World Journal of Fish and Marine Sciences 4 (2): 131-135, 2012
ISSN 2078-4589

DOI: 10.5829/idosi.wjfms.2012.04.02.62165

Effects of Dietary Vitamin C and E and Highly Unsaturated Fatty Acid on Biological Characteristic of Gonad, Hatching Rate and Fertilization Success in Goldfish (*Carassius auratus Gibelio*)

Zeinab Hanaee Kashani, Mohammad Reza Imanpoor, Ali Shabani and Saeed Gorgin

Department of Fishery, Gorgan University of Agricultural Sciences and Natural Resources, Iran

Abstract: A number of biological characteristics of gonad (biological characteristics of egg, hydrated egg, gonad somatic index and gonad's weight), fertilization success and hatching rate determined in 160 female Gold fish in spring of the 2010. The results of the study showed that vitamin C and E and HUFA have significant effect on ovum diameter and Gonad somatic index ($P<0.05$). Fish fed with diets C_{1000}+HUFA, C_{100}+HUFA, C_{100}-HUFA and C_{100}+E_{1000}+HUFA had highest ovum diameter and fish fed with diet C_{100}+E_{1000}+HUFA had lowest value. Surface to volume ratio and hydrated egg diameter were not significant ($P>0.05$) among treatments. Fertilization success and hatching rate were significant ($P<0.05$) and fish fed with C_{1000}+HUFA, C_{100}+HUFA, C_{100}+E_{1000}+HUFA and E_{100}+HUFA had shown the highest ratio and fish fed with C-E+HUFA and E-C-HUFA had the lowest values among treatments.

Key words: Gold Fish % Vitamin C % Vitamin E % Egg

INTRODUCTION

Fish is an important source of n-3 HUFA (highly unsaturated fatty acids) and thus there is a great interest in the beneficial aspects of the consumption of these fatty acids for human health [1, 2]. Moreover, it is known that the tissue fatty acid profile depends on dietary lipid content [3- 6]. This would favour an imbalance between available antioxidant defenses and free-radical production, which could increase lipid oxidation [7-12] and thereby affect the fatty acid profile and, ultimately, the quality of cultured fish for human consumption.

Vitamins E and C protect biological molecules from oxidation, vitamin E requirements being directly related to dietary HUFA levels since they are fatty acids highly prone to oxidation [13-16]. The important antioxidant function of vitamin C can indirectly affect lipid protection, since vitamin E regeneration from its oxidized form is vitamin C dependent [17, 18]. Also, it has been reported that vitamins E and C as well as HUFA have a positive modulator effect on stress response [19-20].

MATERIALS AND METHODS

Experimental Diets: The ingredients and the nutrient composition of the experimental diets are given in Table 1. The 10 dietary treatments consisted of a E_{100}+HUFA, E_{50}+HUFA, -C-E+HUFA, -C-E-HUFA, C_{100}+E_{1000}+HUFA, C_{100}+E_{1000}-HUFA, C_{1000}+HUFA, C_{100}+HUFA, C_{1000}-HUFA, C_{100}-HUFA. The ingredients were mixed with together and were stored at -20°C until fed.

Fish and Feeding Trial: 160 goldfish with an average of 0.69±0.12 g initial weight were divided into 10 groups corresponding combinations of vitamin E (0, 50, 100mg kg diet^{-1}), vitamin C (0, 100, 1000 100 mg kg diet-1) and HUFA and kept at the aquaculture laboratory at the Gorgan University of Agricultural Sciences and Natural Resources, Iran. 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. Fish were hand-fed to apparent satiation (5% of the wet biomass) tree times a day (0600, 1200 and 1800 h) for 1 year.

Corresponding Author: Zeinab Hanaee Kashani, Department of Fishery, Gorgan University of Agricultural Sciences and Natural Resources, Iran. Tel: +989112755359.

