Journal of Aquaculture Feed Science and Nutrition 6 (2): 39-46, 2014
ISSN: 2070-1667

Effects of Vitamin E on Growth Performance, Survival Rate, Hematological Parameters Response to Heat Stress in Rainbow Trout (*Oncorhynchus mykiss*) at Two Stocking Densities

[1]Rahimi Mohammad and [2]Ouraji Hosein
[1]Department of Fisheries,
Khoramshar University of Marian Science and Technology, Khoramshar,
[2]Department of Fisheries,
Gorgan University of Agricultural Sciences and Natural Resources, Sari, Iran

Abstract: The effects of vitamin E were studied in fingerlings rainbow trout (*Oncorhynchus mykiss*) kept at two different densities. Fingerlings (initial weight of 2.1±0.1 g) were reared in triplicate groups in fiberglass tanks at densities of 400 and 800 m^3. The fish were fed with 4 diets containing different levels of vitamin E (0, 100, 200 and 1000 mg vitamin E kg^{-1} diet) for 9 weeks. The at end of feeding experiment fish were exposed to different temperatures (24, 26 and 28°C) and evaluate survival. The growth performance in fish fed diets containing different levels of vitamin E (100, 200 and 1000 mg kg^{-1} diet) was significantly ($p<0.05$) higher than those fed diet without vitamin E. Levels of vitamin E significantly ($p<0.05$) affected the hematocrit, hemoglobin, Red Blood Cells (RBC) count, Mean Corpuscular Hemoglobin (MCH) and eosinophil in rainbow trout. However, it did not change Mean Corpuscular Hemoglobin Concentration (MCHC), lymphocyte and monocyte values. Stocking density had significant effect on eosinophil count only. Survival of the treated fish was significantly ($p<0.05$) higher than the control in response to heat stress. The highest effects of vitamin E was at level of 200 mg kg^{-1} and a density of 400 pieces m^{-3}.

Key words: Vitamin E, growth, *Oncorhynchus mykiss*, hematological parameters, temperature stress

INTRODUCTION

Aquaculture industry is one of the most important industries in the developing world increasing annual aquaculture production. Fishes are normally reared in closed environments, such as pond aquaculture in earthen, cement and cages tanks. Attempts on rising fish stocking density account for one of the main causes of the rise and progress of this industry and also, as one of the key factors in the efficiency and economic viability of aquaculture. Undoubtedly, regardless of the control environment and stressful factors, raising density will adversely affect the health status of fish. This situation creates difficult and stressful environmental conditions, as well as increased susceptibility to fish pathogens. Therefore, beside the control of stressful environmental factors by adopting methods, the survival and growth of fish kept, as well as the immune system in such conditions can be boosted using immune stimulants in diet against infectious diseases (Huang and Huang, 2004; Irwin *et al*.,1999; Montero *et al*., 1999a; Soltani, 2008). Vitamins are important nutrients that stimulate the immune system of fish and vitamin E, as an essential nutrient in aquaculture is proposed to maintain physiological processes, such as normal growth and immune function reproductive processes (De Menezes *et al*., 2006; Andrade *et al*., 2007). Vitamin E is a fat-soluble vitamin and diets with high levels of vitamin E, as opposed to normal levels lead to storage of the vitamin in the body fat tissues. Furthermore, excessive vitamin E in the diet results in hypervitaminosis which reduces growth and increases mortality in response to liver toxicity. Appropriate amounts of this vitamin, therefore should be added in the diet.

The vitamins and antioxidants, as well as macro-molecules such as DNA, lipids, proteins and other molecules form oxidation by free radicals during normal metabolism or under such conditions as pollution, corruption stress. Antioxidant properties of vitamin E may be effective in increasing the tolerance of fishes to environmental stress (Chen *et al*., 2004). Vitamin E is needed to protect fish's immunity, meat quality, resistance of red blood cells against hemolysis, capillary permeability heart muscle (Blaxhall and Daisley, 1973). It also increases

Corresponding Author: Rahimi Mohammad, Department of Fisheries, Khoramshar University of Marian Science and Technology, Khoramshar, Iran

the amount of Thiobarbituric Acid (TBARs) and reduces RBC fragility (Paul *et al*., 2004). Research has also shown that vitamin E is effective on blood parameters of fish (Randall *et al*., 1992; Chen *et al*., 2004; Trenzado *et al*., 2006; Garcia *et al*., 2007). Although, numerous studies have been conducted on the effects of vitamins and the resultant fish immune response, as well as the relationship between dietary vitamin and fish response to stress, there are few studies mostly focusing on stress in fish driven by vitamin C, a small number of studies were conducted on the effect of vitamin E and stress (Montero *et al*., 1999a, 2001).

