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Research Article

Growth Performance, Survival Rate, and Water Quality in an Aquaculture System Using Different Feeding Strategies for Juveniles of Nile Tilapia (*Oreochromis niloticus*)

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ABSTRACT

Aquaculture is a rapidly growing industry worldwide, with Nile tilapia (*Oreochromis niloticus*) being one of the most intensively farmed fish species. This study aimed to evaluate the growth performance and water quality parameters in different culture systems for Nile tilapia. Six treatments were tested, including variations in feed type (commercial or microalgae), aeration, and their combinations. The results showed that the presence of commercial feed and aeration (T2) resulted in the highest weight gain and specific growth rates, while treatments without commercial feed showed lower growth performance. The addition of microalgae supplementation did not significantly improve growth compared to commercial feed alone. Water quality parameters, particularly nitrite levels and dissolved oxygen, played crucial roles in the production of tilapia. It was observed that high nitrite levels were associated with decreased growth and survival rates. Proper monitoring and management of water quality, including nitrite levels and dissolved oxygen, are essential to ensure the survival and growth of tilapia in aquaculture systems. These findings highlight the importance of implementing sustainable practices and appropriate feeding strategies to optimize the growth and well-being of farmed tilapia while minimizing environmental impacts.

Keywords: Fish nutrition, fish production, fish farming, water quality, microalgae, *Oreochromis niloticus*

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INTRODUCTION

Aquaculture is a thriving industry worldwide, characterized by diversity and expansion (Sarker et al., 2016; Verdegem et al., 2023). In developing countries, this sector has experienced significant growth due to ongoing efforts in regulation, professionalization, and modernization. Farmers have become increasingly knowledgeable about management practices and essential inputs (Scorvo-Filho et al., 2010; Moreira et al., 2012).

Fish farming is expanding at a faster rate compared to other livestock commodities (FAO, 2022). However, despite notable progress, the adoption of technology and technical capabilities still restricts overall achievements and expectations. Addressing the reduction of production costs and environmental impact is crucial. These are critical obstacles that need to be overcome for a more optimistic and sustainable outlook (Leonardo et al., 2009).

In Brazil, Nile tilapia (*Oreochromis niloticus*) is the most intensively farmed fish species. This is primarily due to its rapid growth, efficient feed conversion, desirable meat quality, and market acceptability both locally and internationally (Schwarz et al., 2010). In fact, tilapia is the second most cultivated fish worldwide, following carp (Fonseca et al., 2013).

Successful fish farming in ponds relies on effective management of nutrition and water quality. In most cases, the use of complete diets is necessary to meet the nutritional requirements of fish, ensuring growth, high productivity, and profitability (Furuya et al., 2001; Carvalho et al., 2012). This becomes particularly crucial when fish are bred or fattened under confined conditions, such as cages, ponds, and raceways, where high biomass per unit area is present.

In an attempt to increase income, farmers often increase stocking rates (density), but this may negatively impact growth and result in poor water quality for the fish (Lima et al., 2016). Such conditions affect survival rates since suitable water quality is essential for the physiological functions of fish, including breathing, reproduction, feeding, and defecation. Water quality in fishponds may also depend on the availability and nutritional quality of food (Bhatnagar and Devi, 2013).

At the farm level, intensification typically leads to deteriorating water quality in fish ponds, posing hazards and increased risks to surrounding ecosystems. Effluents from aquaculture activities can contain substantial amounts of feces, leftover feed, and bacterial biomass (Gorlach-Lira et al., 2013). Therefore, ensuring constant monitoring is crucial to maintain the sustainability of fish farming, preventing downstream contamination and environmental degradation. In ponds with limited water renewal, the effects of intensification become even more problematic. Aeration techniques offer alternatives to increase the carrying capacity of a pond for fish (Boyd et al., 2018). Nonetheless, water renewal remains important to dilute the concentration of toxic metabolites.

The concentration of microalgae in fishponds also influences fish performance and water quality. Microalgae are responsible for recycling nutrients excreted by fish (Gorlach-Lira et al., 2013). They also contribute to the dissolved oxygen content in the water through photosynthesis and can adsorb significant amounts of heavy metals from contaminated water sources (Coêlho et al., 2014).

