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FERMENTED AND NON-FERMENTED WHOLE RICE BRAN IN THE PRODUCTION OF THE ROTIFER *Brachionus plicatilis**

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ABSTRACT

The objective of the present study was to evaluate the use of fermented and non-fermented whole rice bran for rotifer feeding, based on the effects on growth parameters, antioxidant and oxidative damage responses, and water quality. The study was based on three experiments, which compared the effect of different concentrations of non-fermented whole rice bran, the effect of different concentrations of fermented whole rice bran and the effect of the best concentrations of fermented and non-fermented whole rice bran, as well as the replacement of part of the baking yeast by rice bran. The results showed the best growth performances in treatments with 0.7 g yeast with 1.5 g fermented rice bran, 0.35 g yeast with 0.75 g whole rice bran, and 0.35 g yeast with 0.75 g fermented rice bran. Fermentation of rice bran for 6 hours did not induce oxidative stress in rotifers. This work revealed that the use of 1.5 g of fermented bran and replacement of 50% of yeast with fermented or non-fermented rice bran may be used for rotifer feeding, with the additional benefit of improving the environmental quality due to the lower amount of ammonia released in the water.

Keywords: Rice bran; population growth; production; nutrition; solid-state fermentation.

UTILIZAÇÃO DO FARELO DE ARROZ COM E SEM FERMENTAÇÃO NA PRODUÇÃO DO ROTÍFERO *Brachionus plicatilis*

RESUMO

O objetivo do presente trabalho foi avaliar a utilização do farelo de arroz integral e fermentado na alimentação de rotíferos, baseado nos efeitos sobre os parâmetros de desempenho, respostas antioxidantes e de dano oxidativo, e qualidade de água. O estudo foi baseado em três experimentos, os quais compararam o efeito de diferentes concentrações de farelo de arroz integral na alimentação de rotíferos, o efeito de diferentes concentrações de farelo de arroz fermentado na alimentação de rotíferos e o efeito das melhores concentrações de farelo de arroz integral e fermentado, bem como a substituição de parte da levedura de panificação por esses farelos. Os resultados mostraram um melhor desempenho em crescimento nos tratamentos com 0,7 g de levedura, 1,5 g de farelo fermentado, 0,35 g de levedura + 0,75 g de farelo integral e 0,35 g de levedura +0,75 g de farelo fermentado. A fermentação do farelo de arroz por 6 horas não induziu estresse oxidativo nos rotíferos. Foi constatado que pode ser usado 1,5 g de farelo fermentado e substituído 50% de levedura por farelo de arroz fermentado ou integral na alimentação de rotíferos, com a vantagem de apresentar melhor qualidade ambiental, devido a diminuição da amônia da água.

Palavras-chave: Farelo de arroz; crescimento populacional; produção; nutrição; fermentação em estado sólido.

INTRODUCTION

In aquaculture, live food is essential for the creation of early life stages of marine fish larvae. Advances in the development of artificial microdiets have reduced dependence on *Artemia* (Kolkovski, 2013). However, the use of microdiets to replace or minimize the use of rotifers is not yet a reality for early feeding of most marine fish larvae (Kim et al., 2018).

Rotifers are considered non-selective filtering animals, which facilitates their feeding and enables the offer of many different diets, including microalgae, yeast and, inert foods

(Abdull et al., 2018). Among the different species, the best-known rotifer used in fish larviculture is *Brachionus plicatilis*, due to its small size (120 - 300 µm), reduced mobility, permanence in the water column, large-scale production capacity, easy management in terms of assimilation of enriched and bactericidal substances, and a wide tolerance range for changes in temperature, salinity, and oxygen (Kailasam et al., 2015).

Despite microalgae are often used in the diet of *B. plicatilis* since they are a source of essential fatty acids (Ferreira et al., 2018), microalgae production requires a lot of work and it is considered costly for rotifer feeding (Norsker et al., 2011). Thus, the current trend is to avoid the use of live microalgae and replace them with bioencapsulated diets and yeast (Muller-Feuga, 2000). Baking yeast is very used as a cheap food source for the rotifers. However, yeast presents the problem of rapidly deteriorating the water quality, requiring a higher level of water management attention, and the rotifers produced are unsuitable for feeding marine fish larvae, due to their low nutritional quality (Hamre, 2016).

Rotifers are produced in environments where environmental variables, such as temperature, pH, oxygen, and salinity are subject to variation. These variations can lead to a state of oxidative stress for rotifers (Denekamp et al., 2009). Reactive oxygen species (ROS) are naturally produced in aerobic organisms during oxidative metabolism and include hydroxyl radicals ($HO^{\cdot}$), superoxide anions ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) (Dröge, 2002). At high concentrations, ROS species can promote harmful effects on biomolecules (Navarro-Yepes et al., 2014). The organisms have antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), which react with oxidizing compounds and protect cells and tissues from oxidative stress (Sevcikova et al., 2011).

Rice bran, one of the byproducts that result from rice processing, represents about 5 to 8% of the total grain. It is one of the most nutritious parts of the grain, containing vitamins, minerals (such as iron, phosphorus, and magnesium), 11 to 13% protein, 11% fiber, and over 20% oils, mainly unsaturated fatty acids (Christ-Ribeiro et al., 2017). Rice bran also has antioxidant components, including oryzanol and tocopherols (Massarolo et al., 2017). On the other hand, the fermentation of rice bran by microorganisms may significantly increase the protein content and the antioxidant activity due to the high level of phenolic compounds (Christ-Ribeiro et al., 2019). Due to its abundance, rice bran has a low cost, being the object of different investigations in the areas of feeding, oil component extraction, protein extraction, and use in biotechnology processes to obtain useful metabolites (Massarolo et al., 2016; 2017; Christ-Ribeiro et al., 2019).

Accordingly, this study aimed to evaluate the use of fermented and non-fermented whole rice bran as food sources for the production of *B. plicatilis*. To meet this objective, studies were performed on the zootechnical performance of this species assessing parameters such as population growth rate (r), percentage of ovate female (F), and doubling time (dt). Antioxidant and oxidative stress parameters such as reactive oxygen species (ROS), total antioxidant capacity (ACAP), and lipid peroxidation (TBARS) were also evaluated.

