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The effects of different feeding times and diets on the whole body fatty acid composition of goldfish (*Carassius auratus*) larvae

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Abstract

In this study, the effects on whole body fatty acid levels of goldfish larvae (*Carassius auratus*) caused by different feeding times $T_{(1-4)}$ and diets $D_{(1-3)}$ were investigated during the early larval stages which is the first 15 days' of feeding. Fatty acid levels for all experimental groups were found to be: 1.54 to 3.45% for myristic acid, 17.57 to 22.68% for palmitic acid, 28.81 to 41.16% for total saturated fatty acids-∑SFA, 2.37 to 4.04% for palmitoleic acid, 13.79 to 39.21% for oleic acid, 22.60 to 47.59% for total monounsaturated fatty acids-∑MUFA, 3.62 to 7.69% for linolenic acid-ALA, 0.35 to 1.11% for arachidonic acid-ARA, 7.50 to 9.76% for total n-6 fatty acids-∑ n-6, 1.04 to 2.28% for linoleic acid-LA, 0.81 to 2.52% for eicosapentaenoic acid-EPA and 5.56 to 10.28% for docosahexaenoic acid-DHA. The total n-3 acids-∑ n-3, total polyunsaturated fatty acids-∑PUFA, PUFA/SFA ratio, ∑ n-6/∑ n-3 and DHA/EPA ratio of the experimental groups were determined to be 7.24 to 14.83%, 14.73 to 23.46%, 0.47 to 0.73, 0.58 to 1.04 and 3.21 to 8.7, respectively. In conclusion, the whole body fatty acid levels of goldfish larvae (*Carassius auratus*) determined in this study revealed the significant effects of different feeding times (T_{1-4}) and different diets (D_{1-3}) using ($P < 0.05$).

Keywords: *Carassius auratus*; larvae; fatty acids; feeding times; diets.

Practical Application: Generation of species-specific larval feeding protocols.

1 Introduction

Lipids are the source of essential dietary fatty acids that provide energy for growth and the survival of the fish. Fish require fatty acids with a lower melting temperature and longer-chain unsaturated fatty acids relative to mammals. These fatty acids increase stress resistance and are essential in developing the nerve tissues (Parameshwaran et al., 2002). Freshwater fish need either linoleic (18: 2n-6), linolenic acids (18: 3n-3) or both in their diets, whereas marine fish require eicosapentaenoic (EPA) (20: 5n-3) and docosahexaenoic acids (DHA) (22: 6n-3). However, it has been reported that DHA dietary supplements positively affect the incubation rate in common carps. Nevertheless, the fatty acids requirements of ornamental fish larvae are not sufficiently documented (Sales & Janssens, 2003).

The total lipid amounts and fatty acid composition of fish vary depending on the environmental, physical, chemical and biological conditions of the water (as well as salinity, turbidity, pH, hardness and diversity, and density of phyto and zooplankton) (Bayır et al., 2010). Adequate and balanced feeding of embryos and larvae is very important for their normal development, growth and metamorphosis. All nutrients required for development and growth during incubation and for the yolk-sac fry until first feeding are transferred from the mother through the yolk sac (Zengin et al., 2013). In fish feeds, fish oil is very expensive and can be easily oxidized, so it is therefore, necessary to partially or totally replace it with plant oils. The inclusion of plant oils into larval diets increases growth and survival (Geurden et al., 1999). Dietary requirements of n-3 fatty acids of goldfish (*Carassius auratus*) and common carp are range from 0.05 to 0.1% (Parameshwaran et al., 2002). Goldfish can synthesize n-3 and n-6 PUFAs from 18 carbon fatty acids (Geurden et al., 1999). Low levels of highly unsaturated fatty acids (HUFA) in the diet led to larval mortalities and, but EPA supplements alone, or together with DHA, increased the survival and growth in Asian sea bass. This is also the case for other marine fish larvae (Seiffert et al., 2001). Some researchers have reported that dietary DHA is more effective than EPA (Seiffert et al., 2001). Higher dietary DHA requirement levels – compared to EPA – have been reported in coral fish, yellowtail, turbot and some flatfish. There are some conflicting reports in published literature (Seiffert et al., 2001). For instance, some put forward the idea that dietary n-3 HUFA for larvae is not significant. A study argued that dietary n-3 HUFA did not affect fatty acid compositions of lambuka larvae "mahi-mahi" (*Coryphaena hippurus*) during the first 9 days. This was attributed to the amounts of EPA and DHA deposited in the yolk sac of the larvae. Marine fish eggs were reported to be rich in DHA, but during the first 10 days after incubation, the levels rapidly decreased. *Centropomus parallelus* larvae were not affected in terms of growth rate, survival or swim bladder development by feeding with rotifers, including varying the fatty acid compositions by enriching with different sources (Seiffert et al., 2001). Small changes in the fatty acid levels of total lipids were observed during the 6-day incubation period. The most significant decrease occurred in MUFA levels.

