Supplemented prebiotics in the feeding of *Arapaima gigas* juveniles raised in water-boxes with continuous mechanical aeration and water-boxes flow

Suplementação de prebióticos na alimentação de juvenis de *Arapaima gigas* criados em caixas d'água com aeração mecânica e fluxo de água contínuos

Suplementación prebiótica en la dieta de juveniles de *Arapaima gigas* criados en tanques de agua con aireación mecánica y flujo de agua continuos

DOI: 10.55905/revconv.17n.4-204

Originals received: 03/25/2024
Acceptance for publication: 04/12/2024

**Maria Mirtes de Lima Pinheiro**
Master in Environmental Sciences
Institution: Universidade Federal de Rondônia (UNIR)
Address: Porto Velho - Rondônia, Brasil
E-mail: mmirteslp@gmail.com
Orcid: https://orcid.org/0000-0002-2778-0452

**Carlindo Pinto Filho**
Graduated in Veterinary Medicine
Institution: Superindentência da Pesca e Aquicultura em Rondônia
Address: Porto Velho - Rondônia, Brasil
E-mail: cpfmaranhao@hotmail.com
Orcid: https://orcid.org/0000-0003-4464-7415

**Jerônimo Vieira Dantas Filho**
PhD in Animal Science
Institution: Universidade Federal de Rondônia (UNIR), Centro Universitário São Lucas Ji-Paraná (UNISL - AFYA)
Address: Ji-Paraná - Rondônia, Brasil
E-mail: jeronimovdantas@gmail.com
Orcid: https://orcid.org/0000-0002-5965-9438

**Bruna Lucieny Temponi Santos**
Master in Environmental Sciences
Institution: Universidade Federal de Rondônia (UNIR)
Address: Ji-Paraná, Rondônia - Brasil
E-mail: brunalucienytemponi@gmail.com
Orcid: https://orcid.org/0000-0001-8783-4523
ABSTRACT

The study aimed to evaluate the effect on zootechnical performance and survival of pirarucu juveniles (Arapaima gigas) submitted to prebiotic supplementation in their diet. Were 120 juveniles of A. gigas acquired, acclimated in water-boxes with continuous aeration and water flow, and fed ad libitum four times a day, with a diet containing 55% crude protein. The 25.5 ± 3.5 g fish were distributed in 12 water-boxes of 500L (3 water-boxes/treatment and 10 fish/water-box). Juveniles were subjected to control (without prebiotics) and also subjected to three treatments A, B and C. The parasites found were observed under optical and scanning electron microscopy (SEM). Fish were distributed in completely randomized design water-boxes. The data obtained were evaluated by the Shapiro-Wilk and Bartlett’s tests (α=0.05). Then, they were submitted to ANOVA, and contrasted by the Tukey’s test (α=0.05). While the treatments were contrasted with each other by Dunn's test (α=0.05). The water quality parameters were kept adequate for A. gigas cultivation. In treatments, zootechnical performance and survival rate differed from each other, and were better than the control (p<0.05). Treatment A expressed the highest final weight 77.32 ± 10.23g (p<0.05), and the control had the lowest final weight 20.08 ± 6.64g. The average weight gain was different between treatments (p<0.05), which presented better results than the control (p<0.05). Treatments A and C expressed the highest mean weight gain 49.90 ± 8.95 and 54.17 ± 4.12 g (p<0.05), respectively. The final biomass was different between treatments, which showed better results than the control group (p<0.05). Survival rate
was not different between treatments (p>0.05). However, the control presented the lowest rate of 13.33%, due to trichodinid infestation. Supplemented prebiotics were beneficial for weight gain, final biomass, apparent feed conversion and survival of pirarucu juveniles.

