GROWTH AND SURVIVAL OF TILAPIA *Oreochromis niloticus* (Linnaeus, 1758) SUBMITTED TO DIFFERENT TEMPERATURES DURING THE PROCESS OF SEX REVERSAL

Crescimento e sobrevivência de tilápias *Oreochromis niloticus* (Linnaeus, 1758) submetidas a diferentes temperaturas durante o processo de inversão sexual

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ABSTRACT

The objective of this research was to evaluate the sex reversal technique using 17α--methyltestosterone (MT) hormone, submitted to temperature modification of fry Nilo tilapia storage, aiming to get the data of sex reversal combined with growth performance and fry survival. The experiment was performed at UFLA Fish Culture Station, using tilapia fry (0,008 ± 0.002 g e 0,9 ± 0.1 cm) obeying a totally randomized experimental delineation in a factorial scheme 4x4, in 4 temperatures (26º, 28º, 30º, 32ºC) and 4 hormonal doses (0, 20, 40, 60mg of MT/kg of ration) during 28 days, with 5 repetitions. As temperature raised, weight gain rate, size and survival increased (p<0.01); however, this temperature raise was not effective in modifying males ratio (p>0.01), which occurred only due to the used hormone treatment. The dose of 40 mg of MT/kg of ration provided similar results to those of 60mg of MT/kg of ration. Hence, the temperature band from 26º to 32ºC does not affect sex reversal rate, but temperatures around 30ºC improves the performance of tilapias related to the growth and survival, and the dose of 40 mg of MT/kg of ration is enough to achieve monosex populations.

Index terms: Tilapia, 17α-methyltestosterone, temperature.

RESUMO

Objetivou-se, neste trabalho, avaliar a técnica de inversão sexual utilizando hormônio 17α-metiltestosterona (MT), submetidas à modificação da temperatura de estocagem das pós-larvas de tilápia, visando obter os melhores dados de inversão sexual aliado à performance de crescimento e sobrevivência das pós-larvas. O experimento foi conduzido na Estação de Piscicultura da UFLA, utilizando pós-larvas (0,008 ± 0,002 g e 0,9 ± 0,1 cm) de tilápia em delineamento experimental inteiramente casualizado em esquema fatorial 4x4, com 4 temperaturas (26º, 28º, 30º e 32ºC) e 4 doses hormonais (0, 20, 40 e 60mg de MT/kg de ração) durante 28 dias, com 5 repetições. À medida que se elevou a temperatura, a taxa de ganho de peso, o tamanho e a sobrevivência foram maiores (p<0,01); entretanto, esse aumento na temperatura não foi suficiente para alterar a proporção de machos (p>0,01), que ocorreu apenas em função do hormônio utilizado. A dose de 40 mg de MT/kg de ração proporcionou resultados semelhantes aos da dose de 60 mg de MT/kg de ração. Portanto, a faixa de temperatura entre 26º e 32ºC não influencia na taxa de inversão sexual, mas temperaturas em torno de 30ºC melhoram o desempenho das tilápias quanto ao crescimento e à sobrevivência, e a dose de 40 mg de MT/kg de ração é suficiente para a obtenção de populações monossexo.

Termos para indexação: Tilápia, 17α-metiltestosterona, temperatura.

(Received in April 16, 2007 and approved in April 24, 2008)

INTRODUCTION

Tilapia is the generic name of a large range of fish species of cichlids. The original distribution of this group was south-central Africa northward into Syria, where more than 70 species have been identified (Popma & Phelps, 1998). The Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) is the main genus *Oreochromis* with potential for aquaculture due to its rusticity and rapid growth, reaching commercial weight in small interval of time; great capacity for adaptation to confinement and the various systems of farming, high capacity for hybridization, which allows the characters unite desired; tolerance to wide variations in salinity, temperature and concentrations of dissolved oxygen, high resistance to diseases, high quality meat with its clear color, and high acceptance by the consumer (BOSCOLO et al., 2001; HAYASHI, 1995; LOVSHIN, 1997).

Tilapia males have a higher weight gain and better feed conversion when compared with females under conditions of large-scale commercial. Males grow 18-25% faster than females (Macintosh & Little, 1995). Therefore,

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various techniques of sex reversal, or more specifically the production of masculinization monosex culture, have been advocated. Among the techniques used for sex reversal in tilapia, the most widespread in the world and Brazil is the use of hormone incorporated in the diet, especially the 17 -methyltestosterone (GUERRERO III & GUERRERO, 1997; NAKAMURA, 1975; SHELTON et al., 1978).