Stocking density has been shown to affect behavioral interactions in several fish species (Irwin *et al*., 1999; Chen *et al*., 2004; Trenzado *et al*., 2006) which may ultimately affect growth rates. The effect of stocking density on growth was reported for a range of cultured fish species, such as gilthead sea bream, *Sparus aurata* (Montero *et al*., 1999a), juvenile turbot, *Scophthalmus maximus* (Irwin *et al*., 1999), Arctic charr (Jorgensen *et al*., 1993), Japanese flounder, *Paralichthys olivaceus* (Bolasina *et al*., 2006) rainbow trout *Oncorhynchus mykiss* (North *et al*., 2006; Trenzado *et al*., 2008), silver perch *Bidyanus bidyanus* (Rowland *et al*., 2006). Both positive and negative relationships between stocking density and growth have been reported the pattern of this interaction appears to be species specific. Therefore, the aim of this study was to investigate the effects of different levels of vitamin E on growth, blood parameters resistance against heat stress in rainbow trout fingerlings at two stocking densities.

MATERIALS AND METHODS

Experimental design, procedures and diets: This research was performed for 9 weeks in Tehran Ghezel Ala Co. located in Firoozkooh City, Northern Iran. The 4 diets with similar energy and protein levels were tested (Table 1). The diets contained different levels of vitamin E (0.0, 100, 200 and 1000 mg kg^{-1} diet). The fish were stocked in densities of 400 pieces (typical density in the workshop) and 800 pieces m^{-2} (high density) in a factorial (2×4) experimental design, considering 3 replicates per treatment. Rainbow trout fingerlings with average initial weight of 2.1±0.15 g stored in 24 experimental tanks (0.9×0.3×0.4 m). During the test, the experimental conditions were as: Water flow rate = 0.5 L/S, pH = 7.8, the average dissolved oxygen = 8.5 ppm, water temperature 10-13°C a natural photoperiod (10 L, 14 D). In order to feed the fingerlings, fish meal and meat meal were milled separately twice using tiny-meshed screens to obtain a homogeneous raw material. To mix food items to better work being done in the 1st dry ingredients that the greater the amount of rations were allocated to mixed together and then dry food items that were smaller (supplements, vitamins, minerals and salts), both blended after it's all dry ingredients together mixed well for 5 min with the ratios specified and then vitamin E with oil diet (tofu and soy oil) mixed and later received a higher volume was added to the diet. Then, the resulting mixture with specific humidity was ground using a meat grinder (mesh size from 5.1-2.2 mm) to yield string feeds. The food was dried (10% moisture) under a fan and open air for 24 h. It was then broken manually into small sizes (the size of the fish's mouth) and was stored in separate packages for each treatment at a cool and dry place away from sunlight. It was fed to the juveniles at 3-5% of body weight 6 times a day (8, 11, 13, 15 and 18 h).

Table 1: Diet formulations and proximate composition (protein, lipid, NFE, moisture and energy)

Ingredients	Amount
Fish meal	58
Wheat flour	14
Meat flour	12
Dextrin	5
Fish oil	6
Vegetable oil	2.2
Filler (sawdust)	0.8
Mineral mixturec	1
Vitamin mixture (vitamin E free)	1
Proximate composition (analyzed) moistur	10±0.8
Crude protein	49±1
Crude lipid	15.1±0.8
NFE	16.5±.92
Energy (Kcal g^{-1})	380

[1]1 kg mineral premix contained 130.6 g calcium phosphate dibasic, 327 g calcium lactate, 29.7 g ferric citrate, 137 g magnesium sulfate, 239.8 g potassium phosphate dibasic, 87.2 g sodium phosphate dibasic, 43.5 g sodium chloride, 0.15 g, aluminum chloride hexahydrate, 0.15 g potassium iodine, 0.1 g cupric chloride, 0.8 g manganese sulfate monohydrate, 1 g cobalt chloride hexahydrate and 3 g zinc sulfate heptahydrate; [2]Vitamin E free premix containing 6600 IU vitamin A (retinol palmitate), 2400 IU vitamin D3, 28 mg vitamin K (menadione sodium bisulfate), 47 mg thiamin, 53 mg riboflavin, 38 mg pyridoxine, 115 mg pantothenate, 220 mg niacin, 0.6 mg biotin, 12.7 mg folic acid, 0.06 mg vitamin B12 and 300 mg inositol per kg feed; Vitamin E used in the diets was Dl-all-rac-α-tocopherol (Sigma chemical CO., Germany) which had purity ≥96% and with diet were mixed by vegetable and fish oil

Analyses of growth indices: The fish were biometried in breeding period once every 10 days. To do this, 30 juveniles from each tank were randomly picked and anesthetized using clove powder. The fish weight and length, respectively were measured on a digital scale (accuracy 0.01 g) and on a biometry board (accuracy 1 mm) and transferred to rearing tanks afterwards. At the end of week 9, all the fish in each tank were analyzed for growth indices (weight gain, survival, Specific Growth Rate (SGR), Feed Conversion Rate (FCR) and Condition Factor (CF)).

