Vol.67: e24220239, 2024
https://doi.org/10.1590/1678-4324-2024220239
ISSN 1678-4324 Online Edition

Article - Food/Feed Science and Technology

Growth Performance of Tilapia Juveniles *Oreochromis niloticus* Reared in Two Sustained Swimming Speeds and Two Stocking Densities

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Editor-in-Chief: Paulo Vitor Farago
Associate Editor: Jéssica Caroline Bigaski Ribeiro

Received: 02-Apr-2022; Accepted: 06-Oct-2023

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HIGHLIGHTS

- The high flow of water stimulates sustained swimming exercise of fish.
- Sustained swimming speed of 2 body length per second enhanced fish growth.
- Fish yield in sustained swimming exercise was 3.6 times higher than no exercised fish.
- Tilapia are fish inhabiting shallow ponds and lakes but, the artificial sustained swimming exercise promotes better growth.

Abstract: Tilapia is one of the most important aquaculture species due to the desirable characteristics as easy reproduction and quick growth in several farming systems, including the recirculating aquaculture systems RAS. In this, the high flow of water to remove waste casually stimulates the fish to swim backset, and growth performance may be improved. Increasing the speed of swimming can improve performance within certain limits, in which however if exceeded can be harmful. Fish may be exhausted leading to impaired growth or even mortality. The objective of this work was to evaluate the growth performance of tilapia juveniles under sustained swimming exercise at different speeds. Additionally, the fish stocking density effect was tested in the sustained swimming exercise condition. In the first experiment, Nile tilapia juveniles were submitted in triplicates to sustained swimming exercise in speeds of control (not exercised), 1 BL/s (body length per second), 1.5 BL/s and 2 BL/s. In the second experiment, two fish stocking densities were tested in triplicates in sustained swimming exercise in speed of 2 BL/s and control not exercised. Fish growth performance was improved by sustained swimming exercise in speeds up to 2 BL/s. No expressive fish mortalities were observed in both experiments. The comparison between the two stocking densities does not allow us to safely say tilapia can be reared at stocking densities in order of 1,000 fish/m3, even under sustained swimming exercise. Other densities need to be tested.

Keywords: fish; *Oreochromis niloticus*; sustained exercise.

INTRODUCTION

The Nile tilapia *Oreochromis niloticus* is one of the most important aquaculture fishes in the world. This species exhibits important farming traits such as ease of alevin production, rusticity, and good growth performance in a variety of rearing systems. Tilapia is an omnivore, which is important for feeding and management in farming. This characteristic opens up a range of nutrient sources when handling fish feeding. In addition, fillets are generally well received in fish markets [1]. There are several rearing systems widely used in tilapia farms, but growth performance under sustained swimming conditions still raises some questions [2].

A very practical system for obtaining sustainable swimming conditions is to rear the fish in circular tanks. In such tanks it is easier to keep the water in constant motion and maintaining the basic water characteristics such as degree of aeration, purification and temperature. In addition, the swimming speed of the fish is easy to achieve and easy to monitor. This type of approach is commonly found in the recirculating aquaculture systems (RAS) and this industry has expanded recently [3]. For the above reasons, tilapia is a species of great interest as it is a good source of protein from sustainable fish farming systems. Nowadays, RAS is among the most manageable water systems, because of its ability to use mechanical and biological filtrations related to water recycling. Moreover, RAS efficiency is the only system where water exchange may be only due to evaporative losses [4].

RAS units are usually much smaller than the other traditional fish farming facilities such as the fish ponds. RAS tanks are made in fiberglass or even in brickwork masonry. The high flow of water in the RAS tanks to remove fish wastes also encourage the fish schooling [2, 3]. Better fish growth conditions are provided in RAS, and sometimes the stocking densities can be increased. However, the fish swimming speed values must be selected to the exercise occurs in the edge [5]. Water speed values above the ideal limit can increase the fish metabolism to be overwhelmed reducing the growth rates and even threatening the health and increasing mortality. It is coming to be consensual that fishes have an ideal sustained swimming speed range in the RAS [6, 7, 8, 9, 10].

