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Effect of Dietary Vitamin A and E on Tissues Vitamin Concentrations and Lipid Peroxidation of Juvenile Rainbow Trout at Different Flow Rates

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Abstract

The feed efficiency and biochemical effects of vitamin A and E in diets for juvenile rainbow trout treated with to two different flow rates (0.9 and 2.1 l min^{-1}, respectively) was investigated of some tissues. Four formulated diets ($A_{18}E_0$, $A_{18}E_{60}$, A_0E_{30}, $A_{36}E_{30}$) with different combination of vitamin A and E were tested, fish fed with these experimental diets during 12 weeks. Fish subject to 0.9 l min^{-1} flow rate was determined greater mortality, poorer FCR (feed conversion ratio) and poorer PER (Protein Efficiency Ratio) than fish subject to 2.1 l min^{-1} flow rate. There was no significant difference mortality of all diet groups subjects to 2.1 l min^{-1} flow rate ($P>0.05$). At 2.1 l min^{-1} flow rate trials, muscle and kidney vitamin A concentration was highest in fish fed the diet with 18 mgvitA kg^{-1} and 36 mgvitA kg^{-1}, and lowest in fish fed the diet with vitamin A-free (diet containing 0 mgvitA kg^{-1}). But at 0.9 l min^{-1} flow rate trial, there was no significant difference in muscle and liver vitamin A concentration of all diet groups ($P>0.05$). Tissues malondialdehyde (MDA) level was highest in fish fed the E-free (diet containing 0 mgvitE kg^{-1}) diet ($p<0.05$).

Keywords: Rainbow trout, Hypoxia, Diet, Antioxidant Vitamins, Malondialdehyde.

1. Introduction

Acute decreases in water oxygen concentrations may occur in intensive fish farming, especially when fishes are reared at high densities and insufficient water flow. Considerable attention has been paid to oxygen, as low ambient O_2 concentrations are known to affect growth, food consumption and physiological state of fishes [1]. Hypoxia, or dissolved oxygen below saturation, is considered a major threat to the value of estuarine habitats as nurseries for ecologically and economically important fishes [2]. Some vitamins possessing an antioxidant activity protect the cells from the damage caused by the free radicals and by preventing free radical formation they play an important role in the antioxidant defense. The most significant antioxidant vitamins are vitamins E, A and C (ascorbic acid) [3]. Among these smaller molecules, vitamin E and vitamin A, is regarded as the primary lipid-soluble antioxidant that operates synergistically with vitamin C to protect lipids against per oxidative damage [4].

All aerobic organisms depend on oxygen presence in the environment, using it primarily for energy generation via oxidative phosphorylation [5]. The generation of various by-products of oxygen metabolism, so-called reactive oxygen species (ROS), is the other side of the coin. They include superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (^-OH) and others [5,6,7].

If antioxidant defences are effective in detoxifiying ROS, then no harmful consequence results in the tissues [8]. The paradox of aerobic life is that oxidative damage occurs to key biological sites, threatening their structure and function. Oxygenic threat is met by an array of antioxidants that evolved in parallel with our oxygenic atmosphere. Antioxidant defense system includes a number of radical and peroxide scavenging enzymes and radical-sequestering molecules. In addition there are various antioxidants, some of which are dietary-derived, including tocopherols (vitamin E), ascorbate (vitamin C), retinol (vitamin A), glutathione, urate, flavonoids and caretenoids [9]. Among these smaller molecules, vitamin E and vitamin A, is regarded as the primary lipid-

soluple antioxidant [4]. High vitamin E content in tissues would inhibit tissue lipid peroxidation [10, 11]. Malondialdehyde is the final product of lipid peroxidation. The concentration of MDA is the direct evidence of toxic processes caused by free radicals. The most widely used assay for lipid peroxidation is the MDA formation. Malondialdehyde is the final product of lipid peroxidation.

nutritients [15]. Hypoxia is a common event in aquatic environments. Reductions of oxygen concentration (O_2) in water have resulted in significant changes in antioxidant defense systems in fish. In recent years, physiology and biochemical effects of the hypoxic stress on fish created by various methods have been

The concentration of MDA is the direct evidence of toxic processes caused by free radicals [12,13]. Fish are frequently exposed to stressors under culture conditions, which cause a series of physiological responses, known as stress [14]. Stress leads to more macro and micro nutrients requirements. Therefore, nutritional modifications usually made are the optimization of diets to meet the altered needs of stressed fish for protein and energy and for providing investigated [16]. The primary objectives of this study were to determine the interactions these two vitamins concentrations and lipid peroxidation (MDA levels) in muscle, kidney and liver of rainbow trout at two different flow rate treatment (0.9 and 2.1 l min^{-1}).