MATERIALS AND METHODS

Local of the study

The study was conducted at the Estuarine and Marine Fish Farming Laboratory (LAPEM) of the Marine Aquaculture Station of the Institute of Oceanography of the Federal University of Rio Grande (EMA-FURG), located at the Cassino Beach, Rio Grande, RS, Brazil.

Foods used in experiments

Whole rice bran of the species *Oryza sativa* was donated by the Chemistry and Food School (IQA) of the Federal University of Rio Grande (FURG). The baking yeast (*Saccharomyces cerevisiae*) was purchased in retail shops. The fermented rice bran was obtained by solid-state fermentation performed according to Feddern et al. (2007). The procedure consists in distributing the rice bran in a 1 cm layer of tray bioreactors (29 x 17 x 5.5 cm) and sterilizing the set. Afterward, the previously hydrated yeast (3% pp^{-1}) was added and the moisture adjusted to 30% with sterile water. The process was carried out in an oven with air circulation at 30°C for 6 h.

Rotifer cultivation

The rotifers were produced in a batch system and fed with 0.7 g yeast / 1.0 x 10^6 rotifers, supplied 5 times a day, according to Ferreira (2009).

Experimental design

Experiment 1: Use of different concentrations of non-fermented whole rice bran in the feeding of Brachionus plicatilis

The first experiment compared the effect of different concentrations of non-fermented whole rice bran on the rotifer growth rate. Four treatments were tested, with three repetitions each: Control (0.7 g yeast); 0.5 g non-fermented whole rice bran; 1.0 g non-fermented whole rice bran; 1.5 g non-fermented whole rice bran. The concentration of the food offered for all treatments was 1.0 x 10^6 rotifers.

Experiment 2: Use of different concentrations of fermented whole rice bran in the feeding of Brachionus plicatilis

The second experiment compared the effect of different fermented whole rice bran concentrations on the rotifer growth rate. Four treatments were tested, with three repetitions each: Control (0.7 g yeast); 0.5 g fermented whole rice bran; 1.0 g fermented whole rice bran; 1.5 g fermented whole rice bran. The concentration of the food offered for all treatments was 1.0 x 10^6 rotifers.

Experiment 3: Partial and total replacement of baking yeast by fermented and non-fermented whole rice bran in the feeding of Brachionus plicatilis

The third experiment compared the effect of the best concentrations of fermented and non-fermented whole rice bran, as well as the effect of the replacement of part of yeast by rice bran on the population growth rate and antioxidant and oxidative damage responses of the rotifer. Five treatments were tested with three repetitions each: Control (0.7 g yeast); 1.5 g whole bran; 1.5 g fermented whole rice bran; 0.35 g yeast + 0.75 g whole rice bran; 0.35 g yeast + 0.75 g fermented whole rice bran. The concentration of the food offered for all treatments was 1.0 x 10^6 rotifers.

The experimental design of the three experiments was completely randomized. The cultures were continuously aerated through aeration stones. The water used was pumped from the Casino beach, filtered, chlorinated and dechlorinated for later use. The salinity of the water was 25. The 2 L volume tanks were placed on a water table with thermostats, keeping the temperature at 25°C within the experimental units. The light was kept constant. For each treatment, 200 rotifers mL^{-1} were initially used. The food was dissolved in freshwater and supplied 6 times a day, in a volume of 2 mL, at 4- hour interval. At the end of 3 days, the rotifers were washed, the population growth assessed, and the culture restarted. Two cultivation cycles were performed in each experiment to observe the influence of time over the growth performance in each experiment.

Zootechnical performance evaluation

In the three experiments, the assessment of *B. plicatilis* population growth was made through daily counts of individuals from each experimental unit made in a Sedgewick-Rafter chamber through a sub-sample of each experimental unit, fixed in Lugol. The population density (rotifers mL^{-1}) and the number of ovate females were evaluated to determine the percentage of ovate females (F), calculated according to F = egg rotifers / total rotifers (Kostopoulou and Vadstein, 2007).

Population growth rate (r) was determined by the following formula: $r = (\ln N_t - \ln N_o)/t$, where Nt is the final number of rotifers; No is the initial number of rotifers, and t is the cultivation time (Rioboo et al., 2007). The duplication time (dt) was calculated according to the equation: $dt = \ln(2)/r$.

Water quality parameters

For all experiments, the temperature was measured daily in the morning, along with dissolved oxygen, using an oximeter (550A, YSI, U.S.A). Salinity was measured with a refractometer (ATAGO S / Milli-E, Japan) and pH with bench pH meter (METTLER TOLEDO Five Easy FE20, Swiss). Alkalinity was analyzed by titration according to the APHA method (APHA, 1998). Nitrogen, ammonia, and nitrite analyses were performed daily according to the methods of Koroleff and Palmork (1972) and Aminot and Chaussepied (1983).

Reactive Oxygen Species (ROS) concentration in rotifers

The *in vivo* ROS quantification of the third experiment followed the protocol adapted from Xie et al. (2006), which uses dichlorofluorescein 2'7 'diacetate (H_2DCFH-DA), a compound with high permeability across cell membranes which emits fluorescence when oxidized by the action of ROS. A spectrofluorimeter (Biotek, Synergy HT) was used to perform fluorimetric readings (485 nm excitation and 520 nm emission) every 3 minutes for 60 minutes. The temperature was set at 25ºC (same temperature maintained in rotifer cultivation). The estimation of ROS production was made by calculating the area of the curve after adjusting the second-degree polynomial to the liquid fluorimetric data over time.

Total antioxidant capacity against peroxyl radicals

Rotifer samples from the third experiment were previously diluted with homogenizing buffer and the protein concentration adjusted to 2.0 mg protein mL^{-1}. The antioxidant capacity against peroxyl radicals (ACAP) was determined according to the method described by Amado et al. (2009). Fluorimetry (485 nm excitation; 520 nm emission) was measured by spectrofluorimeter (Biotek, Synergy HT) with readings every 5 minutes for 30 minutes. ACAP values (expressed as relative area) were calculated using the expression proposed by Monserrat et al. (2014) where a larger relative area means a lower antioxidant capacity and vice versa.

