# INFLUENCE OF YEAST AND YEAST DERIVATIVES ON GROWTH PERFORMANCE AND SURVIVAL OF JUVENILE PRAWN

# Macrobrachium amazonicum

Hamilton Hisano<sup>1</sup>, Dario Rocha Falcon<sup>2</sup>, Margarida Maria Barros<sup>3</sup> e Luiz Edivaldo Pezzato<sup>3</sup>

Embrapa Agropecuária Oeste, Rod. BR 163, km 253,6, Caixa Postal 661, CEP 79804-970, Dourados, MS. E-mail: hhisano@cpao.embrapa.br
 Universidade Federal Rural de Pernambuco, Unidade Acadêmica de Serra Talhada – UFRPE/UAST
 Departamento de Melhoramento e Nutrição Animal, UNESP-FMVZ, Botucatu, SP, Brasil.

#### ABSTRACT \_\_\_\_

Supplements of whole yeast spray dried *Saccharomyces cerevisiae* and yeast derivatives (autolyzed yeast, mannanoligosaccharide and  $\beta$ -glucan) in to diets fed to juvenile Amazon River prawn were evaluated. Prawns with initial weight (1.27  $\pm$  0.07 g) and initial length (5.49  $\pm$  0.09 cm) were stocked in to 200 L aquaria (10 prawns/aquarium), in flow-through water system with controlled temperature, during 60 days. The experimental design was completely randomized with five treatments and four replicates. The diets were formulated to be isoproteic (35.0%

CP) and isoenergetic (3500 kcal/kg GE) supplemented with no yeast and yeast derivatives - control, whole yeast - 2.0%, autolyzed yeast - 2.0%, mannanoligosaccharide - 0.2% and  $\beta$ -glucan - 0.2%. Weight gain of prawns fed diet containing mannanoligosaccharide yeast derivative was significantly (P<0.05) higher than prawns fed the others diets. The results indicate that 0.2% of mannanoligosaccharide supplementation provided better growth responses of juvenile *M. amazonicum*.

KEYWORDS: aquaculture, Amazon River prawn, glucan, mannan.

RESUMO \_

# INFLUÊNCIA DA LEVEDURA E DERIVADOS SOBRE O DESEMPENHO E SOBREVIVÊNCIA DE JUVENIS DO CAMARÃO Macrobrachium amazonicum

Avaliaram-se a suplementação de levedura íntegra *Saccharomyces cerevisiae* e derivados do seu processamento (levedura autolisada, mananoligossacarídeo e  $\beta$ -glucano), desidratadas pelo método de *spray dry*, em rações para juvenis de camarão amazônico. Indivíduos com peso inicial  $(1,27\pm0,07~g)$  e comprimento inicial  $(5,49\pm0,09~cm)$  foram alojados em aquários de 200 L (10 camarões/aquário) dotados de recirculação de água com temperatura controlada, durante o período de sessenta dias. O delineamento experimental foi inteiramente casualizado com cinco tratamentos e quatro repetições. Formularam-se as

rações para serem isoproteícas (35 % PB) e isoenergéticas (3.500kcal/kg EB), suplementadas com 2,0 %, de levedura integra, 2,0 % de levedura autolisada, 0,2 % de mananoligosacarídeo e 0,2 % de β-glucano. A ração-controle se caracterizou pela não-suplementação dos diferentes aditivos alimentares. Os juvenis de camarão alimentados com mananoligossacarídeo apresentaram ganho de peso superior (P<0,05) aos alimentados com outras rações. Os resultados indicam que a suplementação de 0,2 % de mananoligossacarídeo proporciona melhores resultados de desempenho aos juvenis de *M. amazonicum*.

PALAVRAS-CHAVES: Aquicultura, camarão amazônico, glucano, manano.

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#### INTRODUCTION

The improvement of prawn culture has focused on the development of intensive management techniques to maximize productivity and consequently optimize financial returns. Animals kept in these conditions generally experience stress at some time or another, and thereby become more susceptible to diseases and potentially pathogenic organisms will cause diseases or infestation problems in weak and stressed animals (LEE & WICKINS, 1992).

