Marine protein hydrolysates as shrimp immune modulators

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Are functional feeds a part of the answer to sustainability of the shrimp aquaculture industry?

Shrimp aquaculture production recently exceeded fish production in global fisheries supply. In return, due to rapid intensification, shrimp industry is now facing critical issues, which may jeopardise its sustainability. Some of the more challenging issues are the depletion of marine resources, the deterioration of the environment and the dramatic production losses resulting from the cyclic emergence of disease outbreaks.

However, shrimp are known to be tolerant to several viruses, as asymptomatic carriers. In addition, most bacterial strains involved in bacterial shrimp diseases are widely present in the environment and remain harmless under normal conditions. This is the case for *Vibrio* species such as *V. alginolyticus, V. harveyi* or *V. parahaemolyticus*. According to Tran et al. (2013), the latter is now identified as the indirect cause of mass mortalities associated to EMS (early mortality syndrome) or AHPND (acute hepatopancreatic necrosis disease). There is currently no generally approved treatment, or practice, which can be implemented to prevent EMS from occurring. Inbreeding, high water pH, feed pollution etc have however been identified as aggravating factors. Besides the implementation of better husbandry and genetic improvement practices, nutrition is the third most important way of improving shrimp culture sustainability, especially through the design of functional feeds.

Several studies refer to the use of beta-glucans, LPS (LipoPolySaccharide), pro and prebiotics, algae and plant extracts as potential shrimp immune modulators (Zhang and Mai, 2010). Fewer studies have been reported on the use of marine protein hydrolysates (MPH). These studies have shown that their palatability, nutritional and health benefits have positive advantages in the culture of several terrestrial and aquatic species. Health benefits shown by MPH are due to their natural high contents in small soluble nitrogen nutrients such as free amino acids and their derivatives (taurine for instance), nucleotides and peptides. These bioactive nutrients have documented anti-oxidative, anti-stress, anti-microbial and/or growth hormone like activities.

The purpose of this trial was to assess the growth and immune benefits of different MPH in white shrimp, when supplemented in a commercial diet.

Experimental trials

Trials were conducted in thirty 400 L tanks (Figure 1), with each tank individually fitted with water supply and aeration devices. Environmental parameters remained within requirements for the Pacific white shrimp *Penaeus vannamei* with an average water temperature of 26-27 °C, dissolved oxygen (DO) of 4-5 ppm and salinity of 35 ppt. Water renewal rate was a minimum of 10% per day; feeding wastes and faeces were removed daily. Specific pathogen free (SPF) certified juveniles (average weight 1.2 g) were randomly stocked in each experimental tank (n=50) and acclimated for 1 week on a commercial diet (a standard shrimp diet commonly found in Ecuador with 28% of crude protein).

Experimental diets consisted of the commercial diet supplemented with either 5% fish hydrolysate (FH), 5% squid hydrolysate (SqH), 3% krill hydrolysate (KH) or 2% shrimp hydrolysate (SH) on a w:w basis. Proximate and peptide profiles of supplemented hydrolysates are illustrated in Table 1.

Experimental facilities

Table 1: Nutritional and peptide size profiles of supplemented hydrolysates

	FH	KH	SqH	SH
Dry matter (% of product)	26.50	58.18	43.81	96.01
Crude protein (% of product)	15.50	44.85	23.16	64.90
Crude fat (% of product)	3.60	1.56	13.45	10.81
Ash (% of product)	6.50	11.59	5.24	10.33
Soluble nitrogen (% of crude protein)	84.73	96.28	80.00	90.02
Peptides < 0,5kd (% of soluble protein)	90.14	63.98	32.08	82.67
0.5kd < Peptides < 1.0kd (% of soluble protein)	5.26	15.95	10.60	8.84
1kd < Peptides < 5kd (% of soluble protein)	4.19	17.74	48.15	7.76
5kd < Peptides < 10kd (% of soluble protein)	0.26	2.03	4.77	0.58
10kd < Peptides < 20kd (% of soluble protein)	0.08	0.26	2.74	0.11
Peptides > 20kd (% of soluble protein)	0.06	0.04	1.66	0.04

FH- fish hydrolysate, KH krill hydrolysate, SqH-squid hydrolysate and SH- shrimp hydrolysate

Dosages of hydrolysate used were adjusted to provide similar amounts of crude protein. The commercial diet, used as the control diet (COM), was initially crumbled and re-pelleted to allow the incorporation of the hydrolysates. Each experimental diet was randomly allocated to six replicates.