Lipid peroxidation

In the third experiment, lipid peroxidation levels in rotifers were measured according to Oakes and Van Der Kraak (2003). This method quantifies the levels of malondialdehyde (MDA), a byproduct of lipid peroxidation, assessing thiobarbituric acid-reactive substances (TBARS). Fluorimetric measurements (520 nm excitation, 580 nm emission) were performed using a spectrofluorimeter (Biotek, Synergy HT) and the results were expressed as nmol TMP mg wet $tissue^{-1}$, where TMP corresponds to tetramethoxypropane (ACROS Organics), used as standard.

Proximal composition of diets

To determine the composition of the diets, the analysis of the proximal composition was performed according to the AOAC (1999) methodology at the Laboratory of Nutrition of Aquatic Organisms (LANOA) of the Federal University of Rio Grande - FURG. Dry matter (DM) analysis was performed in an oven at 60ºC for 5 h; for ashes (MM) the samples were taken to the muffle furnace at 600ºC for 6 h. Crude protein (CP) analysis was performed according to the Kjeldahl methodology. The lipid value was determined using the hot extraction method by the Soxhlet extractor.

Extraction of phenolic compounds

Phenolic compounds from fermented and non-fermented whole rice bran were extracted with methanol 1:10 (W/V) at the School of Chemistry and Food (EQA) of the Federal University of Rio Grande - FURG, following the method described by

Souza et al. (2009). Phenolic compounds were quantified by the spectrophotometric method (765 nm) using gallic acid (Sigma-Aldrich) standard curve (2–30 µg mL^{-1}).

Statistical analysis

In all three experiments, the data obtained were assessed for the assumptions of normality (Shapiro-Wilk) and homogeneity (Levene). The data were submitted to one-way ANOVA and, when statistical differences were detected between treatments, the means were compared by the Newman-Keuls test. To compare the first and second cycles, repeated measures ANOVA was performed. All analyzes were performed with a minimum significance level of 5% (Sokal and Rohlf, 1995).

RESULTS

Proximal composition of diets

There was no significant difference for crude protein, lipid, and moisture between fermented and non-fermented whole rice bran, but a significant difference was detected between them regarding the ash content. Yeast presented statistical differences, with higher crude protein and lower lipid, ash, and moisture compared to all other diets ($p<0.05$). Non-fermented whole rice bran + yeast and fermented whole rice bran + yeast did not present statistical differences between them regarding protein and lipid content but exhibited differences in relation to moisture and ash (Table 1). Regarding the total amount of phenolic compounds, fermented whole rice bran presented a higher amount of total phenolic compounds than non-ferment whole rice bran (Table 2).

Water quality

There were no significant differences ($p>0.05$) in the water quality parameters between treatments, for both first and second cycles, except for ammonia, which presented significantly higher values ($p<0.05$) for the yeast treatments than the other ones (Table 3). All parameters were kept within the safe limits for *B. plicatilis* and here are presented as means ± standard deviation of all values. The dissolved oxygen concentration was 5.89 ± 0.30 mg L^{-1}, the temperature was 25.44 ± 0.19°C, salinity was 25.73 ± 0.36, pH was 7.62 ± 0.16, and alkalinity was 122.96 ± 20.72 mg L^{-1} $CaCO_3$ for all three experiments.

Table 2. Amount of total phenolic compounds found in the crumbs used for rotifer feeding.

Diets	Phenols amount (mg g^{-1})
Fermented whole Rice Bran	16.16±0.03^{a}
Non-fermented Whole Rice Bran	15.71±0.10^{b}

Values are expressed as means ± SD (n = 3). Different letters in the columns show statistical differences according to the Newman Keuls test ($p<0.05$).

Population density (rotifers mL^{-1})

In the first experiment, the rotifer density was significantly higher in the 0.7 g yeast treatment than in the other treatments, both in the first and second cycles. In the first cycle, the treatments with 1.0 g and 1.5 g of non-fermented whole rice bran did not differ from each other, with densities of 486 ± 58 and 479 ± 34 rotifers mL^{-1}, but differed from the treatment with 0.5 g of non-fermented whole rice bran, which presented a lower final density (378 ± 21 rotifers mL^{-1}) ($p<0.05$). In the second cycle, the three treatments with 0.5, 1.0, and 1.5 g with non-fermented whole rice bran did not differ statistically from each other, while 0.7 g yeast treatment differed statistically from the others, presenting a higher density (588 ± 93 rotifers mL^{-1}) (Figure 1A).

For experiment 2, the treatments with 0.7 g yeast and 1.0 and 1.5 g of fermented whole rice bran obtained higher densities (683 ± 76, 642 ± 28 and 738 ± 119 rotifers mL^{-1}, respectively) and did not show statistical differences between them ($p>0.05$). Only the 0.5 g fermented whole rice bran treatment differed statistically from the other treatments, with a lower rotifer density of 496 ± 28 rotifers mL^{-1} ($p<0.05$). In the second cycle, the density of rotifers from the treatment with 0.7 g of yeast + 1.5 g of fermented whole rice bran did not differ statistically with values of 657 ± 81 and 666 ± 109 rotifers mL^{-1}, respectively. However, the treatment with 1.0 g of fermented whole rice bran did not differ statistically ($p>0.05$) from the 0.5 g fermented whole rice bran treatment (Figure 1B).

In experiment 3, the highest value of rotifer density was found in the treatment with 0.35 g yeast + 0.75 g of fermented whole rice bran in the first cycle (866 ± 22 rotifers mL^{-1}), which was statistically different from the other treatments ($p<0.05$). The treatments with 0.7 g yeast + 1.5 g of fermented bran and 0.35 g yeast + 0.75 g of non-fermented whole rice bran showed no statistical differences ($p>0.05$) between them (662 ± 154, 608 ± 75, and 685 ± 150 rotifers mL^{-1}, respectively).

Table 1. Proximal analysis of the diets (% dry weight) used to feed rotifers.