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According to the result of the study, the levels of myristic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, MUFA, linoleic acid, arachidonic acid, Σ n-6, EPA, DHA and Σ n-3 were 1.6 to 1.3%, 19.1 to 18.8%, 4.5 to 3.6%, 4.4 to 5.8%, 12.5 to 13.5%, 29.5 to 32.1%, 3.3 to 4.1%, 3.4 to 3.7%,11.2 to 12.0%, 2.9 to 3.3%, 20.6 to 22.3% and 25.9 to 27.1%, respectively. An increase in ARA and DHA, and a decrease in 18:2 (n-6) and 20:5 (n-3) were noted. SFA levels of polar lipids were increased (Wiegand, 1996). Levels of goldfish larvae 16:0, 18:0, 20:4 (n-6) and 22:6 (n-3) during the yolk sac and following stages were higher than in the eggs. Larvae hatched from low quality eggs were low in n-3 HUFA, whereas those from high quality eggs consumed MUFA for growth. The survival rate of larvae incubated at 22 °C was not dependent on the egg quality but on the total lipid and fatty acid profiles of the eggs (Wiegand et al., 1991). During the first 21 days, carp larvae had higher levels of oleic acid when fed triolein, and higher levels of lauric and myristic acid when fed groundnut oil. In the study mentioned, significant differences were recorded for medium-chain fatty acids (12:0 and 8:0) (Fontagne et al., 1999). A comparison was made of the fatty acid levels in rainbow trout larvae and fry that were fed or starved during the first 16 days. In the starved fish, consumption of oleic acid was preferable, but palmitic acid levels remained unchanged. In trout larvae starved for 29 days, linoleic, eicosanoic (20:0), docosanoic (22:0) and docosatrienoic acids (C22:3ω-3.) were consumed, but EPA was changed little and DHA was retained (Zengin et al., 2013). The larvae of common carp hatched from the eggs of fish distributed in an estuarine region of the South Caspian Sea were fed with rotifer and compounded diets for 15 days, and their fatty acid compositions were determined (Farhoudi et al., 2011). Fatty acid compositions of mature dwarf carp changed depending on the water temperature and the DHA level in the diet (Varga et al., 2013). Larvae fed on 7.5% dietary fish oil had higher MUFA and PUFA levels (Thamizshselvi et al., 2014). Despite a rapid growth in the ornamental aquaculture sector, broodstock collection from the wild is a limiting factor. It is forecast that ornamental aquaculture will put less pressure and damage on the environment than food aquaculture (Tlusty, 2002).

In this study, all trial groups were supplied with live feed (freshwater rotifers) as the first food, but over different periods, and then fed with different foods. The aim of this study was to determine the effects of four different feeding times (T_{1-4}) with three different diets (D_{1-3}) on the whole body fatty acid profiles of goldfish larvae during the early larval stage.

2 Materials and methods

2.1 Experimental conditions

The experiment was conducted in the aquarium section of the Kepez Unit of the Mediterranean Fisheries Research Production and Training Institute, Antalya, Turkey. Post yolk sac larvae used in the study were produced by the Oranda variety of goldfish (*Carassius auratus*).

A total of 2340 goldfish larvae, with an initial weight of 0.97 to 1.13 mg and a total length of 5.77 to 6.05 mm were used in this experiment. Thirteen experimental triplicate treatments – including the control – were tested in thirty-nine 10 L glass aquariums connected to a recirculation system. Each tank was stocked with 60 larvae (6 larvae / L). Each aquarium was supplied with a water flow of 100 mL/min with air stones providing aeration. The water renewal rate and frequency of the recirculation system was 30% and twice a week. Water temperature was thermostatically controlled at 26.8 °C. The photoperiod was set to 12 hours of darkness and 12 hours of light with a timer.

Feeding schedules, diet compositions and formulations were shown in Tables 1 and 2. Experimental dry diets (D_1 commercial feed, D_2 casein-based diet and D_3 egg protein-based diet and R-rotifer, Table 2) were formulated according to Castell & Tiews (1980) and Bandyopadhyay et al. (2005). Freshwater rotifers used in this study were produced in a 12 m^3 pond within a

Table 1. Larval feeding schedule used in the experiment*.

Experimental groups		Feeding times "T" (days)		Diets (D)
1	T_1D_1	T_1	15	D_1
2	T_1D_2			D_2
3	T_1D_3			D_3
4	T_2D_1	T_2	3+3+9	3R+(3R+ D_1)+9 D_1
5	T_2D_2			3R+(3R+D_2)+9 D_2
6	T_2D_3			3R+(3R+D_3)+9 D_3
7	T_3D_1	T_3	5 +3 +7	5R+(3R+ D_1)+7 D_1
8	T_3D_2			5R+(3R+ D_2)+7 D_2
9	T_3D_3			5R+(3R+D_3)+7 D_3
10	T_4D_1	T_4	7 + 3 +5	7R+(3R+ D_1)+5 D_1
11	T_4D_2			7R+(3R+ D_2)+5 D_2
12	T_4D_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 (D_1), casein based dry diet (D_2), egg protein based dry diet (D_3), 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. Nutrient compositions (%) and formulation of experimental diets (D_1 Commercial diet, D_2 Casein based diet and D_3 Egg protein based diet) used in the study.

