

Effect of the feeding ration on growth performance of *Oreochromis mossambicus* (Peters) larvae using decapsulated *Artemia* cysts as dietary supplement

Efecto de la ración alimenticia en el crecimiento de larvas de *Oreochromis mossambicus* (Peters) usando quistes de *Artemia* decapsulados como suplemento dietético

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ABSTRACT

Red tilapia larvae *Oreochromis mossambicus*, were cultured during the 30 days after the onset of the feeding, in order to evaluate different food rations (15, 20, 25, 30 and 35% body-weight/daily) when dried decapsulated *Artemia* cysts (DDC) were included in a commercial diet as a dietary supplement. Each feeding level was tested with three replicates, and given four times a day. Fish were reared in 10-L plastic containers (n = 40 fish per container) in a water recirculation system. Mean standard length, wet weight and survival were determined at the end of the experiment. Fish larvae growth increased with food ration. There were no differences in fry wet weight results for the 25, 30 and 35% body-weight/daily feeding levels (1.64, 2.05 and 1.94 g, respectively), although decrease in weight gain obtained for the 35% BW/d ration suggest that such food level was higher than the optimum. Survival was not affected for the different feeding rations tested.

Keywords: Tilapia larvae, growth, feeding ration, decapsulated *Artemia* cysts

RESUMEN

Se estudió el crecimiento de crías de tilapia roja, *Oreochromis mossambicus*, durante el primer mes de alimentación exógena, evaluando diferentes raciones alimenticias (15, 20, 25, 30 y 35% del peso corporal/día). Se incluyeron quistes secos y decapsulados de *Artemia* (DDC) en la dieta comercial como suplemento alimenticio. Cada ración alimenticia se suministró cuatro veces al día, y fueron examinadas por triplicado. Los peces fueron cultivados en contenedores de plástico de 10-L (n = 40 peces por contenedor), conectados a un sistema de recirculación de agua. Al final del experimento, se evaluaron la longitud estándar y peso húmedo promedio, y la sobrevivencia. El crecimiento de las crías se incrementó con el aumento de la ración alimenticia. No se observaron diferencias en el peso húmedo promedio de las crías para los tratamientos 25, 30 y 35% del peso corporal/d (1.64, 2.05 y 1.94 g, respectivamente), aunque el valor obtenido para la ración 35% del peso corporal/d sugiere que tal nivel alimenticio fue mayor que el óptimo. La sobrevivencia no fue afectada por los niveles alimenticios examinados.

Palabras clave: Crías de tilapia, crecimiento, ración alimenticia, quistes decapsulados de *Artemia*

INTRODUCTION

Food quantity and quality have been identified as critical factors for larval fish growth performance (Halver, 1972; Verreth, 1994). Regardless to the food quantity, there are several feeding strategies commonly applied in fish larval growth trails. It is recommended that fry be fed *ad libitum* or to apparent satiation (Gropp & Tacon, 1994), so that all fish have adequate access to feed. These feeding techniques are widely used for rearing tilapia fry during the first month of exogenous feeding, which represent the culture time when hormonal sex-reversal treatment is applied (Meyer & Smitherman, 1991; Watanabe *et al.*, 1993; Galvez *et al.*, 1996; Siddiqui *et al.*, 1997; Abucay & Mair, 1997). Other studies recommend that due to the rapid growth of tilapia at early stages, feeding ration should be adjusted to better utilization of food and feed waste reduction (Phelps & Popma, 2000), but in many cases, the reported amount of food vary considerably. For instance, MacIntosh and De Silva (1984) fed *O. mossambicus* (Peters) fry at different food rations (6, 12 and 24% of body weight daily, BW/d). De Silva (1985) and De Silva and Perera (1985) mentioned that a food ration of 6 % of BW/d was adequate to fed Nile tilapia fry. Santiago *et al.* (1987) fed up to 60 % of the BW/d to *O. niloticus* (Linnaeus) fry in aquaria, obtaining better growth and survival results fed the larvae with 60% BW/d, when compared with food rations of 30 and 45 % BW/d. Guerrero III *et al.* (1996) fed tilapia fry with a formulated diet at 10-30 % of their body weight daily. Siddiqui and Al-Harbi (1995) reported 30 % of total biomass/d when feeding tilapia larvae for the first week, thereafter, the feeding rate was reduced to 20, 15 and 10 % of biomass in the following weeks. Phelps and Popma (2000) mentioned that young tilapia fry can consume 20 % or more of their BW/d, suggesting the use of a feeding chart based on anticipated growth to adjust the amount of feed daily.