In RAS, the growth performance of tilapia juveniles under sustained swimming exercise was evaluated, and also the possible increase of fish stocking density was investigated. The present data are relevant to the RAS industry and to the biological knowledge of tilapia under the specified conditions.

MATERIAL AND METHODS

General information

The growth performance of tilapia in RAS was studied for three water velocities in comparison to untrained juveniles kept in a tank with standing water (Experiment-I). Two densities of fish were then tested at the best-established water velocity (Experiment-*II*). Tilapia fingerlings were brought from a commercial fish farm in Dourados - Mato Grosso do Sul - Brazil. The fish were transported to Embrapa Western Agriculture facilities, and kept in 600-L self-cleaning, circular fiberglass tanks at 27 °C. The water was recycled by filtration and aerated to saturation.

Experiment I

Twelve circular 1,000-L fiberglass tanks were adapted to an experimental RAS that was equipped with a sump, rotating cylinder, and sand filter. Continuous aeration was provided in each experimental unit by submerged porous aeration stones. Ammonia from fish metabolism was purified in the water by nitrogen bacteria located in bio-beds in a serial container with continuous aeration. The water was heated by a thermal pump and maintained at a temperature of 26 ± 1 °C. One hundred juvenile tilapia were stocked in each tank. The average initial weight (90 ± 15 g) and length (16 ± 0.7 cm) of the fish were estimated from 200 fish taken randomly from the holding tank at the beginning of the experiment. Three velocities at Sustained Swimming — SS (treatments) were set in triplicate and Standing Water — SW (control); 1.0 BL (body length) per second; 1.5 BL/s; and 2.0 BL/s. Fish swimming velocities were achieved with individual flow-through submerged vertical and horizontal pipes with drain holes put in each tank. Water flow for the experimental swimming velocities was adjusted with a flow control valve in each experimental unit (clock sense). Water velocity was monitored with a mechanical flow meter (General Oceanics Inc). In the control group, the standing water did not encourage the fish to swim. Water was replaced approximately at a rate of 0.12 L/s (control), 0.38 L/s (1.0 BL/s), 0.58 L/s (1.5 BL/s), and 0.78 L/s (2.0 BL/s). During the experimental period, the fish were fed commercial pellets with 36% crude protein — six times a day for 30 days near to satiation. Feeding was discontinued one day before the fish harvest. They were counted, and 20 of them picked up randomly from each tank were anesthetized and length and weight were measured. The growth performance over the experimental period was evaluated according to the following parameters: *1)* Initial biomass (IB) = average initial weight x number of fish in each tank; *2)* Final biomass (FB) = average final weight x number of fish in each tank; *3)* Yield (Y) = final biomass — initial biomass; *4)* Daily yield (DY) = Y / 30; *5)* Weight gain (WG) = final weight of fish — initial weight of fish; *6)* Daily weight gain (DWG) = weight gain / 30 feeding period days; *7)* Specific growth ratio (SGR) = [(ln final weight — ln initial weight) × 100]/30; *8)* Survival rate (SR) = [(initial number of fish in each tank — final number of fish in each tank) / initial number of fish in each tank] x 100; *9)* Feed conversion ratio (FCR) = total feed in each tank / Y.

