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Growth of Nile tilapia post-larvae from broodstock fed diet with different levels of digestible protein and digestible energy[1]

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ABSTRACT - This study was carried out to evaluate the influence of different levels of digestible energy and digestible protein in diets of Nile tilapia (*Oreochromis niloticus*) broodstock on offspring growth during sex reversal stage. A total of 2,700 post-larvae (8.2±0.001 mg) derived from breeders fed nine diets with distinct levels of digestible protein (28, 34 and 40% DP) and digestible energy (2,800; 3,400; and 4,000 kcal DE.kg^{-1}) were stored in twenty-seven 70 L tanks. After 30 days of growth, their average final weight (AFW, g), average weight gain (AWG, g), final standard length (SL, cm), condition factor (CF), specific growth rate (SGR, %/day), stock uniformity (UNI, %), survival (SUR, %) and sex reversal rate (SRR, %) were measured. Although female nutrition is thought to influence the performance or quality of progeny during early life stages, no influence of diets supplied to broodstock was detected on any parameter. Nonetheless, the offspring presented commercially satisfactory growth rates. Thus, diets containing 28% of digestible protein and 2,800 kcal of digestible energy.kg of diet^{-1} can be used to feed Nile tilapia broodstock without jeopardizing offspring performance during the sex reversal phase.

Key Words: fry, nutrition, *Oreochromis niloticus*, reproduction, sex reversal

Introduction

The Nile tilapia production outside its native range (El-Sayed et al., 2005) represents about 8% of the total of freshwater fish produced worldwide (FAO, 2010). In this scenario, the Brazilian fishery production increased 15.7% in 2009, reaching 1,240,813 t, of which 39% (132,000 t/year) are from tilapia culture (MPA, 2010).

To supply these growth indexes and the expansion of tilapia farming in Brazil, productive practices able to provide larger and high-quality offspring are necessary (Tsadik & Bart, 2007). For that, improved nutrition, feeding and management of broodstock are essential tools (Bhujel et al., 2007), given that the nutrients used by females during vitellogenesis might impact both the quality and the chemical features of eggs (Tyler & Sumpter, 1996; Wiegand, 1996; Izquierdo et al., 2001) and consequently the progeny development (Pereira et al., 2009).

In spite of the notorious influence of broodstock nutrition on breeding performance of males (Moraes et al., 2004), females (Ng & Wang, 2011) and offspring (Gunasekera et al., 1996; Pereira et al., 2009; Sink et al., 2010), broodstock nutrition is a not well known segment of nutrition in bony fishes (Izquierdo et al., 2001). This lack of information about the specific broodstock nutritional requirements in fish (Bhujel et al., 2001; Lupatsch et al., 2010) has driven the recent development in research studies on both tilapia (El-Gamal et al., 2007; Sink & Lochman, 2008; Sink et al., 2010; Ng & Wang, 2011) and other commercial freshwater (Parra et al., 2008; Navarro et al., 2010) and marine (Fernandez-Palacios et al., 1995; Mazzorra et al., 2003) fish cultures.

Proteins and lipids stand out as the most important nutrients for mobilization and formation of body tissues (Cyrino et al., 2000). Moreover, both nutrients play a major role in reproduction (Çek & Yilmaz, 2009), since they are the main components of yolk and have a direct influence on embryonic development (El-Sayed & Kawanna, 2008). However, there are few studies about the effects of broodstock feeding or nutrition over progeny features in fish (Izquierdo et al., 2001).

The objective of this work was to evaluate the effects of Nile tilapia (*Oreochromis niloticus*) broodstock nutrition using diets with different levels of digestible protein and energy on their offspring performance.

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Material and Methods

The experimental period was from January 21st to February 21st, 2011. The procedures were performed according to protocol #07/2010 and approved by the Ethics Committee of Experimentation and Practices with Animals from Unioeste (CEEAAP/Unioeste).

In this study were used 567 females (161.4±33.7 g; 16.9±1.5 cm) and 189 males (255.1±79.4 g; 19.9±5.4 cm) of Nile tilapia (*Oreochromis niloticus*) at 13 months of age. The breeding management of specimens was carried out from the 9th to the 13th months of age, employing 10 days for resting and four days for mating at a sex ratio of 1:3 (male:female). The broodstock was fed from the 2nd to the 13th months of age with nine diets containing a combination of different levels of digestible protein (28, 34 and 40% of DP) and digestible energy (2,800; 3,400; and 4,000 kcal of DE.kg of diet^{-1}) (Table 1).