Diets	Protein	Lipid	Ash	Moisture
Yeast	49.0±1.66^{a}	1.2±0.33^{c}	5.1±0.26^{e}	1.4±0.07^{b}
Non-fermented Whole Rice Bran	19.8±0.82^{c}	20.4±2.32^{a}	12.2±0.38^{b}	8.1±0.15^{a}
Fermented whole Rice Bran	19.0±0.55^{c}	19.2±0.82^{a}	13.0±0.10^{a}	12.8±5.30^{a}
Non-fermented whole Rice Bran + Yeast	29.1±0.06^{b}	14.2±1.56^{b}	9.8±0.26^{d}	5.9±0.10^{ab}
Fermented whole Rice Bran + Yeast	28.6±0.75^{b}	13.3±0.61^{b}	10.4±0.15^{c}	9.2±3.6^{a}

Values are expressed as means ± SD (n = 3). Different letters in the columns show statistical differences according to the Newman Keuls test ($p<0.05$).

Table 3. Mean values of nitrogen compounds from the different production cycles in the three experiments.

Treatments	$NH_4^+ + NH_3$ (mg L^{-1})		NH_3 (mg L^{-1})		NO_2 (mg L^{-1})	
	Cycle 1	Cycle 2	Cycle 1	Cycle 2	Cycle 1	Cycle 2
0.7 g yeast	23.8±6.3[a]	18.0±3.5[a]	0.29±0.1[a]	0.12±0.04[a]	0.01±0.00 [a]	0.01±0.00[a]
0.5 g non-fermented whole rice bran	0.2±0.1[b]	0.22±0.06[b]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[a]	0.00±0.00[a]
1 g non-fermented whole rice bran	0.1±0.0[c]	0.16±0.01[c]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[a]	0.00±0.00[a]
1.5 g non-fermented whole rice bran	0.1±0.1[c]	0.13±0.03[c]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[a]	0.00±0.00[a]
0.7 g yeast	19.17±2.25[a]	19.33±0.58[a]	0.55±0.04[a]	0.48±0.01[a]	0.12±0.12[a]	0.00±0.00[a]
0.5 g fermented whole rice bran	0.00±0.00[b]	0.07±0.07[b]	0.00±0.00[b]	0.00±0.00[b]	0.01±0.01[b]	0.00±0.00[a]
1 g fermented whole rice bran	0.00±0.00[b]	0.04±0.08[b]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[a]
1.5 g fermented whole rice bran	0.000.00[b]	0.05±0.09[b]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[a]
0.7 g yeast	9.50±1.00[a]	8.83±1.04[a]	0.26±0.02[a]	0.22±0.03[a]	0.00±0.00[a]	0.00±0.00[a]
1.5 g non-fermented whole rice bran	0.13±0.03[c]	0.02±0.04[c]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[a]	0.00±0.00[a]
1.5 g fermented whole rice bran	0.00±0.00[d]	0.00±0.00[c]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[a]	0.00±0.00[a]
0.35g yeast + 0.75 g non-fermented whole rice bran	0.69±0.18[b]	0.50±0.44[b]	0.02±0.00[b]	0.01±0.01[b]	0.00±0.00[a]	0.00±0.00[a]
0.35g yeast + 0.75g fermented whole rice bran	0.75±0.07[b]	1.17±0.65[b]	0.02±0.00[b]	0.03±0.02[b]	0.00±0.00[a]	0.00±0.00[a]

Values are expressed as means ± SD (n = 3). Different letters in the columns show statistical differences according to the Newman Keuls test ($p<0.05$) only between each experiment. Each production cycle corresponded to three days.

The treatment with 1.5 g non-fermented whole rice bran presented the lowest density of rotifers (563 ± 63 rotifers mL^{-1}), and differed statistically from the other treatments ($p<0.05$). In relation to the second cycle, the treatments with 0.35 g yeast + 0.75 g of non-fermented whole rice bran and 0.35 g yeast + 0.75 g of fermented whole rice bran were the ones with the highest rotifer densities (661 ± 135 and 666 ± 13 rotifers mL^{-1}, respectively), being statistically different from the other treatments ($p<0.05$; Figure 1C). Moreover, no statistical differences were observed among the population density obtained in first and second cycles.

Percentage of ovate female (F)

Regarding the percentage of ovate females in Experiment 1, only the treatment with 0.5 g of non-fermented whole rice bran (3.0 ± 1.0%) differed statistically from the other treatments ($p<0.05$). The treatment with 0.7 g yeast and 1.0 and 1.5 g of whole rice bran presented the highest percentages in the first cycle (7.0 ± 2, 8.0 ± 1.0, and 11.0 ± 2.0%, respectively). In the second cycle, no statistical differences were obtained between them ($p>0.05$) (Figure 2A).

No statistical differences were observed among the percentage of ovate female obtained in first and second cycles for all experiments ($p>0.05$). No statistical differences ($p>0.05$) were found regarding the percentage of ovate females between treatments in either the first or the second cycle in experiments 2 and 3 (Figure 2B and C).

Population growth rate (r)

Significant differences were obtained concerning the population growth rate between treatments, both in the first and second cycle of Experiment 1. The 0.7 g yeast treatment showed significantly higher growth rate (0.41 ± 0.05 rotifers day^{-1}) compared to the other treatments ($p<0.05$). In the first cycle, the treatments of 1.0 and 1.5 g of non-fermented whole rice bran showed no differences between them ($p>0.05$) with values of 0.25 ± 0.02 and 0.26 ± 0.02 rotifers day^{-1}, respectively, differing from the treatment of 0.5 g of non-fermented whole rice bran (0.2 ± 0.02 rotifers day^{-1}) ($p<0.05$). In the second cycle, the treatments of 1.0 and 0.5 g of non-fermented whole rice bran showed no statistical differences (0.17 ± 0.02 and 0.18 ± 0.01 rotifer day^{-1}) between them, but differed from the treatment of 1.5 g of non-fermented rice bran (0.22 ± 0.01 rotifers day^{-1}) ($p<0.05$; Figure 3A).

In the first cycle of Experiment 2, the treatment 0.7 g of yeast, and 1.0 and 1.5 g of fermented whole rice bran, presented similar ($p<0.05$) population growth rates (0.39 ± 0.04, 0.37 ± 0.01, and 0.42 ± 0.05 rotifers day^{-1}, respectively). The treatment of 0.5 g of fermented whole rice bran differed statistically from the other treatments, presenting lower population growth rate (0.29 ± 0.02 rotifer day^{-1}, $p<0.05$). In the second cycle, the treatment with 1 g of fermented rice bran presented statistically lower values of population growth rate compared to the treatment fed with 1.5 g of fermented rice bran ($p<0.05$). The treatments with 0.7 g of yeast and with 1.5 g of fermented rice bran were the ones with the highest ($p<0.050$ population growth rates (0.39 ± 0.04 and 0.40 ± 0.02 rotifer day^{-1}), showing no significant differences between them ($p<0.05$; Figure 3B).