2. Materials and methods

2.1. Fish material

Juvenile rainbow trout *Oncorhynchus mykiss* (Walbaum, 1972) obtained from Çırçır Hatchery Farm (Keban/TURKEY) were transferred to Keban Dam Lake General Directorate of State Hydraulic Works Laboratory (Keban/TURKEY). After the acclimation, fish were randomly taken for stocking. Fish (initial weight and length, 41.43±1 g, 15.41±0.62 cm) were distributed to 24 fiberglass rectangular (200×40×40 cm) tanks with a 4×2×3 experimental design (4 diets group ×2 flow rates × 3 replicate groups) with a density of 20 juvenile rainbow trout (*Oncorhynchus mykiss*) per tank (17).

2.2. Experimental desing

Experimental tanks were supplied with well water at two different flow rates, 2.1 and 0.9 l min^{-1}. Several researchers have reported that the concentration of the dissolved oxygen in water should not be allowed to fall below 5 mg/L and that the optimal concentration of dissolved oxygen varies between 7 and 9 mg/L in trout stock farming [18]. Petersen (1987), determined that natural oxygen requirement for the trout as 8 mg/L at concentrations, 4 mg/L difficulties in breathing appeared and 3 mg/l slow deaths occurred [19]. The flow rates that were determined for low and optimal flow rate groups were stabilized through the use of the water inflow control valves [17]. The study was initiated after 1 week-adaptation period of fish to the hypoxic condition at the level of 4.5 mg l^{-1} dissolved oxygen. Water temperature was between 8.8 and 9.4 °C and water pH was 8.4 throughout the experiment. The pH of the water was determined by using a portable Checker brand pH meter and the dissolved oxygen and the temperature values were recorded with a portable YSI 55 Model 51/12 oxygen probe throughout the during the research. Before the fish were anesthesiad (Quinaldin), body weights were measured two times in a month [20].

2.3. Diets Composition of the basal diet (not supplemented vitamin A and E) is given in Table 1. Experimental diets were formulated by the supplementation of vitamin A and E to the four diets. Four experimental diets ($A_{18}E_0$, $A_{18}E_{60}$, A_0E_{30}, $A_{36}E_{30}$) were prepared in the laboratory according to the nutritional requirements of rainbow trout. Diets were formulated with some macronutrient content and considering different levels of vitamin E (Rovimix E-50 adsorbate; min. %50 dl-α-tokopherly acetate) (30 and 60 mg kg^{-1}) and vitamin A (Rovimix A 1000; min. 1 000 000 IU per gram, vitamin A asetat) (18 and 36 mg kg^{-1}) [17]. The vitamins content was suggested as the optimal dietary vitamin A and E requirement for this species [11,21]. Vitamin A and E were supplied from Turkey DSM nutritional productions firm. Vitamin A and E were not present in the vitamin mixture and were

added as a supplemented according to the diet formulation. A small amount of vitamin E in –E diets proved unavoidable since this vitamin was present both in the fish flour and lipid source, likewise in dietary vitamin A.

Diets and tissues were analyzed for vitamin A and E by Cecil 1100 series HPLC (Cecil Inst., Ltd., Cambridge, United Kingdom) equipped with an 1100 series pump and UV absorbance detector. Based on this analysis, level of 13.3, 54.41, 26.23, 25.96 mg vitamin E kg^{-1} diet were determined for, $A_{18}E_0$, $A_{18}E_{60}$, A_0E_{30}, $A_{36}E_{30}$ respectively and 14.24, 13.83, 2.4, 32.45 mg vitamin A kg^{-1} diet were determined for, $A_{18}E_0$, $A_{18}E_{60}$, A_0E_{30}, $A_{36}E_{30}$ respectively.

Ash (650°C, 6 h), crude protein (nitrogen×6.25), ether extract, crude fibre of diets was analyzed by methods of AOAC (1990) [22]. Experimental groups of fish were fed approximately 3% of body weigth per day. The feeding trial was conducted for 12 weeks.