The experimental diets offered to breeders were formulated at the Laboratory of Nutrition of Aquatic Organisms of UFPR. The feeds were ground in a hammer mill, filtered through a 0.5 mm sieve and pelletized (Meurer et al., 2003a) to 3 mm in diameter (Tessaro et al., 2012a).

When the breeders were at 13 months of age, 2,700 post-larvae were obtained from a single mating period. After the reproduction and production of offspring, 30 five-day-old post-larvae derived from the breeders fed the nine diets were collected in each treatment to measure initial values of weight (mg), standard length (cm) and total length (cm). Afterwards, 1,890 post-larvae of same age were divided into 27 tanks (70 L each) with a storage density of 1 fish.L^{-1}. The tanks were equipped with a semi-closed water recirculation system, mechanical filters and automatic controlled temperature using electric heaters (27±1 °C).

A statistical randomized bifactorial design (identical to that applied to breeders), composed of nine treatments and three repetitions was used. The offspring from the broodstock fed the nine diets containing the combination between the three levels of digestible protein (28, 34 and 40% DP) and three levels of digestible energy (2,800; 3,400; and 4,000 kcal of DE.kg of diet^{-1}) were regarded as treatments (Table 1). Each 70 L tank containing 70 tilapia post-larvae was considered an experimental unit.

The post-larvae from each experimental unit were fed commercial bran diets (Meurer et al., 2003b), containing 42% of crude protein, 4,200 kcal of gross energy.kg of diet^{-1} and 60 mg of 17 α-methyltestosterone.kg of diet^{-1} (Kubitza, 2000). The post-larvae were fed *ad libitum* four times a day (8h30, 10h30, 14h30 and 17h30) (Sanches & Hayashi, 2001).

Tanks were siphoned twice daily so as to remove feces and feed wastes. During this process, 20% of total water volume in the experimental units was changed (Meurer et al., 2002).

Table 1 - Percentage composition of ingredients and nutrients of experimental diets supplied to broodstock of Nile tilapia (*Oreochromis niloticus*)

Parameters	Digestible protein level (%)								
	28			34			40		
	Digestible energy level (kcal DE.kg of diet^{-1})								
	2800	3400	4000	2800	3400	4000	2800	3400	4000
Soybean meal	52.92	44.06	46.27	68.57	62.55	63.37	83.99	58.25	26.06
Cornmeal	30.31	32.86	19.52	13.90	15.94	3.00	0.00	5.00	1.00
Fish meal	5.00	12.76	13.13	6.22	11.44	13.02	0.29	30.20	60.81
Inert material	5.24	0.00	0.00	6.00	0.00	0.00	5.00	0.00	0.00
Wheat gluten	0.00	0.00	0.00	0.00	0.00	0.00	4.38	0.00	0.00
Premix[1]	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Dicalcium phosphate	1.92	0.00	0.00	1.30	0.00	0.00	2.84	0.00	0.00
Soybean oil	1.00	6.70	17.45	0.50	6.56	17.10	0.00	3.04	8.62
Common salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
DL-methionine	0.10	0.10	0.12	0.00	0.00	0.00	0.00	0.00	0.00
BHT[2]	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Nutrients (%)									
Linoleic acid	1. 50	4.57	10.14	1.03	4.30	9.75	0.67	2.18	4.90
Starch	26.02	26.42	18.41	17.91	18.37	10.42	11.94	10.98	4.14
Calcium	1.10	1.28	1.32	1.09	1.22	1. 36	1.0	2.86	5.46
Fat	3.34	10.22	20.60	2.83	9.67	20.04	1.36	8.97	19.04
Total phosphorus	1.0	1.0	1.0	1.0	1.0	1.0	1.06	1.92	3.30
Fiber	2.71	2.64	2.01	2.21	2.18	1.54	1.88	1.53	0.62

