

Desempenho de juvenis de tambatinga em sistema recirculação água com diferentes densidades de estocagem

Tambatinga juveniles performance in a recirculation aquaculture system with different stocking densities

Rendimiento de juveniles tambatinga en sistema de recirculación de agua con diferentes densidades de población

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Rebeca Maria Sousa

ORCID: <https://orcid.org/0000-0002-1195-3304>

Universidade Federal de Mato Grosso do Sul, Brasil

E-mail: rebeca_sousa31@hotmail.com

Romério Rodrigues dos Santos Silva

ORCID: <https://orcid.org/0000-0003-2712-4495>

Universidade Federal do Maranhão, Brasil

E-mail: romeriorodrigues95@hotmail.com

Anália Sousa dos Santos

ORCID: <https://orcid.org/0000-0001-5914-3602>

Universidade Federal do Maranhão, Brasil

E-mail: a.luma@hotmail.com

Camila Viera da Silva

ORCID: <https://orcid.org/0000-0001-5118-5381>

Universidade Federal do Maranhão, Brasil

E-mail: camillavieira_milla@hotmail.com

João Avelar Magalhães

ORCID: <https://orcid.org/0000-0002-0270-0524>

Embrapa Meio-Norte, Brasil

E-mail: joao.magalhaes@embrapa.br

Fabiola Helena dos Santos Fogaça

ORCID: <https://orcid.org/0000-0001-8055-4406>

Embrapa Agroindústria de Alimentos, Brasil

E-mail: fabiola.fogaca@embrapa.br

Jane Mello Lopes

ORCID: <https://orcid.org/0000-0003-0396-3104>

Universidade Federal do Maranhão, Brasil

E-mail: janemellolopes@hotmail.com

Resumo

Numa pesquisa de campo e de natureza quantitativa, o objetivo deste trabalho foi determinar a melhor densidade de estocagem para juvenis de tambatinga (*Colossoma macropomum* × *Piaractus brachypomus*) em um sistema de recirculação durante 50 dias de cultivo. Os juvenis de tambatinga ($0,72 \pm 0,02$ g) foram distribuídos em caixas de polietileno de $0,08 \text{ m}^3$ com densidades de 5, 10 e 15 peixes/caixa, com cinco repetições por tratamento. Durante o período experimental foi avaliado a qualidade da água, ganho de peso, conversão alimentar aparente, taxa de crescimento específico, rendimento de carcaça, composição centesimal e sobrevivência final. Os parâmetros da água permaneceram estáveis e na faixa desejada para a espécie durante o período experimental (temperatura $25 \pm 1,52^\circ \text{ C}$, pH $5,85 \pm 0,7$ e oxigênio dissolvido $6,51 \pm 0,05 \text{ mg L}^{-1}$). As análises estatísticas não mostraram diferença significativa para conversão alimentar aparente e taxa de sobrevivência. A menor densidade de lotação (5 peixes/caixa) apresentou maior ganho de peso e taxa de crescimento específica, enquanto que na densidade de 10 peixes/caixa apresentou alta deposição lipídica na carcaça. Visando maior ganho de peso e melhor taxa de crescimento específico, os resultados indicaram que a densidade de 5 peixes/caixa ($0,08 \text{ m}^3$), é a mais adequada para juvenis desta espécie.

Palavras-chave: Composição Centesimal; RAS; Taxa de Crescimento Específico; Ganho de Peso.

Abstract

In a quantitative field research, the objective of this work was to determine the best stocking density for tambatinga juveniles (*Colossoma macropomum* × *Piaractus brachypomus*) in a recirculation system during 50 days of cultivation. Tambatinga juveniles (0.72 ± 0.02 g) were distributed in 0.08 m^3 polyethylene boxes with densities of 5, 10 and 15 fish/box, with five replicates per treatment. During the experimental period, water quality, weight gain, apparent feed conversion, specific growth rate, carcass yield, proximate composition and final survival were evaluated. The water parameters remained stable and in the desired range for the species during the experimental period (temperature $25 \pm 1.52^\circ \text{ C}$, pH 5.85 ± 0.7 and dissolved oxygen $6.51 \pm 0.05 \text{ mg L}^{-1}$). Statistical analyzes showed no significant difference for apparent feed

conversion and survival rate. The lowest stocking density (5 fish/box) showed greater weight gain and specific growth rate, while the density of 10 fish/box showed high lipid deposition in the carcass. Aiming at greater weight gain and better specific growth rate, the results indicated that the density of 5 fish/box (0.08 m³), is the most suitable for juveniles of this species.