**Keywords**: fish farming, *Trichodina spp.*, zootechnical performance.

**RESUMO**
O estudo teve como objetivo avaliar o efeito no desempenho zootécnico e na sobrevivência de juvenis de pirarucu (*Arapaima gigas*) submetidos à suplementação de prebióticos em sua dieta. Foram adquiridos 120 juvenis de *A. gigas*, aclimatados em caixas d’água com aeração e fluxo de água contínuos, e alimentados ad libitum quatro vezes ao dia, com dieta contendo 55% de proteína bruta. Os peixes de 25,5 ± 3,5 g foram distribuídos em 12 caixas d’água de 500L (3 caixas d’água/tratamento e 10 peixes/caixa d’água). Os juvenis foram submetidos ao controle (sem prebióticos) e também aos três tratamentos A, B e C. Os parasitas encontrados foram observados em microscopia óptica e eletrônica de varredura (MEV). Os peixes foram distribuídos em caixas d’água de delineamento inteiramente casualizado. Os dados obtidos foram avaliados pelos testes de Shapiro-Wilk e Bartlett (α=0,05). Em seguida, foram submetidos à ANOVA e contrastados pelo teste de Tukey (α=0,05). Enquanto os tratamentos foram contrastados entre si pelo teste de Dunn (α=0,05). Os parâmetros de qualidade da água foram mantidos adequados para o cultivo de *A. gigas*. Nos tratamentos, o desempenho zootécnico e a taxa de sobrevivência diferiram entre si e foram melhores que o controle (p<0,05). O tratamento A apresentou o maior peso final 77,32 ± 10,23g (p<0,05), e o controle o menor peso final 20,08 ± 6,64g. O ganho médio de peso foi diferente entre os tratamentos (p<0,05), que apresentou melhores resultados que o controle (p<0,05). Os tratamentos A e C expressaram o maior ganho médio de peso 49,90 ± 8,95 e 54,17 ± 4,12 g (p<0,05), respectivamente. A biomassa final foi diferente entre os tratamentos, apresentando melhores resultados que o grupo controle (p<0,05). A taxa de sobrevivência não foi diferente entre os tratamentos (p>0,05). Porém, o controle apresentou o menor índice de 13,33%, devido à infestação por tricodínidos. Os prebióticos suplementados foram benéficos para ganho de peso, biomassa final, conversão alimentar aparente e sobrevivência de juvenis de pirarucu.

**Palavras-chave**: desempenho zootênico, piscicultura, *Trichodina spp.*

**RESUMEN**
El estudio tuvo como objetivo evaluar el efecto sobre el desempeño zootécnico y la supervivencia de juveniles de paiche (*Arapaima gigas*) sometidos a suplementación prebiótica en su dieta. Se adquirieron 120 juveniles de *A. gigas*, se aclimataron en tanques de agua con aireación y flujo de agua continuos, y se alimentaron ad libitum cuatro veces al día, con una dieta que contenía 55% de proteína cruda. Los peces de 25,5 ± 3,5 g se distribuyeron en 12 tanques de agua de 500 L (3 tanques de agua/tratamiento y 10 peces/tanque de agua). Los juveniles fueron sometidos al control (sin prebióticos) y también a los tres tratamientos A, B y C. Los parásitos encontrados se observaron bajo microscopía óptica y electrónica de barrido (MEB). Los peces fueron distribuidos en tanques de agua en un diseño completamente al azar. Los datos obtenidos fueron evaluados mediante las pruebas de Shapiro-Wilk y Bartlett (α=0,05). Luego fueron sometidos a ANOVA y contrastados mediante la prueba de Tukey (α=0,05). Mientras que los tratamientos se contrastaron entre sí mediante la prueba de Dunn (α=0,05). Se mantuvieron parámetros de calidad del agua adecuados para el cultivo de *A. gigas*. En los tratamientos, el desempeño zootécnico y la tasa de supervivencia difirieron entre sí y fueron mejores que el control (p<0,05). El tratamiento A tuvo el mayor peso final de 77,32 ± 10,23 g (p<0,05), y el control tuvo el menor peso final de 20,08 ± 6,64 g. La ganancia de peso promedio fue diferente entre los tratamientos (p<0,05), los cuales presentaron
mejores resultados que el control (p<0,05). Los tratamientos A y C expresaron la mayor ganancia de peso promedio, 49.90 ± 8.95 y 54.17 ± 4.12 g (p<0,05), respectivamente. La biomasa final fue diferente entre tratamientos, mostrando mejores resultados que el grupo control (p<0.05). La tasa de supervivencia no fue diferente entre tratamientos (p>0,05). Sin embargo, el control presentó la tasa más baja de 13,33%, debido a la infestación por tricodinidos. Los prebióticos suplementados fueron beneficiosos para el aumento de peso, la biomasa final, la conversión alimenticia aparente y la supervivencia de los juveniles de paiche.

Palabras clave: desempeño zootécnico, piscigranja, Trichodine spp.

1 INTRODUCTION

The world demand for products from aquaculture has grown relatively significantly in recent years, mainly due to population growth and consumer demand for healthier foods (Oliveira et al., 2021). In this context, fish farming emerges as a viable alternative to continue increasing food supply in the coming years (Dantas Filho et al., 2021). Furthermore, the Rondônia state is the largest producer of native fish in Brazil, corresponding to about 47% of the production of native species of a total of 65.5 thousand tons of fish produced in 2020 (Peixe BR, 2021), and the tambaqui Colossoma macropomum (Cuvier, 1818) and pirarucu Arapaima gigas (Schinz, 1822) are the most cultivated fish, which together represent about 85% of fish cultivated in Rondônia state (Meante and Dória, 2017; Cavali et al., 2020).