$$\text{Weight gain}(g) = \text{Final weight} - \text{Initial weight}$$

$$\text{FCR} = \text{Dry feed intake}/\text{Wet weight gain}$$

$$\text{SGR} = \frac{\text{Ln final weight} - \text{Ln initial weight}}{63 \text{ days}} \times 100$$

$$\text{Survival}(\%) = \frac{\text{Initial fish number} - \text{Dead fish number}}{\text{Initial fish number}} \times 100$$

$$\text{CF} = \left(W/L^3\right) \times 100$$

Hematological measurements: At the completion of the experiment, changes in the blood factors (hemoglobin, hematocrit, Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), White Blood Cells (WBC) Red Blood Cells (RBC)) of 15 fish per treatment were determined after they had anesthetized with clove powder. Blood samples were taken through the caudal fin (Haghighi, 2009) and stored in EDTA tubes until use. WBC and RBC were counted under a Neubaur hemocutometer using diluents Gaur and torque (Blaxhall and Daisley, 1973). Hemoglobin was measured by a test kit (Pars Azmun Co., Tehran, Iran) with colorimetric method on a spectrophotometer (in 540 nm). Hematocrit was estimated through centrifuge (Haghighi, 2009). MCH and MCHC were calculated using the following equations (Haghighi, 2009):

$$\text{MCH} = \left(\text{Hb}/\text{RBC}\right) \times 10$$

$$\text{MCHC} = \left(\text{Hb}/\text{Ht}\right) \times 100$$

Experimental procedures for thermal stress: After the end of the rearing period to evaluate resistance of rainbow, trout fingerlings against thermal stress, thermal points of 24, 26 and 28°C were employed. To perform this evaluation, 5 fish from low-density treatments and 10 fish from those with high density were randomly selected and stored separately in 12 L tanks. The water temperature in each tank was set using an aquarium heater and survival of juveniles was assessed after 35 min.

Statistical analyses: For statistical evaluation of the data, normality of the data was analyzed by one-sample Kolmogorov-Smirnov test. In the case of normal data, two-way ANOVA analyzed the data by the use of SPSS statistical software. The data were compared using Duncan's test at a significance level of 5%.

RESULTS

The effect of vitamin E on growth: After 9 weeks of the experiment, fish fed diets containing different levels of vitamin E significantly showed increased body weight, SGR and lower FCR than fish fed diets without supplemental vitamin E. As shown in Table 2, most of the weight gain (average 49.16 g) was associated with 200 mg of vitamin E supplementation and the lowest value (average 6.13 g) was obtained by fish in the control. The highest level (average 43.3 g) of SGR was also resulted from a dietary vitamin E of 200 mg and the lowest estimation (average 1.3 g) obtained by fish fed control diets. The best FCR from 200 mg of vitamin E was not significantly different from the diets containing 100 and 1000 mg of vitamin E but had significant differences with diets without vitamin E. Supplementation of 200 mg vitamin E led to significant differences ($p<0.05$) in the CF values with the non-complemented diets with average values of 1.16 and 0.9, respectively. The examined levels of vitamin E did not differ significantly ($p>0.05$) in respect of survival rates (Table 2). In addition, the fish not fed with vitamin E showed symptoms such as sclerosis, lordosis muscle and skin atrophy.

The effects of vitamin E on hematological indices: Vitamin E had significant effects on hematological

Table 2: Growth indicators of rainbow trout fed different levels of vitamin E at two stocking densities

Vitamin E (mg kg^{-1} diet)	Density (m)	Weight gain (g)	SGR	FCR	CF	Survival (%)
0	400	13.73±1.4^{ab}	3.15±0.08^{a}	1.39±0.05^{b}	0.99±0.5^{a}	87±9.8^{a}
	800	13.6±0.72^{a}	3.14±0.07^{a}	1.39±0.04^{b}	1.01±0.07^{ab}	89±6.7^{a}
100	400	15.96±1.8^{ac}	3.5±0.09^{bc}	1.22±0.08^{a}	1.06±0.017^{abc}	92±6.4^{a}
	800	15.29±0.74^{abc}	3.3±0.05^{abc}	1.27±0.01^{a}	1.07±0.2^{abc}	89±5.8^{a}
200	400	16.48±0.57^{c}	3.4±0.08^{bc}	1.21±0.01^{a}	1.14±0.1^{bc}	92±6.2^{a}
	800	16.06±0.62^{c}	3.43±0.03^{c}	1.21±0.05^{a}	1.16±0.09^{c}	88±8.8^{a}
1000	400	15.7±1.2^{abc}	3.13±0.09^{abc}	1.26±0.01^{a}	1.1±0.11^{abc}	90±4.5^{a}
	800	14.23±1.6^{abc}	3.22±0.18^{ab}	1.29±0.03^{a}	1.07±0.05^{abc}	86±9.7^{a}
The effect of each variable and their interactions						
Vitamin		0.006*	0.002*	0.00*	0.02*	0.2
Density		0.25	0.39	0.26	0.86	0.9
Interactions		0.69	0.74	0.77	0.92	0.11

*Means significance at a level of 0.05

Table 3: Hematological indicators of rainbow trout fed different levels of vitamin E at two different densities