Keywords: Centesimal Composition; RAS; Specific Growth Rate; Weight Gain.

Resumen

En una investigación cuantitativa y de campo, el objetivo de este trabajo fue determinar la mejor densidad de población para juveniles de tambatinga (*Colossoma macropomum* × *Piaractus brachypomus*) en un sistema de recirculación durante 50 días de cultivo. Tambatinga juveniles (0,72±0,02 g) se distribuyeron en cajas de polietileno de 0,08 m³ con densidades de 5, 10 y 15 peces/caja, con cinco repeticiones por tratamiento. Durante el período experimental, se evaluaron la calidad del agua, el aumento de peso, la conversión aparente del alimento, la tasa de crecimiento específica, el rendimiento de la canal, la composición próxima y la supervivencia final. Los parámetros del agua permanecieron estables y en el rango deseado para la especie durante el período experimental (temperatura 25±1.52 ° C, pH 5.85±0.7 y oxígeno disuelto 6.51±0.05 mg L⁻¹). Los análisis estadísticos no mostraron diferencias significativas para la conversión aparente de alimento y la tasa de supervivencia. La menor densidad de población (5 peces/caja) mostró un mayor aumento de peso y una tasa de crecimiento específica, mientras que en la densidad de 10 peces/caja mostró una alta deposición de lípidos en la carcasa. Con el objetivo de un mayor aumento de peso y una mejor tasa de crecimiento específico, los resultados indicaron que la densidad de 5 peces/caja (0.08 m³) es la más adecuada para los juveniles de esta especie.

Palabras clave: Composición centesimal; RAS; Tasa de crecimiento específico; Ganancia de peso.

1. Introduction

Tambatinga, one of the fish most produced in the Brazilian Northeast, is a hybrid between the tambaqui (*Colossoma macropomum*) and pirapitinga (*Piaractus brachypomus*) which stands out in commercial aquaculture from tambaqui due to its rapid growth and from pirapitinga due to its high deposition of dorsal meat (Hashimoto et al. 2002). Among the production systems used for tambatinga are earthen ponds and net cages. However, recirculation aquaculture systems (RAS) can be an alternative to reduce water consumption

and the disposal of effluents and, consequently, to generate less environmental impact (Azevedo et al. 2014). An RAS also provides greater control over water quality and isolation from pathogens; but, when management is not adequate, it can promote high levels of ammonia and nitrite, and low levels of dissolved oxygen (Masser et al. 1999).

To provide better aquaculture management and promote fish welfare, it is necessary to determine an optimal stocking density (Samad et al. 2014). Stocking density is defined as the amount or biomass of fish per unit area or volume. It is specific for each species; production system, age and weight of fish (Merino et al. 2007), and can interfere with fish performance (Jobling & Baardvik 1994) and change fish behavior (Martins et al. 2012). That is why many studies on stocking density in RAS have been conducted. Lima et al. (2019) suggested a density up to 1.4 kg m^{-3} for better tambaqui juvenile's (*Colossoma macropomum*) performance and lower parasites presence in RAS. Sgnauli et al. (2018) described a Biofloc technology for piracanjuba juvenile's (*Brycon orbignyianus*) cultivation with a density of 1 kg m^{-3} . Samad et al. (2014) observed better growth performance in grouper (*Epinephelus coioides*) reared at high density ($25 \text{ fish } 100 \text{ L}^{-1}$) in an RAS. Pedrosa et al. (2019) developed a RAS for production of arapaima (*Arapaima gigas*) juveniles with 500 g of weight, in a density of 6 kg m^{-3} .

Thus, a stocking density study for tambatinga produced in RAS is necessary to develop management strategies as an alternative for regional aquaculture. In view of the above, the objective of this work was to evaluate the productive performance of tambatinga juveniles in a water recirculation system with different storage densities.

2. Methodology

In a field and quantitative research, this work was based on studies by Brandão et al (2004), Da Silva et al (2015) and Rodrigues et al (2016) who tested different stocking densities for tambaqui and tambatingas juveniles.