However, according to Dias et al. (2018), the development of intensive fish farming requires high stocking densities and the intensification of the handling routine. These practices inevitably cause fish handling stress and consequently the emergence of opportunistic pathogens, factors that can negatively affect fish health and performance (Hisano et al., 2018). Previous studies have shown in fish a significant improvement in zootechnical performance, resistance and disease survival using food additives such as probiotic and prebiotic immunostimulants (Amin et al., 2015; Ramos et al., 2015). Supplementation of prebiotics in the feed of fishes is an alternative to enable fish welfare in intensive farming. In addition to reducing the possibility of animals contracting gastrointestinal diseases (Azevedo et al., 2016). However, several countries have strict legislation on the use of feed additives (Baets et al., 2009). Thus, food additives based on the yeast Saccharomyces cerevisiae or its co-products were tried in order to stimulate growth and act as an immunomodulator in Labeo rohita (Twary and Patra, 2011), Huso huso (Hoseinifar
et al., 2011), Oreochromis niloticus (Abdel-Tawwab et al., 2008; Amin et al., 2015), Piaractus mesopotamicus (Biller-Takahashi et al., 2014; Hisano et al., 2018), Rutilus frisii (Rufchaie and Hoseinifar, 2014), Solea senegalensis (Batista et al., 2016), Oncorhynchus mykiss (Huyben et al., 2017), Brycon amazonicus (Montoya et al., 2018) and Arapaima gigas (Dias et al., 2019). Much of this research has expressed not only the importance of the effects of immunostimulants, but also improvements in performance, survival and feed conversion (Twary and Patra 2011; Batista et al. 2016).

The walls of the yeast S. cerevisiae contain glycoproteins, glucan (branch of β-1,3-d-glucan and β-1,6-d glucan) and a small amount of chitin, which provides favorable energy and protein support in relation to other food supplements (Amin et al. 2015). Thus, there is considerable interest from companies and fish farms to administer S. cerevisiae and its derivatives to mitigate the harm caused by the stress of handling and/or intensive cultivation. Therefore, according to Batista et al. (2016) S. senegalensis fed diets supplemented with products derived from S. cerevisiae, presented better utilization of dietary protein, feed conversion and longer intestinal villi. A. gigas is an excellent candidate for the most desirable species for aquaculture, thanks to its rapid growth rates, high market price for regional marketing and export, captive breeding, tolerance to low water quality, high memory immunological, high pulp quality – mechanically separated meat (CMS) and fillet yield (<45%), which outperforms other species (Oliveira et al., 2012; Silva and Duncan, 2016). However, parasitic diseases outbreaks are frequent, which makes their cultivation a primary restriction (Lima et al., 2017).

Given these assumptions, the study aimed to evaluate the effect on the zootechnical performance and survival of juvenile A. gigas submitted to prebiotic supplementation in the feed and cultivated in water-boxes with continuous aeration and water flow.

2 MATERIAL AND METHODS

A total of 120 juveniles A. gigas were acquired in a commercial fish farming in the city of Porto Velho, RO – Brazil (Figure 1 A). The fish were transported to the hatchery laboratory, acclimated in 500L water-boxes with, continuous aeration and water flow and fed ad libitum four times a day, with a diet containing 55% crude protein. Four experimental diets formulated to be isoprotein (55% crude protein) and isolipid were provided. (9%) (Table 1), which were
supplemented in different treatments with prebiotics. For 90 days, the fish were fed the experimental diets four times a day (8 am, 11 am, 2 pm and 5 pm), at 10% of the biomass.

Table 1: Guarantee levels of the feed provided to juvenile A. gigas for 90 days (n=120).

