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Effect of dietary vitamin C, E and highly unsaturated fatty acid on growth and survival of goldfish (*Carassius auratus*)

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Abstract. A 10 weeks growth experiment was conducted to determine the effects of dietary vitamin C, E and highly unsaturated fatty acid on growth and survival of goldfish (*Carassius auratus*) with initial weight of 0.69±0.12 g. They were fed with ten experimental diets having different level of vitamin E (50, 100, 1000 mg kg diet^{-1}), C (100, 1000 mg kg diet^{-1}), HUFA (highly unsaturated fatty acid): the test treatments were as follow: C100+E1000-HUFA, C1000+HUFA, C100+HUFA, C100+E1000+HUFA, E100+HUFA, E50+HUFA, C1000-HUFA, C100+HUFA, -C-E+HUFA, -C-E-HUFA. Final weight, specific growth rate, feed conversion rate and condition factor were evaluated at the end of experimental period. Final weight, specific growth rate were higher in fish fed the diet with C100+E1000+HUFA. The lowest final weight and specific growth rate were observed in -C–E-HUFA; whereas food conversion rate (FCR) and condition factor (K) were not significantly different between groups. These results indicate that the diet with C100+E1000+HUFA can effect some growth factors in goldfish, *Carassius auratus*.
Key words: Vitamin C and E, survival and growth, HUFA, goldfish.

Introduction. Goldfish, *Carassius auratus* (Bloch 1783) are widespread throughout the world as the most famous companion fish. Goldfish were derived by the traditional breeding on the oriental cultural background to appreciate fish with beautifully unique phenotypes (Matsui 1963; Kojima & Takai 1995; Suzuki 1997; Smartt 2001). Goldfish were thought to be originally for social and religious ceremonies (Smartt 2001). Goldfish is a relatively small member of the carp subfamily (Cyprininae) that also includes the common carp (*Cyprinus carpio*) and Crucian carps (Genus: *Carassius*) (Zhen 1988).

Vitamins C (ascorbic acid, AA) and E (tocopherols) are strong antioxidants. These two vitamins have been extensively studied in fish nutrition (Wilson et al 1984; Dabrowski & Ciereszko 2001; Halver 2002), as well as in humans and other animals (Frei et al 1990; Liu & Lee 1998; Hamilton et al 2000). Vitamin C plays an important role in growth and immunity of fish (Al-Amoudi et al 1992; Lin & Shiau 2005). Most teleosts are unable to synthesize ascorbic acid due to the lack of L-gulonolactone oxidase which is necessary to convert L-gulonolactic acid to AA; therefore, an exogenous source of vitamin C is required in fish diet (Wilson et al 1973; Fracalossi et al 2001). The quantitative requirements on vitamin C have been determined for several species and the recommended values varied by various studies (NRC 1993).

Vitamin E is a lipid-soluble vitamin that comprises four tocopherols and four tocotrienols in nature. Among them, α-tocopherol has the highest vitamin E activity (NRC 1993). Vitamin E requirement are directly related to dietary HUFA levels since they are fatty acids highly prone to oxidation (Chow 1991; Stahl & Sies 1997; Gökkuşu & Mostafazadeh 2003; Udilova et al 2003).

One factor that may affect the dietary vitamin E requirement is the oxidative stability of the diets (Huang & Huang 2004). Addition of vitamin E to rancid diets significantly improved growth performance of the fish (Baker & Davies 1996).

Fish are an important source of n-3 HUFA (highly unsaturated fatty acids), and thus there is great interest in the beneficial aspects of the consumption of these fatty acids for human health (Kroes et al 2003; Moreno & Mitjavila 2003). Moreover, it is known that the tissue fatty acid profile depend on lipid content (Olsen & Henderson 1997; Olsen et al 1999; Mourente et al 2000; Montero et al 2001).

The present study was designed to determine the effects of dietary vitamin C, E and highly unsaturated fatty acid on survival and growth of this fish.

Materials and Methods

Experimental Diets. Ten experimental diets having different levels of vitamin C, E and HUFA were formulated and manufactured in the laboratory according to goldfish nutritional requirements; the test diet were as follow: C100+E1000-HUFA, C1000+HUFA, C100+HUFA, C100+E1000+HUFA, E100+HUFA, E50+HUFA, C1000-HUFA, C100+HUFA,-C-E+HUFA, -C-E-HUFA. Fish or soybean oil was used as a lipid source for +HUFA and -HUFA diets, respectively (see Table 1).

