

Effect of vitamin E and highly unsaturated fatty acids supplementation on sperm quality of goldfish (*Carassius auratus*)

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Abstract. The effects of dietary vitamin E and highly unsaturated fatty acid (HUFA) supplementation on sperm quality was studied in goldfish (*Carassius auratus* (Linnaeus, 1758)), for one year. Fish fed experimental diets had no significant differences in sperm concentration, spermatocrit, motility duration and percent motility of each sperm ($P > 0.05$). Fish fed with E100+HUFA had the highest sperm concentration and highest spermatocrit. Motility duration and percent motility of each sperm were not significantly different ($P > 0.05$) although the control group had the lowest value.

Key words: vitamin E, HUFA, sperm, motility, goldfish.

چکیده: اثرات غذایی ویتامین E و اسیدهای چرب غیر اشباع بر کیفیت اسپرم ماهی قرمز (*Carassius auratus*) (Linnaeus, 1758) در طی یکسال مطالعه شد. ماهیان تغذیه شده با جیره های آزمایشی تفاوت معنی داری را در تراکم اسپرم، اسپرماتوکریت، طول دوره تحرک و درصد اسپرم های متحرک نشان ندادند ($P > 0/05$). ماهیان تغذیه شده با جیره E100+HUFA بالاترین تراکم اسپرم و اسپرماتوکریت را داشتند. طول دوره تحرک و درصد اسپرم متحرک در بین تیمارهای آزمایشی تفاوت معنی داری نداشتند ($P > 0/05$) اما گروه شاهد کمترین میزان را داشت.

کلمات کلیدی: ویتامین E، HUFA، اسپرم، دوره تحرک، ماهی قرمز

Introduction. Vitamin E activity is present in a group of naturally occurring closely related tocopherols. Among them, α -tocopherol has the highest vitamin E activity. DL- α -tocopherol acetate, a stable vitamin of α -tocopherol, is the most commonly used form in animal feeds (NRC 1983). As a fat-soluble antioxidant, the major function of vitamin E is to prevent peroxidation of polyunsaturated fatty acids of phospholipids and cholesterol in cellular and sub cellular membranes. Vitamin E has been known to improve the reproductive performance of fish. Vitamin E deficiency hindered ovarian growth in common carp (*Cyprinus carpio* Linnaeus, 1758) and led to a reduction in spawning success (Watanabe & Takashima 1977).

Another important dietary component are the highly unsaturated fatty acids (HUFAs). Marine fish generally lack, or have low activity of the desaturase necessary to synthesize n-3 HUFAs from C18 fatty acids. Therefore, n-3 HUFAs are considered essential fatty acids (EFA) in the diets of marine fish as they are required for normal growth and survival. High dietary levels of HUFA, however, can negatively affect fish growth as HUFAs are readily oxidized by reactive oxygen species to lipid peroxides (Porter et al 1995) and HUFA tend to be less available for energy (Murata 1983).

Many authors have studied the impact of vitamin E on sperm and egg quality and reproduction (Canyurt & Akhan 2008), but studies related to dietary vitamin E requirement, fish performance and reproduction in ornamental fishes are scanty (most notable of them being that of Mehrad & Sudagar 2010 and Kashani et al 2010). Hence, the present research was undertaken to study the effect of different levels of dietary vitamin E and HUFA on sperm quality in the goldfish, *Carassius auratus* (Linnaeus, 1758), being similar to the one of Canyurt & Akhan (2008).

Material and Method

Fish and maintenance: 160 goldfish with an average of 0.69 ± 0.12 g initial weight were divided into 4 groups corresponding combinations of vitamin E (0, 50, 100mg kg diet⁻¹) and HUFA. Each diet was tested in triplicate groups of fish that reared in fiberglass tanks. Temperature ranged from 21.5 to 22 °C; the pH was approximately 7.9 to 8.1.

Feed and feeding: the diets were formulated and manufactured in the laboratory according to goldfish nutritional requirements; the test diets were as follow: E100+HUFA, E50+HUFA, -E+HUFA, -E-HUFA. Vitamin E inclusion levels were 0, 50 or 100 mg kg diet⁻¹ (vitamin E used as α -tocopherol). Fish or soybean oil was used as a lipid source for +HUFA and -HUFA diets. The general composition of each experimental diet is given in Table 1. Fish were fed three times a day for one year.