In relation to the food quality for culturing small tilapia, formulated diets with at least 40 % protein content are commonly used for rearing tilapia larvae and are considered to fulfill the tilapia fry nutritional requirements (National Research Council, 1993). However, there are constraints on the development of tilapia culture in some countries due to the lack of high quality seed for farmers (MacNiven & Little, 2001). Dietary inclusion of high quality supplements in commercial fry diets might improve its growth responses. Although there are some successful feeding experiments on the use of high quality food supplements as live or dried live food for rearing freshwater larvae of several commercial fish species (Verreth *et al.*, 1987; Pector *et al.*, 1994; Weirich *et al.*, 2000), only few reports are found for growing tilapia fry fed with high quality food items (Cruz & James 1989; Lu *et al.*, 2002). Recently, García-Ulloa and Hernández-Garciabada

(2003) reported that growth of *O. mossambicus* fry was improved with the utilization of dried decapsulated *Artemia* (Kellogg) cysts (DDC) as food supplement, suggesting that DDC could be included in the tilapia starter commercial diet, if feeding strategies and techniques, as well as economic considerations, are carefully reviewed. Preliminary results of our research indicated that feeding the tilapia larvae *ad libitum* with an inclusion of at least 25% of DDC in its commercial diet, produced tilapia juveniles with a mean wet weight of 1.8 g after the first 30 days of feeding, which was similar than the growth obtained for the group of fish fed solely with DDC. However, it is necessary to improve the possible use of DDC in the rearing of tilapia larvae by applying different feeding strategies.

Therefore, the purpose of this study was to find out the effects of different feeding rations (from 15 to 35 % of BW/d) on growth and survival of red tilapia (*O. mossambicus*) fry, fed with an inclusion of 25% of DDC as a dietary supplement in a commercial diet.

MATERIALS AND METHODS

O. mossambicus larvae were produced in indoor fiber glass tanks, by removing fertilized eggs (1-2 d after spawning) from the mouth of 20 incubating adult females of the brood stock held at the Laboratorio de Ciencias Marinas (Universidad Autonoma de Guadalajara, Jalisco, Mexico). Eggs were transferred to McDonald jars until hatching and total yolk absorption occurred. The experiment was conducted using 10 day-old tilapia larvae (initial mean standard length of 7.48 ± 0.38 mm and wet body weight of 8.5 ± 1.7 mg), when larvae were at the onset of the feeding, and lasted for the first 30 d after total yolk absorption. Fifteen plastic containers of 10-L each (filled at 8-L with freshwater) in a closed recirculation system (water flow rate = 0.25 L/min), were stocked with 40 larvae each (García-Ulloa & Hernández-Garciabada, 2003). Temperature and dissolved oxygen were daily measured and maintained at 27 ± 1.2 °C and higher than 4 mg/L (Chervinski, 1982), respectively, throughout the 30 experimental days. Five feeding rations were tested with three replicates: 15, 20, 25, 30 and 35% of the total BW/d. A tilapia starter diet, TS (API-ABA® 40 % protein, Malta Texo de Mexico, S.A. de C.V., Mexico, D.F.) was grounded and sieved to obtain food particles around 600 to 800 µm diameter. Dried *Artemia* cysts from the Salt Lake (GREAT LAKE ARTEMIA, Utah, USA) with a hatching efficiency of 85%, were used to prepare the experimental diet. Cysts were decapsulated with a solution of NaOCl, NaOH and water following standardized techniques (Vanhaecke & Sorgeloos, 1982; Sorgeloos *et al.*, 1983). In order to stop the embryo metabolic activity, decap-

Table 1. Proximate composition of the used diet (75% starter tilapia diet + 25% dried decapsulated *Artemia* cysts) 75TS/25DDC (n = 3)

Proximate composition	75TS/25DDC
Moisture	6.76 ± 0.31
Crude protein	54.05 ± 1.62
Ether extract	4.23 ± 0.51
Fiber	4.07 ± 0.32
Ash	7.05 ± 0.29
Nitrogen-free extract	30.6 ± 2.01

sulated cysts were dried in a laboratory oven (FELISA®, Guadalajara, Mexico) at 40°C (Sorgeloos *et al.*, 1986), until their water content decreased and kept below a level of 9%, which was confirmed by weighing the cysts every 12 hours for 3 days after decapsulation. Finally, TS and dried decapsulated cysts (DDC) were mixed at a proportion of 75%TS/25%DDC, as mentioned by García-Ulloa and Hernández-Garciabada (2003). The proximate composition of the tested diet is given in Table 1.