Table 1

Ingredient (g /100 g diet) and chemical proximate composition (% dry matter) of experimental diets

Ingredient	*C100+E1000 HUFA-*	*E50 + HUFA*	*-E-C + HUFA*	*-E-C - HUFA*	*C100 + HUFA*	*C100 - HUFA*	*C1000 - HUFA*	*C1000 + HUFA*	*E100 + HUFA*	*C100+ E1000 +HUFA*
Corn meal	6	6	6	6	6	6	6	6	6	6
Fish meal	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5
Soybean meal	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5
Bread flour	10	10	10	10	10	10	10	10	10	10
Rice bran	18.75	18.75	18.75	18.75	18.75	18.75	18.75	18.75	18.75	18.75
Fish oil	-	0.5	0.5	-	0.5	-	-	0.5	0.5	0.5
Soybean oil	0.5	-	-	0.5	-	0.5	0.5	-	-	-
Mineral mixture[1]	2	2	2	2	2	2	2	2	2	2
Vitamin mixture[2]	2	2	2	2	2	2	2	2	2	2
Lysine	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Metionin	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Anti fungi	0.25	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
									Proximate composition	
									Moisture	6
									Protein	39
									Lipid	10.8

[1]Mineral mixture(g/kg) according to De la Higuera et al 1998: Ca(PO4H2)2·H2O(30), CaCO3 (6.5), KCl (2.5), NaCl (4), MnSO4·H2O (0.2), FeSO4·7H2O (1.5), MgSO4 (4.6), KI (0.02), CuSO4·5H2O (0.05), ZnSO4·7H2O (0.2), CoSO4·7H2O (0.05), Na2SeO3 (0.218·10−2), Al2(SO4)3·18H2O (1·10−2).
[2]Vitamin mixture (mg/kg diet): Thiamine (40), Riboflavin (60), Pyridoxine (30), Panthotenic acid (150), Niacin (25), Folic acid (15), Inositol (1000), Choline (5000), Biotin (3), Cyanocobalamin (0.05), Vitamin A (1), Menadion (25).

Experimental Design. Goldfish (*Carassius auratus*), average 0.69±0.12g initial weight, were used as an experimental fish and the feeding trial was conducted in the Aquaculture Laboratory, Aquaculture Research Center of Fisheries Department in Gorgan University of Agricultural Sciences and Natural Resource. Prior to the start of the experiment, the goldfish were reared into 400 L fiberglass tanks for 2 weeks to acclimate to the experimental diet and conditions. At the start of the experiment, feeding was stopped 24 h prior to weighing. Fish of similar sizes were randomly distributed in to 30 fiberglass tanks (400 L), and each tank was stocked with 8 fish. Each diet was randomly assigned to triplicate tank. Fish were hand-fed to apparent satiation twice daily for 10 weeks. During the experimental period, the temperature ranged from 21.5 to 22 °C, the pH was approximately 7.9 to 8.1.

At the termination of the experiment, the feeding was stopped for 24 h before harvest. Total number and mean body weight of fish in each tank were measured. After feeding for 10 weeks, fish fed the diets with different level of vitamin E (50, 100, 1000 mg kg diet^{-1}), C (100,1000 mg kg diet^{-1}), HUFA (highly unsaturated fatty acid) were selected to investigate the effects of dietary vitamin C, E and highly unsaturated fatty acid on growth and survival of goldfish.

Analysis of Dietary Composition. The samples of diets were dried to a constant weight at 105 °C to determine the dry matter content. Protein was determined by measuring nitrogen (N×6.25) using the Kjeldahl method; lipid by ether extraction using Soxhlet (AOAC 1995).

Calculations. The following variables were calculated:
Specific growth rate (SGR) = (LnWt−LnW0) ×100/ t
Survival rate = Nt * 100/N0
Condition factor (K) = W/L^3*100
FCR = FED DIET/ W2 Feed conversion rate

where,

Wt and W0 were final and initial fish weights, respectively; Nt and N0 were final and initial numbers of fish in each replicate, respectively; t_2-t_1 is the experimental duration in day.

