficient.

Experimental setup

White leg shrimp *Penaeus vannamei* juveniles (2-3 g/individual) were spawned and nursed at the shrimp hatchery and nursery of College of Aquaculture and Fisheries, Can Tho University, Vietnam. Shrimp were screened for white spot syndrome virus (WSSV), taura syndrome virus (TSV) and *V. parahaemolyticus* to make sure they are free from these pathogens prior to distribution to experimental aquaria.

The experiment was set up in the wet laboratory at Can Tho University. Experimental glass aquaria (30L in volume) were used. The aquaria contained 70% volume of seawater (25 ppt) with aeration system and temperature control around 28-30°C. Experimental shrimp were stocked at a density of 30 individuals/aquarium. Each treatment was set up in triplicates as detailed in Table 1.

Feeding

The treatment diets were commercial pelleted feed supplemented with the parietal fraction Safmannan® at an inclusion rate of 0.5, 2 and 5 g/kg of feed (Table 1). Shrimp were fed these diets *ad libitum* for two weeks before being challenged with *V. parahaemolyticus*. The control treatment diet was a commercial pelleted feed. These feeds were also fed to the juveniles for the three-week post-challenge tests as detailed in Figure 1.

Bacteria challenge

The bacterial strain, *V. parahaemolyticus* (identified by using conventional biochemical tests, API 20E and PCR) which is responsible for AHPND, was used for the challenge experiment (Nguyen et al., 2014). Strains were stored at -80°C in iso-sensistest broth (Oxoid) containing 25% glycerol. Bacterial cultures were grown 24 hours in 150 ml of tryptone soya broth (Oxoid) at -28 °C. A 150 ml of bacterial culture was prepared for each tank. The optical density of bacterial cells was estimated using spectrophotometer. Bacterial suspensions were adjusted to the concentrations of 10^8 cells/ml.

Shrimp were immersed for 15 minutes in bacterial culture with continuous aeration and then both bacterial solution and shrimp were transferred to individual experimental aquarium containing sea water to reduce the bacteria density to 10^6 cells/ml. Shrimp were observed for 3 weeks post challenge to record mortality and clinical signs. There was no water exchange for 2 days after the challenge. Water exchange was subsequently increased to 20% daily for the rest of the experimental period. Shrimp were fed with normal feeds after the challenge.

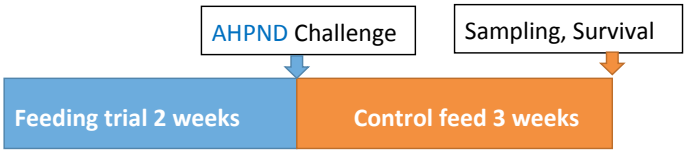

Figure 1: Experimental set up

Table 1. Experimental set up to test the response to EMS/AHPND of juvenile shrimp *P. vannamei* fed with parietal fraction.

Name of treatment	Safmannan® (g/kg feed)	Application to shrimp	Treatment of Shrimp
S500	0.5	Product mixed in shrimp diet	Challenge with *Vibrio**
S2000	2	Product mixed in shrimp diet	Challenge with *Vibrio**
S5000	5	Product mixed in shrimp diet	Challenge with *Vibrio**
Negative Control	0	Normal shrimp diet, no product	No challenge
Positive Control	0	Normal shrimp diet, no product	Challenge with *Vibrio**
Safmannan® control	2	product mixed in shrimp diet	No challenge
(*) *V. parahaemolyticus* infected by the phage			

Data collection

After the performance of the test the following parameters were analysed: (1) gross signs of AHPND; (2) survival rate (%) after 21 days post challenge; (3) total haemocyte count; (4) prophenoloxidase (PO) activity and (5) respiratory burst activity. All the data were analysed by using Excel and SPSS programs.

Gross signs of EMS

Gross signs and hepatopancreas (HP) of experimental shrimp at 3 days post challenge are shown in Figures 2 and 3. Shrimp in the control group (negative control and Safmannan® control) looked apparently healthy with good HP and full gut content (Figure 2).

