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Short Communication

Performance and stress resistance of Nile tilapias fed different crude protein levels

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ABSTRACT - The objective of this study was to evaluate the effect of different levels of diet crude protein on the performance and stress resistance rate (Re) of *Oreochromis niloticus* larvae and fingerlings. In the first experiment, 5, 15 and 25 day-old animals were submitted to 1, 5, 7, 10, 15, 20, 30 and 40 minutes of air exposure on a sieve. In the second experiment, tilapia larvae were fed with 32, 40 and 55% crude protein (CP) diets. Animals after 15 and 30 days of feeding (21 and 36 days of life, respectively) were submitted to the air exposure test for 7 and 10 minutes. Re was estimated based on survival 24 hours after the tests. In the first experiment, it was observed that 5-day-old animals were more resistant than animals with 10 and 20 days of feeding (15 and 25 days of life, respectively), when Re starts to decrease for longer than 7 minutes. In the second experiment, the different diets affected survival, performance and Re, and, in general, the worst results observed were the ones for the animals which received the 55% CP diet. The air exposure tests were efficient to evaluate the effect of diet on the resistance rate of Nile tilapia.

Key Words: air exposure, feeding, larviculture, *Oreochromis niloticus*

Introduction

Larviculture is considered to be the limiting phase in the production of several fish species due to the lack of information regarding the best handling procedures to be adopted (Santos & Luz, 2009). Nile tilapia, contrary to several species which need live food, may be fed exclusively with formulated diet from the first feeding (Sanches & Hayashi, 1999; Toyama et al., 2000; Hayashi et al., 2002; Meurer et al., 2005; Boscolo et al., 2008), thus facilitating its larviculture.

Currently, diet with more than 50% of crude protein for thie larviculture of this species is available on the Brazilian market. However, it is known that food may represent more than 50% of the total production cost in semi-intensive systems, and that it may reach 80% in superintensive systems. Furthermore, inappropriate diets are not efficiently utilized by the animals, resulting in losses to both the economic value and water quality, besides decreasing the production potential significantly. Thus, studies examining the correct levels of nutritional requirements of fish are fundamental to the improvement of commercial production (Toyama et al., 2000).

During larviculture, performance variables such as growth and survival are the main indicators of feeding management quality. Resistance to stress tests may, along with these variables, be a support tool to evaluate the quality of larvae and fingerlings production (Dhert et al., 1992). Amongst the stress tests, air exposure is usually used for several species (Sakakura et al., 1998; Arends et al., 1999; Benfey & Biron, 2000; Martins et al., 2000; Koven et al., 2001; Van Anholt et al., 2004; Luz & Portella, 2005; Luz, 2007; Trushenski et al., 2010), and it is efficient for the evaluation of food quality on the stress resistance rate (Ako et al., 1994; Kanazawa, 1997; Luz, 2007). These tests may point to the effects of diet on the improvement of the health of the animals (Ako et al., 1994) and indicate the lot quality through the relationship between stress resistance, growth and survival results (Luz, 2007).

This study aimed at assessing the effect of different crude protein levels in the diets on the performance and stress resistance rate (Re) of *Oreochromis niloticus* larvae and fingerlings.

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Material and Methods

The research was done at the Aquaculture Laboratory of the Veterinary School of Universidade Federal de Minas Gerais, and was divided into two experiments.

In the first experiment, which was intended to determine the best air exposure time for tilapia, five-day-post-hatching larvae were stocked at the density of 30 larvae/L in two 30-L tanks, and kept in a recirculating aquaculture system. Acrylic wool was used for the mechanical filtration and, for biological filtration, plastic tapes were used as biofilm. The water flow in the tanks was 40 mL/min, at a temperature of 27.5±0.5 °C and the dissolved oxygen was kept above 4.5 mg/L.

The larvae were fed until satisfied, five times per day (8, 10, 12, 14 and 16 h) with commercial diet with 55% crude protein for 20 days of exogenous feeding. The tanks were siphoned daily to remove food residues and excreta.

