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RESEARCH ARTICLE

Effects of vitamins E and Riboflavin (B_2) and combinations of them on the hematological parameters of common carp, *Cyprinus carpio* L., fingerlings

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Abstract. In the present study, the effects of vitamins E, B_2 and combinations of them on hematological parameters of common carp, *Cyprinus carpio* L., were investigated during a 56-day experiment. The various dietary levels of vitamins E, B_2 and their combination were used as experimental treatments, as follows: T_1: vitamin E (80 mg), T_2: vitamin E (160 mg kg diet^{-1}), T_3: vitamin E (240 mg kg diet^{-1}), T_4: vitamin B_2 (7 mg kg diet^{-1}), T_5: vitamin B_2 (15 mg kg diet^{-1}), T_6: vitamin B_2 (20 mg kg diet^{-1}), T_7: vitamin E (80 mg kg diet^{-1}) + vitamin B_2 (7 mg kg diet^{-1}), T_8: vitamin E (160 mg kg diet^{-1}) + vitamin B_2 (15 mg kg diet^{-1}), T_9: vitamin E (240 mg kg diet^{-1}) + vitamin B_2 (20 mg kg diet^{-1}). One group not given vitamin supplements was the control. The values of red blood cells (RBC), hemoglobin (Hb), and hematocrit (Hct) were higher in T_7 than in the other experimental treatments. The values of white blood cells (WBC) were higher in T_2 than in the other treatments. The concentration of immunoglobulin (IgM) was also lower in T_{10} than in the other experimental treatments. The highest values of mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV) were observed in T_2. No significant differences were noted among experimental groups in the values of mean corpuscular hemoglobin concentration (MCHC). This study showed that vitamin E and B2 supplements alter the hematological parameters of common carp.

Keywords: blood, cyprinids, hematology, vitamins

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Introduction

Vitamin supplementation in fish diets has been used widely to improve fish production in aquaculture. Vitamins improve the immunity system, meat quality, survival, growth, resistance against diseases and stressors, fecundity, and reproductive efficiency (Conklin 1989, Gapasin et al. 1998, Samocha et al. 1998, Racotta et al. 2004). Vitamin E (α-ocopherol) is an essential vitamin, to which numerous functions are attributed, including increased spawning success, egg survival, hatchability, larval survival, gonadosomatic index, and vitellogenesis in many fish species (Watanabe and Takashima 1977, Kanazawa 1985, Santiago and Gonzal 2000). Vitamin E also prevents the peroxidation of the polyunsaturated fatty acids of phospholipids and cholesterol in cellular and subcellular membranes. Most deficiency

signs observed in fish, such as nutritional muscular dystrophy, fatty liver degeneration, anemia, erythrocyte hemolysis, hemorrhage, depigmentation, and reduced 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. Riboflavin is an essential nutrient for all animals including fishes (NRC 1993). Anorexia, poor growth, and high mortality are common signs of riboflavin (vitamin B_2) deficiency in various species of fish (Murai and Andrews 1978, NRC 1993). Generally, hematological parameters are used as indicators of health in fish. Considering the significance of hematological parameters as indicators of fish health, we decided to investigate the effect of vitamins E and B_2 on some hematological parameters of common carp, *Cyprinus carpio* L. As in many countries, common carp is one of the most important fish species in Iranian aquaculture.

Materials and methods

Three thousand fingerling common carp (fish weight: 10-15 g) were obtained from 30 experimental tanks containing 1000 l of dechlorinated, gently aerated water with a stocking density of 100 fingerlings per tank. The fish were fed a commercial diet (SFC) containing various dietary levels of vitamins E and B_2 and combinations of them for 56 days. The nine experimental treatment variants were as follows: T_1: vitamin E (80 mg), T_2: vitamin E (160 mg kg diet^{-1}), T_3: vitamin E (240 mg kg diet^{-1}), T_4: vitamin B_2 (7 mg kg diet^{-1}), T_5: vitamin B_2 (15 mg kg diet^{-1}), T_6: vitamin B_2 (20 mg kg diet^{-1}), T_7: vitamin E (80 mg kg diet^{-1}) + vitamin B2 (7 mg kg diet^{-1}), T_8: vitamin E (160 mg kg diet^{-1}) + vitamin B_2 (15 mg kg diet^{-1}), T_9: vitamin E (240 mg kg diet^{-1}) + vitamin B_2 (20 mg kg diet^{-1}); one control group (T_{10}) without vitamin supplements. The vitamin doses were determined according to Halver and Hardy (2002). Before vitamins E and B_2 were added to SFC, the content of them in SFC was measured with HPLC. The values of experimental vitamin supplements were regulated based on the values in SFC. Vitamin E (5500 IU α-Tocopherol acetate kg^{-1}) and B_2 (4 g Riboflavin kg^{-1}) premix were obtained from RSHT-DANEH company, Gorgan, Iran. The general composition of the experimental diet is presented in Table 1. Feeding frequency and rations during the experiment were those of the standard feeding schedule suggested by the manufacturer.

