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Effect of Dietary Vitamin C and Highly Unsaturated Fatty Acids on Some Biochemical Blood Parameters in Goldfish (*Carassius auratus gibelio*)

[1]Zeinab Hanaee Kashani, [2]Mohammad Reza Imanpoor, [2]Ali Shabani and [2]Saeed Gorgin

[1]Payamenor University of Gorgan, Iran
[2]Department of Fishery, Gorgan University of Agricultural Sciences and Natural Resources, Iran

Abstract: This study evaluated the influence of diets supplemented with 0, 100, 1000 mg kgG[1] of vitamin C (ascorbic acid or AA) and highly unsaturated fatty acids (fish oil and soy bean oil) on the physiological responses of goldfish fed for 1 year. The results of this study showed that vitamin C and HUFA have significantly ($P<0.05$) effect on some biochemical blood parameters. Fish fed with the diet C_{1000}-HUFA had highest cholesterol. The highest glucose concentration was observed in treatment C_{100}+HUFA. Total protein content was significant ($P<0.05$) and the lowest value was observed in C_{100}+ HUFA treatment.

Key words: Goldfish % Vitamin C % Blood Parameter % Highly Unsaturated Fatty Acids

INTRODUCTION

Vitamin C (ascorbic acid) is among the most important nutrients influencing the organism immune system and their supply can reduce fish mortality and improve performance [1-4]. Vitamin C is a potent antioxidant that offers protection against oxidative damage to various fish tissues [5].

Teleost fish unable to synthesize vitamin C [6] due to the lack of L-gulonolactone oxidase enzyme (GLO, EC 1.1.3.8), which catalyzes the conversion of L-gulonolactone into ascorbic acid [7]. Fish are also unable to synthesize á-tocopherol [8].

Although commercial fish feeds contain adequate amounts of this vitamin for fish growth in intensive culture systems, the vitamins C requirement for immunostimulation is much higher than the level required for prevention of deficiency signs [8-10]. The purpose of this study was assess the physiological responses when goldfish fed with diets supplemented with vitamin C (ascorbic acid) at 0, 100, 1000 mg kgG[1] for 1 year.

MATERIALS AND METHODS

Goldfish (0.69±0.12 g) were obtained from a farm from Agh ghala, Golestan, Iran and kept in tanks at the Aquaculture Laboratory, Aquaculture Research Center of Fisheries Department in Gorgan University of Agricultural Sciences and Natural Resource, Iran for two weeks to acclimatize to the experimental diets and conditions.

After the initial biometry, fish were distributed in 12 groups of 8 fish in 400-L fiberglass tanks. The experimental design consisted of sex treatments: C_{1000}+HUFA, C_{100}+HUFA, C_{1000}-HUFA, -C- HUFA, -C+HUFA and C_{100}-HUFA (Table 1). Each treatment had 3 replicates and fish were fed twice a day to apparent satiation during 1 year.

At the end of the experiment, the fish were fasted for 24 h. blood samples were drawn from the caudal vessel from seven fish per tank using heparinized syringes for determining blood parameters. Blood samples were centrifuged (for 15 min at 3000 rpm) and the plasma was used for determining glucose, total protein and cholesterol. The contents of glucose, total protein and cholesterol were measured by using commercial clinical investigation kits (Pars Azmun, Iran).

Water temperature, dissolved oxygen and pH were measured daily in each experimental tank, (Water Checker, Horiba U10, Japan). The average water temperature and pH in all treatments were 21.5 to 22°C, 7.9 to 8.1 respectively.

Corresponding Author: Zeinab Hanaee Kashani, Payamenor University of Gorgan, Iran
Tel: +989112755359.

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

Tretments	Corn meal	Fish meal	Soybean meal	Bread flour	Rice bran	Fish oil	Soybean oil	Mineral mixture?	Vitamin mixture|	Lysine	Metionine	Anti fungi
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
-C +HUFA	6	20.5	38.5	10	18.75	0.5	-	2	2	0.75	0.75	0.25
-C-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												

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 [11].

Statistical Analyze: 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.

RESULTS

Water quality parameters in the tanks during the experiment are shown in Table 2. DO, Temperature and pH) did not show any statistical difference throughout the rearing period. These values were within the limits considered to be suitable for production performance and health maintenance of goldfish.

The factors related to the blood parameter in goldfish fed with diet containing different levels of vitamin C and highly unsaturated fatty acids are presented in Table 3.