2.1. Experimental Fish

Tambatinga juvenile's ($0.72 \pm 0.02 \text{ g}$; $2.76 \pm 0.07 \text{ cm}$, 50 days), were acquired from the aquaculture station "Ademar Braga" of Departamento Nacional de Obras Contra a Seca – DNOCS (National Department of Works Against Drought) in Piripiri, Piau  State, Brazil.

2.2. Experimental Design

The study was conducted in the aquaculture laboratory of the Centro de Ciências Agrárias e Ambientais, Universidade Federal do Maranhão, Chapadinha, Maranhão State, Brazil. The experiment was performed in recirculation aquaculture systems (RAS) which consisted of 15 circular indoor tanks (0.08 m^3 each), supplied with water from an artesian well and maintained by a biological filter. The flow was 720L/h, maintained by peripheral water pump (FERRARI, Brazil; 2400L/h). Aeration was maintained by a central air blower ($\frac{1}{2}$ HP) and kept constant throughout the experimental period.

Tambatinga juvenile's ($0.72 \pm 0.02 \text{ g}$) were stocked at D5=5 fish 0.08 m^{-3} (D10=10 fish 0.08 m^{-3} and D15=15 fish 0.08 m^{-3}) with five replicates for each density, and fed four times a day for 50 days, *ad libitum*. Fish were fed a commercial extruded feed (40% crude protein and 3400 kcal kg^{-1} metabolizable energy). Siphoning of the boxes for feces and feed debris withdrawal was carried out daily, as was water replenishment for evaporation losses and to maintain the water level (30% per day).

2.3. Water Quality Parameters

Dissolved oxygen levels, temperature (Y5512 oxymeter, YSI Inc., Yellow Springs, USA) and pH (pHmeter DMPH-2, Digimed, São Paulo, Brazil) were determined daily. Nitrite (Boyd 1998) and total ammonia (Verdouw et al. 1978) levels were determined daily. Water hardness and total alkalinity levels were calculated weekly following the method of Eaton et al. (2005). All water samples were collected in triplicate and analyzed in the laboratory.

2.4. Fish Performance

Measurement of parameters such as growth performance, feeding activity and survival rate was carried out based on the data required. Data on growth rate was recorded regularly every 2 weeks by weighing and measuring individual fish from each box. Fish were removed from the test tanks and anesthetized by immersion in a MS222 solution (15 mg L^{-1} ; Sigma-Aldrich, USA) for 30 seconds and padded dry for fish measurements (total length and weight). At the end of trial, fishes were euthanized again and harvest by spinal cord sectioning according to the Ethics Committee recommendations. Wet body weight was measured using an analytical balance (Shimadzu), and total length using a digital caliper (Mitutoyo, Absolute Digimatic, Japan).

Growth performance was calculated according to Samad et al. (2014) as follows:

- a) Weight gain (WG, g) = [final weight (g) – initial weight (g)];
- b) Total biomass (TB, g) = number of fish × average weight;
- c) Feed consumption (FC, g) = g of feed consumed by each fish;
- d) Apparent food conversion (CAA) = feed consumption/weight gain;
- e) Specific growth rate (SGR, % day⁻¹) = 100 × (ln final weight – ln initial weight)/T (days of feeding);
- f) Condition factor (K) = [10⁵ × weight of fish (g)/ (length of fish)³ (cm)]
- g) Survival rate (SR, %) = (final no. of fish/initial no. of fish) × 100

After the experiment period (50 days), the carcass parameters were determined: total weight, weight of whole eviscerated fish, fish weight without head, fillet weight, viscera and carcass weight. Based on the data collected, the following yield parameters were calculated, related to the initial weight of the whole fish (total weight):

- a) Eviscerated whole fish yield (RPIE, %) = (eviscerated fish weight × 100)/total weight;
- b) Head yield (%) = head weight/total weight;

2.5. Centesimal Composition Analysis

All samples used were stored at –18°C and maintained at 5° C for 24 h prior to analysis of composition. Fillet samples of five fish in each weight class were removed and homogenized, with analysis performed in triplicate. The composition was determined by standard analytical methods (AOAC 2005). Moisture was determined by drying a sample of 2.0 g at 105° C for 24 h. Ash content was determined by incineration at 550° C for 6 h in a muffle furnace. Lipids were determined by extraction in petroleum ether in Soxhlet apparatus. Crude protein was determined by a semimicro-Kjeldahl method. The protein nitrogen conversion factor considered was 6.25.