Specimens aged five days after hatching (unfed) (8.4±0.3 mg and 8.7±0.3 mm long), 10 days of feeding (15 days of life) (31.9±5.5 mg and 12.3±0.8 mm long) and 20 days of feeding (25 days of life) (112.8±48.5 mg and 19.2±2.7 mm long) were submitted to different air exposure times, on a sieve, as follows: control (no air exposure); 1 minute; 5 minutes; 7 minutes; 10 minutes; 15 minutes; 20 minutes; 30 minutes and 40 minutes. Each treatment was comprised of three replicates.

In order to perform the tests, the animals were carefully siphoned into plastic bowls, counted and kept in containers with 2 L of cleaned water in a thermostatic bath system (27 °C). Each container received 10 specimens which were fasted for 12 hours. After fasting, the volume of each container, along with the animals, were poured onto a 0.5 mm diameter sieve, submerged in water to prevent injuries due to the direct contact with the sieve mesh. After that, the sieve was removed from the water, covered with a screen to prevent escape and put on drying paper to remove the excess of humidity. From this moment on, the different air exposure times were counted.

After the respective air exposure times, each sieve was submerged and the animals were again placed in the 2-L containers. After 24 hours, the stress resistance rate (Re in %) was determined, based on survival percentage. In order to calculate this rate, the live animals of all replicates of each treatment were counted. The comparison among treatments was done by a frequency dispersion study, using the Fisher exact test, at a 5% probability.

For the second experiment, tilapia larvae aged six days (10.7±2.7 mg and 9.5±0.7 mm long) were stocked in 18 circular 30-L tanks, on a 5 larvae/L density, and kept at the same temperature, water quality, dissolved oxygen and feeding described for the first experiment. They were fed commercial fish food with 32, 40 and 55% of crude protein (CP). The extruded diets with 32% and 40% crude protein were crumbled to present grain sizes similar to the 55% crude protein diet. The amount of food was the same for all tanks. Twice a day, the tanks were siphoned to remove the food residues and excreta.

After 15 and 30 days of feeding (21 and 36 days of life, respectively), 10 animals from each tank were collected and submitted to the air exposure test for 7 and 10 minutes; the times were based on the results of the first experiment. The test procedures and statistics were as described for the first experiment. After 30 days, survival, length, weight and final biomass for the different treatments were assessed.

The performance data were submitted to ANOVA and, subsequently, the means were compared using the Tukey test at 5% probability. In order to calculate the survival rate, the live animals of every replicate of each treatment were summed. The comparison between treatments was done by a frequency dispersion study, using the Chi-squared test, at 5% probability.

Results and Discussion

The different air exposure times tested in the first experiment affected the stress resistance of fish with significant interaction between the air exposure times and animal age ($P<0.05$; Table 1).

At five days of age, the stress resistance (Re) was 100% for air exposure periods of 0, 1, 5, 7, 10 and 15 minutes. With air exposure of 20 minutes, Re decreased to 90%, but with no significant difference ($P>0.05$) between the above mentioned treatments. From this treatment on, there is a progressive decrease with the increase of exposure time. On the other hand, animals with 10 and 20 days of feeding (15 and 25 days of life, respectively) presented 100% of Re, when submitted to air exposure for up to five minutes ($P>0.05$). However, from 7 minutes on there was a decrease in Re. Analysing Re for the different ages, it can be observed that larvae aged five days were more resistant than animals aged 15 and 25 days for air exposure times longer than 7 minutes. These results are probably due to the fact that at five days of life, when larvae still do not present fully formed gills, breathing occurs through body diffusion over the animal surface. Furthermore, even when placing the animals on drying paper, some humidity is observed on the top surface of the larvae. Another factor to be taken into consideration is that, at this age, larvae receive nutrients from their yolk, whereas older specimens are fed