Vitamin E (mg kg^{-1} diet)	Density (m^2)	MCH (pg)	Hct (%)	RBC (10^6 L^{-1})	Hb (g L^{-1})	MCHC (g L^{-1})	EOS (%)	MON (%)	LYM (%)
0	400	119.06±4.9^a	29.73±4.83^a	0.51±0.06^a	6±0.36^a	20.76±2.7^a	5.9±0.6^{cd}	5.06±1.5^{ab}	76±4.3^a
	800	107.5±5.9^a	31.5±4.73^{ab}	0.56±0.05^a	6±0.27^a	19.3±2.08^a	6.2±0.6^d	5.4±1.2^b	78.6±4.04^{ab}
100	400	97.1±2.1^{ba}	34.6±5.2^{ab}	0.65±0.11^b	6.23±0.3^{ab}	18.1±1.78^a	5.6±0.4^{bcd}	4.7±1.3^{ab}	76.3±2.3^a
	800	92.42±1.6^a	32.3±6.86^{ab}	0.65±0.06^b	6.0±0.45^a	18.9±2.6^a	6±0.7^{cd}	3.5±1.4^{ab}	80±3.6^{ab}
200	400	100.1±8.1^a	42.06±2.95^b	0.74±0.13^c	7.40±.05^c	17.52±0.27^a	4.3±0.5^a	2.8±1.2^a	85.3±4.1^b
	800	98.1±6.1^a	38.3±6.02^{ab}	0.71±0.09^{bc}	6.93±0.4^{bc}	18.25±1.7^a	5.3±0.6^b	3.9±1.6^{ab}	82±4.3^{ab}
1000	400	100.7±8.1^a	37.96±5.7^{ab}	0.96±0.1^{bc}	6.9±0.4^{bc}	18.3±1.6^a	5.1±0.6^{bc}	3.8±1.3^{ab}	80.3±6.6^{ab}
	800	100.9±9.9^a	41.23±6.02^b	0.72±0.1^{bc}	7.2±0.3^c	17.6±1.89^a	6.1±0.3^d	4.9±1.5^{ab}	80.3±2.9^{ab}
The effect of each variable and their interactions									
Vitamin		0.03*	0.018*	0.009*	0.00*	0.78	0.63	0.48	0.15
Density		0.29	0.92	0.77	0.54	0.86	0.014*	0.55	0.67
Interactions		0.78	0.64	0.85	0.39	0.78	0.63	0.48	0.51

*Means significance at a level of 0.05

parameters, such as Hb, Hct, RBC, eosinophils MCH but not on MCHC, monocytes lymphocytes, however there was not significant changes among the treatments ($p<0.05$). Values of Hb, Hct, erythrocytes and lymphocytes in fish fed diets containing vitamin E (100, 200 and 1000 mg kg^{-1}) were significantly higher than the control diet. Levels of monocytes, eosinophils, MCH MCHC in the fish fed diets containing vitamin E were less than those in fish fed the control diet. The fish fed 1000 and 200 mg of vitamin E were not significantly different whereas groups fed levels of 1000 and 200 mg differed significantly from both 100 mg and control groups (Table 3).

The effects of stocking density on growth and hematological parameters: Although, the effect of crowding on growth was not significant, the fish stored in a density of 400 m^{-2} had relatively better growth performance (Table 2). The storage density had a significant effect on eosinophil ($p<0.05$), other blood parameters were not significantly different ($p>0.05$) (Table 3).

The interaction of vitamin E and stocking density on growth and hematological parameter: The highest weight gain, CF, SGR, FCR survival rate were found in fish fed 200 mg kg^{-1} of vitamin E stocked at low density (400 m^{-2}). These factors were lowest in fish without vitamin E feeding at high density (800 m^{-2}). The interaction of vitamin E and stocking density on hematological indices did not give rise to significant effects ($p>0.05$).

The effect of vitamin E on thermal stress: Thermal stress showed that regardless of the density, survival rates in fish fed all levels of vitamin E (1000, 200 100 mg kg^{-1}) in 24 and 26°C yielded no significant differences. However, survival rates in the control group was significantly lower than those in the supplemented levels ($p<0.05$). Fish fed diets containing 200 mg kg^{-1} of vitamin E at both densities (400 and 800 m^{-2}) differed statistically from fish fed diets containing 100 mg kg^{-1} of vitamin E and also the control group but not from fish fed 1000 mg kg^{-1} of vitamin E. Density affected significantly in 24, 26 and 28°C with the highest survival rate in 28°C and a density of 400 pieces m^{-2} (Table 4).

