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

Zeinab Hanaee Kashani, Mohammad R. Imanpoor, Ali Shabani, and Saeed Gorgin

Department of Fishery, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran. Corresponding author: Z. Hanaee Kashani, Z.h.kashani@gmail.com

Abstract. The aim of the work was to determine the effects of three different doses of ascorbic acid (0, 100, 1000 mg kg diet^{-1}) and highly unsaturated fatty acid (HUFA). They were evaluated on sperm quality of goldfish. The results of the study shown that vitamin C and HUFA have no significant ($P>0.05$) effect on sperm concentration, spermatocrit and sperm motility duration. Percentage motility of sperm in fish fed with diet C1000+HUFA was significantly higher than other groups ($P<0.05$) and there were no significant differences between other groups.
Key Words: vitamin C, sperm quality, HUFA, goldfish.

چکیده : هدف از این کار تعیین اثرات سه دوز مختلف از اسید اسکوربیک (0.100.1000) و اسیدهای چرب غیر اشباع بود. این مواد روی کیفیت اسپرم گلدفیش ارزیابی شدند. نتایج این مطالعه نشان داد که ویتامین C و HUFA تاثیر معني داري روي تراکم اسپرم، اسپرماتوکریت و طول دوره تحرک اسپرم ندارند ($P>0/05$). درصد اسپرم متحرک در ماهیان تغذیه شده با جیره HUFA + C_{1000} بطور معني داري بالاتر از سایر تیمارها بود ($P<0/05$) و تفاوت معني داري بین سایر گروه ها مشاهده نشد.

کلمات کلیدی: ویتامین C، کیفیت اسپرم، اسیدهای چرب غیر اشباع و گلدفیش.

Introduction. There are several agents affecting gamete quality in fish such as environmental conditions and genetic factors. Nutrition and content of feed (fatty acids, amino acids, minerals and vitamins) are environmental cues that influence reproduction in fish (Bromage 1995; Izquierdo et al 2001; Mehrad & Sudagar 2010). Acid ascorbic (AA) also plays a critical role on gamete quality of fish like other vertebrates (Ciereszko & Dabrowski 2000). Long term feeding, using diets without AA resulted in decreases of sperm concentration, motility and fertilizing ability, and an increase in sperm lipid peroxidation value (Ciereszko & Dabrowski 1995; Ciereszko et al 1996; Dabrowski & Ciereszko 1996; Liu et al 1997). Positive effect of AA on sperm quality of men was demonstrated (Dawson et al 1992). Antioxidants such as AA protect germ cells against to DNA damage and oxidation seminal plasma proteins with reactive oxygen radicals (Fraga et al 1991; Liu et al 1995).

Since sperm fatty acid composition depends on the essential fatty acid content of brood stock diet in species such as rainbow trout (*Oncorhynchus mykiss* (Walbaum, 1792)) (Watanabe et al 1984; Labbe et al 1993) and it is possible that sperm motility and in turn fertilization would be affected. Particularly in salmonids, where cryopreservation of sperm is currently utilized, sperm fatty acid composition could be a factor that determines the membrane integrity after freeze-thawing. However, Labbe et al (1993) did not find any effect of dietary fatty acids n-3 and polyunsaturated fatty acids n-6 on sperm freeze–thaw fertilizing ability, whereas low membrane cholesterol–phospholipids ratios were correlated with a better sperm freezing resistance (Labbe & Maisse 1996).

Material and Method. A number of 160 goldfish (*Carassius auratus* (Linnaeus, 1758)), average 0.69±0.12 g initial weight, from larval stage to broodstock 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 Resources.

Fish were fed with diets containing different level of vitamin C and highly unsaturated fatty acid: C100+HUFA, C1000+HUFA, -C-HUFA, -C+HUFA, C100-HUFA, C1000–HUFA.

The general composition of each experimental diet is given in Table 1.