Table 1 - Stress resistance of Nile tilapias (*Oreochromis niloticus)* at different ages, submitted to the air exposure test on a sieve[1]

Treatments	Stress resistance rate (Re %)			Means
	5 days of life	15 days of life	25 days of life	
R_0	100.0aA	100.0aA	100.0aA	100.0
R_1	100.0aA	100.0aA	100.0aA	100.0
R_5	100.0aA	100.0aA	100.0aA	100.0
R_7	100.0aA	53.3bB	62.5bB	71.9
R_{10}	100.0aA	16.7cB	20.8cB	45.8
R_{15}	100.0aA	6.7cB	0.0dB	35.6
R_{20}	90.0aA	0.0cB	0.0dB	30.0
R_{30}	46.7bA	0.0cB	0.0dB	15.6
R_{40}	43.3bA	0.0cB	0.0dB	14.4
Means	86.7	41.8	42.6	

R+ no. = minutes of air exposure.
[1]Survival values, followed by different letters (lower case in columns and capital letter in rows) significantly differ (P<0.05) by Fisher exact test.

exclusively from the food, which may not meet all the animals needs. Thus, testing larvae which did not receive exogenous feed may not be the right moment to test air exposure.

The survival and performance results of the second experiment were significantly affected (P<0.05) by the levels of crude protein in the diet (Table 2). Survival was higher for animals fed diet with the 32% CP, whereas the weight and length were larger for animals fed 40% CP diet. Biomass, however, was similar for the groups fed 32 and 40% CP diet. In general, animals fed 55% CP diets presented the worst performance, a fact that is confirmed by the air exposure tests (Table 3). These results are in accordance with the ones recorded by Hayashi et al. (2002) who observed a decrease in growth and survival for crude protein levels over 43% on this species larviculture.

Table 2 - Nile tilapia (*Oreochromis niloticus*) performance after 30 days of feeding (36 days of life) with different levels of crude protein (CP) feedstuffs

Parameters	Levels of crude protein		
	32% CP	40% CP	55% CP
Survival (%)[1]	68.6a	37.9b	40.7b
Final length (mm)[2]	28.2±0.7b	34.9±1.4a	26.4±0.7c
Final weight (g)[2]	0.4±0.05b	0.8±0.1a	0.3±0.02c
Final biomass (g)[2]	40.8±5.2a	42.7±7.7a	18.0±3.8b

[1] Values followed by different letters (lower case in rows) significantly differ (P<0.05) by Chi-squared test.
[2] Means ± standard deviations followed by different letters significantly differ (P<0.05) by Tukey test.

The data from this study show that the levels of crude protein which provide the best performance and Re (Table 3) are close to those recommended by literature for this species larviculture, between 35 and 45% CP (El-Sayed & Kawanna, 2004; Meurer et al., 2005; Boscolo et al., 2008), with the ideal level of 41.3% (Hayashi et al., 2002).

It is important to stress that after the end of the intensive larviculture, such as in this study, the fish are submitted to handling, including transportation and transfer to other tanks, either recirculating systems or ponds. According to Koven et al. (2001), feeding is important at this stage, since the correct diet management may raise fish survival rates after tank transfer, reducing the stress effects caused by this handling.

When animals were fed different diets for 15 days (21 days of life), a significant interaction (P<0.05) between protein levels and air exposure times was recorded (Table 3). It was observed that fish fed 55% CP diet showed smaller Re for both exposure times. Furthermore, the 10-minute time decreased the Re compared to the 7-minute time, regardless of the diet.