Table 4: Percentage survival of rainbow trout under heat stress at different levels of vitamin E and stocking density

Vitamin E (mg kg^{-1} diet)	Density (m^2)	24°C	26°C	28°C
0	400	33.30±6.7^a	33.30±6.7^a	19.86±6.8^{ab}
	800	47.7±5.09^{ab}	44.04±1.09^b	17.73±1.96^a
100	400	53.26±11.54^{bc}	46.63±6.65^{bc}	26.63±6.65^{ab}
	800	63.30±3.30^c	56.63±6.65^{cd}	29.93±5.7^{bc}
200	400	59.93±11.54^{bc}	55.53±7.73^{bcd}	42.16±7.67^d
	800	53.30±3.30^{bc}	63.30±3.30^d	31.06±3.8^{bc}
1000	400	46.6±6.65^{ab}	59.96±6.65^d	39.69±6.65^{cd}
	800	55.5±1017^{bc}	53.30±6.7^{bcd}	28.83±6.9^{abc}
The effect of each variable and their interactions				
Vitamin		0.006*	0.00*	0.00*
Density		0.057	0.04*	0.049*
Interactions		0.155	0.076	0.145

*Means significance at a level of 0.05

DISCUSSION

The present study indicates that dietary vitamin E is essential for normal growth of rainbow trout which showed significant effects on growth, blood parameters thermal stress mitigation. Vitamin E requirements were evaluated in several species and the results of the present study are in agreement with the reports of the earlier works, such as common carp requirements of 100, 80 and 100 mg kg^{-1} (Halver, 1995), Atlantic salmon demand of 120 mg kg^{-1} (Hamre *et al*., 1994), rohu up to 131.19 mg (Sau *et al*., 2004), grouper 100 mg (Lin and Shiau, 2005; North *et al*., 2006) rainbow trout 120 mg (Cowey *et al*., 1981).

Because fish growth is related to food quality and food intake, feeding is done on the basis of growth rates and percent body weight. Thus, weight gain and growth

rate of rainbow trout in this study was found to be related to the food quality that is changes in the supplemented levels of vitamin E in diet. Also, it was shown that with increasing vitamin E levels up to levels needed for fish, fish weight increased, so that with supplementing from 200-1000 mg kg^{-1} no weight gain was observed. This is in agreement with those of Sau *et al.* (2004), Paul *et al.* (2004), Lin and Shiau (2005) and Chen *et al.* (2004). However, studies of Cowey *et al.* (1981, 1983) on salmon showed that with increasing vitamin E, weight gain was not observed. Blazer (1982) demonstrated that rainbow trout reared without vitamin E for 4 months showed no differences in growth compared with fish fed 400 mg of dietary alpha-tocopherol. Similar results were obtained for other species, such as Atlantic salmon (Raynard *et al*., 1991; Hardie *et al*., 1990), gilthead seabream (Montero *et al*., 2001), rainbow trout (Blazer and Wolke, 1984; Furones *et al*., 1992). Nevertheless, some researchers reported effects of deficiency in dietary vitamin E on the growth and survival of fish. For instance, consequences observed in Atlantic salmon (Hamre *et al*., 1994) rainbow trout (Cowey *et al*., 1983) chinook salmon (Thorarinsson *et al*., 1994) agree with the results of present study.

The vitamin E requirement in this study was higher than that in other reports (Lin and Shiau, 2005; Sau *et al*., 2004; North *et al*., 2006) in that the best performance was obtained with 200 mg of vitamin E. The differences in vitamin E requirements may be dependent on ration formulation such as the type and quality of used oils, species, fish size, form of vitamin E and/or the experimental conditions (Montero *et al*., 2001). Besides, fluctuations and/or high FCR estimations can reflect difficulties in the diet or feeding methods (Montero *et al*., 1999b). In this investigation, as feeding method was similar for all treatments, therefore different FCR values depended on the type of feed rations, i.e., differences in vitamin E levels.

Some symptoms of vitamin E deficiency have been reported, such as reduced growth, damage to collagen, darkened skin, lordosis, sclerosis mortality (Fracalossi *et al*., 1998; Montero *et al*., 1999a; Wang *et al*., 2003; Chen *et al*., 2004). The present study revealed some symptoms of vitamin E deficiency, such as reduced growth, atrophy of muscle darkened skin. Many deficient fish showed darkened skin which might be caused by impaired production and/or malfunction of melanocyte-stimulating hormones or broken tyrosine phosphorylation affecting the regulation of melanosome distribution in melanocytes (Chen *et al*., 2004). Melanin concentration was not measured in this study and further work is needed to verify the cause of the darkened skin associated with vitamin E deficiency in the rainbow trout. Feeding the fish with ration without vitamin E rendered thinner (atrophied muscle) than other groups of fish. Muscle atrophy in fish and other animals may be caused by lack of antioxidants, particularly vitamin E in the diet because dietary vitamin E deficiency can cause severe oxidative stress (Puangkaew *et al*., 2004) which plays a role in animals having muscle atrophy.

After 9 weeks, despite signs of vitamin E deficiency in the control fish, there was no significant differences in survival rates since the lowest survival was observed in the control; this is likely due to the short duration of the test period leading to non-significant fish death.