Ingredients	D_2 (%)	D_3 (%)	D_1 (%)	R (rotifer)
Casein	45	33	(Fish meal, Hydrolyzed fish protein, Fish oil, soybean products, wheat products, vitamin premix, mineral premix, natural mold inhibitor and antioxidant)	
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		
Crude protein*	59.85	59.55	60.8	65.85
Crude oil*	10.81	10.39	11.28	10.03
Crude ash*	3.86	4.26	11.91	10.11

[1]Vitamin premix: 12.000.000 IU A, 2.500.000 IU D_3, 200.000 mg E, 10.000 mg K_3, 20.000 mg B_1, 24.000 mg B_2, 100.000 mg B_5 (Ca D-pantothenate), 20.000 mg B_6, 200 mg B_{12}, 150.000 mg B_3 (niacin), 10.000 mg B_9 (folic acid), 1.000 mg B_7 (biotin), 200.000 mg C, 200.000 mg inositol, 1.000.000 mg choline; [2]Mineral premix: 5.000 mg Cu, 20.000 mg Mn, 2.000 mg Co, 2.500 mg I, 30.000 mg Zn, 250 mg Se; *Proximate compositions of dry matter of experimental diets (%).

greenhouse. All trial groups were supplied live feed as the first food, but over different periods of 15, 7, 5 and 3 days, and then fed with different foods. In the experiment, 4 feeding times and 3 different diets (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 a minimum rate 10 ind/mL, whereas dry diets were given *ad lib.* At the end of the experiment, all larvae in each tank were collectively weighed and then killed with an overdose of anesthesia (phenoxyethanol). The samples were dried using tissue paper and then kept in a freezer at −20 °C prior to fatty acid analysis.

2.2 Chemical analysis

Proximate analysis – except for crude lipids – of the experimental diets was performed according to the methods for (Association of Official Analytical Chemists, 1990; James, 1999) dry matter at 104 °C until a constant weight; for ash content by incineration in a muffle furnace at 600 °C for 2 hours and for crude protein (N × 6.25) using the Kjeldahl method after acid digestion. Lipid content was determined by ether extraction using an automatic extraction system (ANKOMXT15 Extractor, ANKOM Technology, Macedon, USA) (American Oil Chemists Society, 2009). Lipids were extracted from the larvae and feed samples according to Bligh & Dyer (1959). Fatty acids were esterified according to the method of Ichihara et al. (1996). Briefly, 4 mL 2N of KOH and 2 mL of n-hexane were added to 10 mg of extracted lipids. The mixture was then vortexed for 2 minutes, centrifuged at 4000 rpm for 10 minutes and the hexane layer was used for fatty acid analysis using a Thermo Focus GC (Thermo Fischer Scientific Inc., Italy). The injector and detector temperatures were set at 220 and 280 °C, respectively. During this time, the oven temperature was kept at 140 °C for 5 minutes. The temperature was increased by 4 °C/min up to 200 °C and then to 220 °C at increments of 1 °C/m. A 5 µL sample amount was used and the pressure of the carrier gas was 6ps. The split ratio was 1:40. Fatty acids were detected based on the Supelco standard and the results were expressed as a percentage of the total. The fatty acid compositions of the experimental diets are given in Table 3.

2.3 Statistical analysis

Statistical analysis of data was performed using JMP software v8 for windows. The effects of treatments on variables were analyzed by a factorial design (4 feeding times and 3 different diets) and the treatments were distinguished by using the Tukey multiple comparison test (P = 0.05). On the other hand, a comparison between the control and the other diets was made using Dunnett's test (Özdamar, 2001; Yıldız et al., 2011).

3 Results

The larvae were fed with live feed for 15, 7, 5 and 3 days. Larval trial feeds were used for the rest of the 15-day feeding period. The amount of body fatty acidity of the larval groups fed with live feed for the same period varied depending on the test diet ($P < 0.05$). The body fatty acidity of larval groups fed with the same trial diet varied depending on the different feeding times ($P < 0.05$).

The influence of the experimental (D_{1-3}) diets used in this study was generally lower than those fed with rotifers. The levels of fatty acid 14:0 and 16:0 were significantly higher, but not that of 18:0. The diets (D_{1-3}) and feeding times (T_{1-4}) also had significant effects on ∑SFA concentrations. All of the group's 16:1 and 18:1n-9 were significantly higher, except for the T_1D_{1-3} groups ($P < 0.05$). The effects of experimental treatments on the fatty acid profiles of goldfish larvae are given

Table 3. Fatty acid profiles of the experimental diets and rotifer.

Fatty acids	D_1	D_2	D_3	Rotifer
C14:0	5.29	2.69	2.9	7.97
C16:0	14.75	8.98	9.39	12.64
C18:0	2.58	2.34	2.34	2.91
∑SFA	22.62	14.01	14.63	23.52
C16:1	4.46	2.77	2.87	7.74
C18:1n-9	20.29	40.43	41.14	10.22
∑MUFA	24.75	43.20	44.01	17.96
C18:2n-6 (LA)	9.2	14.06	14.14	6.9
C18:3n-3 (ALA)	3.89	5.25	5.30	10.55
C20:4n-6 (ARA)	0.06	0.17	0.15	0.79
C20:5n-3 (EPA)	7.02	2.73	2.59	6.07
C22:6n-3 (DHA)	7.64	3.9	3.43	1.41
∑PUFA	27.81	26.36	25.61	25.41
TOTAL	75.18	83.57	84.25	66.89
PUFA/SFA	1.23	1.88	1.75	1.08
∑n-6	9.26	14.76	14.29	7.69
∑n-3	18.55	11.88	11.32	18.03
∑n-6/∑n-3	0.50	1.24	1.26	0.43
DHA/EPA	1.09	1.43	1.32	0.23
Others	24.82	16.43	15.75	33.11

C14:0 = myristic acid; C16:0 = palmitic acid; C18:0 = stearic acid; ∑SFA = total saturated fatty acids; C16:1 = palmitoleic acid; C18:1n-9 = oleic acid; ∑MUFA = total monounsaturated fatty acids; C18:2n-6 (LA) = linoleic acid; C18:3n-3 (ALA) = linolenic acid; C20:4n-6 (ARA) = arachidonic acid; C20:5n-3 (EPA) = eicosapentanoic acid; C22:6n-3 (DHA) = docosahexaenoic acid; ∑PUFA = total polyunsaturated fatty acids; ∑n-6 = total n-6 fatty acids; ∑n-3 = total n-3 fatty acids.

in Table 4. The palmitoleic acid proportion of larvae fed with the D_1 commercial feed and the D_2 casein-based diet tended to decrease with feeding times. However, the 16:1 level for the larval group fed with rotifer was significantly higher than those on the experimental diets ($P < 0.05$). Also, the effect on the 18:1n-9 proportion of larvae was similar to those fed with the D_3 egg protein-based food with feeding times (T_2, T_3 and T_4), but not to any others. The ∑MUFA was seen to be the lowest in the $T_{2-3}D_3$ groups.

