



Development of a Feeding Program for Early Larval Stage of Goldfish (*Carassius auratus*)

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Abstract

The aim of this study was to develop a feeding program for early larval stage of goldfish (*Carassius auratus*) with an initial weight of 0.97-1.13 mg and total length of 5.77 - 6.05 mm. The study was conducted with totally 2340 larvae during 15 days, and included 13 groups with three replications. Four different feeding times (T₁₋₄), three different dry (D₁₋₃ “one commercial feed and two test”) diets plus continuous live food were the treatments. Larval growth were checked on 8th day and at the end of the experiment. Dry diets were fed to larvae continuously (T₁) or after 3 (T₂), 5 (T₃) and 7 (T₄) days of rotifer feeding. Also, in the control group only rotifer (R) was fed for 15 days. Results were analyzed with the factorial analysis design (feeding times × diets) and compared using the Tukey multiple comparison test. A comparison of the control group R with subgroups were also made using the Dunnett’s test. The present results indicate that various combinations of “live and dry foods” administered at different durations significantly effected growth and survival rates of goldfish larvae (P<0.05). Briefly, continuous feeding with all dry diets were not as successful as the others. As the duration of rotifer feeding as first food increased, larval growth and survival also increased even if still worse than the control (15 days rotifer). The best practice for early larval feeding of goldfish could be T₄D₁₋₂₋₃ (7 days rotifer, 3 days rotifer + dry diet and 5 days dry diet) during the first 15 after hatching.

Keywords: Goldfish, early larval feeding, rotifer, dry diet.

Japan Balıklarının (*Carassius auratus*) Erken Larva Dönemi Besleme Programının Geliştirilmesi

Özet

Bu çalışmada, Japon balıklarının (*Carassius auratus*) erken larva dönemi besleme programının geliştirilmesi için canlı ağırlıkları 0,97-1,13 mg ve total boyu 5,77-6,05 mm olan 2340 adet besin kesesi çekilmiş larva kullanılmıştır. Larvalar farklı yemleme zamanı (T₁₋₄) ve farklı yemlerle (Y₁₋₃; biri ticari karma yem, diğer ikisi deneme yemi) on beş gün süresince beslenmiştir. Kontrol gurubu (KY) da dahil olmak üzere 13 farklı muamele 3 tekerrürlü olarak denenmiştir. Denemede 8. ve 15. günlerde periyodik ölçümler yapılmıştır. Veriler faktöriyel (T₁₋₄ X Y₁₋₃) düzende analiz edilerek grup ortalamaları Tukey yöntemiyle, kontrol ve alt gruplar ise Dunnett testiyle karşılaştırılmıştır. Elde edilen yaşama ve büyüme oranları, farklı yemleme zamanı ile canlı ve kuru yem kombinasyonlarından önemli derecede etkilenmiştir. Kısaca, sürekli olarak kuru yemlerle besleme, rotifer ve kuru yem kombinasyonları kadar başarılı olmamıştır. Beslenmeye rotiferle başlanan gruplarda canlı yemle besleme süresi arttıkça, büyüme ve yaşama oranları da artmıştır. Buna göre 15 günlük toplam besleme sürecinde ilk 7 gün canlı yem (rotifer) sonra 3 gün süreyle canlı yem+kuru yem ve daha sonra 5 gün süreyle de tamamen kuru yemle beslenen grupların (T₄Y₁₋₂₋₃) daha başarılı olduğu görülmüştür. Böyle bir program Japon balığı erken larva dönemi besleme programı olarak önerilebilir.

Anahtar Kelimeler: Japon balığı, erken larva dönem, canlı yem, besleme programı.

Introduction

Aquariums can be both a living environment for maintaining beautiful fish and a place that reduces the impact of daily stress due to their visual beauty (Hekimoğlu, 2006; Savaş *et al.*, 2006). While there

were only 30 countries involved in this sector in 1976, the number increased to 146 in 2004 (Ploeg, 2007). While the number of exotic and cultured aquarium fish species was 410 and 40 in 1955 respectively, today thousands of species are available (Fossa, 2003). Turkey, like many other countries, has recently witnessed a drastic development of ornamental fish

culture (Alpbaz, 1993; Hekimoğlu, 1997; Tlustý, 2002; FAO, 2011).