Figure 2: Control shrimp look apparently healthy. A: Negative control; B: Safmannan® control

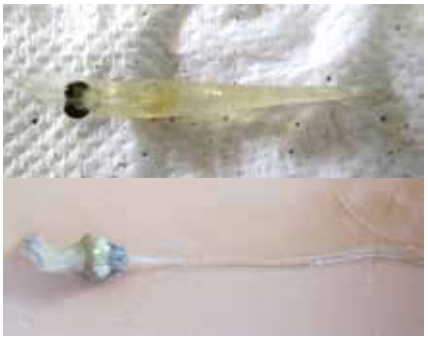

Figure 3: Gross signs and HP of shrimp in positive control (challenged with *V. parahaemolyticus*)

However, infected shrimp in the positive control and other tests which were challenged with EMS showed typical gross signs of EMS including abnormal and lethargic swimming behaviour, animals are relatively weak, with soft shell, gut with discontinuous content and the HP became darkened or pale colour (Figure 3). A few shrimp sank to the bottom of the aquarium.

Histological examination

Healthy shrimp from the control group and moribund shrimp from challenged groups were collected and subjected to histological analysis. As a result, shrimp that were apparently healthy had healthy HP with E, B, R and F cells (Figure 4), whereas, shrimp that had displayed typical histopathology of AHPND including dysfunction of hepatopancreatic cells, lack E, B and R cells, exhibit HP tubule epithelium sloughing and significant proximal haemocytic inflammation (Figure 5). Histopathological lesions were similar to typical EMS pathology described by Lightner (2012) and Loc et al. (2013).

Survival at 3 weeks post challenge

At 3 weeks post challenge, the positive control group (normal feed and challenge with *Vibrio*) had a significant lower survival rate in comparison to the remaining groups (Figure 6). We did not see any significant difference in survival of shrimps in the negative control and Safmannan® control (fed with normal feed mixed with parietal fraction and no *Vibrio* challenge). The survival in these groups ranged from 90.3-90.7%. In groups fed with the parietal fraction, there was a significant difference with the positive control treatment but no difference in survival between shrimp receiving treatments at 2 g/kg feed and 5 g/kg feed whereas, a significant difference was observed between treatment 0.5 g/kg feed and 5 g/kg feed.

In general, shrimp fed with the products had higher survival rates after being challenged with *V. parahaemolyticus* compared to the control (without feeding the products but challenged with *V. parahaemolyticus*). The survival rate of infected shrimp treated without the product dropped to 38.9% on the 21st day of the

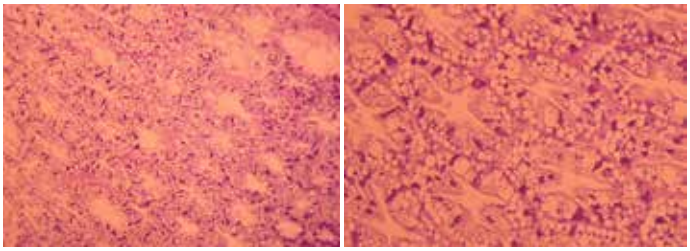

Figure 4: Healthy HP of experimental shrimp (20X and 40X)

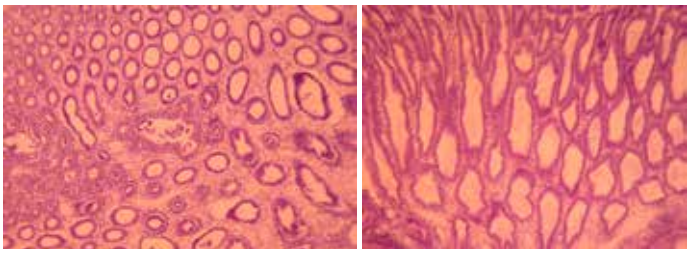

Figure 5: HP of infected shrimp collected from experimental tanks. Dysfunction of hepatopancreatic cells, lack of E, B and R cells. HP tubule epithelium sloughing, significant proximal haemocytic inflammation (10X & 20X).

challenge, which was significantly lower than that of Safmannan®-treated shrimp (survival rates ranged from 54.2% – 63.9%).

Total haemocytes count

At different sampling times

No significant difference was found in total haemocyte count (THC) of experimental shrimp in all the tested groups except for groups which were fed with normal feed and challenged with *Vibrio*. There was no significant difference in THC before feeding and before challenge. However, THC decreased significantly after the challenge test.

Between treatment groups

There was no significant difference in THC of experimental shrimps in different tests before feeding, and before and after challenge (Figure 7).

Prophenoloxidase

At different sampling times

We did not see any significant difference in PO in negative control shrimp group before feeding, before being challenged and at sampling time. However a significant decrease was observed in PO of experimental shrimp in positive control after being challenged compared to shrimp before feeding and before being challenged. We can also see a significant increase in all treatment groups after feeding.

