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Full Length Research Paper

The effect of vitamin E and highly unsaturated fatty acid on growth, survival and haematocrit of goldfish (*Carassius auratus gibellio*)

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This study was conducted to examine the effects of different content of vitamin E and highly unsaturated fatty acid on growth and survival in goldfish. Goldfish with initial weight 0.69±0.12 g were fed with diets having different levels of vitamin E and highly unsaturated fatty acid (E100 + HUFA, E50 + HUFA, -E + HUFA, -E - HUFA) for 10 weeks. At the end of period, the final weight, specific growth rate, feed conversion ration and condition factor were measured. The result of this study showed that the difference between the final weights, specific growth rate, feed conversion ratio between treatments were not significant (P>0.05) but in control group was less than other groups.

Key words: Fish growth, fish survival, vitamin E, highly unsaturated fatty acid, goldfish.

INTRODUCTION

Goldfish is a relatively small member of the carp subfamily (Cyprininae) that includes the common carp (*Cyprinus carpio*) and Crucian carps (Genus: Carassius) (Zhen, 1988). Vitamin E is a lipid-soluble vitamin that comprises four tocopherols and four tocotrienols in nature. Among them, a-tocopherol has the highest vitamin E activity (NRC, 1993).

One factor that affects the dietary vitamin E requirement is the oxidative stability of the diets (Huang et al., 2004). Addition of vitamin E to rancid diets significantly improved growth performance of the fish (Baker and davis, 1996). Vitamin E is to prevent peroxidation of polyunsaturated fatty acids of phospholipids and cholesterol in cellular and subcellular membranes. Most of the deficiency signs observed in fish, such as nutritional muscular dystrophy, fatty liver degeneration, anemia, erythrocyte hemolysis, hemorrhage, depigmentation, and reduction of fertility, are related to peroxidative damage to cellular membranes (NRC, 1993). As a membrane-bound antioxidant, vitamin E appears to scavenge free radicals at the site of their formation.

High levels of n-3 HUFA, which mainly consist of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are found in marine organisms. These fatty acids in the phospholipids fraction of the biomembrane in marine species are observed to play a critical role in membrane fluidity (Sargent, 1995; Takeuchi, 1997). The n-3 highly unsaturated fatty acids (HUFA) eicosapentaenoic acid (EPA, 20:5n_3) and docosahexaenoic acid (DHA, 22:6n-3) have been reported to be essential for optimal growth of several species of bivalve larvae (Waldock and Nascimento, 1979; Waldock and Holland, 1984; Delaunay et al., 1993) and juveniles (Langdon and Waldock, 1981; Hurd et al., 1989; Parrish et al., 1995; Knauer and Southgate, 1997). Many authors have studied the effect of vitamin E on growth and immune response in various organisms but studies related to effects of vitamin E on growth, survival and haematocrit in ornamental fishes are scanty. Hence, the present study was undertaken to study the effect of different levels of dietary vitamin E and HUFA on growth, survival and haematocrit in the goldfish, *Carassius auratus gibellio*.

MATERIALS AND METHODS

Experimental diets

Ten experimental diets having different levels of vitamin E and HUFA were formulated and manufactured in the laboratory

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Table 1. Ingredient (g /100 g diet) and chemical proximate composition (% Dm[1]) of experimental diets.

Ingredient	E50+ HUFA	-E + HUFA	-E- HUFA	E100+ HUFA
Corn meal	6	6	6	6
Fish meal	20.5	20.5	20.5	20.5
Soybean meal	38.5	38.5	38.5	38.5
Bread flour	10	10	10	10
Rice bran	18.75	18.75	18.75	18.75
Fish oil	0.5	0.5	-	0.5
Soybean oil	-	-	0.5	-
Mineral mixture[2]	2	2	2	2
Vitamin mixture[3]	2	2	2	2
Lysine	0.75	0.75	0.75	0.75
Metionin	0.75	0.75	0.75	0.75
Antifungi	0.25	0.25	0.25	0.25
Proximate composition				
Protein	39	39	39	39
Fat	10.8	10.8	10.8	10.8
Energy	4000	4000	4000	4000