Table 1

Ingredient (g 100g diet⁻¹) and chemical proximate composition
(% Dm) of experimental diets

<i>Ingredient</i>	<i>E50</i> + <i>HUFA</i>	<i>-E</i> + <i>HUFA</i>	<i>-E</i> - <i>HUFA</i>	<i>E100</i> + <i>HUFA</i>
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
Vitamin mixture	2	2	2	2
Lysine	0.75	0.75	0.75	0.75
Metionin	0.75	0.75	0.75	0.75
Anti fungi	0.75	0.75	0.75	0.75

Dm: dry matter.

Mineral mixture (g kg diet⁻¹): 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).

Vitamin Mixture (mg kg diet⁻¹): 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).

Estimation sperm quality of goldfish. We split males and females five days prior to hormonal injection for sperm release. Male fish of each tank were selected and stripped of all milt in a dry and a cool glass vial. Before stripping, the anal regions of the fish were cleaned by a dry towel. The anal and seminal ducts were emptied to avoid the samples contamination with urea and faeces. All sperm manipulations were performed on ice. Semen sampled for quality estimates was used within 6-8 h after stripping. Micro haematocrit tubes (75mm length, 1.1-1.2 internal diameter) were used for determination of spermatocrit. The triplicate tubes for each sample were filled up with milt and one side of the tubes was covered with glass putty and plaster. They were centrifuged 3000g for 8 min and measured using a haematocrit reader (Fitzpatrick et al 2005).

In order to estimate motility, the sperm was diluted using saline solution to a ratio of 1:2000. After that, it was put on the stereomicroscope device (microscop equipped with a CCD camera attached to computer, Panasonic WV-CP240, Japan) (Cosson et al 2000) with magnification number x10 to measure the duration sperm movement and percentage of milt sperms (time started in less than 7 seconds) and a digital camera recorded the sperm movement by high resolution. The shelf life of sperm for each prototype was measured by stopwatch. The stopwatch started to work once milt

was activated by water and stopped when sperm movement stopped (Leach & Montgomery 2000; Turner & Montgomerie 2002). Then adobe primer software (version 6) was used to get pictures every 10, 20, 30, 40 seconds after the activation of sperms. These pictures changed to 30 formes (slides) and we randomly selected four forms (i.e. 1, 4, 7 and 10). Positions of 10 spermatozoa in these randomly selected pictures and also the percentage of motile sperms were calculated.

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).

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. The values of P<0.05 were considered significantly different.

Results and Discussion. After feeding fish with experimental diets motility duration and percent motility of each sperm were not significant across treatments (P>0.05) (Fig. 1), although the control group tended to be lower than other groups. Spermatocit and sperm concentration were not different (P>0.05) in treatment groups but fish fed with E100+HUFA had highest sperm concentration and highest spermatocrit.