<table>
<thead>
<tr>
<th>Composition</th>
<th>Content (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein (g)</td>
<td>450.0</td>
</tr>
<tr>
<td>Crude fiber (g)</td>
<td>40.0</td>
</tr>
<tr>
<td>Calcium (Min, g)</td>
<td>20.0</td>
</tr>
<tr>
<td>Calcium (Max, g)</td>
<td>30.0</td>
</tr>
<tr>
<td>Phosphorus (g)</td>
<td>8.0</td>
</tr>
<tr>
<td>Vitamin C (g)</td>
<td>0.6</td>
</tr>
<tr>
<td>Ethereal extract (g)</td>
<td>80.0</td>
</tr>
<tr>
<td>Mineral matter (g)</td>
<td>1150.0</td>
</tr>
<tr>
<td>Moisture (g)</td>
<td>120.0</td>
</tr>
</tbody>
</table>

1Amount of nutrients per kg for the crude protein ration (55%). Pantothenic Acid (min) – 3.00 mg; Biotin (min) – 50 mg; Choline (min) – 290 mg; Vitamin A (min) – 28,000 IU; Vitamin B1 (min) – 2.00 mg; Vitamin B12 (min) – 4.00 mg; Vitamin B2 (min) – 3.00 mg; Vitamin B6 (min) – 2.00 mg; Vitamin D3 (min) – 5,000 IU; Vitamin E (min) – 45.00 IU; Vitamin K3 (min) – 2.00 mg; Copper (min) – 10.00 mg; Iron (min) – 90 mg; Iodine (min) - 0.40 mg; Niacin (min) – 50.00 mg; Manganese (min) – 10.00 mg; Zinc (min) – 180 mg; Selenium (min) – 0.60 mg.
Source: authors’ archive.

Following the acclimatization process, fishes of 25.5 ± 3.5g were distributed in 12 water-boxes of 500 L (3 water-boxes/treatment and 10 fishes/water-boxes) (Figure 1B). Therefore, juvenile A. gigas were submitted to the control (without prebiotics) and submitted to three treatments A, B and C with different prebiotic ingredients (Table 2). All treatments, equally, received preventive measures, such as prophylactic measures.

Table 2: Treatments with prebiotics ingredients for juveniles A. gigas, for 90 days (n=120).

<table>
<thead>
<tr>
<th>Prebiotics ingredients</th>
<th>Yeast (Sccharomyces cerevisiae) (%)</th>
<th>Beta Glucans (min., g/kg)</th>
<th>Glucamanans (min., g/kg)</th>
<th>Mannanoligosaccharides (min., g/kg)</th>
<th>Fructooligosaccharides (min., g/kg)</th>
<th>Galactooligosaccharides (min., g/kg)</th>
<th>Crude Protein (mi., g/kg)</th>
<th>Calcium (g/kg)</th>
<th>Magnesium (min., g/kg)</th>
<th>Selenium (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>71.4</td>
<td>221.0</td>
<td>309.0</td>
<td>88.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>30.0</td>
<td>5.0</td>
<td>160.0</td>
</tr>
<tr>
<td>B</td>
<td>50.0</td>
<td>150.0</td>
<td>-</td>
<td>60.0</td>
<td>120.0</td>
<td>72.0</td>
<td>150.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C</td>
<td>85.0</td>
<td>300.0</td>
<td>420.0</td>
<td>120.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>150.0</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

1Treatments were administered by purchasing three commercial prebiotic formulations available in the local market.
Source: authors’ archive.
The water quality parameters checked daily in the water-boxes were hydrogen potential (pH), dissolved oxygen (mg/L), conductivity (µs/cm) and temperature (ºC) in situ, using a multiparameter probe (HSY, Kyoto, Japan) previously calibrated following the recommendations of Galvão et al. (2014) and Cavali et al. (2020). Also, the variables Total ammonia concentration (mg/L), alkalinity (mg/L CaCO₃), hardness (mg/L CaCO₃), nitrite (NO₂) and nitrate (NO₃) were verified weekly. Data which were obtained in the laboratory, following the methodologies of Scorvo Filho et al. (2004) and Davidson et al. (2014).

After the feeding trial, some parameters of zootechnical performance and survival rate were evaluated, as suggested by Yamamoto et al. (2002) and Dias et al. (2019). These were initial weight (g), final weight (g), average weight gain (g), biomass gain per water-box (g), amount of feed in the period (g), apparent feed conversion and survival rate (%). In order to standardize sanitary conditions, the control group and prebiotic treatments received the same prophylactic measures. Before the tests, the fish received salt baths/concentration 4.0 g/L, for 30 minutes, to prevent stress and improve mucus production (Tavares-Dias and Montagner, 2015).

If parasites are found during the experimental period, they will be observed by light optical microscopy and then by scanning electron microscopy (SEM). Protocols for optical microscopy, sanitary conditions, sampling, monitoring, laboratory analysis (scraping, slides, and parasitological analysis) were performed as presented by Ishikawa et al. (2016).