To minimize susceptibility to disease antibiotics and chemotherapeutics are routinely used for therapeutic, prophylactic or growth promoting purposes, however the supplementation of subtherapeutic levels can cause microorganism to develop resistance and a part of the original gut microbiota can be damaged (MULDER, 1993). TEUBER (2001) reported antibiotics resistance to aquaculture system with oxytetracycline and tetracycline and proposed considerable changes in the management of farming animals that are cultured in intensive systems.

The great adverse impact of some diseases that recently occurred in shrimp culture has stimulated studies to find alternatives to replace antibiotics and chemotherapeutics in aquaculture diets. Natural ingredients, like yeast and yeast derivatives, may be effective substitutes. Previous studies conducted with fishes and shrimps have shown that yeast and yeast derivatives are effective growth enhancers and immunostimulants (SCHOLZ et al., 1999; LI & GATLIN, 2003, 2004).

Additionally, other strategies are attractive, such as the production of native species that could minimize diseases, and the Amazon River prawn *M. amazonicum* has been studied and considered a potential candidate species for aquaculture in Brazil (VALENTI, 2002). Currently this species is principally exploited by artisan fisheries and extensively consumed by populations in the Amazon region (MORAES-RIODADES & VALENTI, 2001) and in the Brazilian Northwest (NEW et al., 2000).

Information derived from comparative studies of growth and survival response to yeast

and yeast derivatives in diets for freshwater prawns is limited. The purpose of this study was to evaluate the growth performance and survival of M. amazonicum fed pelleted diets containing spray dried whole yeast and yeast derivatives (autolyzed yeast, mannanoligosaccharide and  $\beta$ -glucan).

# MATERIALS AND METHODS

Diets

The experimental diets were formulated to provide all known nutrient requirements for prawns and shrimps (ZIMMERMAN, 1998; D'ABRAMO & NEW, 2000; PEZZATO et al., 2004) and based in the chemical-bromatological feedstuffs composition (NRC, 1993; ROSTAGNO, 2000). Diets with primary ingredients of soybean meal, fish meal, wheat middlings and corn were formulated to be isoproteic (35.0% CP) and isoenergetic (3500 kcal/kg GE) containing no yeast control (C) or supplemented with yeast derivatives whole yeast 2.0% (WY), autolyzed yeast 2.0% (AY) mannanoligosaccharide 0.2% (MOS) and β-glucan 0.2% (G) (Table 1). Whole yeast and autolyzed yeast were obtained from sugar cane fermentation and then spray dried. Mannanoligosaccharide and glucan sources utilized were BioMOS® and Mycosorb® respectively. Technical specification of Alltech Company described BioMOS® as based on yeast cell wall (S. cerevisiae strain phosphorylated mannanoligosaccharide and Mycosorb® based on β-glucan from the same strain. All ingredients were grounded to 0.5 mm, mechanically mixed, moistened with water (55.0°C) and pressured pelleted through a meat grinder (Ação Científica, Piracicaba-SP, Brasil). The pellets were dried in a forced-draft oven for 24-h (55.0°C) and processed to obtain 1.0 mm diameter pellets. Juveniles were fed ad libitum four times a day at 8:00, 12:00, 14:00 and 18:00-h and weekly faeces were removed by siphon.

# Feeding trial

Juveniles of *M. amazonicum* provided from the spawn of one female (Crustacean Laboratory

of the Aquaculture Center, CAUNESP, São Paulo State University, Jaboticabal, SP) were transported to Aquanutri (Laboratory of Aquaculture Nutrition, São Paulo State University, Botucatu, SP), where they were adapted for one week in a 1000-L circular aquarium equipped with recirculating system, controlled temperature, and fed *ad libitum* with control diet. After this pre-experimental period, 50 individuals were selected for measurement of initial weight and

initial length, with previous 24 h fasting  $(1.27 \pm 0.07 \text{ g})$  and  $5.49 \pm 0.09 \text{ cm}$ . Groups of 10 prawns were stocked in to 200 L aquaria in flow-through recirculating system, with controlled temperature and photoperiod (12 h light and 12 h dark) and equipped with mechanical and biological filters. The duration of the feeding trial was 60 days. Ammonia and pH were measured weekly and temperature twice a day at 8:00 and 14:00 h.