Fish larvae were fed four times a day between 8:00 and 19:00 hours. Before each feeding, dead animals, feed remnants and faeces were extracted out from the containers by siphoning. The initial total biomass in each container (340 mg) was used to adjust the food ration at experimental day 1. The daily average wet weights of *O. mossambicus* fry for the first 30 days of feeding shown in Table 2, were obtained by conducting a previous study in which larvae were fed at satiation for these first 30 days of feeding with the experimental diet (75%TS/25%DDC), and sampled each day (n = 60) to obtain the daily mean weight values. Mean weight values were used to calculate the daily food ration.

Total larvae from each replicate per treatment, were sampled at the end of the experiment to obtain the mean wet weight and standard length. Prior to weighing, fish were placed on absorbent paper to remove excess of water. The daily weight gain (g/d) was calculated from DWG = (final body weight – initial body weight)/days of culture (Mbahinzireki *et al.*, 2001). The specific growth rate (SGR, % body weight/d) was obtained from $SGR = 100 \times (\ln W_f - \ln W_i) / t$, where W_f = mean weight at the end of the period, W_i = mean weight at the beginning of the period, and t = time in days of the period (Ricker, 1979). Survival was calculated by counting total animals per replicate for each diet, after the 30 experimental days. Food conversion ratio (FCR) was obtained from $FCR = g \text{ feed consumed} / g \text{ wet weight gained}$ (Al Hafedh *et al.*, 1999). All data were tested for normal distribution and homogeneity of variance before ANOVA was performed (Sokal & Rohlf, 1969). A simple ANOVA analysis was performed and differ-

ences between means were compared for significance ($\alpha = 0.05$) using the Tukey's multiple range test (Reyes, 1982). The statistical analyses were performed using Jandel SigmaStat 2.0 statistical software (Jandel Co., USA).

RESULTS

The better growth performance of *O. mossambicus* fry was observed for the fish fed with the 30% BW/d feeding ration (Figure 1). Significantly higher wet weight ($P < 0.05$) was found for fish fed 30, 35 and 25% BW/d feeding levels than for fish fed lower feeding levels (20 and 15% BW/d). Same trend was observed for the standard length. Significant differences ($P < 0.05$) were found for the DWG, SGR and FCR among the treatments (Table 3). DWG ranged from 0.0683 g/d for the 30% BW/d treatment, to 0.0428 g/d obtained for the

Table 2. Daily mean individual wet weight (MIWW) of red tilapia *O. mossambicus*, fry fed *ad libitum* with the experimental diet 75% TS + 25% DDC. (N= 60 org/d). Results obtained from previous experiment (García-Ulloa, 2002).

Culture day	MIWW (g)
Initial day	0.0084 ± 0.0013
1	0.0085 ± 0.0017
2	0.0087 ± 0.0020
3	0.0221 ± 0.0060
4	0.0305 ± 0.0078
5	0.0397 ± 0.0060
6	0.0465 ± 0.0092
7	0.0561 ± 0.0142
8	0.0668 ± 0.0140
9	0.1060 ± 0.0291
10	0.1179 ± 0.0270
11	0.1254 ± 0.0274
12	0.1457 ± 0.0371
13	0.1724 ± 0.0520
14	0.2028 ± 0.0586
15	0.2225 ± 0.0592
16	0.2720 ± 0.0820
17	0.3164 ± 0.1023
18	0.3612 ± 0.1045
19	0.4295 ± 0.1267
20	0.4902 ± 0.1558
21	0.6085 ± 0.2693
22	0.7302 ± 0.2935
23	0.7733 ± 0.1976
24	0.8191 ± 0.2214
25	0.9049 ± 0.2641
26	1.0374 ± 0.1663
27	1.0676 ± 0.3968
28	1.2178 ± 0.3242
29	1.2900 ± 0.3428
30	1.3700 ± 0.3648

Table 3. Mean final DGW (g/d), SGR (% wet gain/d), survival (%) and FCR of *O. mossambicus* fry fed with 75% TS + 25% DDC at different feeding rations (% BW/d). Culture time = 30 days after the onset of the exogenous feeding. In each line, values with different letter are significantly different ($\alpha = 0.05$). Values between parenthesis = s. d.