Table 1: Ingredient (g /100g diet) and chemical proximate composition (% Dm*) of experimental diets

Parameter	Corn meal	Fish meal	Soybean meal	Bread flour	Rice bran	Fish oil	Soybean oil	Mineral mixture[I]	Vitamin mixture[II]	Lysine	Metionin	Anti fungi
C_{100}+E_{1000}+HUFA	6	20.5	38.5	10	18.75	0.5	-	2	2	0.75	0.75	0.25
C_{100}+E_{1000}-HUFA	6	20.5	38.5	10	18.75	-	0.5	2	2	0.75	0.75	0.25
C_{100}+HUFA	6	20.5	38.5	10	18.75	0.5	-	2	2	0.75	0.75	0.25
C_{100}-HUFA	6	20.5	38.5	10	18.75	-	0.5	2	2	0.75	0.75	0.25
C_{1000}+HUFA	6	20.5	38.5	10	18.75	0.5	-	2	2	0.75	0.75	0.25
C_{1000}-HUFA	6	20.5	38.5	10	18.75	-	0.5	2	2	0.75	0.75	0.25
E_{100}+HUFA	6	20.5	38.5	10	18.75	0.5	-	2	2	0.75	0.75	0.25
E_{50}+HUFA	6	20.5	38.5	10	18.75	0.5	-	2	2	0.75	0.75	0.25
-C-E+HUFA	6	20.5	38.5	10	18.75	0.5	-	2	2	0.75	0.75	0.25
-C-E-HUFA	6	20.5	38.5	10	18.75	-	0.5	2	2	0.75	0.75	0.25
Proximate composition												
Moisture 6												
Protein 39												
Lipid 10.8												

*Dm: dry matter. [I]Mineral mixture (g kg^{-1} diet): $Ca(PO_4H_2)2•H_2O(30)$, $CaCO_3$ (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), $Al_2(SO_4)3•18H_2O$ (1•10-2).

[II]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).

The biomass tanks were weighed every fortnight, in order to update the daily ration. The natural photoperiod was used.

Estimation Surface-to-volume Ratio, Gonad Somatic Index, Hatching Rate and Fertilization Rate: We split males and females five days prior to hormonal injection for sperm and ovum release. Male and female fish of each tank were selected and stripped. After each female stripped separately, attaining ovum fertilized with mixed sperm and the fertilization success was determined. For determination of fertilization success, due to the thick chorionic layer, fertilized eggs placed in acetic acid for 10 minutes [21]. Then, under a loop equipped with ocular micrometer (with the accuracy of 100 micrometer), the amounts of fertilized eggs determined. Before fertilization, some ovum were taken and after fertilization some hydrated eggs were taken for evaluating the diameter of ovum and hydrated eggs [22]. The surface-to-volume ratio, S/V, was calculated with the following formula [23]:

$$S = 4 : r, V = 4/3 : r^3$$

In the formula, S is surface, V is volume and r is ovum and hydrated diameter.

Gonad somatic Index was calculated with the following formula:

$$\%GSI= WG (Wt\times100)$$

At the end of the experiment, fertilized eggs transferred to aquarium with clean water and good aeration in order to hatching. Upon the hatching, hatching rate was measured. In order to determine the hatching rate of each broodstock, the number of fertilized eggs which transferred to aquarium calculated using the following formula: number of egg = number of egg in gram × the weight of all attaining eggs in gram [24]. Hatching success is calculated by dividing the number of larvae by the ovum number, according to the following formula [24]:

$$\text{Hatching success= number of larvae / number of ovum}\times100.$$

Analytical Methods: 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 [25]. All analyses were performed in triplicate.

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.