Table 1. Composition and proximate analysis of the experimental diets

Ingredient	Percent of dry weight
Fish (anchovy) flour	50
Soybean meal	23.1
Wheat flour	19.8
Sunflower oil	6
Antioxidant[a]	0.10
Vitamin premix[b]	0.90
Mineral premix[c]	0.10
Proximate compasition (% dry basis)	
Crude protein	43.48±1.19
Crude lipid	13.02±2.8
Crude ash	4.04±1.14

[a]Antioxidant (mg kg^{-1} dry diet): Butilen Hydroxytoluene (BHT); 125.000 mg $kg^{-1.}$

[b]Vitamin premix (IU or mg kg^{-1} dry diet): Menadion 3.000, Riboflavin 6.000, Pridoksin 5.000, Kobalamin 15, Askorbik asit 150.000, Niasin 25.000, Biotin 40, Folik asit 1.000, Kolin klorid 300, Kalsiyum D-pantothenat 8.000, Kalsiferol 2.000.000 IU.

[c]Mineral premix (mg kg^{-1} dry diet): Mn 80.000, Fe 35.000, Zn 50.000, Cu 5.000, I 2.000, Co 400, Se 150.

2.4. Determination of vitamin A and E levels in tissues

The homogenized tissues were transferred into polyethylene tubes and 2 ml of ethanol was added to the tubes. Following the addition of 0.3 ml n-hexane that is required for the vitamin extractions into the tubes, they were centrifuged. This step was repeated for two times. N-hexane in the tubes was evaporated using nitrogen. Then the residues were dissolved in the mobile phase (methanol: acetonitrile: chloroform; 47:42:11,v/v/v). The chromatographs were monitored at 326 and 296 nm for vitamins A and E, respectively and the injection volume was set to 50 ml. Techsphere ODS-2 packed column (5 mm particle, 250× 4.6 ID) was used and the flow rate was 1.0 ml min^{-1} [23].

2.5. Determination of MDA levels in tissues

A 1 g tissue samples were taken from 120 fish; 30 from each one of the groups and the samples were stored in the freezer at -20ºC until analysis Fish tissue samples were homogenized. The homogenization mixture consisted of 0.5 ml $HClO_4$ (0.5 M), 4.5 ml distilled water and 100 ml-500 ppm 2(6)-di-tert-butyl-p-cresol (BTH). Then, the samples were centrifuged at 4500 rpm for 5 min and the supernatants were injected into the HPLC. The mobile phase was 30 mM KH_2PO_4-methanol (82.5+17.5, v/v %, pH 3.6) mixture and the flow rate was 1.2 ml min^{-1}. The chromatograms were detected at 250 nm and the injection volume was 20 ml [24].

2.6. Statistical analysis

All the values were presented as mean±S.E. Differences between group means were assessed by a one-way and two-way analysis of variance ($P<0.05$, ANOVA) and post-

hoc Duncan test used by SPSS/PC (SPSS, 11.5) computer program.

3. Results

3.1. FCR, PER and mortalitiy

The effects of supplemental vitamin A and E on FCR, PER and mortalitiy of rainbow trout at two different flow rates are shown in Table 2. Fish subject to 0.9 l min^{-1} flow rate was greater mortality, poorer FCR and PER than fish subject to 2.1 l min^{-1} flow rate. There was no significant difference between mortality of all diet groups subjects to 2.1 l min^{-1} and 0.9 l min^{-1} flow rate (P >0.05, Table 2). All group in 2.1 l min^{-1} flow rate conditions improved significantly more FCR than from 0.9 l min^{-1} flow rate conditions. FCR and PER values of fish fed with vitamin A and E combination diet was greater than fish fed diets with A-free and E–free subjected to two different flow rates. Both flow trial, improvement in FCR was found fish feed diet containing 18 mg kg^{-1} vitamin A and 60 mg kg^{-1} vitamin E and also worst FCR was found fish feed diet containing 0 mg/kg vitamin A and 30 mg kg^{-1}vitamin E. In 2.1 l min^{-1} flow rate trial, PER value of the $A_{18}E_{60}$ group was significantly higher than the other groups. At 0.9 l min^{-1} flow rate trial, PER value was highest in the $A_{18}E_{60}$ and $A_{36}E_{30}$ diet groups and lowest in fish fed with the diet with vitamin E-free and A-free.

The results of ANOVA test showed that FCR and PER values of fish were negatively affected by low flow rates (Table 2). Considered separately, diet and flow rates (FR) significantly affected the FCR and PER (P<0.05), however no interaction was found between diet and FR value (P>0.05, Table 6).

Table 2. Effects of dietary supplementation with vitamin A and E on FCR, PER and mortalitiy in juvenile rainbow trout reared during at flow rates of 2.1 and 0.9 l min^{-1}.