Shrimp were fed 4 times a day using a commercial feeding table as an indicative reference. During this study, we only focused on growth and health benefits resulting from the MPH supplementation. Feed wastes were therefore not collected and feed conversion ratio (FCR) was not calculated.

During the 8 weeks feeding trial, shrimp were sampled every second week for mean weight assessment.

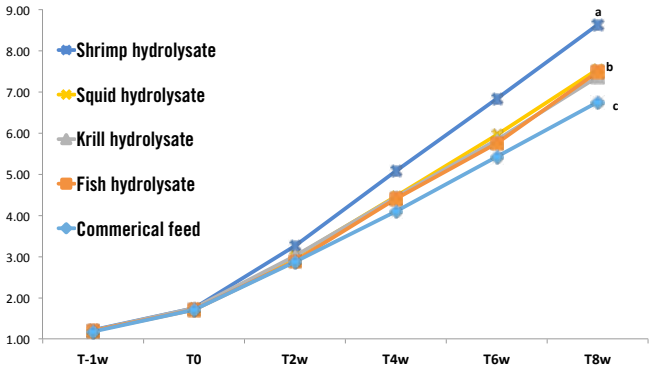
At the end of the feeding trial, shrimp were injected with either a 20 µL saline solution (n=12 shrimp per experimental diet) or a 20 µL suspension of *V. harveyi* at 10^6 UFC/mL (n=24 shrimp per experimental diet). At 3 hours post induction, haemolymph was sampled for the assessment of plasma antimicrobial activity. For each experimental diet, 12 shrimp were sampled before induction, as a control.

Plasma samples were collected after haemolymph micro-centrifugation. Plasma samples were incubated with a suspension of *V. harveyi* (with an optical density (OD) = 0.5 before a 1:10 dilution) and 200µL of growing medium. After an hour of incubation, plates were read for OD for the first time (T0), before a second reading 4 hours later (T4). All plasma samples were analysed in triplicate and compared to blank samples consisting of bacterial suspension incubated with growing medium but without plasma. Plasma antimicrobial activity was defined as the percentage of bacterial growth inhibition resulting from the plasma activity = 100 – 100 x (ΔOD samples/ΔOD blanks). All results were statistically analyzed by ANOVA followed by student t- test.

Better growth performance

There was no mortality observed during the feeding trial. After 56 days of feeding trial, FH, KH and SqH supplemented diets resulted in heavier shrimp (+11% on average) and higher growth rates (+14% - p<0,001; Figure 1). SH supplemented diets resulted in the best growth rates with an increase of 37% of the weekly growth rate. This increase was already significant only 2 weeks after starting the feeding trial.