Table 2: biological factors of gonad in female Goldfish

Parameter	S/V	Ovum diameter	GSI	hydrated egg diameter
C_{100}+E_{1000}+HUFA	2.31±0.08	1.21±0.26^{a}	6.28±4.08^{a}	1.30±0.14
C_{100}+E_{1000}-HUFA	2.26±0.06	1.07±0.007^{b}	8.1±5.7^{a}	1.32±0.3
C_{100}+HUFA	2.06±0.25	1.50±0.28^{a}	7.1±4.3^{abc}	1.46±0.18
C_{100}-HUFA	2.25±0.08	1.14±0.18^{a}	3.54±3.2^{c}	1.33±0.05
C_{1000}+HUFA	2.15±0.22	1.17±0.18^{a}	7.5±4.6^{ab}	1.40±0.14
C_{1000}-HUFA	-	-	2.67±1.8^{bc}	-
E_{100}+HUFA	2.1±0.06	1.09±0.01^{b}	3.26±1.4^{c}	1.37±0.04
E_{50}+HUFA	2.06±0.05	1.08±0.02^{b}	2.8±1.9^{c}	1.45±0.04
-C-E+HUFA	2.02±0.01	0.99±0.01^{b}	2.26±.22^{bc}	1.34±0.01
-C-E-HUFA	2.1±0.01	0.99±0.01^{b}	2.1±0.07^{abc}	1.38±0.08

Table 3: Hatching ratio and Fertilization success of gold fish fed with experimental diets

parameter	Hatching ratio	Fertilization success
C_{100}+E_{1000}+HUFA	97.5±2.5^{a}	96.5±1.5^{a}
C_{100}+E_{1000}-HUFA	87.5±7.5^{ab}	96.5±1.5^{a}
C_{100}+HUFA	97.5±2.5^{a}	98.5±1.5^{a}
C_{100}-HUFA	79±1.00^{ab}	80±19.00^{a}
C_{1000}+HUFA	97.5±2.5^{a}	99±1^{a}
C_{1000}-HUFA	-	-
E_{100}+HUFA	96.5±2.5^{a}	98.5±1.5^{a}
E_{50}+HUFA	60±20^{b}	72.5±22.5^{a}
-C-E+HUFA	0.00±0.00^{c}	0.00±0.00^{b}
-C-E-HUFA	0.00±0.00^{c}	0.00±0.00^{b}

RESULTS

Biological factors of gonad in female Goldfish fed with diets containing different levels of vitamin C and E and highly unsaturated fatty acid for 1 year are presented in Table 2.

There was significant differences in ovum diameter and Gonad somatic index ($P<0.05$). Diets with C_{1000}+HUFA, C_{100}+HUFA, C_{100}-HUFA and C_{100}+E_{1000}+HUFA led to fish that had higher ovum diameter. And fish fed with diets C_{100}+E_{1000}-HUFA, C_{100}+E_{1000}+HUFA had shown highest Gonad somatic index.

Surface to volume ratio and hydrated egg diameter were not affected by dietary vitamin C and E and highly unsaturated fatty acid ($P>0.05$).

Fertilization success and hatching rate were significant ($P<0.05$) and fish fed with C_{1000}+HUFA, C_{100}+HUFA, C_{100}+E_{1000}+HUFA and E_{100}+HUFA had shown highest ratio and fish fed with C-E+HUFA and E-C-HUFA had lowest value between treatments (Table 3).

DISCUSSION

Results of this experiment indicated that dietary vitamin C and E and highly unsaturated fatty acid was effective on some biological factors of gonad, hatching ratio and Fertilization success in female goldfish. In this experiment, egg diameter was not different between treatments. In this experiment, vitamin C and E improved egg quality. The effect of vitamin C on egg production in fish is not clear. [26] Find no significant increase in fecundity of rainbow trout brood stock fed a vitamin C-supplemented diet. Blom and Dabrowski [27] showed a significant increase in fecundity with graded levels of vitamin C. in this experiment, the enhancement of egg hatchability and fertilization success following dietary vitamin C and E and highly unsaturated fatty acids supplementation of fish brood stock are well known. Goldfish fed vitamin C and E and highly unsaturated fatty acids supplemented diets produced eggs with a significantly improved hatching rate than did fish fed the same diets without vitamin C and E and highly unsaturated fatty acids supplementation. These findings are agreeable with results of sandnes *et al*. [28] for rainbow trout. It was even demonstrated that vitamin C in brood stock diet could be transferred via the egg to the hatchlings to support vitamin C activity during early larval stages.