FR 2.1 l/min	$A_{18}E_0$	$A_{18}E_{60}$	A_0E_{30}	$A_{36}E_{30}$
FCR	3.23 ± 0.75^{b}	2.81 ± 0.39^{c}	3.73 ± 0.35^{a}	3.58 ± 0.37^{bc}
PER	1.35^{b}	1.64^{a}	1.13^{c}	1.28^{bc}
Mortality (n)	5	6	5	5
FR 0.9 l/min				
FCR	6.92 ± 1.12^{ab}*	6.54 ± 1.14^{b}*	7.41 ± 1.35^{a}*	6.93 ± 1.40^{ab}*
PER	0.75^{b}*	0.96^{a}*	0.78^{b}	0.96^{a}
Mortality (n)	20	17	19	16

Letters indicate significant differences between groups fed with different diets under the same flow rate (P<0.05). *Significant differences between unstressed and stressed for the same group (P<0.05). Each value is mean±S.E. of 3 replicate tanks.
FR: Flow rate
Mortality (n): Final fish death number (Gupta *et al*., 2014; Gupta *et al*., 2007).
Feed Conversion Ratio (FCR): feed fed (g)/wet weigth gain (g); Protein Efficiency Ratio: wet weight gain/protein intake

3.2. Vitamin A and E levels in tissues

Muscle, liver and kidney vitamin A and E concentrations after 12-week feding trial are shown in Table 3. It was obvious that vitamin A and E concentrations in tissues were negatively affected by flow rates. At 2.1 l min^{-1} flow rate trials, muscle and kidney's vitamin A concentration was highest in fish fed the diet with 18 mg vit A kg^{-1} and 36 mg vit A kg^{-1}, and lowest in fish fed the diet with vitamin A-free. But at 0.9 l min^{-1} flow rate trial, there was no significant difference in muscle and liver vitamin A concentration of all diet groups (P>0.05, Table 3). Both flow rate trials, there were no significant differences in kidney vitamin A concentration of

all diet groups (P >0.05, Table 3). At 2.1 l min^{-1}, muscle vitamin E concentration was the highest in fish fed diets with 60 mg vit E kg^{-1}, followed by 30 mgvitE kg^{-1}, and the lowest in fish fed with the E-free diet (P<0.05, Table 4). Both flow rate trials, muscle and liver vitamin E concentration was the highest in fish fed diets with $A_{18}E_{60}$ and the lowest in fish fed diets with $A_{18}E_0$. 0.9 l min^{-1} and 2 l min^{-1}, the highest level of vitamin E supplementation (60 mg kg^{-1}) did not result increase level of liver vitamin E compared to the A and E diet groups, but both diet groups was no significant differences. Both flow rate trials, there was no significant difference in kidney vitamin E concentration of all diet groups (P>0.05, Table 4). After 12 weeks, muscle and liver vitamin A concentrations were significantly affected by flow rate and diet, and a significant interaction was found between FR value and diet. FR value and diet also significantly affected liver vitamin A concentration, and an interactions were observed between the two parameters. Kidney vitamin A concentrations were not significantly affected by FR value, diet and by not diet and FR interactions (P>0.05). Diet, FR value and the interaction between diet and FR value, significantly affected muscle and liver vitamin E concentrations of fish, but they were not affected by FR value, diet and interaction between the two parametrs for kidney vitamin E concentrations (Table 6).

Table 3. Effects of dietary supplementation vitamin A and E on tissues vitamin A concentrations in juvenile rainbow trout reared during at flow rates of 2.1 and 0.9 l min^{-1}.

Experimental Groups	FR (l min^{-1})	Muscle Vit. A (mg g^{-1})	Liver Vit. A (mg g^{-1})	Kidney Vit. A (mg g^{-1})
$A_{18}E_0$	2.1	2.23±0.3^{b}	6.45±1.41^{b}	0.15±0.13
$A_{18}E_{60}$	2.1	3.08±0.3^{a}*	7.34±1.23^{a}*	0.16±0.41
A_0E_{30}	2.1	1.02±0.12^{c}	4.78±0.56^{c}	0.11±0.31
$A_{36}E_{30}$	2.1	3.07±0.18^{a*}	7.43±1.34^{a}	0.18±0.25
$A_{18}E_0$	0.9	0.91±0.03	2.64±0.93	0.08±0.01
$A_{18}E_{60}$	0.9	0.85±0.07	2.80±0.46	0.08±0.01
A_0E_{30}	0.9	0.43±0.07	2.33±0.39	0.05±0.01
$A_{36}E_{30}$	0.9	1.03±0.12	3.63±0.37	0.12±0.02

$^{a-c}$Letters indicate significant differences between groups fed with different diets under the same flow rate (P<0.05). *Significant differences between unstressed and stressed for the same group (P<0.05). Each value is mean±S.E. of 3 replicate tanks.