With their ability to adapt to Mediterranean climate conditions, gold fish are the most widely cultured species both in the world and Turkey (Türkmen and Çelik, 2014; Gümüş *et al.*, 2014). However, goldfish larval survival under culture conditions is low due to inappropriate feeding practices (Watson *et al.*, 2004; Korkmaz, 2008). Although artemia (*Artemia nauplii*), daphnia (*Daphnia magna*) and tubifex (*Tubifex tubifex*) worms are used during goldfish larval rearing in Turkey (Gümüş *et al.*, 2014), it has been reported that freshwater rotifer (*Brachionus calyciflorus*) is the most suitable live food for goldfish larvae because of their size, slow movement, capability of remaining suspended in the water and high reproduction capacity (Lim and Wong, 1997; Harzevili *et al.*, 2003; Arimoro, 2006).

Present study was aimed to determine the optimal weaning time, live and dry feed requirements of early larvae of goldfish.

Materials and Methods

The experiment was conducted in aquarium section of the Kepez Unit of Mediterranean Fisheries Research Production and Training Institute, Antalya, Turkey in 2014. Post yolk sac larvae used in the study was produced from Oranda variety of goldfish (4 female and 6 male).

A total of 2340 larvae early larval stage of goldfish (initial weight of 0.97-1.13 mg and total length of 5.77 - 6.05 mm.) were used. Thirteen experimental treatments including the control were triplicated and tried in 39 10 L glass tanks connected in a recirculation system. Each tank was stocked with 60 larvae (6 larvae/L). Each aquarium was given 100 mL/min water flow and provided with aeration using air stones. Water renewal rate and frequency of the recirculation system was 30% and twice a week. Water temperature was kept at an optimum of 27.0°C according to Kestemont (1995) using a thermostat, and changed between 26.0 and 27.4°C over the experiment. Photoperiod was set on 12 hours darkness and 12 hours lightness with a timer.

Feeding schedules, diet compositions and formulation are shown in Table 1-3. In the experiment, 4 feeding times "T₁₋₄" and 3 different diets "D₁₋₃" (4×3=12) were used. Feeds were offered at 07:30, 10:00, 12:30, 15:30 and 17:30. Rotifers were given at a rate of at least 10 ind./mL whereas dry diets were given *ad libitum*.

Experimental dry diets (D2 and D3) were formulated according to Castell and Tiews (1980). Freshwater rotifer used in the present study was produced in a 12 ton pond (3×4×1) within a greenhouse.

Length specific growth rate (SGR_L, % / day) = $[(\ln L_t - \ln L_{t0}) / \text{day}] \times 100$

Weight specific growth rate (SGR_w, % / day) = $[(\ln W_t - \ln W_{t0}) / \text{day}] \times 100$

Survival rate = $(N_t / N_{t0}) \times 100$

Condition factor (CF) = $(W / L^3) \times 100$

Where L_t is larvae length at day t, L_{t0} is length at day 0, W_t is the weight at day t, W_{t0} weight of larvae at day 0, N_t and N_{t0} are the number of larvae at day t and 0.

Variation coefficient (VC) of individual larval lengths at 8th day and at the end of the experiment was also calculated using the formula below to determine the effects of the feeding treatments on size homogeneity of goldfish larvae.

VC (%) = $(\text{Standard deviation} / \text{arithmetic mean}) \times 100$

Twenty larvae were used in periodical measurements. They were weighed using a balance with a precision of 0.0001 g. Lengths of larvae was determined with a software (ImageJ 1.44p, National Institutes of Health, Bethesda, MD) after photographing. Temperature and dissolved oxygen of water were measured with a portable device (YSI 55 12 FT, Yellow Springs Instrument CO., Inc., Yellow Spring, Ohio) whereas pH, salinity and conductivity were recorded using a pH meter (YSI 63-10 FT, Yellow Springs Instrument CO., Inc., Yellow Spring, Ohio). The parameters were checked three times a week.