To macroscopically observe the different structures, the images were photographed with the aid of a professional photographic camera - Digital Camera NIKON 7557 Coolpix P950. After complete fixation, these were processed for scanning electron microscopy (SEM). The resolutions established for observation in SEM were 100x and 800x, according to standardization of observation suggested by Gomes et al. (2017). The fragments were analyzed (in SEM) at the Advanced Diagnostic Imaging Center – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo (CADI/FMVZ/USP).

Animals were distributed in water-boxes in a completely randomized design. The data obtained were evaluated under the assumptions of homoscedasticity and normality using the Shapiro Wilk and Bartlett’s tests (α=0.05). Then, the data were submitted to ANOVA (One Way), and later the control group and treatments were contrasted using the Tukey’s test (α=0.05). While the treatment data were contrasted with each other by the Dunn’s test (α=0.05) (Zar, 2010). The
software used to perform the statistical analyzes was the Genes Program made available by the Universidade Federal de Viçosa (UFV), version 13.3 (Cruz, 2013).

The study was conducted by the Universidade Federal de Rondônia (UNIR) and approved by the Ethics Committee on the Use of Animals (CEUA) of UNIR, with protocolled assent under registration number 012/2021. The research was carried out in the hatchery laboratory of a fish farm located in the city of Porto Velho, RO - Brazil.

3 RESULTS

Firstly, in relation to water quality, pH, dissolved oxygen (mg/L), temperature (°C), hardness (mg/L CaCO$_3$), nitrate (NO$_3$) and nitrite (NO$_2$) values presented no statistical difference (p>0.05) between the control and treatments, and there was also no difference (p>0.05) between treatments. However, total ammonia concentration (mg/L) and alkalinity (mg/L CaCO$_3$), presented statistical differences (p<0.05) between treatments. More specifically, control and treatment C had the highest values of total ammonia concentration, 0.23 ± 0.3 and 0.20 ± 0.03 mg/L, respectively. treatments A and B had the highest values of alkalinity, 16.67 ± 2.50 and 20.00 ± 3.20 mg/L CaCO$_3$, respectively. The control and treatments B and C presented the highest values of hardness, 23.00 ± 4.90, 26.67 ± 3.47 and 20.0 ± 2.60 mg/L CaCO$_3$, respectively. And finally, the control group and treatment C had the highest Nitrate values, 0.90 ± 0.18 and 0.10 ± 0.02 NO$_2$, respectively (Table 3).

Concerning the results of zootechnical performance and survival rate, except for the initial weight (p>0.05), the other variables of the treatments presented differences between them (p<0.05), and their results of performance and survival were better than the control (p<0.05). Treatment A expressed the highest final weight 77.32 ± 10.23g (p<0.05), and the control had the lowest final weight 20.08 ± 6.64g. The average weight gain was different between treatments (p<0.05), which presented better results than the control (p<0.05). Treatments A and C expressed the highest mean weight gain 49.90 ± 8.95 and 54.17 ± 4.12g, respectively (p<0.05), and the control had the lowest mean weight gain 27.00 ± 3.25g. The final biomass was different between treatments (p<0.05), which presented better results than the control (p<0.05). Treatment A expressed the highest final biomass 655.67 ± 112.17g (p<0.05), and the control had the lowest final biomass 80.33 ± 26.78. Biomass gain per water-box was different between treatments
which showed better results than the control (p<0.05). Treatment C expressed the greatest gain in biomass per water-box 428.33 ± 97.84g, and the control group presented the smallest gain in biomass per water-box 9.00 ± 3.88g (Table 4).

The amount of feed consumed in the experimental period was not different between treatments (p>0.05), but presented higher amounts compared to the control (p>0.05). Treatments A, B and C expressed values of 1.56 ± 0.022, 1.55 ± 0.015 and 1.58 ± 0.035g, respectively. However, the control presented a value of 1.34 ± 0.034g for feed consumed. The apparent feed conversion index was not different between treatments (p<0.05). However, they expressed better results compared to the control (p<0.05). Treatments A, B and C expressed apparent feed conversion of 5.27 ± 2.55, 5.84 ± 0.94 and 4.66 ± 1.64, respectively. However, the control group presented an apparent feed conversion of 0.00 ± 0.00g, due to a low survival rate (Table 4).

Survival rate was not different between treatments (p>0.05). However, they had better rates compared to the control (p<0.05). Treatments A, B and C expressed survival rates of 83.33 ± 6.67, 80.00 ± 10.00 and 70.00 ± 15.00%. However, the control had a low survival rate of 13.33 ± 6.67% (Table 4), due to *Trichodina* spp. (Protozoa) infestations (Figure 1).