	Feeding rations				
	15	20	25	30	35
DWG	0.0428 ^a (0.0033)	0.0436 ^a (0.0037)	0.0543 ^{ab} (0.0068)	0.0683 ^b (0.0070)	0.0644 ^b (0.0057)
SGR	16.46 ^a (0.31)	16.77 ^{ab} (0.48)	17.37 ^{abc} (0.43)	18.20 ^c (0.36)	17.96 ^{bc} (0.25)
Survival	85.83 (5.13)	95.83 (4.24)	90.83 (5.13)	84.16 (4.71)	86.66 (9.20)
FCR	1.61 ^a (0.14)	2.02 ^{ab} (0.37)	2.15 ^{ab} (0.34)	2.08 ^{ab} (0.24)	2.50 ^b (0.28)

15% BW/d feeding ration. The higher SGR final value was observed for the 30% BW/d feeding level (18.20%/d). For the FCR, the lowest value (1.61) was shown for the *O. mossambicus* fry fed with the lowest experimental feeding ration (15% BW/d). The rest of the tested treatments obtained FCR values above 2.00. Survival among treatments did not show differences ($P > 0.05$) after the 30 experimental days, and fluctuated from $84.16 \pm 4.71\%$ for the fry fed with the 30% BW/d feeding ration, to $95.83 \pm 4.24\%$ for the tilapia larvae fed with the 20% BW/d feeding level.

DISCUSSION

Feed supply influences the growth of fish (Jobling, 1994), but it turns more critical for the early larval stages since its metabolic requirements vary rapidly with time (Bromage, 1995). One of the most important factors for assessing larval fish growth performance is the study about the relationship between growth and feeding ration. Determining the optimum feed ration should contribute to improving fish larvae production since food is better utilized (Mihelakakis *et al.*, 2001). There are some studies about the effect of feeding ration on the larval growth of various tilapia species. However, growth output results vary considerably. For instance, MacIntosh and De Silva (1984) observed that after the first 30 culture days, growth of *O. mossambicus* larvae increased with higher food rations (6, 12 and 24% BW/d). They obtained mean weight values that fluctuated from 0.06 to 0.38 g at different stocking densities. Early studies carried out by De Silva and Perera (1984, 1985) and De Silva (1985) con-

sidered that 6% BW/d food ration to be more than adequate for rearing of different commercial tilapia species, but growth results varied considerably. In other experiment, Ron *et al.* (1995) fed *O. mossambicus* fry at an initial rate of 18% BW/d from day 1 to day 21. Then, ration decreased every 21 days until 4% BW/d. After the first month of feeding, mean weight value was below 0.5 g. Siddiqui and Al-Harbi (1995) worked with *O. mossambicus* larvae (18.5 mg initial weight) fed them at 30% of total biomass/d in the first culture week, thereafter, it was gradually reduced from 20 to 10% BW/d in the following 5 weeks. In that case, the final individual weight was 0.958 g after 42 culture days. In the Philippines, tilapia fry are commercially reared in net enclosures, concrete tanks and earthen ponds, and fed them with feeding rations varying from 5 to 30 BW/d (Guerrero III *et al.*, 1996), consequently, production output results differ among the different applied culture techniques. Working with blue tilapia (*O. aureus*, Steindachner) fry, Galvez *et al.* (1996) obtained a final average weight of 0.65 g after the first 28 days of feeding with a commercial diet, adjusting the feed amount at 15% BW/d.

In the present experiment, food rations tested did influence growth performance of red tilapia larvae after the first month of feeding, using DDC as dietary supplement. Figure 1 indicates that bigger animals were obtained at the 30% BW/d feeding ration (2.05 g mean individual wet weight), showing no differences ($p > 0.05$) compared with the 25 and 35% BW/d feeding levels. Nevertheless, final weight for the 35% BW/d was lower than the value observed for the 30% BW/d ration. It is well documented that fish growth increases with food ration above the maintenance until it reaches a maximum value at the optimum ration. Then, in rations higher than the optimum, the growth rate declines (Halver, 1972; Hogendoorn, 1983; García-Ulloa, 2000), which could partially explain the reduced mean weight value at the 35% BW/d ration. Generally, plateaus or decreases in weight gain of fish fed diets containing energy levels above the requirement have been observed in some species (Siddiqui *et al.*, 1988; El-Sayed & Teshima, 1992; Lee *et al.*, 2000), coinciding with our results at the higher food ration tested. On the other hand, the highest FCR was observed for the 35% BW/d feeding level (2.50) suggesting that such ration was above the larvae requirement and consequently, food was not well utilized for larval growth (Phelps & Popma, 2000). There were no differences ($p > 0.05$) in survival among the tested rations. Final survival ranged from 84% for the 30% BW/d ration, to 95% observed for the 20% BW/d feeding level, and were similar to those reported by Galvez *et al.* (1996), Al-Hafedh *et al.* (1999) and Olvera-Novoa *et al.* (2002) working with larvae of different tilapia species. However, our survival results were higher compared with the value reported by Siddiqui and Al-Harbi (1995) for *O. mossambicus* larvae. Mean DWG and SGR values

obtained in our experiment (0.054 g/d and 17.34% weight gain/d, respectively) for the experimental rations, were higher than those reported for the above mentioned works. Such differences in the biological parameters evaluated when compared with other studies could be attributed to several factors as tilapia species, type of food and different food ration used, among others.

