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Mannoprotein dietary supplementation for Pacific white shrimp raised in biofloc systems

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ABSTRACT

In order to reduce disease outbreaks in shrimp aquaculture, biosafe farming practices have been created, such as the biofloc system and the use of feed additives to improve shrimp immune response and growth performance. Many studies with feed additives have tested probiotics in biofloc rearing systems; yet to date, no studies have reported on the use of prebiotics in the feed of farmed shrimp in biofloc systems. Moreover, the effects of mannoprotein (MP) prebiotics on shrimp and shrimp farming are also unknown. Therefore, this study aimed to evaluate the effects of dietary supplementation with mannoproteins (MP) on the performance, immune response, and midgut intestinal villi of *L. vannamei* raised in a biofloc system. Three diets with different MP concentrations were formulated (0.02%; 0.08%; 0.12%) and one control group without MP additive. The experiment utilized 12 experimental units (800 L) stocked with 400 shrimp/m^3, with 3.64 ± 0.07 g of initial weight, in triplicate. Shrimp were fed four times per day, and both histology of the midgut and growth indices were assessed after 65 days. Intestinal villi presented a larger internal surface area in animals that were fed dietary supplements of 0.08% and 0.12% MP ($p < .05$). Shrimp fed with MP showed an approximate 10% increase in survival compared to animals in the control treatment ($p < .05$). No statistical differences were found in the growth parameters (final weight, weekly weight gain, final biomass, feed efficiency) or survival after challenge with *Vibrio parahaemolyticus*. Prior to infection, no differences were found in any of the immunological parameters analyzed. However, after infection, the production of superoxide anion after feeding with 0.12% MP showed higher ROI values. Therefore, dietary supplementation with different concentrations of mannoproteins resulted in an increase in survival and average surface area of shrimp midgut, as well as higher superoxide anion production in shrimp fed with 0.12% MP after infection with *V. parahaemolyticus*.

1. Introduction

Among today's farmed shrimp species, the Pacific White shrimp *Litopenaeus vannamei* made up 74% of all shrimp farmed in 2013 (FAO-FISHSTAT, 2013). Despite significant growth in global production, it is common to have regional decreases in production, thus affecting producers and the region's economy (Shekar et al., 2012). Viral and bacterial diseases are the main causes of decreased shrimp production. Such diseases are triggered by the use of infected post-larvae, adverse climatic conditions, dietary deficiency, toxic compounds in water, eutrophication, and accumulation of organic matter (Kautsky et al., 2000).

Recent findings have identified a disease known as Acute Hepatopancreatic Necrosis Syndrome (AHPNS), which is caused by *Vibrio parahaemolyticus* (Tran et al., 2013). This disease has led to a massive mortality rate of Pacific white shrimp in captivity, affecting China, Vietnam, Malaysia, Thailand (Lightner et al., 2012) and Mexico (Nunan et al., 2014). The use of unknown antibiotics as treatment for

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bacterial infections can promote the development of resistant strains, thus complicating the control of such diseases (Defoirdt et al., 2011). One alternative is the use of feed additives (Silva et al., 2013). Feed additives are given in small amounts, and they are known to improve the characteristics of the feed or the animal products (Rosen, 1996; FAO, 2001). Among the many types of additives used, prebiotics can act as modulators of intestinal mucus, promoting the development of healthy bacteria and reducing colonization of pathogenic species (Merrifield et al., 2010).

Prebiotics are nondigestible ingredients that benefit the host's intestinal microbiota, selectively stimulating growth and/or activity of certain bacteria and improving the host's health (Gibson and Roberfroid, 1995). The most commonly used prebiotics in aquaculture are Fructooligosaccharides (FOS), Galacto-oligosaccharides (GOS), and Mannan-oligosaccharides (MOS), as well as inulin polysaccharides and β-glucanase (Song et al., 2014). They can increase intestinal villi and microvilli, promote immune response, and positively impact the growth parameters of fish and shrimp (Zhang et al., 2012; Torrecillas et al., 2013; Sang et al., 2014). Despite preliminary studies on the use of prebiotics in aquaculture, the mannoprotein (MP) derived from yeast cell walls of *Saccharomyces cerevisiae* (Morales-López et al., 2009) has not yet been studied in shrimp farming.

Apart from the use of feed additives to control disease rates and increase shrimp and fish farming productivity, biofloc systems are another option (Avnimelech, 1999). By increasing rearing density, this system increases productivity since heterotrophic bacteria control the buildup of nitrogen compounds (Avnimelech, 1999; Ebeling et al., 2006), along with chemoautotrophic bacteria in microbial bioflocs (Ebeling et al., 2006). In this rearing system, the chance of introducing pathogens and disease vectors is reduced because less water renewal is needed to maintain water quality for the reared animals (Crab et al., 2010).

Many studies with feed additives have tested probiotics in biofloc rearing systems; yet to date, no studies have reported on the use of prebiotics in the feed of farmed shrimp in biofloc systems. Moreover, the effects of mannoprotein (MP) prebiotics on shrimp and shrimp farming remain unknown. Therefore, this study aimed to evaluate the effects of dietary supplementation with mannoproteins on the performance, immune response, and midgut intestinal villi of *L. vannamei* raised in a biofloc system.

2. Material and methods

The experiment was conducted in the Laboratório de Camarões Marinhos (LCM), Departamento de Aquicultura, Universidade Federal de Santa Catarina (UFSC), in the southern region of Brazil, from July to September 2014, for a total duration of 65 days.