Experiment II

This experiment was performed with 9,000 tilapia fingerlings with an average weight and length of 5.3 ± 0.2 g and 6.3 ± 0.2 cm. The fish were randomly distributed in twelve 1,000-L tanks like those described in Experiment-*I*. Three thousand fish were equally distributed into six tanks (LD — low density) and six thousand fish into the other six tanks (HD — high density). Both fish stock densities were compared in sustained swimming exercise (SS) of 2.0 BL/s and in still water (SW). The experimental design was 2×2 (HD and LD treatments *versus* SS and SW conditions) in triplicates. Fish were fed six times a day near to satiation with commercial pellets of 45% crude protein for 35 days. Feed intake was daily estimated. Water speed was monitored by a mechanical flow meter (General Oceanics Inc) every day. Feeding was discontinued one day before the fish sampling. All the remaining fish were counted and 10% were measured for the growth performance evaluation as described in *Experiment-I.*

Water quality and management

Water temperature and dissolved oxygen were daily measured (YSI model 55), and pH and total ammonia, twice a week. The flow was daily and individually interrupted for a few minutes for siphoning. The water loss and replacement for such procedure were about 10%. These procedures were enough to keep its quality in all experimental tanks at 27 ± 1 °C; pH 7.2 ± 0.3; [NH3-NH4+] 0.5 ± 0.24 mg L^{-1} and [O2] 7.6 ± 0.4 mg L^{-1}.

Statistics analyses and data availability

The normality of data from each condition was verified through the Kolmogorov-Smirnov analysis. Since the symmetry of the variables was accepted, the two-way ANOVA was applied. The Tukey's test was done to compare the means between each experimental condition. Differences were accepted at a significant level of $p < 0.05$. All values are reported as mean ± SD.

Ethics, care and use of laboratory animals

The experimental procedures were evaluated by the Ethics in the use of laboratory animals (CEUA 02/2016) from Embrapa Western Agriculture.

RESULTS

The growth of juvenile tilapia was remarkable after 30 days of living under sustained-swimming. The effects of the growing swimming velocity were distinct and gradual. Except for the FCR value, all other studied growth performance parameters yielded their highest values at the maximum swimming speed of 2 BL/s. No suffering or exhaustion was observed in the fish encouraged to swim at sustained-swimming, even at the highest speed. No significant mortality was observed at any of the experimental swimming velocities. These results are shown in Table 1.

Table 1. Productive response of tilapia to sustained swimming exercise.

Parameter	Swimming speed (BL/s)*			
	0	1.0	1.5	2.0
Final length (cm)	18.1±0.4^{B}	18.5±0.8^{B}	19.3±0.4^{B}	19.9±0.3^{A}
Final weight (g)	107.8±8.8^{C}	130.1±16.7^{B}	142.6±8.7^{B}	154.5±7.8^{A}
Final Biomass (kg/m^3)	10.8±0.9^{C}	13.0±1.7^{C}	14.3±0.9^{B}	15.5±0.8^{A}
Yield (kg/m^3)	1.8±0.9^{A}	4.0±1.7^{B}	5.3±0.9^{C}	6.5±0.8^{D}
Daily yield (g/m^{3}day)	59.8±29.2^{D}	134.2±55.8^{C}	175.5±28.9^{B}	215.2±26.0^{A}
Fish weight gain (g)	17.9±8.8^{D}	40.2±16.7^{C}	52.7±8.7^{B}	64.5±7.8^{A}
Daily fish weight gain (g/day)	0.6±0.3^{D}	1.3±0.5^{C}	1.7±0.3^{B}	2.1±0.3^{A}
Specific growth ratio (%)	0.6±0.3	1.2±0.4	1.5±0.2	1.8±0.2
Survival (%)	98±1.0	97±3.0	99±1.7	98±1.8
Feed conversion ratio	1.5±0.1	1.4±0.2	1.5±0.3	1.4±0.2

Fish were submitted to four different swimming velocities for 30 days; *Swimming speed is expressed in fish body length per second (BL/s). Table values are expressed as mean ± SD (1). Initial weight = 90 ± 15 g; Initial length = 16 ± 0.7 cm; Stocking density = 100 fish/m^3. (1) Means followed by equal letters do not differ by Tukey's test (P<0.05).