The significance of vitamin E in fish reproduction has been confirmed by several authors. In this experiment, diets with 100 and 1000 mg vitamin E/Kg diet have high Hatching ratio, Fertilization success and egg diameter. This finding is in agreement with takeuchi *et al*. and

Gammanpila *et al*. [29, 30]. They pointed out a reduced "-tocopherol level in broodstock diet of ayu resulted in low survival rates eggs to eyed stage and decreased hatching. It was concluded that these supplements acted as free radical scavengers and enhanced egg and larval quality.

REFERENCES

1. Kroes, R., E.J. Schaefer, R.A. Squire and G.M. Williams, 2003. A review of the safety of DHA45-oil. Food Chemistry and Toxicology, 41: 1433-1446.
2. Moreno, J.J. and M.T. Mitjavila, 2003. The degree of unsaturation of dietary fatty acids and the development of atherosclerosis (Review). J. Nutrition and Biochemistry, 14: 182-195.
3. Olsen, Y.A. and R.J. Henderson, 1997. Muscle fatty acid composition and oxidative stress indices of Arctic charr, *Salvelinus alpinus* (L.) in relation to dietary polyunsaturated fatty acid levels and temperature. Aquaculture Nutrition, 3: 227-238.
4. Olsen, R.E., E. Løvaas and Ø. Lie, 1999. The influence of temperature, dietary polyunsaturated fatty acids, a-tocopherol andspermine on fatty acid composition and indices of oxidative stress in juvenile Artic char, *Salvelinus alpinus* (L.). Fish Physiology and Biochemistry, 20: 13-29.
5. Mourente, G., E. Díaz-Salvago, D.R. Tocher and J.G. Bell, 2000. Effects of dietary polyunsaturated fatty acid/vitamin E (PUFA/tocopherol) ratio on antioxidant defense mechanisms of juvenile gilthead sea bream (*Sparus aurata* L., Osteichthyes, Sparidae). Fish Physiology and Biochemistry, 23: 337-351.
6. Montero, D., L.E. Robaina, J. Socorro, J.M. Vergara, L. Tort and M.S. Izquierdo, 2001a. Alteration of liver and muscle fatty acid composition in gilthead sea bream (*Sparus aurata*) juveniles held at high stocking density and fed an essential fatty acid deficient diet. Fish Physiology and Biochemistry, 24: 63-72.
7. Watanabe, T., 1987. Requerimientos de ácidos grasos y nutrición lipídica en peces. In: Espinosa de los Monteros, J., Labarta, U. (Eds.), Nutrición en Acuicultura II. CAICYT, Madrid, pp: 99-149.
8. Roberts, R.J. and A.M. Bullock, 1989. Nutritional pathology, In: Halver, J.E. (Ed.), Fish Nutrition, 2a ed. Academic Press, London, pp: 424-469.
9. Sargent, J., R.J. Henderson and D.R. Tocher, 1989. The lipids, In: Halver, J.E. (Ed.), Fish Nutrition, 2a ed. Academic Press, London, pp: 154-209.
10. Stéphan, G., J. Guillaume and F. Lamour, 1995. Lipid peroxidation in turbot (*Scophthalmus maximus*) tissue: effect of dietary vitamin E and dietary n-6 or n-3 polyunsaturated fatty acids. 1995. Aquaculture, 30: 251-268.
11. Morales, A.E., G. Cardenete, E. Abellán and L. García-Rejón, 2005. Stress-related physiological responses to handling in the common dentex (*Dentex dentex*). Aquaculture Research. 36: 33-40.
12. Martínez-Álvarez, R.M., A.E. Morales and A. Sanz, 2005. Antioxidant defenses in fish: biotic and a biotic factors. Reviews in Fish Biology and Fisheries, 15: 75-88.
13. Chow, C.K., 1991. Vitamin E and oxidative stress. Free Radical Biology and Medicine, 11: 215-232.
14. Stahl, W. and H. Sies, 1997. Antioxidant defense: vitamin E and C and carotenoids. Diabetes, S1: 4-8.
15. Gökkuů, C. and T. Mostafazadeh, 2003. Changes of oxidative stress in various tissues by long-term administration of vitamin E in hypercholesterolemic rats. Clinica Chimica Acta, 328: 155-161.
16. Udilova, N., D. Jurek, B. Marian, L. Gille, R. Schulte-Hermann and H. Nohl, 2003. Induction of lipid peroxidation in biomembranes by dietary oil components. Food Chemistry and Toxicology, 41: 1481-1489.
17. Upston, J.M., A.C. Terentis and R. Stocker, 1999. Tocopherol-mediated peroxidation of lipoproteins: implications for vitamin E as a potential antiatherogenic supplement. FASEB J., 13: 977-994.
18. Moreno, J.J. and M.T. Mitjavila, 2003. The degree of unsaturation of dietary fatty acids and the development of atherosclerosis (Review). J. Nutrition and Biochemistry, 14: 182-195.
19. Henrique, M.M.F., E.F. Gomes, M.F. Gouillou-Coustans, A. Oliva-Teles and S.J. Davies, 1998. Influence of supplementation of practical diets with vitamin C on growth and response to hypoxic stress of sea bream, *Sparus aurata*. Aquaculture. 161: 415-426.
20. Sakakura, Y., S. Koshio, Y. Iida, K. Tsukamoto, T. Kida and J.H. Blom, 1998. Dietary vitamin C improves the quality of yellowtail (*Seriola quinqueradiata*) seedlings. Aquaculture, 161: 427-436.