2.1. Biological material

We used a Specific-pathogen-free (SPF) species (as mandated by the World Animal Health Organization), namely the marine shrimp *L. vannamei*, as our model animal. Animals were obtained from Aquatec LTDA, located in Canguaretama, Rio Grande do Norte State, Brazil. The shrimp were reared in a biofloc system until reaching an average initial weight of 3.64 ± 0.07 g.

2.2. Preparation of the experimental diets

Five dietary experiments were formulated using 39% brut protein (Table 1), based on the nutritional recommendations and requirements of *L. vannamei* (Fox et al., 1995; NRC, 2011; Zhou et al., 2012). Such diets were formulated using Feedsoft® Professional software, version 3.14 (Feedsoft Corporation, Richardson, TX, USA).

Table 1
Formulation and composition of experimental diets for the juvenile marine shrimp *L. vannamei* with increasing supplementation of mannoprotein (MP).

Ingredient ($g \cdot kg^{-1}$)	Mannoprotein %			
	Control (0.0)	0.02	0.08	0.12
Soybean meal	450.0	450.0	450.0	450.0
Salmon meal by product	190.0	190.0	190.0	190.0
Wheat flour	150.0	150.0	150.0	150.0
Kaolin	90.0	89.8	89.2	88.8
Soybean lecithin	30.0	30.0	30.0	30.0
Monocalcium phosphate	20.0	20.0	20.0	20.0
Sodium chloride	15.0	15.0	15.0	15.0
Magnesium sulfate	15.0	15.0	15.0	15.0
Vitamin-mineral Premix[1]	13.5	13.5	13.5	13.5
Oil. cod liver	10.8	10.8	10.8	10.8
Oil. soybean	10.0	10.0	10.0	10.0
Carboxymethyl cellulose	5.0	5.0	5.0	5.0
Vitamin C	0.7	0.7	0.7	0.7
Mannoprotein[2]	0.0	0.2	0.8	1.2
Chemical composition (%, expressed as dry matter)				
Ash	9.4	8.0	8.4	6.9
Crude protein	43.7	43.3	42.7	42.8
Ashes	18.7	18.6	18.7	18.7
Ethereal extract	8.7	8.4	8.6	9.0
Crude fiber	4.4	5.4	6.3	3.7

[1] Vitamin-mineral Premix: vitamin A, 1250,000 UI; vitamin D_3, 350,000 UI; vitamin E. 25,000 UI; vitamin K_3, 500.0 mg; vitamin B_1, 5000.0 mg; vitamin B_2, 4000.0 mg; vitamin B_6, 10.0 mg; nicotinic acid, 15,000.0 mg; pantothenic acid, 10,000.0 mg; biotin,150.0 mg; folic acid, 1250.0 mg; vitamin C, 25,000.0 mg; choline, 50,000.0 mg; inositol, 20,000.0 mg; iron. 2000.0 mg; copper, 3500.0 mg; copper chelate, 1500.0 mg; zinc, 10,500.0 mg; zinc chelate, 4500.0 mg; manganese, 4000.0 mg; selenium, 15.0 mg; selenium chelate, 15.0 mg; iodine, 150.0 mg; cobalt, 30.0 mg; chromium, 80.0 mg.
[2] Alltech Brasil Agroindustrial Ltda (Araucária, PR, Brasil).

2.3. Experimental design and units

The experiment was performed in a biofloc system. The experimental design was completely randomized with three replicates, and three concentrations of mannoprotein (Actigen®, Alltech Brasil Agroindustrial Ltda, 0.02%, 0.08% and 0.12%) were tested in the diet with an additional control group without inclusion of mannoprotein. Rearing was carried out inside a greenhouse covered with a 20-μ polyethylene film. Twelve experimental units, consisting of polyethylene circular tanks with a flat bottom and 800 L volume capacity, were used. Each tank was equipped with a titanium electric heater (800 W), controlled by a thermometer (Fullgauge®, model MT511Ri); a central air ring at the bottom with a 40 cm microporous hose and powered by an electric radial blower; a shrimp feeding tray (Wasielesky et al., 2006); six artificial submersed substrates, equivalent to 100% of a tank's surface area (Schveitzer et al., 2013a); and a 20 L volume solids settling tank.

All tanks were filled with water from bioflocs of a matrix tank, with salinity levels of 35 $g \cdot L^{-1}$, alkalinity 156 $mg \cdot L^{-1}$, pH 7.86, ammonia 0.4 $mg \cdot L^{-1}$ and nitrite 0.1 $mg \cdot L^{-1}$. Each unit was stocked with 320 shrimp with an average weight of 3.64 ± 0.07 g, for a total initial rearing density of 400 shrimp m^{-3}. In the first week, tanks were fertilized daily with sugarcane molasses to maintain a carbon/nitrogen ratio (C/N) of 12/1 (Avnimelech, 2012). The molasses was comprised of 55% reducing carbohydrates and 2.7% protein, and the feed that was used contained 42.7% to 43.7% crude protein. The content was thus regarded as 40% carbon in carbohydrates, 50% carbon in the feed and 16% nitrogen in the proteins of the molasses feed (Ebeling et al., 2006). The quantity of daily molasses was split into two feedings, one in the morning and the other in the afternoon. Alkalinity was kept above 120 $mg \cdot L^{-1}$ $CaCO_3$, using hydrated lime.

Feeding was administered in accordance with the Van Wyk and Scarpa Table (1999). Initially, the animals were fed at levels of 6% of