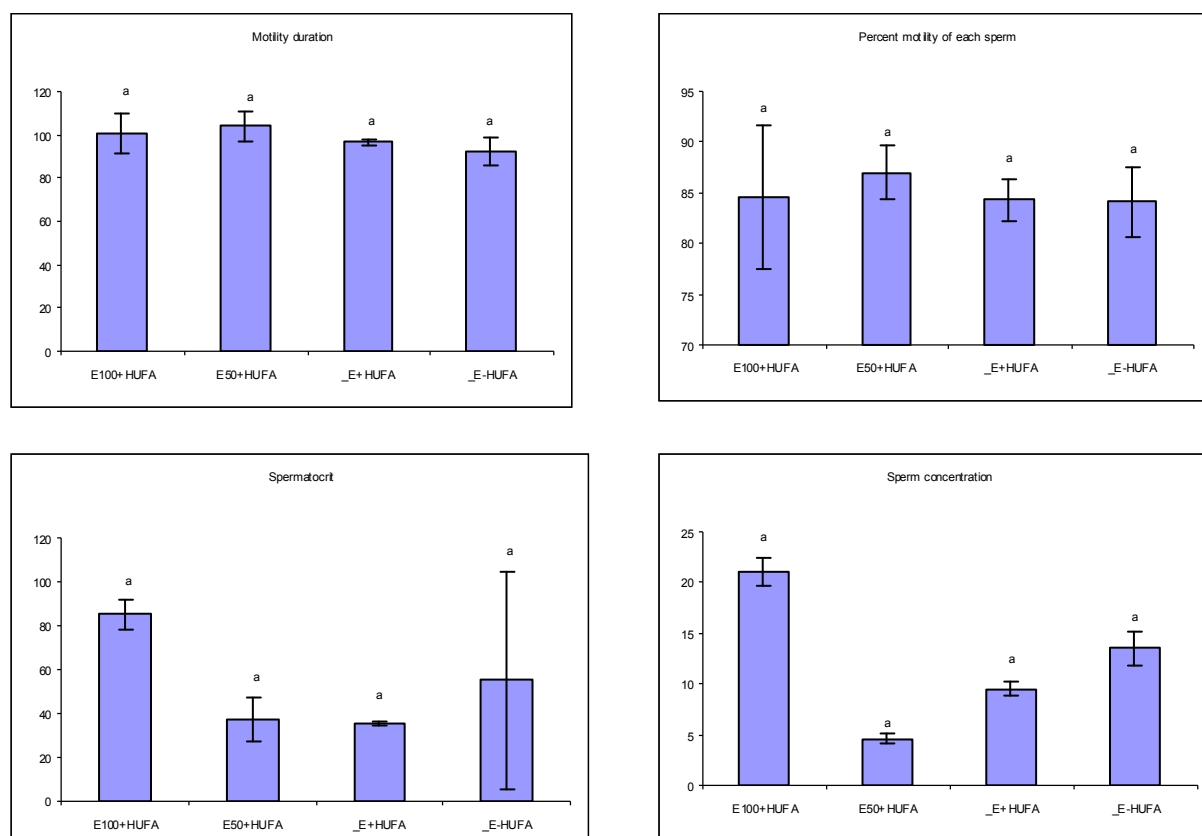


Figure 1. A – Sperm motility duration (s) in different treatments of goldfish;
 B – Percentage motility of sperm (%) in different treatments of goldfish;
 C – Spermatocrit (%) in different treatments of goldfish;
 D – Sperm concentration in different treatments of goldfish.

The study shown that vitamin E and HUFA had no significant influences on sperm quality such as spermatocrit, sperm concentration, motility duration and percent motility of each

sperm in goldfish. Fish fed with diets supplemented with 50 mg kg⁻¹ diet vitamin E and HUFAs showed a trend toward highest motility duration and percent motility of each sperm. This experiment confirms the results of Canyurt & Akhan (2008), which suggested there were no significant differences between the control group and rainbow trout (*Oncorhynchus mykiss* (Walbaum, 1792)) fed with 0.03% vitamin E. *C. auratus* is a continuous spawner with a short vitellogenic period (James & Sampath 2003, 2004). A low level of vitamin E probably reduces gonad development and fecundity. The significance of vitamin E in fish reproduction was confirmed in earlier studies. In a study of the effects of vitamin E and growth hormone on gonadal maturity in the common carp, dietary vitamin E resulted in a higher gonadosomatic index, larger ova, and more eggs with higher hatchability than the control (Gupta et al 1987). On the other hand, beneficial effects of dietary vitamin E on fish reproduction were not observed in some other studies.

Since sperm fatty acid composition depends on the essential fatty acid content of brood stock diet in species such as rainbow trout (Watanabe et al 1984; Labbe et al 1993) it is possible that sperm motility and in turn fertilization would be affected. Dietary eicosapentaenoic (EPA) and arachidonic acid (AA) levels show a correlation with fertilization rates in gilthead sea bream brood stock (Fernandez-Palacios et al 1995). However, in this study, HUFA affected motility duration and percent motility of each sperm in goldfish but they were not significantly higher ($P>0.05$).

Spermatocrit and sperm concentration were not significant different across treatments. Spermatocrit is directly related to sperm concentration, and so spermatocrit values fit to sperm concentrations of the groups respectively. A direct relationship between sperm density and spermatocrit of fish sperm has been established in coho salmon - *Oncorhynchus kisutch* (Walbaum, 1792) (Bouck & Jacobson 1976), Atlantic salmon - *Salmo salar* Linnaeus, 1758 (Piironen & Hyvrinen 1983) and Atlantic halibut, *Hippoglossus hippoglossus* (Linnaeus, 1758) (Tvedt et al 2001).

Conclusions. Our research results did not show a negative effect of vitamin E on male goldfish. In our study, vitamin E was effective but it was not significant even if control group had lower quality than treatment groups. Vitamin E influenced the reproductive performances of goldfish as much as other authors reported in cases of other fish species.

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