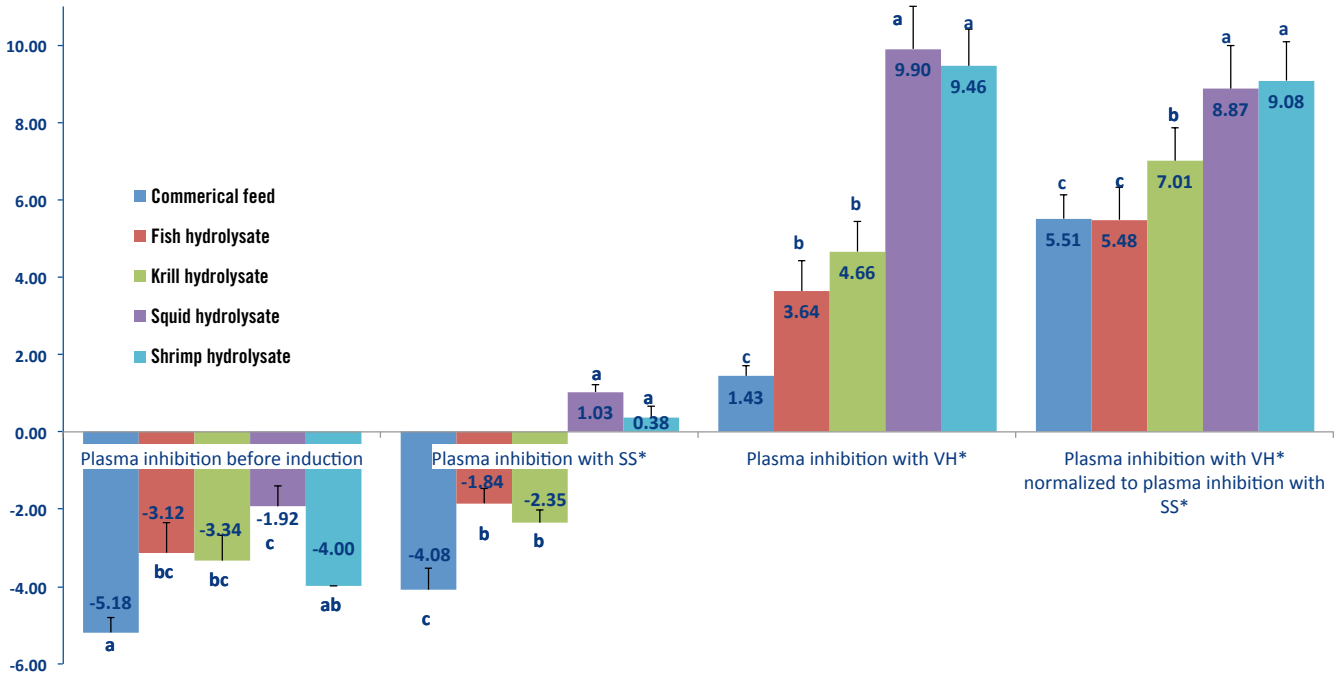
Figure 1. Shrimp mean weights (g) during 8 weeks feeding trial



MPH have been shown to enhance growth of many farmed species. Growth enhancement is usually related to MPH inherent properties, which make feeds supplemented with MPH highly palatable and digestible. Spared energy from homeostasis and feed digestion process are most likely re-allocated to growth process.

Once the feeding trial had been completed, shrimp were sampled for the plasma antimicrobial assay.

Figure 2. Shrimp plasma inhibition before and after induction (in % of OD) with either saline solution (SS) or *V. harveyi* suspension (VH)



*SS : Saline solution
*VH: *Vibrio harveyi*

Shrimp plasma antimicrobial activity was found to significantly increase ($p<0.01$; Figure 2) with all dietary MPH, before or after inductions, when compared to the control diet. While plasma antimicrobial activity was non existent before induction, injection of saline solution was enough to stimulate plasma inhibition of bacterial growth, most likely through the healing process of the injection site. This process was visibly enhanced by dietary supplementation of MPH.

As expected, the immune response resulting from *V. harveyi* induction was much higher than the one observed after an induction with saline solution. Dietary MPH supplementation significantly improved this immune response as was seen by the comparison with the control diet. We normalised plasma inhibition results to reduce the impact of the injection process on shrimp immune response. Once this normalization process was done, relative plasma antimicrobial activity remained significantly higher for all supplemented diets, but the diet FH. The most effective MPH were diets SqH and SH (+65%). These results show that all MPH most likely act at different stages and levels of shrimp immune process. Before bacterial infection, they may increase shrimp plasma levels into 'non specific' antimicrobial compounds while after bacterial induction, some MPH, including SqH and SH, seemed to stimulate an immune response through the production and release of high levels of non specific and/or specific antimicrobial compounds.

Perspectives

Results of this study suggest that MPH could be a cost and nutritionally effective alternative to antibiotics or other health additives when dealing with opportunistic pathogens. Besides enhancing shrimp productivity through higher growth rates, MPH could improve shrimp health and resistance to opportunistic infections. By supplementing shrimp commercial diets with MPH, it is therefore possible to design functional or bioactive feeds, which could be helpful in this context of sanitary concerns. Last but not least, reducing the rearing periods by increasing growth rates will also allow shrimp farmers to reduce the risk of facing an acute disease outbreak before harvesting. Further investigations have to be made to get a mechanistic understanding of the immune system activation induced by dietary hydrolysate.

References

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