Huang and Huang (2004), suggested that decreased survival at high stocking density is related to low quality of the water physicochemical and also to competition for food and space which will increase the stress on fish. The results obtained in this study are in accordance with research on stocking densities of rainbow and brown trout in which fish growth and survival were not affected (North *et al*., 2006). When the effects of stocking density on the growth of fish are examined, different terms of expression are normally used and density is expressed in terms of either biomass or numbers of fish per unit area or volume. Sometimes, numbers of fish are reduced as the fish grow, so as to maintain constant biomass stocking densities (Irwin *et al*., 1999). In this research, numbers of rainbow trout were held constant for the duration of the experiment and therefore, biomass density was allowed to increase with time due to the growth of the fish. Accordingly, maximum biomass was observed in treatment with a density of 400 piece m^{-2} rationing with 200 mg of vitamin E on the other hand, the lowest biomass was noticed in fish fed without vitamin E with a density of 800 pieces m^{-2}.

Many researchers have suggested that increased density has negative effects on growth factors (Irwin *et al*., 1999; Montero *et al*., 1999a; Farhad *et al*., 2006; Trenzado *et al*., 2006, 2007; Andrade *et al*., 2007; Trenzado *et al*., 2007, 2008). Overcrowding is a common chronic stressor in aquaculture (Cristana *et al*., 2006; Chen *et al*., 2004), hence suppressed growth were reported as a result of high stocking density (Ross and Watten, 1998; Irwin *et al*., 1999; Rowland *et al*., 2006). This effect has been attributed to several factors including decreased food consumption. High stocking density also imposes increased energy demands that require fish to cope with metabolic adjustments, such as changes of gluconeogenic and glucolytic enzyme activities. This is because under such conditions, food consumption is reduced and the extra expenditure of energy has to be met by the body reserves resulting

in reduced growth (Trenzado *et al*., 2007, 2008; Garcia *et al*., 2007; Trenzado *et al*., 2006, 2007, 2008).

Agradi *et al*. (1993), evaluated positive effect of dietary vitamin E on sturgeon growth and expressed that vitamin E indirectly increased growth by controlling or reducing metabolic costs resulting in reduced tissue damage during stress. In the study, juveniles reared at low density had higher growth rates than those reared at high density, nonetheless density impact on fish growth was not significant. Yet, weight gain and SGR of fish reared at low density (400 m^{-2}, 16.48 g) was greater than the fish raised at high density (800 m^{-2}, 13.6 g) this may partially confirm the hypothesis that increased fish density will reduce growth. However, there were no significant differences in weight gain between treatments after 9 weeks which probably needs longer testing times to be verified.

It has also been reported that high stocking density affects some hematological parameters. Though, the results are widely variable and not decisive all the time, many researchers reported haemoconcentration, as a common effect of fish crowding evidenced as increases in both Hct and Hb values. This response may be a strategy for increasing the oxygen carrying capacity of blood under high energy demand situations such as chronic stress (Trenzado *et al*., 2006; Montero *et al*., 2001; Trenzado *et al*., 2007). In this study, Hct and Hb concentrations were not significantly affected which may be related to regulative role of vitamin E for metabolic activities in fish and also to reduced oxygen consumption under conditions of oxygen deficiency (Randall *et al*., 1992; McKenzie *et al*., 1995). The fish fed without vitamin E under conditions of high density probably increased oxygen consumption rising amount of Hb, RBC, MCHC and MCH, as opposed to the fish kept at low density indicating likely elevated oxygen demand at high density (Srivastava and Sahai, 1987). Significantly lower Hct, Hb RBC was determined in the control fish than fish fed with different levels of vitamin E. The small amount of blood parameters in the control refers to anemia in fish, as was demonstrated by Taveekijakarn *et al*. (1996) and Chen *et al*. (2004) as well.

Chen *et al*. (2004), suggested that antioxidant properties of vitamin E might also influence temperature tolerance in fish. Vitamin E is a component of cell membranes that prevents long-chain, highly unsaturated fatty acids in phospholipids from oxidative damage these fatty acids maintain cell membrane fluidity. Vitamin E deficient fish might have had impaired membrane structure and function due to lipid oxidation because of heat stress, hence those fish received less vitamin E showed greater mortality rates.

CONCLUSION

This study reveals that different levels of vitamin E can affect growth and hematology of rainbow trout fed 200 mg kg^{-1} dietary vitamin E and stocked in a density of 400 pieces m^{-2}. The results of thermal stress show that fish fed different levels of vitamin E had better survival rate than the non-supplemented fish.

ACKNOWLEDGEMENTS

This research was part of a master's thesis sponsored by Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran. The researchers are deeply grateful to the management of Tehran Ghzel Ala Co. and all the people who helped us to complete this research.

REFERENCES

Agradi, E., G. Abrami, G. Serrini, D. Mckenzie, C. Bolis and P. Bronzi, 1993. The role of dietary N-3 fatty acid and vitamin E supplements in growth of sturgeon (*Acipenser naccarii*). Comp. Biochem. Physiol. Part A Physiol., 105: 187-195.