Table 4. Effects of dietary supplementation vitamin A and E on tissues vitamin E concentrations in juvenile rainbow trout reared during at flow rates of 2.1 and 0.9 l min^{-1}.

Experimental Groups	FR (l min^{-1})	Muscle Vit. E (mg g^{-1})	Liver Vit. E (mg g^{-1})	Kidney Vit. E (mg g^{-1})
$A_{18}E_0$	2.1	2.07±0.10^{c}*	9.29±0.32^{b}*	0.53±0.11
$A_{18}E_{60}$	2.1	7.75±0.23^{a}*	16.06±2.27^{a}*	0.88±0.14
A_0E_{30}	2.1	4.05±0.12^{b}*	14.84±1.10^{ab}*	0.75±0.16
$A_{36}E_{30}$	2.1	4.09±0.03^{b}*	13.85±2.03^{ab}*	0.73±0.21
$A_{18}E_0$	0.9	0.34±0.08^{b}	3.53±1.30^{b}	0.13±0.04
$A_{18}E_{60}$	0.9	0.78±0.11^{a}	3.93±1.07^{ab}	0.51±0.06
A_0E_{30}	0.9	0.65±0.12^{ab}	4.43±1.26^{a}	0.39±0.03
$A_{36}E_{30}$	0.9	0.51±0.18^{ab}	3.79±1.32^{ab}	0.42±0.04

$^{a-c}$Letters indicate significant differences between groups fed with different diets under the same flow rate (P<0.05). *Significant differences between unstressed and stressed for the same group (P<0.01). Each value is mean±S.E. of 3 replicate tanks.

3.3. Tissues MDA levels

The results of ANOVA test showed that MDA levels of fish tissues were positively affected by dietary supplementation of vitamin E (Table 5). At 2.1 l min^{-1} flow rate trial, muscle and kidney MDA level were similarly affected by 60 mg kg^{-1} and 30 mg kg^{-1} vitamin E supplemented diet and lowest in fish fed diets containing E-free, 18 mg kg^{-1} vitamin A ($p<0.05$). Liver MDA level was highest in fish fed the diet with $A_{18}E_0$, followed by A_0E_{30} and lowest in fish fed the diet with $A_{18}E_{60}$ and $A_{18}E_{60}$ (Table 5).

At 0.9 l min^{-1} flow rate trial, muscle MDA level was highest in fish fed diets with $A_{18}E_0$ and lowest in fish fed diets with $A_{18}E_0$ ($p<0.05$). Liver MDA level was highest in fish fed the diet with $A_{18}E_0$ (E-free) , followed by A_0E_{30} (A-free) and $A_{30}E_{36}$. Lowest in fish fed the diet with $A_{18}E_{60}$ ($p<0.05$, Table 5). The interaction between diet and FR value were not affected tissues MDA levels of fishes ($P>0.05$, Table 4). But, diet and FR values were significantly affected the tissues MDA levels ($P<0.05$, Table 6).

Table 5. Effects of dietary supplementation vitamin A and E on tissues MDA level in juvenile rainbow trout reared during at flow rates of 2.1 and 0.9 l min^{-1}.

Experimental Groups	FR (l/min^{-1})	Muscle MDA (nmol ml^{-1})	Liver MDA (nmol ml^{-1})	Kidney MDA (nmol ml^{-1})
$A_{18}E_0$	2.1	6.23 ± 1.06^{a}	4.45 ± 1.92^{a}	$1.85\pm0.44^{a*}$
$A_{18}E_{60}$	2.1	2.18 ± 0.76^{b}	1.47 ± 0.21^{c}	0.76 ± 0.12^{b}
A_0E_{30}	2.1	$2.82\pm0.31^{b*}$	2.43 ± 0.73^{b}	1.11 ± 0.14^{b}
$A_{36}E_{30}$	2.1	2.07 ± 0.25^{b}	$1.53\pm0.22^{c*}$	1.02 ± 0.12^{b}
$A_{18}E_0$	0.9	10.71 ± 2.34^{a}	7.64 ± 2.32^{a}	5.08 ± 1.52^{a}
$A_{18}E_{60}$	0.9	5.15 ± 2.16^{b}	2.34 ± 0.34^{c}	3.08 ± 1.26^{b}
A_0E_{30}	0.9	8.30 ± 3.11^{ab}	4.07 ± 1.07^{b}	4.85 ± 1.34^{a}
$A_{36}E_{30}$	0.9	7.33 ± 2.22^{ab}	4.53 ± 1.44^{b}	3.49 ± 1.23^{ab}