Moreover, certain microalgae serve as direct or indirect sources of food for fish and their ecosystem (Sarker et al., 2016). This is particularly interesting considering that production costs, especially the high cost of feed, pose major constraints in the global fish farming scenario (Taelman et al., 2013). Consequently, research efforts have focused on identifying alternative feeding strategies (Leonardo et al., 2009; Carvalho et al., 2012). In general, these alternatives aim to promote growth, minimize stress, and enhance the efficiency of the fish immune system (Ungsethaphand et al., 2010; Sarker et al., 2016; Zeinab et al., 2015).

The aim of this study was to evaluate the growth performance and the survival rates of Nile tilapia (*Oreochromis niloticus*) and the water quality in an aquaculture system using different feeding strategies. For that, we examined the ability of fish to thrive in ponds without water renewal under various experimental conditions and monitored water quality to assess variations based on treatments involving feed, aeration, and microalgae supplementation.

MATERIALS AND METHODS

An aquaculture system consisting of circular open ponds without water renewal was utilized for raising Nile tilapia (*Oreochromis niloticus*). The ponds were made of polyethylene plastic, with a capacity of 1000 L, filled with 500 L of water. Juvenile Nile tilapia (53.2 ± 5.4 g; 8.0 ± 1.0 cm) were bred at an initial stocking density of 30 fish per pond (n=30), resulting in an average density of 3,192 g m^{-3}. The system was built on a fish farm located in Dourados, MS, Brazil.

Six treatments were designed to evaluate the impact of different factors on the growth and development of juvenile Nile tilapia. These treatments included: commercial feed (T1), commercial feed with aeration (T2), microalgae supplementation (T3), microalgae supplementation with aeration (T4), commercial feed with microalgae supplementation (T5), and commercial feed with microalgae supplementation and aeration (T6) (Table 1).

The commercial feed contained 40% crude protein. The microalgae used in the study was *Chlorella sorokiniana* CTT 7727 (3.9 x 10^6 cells mL^{-1}; 0.39 g L^{-1}), obtained from the André Tosselo Foundation (FAT). The strain was cultivated in a separate open pond using the Bold Basal medium (Bischoff and Bold, 1963). The temperature of the ponds was maintained at 28 ± 2°C using thermostats, while the photoperiod followed a 12 h light/12 h dark cycle.

The experiment was conducted for two weeks. In the first week, the amount of commercial feed provided was fixed at 4% of the initial live weight (g) for treatments T1 and T2. For treatments involving multiple food sources (T5 and T6), the amount of feed was reduced to 50% of the other treatments as per dilution criteria (Table 1). In the second week, the feed amount was halved for all treatments. Feed was administered daily during four feeding periods throughout the entire experiment.

Table 1. Treatments offered to juvenile tilapia bred in open ponds with no water renewal during the early stages of development.

	Treatment	Day 0 – 7	Day 8 – 14
T1	Commercial feed	64 g	32 g
T2	Commercial feed and aeration	64 g	32 g
T3	Microalgae	2,000 mL	1,000 mL
T4	Microalgae and aeration	2,000 mL	1,000 mL
T5	Commercial feed and microalgae	32 g + 1,000 mL	16 g + 500 mL
T6	Commercial feed, microalgae, and aeration	32 g + 1,000 mL	16 g + 500 mL

Water parameters such as pH, hardness, dissolved oxygen, toxic ammonia, and nitrite were measured daily using a commercial colorimetric disk kit. At the end of the experiment (day 14), water samples were collected for microbiological analysis, including total thermo-tolerant coliforms, *Escherichia coli*, and mesophilic bacteria, using the classical multiple-tube technique (APHA, 1998).

To evaluate the growth performance, fish were length measured and weighed before being immediately released into the ponds without inflicting any harm. The other parameters monitored were the specific growth rate (SGR) (Eq. 1), the daily weight gain (DWG) (Eq. 2) (Haque et al., 2023), and the condition factor (K) (Eq. 3) (Pauly, 1983):

$$SGR\ (\%\ day^{-1}) = [(W_2 - W_1) / W_1] \times (100 / T) \quad 1$$

$$DWG\ (\%\ day^{-1}) = [(W_2 - W_1) / W_1] \times 100 \quad 2$$

$$K\ (g\ cm^{-3}) = 100\ W / L^3 \quad 3$$

Where: W = fish weight (g); W_1 = initial fish weight (g); W_2 = final fish weight (g); L = fish length (cm); T = the experimental period in days.