Although it was shown that the use of hormone does not result in the accumulation of residues in tissues of fish treated (ABUCAY & MAIR, 1997; CURTIS et al., 1991), there are still concerns about their release into the environment and the reaction of consumers. It is believed that the indiscriminate use of the hormone causing environmental impact and that this may bring some damage in short and long term in health of humans and animals.

Besides the use of hormones for a male population monosex in tilapia, the increase in water temperature can also be used as an alternative of sex reversal (ABUCAY et al., 1999; BAROILLER & D'COTTA, 2001; BORGES et al., 2005). However, the rates of sex reversal by temperature of the water are not as satisfactory as the technique of sex reversal through hormones. Another alternative to try decreasing the hormone dose utilized could be the use of hormones linked to increased water temperature of rearing, in an attempt to obtain high rates of male of tilapia, and a lower formation of waste released into the environment, decreasing further, the risks of handling during treatment.

The objective of this research was to verify the effects of different temperatures and hormonal doses on sex reversal rate, growth and survival of fry Nilo tilapia.

MATERIALAND METHODS

Were used 16,400 fry of tilapia from breeding of Nile tilapia (*Oreochromis niloticus*) Thailand line, six days after hatching and distributed in density of 4100 fry/temperature. The fry were stocked in 20 plastic trays with a volume of 5L (40 fry/L) with side holes covered with mesh to allow exchange of water. The trays were randomly distributed in 4 cups water of 500L (5 trays/cup) with constant aeration and temperature according to each treatment. Before the fry are transferred into the trays, was removed a sample of 100 fry to determine the mean weight and length, which were 0.008 ± 0.002 g and 0.9 ± 0.1 cm, respectively.

The experiment was conducted at the Aquaculture Station of UFLA in a completely randomized design in a factorial scheme 4x4, with 4 temperatures (26, 28, 30 and 32 ºC) and 4 hormone doses (0, 20, 40 and 60 mg of MT/kg ration) for 28 days with 5 replicates. Each plot consisted of 200 fry.

The diet of fry was initiated seven days after hatching with a commercial diet, which met the nutritional requirements of the species at that stage. The levels of MT (20, 40, 60 mg/kg) were diluted in 500 mL of ethyl alcohol and mixed in diet. The ration was offered at fry six times by day. In the first week of treatment, the ration was offered at 30% of body weight, in the second week 25%, in the third week 20% and in the fourth week 15% of body weight.

During hormonal treatment there were daily renewal water in boxes (the entry in top and exit through the bottom of taps), provides a water renewal rate of 20% of total volume. The trays were cleaned two times/day for removal of the remains of food and excreta, with a renewal rate of approximately 40% of the total volume of water in the tray. The water temperature was measured 2 times per day, with a thermometer in scale of 1 ºC.

After 28 days of treatment was removed a sample of 20 fry by tray for measuring the weight (mg) and size (mm). The remaining fry in trays were counted to calculate the survival rate (%) in each treatment. Then, the fry were transferred to nylon hapas outdoors and fed diet without hormones four times daily, in quantities corresponding to 10% of body weight (PIMENTA et al., 2008). The fry remained in hapas for three months to determine the sex reversal rate, through the collection of the gonads of fry. After routine histological techniques were obtained section were stained with Hematoxylin-Eosin.

Differences between treatments were subjected to analysis of variance and regression. The software used to perform the statistical analysis was SISVAR System - Analysis of Variance (FERREIRA, 2004).

RESULTSAND DISCUSSION

The averages weight of fry obtained in the experiment after 28 days of treatment are described in Table 1. There was significant interaction between temperature and hormonal doses for weight gain ($p < 0.01$). In the analysis of unfolding of the temperature within each hormonal dose it was observed that there was a quadratic effect ($p < 0.01$) in temperature inside the hormonal doses 20, 40 and 60 mg, however, in 0 mg hormonal dose there was no significant difference. When analyzed the unfolding of the hormonal dose within the temperature, only the temperature of 28 °C showed significant interaction (Figure 1).

There was no significant interaction between temperature and levels of hormonal doses used in fry after 28 days of treatment (Table 2). However, there was a quadratic effect ($p<0.01$) of temperature, regardless of the hormonal dose used (Figure 2).

Table 1 – Weight of tilapia fry (g) after 28 days of treatment, reared in tanks with different temperatures and fed diets containing different hormonal doses.