$^{a\text{-}c}$Letters indicate significant differences between groups fed with different diets under the same flow rate ($P<0.05$). *Significant differences between unstressed and stressed for the same group ($P<0.05$). Each value is mean±S.E. of 3 replicate tanks.

Table 6. Two-way ANOVA showing the effect flow rate (FR), Diet and FR×Diet interaction on muscle, liver and kidney vitamin A and E concentrations; muscle, liver and kidney MDA level, FCR and PER.

	FR	Diet	FR×Diet
Muscle Vit. A	0.05	0.01	0.05
Muscle Vit. E	0.01	0.05	0.05
Liver Vit. A	0.05	0.05	0.05
Liver Vit. E	0.01	0.05	0.05
Kidney Vit. A	ns	ns	ns
Kidney Vit. E	ns	ns	ns
Muscle MDA	0.05	0.05	ns
Liver MDA	0.05	0.05	ns
Kidney MDA	0.05	0.05	ns
FCR	0.05	0.05	ns
PER	0.05	0.05	ns

ns: no significant differences ($P>0.05$).

4. Discussion

Reductions of oxygen concentration in water have resulted in significant changes in antioxidant defense system in teleosts. Occured stress leads to generation of free radicals, such as O_2 and HO [5]. These free radicals can damage cell membranes by inducing lipid peroxidation of polyunsaturated fatty acids in the cell membrane [25].

Stimulation of non-specific host defense mechanisms using specific biological compounds, called immunostimulants, enhances the disease resistance and growth of the host The innate immune system, comprising physical barriers, and cellular and humoural components, serves as a defense weapon in fishes [20]. Vitamin E (α-tocopherol) function as biological antioxidants to protect cellular macromolecules (DNA, protein, lipids) and other antioxidant molecules from uncontrolled oxidation by free radicals during normal metabolism or under the conditions of oxidative challenge such as infection, stress, and pollution [26,27]. Survival of fish fed the vitamin E supplemented diets was higher in general. Vitamin E functions as a lipid-soluble antioxidant protecting biological membranes and lipoproteins against oxidation, and it has been demonstrated to be an essential dietary nutrient for all fish studied [28,29]. Vitamin A occurs in three forms: the alcohol (retinol), aldehyde (retinal) and acid (retinoic acid) in animal tissues [30]. The main physiological functions of vitamin A are differentiation of epithelial tissues, resistance to infections, proper growth, reproduction and vision [31,32]. Vitamin A is recognised as a key factor in embryonic development through the regulation of cell differentiation and proliferation, and for its effects in vision growth, stress and normal function of the immune system. For freshwater fish, the requirement has been estimated to be from 600 to 1200 ug retinol kg^{-1} diet to maintain growth [21]. In the stressed and unstressed group, WG deteriorated in trout fed the only vitamin A supplemented diet as compared to the fish fed vitamin A and E participate with diets. In our study, two antioxidants (vitamin A and E) significantly decreased negative effects of low flow stress on WG and SUR. These antioxidants also increased WG and SGR values of fishes under the unstressed conditions.

High vitamin E content in tissues would inhibit tissue lipid peroxidation. Similar trends have been reported in rainbow trout [11], turbot, Atlantic salmon, sea bass, and Atlantic halibut [33]. Huang and Huang (2004) [26] determined that WG of the juvenile hybrid tilapia (*Oreochromis niloticus* × *O. aureus)* fed diets containing 0 IU vitamin E/kg was significantly lower ($P<0.05$) than those fed higher vitamin E (>80 IU/kg) diets and improved in SUR. They also determined that both liver and muscle vitamin E contents increased when dietary vitamin E level increased and lipid peroxidation in tilapia tissues was significantly influenced by the dietary vitamin E level ($P < 0.05$). Results from the present study are in consistent with previous studies where the dietary supplementation of vitamin E at elevated levels increased the concentration of tocopherol and decreased lipid peroxidation in blood of fish. Lin and Shiau (2005) [34], reported that dietary vitamin E supplementation increased growth performance and decreased MDA levels in grouper. Similar findigs were obtained in our study. Additionally, only vitamin A supplemented diet was not enough to suppress lipid peroxidation compared to vitamin E supplementation.