Commonly, mean commercial weight of tilapia fry after the first 30 days of exogenous feeding, including an hormone treatment in the diet, vary from 0.2 to 1.0 g (Arredondo-Figueroa *et al.*, 1994; Hulata, 1997; Popma & Rodríguez, 2000; Green & Engle, 2000; MacNiven & Little, 2001). In this study, the mean wet weight of *O. mossambicus* fry at the end of the experiment, fluctuated from 1.29 for the 15% BW/d feeding level, to 2.05 g for the 30% BW/d food ration. Mihelakakis *et al.* (2001) pointed out that quality of the feeding ration is one of the most important factors determining the fate of the food consumed and subsequent growth. Under laboratory conditions, DDC have been successfully used in some commercial aquatic species as food supplement (Verreth *et al.*, 1987; Vanhaecke *et al.*, 1990; Pector *et al.*, 1994; Stael *et al.*, 1995; Weirich *et al.*, 2000), since DDC are able to combine the advantages of live and dry diets (Sorgeloos *et al.*, 1977; Vanhaecke *et al.*, 1983). García-Ortega *et al.* (1998) indicated that the nutritional value and exogenous enzyme contribution of DDC would be responsible dietary factors for successful rearing of different fish larvae. Previous reports showed that our experimental diet (75% commercial diet and 25% DDC) contains 54% crude protein (García-Ulloa & Hernández-Garcibada, 2003), which represents a high protein content. As mention by Church and Pond (1982), the associative or synergistic effects among the dietary elements contained in

the ingredients used to elaborate our experimental diet, could explain the final high mean growth values obtained for all tested food rations. There are few reports rearing tilapia fry with high quality dietary items (Cruz & James, 1989; Lu *et al.*, 2002; Olvera-Novoa *et al.*, 2002), but again, due to differences in diets, culture conditions, culture techniques, species and fish age at the beginning of the studies, our results might not be properly compared with the above mentioned works.

Considering the feeding costs related to the possible use of DDC as dietary supplement in rearing of red tilapia larvae, an estimation in the adjustment of the daily food ration to the quantitative requirement of the larvae is of great importance, and represents a very useful tool to reduce production expenditures. Results from the present study suggest that the optimal feeding ration for the growth of *O. mossambicus* larvae during the first month of exogenous feeding, and including 25% of DDC in its commercial diet, is between 25 and 30% BW/d. Still, much attention should be paid on feeding techniques and strategies, as well as economic considerations, in order to optimize its possible use at commercial level in tilapia hatcheries.

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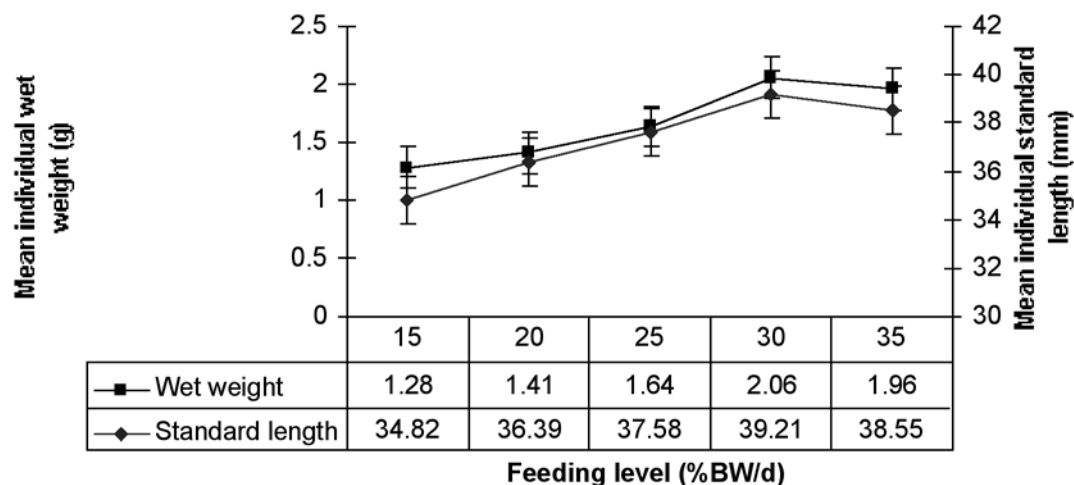


Figure 1. Mean final wet weight (g) and standard length (mm) of *O. mossambicus* fry fed with 75% TS + 25% DDC at different feeding levels.

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