Linoleic acid levels were significantly changed by diet types and their feeding times. The levels of 18:2 n-6 observed in all experimental larvae – except for those fed with D_1 at time T_1 groups – were lower than those that received the rotifer treatment. Also, linoleic acid was detected in the lowest T_4D_3 group. However, the 20:4n-6 level of the rotifer feeding group was lower than those of the diets at all feeding times, except for T_1D_1. The 18:3n-3 level of the D_1 feeding group was significantly reduced compared to those fed diet D_2 and D_3 at times T_2, T_3 and T_4, but not T_1. The effects on the 18:3n-3 levels of larvae fed with the experimental diets were generally lower than those of the rotifer groups. The 20:5n-3 and 22:6n-3 levels of the D_3 group significantly increased at T_2, T_3 and T_4, compared to the other two diets, but not at T_1.

The D_1 and D_2 groups had lower (0.64-1.11%) levels than that of the D_3 group except for T_1, while the 20:5n-3 level of the control group was generally higher than those of the D_1 and D_2 groups. Although significant differences were observed in the results of this study, the 22:6n-3 levels of the group fed with rotifer did not have a clear trend when compared with the other diet groups.

The Σn-3 levels of larvae belonging to the D_3 (egg protein-based food) group was significantly higher than those of D_1 and D_2, except at time T_1. The larvae of the control group were significantly different from the experimental diets without a clear trend for Σn-3.

The ∑PUFA concentrations displayed a similar trend to Σn-3 among the treatments. The PUFA/SFA ratio for the D_1 group was significantly higher than those of the D_2 and D_3 groups at times T_1 and T_4, but significantly lower than at the other feeding times. The Σn-6/Σn-3 ratio changed significantly from 0.58 to 1.04 by diet and feeding times. The DHA/EPA ratios of the experimental diets at all feeding times were significantly higher than those of the control group. The DHA/EPA ratios of larvae were significantly lower than those in D_1 compared to D_2 and D_3 at feeding time T_1.

4 Discussion

In this study, the whole body fatty acid compositions of goldfish larvae (C14:0, C16:0, C18:0, ∑SFA, C16:1, C18:1n9, ∑MUFA LA, ALA, ARA, EPA, DHA, ∑PUFA, PUFA/SFA, Σn6, Σn3, n6/n3, DHA/EPA) were significantly affected by feeding times and diets ($P < 0.05$). The average 14:0 levels (1.54 to 3.45%) of the control group and other treatments were higher than those reported by Wiegand (1996) (1.3%) and Farhoudi et al. (2011) (0.96%), but slightly lower than those of Zengin et al. (2013) (3.48 to 4.17%). Higher amounts of 14:0 in common carp larvae were reported by Fontagne et al. (1999). Goldfish larvae fed continuously with rotifer had significantly higher 14:0 levels than those of the other groups in this study. The average levels of 16:0 (17.57 to 22.68%)

Table 4. Average fatty acid and fatty acid groups of goldfish larvae maintained on different experimental diets administered at varying feeding times during 15 days.

