Prebiotic Evaluation of Copra-Derived Mannooligosaccharides in White-Leg Shrimps

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Abstract

β-Mannooligosaccharides, produced by partial enzymatic hydrolysis of copra pulp residues using recombinant A. niger β-mannanase, were evaluated for its potential use as a prebiotic feed supplement for L. vannamei culture. Results observed in a 30-day feeding trial showed that dietary supplementation of copra-derived MOS, with doses varying from 4 to 10 g kg⁻¹ of dried feed, increased the number of intestinal Lactobacilli and Bifidobacteria of shrimps fed copra-MOS supplemented diets increased approximately from 150-300%. The number of intestinal presumptive Vibrio, Clostridia, and Salmonella reduced to 39.6-54.1%, 56.9-65.9%, 71.8-86.1% and 100-100%, respectively. In addition, copra-MOS supplement in the feed led to an enhancement of the weight gain, specific growth rate, feed conversion ratio and feed intake of shrimps (P<0.05). After a 7-day challenging test with Vibrio harveyi pathogen (~10⁷ CFU mL⁻¹) by immersion, the cumulative mortality of the shrimps fed with the diet supplemented 10 g kg⁻¹ copra-MOS was 3.5%, that obviously decreased compared to the control shrimps (29.5% mortality). Taking advantage of its intestinal microflora modulation towards increasing probiotic-like bacteria and Vibriosis resistance, this cheap oligosaccharide will be valuable in various aquatic animal farming.

Keywords: Copra; Mannooligosaccharides; Prebiotics; Litopenaeus vannamei; Growth; Intestine bacteria; Disease resistance

Introduction

Whiteleg shrimp, Litopenaeus vannamei, is among high commercial value aquatic species being cultured recently worldwide [1]. However, because of the rapid development, coupled with unusual climate change, the shrimp farming has severely suffered significant economic losses due to viruses and vibriosis diseases [2-4]. Traditional uses of antibiotics for shrimp disease control have been criticized due to the potential development of multi-antibiotic resistance of pathogenic bacteria, reduction in the efficacy of antibiotic treatments, residue accumulation in tissues, and immunosuppression [5-9]. Therefore, using probiotics, prebiotics, and medicinal plants as preventive, environment-friendly and economically efficient alternatives to antibiotic in shrimp farming has been received heightened attention in recent years [10-13].

“Prebiotics are defined as nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth of and/or activating the metabolism of one or a limited number of health-promoting bacteria in the intestinal tract, and thus improve host health” [14]. It have been reported that fructooligosaccharides, inulin, and isomaltooligosaccharides showed the prebiotic characteristic in whiteleg shrimp [15-18].

MOS is basically constituted by linear chains of mannose. Several commercial products of MOS were in trade with various purities and they differ in their molecular structure, depending on the source and processing conditions. The MOS recently used to cultivate shrimps was extracted from yeast cell-wall and it consists of the chain of mannose residues linked together via α-1, 6-glycosidic bond [12,19,20]. Another kind of MOS is β-1, 4-mannooligosaccharides (β-MOS) produced by enzymatic hydrolysis of plant mannans via β-mannanases [21]. This β-MOS is generally purer and shows excellent prebiotic efficacy on humans and terrestrial animals at low doses. However, as for aquatic animals, there is little known about the effectiveness of any β-mannooligosaccharides at present.

Defatted copra meal’s carbohydrates is abundant agricultural waste which is discharged by coconut industries. Balasubramanian [22] found that content of copra meal was 23% of mannans and 61% galactomannans. This material is very popular in Vietnam to produce MOS [21].

The aim of this study was to evaluate the effects of copra MOS on growth, survival, intestinal microflora, and bacterial pathogen resistance of whiteleg shrimp.

Materials and Methods

Copra MOS and diet preparation

The prebiotic used in this study, mannooligosaccharides, was produced by partial enzymatic hydrolysis of copra pulp residues obtained from coconut oil processing facilities using a recombinant β-mannanase (from Aspergillus niger BK01) [21]. The minimum level of mannooligosaccharides was 90% ww⁻¹. The copra MOS powder was free of antibiotics (tetracycline, chloramphenicol, and chlorotetracycline), aflatoxin B1 and bacterial pathogens (Vibrio cholera, Vibrio parahaemolyticus, Salmonella, E. coli).

Five diets (control, MOS4, MOS6, MOS8 and MOS10) were formulated with supplementation of various amounts of copra MOS (0, 4, 6, 8, or 10 g) in 1 kg of dried feed. Copra MOS supplied in the powder form was diluted in an adhesive and feed attractant oil (squid liver oil, 30% v/w).

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Kaikoh, Japan, 30% omega 3 acid), and then sprayed onto commercial feed pellets (Hipo 1-4 No, CP Vietnam, Vietnam, 42% protein) in all treatments (including control group). Feed, prepared for 10 days, was dried at room temperature for 4 h and then stored at 4°C.

