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Food consumption of marine shrimp *Litopenaeus vannamei* in closed system with the use of probiotics

ABSTRACT

This study aimed to evaluate the food consumption of marine shrimp *Litopenaeus vannamei* in a closed culture system with application of probiotics. Four treatments were established with a water recirculation system: probiotic (*Bacillus* sp.) added in the water and feed (PWF); only in the water (PW); only in the feed (PF); and without the addition of probiotic (NP). Shrimps with mean weight of 3.48 ± 0.62 g were stocked (200 shrimp m^{-2}) in each of the twelve experimental units (50 L). Pelleted feed was offered in four daily meals at the rate of 7.5% of shrimp biomass. For seven consecutive days, the residual feed of these meals were collected after four hours, placed in pre-weighed filters and dried in an electrical oven at a temperature of 60 °C. The values of food consumption (g of feed per meal) were lower in treatments PWF (0.45 g) and PW (0.32 g), which differ significantly when compared with treatments PF (0.60 g) and NP (0.59 g). The probiotic contributed to reduce the food consumption when added in the water, without compromising the growth rate of *L. vannamei*.

Key words: *Bacillus*, feed, recirculation

Consumo alimentar do camarão marinho *Litopenaeus vannamei* em sistema fechado com a utilização de probióticos

RESUMO

O presente estudo objetivou avaliar o consumo alimentar do camarão marinho *Litopenaeus vannamei* em sistema de cultivo fechado com aplicação de probióticos. Foram utilizados quatro tratamentos, todos com sistema de recirculação de água: probiótico (*Bacillus* sp.) adicionado na água e na ração (PWF); apenas na água (PW); apenas na ração (PF); e sem adição de probiótico (NP). Os camarões com peso médio de $3,48 \pm 0,62$ g foram estocados (200 camarões m^{-2}) em cada uma das doze parcelas experimentais (caixas de 50 L). A ração peletizada foi ofertada em quatro refeições diárias na proporção de 7,5% da biomassa. Durante sete dias consecutivos, as sobras destas refeições foram recolhidas após quatro horas, colocadas em filtros pré-pesados e secas em estufa a uma temperatura de 60 °C. Os valores de consumo alimentar (g de ração por alimentação) foram inferiores nos tratamentos PWF (0,45 g) e PW (0,32 g), os quais diferiram significativamente quando comparados com os tratamentos PF (0,60 g) e NP (0,59 g). O probiótico contribuiu para a diminuição do consumo alimentar quando o mesmo foi adicionado na água de cultivo, sem comprometer o crescimento de *L. vannamei*.

Palavras-chave: *Bacillus*, ração, recirculação

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INTRODUCTION

The term probiotic in aquaculture is applied to the use of live microbial supplements with beneficial effects for the host and the rearing environment. These effects result from changes in the microbial community that provide a better use of artificial food, immunologic response of the host animal to diseases and maintenance of water quality (Verschuere et al., 2000; Decamp et al., 2002). The bacteria commonly used as probiotics in aquaculture are *Vibrio*, *Pseudomonas*, *Flavobacterium*, *Pseudoalteromonas*, *Acinetobacter* and *Bacillus* (Maeda, 2002; Ziaei-Nejad et al., 2006).

In rearing ponds of *Litopenaeus vannamei*, the use of commercial probiotics has shown beneficial effects by improving survival, feed conversion, growth rate and keeping the parameters of water quality at optimum levels (Shariff et al., 2001; Wang et al., 2005).

The microorganisms can use efficiently the unconsumed feed in the culture ponds for the production of bacterial protein. This protein can be further consumed by rearing animals, providing a better feed conversion and reducing the production costs (Horowitz & Horowitz, 2000). The probiotic bacteria can also stimulate appetite and improve nutrition through the production of vitamins (Irianto & Austin, 2002). Moreover, its application in the commercial culture of aquatic organisms has been used as a strategy in the control of several diseases (Gatesoupe, 1999).

Especially in closed culture systems without water renewal, the microbial community must be managed to allow the development of non-pathogenic organisms (Maeda, 2002). In these culture systems the risk of diseases is minimized while the natural productivity provides nutritional benefits to reared shrimp (Mcintosh et al., 2000; Bratvold & Browdy, 2001; Moss et al., 2001; Weirich et al., 2002; Burford et al., 2003).

Information about the feeding behavior of shrimp, as well as the amount of food consumed are important to the farmers improve management practices and increase the economic viability of closed culture systems (Wasielesky et al., 2006). Therefore, this study aimed to evaluate the food consumption of marine shrimp *L. vannamei* in a closed culture system with application of probiotics.