Statistical Analysis. Data were analyzed by one-way analysis of variance (ANOVA). When ANOVA identified differences among groups, multiple comparisons among means were made with Duncan's new multiple range tests. All variances were checked for normality and homogeneity. All percentage values were transformed using arcsin transformation. Data are presented as treatment means ± SD. The values of $P<0.05$ were considered significantly different.

Results. Growth factors are summarized in Table 2. In this study, fish fed the diet with C100+E1000+HUFA had higher final weight and specific growth rates (SGR) and fish fed the diet with –E–HUFA shown lower final weight and specific growth rates than other treatments.

There was no significantly ($P>0.05$) different throughout the treatments ($P> 0.05$). Although condition factor (K) and feed conversion ratio (FCR) were not significantly ($P> 0.05$) different between treatments but lowest (K) and highest (FCR) shown in diets with –E-HUFA and C100+E1000+HUFA (see Figure 1).

Table 2

Response of goldfish to the various test diet after 10 weeks of feeding

Parameter	*Treatment*									
	C100 E1000+ HUFA+	C100 +E1000 HUFA-	E100 + HUFA	C100 + HUFA	C1000 + HUFA	E50 + HUFA	C100 - HUFA	C1000 - HUFA	-E + HUFA	-E - HUFA
SGR	1.17±0.11 a	1.06±0.01 abc	1.11±.54 ab	1.09±0.27 abc	0.91±0.12 abc	0.90±0.48 abc	0.88±0.07 bc	0.88±0.08 bc	0.83±0.1 bc	0.81±0.02 c
Final weight	11.20±0.77 a	10.47±0.08 abc	10.79±0.35 ab	10.72±0.28	9.58±0.48 abc	9.50±0.27 abc	9.42±0.45 bc	9.41±0.38 bc	9.15±0.5 bc	9.02±0.1 c
FCR	3.21±0.09 a	2.39± 0.76 a	2.30±0.47 a	2.31±0.17 a	3.85±0.39 a	2.90±0.4 a	3.04±0.4 a	3.17±0.6 a	3.28±0.46 a	3.33±0.22 a
K	2.30±0.21 a	2.10±0.57 a	2.26±0.15 a	2.14±0.23 a	2.05±0.2 a	1.98±0.1 a	1.95±0.4 a	1.94±0.04 a	1.83±0.2 a	1.63±0.2 a