Fatty Acids	Times	Diets			
		D_1	D_2	D_3	Rotifer
C14:0	T_1	$^{a}3.01 \pm 0.01^{A\star}$	$^{a}2.19 \pm 0.01^{B\star}$	$^{c}2.03 \pm 0.01^{C\star}$	$3.45 \pm 0.05^{\star}$
	T_2	$^{c}1.56 \pm 0.01^{B\star}$	$^{c}1.54 \pm 0.02^{B\star}$	$^{a}2.92 \pm 0.01^{A\star}$	
	T_3	$^{c}1.56 \pm 0.01^{B\star}$	$^{c}1.58 \pm 0.01^{B\star}$	$^{b}2.81 \pm 0.01^{A\star}$	
	T_4	$^{b}1.64 \pm 0.02^{B\star}$	$^{b}2.03 \pm 0.01^{A\star}$	$^{c}1.97 \pm 0.02^{A\star}$	
C16:0	T_1	$^{a}22.68 \pm 0.01^{A\star}$	$^{a}21.19 \pm 0.01^{B\star}$	$^{d}18.75 \pm 0.15^{C\star}$	$22.23 \pm 0.0^{\star}$
	T_2	$^{b}19.32 \pm 0.01^{B\star}$	$^{c}18.37 \pm 0.02^{C\star}$	$^{c}20.23 \pm 0.03^{A\star}$	
	T_3	$^{c}18.45 \pm 0.10^{B\star}$	$^{d}17.61 \pm 0.05^{C\star}$	$^{b}21.06 \pm 0.01^{A\star}$	
	T_4	$^{d}17.57 \pm 0.03^{C\star}$	$^{b}18.55 \pm 0.03^{B\star}$	$^{a}21.85 \pm 0.01^{A\star}$	
C18:0	T_1	$^{a}7.27 \pm 0.01^{C}$	$^{b}9.14 \pm 0.24^{B\star}$	$^{c}9.31 \pm 0.00^{A\star}$	$7 .02 \pm 0.04^{\star}$
	T_2	$^{d}9.65 \pm 0.02^{B\star}$	$^{de}9.33 \pm 0.04^{B\star}$	$^{f}7.27 \pm 0.01^{A}$	
	T_3	$^{g}9.20 \pm 0.10^{A\star}$	$^{h}8.48 \pm 0.02^{B\star}$	$^{hi}8.23 \pm 0.01^{B\star}$	
	T_4	$^{j}8.53 \pm 0.02^{B\star}$	$^{jk}8.27 \pm 0.02^{B\star}$	$^{l}13.85 \pm 0.05^{A\star}$	
∑SFA	T_1	$^{a}33.66 \pm 0.01^{A\star}$	$^{a}32.87 \pm 0.11^{B\star}$	$^{d}30.86 \pm 0.14^{C\star}$	$34.04 \pm 0.0^{\star}$
	T_2	$^{b}31.42 \pm 0.01^{B\star}$	$^{b}30.31 \pm 0.02^{C\star}$	$^{c}31.83 \pm 0.05^{A\star}$	
	T_3	$^{c}30.35 \pm 0.07^{B\star}$	$^{c}28.81 \pm 0.03^{C\star}$	$^{b}33.71 \pm 0.03^{A\star}$	
	T_4	$^{d}29.19 \pm 0.06^{C\star}$	$^{b}30.38 \pm 0.03^{B\star}$	$^{a}41.16 \pm 0.11^{A\star}$	
C16:1	T_1	$^{a}3.70 \pm 0.01^{A}$	$^{a}3.12 \pm 0.01^{B\star}$	$^{c}2.91 \pm 0.01^{C\star}$	$4.04 \pm 0.02^{\star}$
	T_2	$^{b}2.73 \pm 0.02^{B\star}$	$^{b}2.68 \pm 0.01^{B\star}$	$^{a}3.47 \pm 0.02^{A}$	
	T_3	$^{c}2.52 \pm 0.00^{B\star}$	$^{c}2.52 \pm 0.00^{B\star}$	$^{b}3.26 \pm 0.01^{A\star}$	
	T_4	$^{d}2.37 \pm 0.02^{C\star}$	$^{c}2.47 \pm 0.02^{B\star}$	$^{c}2.91 \pm 0.00^{A\star}$	
C18:1n-9	T_1	$^{d}27.51 \pm 0.01^{C\star}$	$^{ab}37.62 \pm 0.02^{B\star}$	$^{a}39.21 \pm 0.05^{A\star}$	$26.95 \pm 0.0^{\star}$
	T_2	$^{a}38.49 \pm 0.05^{A\star}$	$^{a}38.92 \pm 0.45^{A\star}$	$^{b}24.19 \pm 0.05^{B\star}$	