PO in shrimp fed Safmannan® control significantly decreased at sampling time compared to shrimp after two weeks feeding but this was not different when compared to PO in shrimp before feeding the products. We can then suggest that stopping feeding of the product brought back the PO levels to their original values. In treatment groups challenged by EMS, the PO values also dropped, but in S2000 and S5000 they remain high and were not different from the values before feeding (Figure 8).

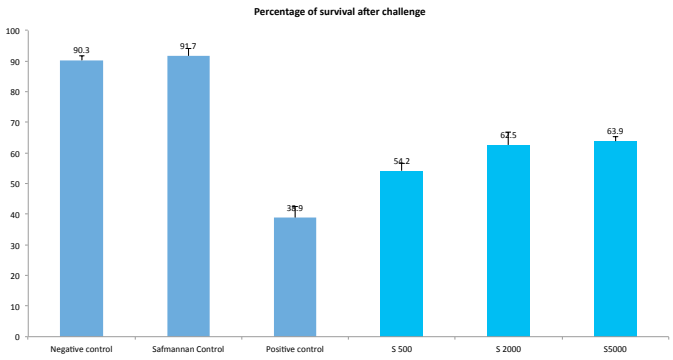

Figure 6: Survival percentage of shrimp at 3 weeks post challenge (mean ± SD). Small letter denotes a significant difference between treatments using one-way ANOVA (P < 0.05).

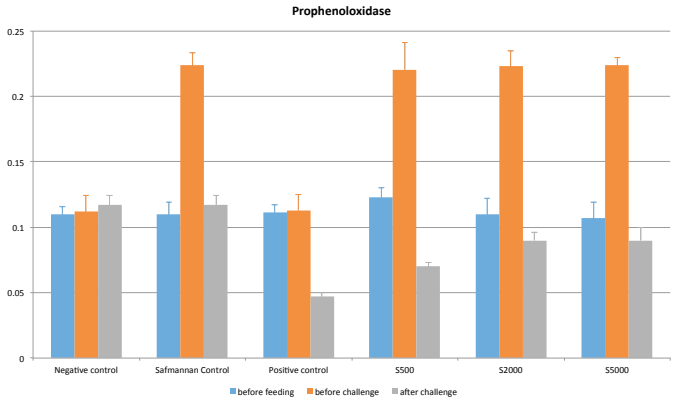

Figure 8: Prophenoloxidase activity (units/min/mg protein) in experimental shrimp at different sampling time intervals. Mean (±SD). Small letter denotes a significant difference between treatments after challenge using one-way ANOVA (P < 0.05). Asterisk shows differences between sampling times (P<0.05).

Between treatment groups

This trial shows that the parietal fraction significantly increases PO activities in all treated groups compared to control groups. We also see that after an AHPND challenge, the parietal fraction at all concentrations tested maintained higher values compared to the positive control. We did not see any difference in respiratory burst between groups and sampling times in this trial.

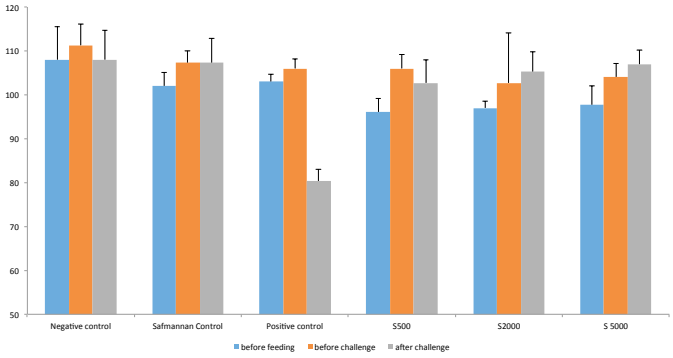

Figure 7: Total haemocytes (10^2 tb/mm^3) in experimental shrimp with different sampling time intervals. Mean (±SD, are not significantly different by One way ANOVA)

Conclusion

The results showed that Safmannan® can maintain high total haemocyte counts after an AHPND challenge. It also maintains higher prophenoloxidase activities, hence increasing the survival of shrimp. It was also shown during this trial that the parietal fraction can have a rapid action on the immune system of shrimp as a two-week feeding program was sufficient to increase survival of shrimp. We can therefore conclude that this product can be a good candidate to be included in feeds to protect against EMS/ AHPND.

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