**TABLE 1.** Ingredient composition of experimental diets

	Composition (%) Treatment						
Ingredient (% dry weight)	Со	WY	AY	MOS	G		
Soybean meal	32.00	31.50	31.50	32.00	32.00		
Fish meal	28.00	27.15	27.15	28.00	28.00		
Corn	6.47	5.70	5.70	6.27	6.27		
Wheat middlings	24.40	24.50	24.50	24.40	24.40		
Salmon oil	2.00	2.00	2.00	2.00	2.00		
Soybean oil	2.00	2.00	2.00	2.00	2.00		
Dicalcium phosphate	2.73	2.73	2.73	2.73	2.73		
L - lysine	0.66	0.66	0.66	0.66	0.66		
DL - methionine	0.42	0.44	0.44	0.42	0.42		
Whole yeast	-	2.00	-	-	-		
Autolyzed yeast	-	-	2.00	-	-		
Mannanoligosacharide	-	-	-	0.20	-		
β-glucan	-	-	-	-	0.20		
Lignin sulphonate	0.10	0.10	0.10	0.10	0.10		
Vitam. min. premix <sup>a</sup>	0.50	0.50	0.50	0.50	0.50		
Soybean lecithin	0.50	0.50	0.50	0.50	0.50		
Cholesterol	0.20	0.20	0.20	0.20	0.20		
BHT	0.02	0.02	0.02	0.02	0.02		
	Analyzed proximate composition (% dry matter)						
Dry matter (%)	90.5	91.2	92.0	91.0	91.4		
Gross energy (kcal/kg)	3550	3610	3520	3570	3490		
Crude protein (%)	35.2	34.8	35.1	35.4	35.7		
Crude lipid (%)	5.7	5.2	5.2	5.0	5.8		
Crude fiber (%)	4.4	4.2	4.0	4.2	4.0		

<sup>&</sup>lt;sup>a</sup> Vit/min premix (kg/premix): Vitamins: A=1.200.000 UI; D3=200.000 UI; E=12.000 mg; K3=2.400 mg; B1=4.800 mg; B2=4.800 mg; B6=4.000 mg; B12=4.800 mg; B12=4.800 mg; B12=4.800 mg; Database Ca=12.000 mg; C=48.000 mg; Database Ca=12.000 mg; Data

# Proximate analysis

Dry matter, gross energy, crude protein, crude lipid and crude fiber composition of the experimental diets were determined according to standard procedures of AOAC (1990). Dry

matter was determined after drying in a laboratory oven at 105.0°C for 24 h until constant weight; gross energy by direct combustion in adiabatic bomb calorimeter (PARR model 1260); crude protein (N x 6.25) by the Kjeldahl method after acid digestion; crude lipid by petroleum ether

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extraction in Soxhlet apparatus, and crude fiber as loss on ignition of dried lipid-free residues after digestion with 1.25% H<sub>2</sub>SO<sub>4</sub> and 1.25% NaOH.

#### **Statistics**

The experimental design was completely randomized with five treatments and four replicates. Weight gain, feed conversion ratio, final length and survival were submitted to a one way analysis of variance followed analyzed by Duncan's multiple range test at significant level of 0.05 was used to compare growth response between treatments. Survival data were transformed by equation  $y=\sqrt{\arcsin x}$  and the results were expressed as percentage. All statistical analysis was carried out using the SAS® software package.

### **RESULTS**

During the experimental period, the water temperature ranged from 27.6±1.4°C (8:00 h) to 28.6±0.9°C (14:00 h), dissolved oxygen from 6.7 to 7.2 mg/L, total ammonia was <0.3mg/L and pH ranged from 6.2 to 7.2, all water quality parameters were considered acceptable for freshwater prawns (SIPAÚBA-TAVARES, 1998).

Juveniles fed the diet supplemented with 0.2% of mannanoligosaccharide, had significantly (P<0.05) higher weight gain (WG) than each of the prawns fed the basal diet, and diets containing whole yeast, autolyzed yeast and β-glucan. Feed conversion ratio (FCR), final length (FL) and survival (S) were not significantly affected by the supplementation of yeast and yeast derivatives (Table 2).