	T_3	$^{b}36.66 \pm 0.56^{A\star}$	$^{b}36.66 \pm 0.56^{A\star}$	$^{c}22.53 \pm 0.03^{B\star}$	
	T_4	$^{c}32.68 \pm 0.05^{B\star}$	$^{b}36.06 \pm 0.03^{A\star}$	$^{d}13.79 \pm 0.10^{C\star}$	
∑MUFA	T_1	$^{d}37.94 \pm 0.01^{C\star}$	$^{ab}45.96 \pm 0.00^{B\star}$	$^{a}47.59 \pm 0.02^{A\star}$	$39.40 \pm 0.0^{\star}$
	T_2	$^{a}47.27 \pm 0.06^{A\star}$	$^{a}47.92 \pm 0.52^{A\star}$	$^{b}35.85 \pm 0.02^{B\star}$	
	T_3	$^{b}45.59 \pm 0.58^{A\star}$	$^{b}45.59 \pm 0.58^{A\star}$	$^{c}33.58 \pm 0.03^{B\star}$	
	T_4	$^{c}41.74 \pm 0.02^{B\star}$	$^{b}44.92 \pm 0.02^{A\star}$	$^{d}22.60 \pm 0.20^{C\star}$	
C18:2n-6 (LA)	T_1	$^{a}7.69 \pm 0.01^{A\star}$	$^{b}6.44 \pm 0.01^{C\star}$	$^{a}6.79 \pm 0.05^{B\star}$	$7.43 \pm 0.02^{\star}$
	T_2	$^{d}5.10 \pm 0.05^{C\star}$	$^{c}5.85 \pm 0.02^{B\star}$	$^{a}6.87 \pm 0.01^{A}$	
	T_3	$^{c}5.76 \pm 0.02^{C\star}$	$^{b}6.58 \pm 0.01^{A\star}$	$^{b}6.40 \pm 0.02^{B}$	
	T_4	$^{b}6.32 \pm 0.03^{B\star}$	$^{a}7.04 \pm 0.06^{A\star}$	$^{c}3.62 \pm 0.06^{C\star}$	
C20:4n-6 (ARA)	T_1	$^{b}0.35 \pm 0.01^{C\star}$	$^{c}0.64 \pm 0.01^{B\star}$	$^{b}0.70 \pm 0.01^{A\star}$	$0.42 \pm 0.00^{\star}$
	T_2	$^{a}0.76 \pm 0.01^{A\star}$	$^{a}0.80 \pm 0.00^{A\star}$	$^{c}0.48 \pm 0.01^{B\star}$	
	T_3	$^{a}0.75 \pm 0.01^{A\star}$	$^{b}0.69 \pm 0.01^{B\star}$	$^{c}0.49 \pm 0.00^{C\star}$	
	T_4	$^{a}0.72 \pm 0.01^{B\star}$	$^{c}0.64 \pm 0.02^{C\star}$	$^{a}1.11 \pm 0.00^{A\star}$	
∑ n-6	T_1	$^{b}9.02 \pm 0.01^{A}$	$^{c}8.49 \pm 0.01^{B\star}$	$^{a}9.12 \pm 0.10^{A}$	$8.97 \pm 0.04^{\star}$
	T_2	$^{d}7.50 \pm 0.04^{C\star}$	$^{c}8.44 \pm 0.02^{B\star}$	$^{ab}8.98 \pm 0.05^{A}$	
	T_3	$^{c}8.37 \pm 0.01^{C\star}$	$^{b}9.08 \pm 0.01^{A}$	$^{ab}8.63 \pm 0.01^{B\star}$	
	T_4	$^{a}9.27 \pm 0.01^{A\star}$	$^{a}9.76 \pm 0.03^{A\star}$	$^{b}8.49 \pm 0.17^{B\star}$	
C18:3n-3 (ALA)	T_1	$^{a}2.37 \pm 0.02^{A\star}$	$^{a}1.77 \pm 0.00^{B\star}$	$^{b}1.88 \pm 0.05^{B}$	$2.07 \pm 0.02^{\star}$
	T_2	$^{d}1.04 \pm 0.03^{C\star}$	$^{c}1.33 \pm 0.01^{B\star}$	$^{b}2.01 \pm 0.02^{A\star}$	
	T_3	$^{c}1.30 \pm 0.01^{C}$	$^{b}1.48 \pm 0.01^{B\star}$	$^{b}2.03 \pm 0.01^{A\star}$	
	T_4	$^{b}1.63 \pm 0.01^{C\star}$	$^{a}1.76 \pm 0.01^{B\star}$	$^{a}2.28 \pm 0.01^{A\star}$	