Statistical analysis of data was performed with JMP v8 software. Effects of treatments on variables were analyzed by a factorial design (4 feeding times and 3 different diets), and the treatments were discriminated with the Tukey multiple comparison test. On the other hand, comparison of the control with other diets was made with the Dunnett's test (Özdamar, 2001; Yıldız *et al.*; 2011).

Results

There were no significant differences among the treatments in terms of initial total length and body weights (P>0.05), (Table 4).

Although length and SGR_L on day 8 were comparable among the diets (P>0.05), these variables were significantly altered by various feeding times (P<0.01). In feeding regimen of T₁, length and SGR_L of larvae on day 8 fed dry diets were significantly lower than the control (P<0.05). At the end of the experiment, there was no significant interaction between feeding times and diet types in terms of larvae length, but feeding times (P<0.01) and diets (P<0.05) significantly affected the size of fish (Table 4). Accordingly, SGR_L values of the treatments displayed a similar pattern, with significant effects of feeding times (P<0.01), i.e. T₂ and T₃ were similar to each other, but significantly higher than T₁ and significantly lower than T₄. All the experimental groups, except T₄D₁, significantly differed from the control in terms of SGR_L.

Final weights of larvae were significantly

Table 1. Larval feeding program used in the experiment*

Experimental groups		Feeding times "T" (days)		Diets 'D'
1	T ₁ D ₁			D ₁
2	T ₁ D ₂	T ₁	15	D ₂
3	T ₁ D ₃			D ₃
4	T ₂ D ₁			3R+(3R+ D ₁)+9 D ₁
5	T ₂ D ₂	T ₂	3+3+9	3R+(3R+D ₂)+9 D ₂
6	T ₂ D ₃			3R+(3R+D ₃)+9 D ₃
7	T ₃ D ₁			5R+(3R+ D ₁)+7 D ₁
8	T ₃ D ₂	T ₃	5 +3 +7	5R+(3R+ D ₂)+7 D ₂
9	T ₃ D ₃			5R+(3R+D ₃)+7 D ₃
10	T ₄ D ₁			7R+(3R+ D ₁)+5 D ₁
11	T ₄ D ₂	T ₄	7 + 3 +5	7R+(3R+ D ₂)+5 D ₂
12	T ₄ D ₃			7R+(3R+ D ₃)+5 D ₃
13	C		15	15R

*Different feeding times (T_{1,4}), dry diets (D_{1,3}), a commercial rainbow trout diet (D₁), casein based dry diet (D₂), egg protein based dry diet (D₃), live food "rotifer" (R) as the control group (C). The figures before the diets in the latest column refer to the number of days that a particular diet was offered.

Table 2. Proximate compositions of experimental diets (%)

Experiment diets	Moisture	Dry matter	Crude ash	Crude oil	Crude protein
Commercial diet (D ₁)	6,07	93,93	11,91	11,28	60,84
Casein based diet (D ₂)	13,18	86,82	3,86	10,81	59,85
Egg protein based diet (D ₃)	10,65	89,35	4,26	9,39	59,55
Live food (R)	92,21	7,79	10,11	10,03	65,83

Table 3. Formulation of experimental diets (D₂ and D₃) used in the study

Ingredients	D ₂ (%)	D ₃ (%)
Casein	45	33
Gelatin	6,7	7
Salmon oil	10,5	10,2
Dextrin	16,5	16,5
Egg protein	9,8	21,8
Vitamin C	0,5	0,5
Vitamin premix ¹	2	2
Mineral premix ²	1	1
Methionine	1,8	1,8
Lisin	1,7	1,7
Carboxymethyl cellulose	3	3
Carofil-red	1	1
Choli chloride (%96)	0,5	0,5
Nutrient compositions (%)		
Crude protein	56,69	56,19
Crude oil	10	10