Dm[1]: dry matter; Mineral mixture[2] (g/kg diet) according to delaHiguera et al. (1998): $Ca(PO4H2)2•H_2O(30)$, $CaCO_3$ (6.5), KCl (2.5), NaCl (4), $MnSO_4•H_2O$ (0.2), $FeSO_4•7H_2O$ (1.5), $MgSO_4$ (4.6), KI (0.02), $CuSO_4•5H_2O$ (0.05), $ZnSO_4•7H_2O$ (0.2), $CoSO_4•7H_2O$ (0.05), Na_2SeO_3 (0.218•10–2), $Al_2(SO4)_3•18H_2O$ (1•10–2); Vitamin mixture (mg/kg diet)[3]: 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).

according to goldfish nutritional requirements; the test diet were as follow: E100 + HUFA, -E + HUFA, -E - HUFA and E 50 + HUFA. Fish or soybean oil was used as a lipid source for +HUFA and -HUFA diets, respectively (Table 1). The general composition of each experimental diet is given in Table 1.

Experimental design

Goldfish (*C. auratus gibellio*), average 0.69±0.12 g 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, Iran. Prior to the start of the experiment, the goldfish were reared in 400 L fiberglass tanks for two weeks to acclimatize to the experimental diet and conditions. In the beginning of the experiment, the fish were fasted for 24 h and weighed. Fish of similar sizes were randomly distributed into 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 7.9 to 8.1.

At the termination of the experiment, the fish were fasted for 24 h before harvest. Blood sampling was carried out from the caudal vasculature with cutting peduncle. The blood was placed in tube containing heparin sodium, then transferred into a 2 ml micro centrifuge tubes and centrifuged for 15 min at 3000 g at 4°C.Haematocrit was read by haematocrit reader.

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 mg kg $diet^{-1}$) and HUFA (highly unsaturated fatty acid) were selected to investigate the effects of them on growth, survival and haematocrit of goldfish.

Analysis and measurement

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; it 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 arcsine transformation. Data are presented as treatment means ± SD. The values of $P<0.05$ were considered significantly different.

Table 2. Response of Gold fish to the various test diet after 10 weeks of feeding.

Treatment	E100+HUFA	E50+HUFA	-E-HUFA	-E+HUFA
SGR	0.19±0.02	0.21±0.004[a]	0.22±0.02[a]	0.18±0.027[a]
FCR	0.45±0.07[a]	0.37±0.01 [a]	0.39±0.05[a]	0.45±0.09[a]
CF	2.3±0.3[a]	2.02±0.4[a]	1.8±0.02[a]	2.01±0.2[a]
BWI	5.8±1.09[b]	6.5±0.41[b]	12.3±0.5 [a]	10.85±2.1[a]
Survival	100[a]	93.7±8.8[a]	100[a]	93.7±8.8[a]

SGR, Specific growth rate; FCR, feed conversion rate; CF, condition factor; BWI, body weight increase.