In the first cycle of Experiment 3, no statistical differences ($p>0.05$) were found between treatments concerning population growth rate. In the second cycle, the treatments of 0.7 g yeast, 0.35 g yeast + 0.75 g of non-fermented rice bran and 0.35 g yeast + 0.75 g of fermented rice bran obtained the highest values ($p<0.05$) of population growth rate (0.37 ± 0.01, 0.39 ± 0.06 and 0.39 ± 0.01 rotifer day^{-1}, respectively) and did not differ from each other ($p>0.05$). The rotifers fed with 1.5 g of

non-fermented rice bran showed the statistically lowest ($p<0.05$) population growth rate (0.30 ± 0.03 rotifer day^{-1}) (Figure 3C). Moreover, no statistical differences were observed among the population growth rates obtained in first and second cycles.

Duplication Time (dt)

In the first cycle of Experiment 1, the 0.5 g of non-fermented whole rice bran treatment presented statistically ($p<0.05$) the longest duplication time (3.51 ± 0.40 h), compared to the other treatments.

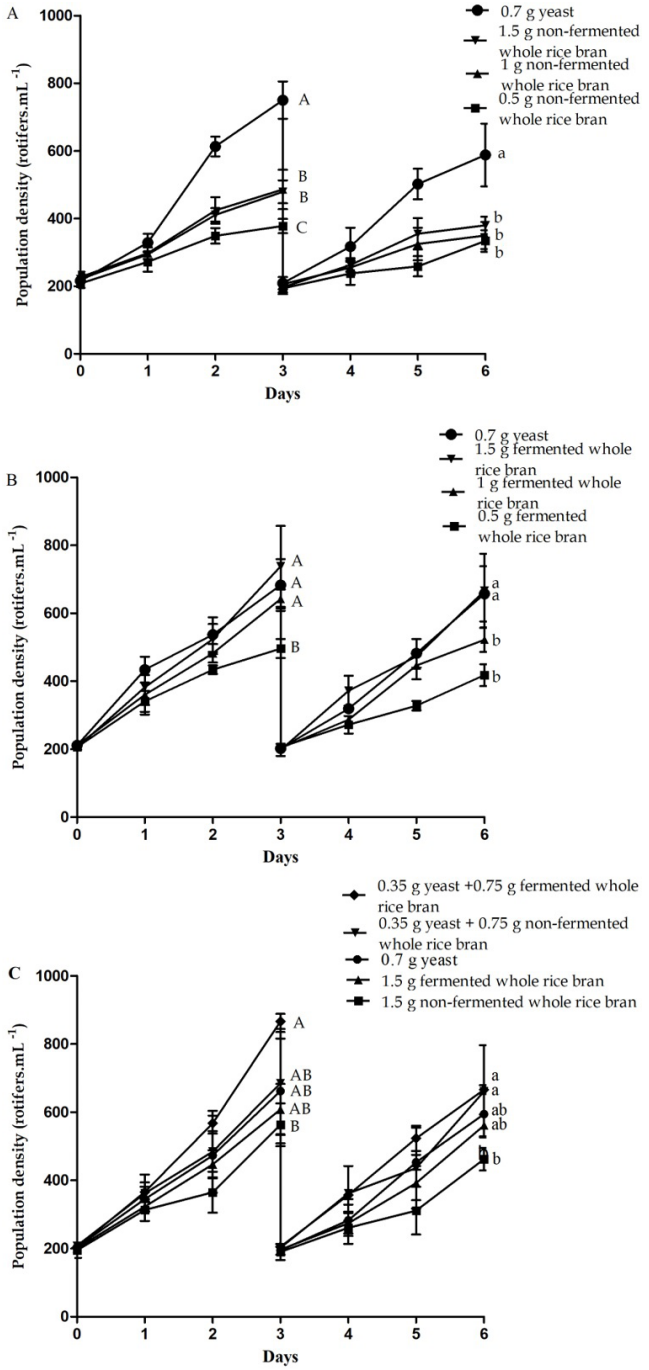

Figure 1. The effect of different diets on the population growth of rotifers (rotifers mL^{-1}) for 6 days in experiments 1, 2, and 3, respectively (A, B, and C). Capital letters show significantly different groups in the first cycle, while lowercase letters show significantly different groups in the second cycle (Newman Keuls; $p<0.05$). Points represent means ± SD (n = 3).