Assessment of hematological parameters

To investigate the hematological parameters of the serum, blood samples were obtained by cutting the caudal peduncle after 56 days of the experiment. Immediately after sampling the blood, the samples were delivered to the laboratory for red blood cell (RBC), white blood cell (WBC), hemoglobin (Hb), and hematocrit (Hct) assays. Mean corpuscular volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) were calculated from RBC, Ht, and Hb. Microhematocrit capillary tubes were used for measurements of Hct values according to Řehulka et al. (2005). Hb values were determined with Cyanmethemoglobin according to Blaxhall and Daisley (1973). A 20 µl amount of uncoagulated blood was mixed with 50 µl Drabkin's solution and then placed in a dark environment for 5-10 min. Then, the Hb concentration was measured with spectrophotometry at a wave length of 540 nm. RBC and WBC values were determined with the chamber method using a Neubauer hemocytometer (Drabkin 1945).

After serum separation in a centrifuge (13,700 g for 10 min), the serum ferritin was analyzed with the Immune Radio Metric Assay (IRMA) technique (Flowers et al. 1986). Serum ferritin was standardized according to the international standards included in the with radioimmunoassay kit (Radim Co, Italy) and a gamma counter (Hewlett Packard, Wilmington, DE, USA). Serum concentrations of IgM were measured nephelometrically with a Binding Site Nephelometry kit.

Table 1
Hematological characteristics and plasma constituents of juvenile common carp (*C. carpio*) fed different dietary vitamin E and B_2 levels for 8 weeks

Groups	Vitamin E content (mg kg^{-1})	Vitamin B2 content (mg kg^{-1})	RBC (n)	Hb (g dl^{-1})	Hct (%)	WBC (n)	IgM (mg dl^{-1})	MCH (pg)	MCV (fl)
T1	80	0	$112\times104\pm295\times103^{abc}$	8.8 ± 2.5^{b}	27 ± 9.5^{bc}	3450 ± 425^{f}	27 ± 9.5^{bc}	78.5 ± 24.5^{b}	247 ± 78.5^{c}
T2	160	0	$62\times104\pm288\times103^{e}$	6.2 ± 1.8^{a}	16 ± 8.5^{e}	9150 ± 921^{a}	32.5 ± 10^{ab}	105 ± 31.2^{a}	315 ± 90.1^{a}
T3	240	0	$108\times104\pm282\times103^{bcd}$	9 ± 2.7^{b}	28.5 ± 10^{bc}	4050 ± 480^{def}	33.2 ± 11.1^{ab}	90 ± 30^{ab}	276 ± 90^{ab}
T4	0	7	$87.5\times104\pm272.5\times103^{d}$	8.2 ± 2.8^{b}	22.5 ± 8^{cde}	5020 ± 502^{bc}	37.5 ± 11^{ab}	93.5 ± 29.5^{ab}	260 ± 85.5^{bc}
T5	0	15	$89.1\times104\pm268\times103$	8.9 ± 2.6^{b}	24 ± 8.2^{cd}	5011 ± 498^{bc}	38.5 ± 11.1^{a}	93.2 ± 30.5^{ab}	274.5 ± 84^{abc}
T6	0	20	$90\times104\pm265\times103^{cd}$	8.7 ± 2.5^{b}	27.2 ± 8.1^{b}	5070 ± 490^{b}	40 ± 12^{a}	91.5 ± 30^{ab}	270 ± 82.5^{abc}
T7	80	7	$120.1\times104\pm271\times103^{cd}$	11.6 ± 3.1^{a}	35.1 ± 12.2^{a}	4352 ± 440^{bcd}	38.1 ± 10.5^{ab}	94.5 ± 32^{ab}	271 ± 85^{abc}
T8	160	15	$118\times104\pm281\times103^{a}$	11.3 ± 3^{a}	32.5 ± 11.7^{ab}	3540 ± 418^{ef}	38 ± 10^{ab}	92 ± 29^{ab}	272.1 ± 84.5^{abc}
T9	240	20	$92\times104\pm248\times103^{ab}$	9.3 ± 2.5^{b}	26.5 ± 10^{cd}	3700 ± 450^{def}	37.5 ± 9.5^{ab}	90 ± 28.8^{a}	269 ± 84^{bc}
T10	0	0	$83\times104\pm235\times103^{d}$	7.8 ± 2.2^{bc}	18 ± 9.1^{de}	4152 ± 501^{cde}	16.5 ± 6.8^{c}	91 ± 31^{ab}	$235.78.5\pm78.5^{c}$