Doses (mg)	Temperature (°C)				Means	p
	26	28 [1]	30	32		
0	0,278	0,285	0,261	0,237	0,265	0,4045
20 [1]	0,206	0,394	0,315	0,209	0,281	0,000
40 [1]	0,226	0,377	0,274	0,201	0,270	0,000
60 [1]	0,266	0,340	0,295	0,195	0,274	0,000
Means	0,244	0,349	0,286	0,211		
P	0,0731	0,0031	0,3151	0,5331		
CV (%)	17,65					

[1]Quadratic Regression ($p<0.01$).

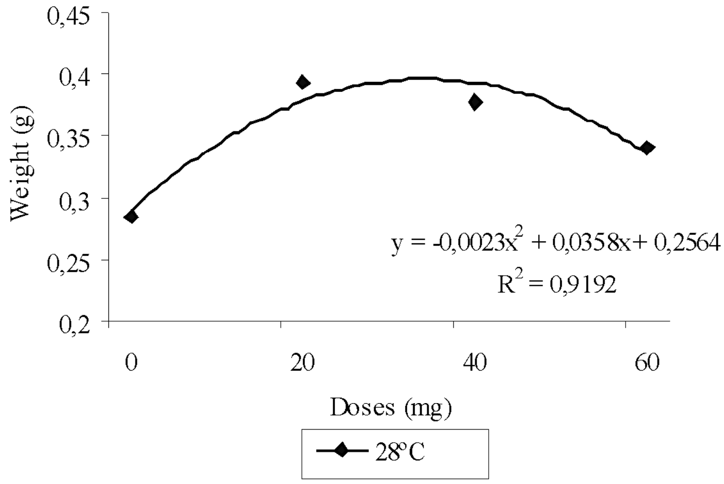

Figure 1 – Average Weight (g) of tilapia fry after 28 days of treatment at temperature of 28 ºC, fed diets containing different doses of 17 α -methyltestosterone/kg ration.

Table 2 – Size of tilapia fry (cm) after 28 days of treatment, reared in tanks with different temperatures and fed diets containing different hormonal doses.

Doses (mg)	Temperature (°C)				Means
	26	28	30	32	
0	2,246	2,482	2,624	2,586	2,485
20	2,171	2,784	2,754	2,488	2,549
40	2,108	2,664	2,650	2,458	2,470
60	2,244	2,670	2,720	2,460	2,524
Means [1]	2,192	2,650	2,687	2,498	
CV %)	7,15				

[1] Quadratic Regression ($p<0.01$).

With respect to the values of survival (%) of fry after 28 days of treatment (Table 3) there was significant interaction of temperature on the hormonal dosages used (p <0.01) but there was not significant difference between the hormonal dose used into the temperature on the survival rate. However, there was a quadratic effect when observed mean of survival in relation of methyltestosterone regardless of rearing temperature (Figure 3).

There was not significant interaction of temperature on the hormonal dosages used (p <0.01) in relation to the males ratio (Table 4). When using the value of final average of male obtained in respective doses, a significant difference was observed (p <0.01) with a quadratic effect (Figure 4).

There was a significant difference in weight gain, size and fry survival according with water temperature of trays. However, for these parameters there was no difference regarding the hormonal doses used in treatments. Similar results were obtained by Cruz & Mair (1994), Guerrero III & Guerrero (1997) and Maine-Pinto et al. (2000), which found that 17á-methyltestosterone has little or no effect on the growth and survival of Nile tilapia during the hormonal treatment. According to Bombardelli & Hayashi (2005), the lack of significant difference for weight, length and survival fry among treatments may be due to rapidly metabolized and excretion of hormone causing no anabolic effect. Guerrero III & Guerrero (1997) cite that low levels of androgens may have a positive effect on growth and fry survival of tilapia during treatment period compared with high levels of hormone. This behavior was also observed in this experiment because there was a decline in weight and survival fry in 40 mg dose of 17á-methyltestosterone.

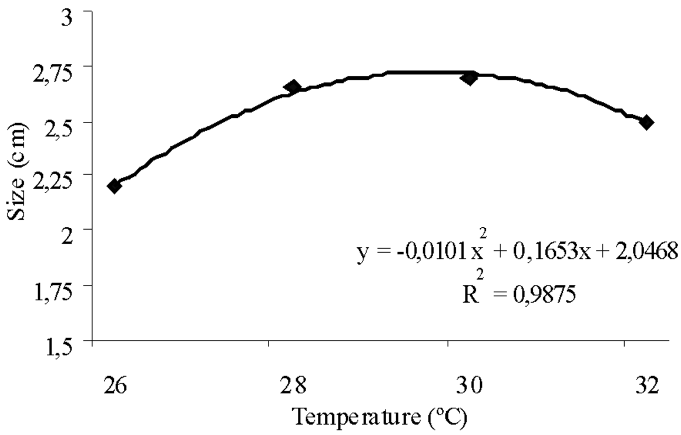

Figure 2 – Average size (cm) of tilapia fry after 28 days of treatment, reared in different temperature of storage regardless of 17-α-methyltestosterone doses in diets.