2.6. Data Analysis

The results were subjected to the ANOVA test at 5% probability. When significant differences were verified (P<0.05), the means were compared using the Tukey test. All statistical procedures were computed in Assistat version 7.7.

3. Results and Discussion

3.1. Water Quality Parameters

The water parameters remained stable and in the desired range throughout the experimental period (Table 1).

Table 1. Water parameters of tambatinga submitted to different stocking densities in a recirculation aquaculture system for 50 days.

Parameter	Stocking density (fish 0,08m ⁻³)		
	5	10	15
Dissolved oxygen (mg L ⁻¹)	6.4±0.03	6.6± 0.13	6.5±0.04
pH	6.0±0.63	5.9±1.0	5.6±0.64
Temperature (°C)	26±0.9	25±1.0	25±0.9
TAN (mg L ⁻¹)	0.93±0.72	0.82±0.77	0.96±0.69
Un-ionized ammonia (mg L ⁻¹)	0.05±0.011	0.03±0.01	0.07±0.016
Nitrite (mg L ⁻¹)	0.05±0.02	0.05±0.02	0.04±0.09
Alkalinity (mg L ⁻¹ CaCO ₃)	43.8±4.6	45±5.0	42.5±4.0
Hardness (mg L ⁻¹ CaCO ₃)	48±5.0	48±4.9	48±5.0

Values are means ± SEM. Source: own study

The temperature was maintained at 25±1.52° C, pH at 5.8±0.7 and dissolved oxygen at 6.51±0.05 mg L⁻¹. Hardness was recorded as 48±5.0 mg L⁻¹ CaCO₃, alkalinity as 43.8±4.5 mg L⁻¹ CaCO₃, nitrite as 0.05±0.01 mg L⁻¹ and total ammonia as 0.91±0.7 mg L⁻¹. There were no significant differences (p>0.05) between treatments.

In general, the water quality variables evaluated were maintained at adequate levels for the tropical fish species (CONAMA 2005), demonstrating that stock density does not influence water quality (p>0.05). As the water came from a well, the temperatures were more stable and slightly below room temperature (25° C), but within the range considered optimal for tambaqui growth of between 24° C and 31° C (Oliveira et al. 2007).

The concentrations of dissolved oxygen (DO) presented constant values (6.51±0.05 mg L⁻¹) over time. In studies with tambatinga juveniles, DO values of 5 mg L⁻¹ are observed in recirculation systems with high densities (López and Anzoátegui, 2012). For pirapitinga in

a bioclarifier (RAS), DO can vary between 2.5 and 6.8 mg L⁻¹ (Poleo et al. 2011). According to these works, when stocking density is high, there is greater consumption of DO by fish, and deterioration of organic material. However, as the tanks were siphoned daily, this effect was not noticed in the present study, ensuring the high DO level during the entire experiment and if the effects on performance came only from stocking density.

According to Thorarensen (2011), RAS offer high-level control over water quality parameters, allowing conditions to remain as close to ideal for fish growth. However, in these systems, the main problem is the constant elimination of toxic metabolites (NH₃⁺ & NO₂⁻) that limit fish growth. This was observed in relation to total ammonia levels, which presented values of 0.91 ± 0.7 mg L⁻¹, five times higher than those determined for tambaqui juveniles stocked at 33 fish m⁻² (Oliveira et al. 2007). However, that study was conducted in earth ponds with constant water flow, which explains the better water quality observed. Azevedo et al. (2014) obtained results higher than those determined in the present study for juvenile tambatinga stocked in net pens at three densities (400, 500 and 600 fish m⁻³) for 60 days. In works with RAS, the ammonia levels vary between 0.20 and 0.98 mg L⁻¹ for Nile tilapia (Gullian-Klanian & Arámburu-Adame 2013); 0.11 and 0.15 mg L⁻¹ for lambari (*Astyanax* sp.) (Jatobá & Silva 2015); and 1.03 and 1.67 mg L⁻¹ for sea bass (Sammouth et al. 2009).