¹Vitamin premix: 12.000.000 IU A, 2.500.000 IU D₃, 200.000 mg E, 10.000 mg K₃, 20.000 mg B₁, 24.000 mg B₂, 100.000 mg B₅ (Ca D-pantothenate), 20.000 mg B₆, 200 mg B₁₂, 150.000 mg B₃ (niacin), 10.000 mg B₉ (folic acid), 1.000 mg B₇ (biotin), 200.000 mg C, 200.000 mg inositol, 1.000.000 mg choline.

²Mineral premix: 5.000 mg Cu, 20.000 mg Mn, 2.000 mg Co, 2.500 mg I, 30.000 mg Zn, 250 mg Se.

affected by feeding times and diet types and their interactions were a significant factor ($P < 0.01$). Larvae fed rotifer were significantly heavier than those fed dry diets. However, dry diets did not significantly affect SGR_W at the end of the study, and there was no a significant interaction term with feeding times. Weaning larvae at different times significantly changed SGR_W values, with lower values in T₁ compared with the others. Feeding with only rotifer

over the study period resulted in significantly higher SGR_W values than dry diets ($P < 0.01$).

Feeding times and their interactions with diet types were significant factors for survival rates of larvae were significantly affected by at end of the experiment ($P < 0.01$). Control larvae showed significantly higher survival than those on T₁D₂ and T₁D₃ ($P < 0.05$), but they were comparable to others ($P > 0.05$).

Table 4. Weight and length growth of gold fish larvae (with an initial weight of 0.97-1.13 mg and total length of 5.77 - 6.05 mm) maintained on various feeding schedules and diets