Table 3 – Survival of tilapia fry (%) after 28 days of treatment reared in tanks with different temperatures and fed diets containing different hormone doses.

Doses (mg)	Temperature (°C)				Means[1]	p
	26	28	30	32		
0[1]	49,1	60,2	80,0	76,9	66.6	0,000
20[1]	50,2	70,6	82,1	83,1	71,5	0,000
40[1]	46,1	64,7	89,9	81,6	70,6	0,000
60[1]	44,0	67,5	85,2	77,1	68,5	0,000
Mean	47,4	65,8	84,3	79,7		
P	0,3398	0,1570	0,0570	0,2464	0,010	
CV (%)	8,56					

[1] Quadratic Regression (p<0.01).

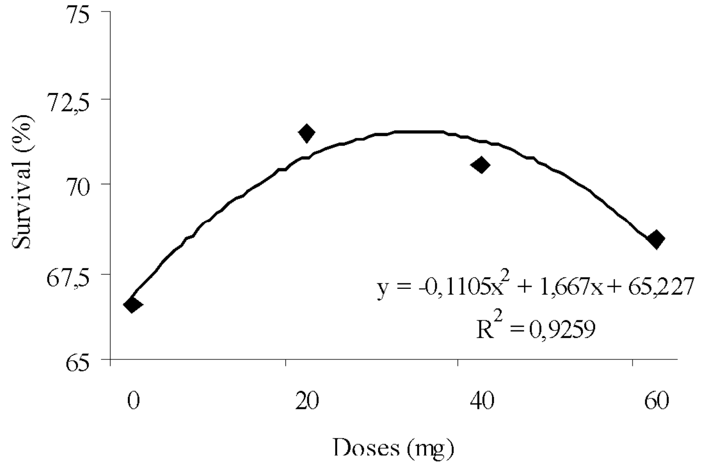

Figure 3 – Average of fry survival (%) after 28 days of treatment depending on the dosage of 17 α -methyltestosterone incorporated in diet regardless of water temperature of storage.

Table 4 – Proportion of males (%) of tilapia identified in histological analysis at final of experiment.

Doses (mg)	Temperature (°C)				Means[1]
	26	28	30	32	
0	55	51	45	57	52,0
20	77	80	74	79	77,5
40	91	78	92	82	85,8
60	91	81	87	94	88,3
Means	78,5	72,5	74,5	78,0	
CV(%)	6,831				

[1] Quadratic Regression ($p<0.01$).

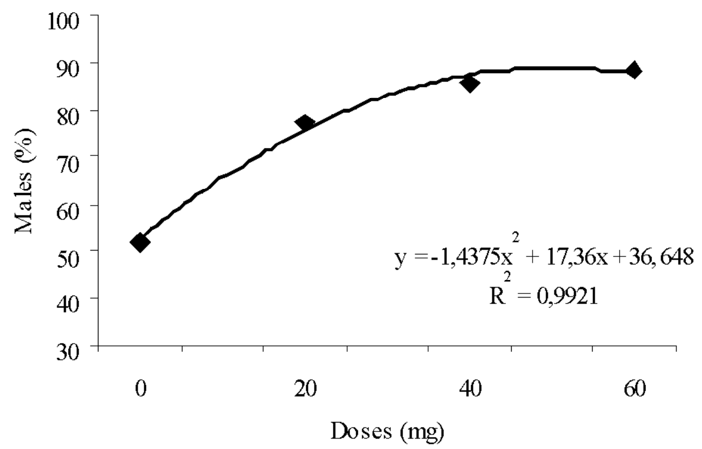

Figure 4 – Average percentage of tilapia males at end of the experiment period depending on the hormone doses used regardless of the temperature of storage.

Regarding temperature, Popma & Lovshin (1996) observed that tilapia prefer waters with temperatures between 29 and 31 ºC for optimal growth. Kubitza (1999) observed that in tilapia culture the zone of thermal comfort is between 28 to 32 °C. Baras et al. (2001) also found effects of temperature on development of tilapia, the range of temperature for better performance in survival and growth is between 27°C and 35°C, and 29.7 °C the ideal temperature. These authors observed also that at temperatures above 35ºC was a significant decrease in survival and growth of fish and in almost all treatments that was used temperature of 39 °C (considered by some researchers as ideal for masculinization) there was 100% of mortality in 3 weeks. This temperature is considered in tilapia, temperature in which mortality begins to tilapia juveniles.