[1] Vitamin and mineral supplement, basic composition: folic acid - 200 mg; pantothenic acid - 4,000 mg; biotin - 40 mg; copper - 2,000 mg; iron - 12,500 mg; iodine - 200 mg; manganese - 7,500 mg; niacin - 5,000 mg; selenium - 70 mg; vitamin A - 1,000,000 IU; vitamin B1 - 1,900 mg; vitamin B12 - 3,500 mg; vitamin B2 - 2,000 mg; vitamin B6 - 2,400 mg; vitamin C - 50,000 mg; vitamin D3 - 500,000 IU; vitamin E - 20,000 IU; vitamin K3 - 500 mg; zinc - 25,000 mg.
[2] Antioxidant - Butylated Hydroxytoluene.

The water temperature was checked twice a day (7h00 and 14h00) prior to cleaning by using a thermometer with a precision of ±1 °C. The dissolved oxygen (digital oximeter YSI® 550A) and pH (digital pHmeter Tecnal® Tec 5) of water were measured weekly at 07h00, prior to cleaning.

At the end of the experimental period, all fish from experimental units were anesthetized and sacrificed by immersion in an aqueous solution with 250 mg of benzocaine.L^{-1} (CFMV, 2008). Then, the tilapias were weighed and the standard and total lengths were measured individually with a digital scale (MARTE® AS2000) with ±0.01 g precision and an ichthyometer with ±0.1 cm precision, respectively. Next, all fries were fixed in 10% formaldehyde (Bombardelli et al., 2007) and the effectiveness of sex reversal was analyzed by staining the gonads with acetocarmine and observation using an optical microscope, according to Pompa & Green (1990).

Based on the obtained data, the average final weight (AFW, mg), average weight gain (AWG, mg), final standard length (SL, cm), condition factor (CF) (Vazzoler, 1996), specific growth rate (SGR %/day) (Bomfim et al., 2005), stock uniformity (UNI, %) (Oliveira, 2010), survival (SUR, %) and sex reversal rate (SRR, %) were calculated.

The results were analyzed using multiple regression based on generalized linear models, using a significance level of 5%. These procedures were performed using the software Statistica 7.0®.

Results and Discussion

The mean values verified for temperature, level of dissolved oxygen and water pH in the experimental tanks were 27.95±1.90 °C; 6.83±1.18 mg.L^{-1} and 8.34±0.22, respectively. These values are within the limits considered satisfactory for proper development of Nile tilapias in early stages (El-Sayed, 2006).

The present results indicate that feeding tilapia broodstock diets containing different amounts of digestible protein and digestible energy had no influence (P>0.05) on either growth or survival of offspring during the first 30 days of development (Table 2). In agreement with the present study, Gunasekera et al. (1996) did not observe difference for average body weight, total length and standard length of Nile tilapia larvae from females fed diets with 20 and 35% CP.

In spite of the inconsistency of the nutrition of breeders on progeny performance, the values observed for average final weight (0.31 to 0.38 g), average weight gain (0.30 to 0.36 g), standard length (2.04 to 2.24 cm), condition factor (3.12 to 3.86), specific growth rate (4.91 to 5.19%/day), stock uniformity (81.7 to 98.5), survival (97 to 100%) and sex reversal rate (100%) in Nile tilapia post-larvae can be regarded as zootechnically adequate for the end of the sex reversal stage (Toyama et al., 2000; Meurer et al., 2003b; Bombardelli & Hayashi, 2005; Boscolo et al., 2005; Meurer et al., 2007; Oliveira, 2010) (Table 2).

Besides the reproductive performance, broodstock nutrition is also important for the quality of offspring. Some studies have reported the relevance of the balance between protein and energy in fish diets (Bomfim et al., 2005; Cotan et al., 2006; Piedras et al., 2006; Ali et al., 2008). If this ratio is unbalanced during fish growth, the proteins in the diet can be used as energy source (Kubitza, 2000) or directed to protein metabolism (Meurer et al., 2002). On the other hand, in the case of broodstock, this balance can be directly related to breeding performance (Lupatsch et al., 2005) or physiology processes such as vitellogenesis (Bromage, 1995).