Figure 1: Trichodinid infestation in juveniles *A. gigas* cultivated in a highly controlled cultivation system. A: A specimen of *A. gigas* parasitized by *Trichodina* spp. at the end of the trial period; B: 100x magnified image of *Trichodina* spp. observed in light optical microscope.; C: 800x magnified image of *Trichodina* spp. observed in scanning electron microscopy (SEM).

Source: authors’ archive.
Table 3: Water quality in control and prebiotic treatments in the cultivation of juveniles A. gigas in water-boxes with continuous aeration and water flow, for 90 days (n=120).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Treatments</th>
<th>Control</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td></td>
<td>6.70 ± 0.80 a</td>
<td>7.0 ± 0.55 a</td>
<td>7.17 ± 0.57 a</td>
<td>6.83 ± 0.67 a</td>
<td>NS1</td>
</tr>
<tr>
<td>Dissolved oxygen (mg/L)</td>
<td></td>
<td>4.70 ± 0.05 a</td>
<td>4.71 ± 0.06 a</td>
<td>4.71 ± 0.06 a</td>
<td>4.70 ± 0.05 a</td>
<td>NS</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td></td>
<td>29.00 ± 0.01 a</td>
<td>29.02 ± 0.01 a</td>
<td>29.82 ± 0.01 a</td>
<td>29.02 ± 0.01 a</td>
<td>NS</td>
</tr>
<tr>
<td>Electrical conductivity (µS/cm)</td>
<td></td>
<td>0.02 ± 0.00 a</td>
<td>0.02 ± 0.00 a</td>
<td>0.02 ± 0.00 a</td>
<td>0.02 ± 0.00 a</td>
<td>NS</td>
</tr>
<tr>
<td>Total ammonia concentration (mg/L)</td>
<td></td>
<td>0.23 ± 0.03 a</td>
<td>1.50 ± 0.02 b</td>
<td>1.33 ± 0.02 b</td>
<td>0.20 ± 0.03 a</td>
<td>0.03333</td>
</tr>
<tr>
<td>Alkalinity (mg/L CaCO₃)</td>
<td></td>
<td>10.00 ± 1.80 b</td>
<td>16.67 ± 2.50 a</td>
<td>20.00 ± 3.20 a</td>
<td>10.00 ± 1.80 b</td>
<td>0.04000</td>
</tr>
<tr>
<td>Hardness (mg/L CaCO₃)</td>
<td></td>
<td>23.33 ± 4.90 a</td>
<td>19.67 ± 2.17 b</td>
<td>26.67 ± 3.47 a</td>
<td>20.00 ± 2.60 a</td>
<td>0.02666</td>
</tr>
<tr>
<td>Nitrite (NO₂)</td>
<td></td>
<td>0.05 ± 0.00 a</td>
<td>0.05 ± 0.00 a</td>
<td>0.05 ± 0.00 a</td>
<td>0.05 ± 0.00 a</td>
<td>NS</td>
</tr>
<tr>
<td>Nitrate (NO₃)</td>
<td></td>
<td>0.20 ± 0.18 a</td>
<td>0.23 ± 0.05 a</td>
<td>0.17 ± 0.03 a</td>
<td>0.10 ± 0.02 a</td>
<td>0.03111</td>
</tr>
</tbody>
</table>

If there are means followed by different letters (a, b) in the columns, they are different by Tukey’s test (p<0.05) and between each other by Dunn’s test (p<0.05);

INS: there was no significant variation.

Source: authors’ archive.