The survival rate (SR) was evaluated at the end of the experiment and expressed as follows (Eq. 4) (Haque et al., 2023):

$$SR\ (\%) = (FH / FS) \times 100 \quad 4$$

Where: FH = number of fish harvested; FS = number of fish stocked.

Variance tests (one-way ANOVA) and comparison of means (Tukey test, 5%) were conducted separately according to data of weight (g) and total length (cm) during the experimental trial. All analyzes were performed in triplicate.

RESULTS AND DISCUSSION

Development of *Nile tilapia*

The weight of the fish varied among treatments on both the 7th and 14th days of the experiment (Fig. 1). During the first week (day 7), treatments T2 and T5 exhibited better results. Subsequently, in the second week, T2 and T6 showed superior outcomes. Conversely, the absence of commercial feed (T3, T4) was associated with lower weight gain in both experimental periods (days 7 and 14) (Fig. 1). Similarly, fish body length was highest in T2 and T6, while it was lowest in T3 and T4 during the final evaluation trial (Fig. 2).

These results showed that the growth performance of Nile tilapia was influenced by various factors, primarily associated with the type of food provided (commercial or natural). However, it was expected that due to the efficient capacity of Nile tilapia to harvest microalgae from the water through their gill rakers (Turker et al., 2003), T6 and T5 would present, respectively, the best results for weight by supplying an extra source of nutrients to the fish diet (T5) in addition to an increment in the oxygen availability (T6), which is critical for fish to achieve their full growth and activity potential (Obirikorang et al., 2020).

The best treatment in terms of total weight gain was obtained by combining feed and aeration (T2), which resulted in an increase

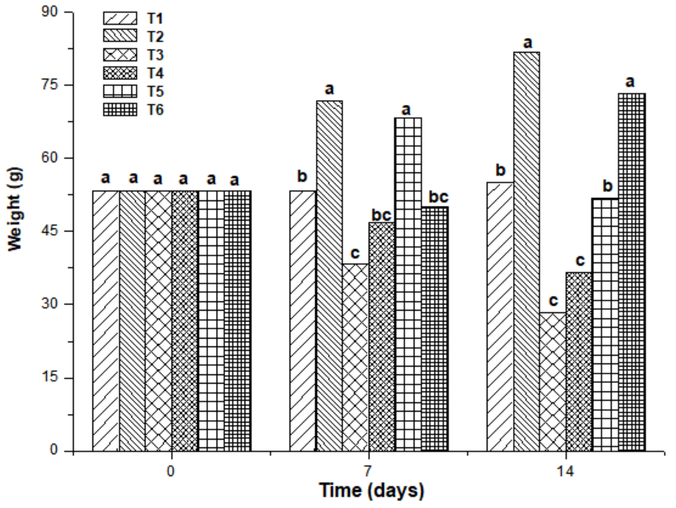

Figure 1. Analysis of variance of the increased weight (g) of Nile tilapia in relation to six treatments and time (Table 1). The results presented are the average of the weighted juveniles (N=5). *Means followed by the same letter do not differ by the Tukey test at the 5% probability level.

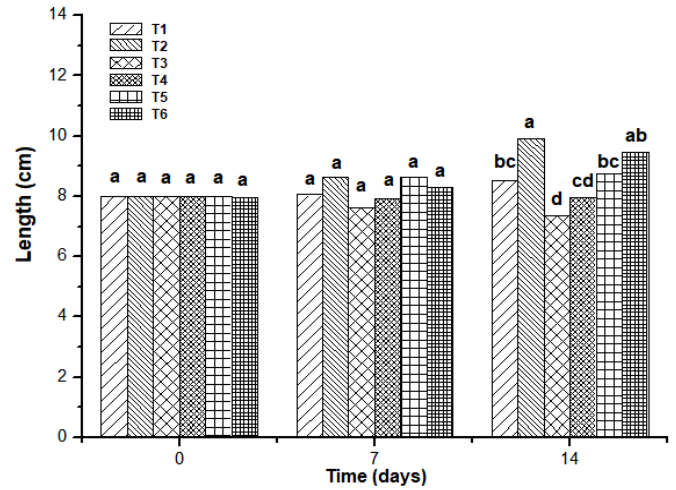

Figure 2. Analysis of variance of the increased total body length (cm) of juvenile Nile tilapia in relation to six treatments and time (Table 1). The results presented are the average of the measured juveniles (N=5). *Means followed by the same letter do not differ by the Tukey test at the 5% probability level.