Variable	Feeding time	Diets			
		D ₁	D ₂	D ₃	R
Length on day 8 (mm)	T ₁	^b 7,74±0,13*	^b 7,65±0,26*	^b 7,65±0,25*	9.53±0.14*
	T ₂	^a 9,26±0,28	^a 9,32±0,42	^a 9,37±0,27	
	T ₃	^a 9,91±0,01	^a 9,67±0,27	^a 9,95±0,33	
	T ₄	^a 10,13±0,22	^a 9,80±0,04	^a 9,59±0,21	
Final length (mm)	T ₁	^d 9,38±0,34*	^{dh} 9,67±0,19*	^h 9,70±0,22*	14.68±0.92*
	T ₂	^c 11,55±0,18*	^{cg} 11,47±0,16*	^g 11,08±0,22*	
	T ₃	^b 12,63±0,32*	^{bf} 11,96±0,24*	^f 11,59±0,29*	
	T ₄	^a 13,30±0,34 ^A	^{ae} 12,88±0,04 ^{AB*}	^e 12,42±0,04 ^{AB*}	
SGR _L on day 8	T ₁	^b 3,07±0,42*	^b 3,13±0,55*	^b 3,45±0,48*	6.15±0.48*
	T ₂	^a 5,41±0,53	^a 5,84±0,51	^a 5,89±0,50	
	T ₃	^a 6,28±0,17	^a 5,90±0,30	^a 6,57±0,51	
	T ₄	^a 6,99±0,33	^a 6,29±0,35	^a 6,37±0,15	
SGR _L at the final	T ₁	^c 2,91±0,14*	^c 3,24±0,05*	^c 3,43±0,13*	6.13±0.27*
	T ₂	^b 4,36±0,19*	^b 4,51±0,18*	^b 4,26±0,21*	
	T ₃	^b 4,96±0,17*	^b 4,57±0,09*	^b 4,53±0,22*	
	T ₄	^a 5,54±0,19	^a 5,18±0,20*	^a 5,18±0,20*	
Final weight (mg)	T ₁	^a 9,29±0,78*	^{ae} 9,74±0,42*	^l 10,05±0,39*	63.43±0.59*
	T ₂	^b 20,03±0,88*	^{bf} 19,08±0,50*	^k 18,58±0,87*	
	T ₃	^c 27,04±0,78*	^{cg} 25,03±0,66*	^k 25,08±0,34*	
	T ₄	^d 37,35±0,89*	^{dh} 32,47±0,97*	^j 29,47±1,73*	
SGR _W at the final	T ₁	^c 14,73±1,46*	^c 15,22±0,92*	^c 15,03±0,53*	27,81*
	T ₂	^b 19,88±1,23*	^b 19,54±0,65*	^b 19,61±0,81*	
	T ₃	^{ab} 21,46±0,73*	^{ab} 21,79±0,82*	^{ab} 21,36±0,76*	
	T ₄	^a 23,38±0,77*	^a 23,03±0,68*	^a 22,45±0,87*	
Survival	T ₁	^b 75,56±8,62	^d 37,20±0,40*	^{df} 30,60±10,30*	93,89*
	T ₂	^a 90,00±3,47	^c 97,22±1,47	^{ce} 95,56±2,94	
	T ₃	^a 95,56±2,94	^c 96,11±1,47	^{ce} 97,78±1,47	
	T ₄	^a 95,56±1,11	^c 97,22±2,00	^{ce} 98,89±1,11	
VC on day 8	T ₁	^a 9,96±2,13	^a 8,37±0,25	^a 10,62±1,69*	7,22±0,24*
	T ₂	^{ab} 9,47±1,47	^{ab} 8,48±0,62	^{ab} 7,84±1,32	
	T ₃	^b 6,63±0,42	^b 7,98±0,06	^b 6,90±0,79	
	T ₄	^a 7,53±0,59	^a 7,10±1,48	^a 7,38±0,28	
VC at the final	T ₁	^a 10,95±1,82*	^a 10,48±0,65*	^a 11,04±0,81*	7,52±1,12*
	T ₂	^b 8,23±0,54	^b 7,32±1,71	^b 7,50±0,69	
	T ₃	^b 7,26±0,14	^b 7,20±0,27	^b 8,51±0,30	
	T ₄	^b 8,86±1,05	^b 7,88±0,58	^b 7,49±1,13	
CF	T ₁	^a 1,13±0,21*	^a 1,07±0,06*	^a 1,11±0,17*	2,10±0,41*
	T ₂	^b 1,31±0,20*	^b 1,26±0,13*	^b 1,36±0,06*	
	T ₃	^{bc} 1,35±0,14*	^{bc} 1,47±0,09*	^{bc} 1,61±0,17*	
	T ₄	^c 1,59±0,16*	^c 1,51±0,08*	^c 1,54±0,15*	

Values sharing common superscripts in the same rows or column are not significantly different (based on Tukey multiple comparison test) ($P < 0,01$; $P < 0,05$), * indicates that these values are significantly different from the control (Dunnett's test) ($P < 0,05$)

There was no an interaction term of feeding times and diet types in terms of VC on day 8 and at end of the experiment. However, final VC values were altered by feeding times ($P < 0,05$), with significantly higher values in T₁ than the others. Average VCs of the control were similar to other treatments, except T₁D₃ on day 8, T₁D₁ and T₁D₃ at the final.

Larval CFs were not affected by diet types and their interactions with feeding times, whereas the later significantly changed, with lowest and highest values in T₁ and T₄ respectively ($P < 0,05$). Rotifer-fed larvae were significantly higher in terms of CF compared with the others ($P < 0,05$).

Discussion

In many previous studies during larval rearing of goldfish, *Artemia*, *Daphnia* spp. and compounded feeds (micro-particle diets etc.) were used as first feed but little attention has been paid to the use of freshwater rotifers (Janakiraman and Altaff, 2015). Single use of dry diets was reported to lower the success of larval rearing when compared to the live foods but their combined use with live foods increased the survival and growth rates (Sales and Janssens, 2003; Demeny *et al.*, 2012). In a 12 day-study with goldfish larvae, best growth and survival were observed with the use of artemia cyst, and when artemia were given, larvae showed cannibalism on

day 5 since the mouth size of these larvae were bigger than other treatments probably due to the cannibalism (Paulet, 2003). However, in the present study, continuous use of dry diets resulted in length differences without any observation of cannibalism.