Table 1

Ingredient (g 100g diet^{-1}) and chemical proximate composition (% Dm) of experimental diets

Ingredient	*-C + HUFA*	*-C - HUFA*	*C100 + HUFA*	*C100 - HUFA*	*C1000 - HUFA*	*C1000 + HUFA*
Corn meal	6	6	6	6	6	6
Fish meal	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
Bread flour	10	10	10	10	10	10
Rice bran	18.75	18.75	18.75	18.75	18.75	18.75
Fish oil	0.5	-	0.5	-	-	0.5
Soybean oil	-	0.5	-	0.5	0.5	-
Mineral mixture	2	2	2	2	2	2
Vitamin mixture	2	2	2	2	2	2
Lysine	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
Anti fungi	0.75	0.75	0.75	0.75	0.75	0.75

Dm: dry matter.

Mineral mixture (g kg^{-1} diet): $Ca(PO_4H_2)2{\cdot}H_2O(30)$, $CaCO_3$ (6.5), KCl (2.5), NaCl (4), $MnSO_4{\cdot}H_2O$ (0.2), $FeSO_4{\cdot}7H_2O$ (1.5), $MgSO_4$ (4.6), KI (0.02), $CuSO_4{\cdot}5H_2O$ (0.05), $ZnSO_4{\cdot}7H_2O$ (0.2), $CoSO_4{\cdot}7H_2O$ (0.05), Na_2SeO_3 (0.218·10−2), $Al_2(SO_4)3{\cdot}18H_2O$ (1·10−2).

Vitamin mixture (mg kg^{-1} 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).

Fish were fed 3 times a day with experimental diets for 1 year. After that male goldfish were selected randomly from each treatment (totally 23 males) 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 contamination of samples with urea and faeces. All sperm manipulations were performed on ice. Semen sampled for quality estimates was used within 6-8 h after stripping. In order to estimate spermatozoa concentration, milt was diluted with saline solution and counted.

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 reder. Sperm was diluted using saline solution a ratio of 1:2. Ten microliters of diluted semen were placed in a bovine serum albumin-coated microscope slide. The percent motility of each sperm sample was estimated using light microscopy at x400 magnification immediately after addition of 20 mL of water used as an activating solution (Figure 1). Motility was determined as the percentage of sperm actively moving forward. Ten microlitres of diluted semen was activated under x100 magnification to determine the motility duration. The time interval from 5 s after activation of sperm to when all the sperm stopped was considered to be the motility duration. 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).