Considering that 2 BL/s was the best sustained-swimming velocity observed in Experiment I, it was chosen to study the stocking density effects. In addition, also the stocking density has been evaluated in fish held in standing water. Then, the results can be understood by reading the effects of swimming conditions at HD and LD or the effects of stocking density at SS and SW. In general, the growth performance effects of SS were positive to tilapia juveniles either at HD or LD. The variable weight gain WG was approximately 24% higher in SS either at HD or LD. Also, the feed conversion improved by SS, but it was more remarkable in fish living at LD. Concerning the stocking densities, the LD was more gainful than the HD either under SS or SW. Uniformity (UI) among the sampled fish improved under SW conditions and was higher in HD (20%) than in LD (10%). However, the uniformity among fish kept in SW remained independent of the stocking density, while fish held in SS showed better uniformity when in HD. The body factor (K) was significantly higher under conditions of HD only for fish kept in SW. However, at HD this index was higher in SS than in SW. In LD the swimming condition had no effect on the body factor. The set of such variables are present in Table 2.

Table 2. Tilapia growth performance in two swimming systems *versus* two stocking densities.

Parameter	SP*	Stocking density (fish/m^3)	
		500	1.000
Initial weight (g)	C	5.1±0.3	5.2±0.2
	E	5.3±0.2	5.3±0.1
Final weight (g)	C	35.01±1.36	28.40±3.95
	E	42.67±1.23(a)	34.01±0.81(b)
Initial biomass (kg/m^3)	C	2.6±0.1	5.2±0.2
	E	2.7±0.1	5.3±0.1
Final biomass (kg/m^3)	C	17.44±0.75	25.04±1.26
	E	20.47±0.66(b)	31.18±3.20(a)
Weight gain (g)	C	29.75±0.65(B-a)	23.24±0.67(A-b)
	E	37.37±0.64(A-a)	28.73±0.53(B-b)
Daily weight gain (g/day)	C	0.89±0.01	0.68±0.01
	E	1.09±0.01	0.84±0.01
Specific growth rate (%/day)	C	5.69±0.19	4.99±0.52
	E	6.14±0.03	5.48±0.09
Feed conversion rate (FCR)	C	1.12±0.04(A)	1.03±0.03(A)
	E	0.86±0.04(B)	0.90±0.08(B)
Feed intake (g/day)	C	0.98±0.02	0.68±0.02Ba
	E	0.95±0.03	0.74±0.03Ba
Survival (%)	C	98.0±1.5	96.0±3.6Aa
	E	90.0±12.	92.0±12.0
Uniformity index (%)	C	57±2.0(B)	52±3.0(B)
	E	63±2.0(A-b)	65±1.53(A-a)
Body factor (k)	C	1.98±0.05(b)	2.01±0.03(a)
	E	2.00±0.03	2.02±0.03

Tilapia juveniles held in RAS for 35 days. SP: system production, LD: Low density-500 fish/m^3. HD: High density-1000 fish/m^3. SW: (Standing-water); SS: (Sustained-Swimming at 2 BL/S). The data are expressed as mean ± (SD). Different capital letters in the columns and lower-case letters in the rows indicate significant differences at $p < 0.05$.

DISCUSSION

Fish farmers usually welcome the possibility of using sustained swimming in the RAS because of the several promising results arising from such practice. Fish living in sustained swimming are encouraged to exercise uninterruptedly, and this fact brings positive outcomes. The utilization of nutrients, for example, is enhanced in fish living under continuous exercise [11, 12, 13, 14]. In addition, the improvement of disease resistance [14], stress reduction [14], and, consequently, an aggressiveness reduction [15, 16, 17] are observed in fish under sustained swimming. The improvement of nutrient utilization in fish living in sustained swimming results in better growth performance [3]. Amazingly, even sedentary species as the Japanese flounder *Paralichthys olivaceus* and the California halibut *Paralychtys californicus* show better growth rates when living in sustained swimming velocities up to 1 BL s^{-1} [8, 18].