of 53.33%. The treatment involving feed, microalgae, and aeration (T6) demonstrated the second-highest weight gain rate of 37.77%. However, this treatment also had the lowest survival rate of 53.3%. Solely relying on commercial feed (T1) was associated with poor performance in daily weight gain. In fact, treatments T3, T4, and T5 exhibited negative daily weight gain throughout the experiment. Thus, weight loss was not correlated with lower survival rates ($S_{\%}$) (Table 2). Weight loss can occur when fish are transitioning to a different feed or adjusting to new dietary conditions. Moreover, environmental stressors, such as water quality issues, handling stress, or competition, can indeed lead to weight loss in fish without negatively impacting survival rates. These stressors can affect fish health and behavior, potentially causing reduced feeding, increased energy expenditure, or physiological changes that result in weight loss. The impact on survival rates will depend on the severity and duration of these stressors (Martins et al., 2012; Toni et al., 2017; Yavuzcan et al., 2017; Hvas et al., 2020; Canosa and Bertucci, 2023).

On the other hand, the specific growth rates of tilapia cultivated in T2 (3.80% day^{-1}) and T6 (2.69% day^{-1}) (Table 2) were higher or comparable to those reported in the literature for juveniles (Table 3).

Finally, the condition factor seems to better explain the growth behavior by combining information from weight and length (Table 2). The results underline that T3 and T4 were the worst treatments for the Nile tilapia development, *i.e.*, microalga is not a good enough source of nutrients to sustain growth, at least at the utilized concentration. However, the other treatments (T1, T2, T5, and T6) did not show statistical differences (P>0.05) between them for this parameter.

Various studies have reported success in the partial replacement of commercial fish feed by microalgae biomass, but the inclusion of *Chlorella* sp. into feed in most cases evolves biomass processing, pre-treatment, and extrusion into pellets (Teuling et al., 2017; Tibbetts et al., 2017; Batista et al., 2018; de Cruz et al., 2018). However, a study conducted to evaluate the use of *Chlorella vulgaris* and *Scenedesmus obliquus* as an autotrophic bio floc technology during Nile tilapia cultivation with commercial feed showed a positive effect on water purification and to prevent fish mortality. Nevertheless, their system utilized water exchange every 10 days, which included the microalgae supply of 0.01 g L^{-1} (equivalent to 2 g per pond per day). Here it was utilized the maximum concentration of 0.78 g L^{-1} (equivalent to 0.78 g per pond per day) (2000 mL of microalgae solution per pond per day; Table 1).

Evaluation of water quality

Table 4 presents the profile of the physical and chemical parameters of the water during two stages of the experimental trial. In the initial stage, the water in all ponds met the appropriate standards outlined in Resolution 357 (Brazil, 2005). This condition persisted until the end of the experiment, with only one exception.

By the end of the experiment, water in T1 was associated with low levels of dissolved oxygen, falling below the recommended thresholds. This is likely due to T1 ponds receiving twice the amount of commercial feed compared to treatments involving commercial feed (T2, T5, and T6). The concentration of dissolved oxygen (DO_2 (mg L^{-1})) may gradually decrease with increased feed input. In terms of nitrite (NO_2^-), treatments exhibited critical levels in the final stage of the experiment, except for T1.

Microbiological indicators of water quality revealed the presence of thermotolerant coliforms in all treatments at the end of the experiment. Regarding fecal coliforms (*E. coli*) and mesophilic bacteria, treatments involving commercial feed (T1, T2, T5, and T6) exhibited the highest values, ranging from 10^4 to 10^5 CFU 100 mL^{-1} (Table 5).

Table 2. Specific growth rate, total weight gain, survival rate, and condition factor of Nile tilapia juveniles during 14 days of cultivation.