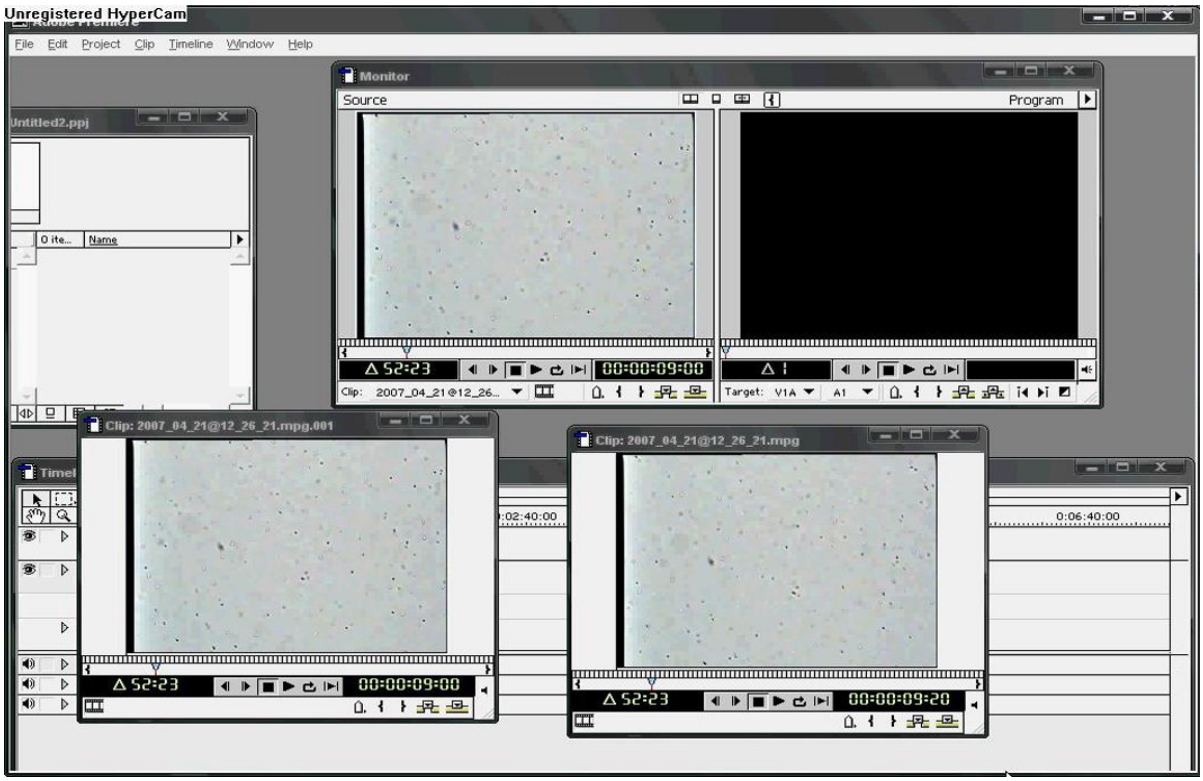

Figure 1. Stereomicroscopic images analyze by special soft.

All data were subjected to one-way analysis of variance. The significance of difference between means was determined using Duncan's multiple range test ($P<0.05$) using SPSS for WINDOWS (Version 16.0). Values are expressed as means ± SE.

Results and Discussion. Sperm concentration, spermatocrit values, motility duration were not significantly different between groups ($P>0.05$). There were significant differences in the percent motility of sperm ($P<0.05$). Highest percent motility of sperm was detected in fish fed with the diet with C1000+HUFA. There was no significant difference between groups (Figure 2).

The present study show that addition of vitamin C and HUFA have no effect on sperm concentration, spermatocrit values and motility duration of goldfish males. Goldfish males given 1000 mg kg diet^{-1} AA supplemented diet for 12 months shown the best percent motility of sperm among C1000+HUFA groups. Although the AA mechanism of how to effect sperm number in animals is not known clearly, there are several researches that reported positive effect of AA on sperm concentration in human and animals. Canyurt & Akhan (2008) suggested that additional AA is essential for male rainbow trout brood stock. AA positively affected some parameters of sperm quality in rainbow trout, such as sperm concentration, motility and spermatocrit values. They showed that motility increased with AA supplementation, but our study revealed that AA has no effect on this parameter. In this study, HUFA has no effect on sperm quality parameters in treatment groups except percent motility of sperm. Addition HUFA with 1000 mg kg diet^{-1} increased percent motility of sperm. 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 by HUFA content.