C14:0 = myristic acid; C16:0 = palmitic acid; C18:0 = stearic acid; ∑SFA = total saturated fatty acids; C16:1 = palmitoleic acid; C18:1n-9 = oleic acid; ∑MUFA = total monounsaturated fatty acids; C18:2n-6 (LA) = linoleic acid; C20:4n-6 (ARA) = arachidonic acid; ∑n-6 = total n-6 fatty acids; C18:3n-3 (ALA) = linolenic acid; C20:5n-3 (EPA) = eicosapentanoic acid; C22:6n-3 (DHA) = docosahexaenoic acid; ∑n-3 = total n-3 fatty acids; ∑PUFA = total polyunsaturated fatty acids. Average fatty acid values sharing common superscripts in the same rows or column are not significantly different (based on the Tukey multiple comparison test) ($P < 0.05$); *Indicates that these values are significantly different from the control (Dunnett's test) ($P < 0.05$).

Table 4. Continued...

Fatty Acids	Times	Diets			
		D_1	D_2	D_3	Rotifer
C20:5n-3 (EPA)	T_1	$^{a}2.20 \pm 0.01^{A\star}$	$^{c}0.80 \pm 0.01^{B\star}$	$^{c}0.81 \pm 0.03^{B\star}$	$2.07 \pm 0.01^{\star}$
	T_2	$^{d}0.64 \pm 0.02^{C\star}$	$^{d}0.73 \pm 0.01^{B\star}$	$^{a}2.46 \pm 0.01^{A\star}$	
	T_3	$^{c}0.88 \pm 0.01^{B\star}$	$^{b}0.90 \pm 0.01^{B\star}$	$^{a}2.52 \pm 0.01^{A\star}$	
	T_4	$^{b}1.18 \pm 0.01^{B}$	$^{a}1.11 \pm 0.01^{C\star}$	$^{b}2.29 \pm 0.00^{A\star}$	
C22:6n-3 (DHA)	T_1	$^{a}9.17 \pm 0.01^{A\star}$	$^{b}6.91 \pm 0.01^{B\star}$	$^{c}6.62 \pm 0.03^{C\star}$	$6.64 \pm 0.01^{\star}$
	T_2	$^{d}5.56 \pm 0.02^{C\star}$	$^{d}6.32 \pm 0.01^{B\star}$	$^{a}9.95 \pm 0.01^{A\star}$	
	T_3	$^{c}6.71 \pm 0.03^{B\star}$	$^{c}6.46 \pm 0.01^{C\star}$	$^{a}10.28 \pm 0.01^{A\star}$	
	T_4	$^{b}8.55 \pm 0.01^{B\star}$	$^{a}7.66 \pm 0.01^{C\star}$	$^{b}9.46 \pm 0.13^{A\star}$	
∑n-3	T_1	$^{a}13.74 \pm 0.00^{A\star}$	$^{b}9.48 \pm 0.02^{B\star}$	$^{d}9.30 \pm 0.05^{B\star}$	$10.78 \pm 0.0^{\star}$
	T_2	$^{d}7.24 \pm 0.02^{C\star}$	$^{d}8.38 \pm 0.02^{B\star}$	$^{b}14.41 \pm 0.01^{A\star}$	
	T_3	$^{c}8.89 \pm 0.02^{B\star}$	$^{c}8.83 \pm 0.02^{B\star}$	$^{a}14.83 \pm 0.03^{A\star}$	
	T_4	$^{b}11.35 \pm 0.00^{B\star}$	$^{a}10.53 \pm 0.01^{C\star}$	$^{c}14.02 \pm 0.12^{A\star}$	
∑PUFA	T_1	$^{a}22.76 \pm 0.01^{A\star}$	$^{b}17.96 \pm 0.02^{B\star}$	$^{c}18.42 \pm 0.05^{C\star}$	$19.75 \pm 0.0^{\star}$
	T_2	$^{d}14.73 \pm 0.05^{C\star}$	$^{c}16.81 \pm 0.03^{B\star}$	$^{a}23.39 \pm 0.06^{A\star}$	
	T_3	$^{c}17.26 \pm 0.01^{C\star}$	$^{b}17.91 \pm 0.02^{B\star}$	$^{a}23.46 \pm 0.04^{A\star}$	
	T_4	$^{b}20.62 \pm 0.01^{B\star}$	$^{a}20.28 \pm 0.02^{B}$	$^{b}22.51 \pm 0.29^{A\star}$	
PUFA/SFA	T_1	$^{b}0.68 \pm 0.00^{A\star}$	$^{d}0.54 \pm 0.00^{C\star}$	$^{c}0.60 \pm 0.00^{B}$	$0.58 \pm 0.00^{\star}$
	T_2	$^{d}0.47 \pm 0.00^{C\star}$	$^{c}0.55 \pm 0.00^{B\star}$	$^{a}0.73 \pm 0.00^{A\star}$	
	T_3	$^{c}0.57 \pm 0.00^{C}$	$^{b}0.62 \pm 0.00^{B\star}$	$^{b}0.70 \pm 0.00^{A\star}$	
	T_4	$^{a}0.71 \pm 0.00^{A\star}$	$^{a}0.67 \pm 0.00^{B\star}$	$^{d}0.55 \pm 0.01^{C\star}$	
∑n-6/∑n-3	T_1	$^{d}0.66 \pm 0.00^{C\star}$	$^{d}0.90 \pm 0.00^{B\star}$	$^{a}0.98 \pm 0.02^{A\star}$	$0.83 \pm 0.00^{\star}$
	T_2	$^{a}1.04 \pm 0.00^{A\star}$	$^{b}1.01 \pm 0.00^{B\star}$	$^{b}0.62 \pm 0.00^{C\star}$	
	T_3	$^{b}0.94 \pm 0.00^{B\star}$	$^{a}1.03 \pm 0.00^{A\star}$	$^{b}0.58 \pm 0.00^{C\star}$	
	T_4	$^{c}0.82 \pm 0.00^{B\star}$	$^{c}0.93 \pm 0.00^{A\star}$	$^{b}0.61 \pm 0.01^{C\star}$	
DHA/EPA	T_1	$^{c}4.17 \pm 0.01^{B\star}$	$^{a}8.69 \pm 0.04^{A\star}$	$^{a}8.23 \pm 0.29^{A\star}$	$3.21 \pm 0.01^{\star}$
	T_2	$^{a}8.70 \pm 0.24^{A\star}$	$^{a}8.66 \pm 0.10^{A\star}$	$^{b}4.04 \pm 0.02^{B\star}$	
	T_3	$^{b}7.67 \pm 0.08^{A\star}$	$^{b}7.17 \pm 0.07^{B\star}$	$^{b}4.08 \pm 0.01^{C\star}$	
	T_4	$^{b}7.27 \pm 0.03^{A\star}$	$^{b}6.90 \pm 0.06^{B\star}$	$^{b}4.13 \pm 0.05^{C\star}$	

C14:0 = myristic acid; C16:0 = palmitic acid; C18:0 = stearic acid; ∑SFA = total saturated fatty acids; C16:1 = palmitoleic acid; C18:1n-9 = oleic acid; ∑MUFA = total monounsaturated fatty acids; C18:2n-6 (LA) = linoleic acid; C20:4n-6 (ARA) = arachidonic acid; Σn-6 = total n-6 fatty acids; C18:3n-3 (ALA) = linolenic acid; C20:5n-3 (EPA) = eicosapentanoic acid; C22:6n-3 (DHA) = docosahexaenoic acid; Σn-3 = total n-3 fatty acids; ∑PUFA = total polyunsaturated fatty acids. Average fatty acid values sharing common superscripts in the same rows or column are not significantly different (based on the Tukey multiple comparison test) ($P < 0.05$); *Indicates that these values are significantly different from the control (Dunnett's test) ($P < 0.05$).

in this study are comparable to those of Wiegand (1996) (18.8%) and Farhoudi et al. (2011) (23.54%), but slightly higher than those of Zengin et al. (2013) (15.46 to 18.46%). The 18:0 levels (7.02 to 13.85%) were similar to Farhoudi et al. (2011) (13.43%) while higher than those of Wiegand (1996) (5.8%) and Zengin et al. (2013) (3.64 to 4.51%). The ∑SFA values of the experimental groups ranged from 28.81 to 41.16%, in harmony with the findings of Farhoudi et al. (2011) (41.52%) and Zengin et al. (2013) (30.43 to 35.81%), but higher than those of Wiegand (1996) (26.7%).