Andrade, J.I.A., E.A. Ono, G.C. Menezes, E.M. Brasil and R. Roubach *et al*., 2007. Influence of diets supplemented with vitamins C and E on pirarucu (*Arapaima gigas*) blood parameters. Comp. Biochem. Phys., 146: 576-580.

Blaxhall, P.C. and K.W. Daisley, 1973. Routine haematological methods for use with fish blood. J. Fish Biol., 5: 771-781.

Blazer, V.S., 1982. The effect of marginal deficiencies of ascorbic acid and α-tocopherol on the natural resistance and immune response of rainbow trout (*Salmo gairdneri*). Ph.D. Thesis, University of Rhode Island.

Blazer, V.S. and R.E. Wolke, 1984. The effects of α-tocopherol on the immune response and non-specific resistance factors of rainbow trout (*Salmo gairdneri* Richardson). Aquaculture, 37: 1-9.

Bolasina S., M. Tagawa, Y. Yamashita and M. Tanaka, 2006. Effect of stocking density on growth, digestive enzyme activity and cortisol level in larvae and juveniles of Japanese flounder, *Paralichthys olivaceus*. Aquaculture, 259: 432-443.

Chen, R., R. Lochmann, A. Goodwin, K. Praveen, K. Dabrowski and K.J. Lee, 2004. Effects of dietary vitamins C and E on alternative complement activity, hematology, tissue composition, vitamin concentrations and response to heat stress in juvenile golden shiner (*Notemigonus crysoleucas*). Aquaculture, 242: 553-569.

Cowey, C.B., J.W. Adron, M.J. Walton, J. Murray, A. Youngson and D. Knox, 1981. Tissue distribution, uptake and requirement for α-tocopherol of rainbow trout (*Salmo gairdneri*) fed diets with a minimal content of unsaturated fatty acids. J. Nutr., 111: 1556-1567.

Cowey, C.B., J.W. Adron and A. Youngson, 1983. The vitamin E requirement of rainbow trout (*Salmo gairdneri*) given diets containing polyunsaturated fatty acids derived from fish oil. Aquaculture, 30: 85-93.

De Menezes, G.C., M. Tavares-Dias, E.A. Ono, J.I.A. de Andrade and E.M. Brasil *et al*., 2006. The influence of dietary vitamin C and E supplementation on the physiological response of pirarucu, *Arapaima gigas*, in net culture. Comp. Biochem. Physiol. Part A: Mol. Integrative Physiol., 145: 274-279.

Farhad, G.P., S.K.A.D. Alameh, M.M. Arani and N.E. Mansour, 2006. Effect of density on growth and feed conversion ratio in rainbow trout (*Oncorhynchus mykiss*). Pajouhesh Va Sazandegi, 70: 23-27 (In Persian).

Fracalossi, D.M., M.E. Allen, D.K. Nichols and O.T. Oftedal, 1998. Oscars, *Astronotus ocellatus*, have a dietary requirement for vitamin C. J. Nutr., 128: 1745-1751.

Furones, M.D., D.J. Alderman, D. Bucke, T.C. Fletcher, D. Knox and A. White, 1992. Dietary vitamin E and the response of rainbow trout, *Oncorhynchus mykiss* (Walbaum), to infection with Yersinia ruckeri. J. Fish Biol., 41: 1037-1041.

Garcia, F., F. Pilarski, E.M. Onaka, F.R. Moraes and M.L. Martins, 2007. Hematology of Piaractus mesopotamicus fed diets supplemented with vitamins C and E, challenged by *Aeromonas hydrophila*. Aquaculture, 271: 39-46.

Haghighi, M., 2009. Laboratory Methods of Fish Hematology. Iranian Fisheries Research Organization Publication, Tehran, Iran.

Halver, J.E., 1995. Vitamin requirement study techniques. J. Applied Ichthyol., 11: 215-224.

Hamre, K., B. Hjeltnes, H. Kryvi, S. Sandberg, M. Lorentzen and O. Lie, 1994. Decreased concentration of hemoglobin, accumulation of lipid oxidation products and unchanged skeletal muscle in Atlantic salmon (*Salmo salar*) fed low dietary vitamin E. Fish Physiol. Biochem., 12: 421-429.

Hardie, L.J., T.C. Fletcher and J.C. Secombes, 1990. The effect of vitamin E on the immune response of the Atlantic salmon (*Salmo salar* L.). Aquaculture, 87: 1-13.

Huang, C.H. and S.L. Huang, 2004. Effect of dietary vitamin E on growth, tissue lipid peroxidation and liver glutathione level of juvenile hybrid tilapia, *Oreochromis niloticus*×*O. aureus*, fed oxidized oil. Aquaculture, 237: 381-389.

Irwin, S., J. O'Halloran and R.D. Fitz Gerald, 1999. Stocking density, growth and growth variation in juvenile turbot, *Scophthalmus maximus* (Rafinesque). Aquaculture, 178: 77-88.

Jorgensen, E.H., J.S. Christiansen and M. Jobling, 1993. Effects of stocking density on food intake, growth performance and oxygen consumption in Artic charr (*Salvelinus alpinus*). Aquaculture, 110: 191-204.