5. Conclusion

In conclusion, dietary supplemen-tations (together supplementary vitamin A and E) of juvenile rainbow trout with antioxidants alleviated the flow stress-induced oxidative damages. Two antioxidants together effects significantly decreased the negative effects of flow stress on FCR and PER values. But there was no significant difference mortality of all diet groups subjects to 2.1 l min^{-1} and 0.9 l min^{-1} flow rate. Moreover, vitamin A and E combinations in diet are more effective on FCR and PER values and fish subject to 0.9 l min^{-1} flow rate was greater mortality. Vitamin E supplementation (60 mg kg^{-1} diet) is more effective in preventing lipid peroxidation in the tissues. And it also improves vitamin E concentration in tissues. The FCR, PER and lipid peroxidations in trout fed the only vitamin A supplemented diet as compared to the fish fed vitamin A and E together supplemented diets.

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References

1. Pichavant P., Ruyet L.P., Bayon L.N., Severe, A., Raux, L.A, Quemener, L., Maxime, V., Nonntte, G. and Boeuf, G. (2000). Effects of hypoxia on growth and metabolism of juvenile turbot. *Aquaculture*, **188,** 103–114.

2. Stierhoff, K.L., Tyler, R.M. and Targett, T.E. (2009). Hypoxia tolerance of juvenile weakfish (Cynoscion regalis): Laboratory assessment of growth and behavioral avoidance responses. *J Exp Marine Biol and Ecol*, **381**, 173–179.

3. Zhang P. and Omaye S T. (2001). ß-Carotene: Interactions with α-tokoferol and ascorbic asid in microsomal lipid peroxidation. *J Nut Biochem*, **12** (1), 38-45.

4. Choi, S. W, Benzic, I. F .F, Collins, A. R, Hannigan, B.M. and Strain, J. J. (2004). Vitamin C and E: acut interactive effects on biomarkers of antioxidant defance and oxidative stress. *Mutation Research*, **551**(1-2), 109–117.

5. Lushchak, V.I. and Bagnyukova, T.V. (2006). Effects of different environmental oxygen levels on free radical processes in fish. *Comp Biochem and Physiol*, **44**, 283–289.

6. Filho D W. (2007). Reactive oxygen species, antioxidants and fish mitocondria. *Frontiers in Biosci.* 12 (1): 1229-1237

7. Barry, H. (1994). Free radicals and antioxidants. *Nutrition Review*, **52**: 253–265.

8. Fang, Y. Z., Yang S. and Wu, G. (2002). Free radicals, antioxidants and nutrition. *Nutrition*, **18**, 872-879

9. Quiles, J, Huertas, J., Batine, M., Mataix, J. and Tortosa, C. (2002). Antioxidant nutrients and adriamycin toxicity. *Toxicology*, **180**, 79–95.

10. Frigg, M., Prabucki, A.L. and Ruhedl, E.U. (1990). Effects of dietary vitamin E levels on oxidative stability of trout fillets. *Aquaculture*, **84**, 145–158.

11. Sau, S.K, Paul, B.N., Mohanta, S.N. and Mohanty, S.N. (2004). Dietary vitamin E requirement, fish performance and carcass composition of rohu (*Labeo Rohita*) fry. *Aquaculture*, **240**, 359-368.

12. Talas, S.Z., Orun, I., Ozdemir, I., Erdogan, K., Alkan, A. and Yılmaz, I. (2008). Antioxidative role of selenium against the toxic effect of heavy metals (Cd+2, Cr+3) on liver of rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792). *Fish Physiol and Biochem*, **34,** 217–222.

13. Tatlı Seven P, Yılmaz S, Seven I, Cercı I H, Azman M A. and Yılmaz M. (2009). Effects of propolis on selected blood ındicators and antioxidant enzyme activities in broilers under heat stress. *Acta Veterinaria Brno*, **78**, 75–83.

14. Andrade, J.I.A., E.A. Ono, G.C. Menezes, E.M. Brasil, R. Roubach, E.C. Urbinati, M.T. Dias, J.L. Marcon and E.G. Affonso. (2007). Influence of diets supplemented with vitamins C and E on pirarucu (Arapaima gigas) blood parameters. *Comp Biochem and Physiol*, Part A, **146,** 576–580.