Treatment	Specific growth rate (% day^{-1})	Weight gain (% day^{-1})	Survival rate (%)	Condition factor (g cm^{-3})
T1	0.34 (± 0.29)^b	4.80 (± 3.1)^b	86.7^a	8.96^a
T2	3.80 (± 0.37)^a	53.33 (± 5.25)^a	96.7^a	8.42^{ab}
T3	-3.26 (± 0.60)^c	-45.70 (± 8.41)^c	93.3^a	7.11^c
T4	-2.18 (± 0.52)^c	-30.55 (± 7.40)^c	93.3^a	7.58^c
T5	-0.19 (± 0.60)^b	-2.72 (± 8.48)^b	83.3^{ab}	7.67^{bc}
T6	2.69 (± 0,58)^a	37.77 (± 8.14)^a	53.3^b	8.64^{ab}

Different letters in the same column differ (Tukey test, 5% significance level). Treatments are according to Table 1.

Table 3. Specific growth rates (% day^{-1}) of juvenile Nile tilapia depending on treatment (feed), time period (days), and culture system reported elsewhere.

Feed	Time (days)	System	Specific growth rates (% day^{-1})		Reference
			Min.	Max.	
Microalgae supplementation	28	pond/constant aeration	0.21	0.35	Costa et al. (2011)
Organic wheat	15	pond/constant aeration	0.3	0.3	Lui et al. (2012)
Alternative protein	180	pond/constant aeration	1.0	1.1	Assano et al. (2011)
Commercial fed (CF)	29	pond/constant aeration	1.34	1.82	Coêlho et al. (2014)
Agroindustrial byproducts	60	cages/water recirculation	2.03	2.27	Carvalho et al. (2012)
CF and enzyme complex	62	pond/water recirculation	2.28	2.37	Signor et al. (2010)
Agroindustrial byproducts	70	pond/constant aeration	3.43	3.96	Workagegn et al. (2014)

Table 4. Physical and chemical parameters of water depending on feed and the presence of microalgae and aeration in Nile tilapia culture systems.

Treatment	pH		DO_2 (mg L^{-1})		NH_3 (mg L^{-1})		NO_2^- (mg L^{-1})	
	Day 1	Day 14	Day 1	Day 14	Day 1	Day 14	Day 1	Day 14
T1	7.2	6.6	7.9	8.3	< LD	0.02	< LD	0.2
T2	7.2	6.2	7.9	7.9	< LD	0.001	< LD	2.8
T3	7.2	6.2	7.9	8.0	< LD	0.02	< LD	2.8
T4	7.2	6.2	7.9	7.9	< LD	0.02	< LD	2.8
T5	7.2	6.6	7.9	7.9	< LD	0.003	< LD	2.8
T6	7.2	6.4	7.9	7.9	< LD	0.02	< LD	2.8
Rv	6.0-9.0		≥ 5 mg L^{-1}		≤ 2.0 mg L^{-1}		≤ 1.0 mg L^{-1}	

< LD = Values below the limit of detection. RV = reference values (guidelines of CONAMA 357/05); pH = hydrogen potential; DO_2 = dissolved oxygen; NH_3 = toxic ammonia; NO_2^- = nitrite. Treatments are according to Table 1.

Table 5. Counts of mesophilic total bacteria present in the water from ponds used to raise tilapia fish under different experimental conditions.

Treat-ment	TTC		*E. coli*	Mesophilic bacteria
	24 h	48 h	(CFU 100 mL^{-1})	(CFU 100 mL^{-1})
T1	+	+	6.06 x $10^{4\,a}$	8.67 x $10^{4\,c}$
T2	-	+	7.00 x $10^{4\,a}$	4.70 x $10^{5\,a}$
T3	+	+	1.45 x $10^{4\,b}$	5.20 x $10^{4\,d}$
T4	-	+	1.04 x $10^{4\,b}$	6.50 x $10^{4\,d}$
T5	+	+	1.80 x $10^{4\,b}$	2.28 x $10^{5\,b}$
T6	+	+	1.30 x $10^{4\,b}$	2.23 x $10^{5\,b}$

TTC: total thermo-tolerant coliforms after 24 and 48 h (+ presence or - absence); CFU: Colony-Forming Unit. *Means followed by the same letter do not differ by the Tukey test at the 5% probability level. Treatments are according to Table 1.