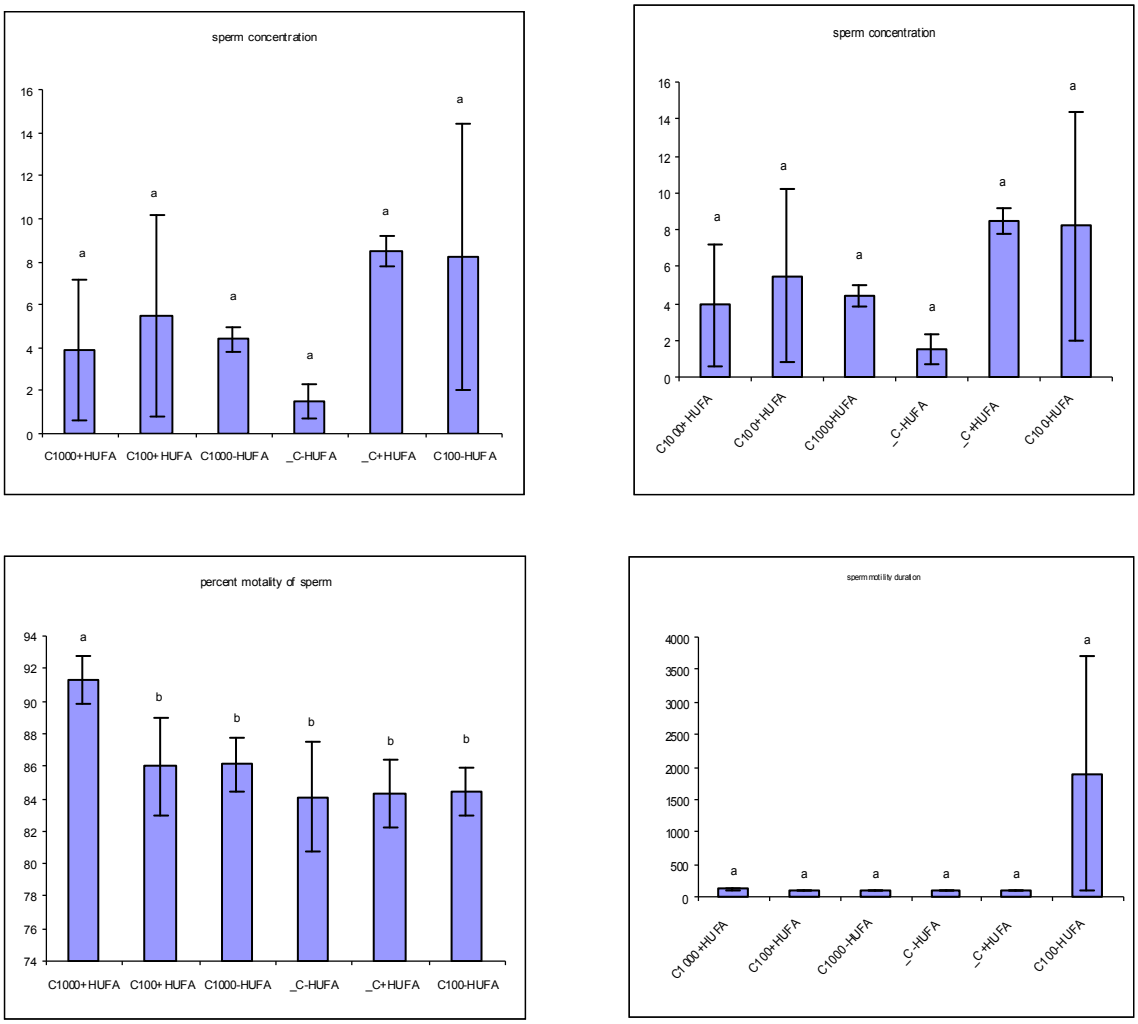

Fig. 2. A – Spermatocrit (%) in different treatment groups of goldfish; B – sperm concentration in different treatment groups of goldfish; C – percentage motility of sperm (%) in different treatment groups of goldfish; D – sperm motility duration (s) in different treatment groups of goldfish.

Conclusions. The antioxidant capacity of L-ascorbic acid (LAA) effects sperm concentration, motility and fertilizing ability positive protective role of LAA on male germ cells are known, but later researches may be designed to explain how LAA increase sperm concentration in male goldfish. Generally LAA increase the maturation of germ cells which lead to increase milt quality such as sperm concentration, motility duration and fertility. In conclusion, the antioxidant capacity of L-ascorbic acid positively effects sperm concentration, motility and fertilizing ability.

Results of this study showed that additional AA supplementation is useful for improving percent motility of sperm. Protective role of AA on male germ cells are known, but later researches may be designed to explain how AA increase sperm viability in male goldfish.

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Authors:
Zeinab Hanaee Kashani, Department of Fishery, Gorgan University of Agricultural Sciences and Natural Resources, Iran, Golestan, Gorgan, Shahid Beheshti Avenue, Postal code: 49138-15739, e-mail: z.h.kashani@gmail.com
Mohammad Reza Imanpoor, Department of Fishery, Gorgan University of Agricultural Sciences and Natural Resources, Iran, Golestan, Gorgan, Shahid Beheshti Avenue, Postal code: 49138-15739, e-mail: mrimanpoor@yahoo.com
Ali Shabani, Department of Fishery, Gorgan University of Agricultural Sciences and Natural Resources, Iran, Golestan, Gorgan, Shahid Beheshti Avenue, Postal code: 49138-15739, e-mail: ali_shabani@yahoo.com
Saeed Gorgin, Department of Fishery, Gorgan University of Agricultural Sciences and Natural Resources, Iran, Golestan, Gorgan, Shahid Beheshti Avenue, Postal code: 49138-15739, e-mail: s_Gorgin@gmail.com
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