15. Seven, I., Aksu, T. and Tatlı Seven, P. (2010). The effects of propolis on biochemical parameters and activity of antioxidant enzymes in broilers exposed to lead-induced oxidative stress. *Asian-Australasian J Anim Sci*, **23**(11), 1482-1489.

16. Randall, D.J. (1982). The control of respiration and circulation in fish during exercise and hypoxia. *J of Exp Biol*, **100**, 275-288.

17. Tuna Kelestemur, G. and Özdemir, Y. (2013). Effects of dıetary vitamin A and E on growth performance and antioxidant status in blood of juvenile raınbow trout (*Oncorhynchus mykıss*, w. 1792) exposed to flow rate stress. *The Journal of Animal and Plant Sciences*, **23**(3), 821-827.

18. Clark, M.L. (2003). Compariyson of water qualitiy, rainbow trout production and economics oxygenated and aerated raceways oxygenated and aerated raceways Thesis Virginia Polytechnic Institute and State University, 66 p.

19. Pedersen, C.L. (1987). Energy budgets for juvenile rainbow trout at various oxygen concantration. *Aquaculture***, 62,** 289-298.

20. Gupta, A., Gupta, P. and Dhawan, A. (2014). Dietary supplementation of probiotics affects growth, immune response and disease resistance of *Cyprinus carpio* fry. *Fish and Shellfish Immunol.* **41**, 113-119

21. Hemre, G, Deng, D., Wilson, R.P. and Berntssen, M.H.G. (2004). Vitamin A metabolism and early biological responses in juvenile sunshine bass (*Morone chrysops M. saxatilis)* fed graded levels of vitamin A. *Aquaculture*, **235**, 645–658.

22. AOAC (1990). Official Methods of Analysism Association of Agricultural Acedemy Press. Washington, 1158 p.

23. Miller, K.W, Lorr, N.A. and Yang C.S. (1984). Simultaneous determination of plasma retinol, α-tocopherol, lycopere, β-carotene, and β-carotene by high performance liquid chromatography. *Anal Biochem*, **138**, 340–345.

24. Karatepe, M. (2004). Simultaneous determination of ascorbic acid and free malondialdehyde in human serum by HPLC/UV. *LC-GC North America*, **32**(4), 362–365.

25. Sorg, O. (2004). Oxidative stress: a theoretical model or a biological reality? *Comptes Rendus Biologies*, **327,** 649–662.

26. Huang, C.H. and Huang, S.L. (2004). Effect of dietary vitamin E on growth, tissue lipid peroxidation and liver glutathione level of juvenile hybrid tilapia, *Oreocromis niloticus x O. aureus* fed oxidized oil. *Aquaculture*, **237,** 381–389.

27. Lukaski, H.C. (2004). Vitamin and mineral status, effect on physical performance. *Nutrition*, **20**, 632-64.

28. NRC, (1993). Nutrient requirements of fish. National Academy of Sciences DC, USA Washington, 114p.

29. Dandapat, J., Chainy, G.B.N. and Rao, K.J. (2000). Dietary vitamin-E modulates antioxidant defence system in giant freshwater prawn, Macrobrachium rosenbergii. *Comp Biochem and Physiol*, **127**, 101–115.

30. Goodman, D.S. (1980). Vitamin A metabolism. *Federation Proceeding*, **39**, 2716-2720.

31. Mohamed, J.S., Sivarama, V., Roy, T.S.C., Mariana, M.P., Murugadass, S. and Hussain, M.R.

(2003). Dietary vitamin A requirement of juvenile greasy grouper (Epinephelus tauvina). *Aquaculture*, **219**, 693–701.

32. Hu, C.J, Chen, M., Pan H.C. and Huang, H.C. (2005). Effect of dietary vitamin A or ß- carotene concentrations on growth of juvenile hiybrit tilapia, *Oreohcromis niloticus x O. Aureus. Aquaculture*, **4**, 233-245.

33. Tuna Kelestemur, G., Tatlı Seven, P. and Yılmaz, S. (2012). Effects of dietary propolis and vitamin E on growth performance and antioxidant status in blood of juvenile rainbow trout (Oncorhynchus mykiss) under different flow rates. *Zoologia*, **29**(2):99-108.

34. Lin, Y.H. and Shiau, S.Y. (2005). Dietary vitamin E requirement of grouper, Epinephelus malabaritus, at two lipid levels and their effects on immune responses. *Aquaculture*, **248**, 235-244.