**TABLE 2.** Growth performance of juvenile *M. amazonicum* fed diets containing yeast and yeast derivatives for 60 days (mean±s.d)

Parameter	Со	WY	AY	MOS	G
WG (g)	1.07±0.27 b	0.93±0.12 b	1.08±0.09 b	1.37±0.05 a	1.12±0.08 b
FCR	$3.09\pm0.93$	$2.93\pm0.56$	$2.97\pm0.20$	$3.13\pm0.64$	$3.12\pm0.54$
FL (cm)	$7.16\pm0.11$	$7.56 \pm 0.47$	$7.24\pm0.25$	$7.42\pm0.24$	$7.39\pm0.18$
S (%)	97.50±5.00	95.00±5.77	95.00±10.00	97.50±5.00	92.50±9.57

Means followed by same letters are not significantly different by Duncan's multiple range test.

# **DISCUSSION**

The supplemented level of mannano-ligosaccharide was effective to provide better responses in weight gain for juvenile Amazon River prawns. LI & GATLIN (2004) observed generally enhanced weight gain in hybrid striped bass fed diets supplemented with 1.0% and 2.0% of autolyzed yeast compared to those fed a basal diet. In contrast, SCHOLZ et al. (1990) incorporated different yeast products *S. cerevisiae*, *Phaffia rhodozyma*, experimental yeast (HPPR1) and β-glucan, to diets fed to juvenile *Litopenaeus vannamei* diets and the results indicated no significant difference in weight gain. However, there was a statistically significant difference between the final biomass of shrimp fed diets

with *P. rhodozyma* and β-glucan. BURGENTS et al. (2004) assessed 1.0% commercial yeast feed supplement (Diamond V XP Yeast Culture®) in shrimp diets and after 4 weeks observed no significant difference in weight gain in comparison to that of the control.

In the present research, the addition of whole yeast and yeast derivatives did not influence (P>0.05) feed conversion ratio and final length. The results are consistent with those observed by SCHOLZ et al. (1990) with marine shrimp and in contrast to those observed by LI & GATLIN (2004), who found a significant difference (P<0.05) in feed efficiency among hybrid striped bass fed diets supplemented with yeast and autolyzed yeast.

Survival was not significantly influenced by the supplementation of experimental ingredients; however studies with other yeast derivatives demonstrated immunostimulatory effects and a decrease in mortality by pathogens. The β-glucan derived from yeast cell wall is most utilized for its immunostimulant properties that enhance the innate immune system of shrimp (SUNG et al., 1994; SONG et al., 1997; CHANG et al., 2000) and has been supplemented in shrimp diets to decrease mortality that results from diseases and handling stress (DUGGER & JORY, 1999). SCHOLZ et al. (1999) observed that dietary inclusion of S. cerevisiae, P. rhodozyma and experimental yeast-HPPR1, especially the *Phaffia* diet improved survival of shrimp inoculated with pathogenic Vibrio harveyi (strain BP05). Highest survival rates of L. vannamei were found by BURGENTS et al. (2004), when supplement yeast (0.5% or 1.0%) was included in diets. LI & GATLIN (2003, 2004) reported that survival of hybrid striped bass fed diets supplemented with yeast and partially autolyzed yeast was significantly higher than those fed the control diet after chalenged with Streptococcus iniae.

The proximate composition of yeast cell wall is 40.0% of  $\beta$ -glucans, 40.0%  $\alpha$ -mannans 8.0 % protein, 7.0% lipid, 3.0% inorganic substances and 2.0% of hexosamines and chitin (HOUGH, 1990). The polysaccharide balance of BioMOS® may have provided positive prebiotic effects, enhanced the juvenile health and thereby provided better weight gain. Numerous human feeding studies have shown that human gut microbiota can be modulated with a prebiotic to increase the number and activity of some probiotics bacteria (TUOHY et al., 2003). Supplementation of (25 or 50g/kg) of chitin in diets fed to gilthead seabream Sparus aurata resulted an increase innate immune system (ESTEBAN et al., 2000). Future studies (in laboratory conditions and field trials) are essential to know how glucans and mannans or other polysaccharides positively influence the gut microbiota and provide immunostimulatory effects in Amazon River prawns.

Research efforts devoted to the replacement of antibiotics and chemotherapeutics as well as the search to develop a responsible and sustainable aquaculture are increasing and could amplify the use of yeast and yeast derivatives in aquaculture diets. Based on results of the present study, it can be concluded that dietary supplementation of 0.2% of mannanoligosaccharide resulted in significantly higher growth performance for juvenile *M. amazonicum*.

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