Nitrite (NO₂⁻) is produced by the oxidation of ammonia; the main nitrogenous compound excreted by fish, and can reach very high levels in systems with high stocking densities and/or when an imbalance occurs to disrupt the normal function of biological filters in recirculating systems (Jensen 2003). In this study, nitrite levels remained low (0.05 ± 0.01 mg L⁻¹) and were not altered by stocking density. The low nitrite level is the result of proper water quality management.

Total alkalinity (43.8 ± 4.5 mg CaCO₃ L⁻¹) and total hardness (48 ± 5.0 mg CaCO₃ L⁻¹) remained within the levels recommended for tropical fish species (Moro et al. 2013). These parameters may vary with the water provided. Alkalinity of supply channels in the North of Brazil varies between 27 and 28 mg L⁻¹ (Silva et al. 2013), while earth ponds from the Midwest of Brazil present values between 39.9 and 43.10 mg L⁻¹ (Oliveira et al. 2007).

The pH (5.85 ± 0.7) was also below the range of 6.5 to 9.0 recommended for tropical fish (Baldisseroto 2013). However, Aride et al. (2004) showed better growth for tambaqui raised in acidic water with pH between 4 and 6. This was corroborated in our work, as pH did not have a negative effect on tambatinga growth.

3.2. Effects of Stock Density on Fish Performance

In this study, significant differences ($p < 0.05$) were observed in relation to weight gain, feed intake, specific growth rate and final biomass of tambatinga juveniles among the densities evaluated (Table 2). There were no significant differences between treatments for condition factor or final survival ($P > 0.05$).

Table 2. Performance of tambatinga submitted to different stocking densities in a recirculation aquaculture system for 50 days.

Performance parameters	Stock density (fish 0.08 m ⁻³)			
	5	10	15	SD
Initial weight (g)	0.72	0.72	0.73	0.006
Final length (cm)	7.19 ^a	6.20 ^b	6.26 ^b	0.992
Final Weight (g)	10.88 ^a	7.23 ^b	7.29 ^b	1.101
Weight gain (g)	11.60 ^a	7.95 ^b	8.02 ^b	1.707
Feed consumption (g)	11.47 ^a	8.01 ^b	7.58 ^b	1.746
Final biomass (g)	57.21 ^b	74.75 ^b	102.97 ^a	18.852
¹ CAA (g g ⁻¹)	1.08	1.14	1.16	0.024
² SGR (%)	5.65 ^a	4.96 ^b	4.90 ^b	0.340
Condition factor	3.32	3.63	3.63	0.226
³ SR (%)	92.00	86.00	81.33	4.36

Means followed by different letters differ from each other according to the Tukey test ($p < 0.05$). SD = standard deviation; ¹Apparent food conversion; ²Specific growth rate; ³Survival rate. Source: own study

Weight gain presented higher values at lower density (5 fish 0.08 m⁻³), probably due to less competition among the fish for food and space, directly reflected in the tambatinga weight gain (Table 2). This trend was also observed in relation to feed consumption (FC) and specific growth rate (SGR). Gullian-Klanian & Arámburu-Adame (2013) also observed higher SGR for treatments with lower densities of tilapias cultivated in RAS. That is because weight gain has a linear correlation with stock density (Jatobá & Silva 2015). The opposite occurs with final biomass (FB); higher density (15 fish 0.08 m⁻³) was, consequently, statistically superior to the other treatments due to the higher number of fish. According to

Baldiasseroto (2013), final biomass is an important parameter for performance, since it allows evaluation of productivity per area. It is worth mentioning that the direct relationship between density and biomass production has been considered an important factor in aquaculture; therefore, when there is a high initial density, the final amount of total biomass will be higher (Souza-Filho & Cerqueira 2003), but RAS can limit stock density as a function of the systems carrying a capacity for organic load, and the resulting water quality (Gullian-Klanian & Arámburu-Adame 2013).

The apparent feed conversion (CAA) of the tambatinga juveniles was not influenced by the stocking density; however, dietary intake was higher at the lower density (5 fish 0.08 m⁻³) (Table 2). That means that increases in density influence consumption but do not affect feed conversion, because partial harvest of standing fish has been used to decrease competition or for social dominance in treatments with high density (Gullian-Klanian & Arámburu-Adame 2013). The condition factor (CF) of a species indicates the physiological state of fish (Felizardo et al. 2011). In this case, more than 80% SR and no differences between CF showed the good health of tambatinga provided by RAS and feed.