The level of dissolved oxygen (DO_2) in ponds with low water renewal is likely the major constraint in tilapia production. Thus, monitoring the feed ratio and regularly testing for dissolved oxygen levels is advisable. Tilapia can survive at low levels of dissolved oxygen as long as temperature, pH, and stocking density remain favorable (Caldini et al., 2013; Abdel-Tawwab et al., 2015).

High levels of nitrite can challenge the immune system of fish, leading to diseases and sudden death (Kroupova et al., 2005). Surprisingly, the nitrite levels in this study were at least twice as high as the reference values (RV) (Table 2). However, the reported levels of nitrite in the literature vary significantly and are frequently higher than the recommended values. Pereira and Lapolli (2009), Gorlach-Lira et al. (2013), Coêlho et al. (2014), and Lima et al. (2016) found lower values ranging from 0.1-2.0, 0.004-0.005, 0.0-0.27, and 0.26-0.46 mg L^{-1}, respectively. Interestingly, Santos et al. (2013) reported higher values ranging from 1.03-3.61 mg L^{-1} in cultivation systems using water recirculation.

Overall, similar to the results observed in this study, the data reported in the literature show a discreet association between high nitrite values and mortality or weight trends in tilapia (Pereira and Lapolli, 2009; Gorlach-Lira et al., 2013; Santos et al., 2013; Coêlho et al., 2014; Lima et al., 2016). However, this association may not be straightforward, limiting our understanding and comparisons between the cultivation strategies.

Recirculation systems are often more effective in controlling nitrite levels in fishponds. However, these systems require larger amounts of water, which can increase environmental footprints. Exploring alternatives to remove nitrite from the water may enhance production while also limiting the adoption of technology for family farmers. On the other hand, biotransformation techniques such as denitrification may reduce the investment required to mitigate the toxicity of effluents derived from tilapia production.

As per the reference values established in Resolution 357 (Brazil, 2005), confined animals should not be exposed to thermotolerant coliforms beyond 1000 per 100 milliliters (1.0 x 10^3 CFU 100 mL^{-1}); *Escherichia coli* may be used as a substitute for thermotolerant coliforms based on the limits set by the competent environmental agency. The contamination of aquaculture systems and fish by human pathogens such as *E. coli* is usually attributed to infected handlers or storage (Rocha et al., 2014; Dewi et al., 2022).

The relatively high bacterial contamination observed in the experiment reflects the nature of fish farming conducted in ponds, which involves a considerable volume of dietary inputs in a relatively small area (Gorlach-Lira et al., 2013). Bacterial contamination was higher than the values recorded by Ahmed and Naim (2003) in tilapia culture ponds and by Gorlach-Lira et al. (2013) in tilapia culture floating net cages, ranging from 10^3-10^4 and 10^4-10^5 CFU 100 mL^{-1}, respectively. The results were similar to the values reported by Ntengwe and Edema (2008) in tilapia culture ponds (10^6 CFU 100 mL^{-1}).

However, bacterial contamination is comparable to the critical values observed for dissolved oxygen and nitrite. The low levels of dissolved oxygen may be attributed to the decomposition of leftover feed (excess of commercial feed). The levels of total mesophilic aerobic bacteria support the evaluation of water in terms of decomposition activity (Gorlach-Lira et al., 2013). Microbiological decomposition requires dissolved oxygen from the water, which can lead to critical levels of DO_2 that threaten the survival of fish. High nitrite values may result from this type of decomposition. Lima et al. (2016) observed that higher loads of organic matter are associated with greater amounts of total ammonia nitrogen, which serves as a substrate for *Nitrossomonas* to release nitrite into the water.

CONCLUSION

In conclusion, this study examined the growth performance and water quality parameters of Nile tilapia (*Oreochromis niloticus*) in different culture systems. The results demonstrated the significant impact of various factors, particularly the type of food provided, on the growth and development of the fish. The treatments combining commercial feed with aeration (T2) and commercial feed with microalgae supplementation and aeration (T6) showed the highest weight gain rates, while treatments without commercial feed exhibited lower weight gains. The specific growth rates of tilapia in T2 and T6 were comparable to those reported in the literature for juveniles. Proper monitoring and management of water quality, including nitrite levels and dissolved oxygen, played a crucial role in the survival and growth of Nile tilapia.

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Ethics Committee Approval: All procedures used in experiments with animals followed the ethical standards of the Ethical Committee from the Federal University of Grande Dourados, Brazil.

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