**Feeding trials**

Juvenile white-leg shrimp (*Litopenaeus vannamei*) was supplied by UNI-President Company (Ninh Thuan, Vietnam) and shipped to the farm of NACMAN (Hai Thanh, Duong Kinh, Haiphong, Vietnam) in a plastic container (250 L) provided with seawater and aeration. The collected shrimp was passed the tests of WSSV, TSV, IHHNV, YHV, and MBV and had no sign of bacterial infections.

Prior to the start of the feeding trials, experimental shrimps were fed with control diet for 30 days to acclimate the experimental diet and condition. After the acclimation period, the experimental design was completely randomized with five treatments. Each diet was randomly assigned to duplicate tanks of shrimp, and each 3 m³ composite tank (2 m³ water capacity) was stocked with 200 shrimps per tank (density 100 individual m⁻³). The mean weight of shrimps used in the experiments was 1.65 ± 0.01 g. Shrimps were fed four times daily at 06:00, 11:00, 16:00, and 21:00 h at about 6-10% body weight, and feeding ration was regulated according to the feed consumption.

Every seven days in the feeding experiment, 10 shrimps per tank were sampled after 3 h feeding to measure the weight of shrimp on a digital balance (Metttler Toledo, AT 200, Greifensee, Switzerland) and to obtain their intestines for the determine of bacterial counts. The cumulative mortality of shrimp during the whole experimental period was recorded.

During the experimental period of feeding experiment (from the start to 30th day), water temperature and water quality parameters were monitored: temperature, 28 ± 2°C, salinity 22 ± 2‰, pH 7.9 ± 0.3, dissolved oxygen, >5 mg L⁻¹. The photoperiod was 12 h light and 12 h dark.

**Bacterial quantification**

The intestines of ten shrimps collected from each tank were homogenized in sterilized peptone salt buffer (0.1% w/v casein peptone and 0.85% w/v sodium chloride) with the ratio of 1:1 (w/v). Bacterial quantifications for homogenized liquid were made using serial 10 fold dilution in the buffer, followed by plating on the medium agar. Seven types of bacteriological media were used in total plate counts determinations. Among them, tryptone glucose agar (TGA) was used to enumerate the total aerotrophic heterotrophic bacteria which were expressed as total viable bacterial counts (TBC), while thiosulphate citrate bile salt sucrose (TCBS) agar, Man Rogosa Sharpe (MRS) agar, BD TM Bifidobacterium agar, violet red bile lactose (VRBL) agar and tryptone sulfite cycloserine (TSC) agar, Bismuth Sulphate Agar (BSA) were used to determine selectively the presumptive *Vibrio*, *Lactobacilli*, *Bifidobacteria*, *Coliforms*, *Clostridia* perfringens, and *Salmonella* counts respectively. After 48 h of incubation at 30°C for total aerotrophic heterotrophic bacteria, *Vibrio* and 37°C for the rest, the number of bacterial colonies was counted and the amount of bacteria was calculated as colony forming unit (CFU). Bacterial counts (BC) were calculated by the formula:

\[
\text{BC (CFU per gram of shrimp intestine)} = \text{Number of colonies (CFU)/(Dilution×Weight of shrimp intestine)}
\]

**Challenge test**

A bacterial pathogen, *Vibrio harveyi* associated with whiteleg shrimp’s vibriosis disease was obtained from Institute of Biotechnology, Vietnamese Academy of Science and Technology. *V. harveyi* was grown in BOSS broth for 24 h at 30°C. Two hundred whiteleg shrimps (mean weight of 1.65 ± 0.01 g) from each duplicate were immersed in infected seawater (containing final bacteria concentration at 10⁶ CFU/ml) for 2 h with a constant aeration in an 8 L plastic container. Then the infected culture was transferred into a 3 m³ composite tank (2 m³ water capacity). Shrimps were cultured in conditions described in the feeding trials. The cumulative mortality was calculated for 7 days.

**Calculations and statistical analysis**

Growth rate was calculated and expressed specific growth rate (SGR) and weight gain (WG) according to the following equation:

\[
\text{SGR} (\text{day}^{-1}) = \frac{100}{d} \times \left( \ln W_f - \ln W_i \right) / W_i
\]

where \(W_f\) and \(W_i\) are the weights of the shrimps at the start and end of the growth period, respectively, and \(d\) is the number of days, in the growth period.

Feed conversion ratio (FCR)=diet consumed (g)/(Wf-Wi)

The survival rate in each tank was measured using the following formula:

\[
\text{survival rate (%)} = \frac{n_t}{n_0} \times 100
\]

where \(n_t\) is the number of shrimps at the time \(t\) and \(n_0\) is the number of shrimp at the commencement.

Cumulative mortality was calculated according to the following equation:

\[
\text{cumulative mortality (%) } = \frac{n_t}{n_0} \times 100
\]

where \(n_t\) is the number of dead shrimps in the challenge period and \(n_0\) is the number of shrimp at the commencement.

Data were subjected to one-way ANOVA in SPSS 18 (IBM, USA). When appropriate, the Duncan’s multiple-range test (\(P<0.05\)) was applied to evaluate the differences between the means. Data in tables were presented as means ± standard deviation of five tanks.