21. Imanpoor, M. and T. Bagheri, 2011. Correlations between Biochemical Factors of Coelomic Fluid with Biological Characteristics of Gonad, Fertilization Success, Hatching Rate and Larval Size in Caspian Kutum, (*Rutilus frisii Kutum*). World J. Fish and Marine Sciences, 3(2): 107-111.
22. Imanpoor, M. and T. Bagheri, 2011.The Correlation between Biochemical Compounds of Blood and Coelomic Fluid of Caspian Kutum (*Rutilus frisii Kutum)*, World J. Fish and Marine Sciences, 3(1): 16-20.
23. Imanpoor, M. and T. Bagheri, 2010. The Correlation between blood Biochemical factors with some Biological Characteristics of Gonad, Fertilization Success, Hatching Rate and Larval Size in in Caspian Kutum, (*Rutilus frisii Kutum*). World J. Fish and Marine Sciences, 5(4): 278-281.
24. Imanpoor, M. and T. Bagheri, 2010. Relationship between biological characteristics of egg with fertility success, hatching rate and larval size in female kutum, (*Rutilus frisii Kutum*). World J. Fish and Marine Sciences, 2(5): 404-409.
25. Tago, A., Y. Yamamoto, S. Teshima and A. Kanazawa, 1999. Effects of 1, 2-di-20:5-phosphatidylcholine (PC) and 1, 2-di-22:6-PC on growth and stress tolerance of Japanese flounder (*Paralichthys olivaceus*) larvae. Aquaculture, 179: 231-239.
26. (AOAC), 1995. 17th Edition, Association of Official Analytical Chemists (AOAC), Washington DC, 21: 447.
27. Sandnes, K., Y. ulgenes, O.R. breakkan and F. Utne, 1984. The effects of ascorbic acid in brood fish seed on reproduction of rainbow trout (*Salmo guirdneri*). Aquaculture, 43: 167-177.
28. Blom, J.H. and K. Dabrowski, 1995. Reproduction success of female rainbow trout (*Oncorhynchus mykiss*) in response to graded dietary ascorbyle monophosphat levels. Biology of Reproduction, 52: 1073-1080.
29. Takeuchi, M., S. Ishii and T. Ogino, 1981. Effect of dietary vitamin E on growth, vitamin E distribution and mortalities of fertilized eggs and fry in ayu, *Plecoglossus altivelis*. Bulletin of the Tokai Regional Fisheries Research Laboratory, 104: 111-122.
30. Gammanpila, M., A. Yakupitiyage and A.N. Bart, 2007. Evaluation of the effects of dietary vitamin C, E and zinc supplementation on reproductive performance of Nile tilapia (*Oreochromis niloticus*). Aquaculture Science, 12: 39-60.