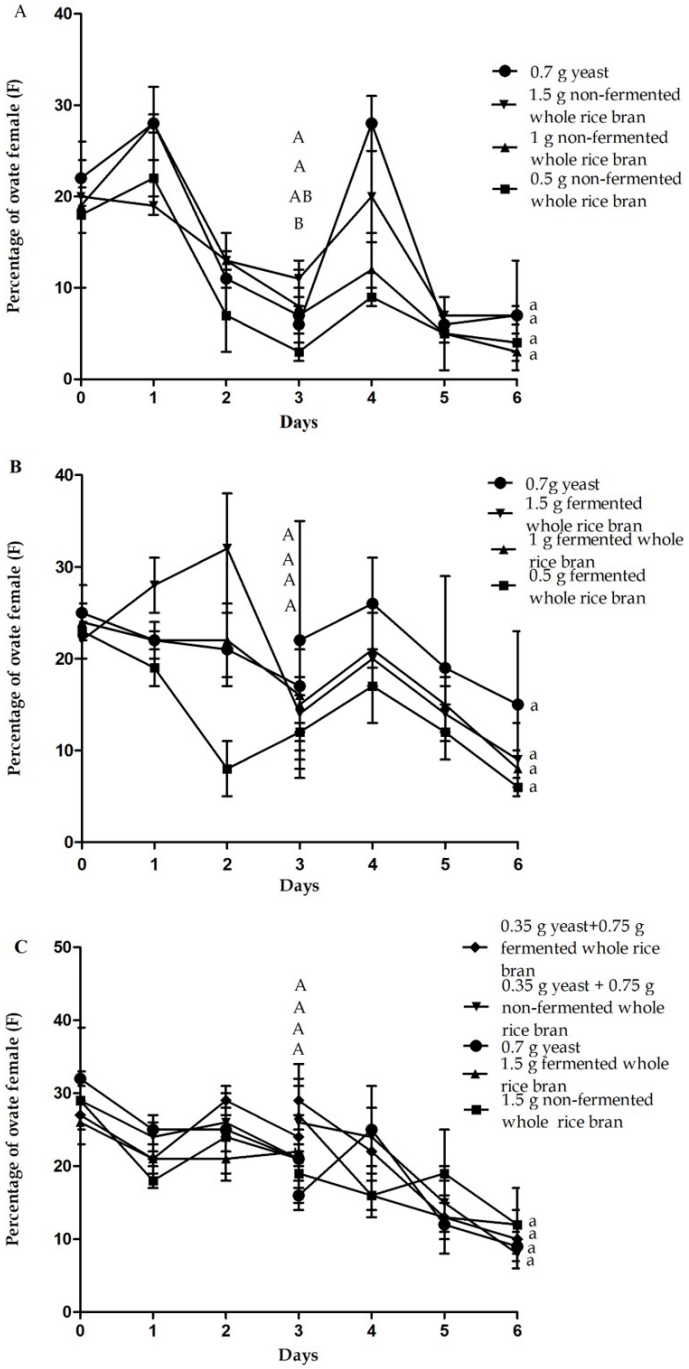

Figure 2. The effect of different diets on the percentage of ovate females (egg rotifers/total rotifers) over 6 days in experiments 1, 2, and 3 respectively (A, B, and C). Capital letters show significantly different groups in the first cycle, while lowercase letters show significantly different groups in the second cycle (Newman Keuls; $p<0.05$). Points represent means ± SD (n = 3).

The 1.0 and 1.5 g non-fermented whole rice bran treatments presented similar duplication time (2.75 ± 0.22 and 2.72 ± 0.27 h, respectively), and showed no statistical difference ($p>0.05$) between them. The treatment with 0.7 g yeast presented the lowest value in both the first and second cycles (1.69 ± 0.24 and 2.04 ± 0.2) ($p<0.05$). In the second cycle, the duplication time of the treatments with 0.5 and 1.0 g of non-fermented whole rice bran treatments was similar (3.8 ± 0.16 and 4.02 ± 0.54 h, respectively; $p>0.05$) (Figure 4A).

In experiment 2, the 0.5 g of fermented whole rice bran treatment obtained the highest duplication time value in both the first and second cycles, differing statistically ($p<0.05$) from the other treatments (2.39 ± 0.20 and 2.97 ± 0.24, respectively). No statistical differences ($p>0.05$) were observed between the treatments with 0.7 g yeast, 1.0 and 1.5 g of fermented whole bran rice in the first cycle (1.79 ± 0.19, 1.87 ± 0.08, and 1.66 ± 0.21 h respectively). For the second cycle, the duplication time of treatments with 1.0 and 1.5 g of fermented whole rice bran were statistically different ($p<0.05$) from each other (2.22 ± 0.15 and 1.73 ± 0.10,

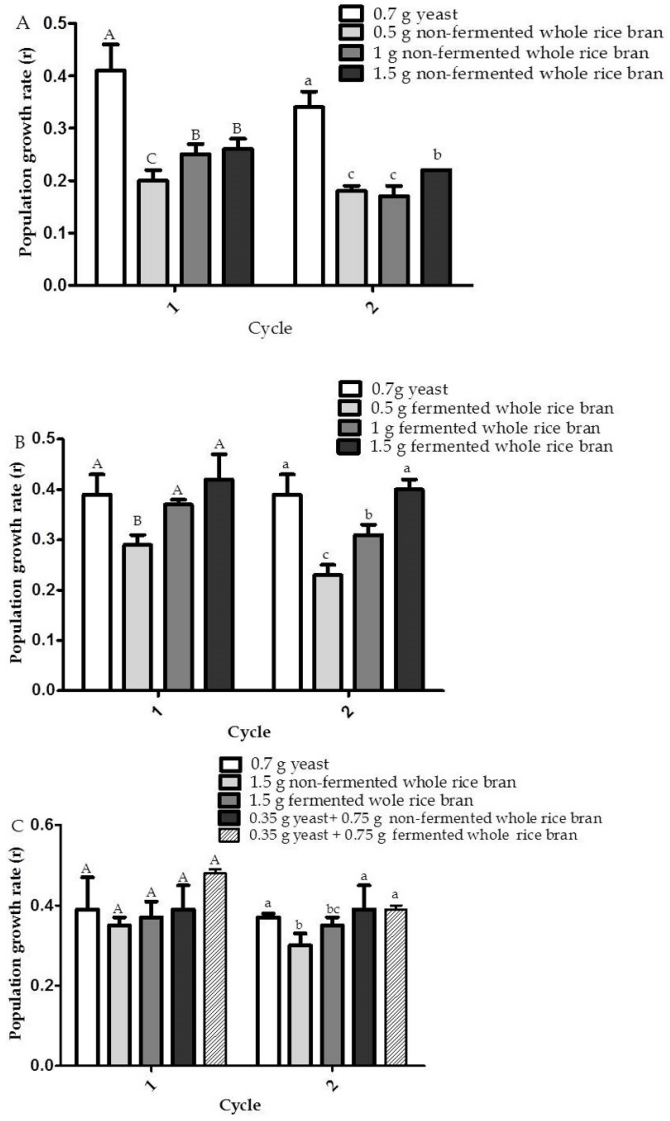

Figure 3. The effect of different diets on the population growth rate of rotifers in cycle 1 and cycle 2 for 6 days in experiments 1, 2, and 3 respectively (A, B, and C). Capital letters show significantly different groups in the first cycle, while lowercase letters show significantly different groups in the second cycle (Newman Keuls; $p<0.05$). Bars represent means ± SD (n= 3).