MATERIAL AND METHODS

The experiment was conducted in the Laboratory of Sustainable Mariculture (LAMARSU) of the Department of Fisheries and Aquaculture (Universidade Federal Rural de Pernambuco). Four treatments with three replicates each were designed, corresponding to the addition of commercial probiotic in the water and feed (PWF), only in the water (PW), only in the feed (PF) and without the addition of probiotic (NP).

All four treatments used an independent system of water recirculation, including a matrix tank (500 L) that supplied all three experimental tanks (50 L) through an underwater pump (Resun SP-3800). The rate of water recirculation was 150 L h⁻¹ and all the tanks were kept under constant aeration.

Two different types of commercial probiotics were used (INVE Sanolife®), one specific to be added in the water (Sanolife® PRO-W) and the other to be added in feed (Sanolife® PRO-2). The probiotic used in the water was composed by strains of *Bacillus subtilis* and *Bacillus licheniformis* in the concentration of 5x10¹⁰ CFU g⁻¹, while the probiotic added to the feed was composed by strains of *Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus pumilus* in the concentration of 2x10¹⁰ CFU g⁻¹. The probiotics were added daily in quantities specified by the manufacturer directly into water (0.001 g L⁻¹) or in the feed (5 g kg⁻¹ of feed).

Before the beginning of the experiment, shrimp were randomly stocked in the experimental units (40 shrimps per tank) in a density of 200 shrimp/m², where they were exposed to the probiotics for seven days.

Pelleted feed (35% crude protein - Purina® MR35) was offered in four daily meals (0700, 1100, 1500 and 1900 h) through the use of feeding trays. The activation of the probiotic used in the feed was performed by humidification of the feed with the product diluted in distilled water during ten minutes, as required by the manufacturer. The process of humidification of the feed was also performed in the treatments without the addition of probiotics in the feed, only to match the texture of the food offered.

The physico-chemical variables of water such as temperature (° C), salinity, pH and dissolved oxygen (mg L⁻¹) were measured twice daily (0800 and 1700 h) using a multiparameter (YSI 556). Transparency (cm) was measured daily with Secchi disc directly from the matrix tanks at 1200 h.

In the day before the start of the food consumption test, ten shrimps of each experimental unit were weighted. Their mean initial weight was 3.48 ± 0.62 g and did not differ among the treatments. At the end of the experiment (seven consecutive days), the final weight was recorded to estimate weight gain (g).

To evaluate the food consumption, the pelleted feed was weighted and offered daily in the rate of 7.5% of shrimp biomass. The residual feed was collected four hours later (corresponding to the interval between the meals) and then put in pre-weighted filters. Moreover, three experimental units without shrimp were used to determine the percentage of feed lost in the water during four hours.

The filters containing the residual feed were taken to the electrical oven in a temperature of 60° C, remaining under these conditions until a constant weight. The food consumption was calculated based on the following equation: FC = (F₀ - F_c) x F₁ (Soares et al., 2005), where FC = food consumption (grams of feed per meal), F₀ = dry weight of feed offered (g), F_c = dry weight of feed collected (g) and F₁ = feed lost in the water (%).

The values of water quality, food consumption and weight gain were analyzed by one-way analysis of variance (ANOVA), followed by Tukey test to determine differences among treatments (p<0.05).

RESULTS AND DISCUSSION

During the experimental period no mortality occurred in any of the treatments. The values of temperature and salinity

showed no significant differences between treatments. However, variables such as pH, dissolved oxygen and transparency differed significantly for the treatment without the use of probiotics (NP) compared with other treatments (Table 1).

Table 1. Mean values of water parameters during the experimental period for different treatments with *L. vannamei*

Tabela 1. Valores médios dos parâmetros analisados na água durante o período experimental para os diferentes tratamentos com *L. vannamei*

	Treatments			
	PWF	PW	PF	NP
Temperature (°C)	25.8±0.6 ^a	25.7±0.6 ^a	25.8±0.7 ^a	25.8±0.8 ^a
pH	7.6±0.1 ^a	7.6±0.2 ^a	7.5±0.2 ^a	8.1±0.1 ^b
Dissolved oxygen (mg L ⁻¹)	4.0±0.8 ^a	4.1±0.9 ^a	4.0±0.8 ^a	5.5±0.4 ^b
Salinity	32.5±1.0 ^a	33.2±0.5 ^a	33.2±0.5 ^a	34.0±1.5 ^a
Transparency (cm)	4.0±0.0 ^a	4.0±0.0 ^a	4.0±0.0 ^a	16.7±1.5 ^b