Table 4: Zootechnical performance and survival rate of juveniles A. gigas in control and prebiotic treatments for 90 days (n=120).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Treatments</th>
<th>Control</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>n</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting weight (g)</td>
<td></td>
<td>24.77 ± 2.03 a</td>
<td>27.43 ± 2.57 a</td>
<td>24.50 ± 3.55 a</td>
<td>25.83 ± 3.83 a</td>
<td>10</td>
<td>NS1</td>
</tr>
<tr>
<td>Final weight (g)</td>
<td></td>
<td>20.08 ± 6.64 c</td>
<td>77.32 ± 10.23 a</td>
<td>64.13 ± 3.35 b</td>
<td>54.17 ± 4.12 ab</td>
<td>10</td>
<td>0.00965</td>
</tr>
<tr>
<td>Average weight gain (g)</td>
<td></td>
<td>27.00 ± 3.25 c</td>
<td>49.90 ± 8.95 a</td>
<td>39.63 ± 2.19 b</td>
<td>54.17 ± 4.12 a</td>
<td>10</td>
<td>0.01333</td>
</tr>
<tr>
<td>Final biomass (g)</td>
<td></td>
<td>80.33 ± 26.78 c</td>
<td>655.67 ± 112.17 a</td>
<td>513.23 ± 81.77 b</td>
<td>559.70 ± 119.93 b</td>
<td>10</td>
<td>0.00167</td>
</tr>
<tr>
<td>Biomass gain per box (g)</td>
<td></td>
<td>9.00 ± 3.88 c</td>
<td>381.33 ± 66.22 b</td>
<td>268.33 ± 31.11 ab</td>
<td>428.33 ± 97.84 a</td>
<td>10</td>
<td>0.01111</td>
</tr>
<tr>
<td>Amount of feed in period (g)</td>
<td></td>
<td>1.34 ± 0.034 b</td>
<td>1.56 ± 0.022 a</td>
<td>1.55 ± 0.015 a</td>
<td>1.58 ± 0.035 a</td>
<td>10</td>
<td>0.00398</td>
</tr>
<tr>
<td>Apparent food conversion (%)</td>
<td></td>
<td>0.00 ± 0.00 b</td>
<td>5.27 ± 2.55 a</td>
<td>5.84 ± 0.94 a</td>
<td>4.66 ± 1.64 a</td>
<td>10</td>
<td>0.03766</td>
</tr>
<tr>
<td>Survival rate (%)</td>
<td></td>
<td>13.33 ± 6.67 b</td>
<td>83.33 ± 6.67 a</td>
<td>80.00 ± 10.00 a</td>
<td>70.00 ± 15.00 a</td>
<td>10</td>
<td>0.04133</td>
</tr>
</tbody>
</table>

If there are means followed by different letters (a, b) in the columns, they are different by Tukey’s test (p<0.05) and between each other by Dunn’s test (p<0.05);

INS: there was no significant variation.

Source: authors’ archive.
4 DISCUSSION

The values of water quality, pH, dissolved oxygen (mg/L), temperature (°C), total ammonia concentration (mg/L), alkalinity (mg/L CaCO₃), hardness (mg/L CaCO₃), nitrite (NO₂) and nitrate (NO₃) in the control and in the treatments with probiotics. During the study, they remained in accordance with the mean values recommended for the cultivation of A. gigas, according to Davdson et al. (2014), Leira et al. (2017) and Cavali et al. (2020).

Based on the results, it could be observed that treatments A and C were a little better than treatment B and better than the control. Especially in relation to the best average weight gain and final biomass (Table 4). Therefore, it is important to emphasize that according to the prebiotic ingredients presented (Table 2), treatments A and C contained the highest levels of Beta Glucans (221.0 k/kg and 300 g/kg), Glucamanans (309g/kg and 420g/kg) and Mannanoligosaccharides (88g/kg and 120g/kg). Pryor et al. (2003), Selim and Reda (2015) and Sado et al. (2018) also observed these benefits in their experiments, which better results in weight gain and survival occur thanks to prebiotic ingredients supplemented in commercial feed.

One of the main challenges of fish farming is to increase weight gain, survival rates and fish growth. The yeast Sacharomyces cerevisiae has been recommended as a dietary supplement due to its high protein content, immunostimulating effects and improvements in the performance of farmed fish (Huyben et al., 2017; Dias et al., 2019). In the present study, A. gigas received different treatments with prebiotics derived from the yeast S. cerevisiae. These treatments were able to improve not only the survival rate, but also weight gain, gain and final biomass, amount of feed consumed and apparent feed conversion (Table 4).