Table 3 - Stress resistance rate of Nile tilapia (*Oreochromis niloticus)* after 15 feeding days (21 days of life) and 30 feeding days (36 days of life) with different levels of crude protein[1]

Air exposure		Crude protein levels			Means
		32%	40%	55%	
Air exposure times after 15 days of feeding	7 minutes	100.0aA	92.85abA	82.75bA	91.95
	10 minutes	57.69aB	57.14aB	17.85bB	43.9
	Means	80.35	75.0	50.87	
Air exposure times after 30 days of feeding	7 minutes	96.7aA	92.6aA	60.7bA	83.5
	10 minutes	76.7aB	86.7aA	6.7bB	56.7
	Means	86.7	89.5	56.7	

[1] Stress resistance values followed by different capital letters in columns and letters lower case in rows significantly differ (P<0.05) by Fisher exact test.

After 30 days of feeding (36 days of life), a significant effect of the interaction between levels in the diet crude protein and air exposure time ($P<0.05$) was also recorded. The lowest Res were registered for the 55% CP diet for both air exposure times. It was also registered that, for the 32% CP diet and 55% CP, there is a decrease in Re with increasing air exposure time, confirming that the 40% CP diet may be the best for this stage. It is known that excess protein in the diet may be used as energy source (Cotan et al., 2006; Alencar Araripe et al., 2011), but it is not used for muscle formation. Another factor relating to protein is its balance in amino acids. Unbalanced diets may lead to an increase in ammonium excretion (Saavedra et al., 2009). These factors may have contributed to worsening the performance and Re.

As was also recorded in this study, the air exposure test was efficient in evaluating the diet effect on stress resistance of *Pargus major* (Kanazawa, 1997), where the use of 2% docosahexaenoic acid (DHA) in the diet was better than lower levels and the use of soy lecithin. Ako et al. (1994) used enriched and non-enriched *Artemia* sp. nauplii and a combination of this food with *Brachionus plicatilis* rotifers, with *Mugil cephalus* larvae. The authors observed greater stress resistance when the larvae were given enriched nauplii or a mixture of enriched nauplii and rotifers. Pintado (*Pseudoplatystoma corruscans*) larvae presented greater Re when fed *Artemia* nauplii and, later, another fish larvae when compared to larvae which were given only *Artemia* or dry diet (Luz, 2007). In pacu larvae (*Piaractus mesopotamicus*), the 20-minute air exposure test signalled that the use of *Artemia* nauplii or *Artemia* + dry diet provides greater Re when compared with the exclusive use of dry diet or animal kept fasting (Luz, 2007).

The 7 and 10-minute air exposure times used are superior to the times observed for some marine species, such as 15 seconds for *Mugil cephalus* (Ako et al., 1994), 30 seconds for *Pargus major* (Kanazawa, 1997), 60 seconds for *Rachycentron canadum* (Trushenski et al., 2010) and 90 seconds for *Sparus aurata* (Van Anholt et al., 2004); and inferior to the 20 minutes that two fresh water species, *P. mesopotamicus* and *P. corruscans*, can endure when exposed to air on a sieve (Luz, 2007). However, before being submitted to the air exposure test, these two fresh water species were fed live food during the larviculture phase, suggesting the importance of food during this initial phase. For *Hoplias lacerdae* larvae and fingerlings, when exposed to air on drying paper, Luz & Portella (2005) observed that times similar to those for tilapia could be used; they were between 7 and 10 minutes. However, according to these authors, air exposure on drying paper is a more stressful method, due to the fact that the animals dry up.

The results presented and discussed above show the need to determine the best air exposure time and the kind of test to be applied for each species. Other tests, such as osmotic stress (Kanazawa, 1997; Tago et al., 1999; Kolkovski et al., 2000; Lim et al., 2002; Koven et al., 2003), temperature changes (Tago et al, 1999), exposure to low levels of oxygen (Kanazawa, 1997) and air exposure on drying paper (Luz, 2007) were also efficient to assess the effects of the diet on stress resistance of fish, and they may be applied to tilapia larvae and fingerlings.

Conclusions

The amount of crude protein in the diet plays an important role on survival, performance and stress resistance rate of Nile tilapias larvae and fingerlings. The 7-minute air exposure on sieve time is an efficient test to infer the quality of the fish lot based on diet quality.

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