Our findings related to 16:1 n-7 (2.37 to 4.04%) are consistent with the results of Wiegand (1996) (3.6%) and Farhoudi et al. (2011) (3.9%), but lower than those of Zengin et al. (2013) (7.45 to 8.66%). The later author (Zengin et al., 2013), however, reported similar levels of 18:1 n-9: 18.74 to 24.45% compared to ours from 13.79 to 39.21%, which are higher than those found by Wiegand (1996) at 12.5% and Farhoudi et al. (2011) at 12.61%. The ∑MUFA concentrations in this investigation varied from 22.60 to 47.59%, which are higher than the average value (20.06%) of Farhoudi et al. (2011), but comparable to those of Wiegand (1996) (29.5%) and Zengin et al. (2013) (31.48 to 35.42%). Our ∑MUFA values are also consistent with those of common carp and catfish larvae (Stancheva et al., 2014). Thamizshselvi et al. (2014) reported the highest MUFA content in fish-fed experimental diets with 7.5% of fish oil.

Wiegand (1996), Farhoudi et al. (2011) and Zengin et al. (2013) reported 18:2n-6 levels between 3.3 and 5.33%, which are within the range of our findings. The 20:4n-6 levels of goldfish larvae were 0.35 to 1.11% in this study, and were remarkably lower than those reported in published literature (Wiegand, 1996; Farhoudi et al., 2011; Zengin et al., 2013). Wiegand et al. (1991) found higher levels of 20:4 n-6 in goldfish larvae when compared

with the eggs, and also underlined the importance of total lipid and fatty acid composition in terms of survival of the larvae.

The ∑ n-6 levels (7.50 to 9.76%) are lower than those of the findings of previous reports (Wiegand, 1996; Zengin et al., 2013), but similar to those reported by Farhoudi et al. (2011). The amounts of the group fatty acids tended to increase with the duration of transition from live feed to dry feeds.

Our findings related to 18:3n-3 levels were supported by Farhoudi et al. (2011). Rainbow trout larvae consumed high levels of this fatty acid during their 29-day starvation period (Zengin et al., 2013). EPA levels reported at 2.9% by Wiegand (1996), 3.93% by Farhoudi et al. (2011) and 4.2 to 5.02% by Zengin et al. (2013) were higher than those of this study. Zengin et al. (2013) recorded that EPA was largely retained by trout larvae during the starvation period. In this study, EPA levels in larvae tended to increase in parallel with the rotifer feeding period.

DHA concentrations in this study appear to be lower than those of the literature findings (Wiegand, 1996; Farhoudi et al., 2011). According to information reported by from studies in which dietary DHA concentrations had a profound effect on the hatching rate in common carp (Sales & Janssens, 2003). As mentioned for EPA levels, DHA was also retained by trout larvae during the starvation period (Zengin et al., 2013). Results revealed that the levels of ∑ n-3 (7.24 to 14.83%) in goldfish larvae were lower than those of the previous studies (Wiegand, 1996; Farhoudi et al., 2011; Zengin et al., 2013). In addition, ∑PUFA levels observed in the study were lower than those of published literature (Wiegand, 1996; Farhoudi et al., 2011; Zengin et al., 2013; Stancheva et al., 2014). Low quality fish eggs were reported to have lower levels of n-3 PUFA (Wiegand et al., 1991). The ratios of ∑PUFA/∑SFA and ∑ n-6/∑ n-3 in goldfish larvae were consistent with those reported by Wiegand (1996), Farhoudi et al. (2011) and Zengin et al. (2013). However, the DHA/EPA ratios of the study are different than those reported by Farhoudi et al. (2011) and Zengin et al. (2013). As mentioned in published literature, larval fatty acid concentrations and profiles changes depend on species, egg quality, larval stage, water temperature, diet and other biotic–abiotic factors.

5 Conclusion

The amount of body fatty acid of larval groups fed with live feed for the same period varied depending on the test diets. The body fatty acid of larval groups fed with the same trial diet varied depending on the different feeding times ($P < 0.05$). Also, in the experiment, it was observed that the fatty acid components of the larvae were changed according to the fatty acid structure of the feed. The ∑SFA, ∑MUFA, ∑ n-6/∑ n-3, DHA/EPA and ARA rates of larvae fed on commercial feed D_1 increased; however, the ∑PUFA, ∑n-3, and ∑PUFA/∑SFA ratios were observed to decrease. The ∑SFA, ∑MUFA, DHA/EPA rates of larvae fed on the D_2 casein-based diet increased; however, the ∑PUFA, ∑n-3, ∑n-6, ALA and ∑PUFA/∑SFA ratios were observed to decrease. The ∑SFA, ∑n-3 and DHA/EPA rates of larvae fed on the egg protein-based diet D_3, increased. Nevertheless, the ∑MUFA, ∑PUFA, ALA, LA, ∑n-6, ∑ n-6/∑ n-3 and ∑PUFA/∑SFA ratios were observed to decrease. The whole body fatty acid levels of goldfish larvae determined in this study revealed the significant effects caused by the different diets used and the different feeding times. The different feeding times and the different diets have been recommended for the whole body fatty acid this way 14:0, 16:0, 16:1, 18:2 n-6 and 18:3 n-3 to T_1D_1; 18:0, ∑SFA and 20:4 n-6 to T_4D_3; 18:1 n-9 to T_1D_3; 20:5 n-3, 22:6 n-3, ∑n-3 and ∑PUFA to T_3D_3; ∑n-6 to T_4D_2; ∑ n-6/∑ n-3 and DHA/EPA to T_2D_1; ∑MUFA to T_4D_2; ∑PUFA/∑SFA to T_2D_3 (Table 4).

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