Borges et al. (2005) studying the effect of temperature on the proportion of sex in tilapia observed that with increasing temperature survival rates are directly related with the occurrence of cannibalism, significantly higher at 35 ºC. The data obtained in this experiment are consistent with those observed by these authors, as reported that the ideal temperature for survival, weight gain and size is between 28.5 and 31ºC and that above this temperature point start to decrease both the survival and fry growth.

Cruz & Mair (1994) mention that fry mortality observed during the hormonal treatment may be explained by the establishment of feed hierarchy among fish. Dominant individuals within the population may consume more food and grow faster leaving less food for submissive individuals who have less growth and become, consequently, vulnerable to cannibalism and death by starvation.

The higher rate of mortality was observed in treatments with temperatures of 26 ºC and 28 ºC. During the experiment was observed that the fry size was not uniform in this temperature and at temperatures of 30 ºC and 32 ºC the fry showed homogeneous sizes. It is suggested that difference in size between the fish may have influenced the high mortality rate through the establishment of feed hierarchy since the diet was the only source of food. Moreover, at temperatures of 30 ºC and 32 ºC with homogeneous size, the survival rate was around 79 to 85% in accordance with Popma & Lovshin.

Another possible hypothesis for mortality in all treatments is the form of cultivation. According Phelps & Popma (2000), when sex reversal was practice in indoor tanks, the mortality of fish was greater than in outdoor tanks. Popma (1987) reported a rate of only 40% survival when Oreochromis niloticus was subjected to sex inversal in indoor tanks.

The rate of sex reversal in groups that no received hormone there was no significant difference in proportion of male. This demonstrated that the temperatures used in treatments were not sufficient to induce the sex reversal because there was no change in the proportion of males in these groups. Similar results were observed by Baras et al. (2001), who observed that in the range of temperature between 20ºC and 33 ºC occurs the same proportion of males (42 to 60%) than in temperature of 27 ºC. These results are similar with other authors who observed that low temperatures do not affect the sex ratio (ABUCAY et al., 1999; BAROILLER et al., 1996a, b, c; DESPREZ & MÉLARD, 1998).

According to several authors the temperature that occur change in sex ratio is 35 º C regardless of the use of hormone. Tessema et al. (2006) found that temperature of 36 ºC and 38 ºC increased the proportion of males (78%) when compared to the control (1:1), but in the temperature of 38 ºC the mortality rate was higher. These authors observed also that there are differences in the response to temperature according to the sensitivity of the fish population. Furthermore, Baroiller et al. (1996b, c) observed a strong influence on the phenotypic sex and a significant masculinization are only obtained at temperatures around 37 ºC, but according to Abucay et al. (1999), this temperature can induce the femininity of some genotypic males.

Therefore, the rate of sex reversal in this study was obtained only by the action of the hormone used. Typically, the rate of sex reversal is 95% but there is also a rate of 80 to 90%. The rapid growth and high temperature combined with good quality of the diet the time of susceptibility to sex reversal may move faster.

The response of rate of fry sex reversal has been similarly in all temperatures and in doses of 40 and 60 mg of methyltestosterone similar results were obtained (86 and 88%, respectively). The best dose of hormone found for sex reversal is 50 mg, but the proportion of males in this dosage (89%) would also be very close to the obtained at doses of 40 and 60 mg.

When analyzing the results obtained by the dose of 40 mg in the process of sex reversal the best result occurred at a temperature of 30 ºC. Temperature of 30 °C is within the range considered as the best performance of fish in terms of growth and survival.

Therefore, if the producers raise the temperature (30 °C, coupled with the use of a lower hormonal dose (40 mg) in the process of sex reversal, is obtained a good rate of reversal, the better performance of fish and a lower rate mortality in its tanks, resulting in higher profits. Furthermore, the use of a lower dose hormone reduce contamination and accumulation of hormone in tissues of treated fish and reduce release into the environment, thus reducing environmental impact and risks that these hormones can cause to human health.

CONCLUSIONS

The increase of water temperature in fry of Nile tilapia caused better weight gain, size and survival. The water temperature for the best performance of growth and survival rate of tilapia in the process of sex reversal is 30 ºC. The water temperature between 26 ºC to 32 ºC is not capable of caused sex reversal of Nile tilapia only occurring alteration in sex proportion with application of hormone and the dose of 40 mg is already sufficient to obtain monosex populations.

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