**Results**

**Bacterial counts in shrimp intestines**

Beneficial and harmful bacteria counts were significantly affected by both supplemented-MOS doses (\(P<0.05\); Table 1 and Figure 1). The intestinal presumptive *Vibrio* counts (VBC) significantly showed the decreasing tendency with the increasing administration doses of MOS in diets. While the differences in *Lactobacilli*, *Bifidobacteria*, *Coliforms* and *Clostridia* counts among shrimp groups showed dissimilar trends to that in VBC. When MOS supplementation was higher than an appropriate level (depending on the group of bacteria in shrimp), the increasing/decreasing trend of these beneficial/harmful bacteria counts was inverted. The maximums of *Lactobacilli* and *Bifidobacteria* counts and the minimums of *Coliforms* and *Clostridia* counts were detected in the shrimp fed with 6 gkg⁻¹ MOS-supplemented diet. Interestingly, intestinal *Salmonella* did not present in shrimps fed MOS-supplemented diet at any dose in whole experimental period, while these bacteria appeared in the gut of control shrimps after 1 week of cultivation (data not shown) and then reached cell number up to 80 CFUg⁻¹ (at the end of feeding trial).
the 7-day challenge test with *Vibrio harveyi* were high (93-99%) in all treatments. Mortality of the shrimps challenged with *Vibrio harveyi* decreased compared to shrimps fed basal diet (control group). The cumulative mortalities of experimental group and control group were 3.5 ± 2.1% and 29.5 ± 4.2%, respectively (Table 3). The numbers of *Vibrio* in the shrimps of experimental and control groups were 200 ± 46 (CFUg⁻¹) and 250,000 ± 94,328 (10³ CFUg⁻¹), respectively.

**Discussion**

Results from the present study clearly indicate that the dietary supplementation of copra MOS exerted positive effect on growth performance and feed utilization of whiteleg shrimps, with shrimps fed 10 gkg⁻¹ MOS-supplemented diet exhibiting the highest weight gain and specific growth rate (P<0.05) in all experimental groups. The improved growth observed in this study was consistent with that reported for shrimps fed diet supplemented other prebiotics including MOS extracted from cell wall of yeast [18]. However, the copra mannooligosaccharides’ growth-promoter effect distinguishes from previous studied prebiotics (fructooligosaccharides, isomaltooligosaccharides) [15,16], because in this study, the copra MOS led to an enhancement of weight gain and specific growth rate as well as survival obviously in the conditions in which shrimps have excellent growth and survival rate. While both Li et al. [16] and Luna-González et al. [17] found no significant increase in weight and survival of *L. vannamei* fed with fructans (scFOS, inulin) dose varying from 0.025 to 1.00% of diet in the similar high growth conditions. Li et al. [15] found dietary isomaltooligosaccharides (IMO) alone had no obvious effect on survival of shrimp as IMO dose increased from 0 to 0.2% [16]. In addition, copra MOS supplementation up to 1% w/w didn’t show any inhibition in growth, survival and feed efficiency of shrimps.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>WG (%)</th>
<th>SGR (%/d)</th>
<th>FCR</th>
<th>FI (g/d)</th>
<th>SR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>135.43 ± 0.82</td>
<td>2.99 ± 0.01</td>
<td>1.46 ± 0.01</td>
<td>3.48 ± 0.04</td>
<td>93.5 ± 0.71</td>
</tr>
<tr>
<td>MOS4</td>
<td>238.11 ± 0.76</td>
<td>4.06 ± 0.01</td>
<td>1.17 ± 0.01</td>
<td>4.55 ± 0.00</td>
<td>96.2 ± 0.28</td>
</tr>
<tr>
<td>MOS6</td>
<td>239.04 ± 3.79</td>
<td>4.07 ± 0.04</td>
<td>1.11 ± 0.01</td>
<td>4.35 ± 0.03</td>
<td>96.4 ± 0.42</td>
</tr>
<tr>
<td>MOS8</td>
<td>240.87 ± 3.80</td>
<td>4.09 ± 0.04</td>
<td>1.08 ± 0.02</td>
<td>4.25 ± 0.05</td>
<td>99.2 ± 0.28</td>
</tr>
<tr>
<td>MOS10</td>
<td>248.49 ± 3.44</td>
<td>4.16 ± 0.03</td>
<td>1.03 ± 0.01</td>
<td>4.20 ± 0.04</td>
<td>98.8 ± 0.07</td>
</tr>
</tbody>
</table>

(*) Data (mean ± SD) in the same column not sharing a common superscript was significantly different (Duncan’s multiple-range test, P<0.05).

**Table 1:** The bacterial counts in the intestine of *Litopenaeus vannamei* fed with copra MOS-supplemented diets for 4 weeks (*).
In conclusions, this is the first determination on the impact of mannoooligosaccharides derived from copra on whiteleg shrimp's health. Supplementation of 4 to 10 gkg⁻¹ copra MOS in diet can significantly improve growth, feed conversion, modulating intestinal microflora and enhance the resistance against *Vibrios, E. coli* and *Salmonella* of whiteleg shrimp. This kind of prebiotic will gain a great deal of interest for aquaculture applications in large scale due to an increasing demand towards food safety and sustainable development.

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**References**


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