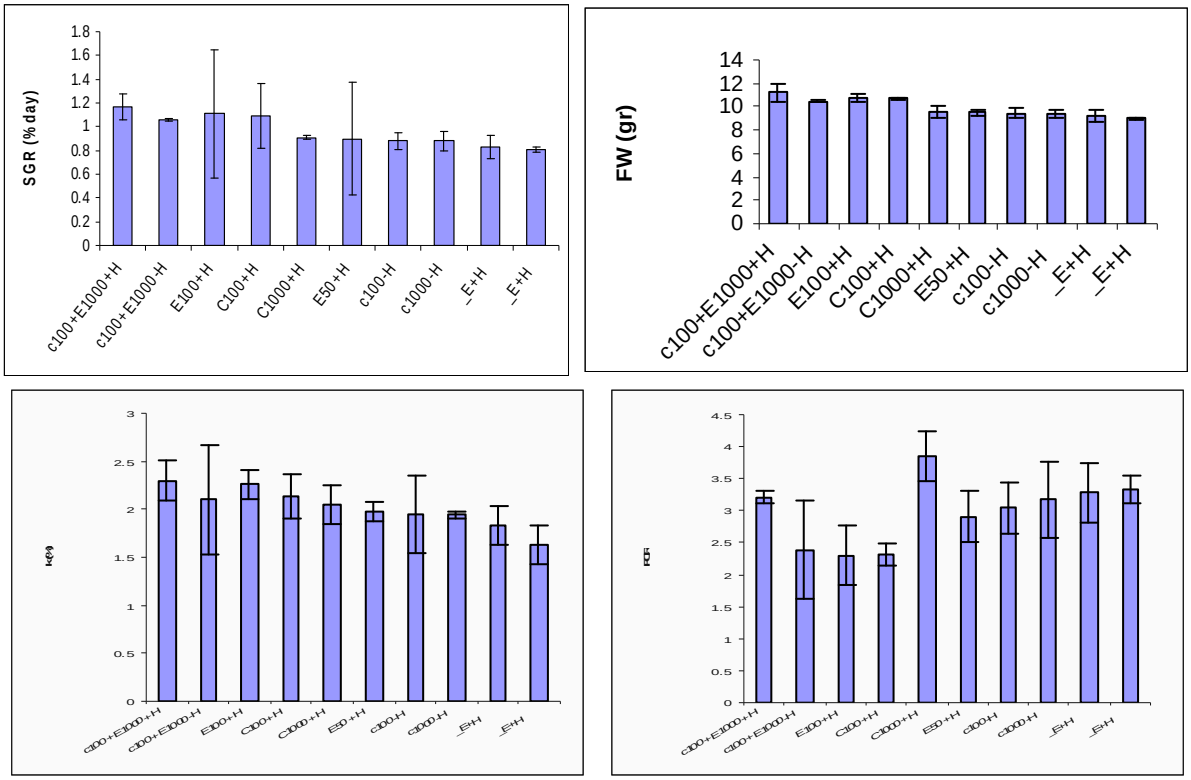

Figure 1. A - SGR (% day) in different treatments of goldfish; B - FW (gr) in different treatments of goldfish; C - (%) in different treatments of goldfish; D - FCR in different treatments of goldfish.

Discussion. In this study, the supplementation of dietary C100+E1000+HUFA had a significant effect on goldfish (*Carassius auratus*) growth. There is no study to examine the effects of dietary vitamin C, E and highly unsaturated fatty acid on the growth and survival in goldfish (*Carassius auratus*). Many marine larvae are believed to require HUFAs, especially EPA and DHA (Watanabe 1982; Sargent et al 1989; Sargent et al 1995). The nutritional value of high levels of HUFA was confirmed by enhanced growth in many marine fish larvae such as turbot (*Scophthalmus maximus*) (Linnaeus, 1758) (Gatesoupe & Le Milinaire 1985), Japanese flounder (*Paralichthys olivaceus*) (Temminck & Schlegel, 1846), red sea bream (*Pagrus major*) (Temminck & Schlegel, 1843) (Izquierdo et al 1989), gilthead sea bream (*Sparus aurata*) (Linnaeus, 1758) (Koven et al 1993) and *Limanda ferruginea* (Storer, 1839) (Copeman et al 2002). Different results were reported by Rainuzzo et al (1994) and Dickey-Collas & Geffen (1992) because they did not find a relationship between (n-3) HUFA levels in live food fed to larval turbot and plaice (*Pleuronectes platessa*) (Linnaeus, 1758) and larval growth. Sau et al (2004) and Paul et al (2004) demonstrated that dietary vitamin E increased growth of rohu (*Labeo rohita*) (Hamilton, 1822) and mrigal (*Cirrhinus mrigala*) (Bloch, 1795). On the contrary, supplementation of dietary *α* -tocopherol in the *Artemia* enrichment did not have a significant effect on walleye (*Stizostedion vitreum*) (Mitchill, 1818) larvae growth (Kolkovski et al 2000). In our study, vitamin E supplementation caused a significant increase in growth factors in fish fed the dietary with C100+E1000+HUFA as compared with the control. The increase in growth of goldfish with a supplementation of 1000 mg vitamin E in the dietary may be due to the level of vitamin E used in diet.

As mentioned before, the food conversion rate (FCR) and condition factor of goldfish fed the diet with C100+E1000+HUFA did not show a significant difference as compared with control group.

However, the dietary with C100+E1000+HUFA increased the growth of goldfish as compared with the control group.

In this study, when we used 100 mg vitamin C plus vitamin E and HUFA, specific growth rate and final weight were significantly different. In other treatment it did not show significant effect.

Conclusions. In summary, data from this study showed that the dietary with C100+E1000+HUFA can improve some growth factors of goldfish (*Carassius auratus*). Further research is needed to examine the supplementation of vitamin E, C and HUFA in dietary and their effects on growth and survival of goldfish at different stages and the effects of these on vitamin E, C and HUFA levels in the fish body.

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