The rearing of commercial fish under sustained swimming has been studied and gradually introduced due to its many advantages. Salmonids are the most studied fish under exercise conditions. They generally have better growth performance in sustained swimming at velocities up to 2.5 BL s^{-1} [3], while matrinxã *Brycon amazonicus*, a typical tropical migratory fish, showed increased growth performance in sustained swimming speeds between 1.0 – 1.5 BL/s [19]. Some species have much higher swim speeds, such as zebrafish *Danio rerio*, whose growth performance rates are better at swim velocities higher than 4 BL/s [5].

Tilapia are mainly freshwater fish inhabiting shallow streams, ponds, rivers, and lakes. Despite being unusual, they also live in brackish water. Nowadays, the importance of tilapia in several aquaculture and aquaponics systems are unquestionable. Like all carangiforms, its swimming category involves the entire body and the tail fin [20]. So, it would expect that sustained swimming would influence their physiology and consequently their growth performance. In fact, an improvement in growth parameters of fish maintained at all tested sustained swimming speeds was observed. Before any discussion or detailed data analyses on tilapia growth performance, it is necessary to consider three relevant points. The first point is the size of the fish in the experiments I and II. The second issue is the lack of knowledge about the point, or the range, in the growth curve where the fish were during the experimental period. And finally, the extension of the trial period. The fish in Experiment I were three times larger than those in Experiment II. This difference in size and weight does not allow for a reliable comparison between the two experiments. However, this does not invalidate the discussions or the conclusions on the results of both experiments singly.

The great difficulty due to the lack of knowledge of the fish's growth phase is the correct interpretation of the specific growth rate SGR data. Even though this variable computes the instantaneous growth rate (SGR day^{-1}), it assumes that the fish are growing exponentially. Furthermore, the trial period was relatively long, and then the incoming of errors in such variable interpretation, which has the characteristic of being instantaneous. These considerations may explain the non-significant differences observed between the SGR of both experiments. It is interesting to note that all the swimming conditions experienced did not harm the animal welfare. Either tilapia kept in standing water or submitted to sustained swimming, they had easy access to food and did not present any health problems. These conclusions come from the fact that we do not have significant variations in the values of FCR, which suggests that all the swimming conditions used are good management in tilapia rearing. The high survival rates under all experimental conditions support such conclusions.

Considering that the variables fish length-FL, fish weight-FW, fish biomass-FB, Yield, and fish weight gain-FWG do not assess punctual values in the growth curves, but rather the performance over a long-term period, it was possible to observe the advantage of tilapia rearing under sustained swimming over that in standing water. The yield of fish reared in sustained swimming was around three times higher than those held in standing water. Therefore, the choice of the two distinct conditions, sustained swimming at 2 BL/s and still water, was thus justified for the Experiment II (for the stocking density test).

Reports about sustained swimming as fish farm practice that allows stocking densities higher than the conventional ones are growing. This fact is reported, for example, for matrinxã [16]. However, there are no reports of the rearing of tilapia in high density under sustained swimming. The data obtained with tilapia kept in two different stocking densities show significant differences between the two tested swimming conditions, SS and SW. The considerations elaborated above regarding the specific growth rate are equally applied to Experiment II. Therefore, it is understandable that the values obtained for this variable were similar under all experimental conditions. Two distinct outcomes must be analyzed separately in the stocking density experiment; the effects of the fish density under both SS and SW; and the effects of the swimming conditions on each stocking density.

The high stocking densities limited growth in both the experimental swimming conditions. When comparing the stocking densities concerning growth performance variables, it is possible to observe that the lower one is better than the high stocking density. Thus, weight gain, for example, is lower in tilapia kept in high density. This effect is most evident in fish kept in SS. In this condition, there is also a reduction in final weight and final biomass. Probably, this should be due to the cronic stress from the population density associated with uninterrupted exercise from sustained swimming. The energy demand caused by continuous physical activity likely masked the positive effects of sustained swimming. Therefore, the lower stocking density used in the present experimental conditions should be indicated in the rearing of tilapia in sustained swimming. Alternatively, other stocking densities need to be tested.