Treatments with probiotics added in the water and feed (PWF), only in the water (PW), only in the feed (PF) and without the addition of probiotic (NP); Different letters among columns indicate significant differences ($p < 0.05$)

The temperature and salinity were in adequate levels to the culture of *L. vannamei*, with averages of 25.7 °C and 33.2, respectively. Nunes (2002) suggests that the range between 26 and 33 °C of temperature is ideal to the rearing of this species, and that temperatures superior to 35 °C and inferior to 25 °C can affect negatively its zootechnical performance. According to Ponce-Palafox (1997) an adequate growth performance of penaeids can be obtained at salinities between 25 and 45. The levels of dissolved oxygen in water observed in this study were within acceptable limits for the shrimp rearing, with an average of 4.40 mg L⁻¹ (Kubitza, 2003). Similarly, mean pH value (7.73) was considered within the ideal range for the shrimp culture ranging from 7 to 9 (Kubitza, 2003).

Water quality variables of dissolved oxygen, pH and transparency were significantly higher in NP treatment. These differences were probably related to the absence of probiotic addition, which in turn reduced the formation of microbial flakes as observed in the other treatments. This microbial community developed in the tanks is responsible not only by the largest decrease in the values of dissolved oxygen and pH due to the process of respiration, but also by reducing the transparency of the water with the formation of microbial aggregates in suspension (Wyk & Scarpa, 1999). Nevertheless, such differences in the physical-chemical water variables were within acceptable limits for the rearing of this species.

For food consumption, significantly lower values were observed for the treatments PWF (0.45 g of feed per meal) and PW (0.32 g of feed per meal) compared with the treatments PF and NP that showed mean values of 0.60 and 0.59 g of feed per meal, respectively (Table 2). The shrimp weight gain in the PW treatment did not differ from the other treatments, but this value in the PWF treatment was significantly lower than the treatments PF and NP (Table 2).

Venkat et al. (2004) observed a better growth rate of freshwater shrimp *Macrobrachium rosenbergii*, when they were submitted to a treatment with strains of the bacteria *Lactobacillus acidophilus* and *L. sporogenes*. According to

Table 2. Food consumption (g of feed per meal) and weight gain (g) of *L. vannamei* in the different treatments

Tabela 2. Consumo alimentar (g de ração por alimentação) e ganho de peso (g) de *L. vannamei* nos diferentes tratamentos

	Treatments			
	PWF	PW	PF	NP
Food consumption	0.45±0.25 ^a	0.32±0.24 ^a	0.60±0.28 ^b	0.59±0.27 ^b
Weight gain	0.27±0.16 ^b	0.31±0.31 ^{ab}	0.37±0.39 ^a	0.37±0.25 ^a

Treatments with probiotics added in the water and feed (PWF), only in the water (PW), only in the feed (PF) and without the addition of probiotic (NP); Different letters among columns indicate significant differences ($p < 0.05$)

these authors, this result may be associated with an improvement in the digestibility of the diet or a stimulus in the production of digestive enzymes. Lin et al. (2004) reported that the inclusion of the bacteria *Bacillus* sp. in the diet of *L. vannamei* improved the digestibility of feed. Ziaei-Nejad et al. (2006) observed for *Fenneropenaeus indicus*, that the activity of digestive enzymes lipase and amylase were higher in the treatments were the shrimp fed with *Bacillus* sp., showing a higher growth rate and lower feed conversion.

Similarly, the results of this study suggest that the addition of probiotic *Bacillus* sp. contributed to the decrease of shrimp food consumption, but only when the product was added in the water (PWF and PW). Furthermore, even with lower food consumption values, shrimp weight gain was similar to the other treatments when the probiotic was added only in the water (PW). The similarities found in the food consumption of shrimp in the treatments PF and NP suggest that the addition of probiotics in the feed is less efficient, at least during this life stage and culture conditions. Therefore, it is suggested that the direct application of the probiotic in the water allowed a greater dispersion and increased the quality of microbial aggregates available for the shrimp ingestion.

Studies indicate that the microbial community is a significant source of nutrients in the rearing of shrimp in closed or semi-closed system (Moss et al., 1992). Microbial aggregates can maintain reasonable rates of growth of *L. vannamei* in the absence of another source of food in heterotrophic systems, depending on the stocked density, biomass of shrimp and water quality (Waselesky et al., 2006). Burford et al. (2004) reported that over 29% of food consumed daily by shrimp *L. vannamei* in the heterotrophic system is microbial flake.

CONCLUSION

The application of probiotics with strains of *Bacillus* in the rearing water of *L. vannamei* allows a reduction in the food consumption without compromising the growth performance in heterotrophic systems.

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