Kaiser *et al.* (2003) observed the best length growth in goldfish larvae fed with artemia 15.8 mm size followed by those on artemia + a commercial diet with 14.8 mm and those on only the commercial diet with 10.8 mm. These researchers recorded a lower survival in larvae fed only dry diet (61.9%) than those fed only either artemia (96.4%) or artemia + dry diet (94.7%). In the present study, we observed the best survival in larvae fed T₄D₃ with 98.89% and although there were no differences among feeding times, continuously feeding with dry diets resulted in low survival rates (75.56% in T₁D₁, 37.20% in T₁D₂ and 30.60% in T₁D₃), which are consistent with the findings of Sales and Janssens (2003). In the present study, low survival of larvae on dry diets could be resulted from undeveloped digestive system and enzyme activities and incomplete digestion of dry foods, which was previously verified by Abi-ayada and Kestemont (1994). However, when rotifer was used as first feed in the current experiment, even only for 3 days (T₃), survival rate increased up to over 90%. The study indicated that a further increase in the duration of rotifer usage resulted in a concomitant elevation of survival and the highest survival was recorded in T₄D₃. Kaiser *et al.* (1994) found that a decrease of weaning day from 24 to 6 did not affect survival rate, which complies with the data of T₁D₁₋₃ treatment of the present study that included a 3 day only rotifer followed by another 3 day co-feeding with dry diets. Therefore, we can suggest a 6-7 day live food feeding in goldfish larval rearing.

In a 21 day study with goldfish larvae, Abi-ayad and Kestemont (1994) investigated the effects of *Artemia nauplii* (D1), *Artemia nauplii*+50% dry food (D2) and only dry food (D3) on survival and growth rates and proteolytic enzyme activities. They found that survival rates in D1 and D2 were higher than D3. The highest trypsin activity and SGR values were in D2 where as the lowest was in D3. In the present experiment, the best length and SGR_L was in rotifer-fed treatment, which was followed by T₂₋₄D₁₋₃ treatments. SGR_L values of T₁ on day 8 were significantly lower than T₁, T₃ and T₄ with the highest value 6.99% in T₄D₁. At the end of the experiment, the highest SGR_L was observed in rotifer-fed larvae with 6.13%. Although continuous feeding of three diets resulted in poor length growth and SGR_L, rotifer administration as first feed with these diets at T₂, T₃ and T₄ remarkably ameliorated growth rates. On the other hand, the fact that only rotifer feeding over the experiment showed the highest growth rates reveals the importance of live feeds in early goldfish larval rearing. In this study, when the same diet was given at different times, responses of larval growth were also significantly different. A similar tendency was also

the case in SGR_w values. Our larval length, SGR_L and CF values are similar to the growth rate reported by Abi-ayad and Kestemont (1994). CF of larvae in T₁D₂ treatment was the lowest (1.07). But the increase in duration of rotifer feeding elevated CF values and it reached the highest in larvae fed only rotifer with 2.10. These values are within the range of literature data (Kashani *et al.* (2010).

One interesting finding in the present study is that using only dry diets resulted in significantly higher VC compared to the other treatments, suggesting that rotifer feeding prior to dry diets would increase the homogeneity of larval individuals or reduce the inter-individual size variation.

Briefly, in this experiment a combination trial of freshwater rotifer and dry diets during early larval feeding of goldfish was evaluated in terms of growth and survival rates. We can suggest that only dry diet should not be applied in goldfish larval rearing and should be fed after rotifer feeding for at least 3 days. Moreover, a transition period from rotifer to dry diets should be performed by co-feeding for at least 3 days. In light of the present findings, one appropriate protocol can be that 7 days rotifer followed by a 3 day co-feeding with dry diets and then a complete weaning.

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