The comparisons between the test condition, sustained swimming or standing water, show few changes in growth performance, both in high and low stocking densities. Sustained swimming had an advantage over standing water rearing condition only when considering tilapia weight gain. Furthermore, this advantage was only observed in fish held at low density. The high stocking density impaired weight gain, and this fact was discussed above. The lower weight gain was aggravated by the sustained swimming condition at high density. An advantage observed in fish reared in sustained swimming was the size uniformity. And this effect was observed both in high and low stocking density. However, the sustained swimming condition markedly impaired the feed conversion rate. Considering that eighty-five percent of the variables studied in low stocking density and ninety-two percent in high density showed no difference between the swimming conditions, it is inconsistent to affirm the advantages of sustained swimming in the present experimental condition.

The benefits of sustained swimming measured through growth performance variables and “condition factors” in these tilapia experiments may not be evident for some factors worth considering. In general, condition factors have created constant confusion regarding their correct application and interpretation [21]. These conditions depend on several variables. For example, the K factor can vary with season [22], health status [23], differences in growth rates between juveniles and adults [24], animal body shape due to variations in life-history (e.g., during the breeding season), and variations in food availability [24, 25]. However, condition indices can be appropriate if used correctly in minimizing the sources of variability. Thus, the K factor, for example, has been used as an indicator of feeding activity [26], body energy reserve [23, 26, 27], and physiological homeostasis [24]. Discussions concerning the requirements demanded by growth factors as a point measure in the growth curve have been done above.

The intensive rearing of fish in standing water conditions with high density of fish can result in decreased growth rates and higher deterioration of water quality [28], increased feed competition [29], and social stress [30]. Moreover, tilapia is a species known for its high aggressiveness when living in low-density populations. Under these conditions, this species forms dominance hierarchies [31]. Increasing stocking densities can be a way to control this characteristic of the species, reducing its aggressiveness in fish farms. The increase in population density could reduce the hierarchical dominance. For these reasons, the results of studies with stocking density in tilapia are unpredictable. From the standpoint of fish farming management, the aggressiveness reduction would lead to a higher yield of the consumed food, increasing its conversion into protein mass. It is known that increasing stocking density improves feed conversion and equalizes the size of fish in the sample [15]. However, our observations with tilapia reared in sustained swimming and stocking density of 500 fish/m^3 showed the best growth performance. This observation is interesting from the perspective of tilapia production.

CONCLUSION

The comparison between the two stocking densities does not allow us to safely say tilapia can be reared at stocking densities in order of 1,000 fish/m^3, even under sustained swimming exercise. Furthermore, intermediate stocking densities may lead to different conclusions. To state that tilapia can be reared in higher stocking densities than 500 fish/m^3, other variables need to be evaluated. In addition, biological and physiological mechanisms that could confirm better farming conditions under sustained swimming exercise as accumulation of protein in the muscles, body composition, and better energy use have to be studied. In this way, the sustained swimming exercise approach for the RAS industry will be recommendable. The sustained swimming exercise for tilapia is favorable when assessed by the growth performance approach. Further studies on such conditions have to be done to adjust the details at different growth stages specially the final commercial one.

Funding: “Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado do Mato Grosso do Sul (FUNDECT- TO 249/16; TO 11/2022)”, National Council for Scientific and Technological Development (CNPQ 421502/2017-7), the Brazilian Development Bank – BNDES (BRS-Aqua 21.17.02.001.04.09.003), and the Brazilian Agriculture Research Corporation (EMBRAPA 03.17.00.059.00.00).
Acknowledgments: To Mr. Edson da Silva for the technical assistance.
Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study, in the collection, analyses or interpretation of data and writing of the manuscript or in the decision to publish the results.

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