Cholesterol was significant between treatments ($P<0.05$) and fish fed with the diet C_{1000}-HUFA had highest cholesterol.

The highest glucose value was observed in treatment C_{100}+HUFA.

Total protein content showing a significance among goldfish fed the supplemented diets ($P<0.05$) and C_{100}+HUFA treatment had the lowest value between treatments.

Table 2: Water quality of tanks stocked with goldfish undergoing different treatments

Treatments	DO	pH	Temperature (°C)
C_{1000}+HUFA	0.5±6	8±0.08	0.14±21.7
C_{100}+HUFA	0.5±6	8±0.08	0.14±21.7
C_{100}-HUFA	0.5±6	8±0.08	0.14±21.7
C_{1000}-HUFA	0.5±6	8±0.08	0.14±21.7
-C-HUFA	0.5±6	8±0.08	0.14±21.7
-C +HUFA	0.5±6	8±0.08	0.14±21.7

Table 3: Effect of vitamin C and highly unsaturated fatty acid on biochemical blood parameters of goldfish

Parameter	Cholesterol	Glucose	Total protein
C_{1000}+HUFA	249.7^{ab}	$59.8±0.0^{c}$	$5.7±0.4^{a}$
C_{100}+HUFA	154.2^{c}	$120±30.56^{a}$	$3.5±0.2^{b}$
C_{1000}-HUFA	301.1^{a}	$112.8±1.9^{b}$	$6.5±0.8^{a}$
-C-HUFA	242.3^{abc}	$15.9±7.5^{d}$	$5.9±0.8^{a}$
-C +HUFA	183.6^{bc}	$65.6±3.06^{c}$	$5.8±0.7^{a}$
C_{100}-HUFA	264.4^{ab}	$45.6±2.41^{cd}$	$6.05±0.0^{a}$

DISCUSSION

Fish are frequently exposed to stressors under culture conditions, which cause a series of physiological responses, known as stress, which are divided in primary, secondary and tertiary responses [12,13]. Primary responses are mediated by the neuro-endocrine system with a fast release of stress hormones, catecholamine and cortisol into the fish circulatory system. Catecholamines (adrenaline and noradrenalin) are released and synthesized through cromafin cells and cortisol by the inter renal cells. Secondary responses are the various biochemical and physiological effects associated with stress and mediated by the stress hormones. Tertiary responses affect the animal as a whole, compromising growth, disease resistance and reproductive success.

The results of this study on biochemical parameters revealed that dietary of vitamin C influences total protein, cholesterol and glucose in gold fish. [14] Reflected that high vitamins C and E concentrations could stimulate protein production in fish, suggesting an important role of both vitamins in the modulation of plasma proteins. This study showed that plasma protein was significantly affected by dietary vitamin C and fish fed with dietary C_{1000}-HUFA had the highest total protein. But some results were obtained by Sealey and Gatlin [15] for hybrid striped bass, [16] for *Piaractus mesopotamicus* and Chen *et al.* [17] for golden shiner all fed diets supplemented with different levels of vitamins C and E didn't observe significantly effect on total protein, Cholesterol and glucose.

In fish, glucose concentration may vary greatly depending on the physiological status of the animal. According to [18], glucose plasma levels can increase, decrease, or keep constant under high plasma cortisol. In this experiment, the highest glucose was observed in treatment C_{100}+HUFA but [19] didn't observe significantly effect on starlet fed different level of vitamin C and E.

Generally, it is assumed that the nutritional state of a fish can affect the animal health and possibly the way they deal with stress. The stress response in fish is generally mediated by a neuroendocrine response, which includes the release of stress hormones such as cortisol and catecholamines into the circulatory system [20, 21]. These and possibly other hormones, elicit several compensatory physiological responses that help the fish to deal with the stressor. Glucose is one of the most important energy substrates used by fish to cope with physiological stress and therefore plasma glucose levels have been used as an indicator of the stress response. It was demonstrated that cortisol and glucose could increase in teleost exposed to stress [18].

The results of present study showed that different levels of vitamin C used in our trial had significant effect on the cholesterol amount and highest value was in C_{1000}-HUFA treatment. In this study different level of HUFA didn't effect on blood biochemical parameter.

It had been documented that different factors are effective on the hematological and biochemical parameters of fishes, from which the species, environmental condition, age, maturation and nutrition are very important [22].

The obtained results of this study showed that dietary levels of vitamin C have influence on some of biochemical parameters of gold fish.

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