A previous report about feeding Nile tilapia females diets with 10 to 40% of protein showed that protein levels could influence the gonadal maturation but not the chemical composition of eggs (Gunasekera et al., 1995). A

Table 2 - Performance parameters of Nile tilapia (*Oreochromis niloticus*) offspring derived from breeders fed diets containing different levels of digestible protein and digestible energy during sex reversal phase

Parameters	Digestible protein level (%)									P-value
	28			34			40			
	Digestible energy level (kcal DE.kg of diet^{-1})									
	2800	3400	4000	2800	3400	4000	2800	3400	4000	
Average final weight (g)	0.33	0.35	0.34	0.38	0.33	0.32	0.31	0.35	0.35	0.37
Average weight gain (g)	0.33	0.35	0.33	0.37	0.32	0.31	0.30	0.34	0.34	0.36
SL	2.13	2.17	2.09	2.20	2.21	2.09	2.08	2.15	2.16	0.36
Condition factor	3.48	3.46	3.74	3.57	3.13	3.54	3.47	3.52	3.50	0.65
SGR (%/day)	5.17	5.06	5.17	5.20	5.04	4.94	4.84	5.06	5.10	0.16
Stock uniformity (%)	90.0	94.8	85.2	86.6	95.7	89.5	93.9	88.8	85.2	0.70
Survival (%)	94.3	99.0	91.9	97.1	98.1	98.6	100	98.6	94.8	0.60
Sex reversal rate (%)	100	100	100	100	100	100	100	100	100	-

SL - average standard length; SGR - specific growth rate.

similar effect was also recently reported by Lupatsch et al. (2010), when different feeding managements in females of Nile tilapia were tested, with a diet with 23.5 g of digestible protein.MJ of digestible energy^{-1}.

According to Gunasekera et al. (1996), high levels of protein affect the viability of offspring up to the sixth day of development. Apart from protein, other nutritional components might also interfere with the quality of progeny. The increase in the levels of digestible energy in the diet of tilapia broodstock may improve the post-larvae vigor. Such influence of broodstock nutrition on offspring performance seems to be caused by physiological processes (Navas et al., 1998) related to both production and incorporation of vitellogenin in oocytes (Coward et al., 2002). The yolk is the main nutritional source of fries during their first days of development because it makes elements important for molecule formation available (Vassalo-Agius et al., 2001) or acts as an energetic component (Tyler & Sumpter, 1996).

Although the yolk sac ensures the endogenous nutritional storage during the initial life stages (Mazorra et al., 2003), little is known about the influence of maternal nutrition on growth of post-larvae when they no longer depend on their endogenous reserves. Ng & Wang (2011) verified that the utilization of crude palm oil in the diet of tilapia broodstock influenced the gonadal development, egg production, hatchability and larval normality. However, the offspring development up to total yolk consumption was unaffected by the oil source.

Studies on fish broodstock nutrition focusing on longer periods of offspring development, in either fries or juveniles, are even more restricted. Sink & Lochman (2008) fed breeders of *Ictalurus punctatus* diets containing 4 and 10% of lipids and raised the offspring up to 37 days of age, observing no effects on their growth.

The period of experimental feeding of the broodstock might also influence their reproductive and offspring performance. This period possibly had no effects on present results, since breeders were fed for 11 months and most research studies recommend experimental periods of, at least, 165 (El-Sayed et al., 2005) or 175 days (Ng & Wang, 2011) to observe dietary effects on tilapia breeding. Therefore, broodstock diet seems to interfere on offspring performance only during the periods of endogenous feeding of larvae, particularly related to yolk composition.

Another aspect to be remarked concerns the high fat content in diets formulated with the highest levels of digestible energy. Regardless of its effects on offspring, these diets might cause medium-term or long-term physiological damages to the broodstock since they promote liver fat deposition and steatosis (Tessaro et al., 2012b). Thus, furthers studies related to overall dietary effects on both fish breeding performance and reproductive life are recommended.

Evidence suggests that female nutrition might influence the reproductive performance or offspring quality in different manners. Although this management can affect the viability of offspring during early stages, the results of this study indicate that there is no influence of broodstock nutrition on growth or survival of progeny during periods when they no longer depend on endogenous reserves as feed or nutritional sources.

Conclusions

Feeding diets containing different levels of digestible protein and digestible energy to tilapia broodstock according to the experimental design adopted has no effects on growth or quality of offspring during 30 days of development.

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