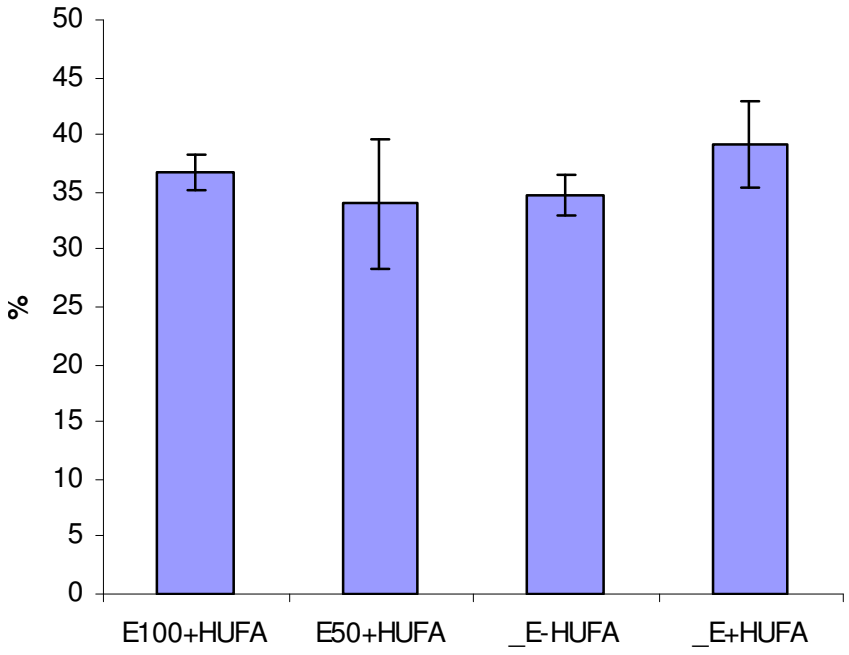

Figure 1. Haematocrit (%) in different treatments of goldfish.

RESULTS AND DISCUSSION

Growth factors are summarized in Table 2. Final weight in fish fed with the diets -E-HUFA, -E + HUFA was significantly higher compared with fish fed with the diet E_{100} + HUFA, E_{50} + HUFA ($P<0.05$). Feed conversion rate (FCR), specific growth rate (SGR) and condition factor (CF) and survival were not significant between groups ($P>0.05$). Haematocrit was not different between groups (Figure 1). Dietary vitamin E requirement of goldfish has been studied with various results. Many factors such as lipid level, source, and other antioxidants or antioxidant enzymes presented in the diet or body system may affect the dietary vitamin E requirement of fish. Satoh et al. (1987) reported that inclusion of 50 to 100 mg α-tocopherol/kg diet was sufficient for tilapia fed diets containing 5% lipid. Our results show that vitamin E addition to diet did not affect the growth of goldfish. Several reports have shown that the supplementation of vitamin E did not improve the growth performance of fish fed oxidized oil. Foster et al. (1988) reported that vitamin E addition to diets containing oxidized herring oil did not affect the growth performance of Coho salmon. Similar results were reported by Mourente et al. (2002) in gilthead sea bream. They found that addition of vitamin E in diets containing oxidized oil did not improve the growth and survival of sea bream although the hepatic antioxidant defense enzyme activities were significantly affected. In contrast, a report from (Baker and Davies, 1996) showed that dietary vitamin E supplementation in rancid diets significantly improved the growth of African catfish. In this study, addition of vitamin E was not significant on growth parameters. Many marine fish larvae are believed to require highly unsaturated fatty acids, especially EPA and DHA (Watanabe, 1982; Sargent et al., 1989, 1995). The nutritional value of high levels of (n-3) HUFA was confirmed by enhanced growth in many marine fish larvae such as turbot *Scophthalmus maximus* (Gatesoupe and Le Milinaire, 1985), Japanese flounder *Paralichthys olivaceus*, Red sea bream, *Pagrus*

major (Izquierdo et al., 1989), and gilthead sea bream, *Sparus aurata* (Koven et al., 1993; Rainuzzo et al., 1997). But in present study, HUFA addition did not have effect on growth, survival, SGR, FCR, CF. Addition of vitamin E and HUFA has no effect on growth parameter and haematocrit. In our opinion, addition of vitamin E and highly unsaturated fatty acids in to diets of goldfish is good for experimental fish but their effects were not significant; suggesting that other author use higher doses of vitamin E such as 200-300-500,…, also they use other source of oil in the diet and they can detect effect of dietary vitamin E and HUFA on other blood parameters, reproduction, etc.

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