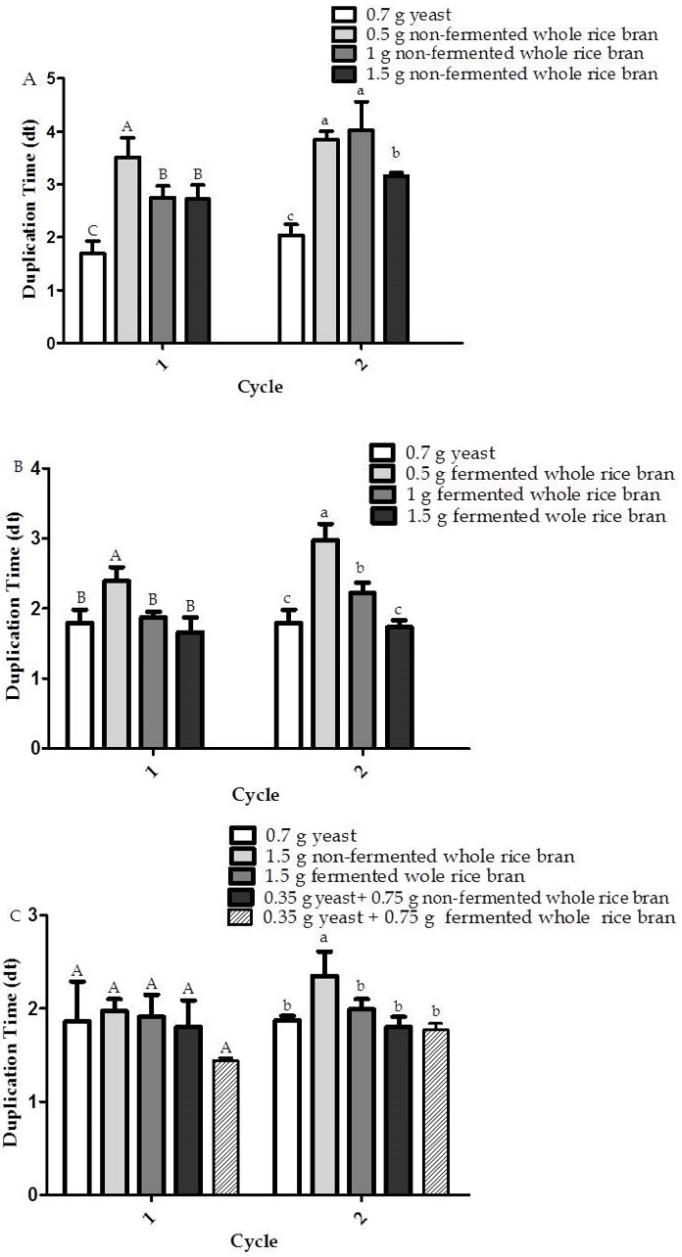

Figure 4. The effect of different diets on the rotifer duplication time in cycle 1 and cycle 2 for 6 days in experiments 1, 2, and 3 respectively (A, B, and C). Capital letters show significantly different groups in the first cycle, while lowercase letters show significantly different groups in the second cycle (Newman Keuls; $p<0.05$). Bars represent means ± SD (n = 3).

respectively), and the treatment with 1.5 g did not differ ($p>0.05$) from the treatment with 0.7 g yeast (1.79 ± 0.19) (Figure 4B).

In the first cycle of Experiment 3, no statistical differences ($p>0.05$) were found between treatments regarding the duplication time. In the second cycle, the duplication time of the treatment with 1.5 g of non-fermented whole rice bran was statistically longer (2.35 ± 0.26 h; $p<0.05$) compared to the other treatments (Figure 4C). No statistical differences ($p>0.05$) were observed for duplication time values obtained in first and second cycles according to the repeated measured ANOVA for all experiments.

Oxidative Stress Analysis

There were no significant differences regarding ROS, ACAP and TBARS between the different treatments in the second production cycle of Experiment 3 (Table 4).

Table 4. Values of reactive oxygen species (ROS), total antioxidant capacity (ACAP), and lipid peroxidation (TBARS) of rotifers fed with different diets, in the second production cycle of Experiment 3.

Treatments	ROS	ACAP	TBARS
0.7g yeast	7.57±3.94	9.18±1.65	0.00535±0.00012
1.5 g of non-fermented whole rice bran	5.27±1.10	10.28±2.42	0.00425±0.00088
1.5 g of fermented whole rice bran	4.53±1.89	5.73±0.64	0.00405±0.00033
0.35 g yeast + 0.75 g of non-fermented whole rice bran	7.63±2.25	8.25±1.87	0.00551±0.00084
0.35g yeast + 0.75g fermented whole rice bran	4.93±3.32	10.90±3.66	0.00439±0.00095

Values are expressed as means ± SD (n = 3).

DISCUSSION

Fermented whole rice bran presented an increase in ash content in relation to non-fermented whole rice bran probably due to the intrinsic contents of the yeast itself, which represented 5.10% of ash. This result corroborated the work of Feddern et al. (2007), who observed an increase in the ash content for whole rice bran after fermentation with *Saccharomyces cerevisiae*. Rice bran presented a high-lipid content since it has not been degreased, and the value found in this work was similar to Oliveira et al. (2010) and Christ-Ribeiro et al. (2017) (18.9%).

The yeast treatment presented a higher percentage of protein, a lower percentage of lipids, ash, and moisture than the non-fermented and fermented whole rice bran. The protein values found were similar to those reported by Hisano et al. (2008) of 49.17%. The ash content was similar to the value reported by Yamada et al. (2003) (4.6%). The lipid content found in this work was higher than the one reported by Yamada et al. (2003) (0.5%). The protein content was higher than that found by several researchers, such as Kupski et al. (2012) (14.8%), Oliveira et al. (2010) (14.7%), and Schmidt et al. (2015) (16.5%). The moisture content is in accordance with the works of Kupski et al. (2012) and Oliveira et al. (2010). The differences found for the proximal composition of fermented whole rice bran might be explained by the different milling techniques employed, as well as to the different types of rice used (Amissah et al. 2003).

High levels of non-ionized ammonia are toxic to rotifers, but their growth with NH_3 concentrations lower than 1 $mg.L^{-1}$ is considered safe (Lubzens and Zmora 2003). In the present study, the highest non-ionized ammonia values found in the yeast treatments were 0.29, 0.55, and 0.26 $mg.L^{-1}$ in Experiments 1, 2, and 3, respectively, which is lower than recommended by those authors. Nevertheless, the total ammonia values were statistically higher in the yeast fed treatments.