3.3. Effects of Stock Density on Carcass Yield

The results obtained for carcass yield are presented in Table 2. In this study, significant differences (p<0.05) were observed for final, eviscerated and head weight of tambatinga juveniles among the densities evaluated (Table 3).

Table 3. Carcass yield for tambatinga submitted to different stocking densities in a recirculation aquaculture system for 50 days.

Carcass yield (%)	Stock density (fish 0.08 m ⁻³)			
	5	10	15	SD
Final weight (g)	12.33 ^a	8.67 ^b	8.75 ^b	0.018
Eviscerated weight (g)	10.57 ^a	7.32 ^b	7.55 ^b	0.020
Head weight (g)	2.70 ^a	1.85 ^b	1.89 ^b	0.080
Eviscerated fish yield (%)	89.62	89.86	91.52	0.001
Head percentage (%)	22.95	22.81	23.22	0.001

Means followed by different letters differ from each other according to the Tukey test (p<0.05). SD = standard deviation. Source: own study

The treatment with the lowest density showed higher final and eviscerated weight, and higher head weight which is not a good parameter (Table 3). However, the fish and head yield were similar between treatments. It means that stock density does not influence carcass yield in tambatinga juveniles. Pirapitinga and tambatinga with a weight below 0.8 kg have a high percentage of head weight which is inversely proportional to the fillet yield (Vasconcelos-Filho et al. 2017).

Intensive systems of fish production (higher stocking densities and high water flow) tend to accumulate a lower fat percentage when compared to semi-intensive systems (static with lower stocking density). This is because fish in RAS have greater energy expenditure, since they are under conditions of continuous exercise, and have greater development of muscle fibers (Arbeláez-Rojas et al. 2002).

3.4. Effects of Stock Density on Fish Composition

In this study, significant differences ($p < 0.05$) were observed only for lipids of tambatinga juveniles among the densities evaluated (Table 4).

Table 4. Centesimal composition of tambatinga submitted to different stocking densities in a recirculation aquaculture system for 50 days

Centesimal composition (%)	Stock density (fish 0.08 m ⁻³)			
	5	10	15	SD
Moisture	75.56	74.76	75.87	0,001
Crude protein	19.44	19.19	20.44	0,004
Lipids	3.58 ^b	4.11 ^a	3.58 ^b	0,026
Ash	2.83	2.88	3.00	0,048

Means followed by different letters differ from each other according to the Tukey test ($p < 0.05$). SD = standard deviation. Source: own study

In our study, fish submitted to an intermediate density presented higher deposition of body fat (Table 4); however, they did not present better performance (Table 2) or carcass yield (Table 3). For juveniles of jundiá (*Rhamdia quelen*), the centesimal composition of the carcasses is not affected by storage density, except for protein content that is lower at intermediate densities (Lazzari et al. 2011). The opposite is observed in Nile tilapia, whose lipid content increases as storage density increases. This means that stocking density could

influence body composition, but in different ways for each species and storage rate. It means that further analysis to determine an optimal stocking density in recirculation systems for tambatinga juveniles must be conducted for better understanding.

4. Final Considerations

The lower stocking density (5 fish/box) showed greater weight gain and specific growth rate, while in the density of 10 fish/box it showed high lipid deposition in the carcass. Aiming at greater weight gain and better specific growth rate, the results indicated that the density of 5 fish/box (0.08 m^{-3}), is the most suitable for tambatinga juveniles in a recirculation system.

It is suggested that in future studies, carried out under conditions similar to this, higher densities and larger animals should be evaluated in order to increase production, since the maximum density may not have been reached in this study.

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Disclosure statement

No potential conflict of interest was reported by the authors.

Ethical statement

Experimental design and fish handling of the current study had been approved by the Ethical and Animal Welfare Committee of UFMA under registration #23115.004974/2016-46.

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Percentage of contribution of each author in the manuscript

Rebeca Maria Sousa – 14,28%

Romério Rodrigues dos Santos Silva – 14,28%

Anália dos Santos Silva – 14,28%

Camila Vieira da Silva – 14,28%

João Avelar Magalhães - 14,28%

Fabiola Helena dos Santos Fogaça - 14,28%

Jane Mello Lopes – 14,28%