Statistical analysis

All data were analyzed with SPSS software. Since percentage data did not have a normal distribution, proportional data were converted with angular transformation (arcsin √p). One-way analysis of variance (ANOVA) was employed to compare the means. When significant F-ratios were calculated with ANOVA, the Tukey test was applied to identify which means were different.

Results

The highest and lowest levels of RBC, Hb, and Hct were observed in T_7 and T_8, respectively (Table 1; $P<0.05$). There were no significant differences in RBC, Hb, or Hct values among the other treatments ($P > 0.05$). The values of WBC were higher in T_2 than in other treatments ($P < 0.05$). The concentration of IgM was lower in T_{10} than in other experimental treatments ($P < 0.05$). No significant differences were noted in IgM values among other treatments ($P > 0.05$). The highest values of MCH and MCV were also observed in T_2 (Table 1; $P < 0.05$). These parameters did not differ significantly among other treatments ($P > 0.05$), and no significant differences among experimental groups were noted for MCHC values ($P > 0.05$).

Discussion

The red blood cell indices (Hb, RBC, Hct) can indicate oxidative status. Erythrocytes are one of the major production sites of free radicals, some of which can trigger the peroxidation of saturated fatty acids in their membrane phospholipids; therefore, altering their quality (integrity, size) and quantity (Pearce et al. 2003, Kiron et al. 2004). In the present study, the values of Hb, RBC, and Hct were higher in fish that received a combination of vitamin B_2 and E, i.e., T_7: vitamin E (80 mg kg diet^{-1}) + vitamin B2 (7 mg kg diet^{-1}); T_8: vitamin E (160 mg kg diet^{-1}) + vitamin B2 (15 mg kg diet^{-1}). The red blood cell indices apparently also decreased when the vitamins were used singly or in a combination of 240 mg kg diet^{-1} vitamin E + 20 mg kg diet^{-1} vitamin B_2. A few studies have reported that hematocrit values decrease with increasing dietary levels of vitamin E (Poston and Livingston 1969, Baker and Davies 1996). However, some studies report increased hematocrit in response to high levels of dietary vitamin E (Bai and Lee 1998, Ispir et al. 2011). The variations in the results of these studies could stem from differences in species and experimental conditions. It seems that some vitamins, especially vitamin E, can alter the production of blood cells from hemopoietic tissues. The response of hemopoietic tissues to dietary vitamin E and vitamin B_2 is likely dose dependent since red blood cell indices decreased at very high dietary levels of vitamins E and B_2 (i.e., T_9). In the present study, WBC concentrations did not differ among experimental treatments except in T_2.

High doses of vitamin E have undesirable immunological effects because of the role this vitamin plays in reducing WBC numbers (Wahli et al. 1998, Sahoo and Mukherjee 2002). On the other hand, in some studies, different dietary levels of vitamin E did not affect WBC concentrations (Blazer and Wolke 1984, De Andrade et al. 2007). It seems that the WBC changes noted in the present and other studies could stem from factors other than dietary levels of Vitamin E. Fish in aquaculture systems are exposed to factors including pathogens and water quality which can influence WBC numbers.

IgM is the only known immunoglobulin in fishes with an important role in immune responses (Ansari et al. 2011). In our study, IgM concentrations were lower in the control group than in groups fed vitamins E and B_2. This study showed that vitamin E and B_2 supplements increased red blood cell indices and IgM concentration in common carp fingerlings over the course of the 56 days of the experiment. This could have stemmed from the protective role of these vitamins on RBC, but it could have also been thanks to the positive impact they have on the immune system.

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Author contributions. S.A.S designed the research and wrote the manuscript, H.K. supervised all stages, from the beginning to the end, of this research, S.G. analyzed the data.

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