The better performance of supplemented fish can be attributed to the benefit of health status, nucleotide supply, high digestibility, stimulation of gastric development and/or enzyme secretion (Hoseinifar et al., 2011; Batista et al., 2016). It should be emphasized that experiences similar to the present study were found in the literature, such as reports for Rohu (Labeo rohita) (Tewary and Patra, 2011), White sturgeon (Huso huso) (Hoseinifar et al. 2011), Nile tilapia (Oreochromis niloticus) (Amim et al. 2015) and Piavuçu (Leporinus macrocephalus) (Batista et al., 2016), in a diet supplemented with different concentrations of S. cerevisiae. These authors showed that treatments with prebiotics led to greater use of protein in the feed, better feed conversion and specific growth rate.
Immunostimulants are compounds that modulate the immune system, increasing adaptive resistance (Bricknell and Dalmo, 2005) against fish diseases and stress. In fish, the ingestion of lipopolysaccharide-based immunostimulates from the yeast cell wall has advantages and is a useful method of exposure in large-scale fish farming. And then it was evaluated in some research (Hoseinifar et al., 2011; Tewary and Patra, 2011; Rufchaie and Hoseinifar, 2014; Amin et al. 2015; Mannopo et al., 2015; Huyben et al. 2017; Hoshino et al. 2017). This study revealed that a dietary content with prebiotic ingredients derived from S. cerevisiae had significant effects on the survival of juveniles A. gigas to stress of cultivation treatment. Similar results have been reported for rainbow trout (Oncorhynchus mykiss) when subjected to cannulation stress (Huyben et al., 2017) and pavuçu (Leporinus macrocephalus) subjected to capture stress (Lima et al. 2015). However, to prevent stress in intensive A. gigas fish farming, an intervention with S. cerevisiae could be necessary in relation to immunostimulants. Beneficial yeasts that manipulate the intestinal microbiota through dietary supplementation are a good nutritional and immunological approach (Dias et al., 2019).

The evolution of fish farming depends on the intensification of production systems, so factors such as water quality, stocking density, food and health are relevant to the system's productivity (Aubin et al., 2017). In intensive fish farms, the nutrition and health of aquatic organisms are essential, as the opportunistic bacteria found in the environment can modify the microbiota of fish and cause outbreaks of mortality in production, as occurred in the present study. And, one of the alternatives that is constantly researched is the use of probiotics that have positive results in the zootechnical performance and in the health of fish by improving the immune system (Mouriño et al., 2012). Finally, in diets supplemented with Lactobacillus plantarum, differences were observed in weight gain and survival rate of Nile tilapia (O. niloticus) cultivated in polyculture of marine shrimp (Litopenaeus vannamei) compared to the control (Jatobá et al., 2011). In the studies by Sousa (2015) and Jatobá et al. (2011), L. plantarum isolated from A. gigas, presented results similar to those of Jatobá et al. (2011) on the survival performance of fingerlings, adding improvement in apparent feed conversion and uniformity of fish fed with probiotic at a concentration of 10^8 CFU/g.

Even though the preventive measures were standardized for all treatments, the control suffered strong parasitic infestation. Confirming the claims of fish farmers in the region, which currently have fish with parasitic diseases. Regarding Trichodinidae infestation in the control of
the present study, the gills and osmoregulation are direct targets of these parasites (Tavares-Dias et al., 2021). The branchial alterations, which characterize the host's responses to these parasites, present a pattern characterized in acute lesions of hypertrophy, edema, necrosis and epithelial desquamation, and in chronic lesions there is hyperplasia, fusion of the lamellae and necrosis (Onaka, 2009). In addition, there are hemodynamic, inflammatory and degenerative changes associated with behaviors that demonstrate respiratory incapacity of fish (Schalch et al., 2006). The histopathological changes observed were mononuclear inflammatory infiltration, epithelial detachment, primary lamella congestion, hemorrhage and secondary lamella epithelial hyperplasia, eosinophilic granular inflammatory infiltrate, lamellar fusion, telangiectasia, epitheliocystis, sinus venous dilatation, among others (Steckert et al., 2018). To avoid this problem in a closed cultivation system like the one carried out in this study, tracking the provenance of fingerlings, disinfection of fishing nets, as well as frequent monitoring of the presence of parasites and water quality, adequate stocking densities and systematization of good management practices. Furthermore, it is suggested that cropping systems be designed with individualized supply and drainage structures.

5 CONCLUSIONS

Supplemented prebiotics, especially Beta Glucans, Glucamanans and Mannanoligosaccharides derived from the yeast Sacharomyces cerevisiae, in the diet were beneficial for weight gain, final biomass, apparent feed conversion and survival of juveniles A. gigas. It is suggested as an emerging preventive measure to avoid Thrichodinine infestations, frequent monitoring of the presence of parasites and water quality, adequate stocking densities and systematization of good management practices. Furthermore, it is suggested that cropping systems be designed with individualized supply and drainage structures.
ACKNOWLEDGMENTS

CAPES/Brazil through the National Program for Academic Cooperation in the Amazon (PROCAD-AM - UNIR/UFAC/USP), for the master's and post-doctoral scholarships. To the National Council for Scientific and Technological Development (CNPq) and the Rondônia Foundation for the Development of Action and Scientific and Technological Research in the State of Rondônia (FAPERO), PAP/Piscicultura 2021 project, for the financial support and for awarding a post-doctoral scholarship to Jerônimo Vieira Dantas Filho [167879/2022-7].
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