In the treatments with non-fermented and fermented whole rice bran, the ammonia remained practically absent. According to Ferreira (2009), bakery yeast presents the problem of rapidly deteriorating water quality in rotifer production through the accumulation of organic matter. Khalil et al. (2018), employing biochar obtained from rice straw to improve water quality in fish farms, obtained 43% ammonia removal efficiency at 25ºC and pH 7.5. Haiwei et al. (2010) evaluated ammonia adsorption from the solution using agricultural residues or plant materials, concluding that these agricultural residues might be used for ammonia removal. Yusof et al. (2010) studied the removal of ammonium ions from aqueous solution with ash obtained from rice husk, concluding that this ash might be used as a cheap adsorbent to remove ammonia from the water. Thus, it might be inferred that the absence of ammonia in the water in the treatments that used non-fermented and fermented whole rice bran was due to adsorption. Coelho et al. (2014) stated that the adsorption process is a physical phenomenon of mass transfer that occurs at the interface of the fluid-solid systems, consisting in the selective adsorption of some components of the fluid phase to the solid surface.

In Experiment 1, the growth parameters presented better results with the baking yeast than with the different concentrations of whole rice bran although it presents a considerable amount of nutrients, such as 19.8% protein and 20.4% lipids. According to Zdradek (2001), the nutrient availability of cereal bran in diets might be considered low, because in the outer layer of the grains, proteins and other micronutrients are strongly bound to cellulose, hemicellulose, and some minerals that make it difficult to digest the nutrients during the digestive processes of animals. On the other hand, Dhert et al. (2001) reports that baking yeast may support a large rotifer biomass.

In Experiment 2, it was observed that the 1.5 g fermented whole rice bran presented values similar to those found with yeast for all production performance variables. Also, when comparing the

variables between experiments 1 and 2, the fermented whole rice bran improved the population performance of rotifers. Pelizer et al. (2007) mention that the use of fermentative processes to alter substrates during their metabolic activity is a good way to increase nutrient availability in raw materials. The growth of the microorganism on a substrate alters the chemical composition of the substrate due to the production of extracellular enzymes, as well as the production of proper metabolites by the fermenting agent. Thus, the substrate may be enriched, depending on the intrinsic components of the fermenting agent, or on the availability of nutrients present in it, which before the microbial action were not accessible to chemical or enzymatic extractive processes (Oliveira et al., 2010). A fact that draws attention is that most of the lipids were present in the bran (fermented or not), because according to Gilbert (2004), lipid is important for the reproduction of rotifers, and diets should promote the synthesis of lipid reserves. According to Wacker and Martin-Creuzburg (2012), rotifer population growth rates are limited by low lipid availability. In this context, rice bran presents a large amount of nutrients such as vitamins, minerals, and also high lipid content that should improve the reproduction process. Further, rice bran presents B vitamins (Park et al., 2017) and Hirayama and Funamoto (1983) showed the importance of vitamin B12 supplementation in baking yeast for rotifer feeding, increasing at population growth rates.

Experiment 3 evaluated the partial and total substitution of baking yeast by whole or fermented rice bran in the diet of rotifers. All growth parameters (population density, growth rate, and duplication time) presented similar results in the treatments with replacement of 50% of yeast with fermented and non-fermented whole bran in the treatment with 1.5g fermented bran. The combination of fermented rice bran and yeast was expected to result in even better population performance parameters, a fact that did not occur in this study.

Yeast has no essential fatty acids that are important for rotifer reproduction (Vijayagopal et al., 2012), but it is an excellent source of protein. Wacker and Martin-Creuzburg (2012) added amino acids such as leucine and isoleucine to the diet of the rotifer *B. calyciflorus* and obtained an increase in the population growth rate, including the limitation of certain aminoacids that impair population growth. Rice bran has a wide variety of amino acids, including leucine and isoleucine (Junqueira et al., 2009).

Generally, the population growth rate values of most species of rotifers range from 0.2 to 2.0 per day, depending on the species and the quality of food provided (Sarma et al., 2001). In the present study, the growth rate values obtained in the first production cycles were all above 0.2. In the second cycle, two treatments, which were the lowest concentrations of non-fermented whole rice bran, were slightly below this range (0.17 ± 0.02 and 0.18 ± 0.01), which shows that the growth in these treatments was not satisfactory. Despite this, when comparing growth at the end of the first cycle with the end of the second cycle, no statistical differences were found between them for any of the three experiments.

The general trend in the percentage of ovate females for all groups of rotifers during the 6-day experiments, was to vary daily, and on the last day of cultivation, most treatments in all experiments fell below 15%, which show that the population in these treatments were no longer growing properly, i.e., it was the period of ending cultivation. According to Dhert et al. (2001), *B. plicatilis*, has a short life cycle with the durability of 3.4 to 4.4 days at a temperature of 25ºC although in optimum conditions they can reach an average of 6 to 8 days (Ferreira, 2009).

Regarding reactive oxygen species (ROS), antioxidant capacity (ACAP), and lipid peroxidation (TBARS), no significant differences were found between treatments. Perhaps the fermentation time was not enough to increase the amount of phenolic compounds in order to improve the antioxidant capacity of rotifers, or the amount of polyphenols produced did not present good bioavailability for rotifers.

The use of 1.5 g of fermented whole rice bran for rotifer feeding resulted in the same population growth as with the baking yeast. Therefore, it might replace baking yeast obtaining a similar growth, being more environmentally friendly, since the bran reduced the ammonia present in the water of the breeding tanks.

CONCLUSIONS

- The use of fermented whole rice bran in the concentration of 1.5 g for one million rotifers, can be an alternative or a substitute for yeast in rotifer feeding;
- It can replace 50% of the baker's yeast with non-fermented and fermented whole rice bran in the food of rotifers and obtain good population growth rates;
- Non-fermented and fermented whole rice bran adsorb ammonia from water for growing rotifers, improving water quality;
- Whole rice bran fermented for 6 hours with *Saccharomyces cerevisiae* did not improve the antioxidant capacity of the rotifers;
- The fermentation of whole rice bran, did not increase the nutrient content, but probably increased its availability.

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