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

McKenzie, D.J., G. Piraccini, J.F. Steffensen, C.L. Bolis, P. Bronzi and E.W. Taylor, 1995. Effects of diet on spontaneous locomotor activity and oxygen consumption in Adriatic sturgeon (*Acipenser naccarii*). Fish Physiol. Biochem., 14: 341-355.

Montero, D., M.S. Izquierdo, L. Tort, L. Robaina and J.M. Vergara, 1999a. High stocking density produces crowding stress altering some physiological and biochemical parameters in gilthead seabream, *Sparus aurata*, juveniles. Fish Physiol. Biochem., 20: 53-60.

Montero, D., M. Marrero, M.S. Izquierdo, L. Robaina, J.M. Vergara and L. Tort, 1999b. Effect of vitamin E and C dietary supplementation on some immune parameters of gilthead seabream (*Sparus aurata*) juveniles subjected to crowding stress. Aquaculture, 171: 269-278.

Montero, D., L. Tort, L. Robaina, J.M. Vergara and M.S. Izquierdo, 2001. Low vitamin E in diet reduces stress resistance of gilthead seabream (*Sparus aurata*) juveniles. Fish Shellfish Immunol., 11: 473-490.

North, B.P., J.F. Turnbull, T. Ellis, M.J. Porter, H. Migaud, J. Bron and N.R. Bromage, 2006. The impact of stocking density on the welfare of rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 255: 466-479.

Paul, B.N., S. Sarkar and S.N. Mohanty, 2004. Dietary vitamin E requirement of mrigal, *Cirrhinus mrigala* fry. Aquaculture, 242: 529-536.

Puangkaew, J., V. Kiron, T. Somamoto, N. Okamoto, S. Satoh, T. Takeuchi and T. Watanabe, 2004. Nonspecific immune response of rainbow trout (*Oncorhynchus mykiss* walbaum) in relation to different status of vitamin E and highly unsaturated fatty acids. Fish Shellfish Immunol., 16: 25-39.

Randall, D.J., D.J. McKenzie, G. Abrami, G.P. Bondiolotti and F. Natiello *et al*., 1992. Effects of diet on responses to hypoxia in sturgeon (*Acipenser naccarit*). J. Exp. Biol., 170: 113-125.

Raynard, R.S., A.H. McVicar, J.G. Bell, A. Youngson, D. Knox and C.O. Fraser, 1991. Nutritional aspects of pancreas disease of Atlantic salmon: the effects of dietary vitamin E and polyunsaturated fatty acids. Comp. Biochem. Physiol., 98A: 125-131.

Ross, R.M. and B.J. Watten, 1998. Importance of rearing-unit design and stocking density to the behavior, growth and metabolism of lake trout (*Salvelinus namaycush*). Aquacult. Eng., 19: 41-56.

Rowland, S.J., C. Mifsud, M. Nixon and P. Boyd, 2006. Effects of stocking density on the performance of the Australian freshwater silver perch (*Bidyanus bidyanus*) in cages. Aquaculture, 253: 301-308.

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

Soltani, M., 2008. Fish and Shellfish Immunology. Tehran University Publication, Tehran, Iran.

Srivastava, A.K. and I. Sahai, 1987. Effects of loading density on carbohydrate metabolism and hematology in the Indian freshwater catfish, *Heteropneustes fossilis*. Aquaculture, 66: 275-286.

Taveekijakarn, P., T. Miyazaki, M. Matsumoto and S. Arai, 1996. Study on vitamin E deficiency in amago salmon. Bull. Fac. Bioresources Mie Univ., 16: 17-24.

Thorarinsson, R., M.L. Landolt, D.G. Elliot, R.J. Pascho and R.W. Hardy, 1994. Effect of dietary vitamin E and selenium on growth, survival and the prevalence of *Renibacterium salmoninarum* infection in chinook salmon (*Oncorhynchus tshawytscha*). Aquaculture, 121: 343-358.

Trenzado, C.E., A.E. Morales and M. de la Higuera, 2006. Physiological effects of crowding in rainbow trout (*Oncorhynchus mykiss*), selected for low and high stress responsiveness. Aquaculture, 258: 583-593.

Trenzado, C.E., M. de la Higuera and A.E. Morales, 2007. Influence of dietary vitamins E and C and HUFA on rainbow trout (*Oncorhynchus mykiss*) performance under crowding conditions. Aquaculture, 263: 249-258.

Trenzado, C.E., A.E. Morales and M. de la Higuera, 2008. Physiological changes in rainbow trout held under crowded conditions and fed diets with different levels of vitamins E and C and highly unsaturated fatty acids (HUFA). Aquaculture, 277: 293-302.

Wang, X., K.W. Kim, S.C. Bai, M.D. Huh and B.Y. Cho, 2003. Effects of the different levels of dietary vitamin C on growth and tissue ascorbic acid changes in parrot fish (*Oplegnathus fasciatus*). Aquaculture, 215: 203-211.