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Effect of High Vitamin E Dosages on Lipid Peroxidation and Fatty Acid Profile of *Labeo rohita* Fingerlings

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ABSTRACT

A 60 days feeding trial was conducted to investigate the effect of dietary high doses of vitamin E on *Labeo rohita* fingerlings. Five experimental diets were formulated to contain one control (VE_0), two adequate levels of vitamin E supplementation, *i.e.* 100 and 150 mg/kg diet (VE_{100}, VE_{150}) and two high levels of vitamin E supplementation, *i.e.* 1000 and 1500 mg/kg diet (VE_{1000}, VE_{1500}). At the onset of feeding trial, 375 fish of nearly uniform initial body weight (3.58±0.04 g) were distributed in fifteen experimental tanks in the way that each dietary treatment was fed in triplicates and each tank contained 25 fish. Upon termination of feeding trial, no significant differences were found for dry matter, crude protein, crude fat and ash content in fish body. Lipid peroxidation was determined in terms of thiobarbituric acid reactive substances (TBARS) and antioxidant enzyme activities. The minimum value of TBARS was recorded in VE_{150} group, which was increased again with supplementation of high vitamin E levels. Similarly, adequate supplementation levels (VE_{1000}, VE_{1500}) reduced the superoxide dismutase (SOD), catalase and glutathione peroxidase activities, which were increased by feeding high vitamin dosages. Supplementation of vitamin E decreased the saturated fatty acids (SFA) and monoenes while increased the poly unsaturated fatty acids (PUFA) compared to control group. Similarly, eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), docosahexanoic acid (DHA), total n-3 PUFA and total n-6 PUFA, arachidonic acid (ARA)/EPA, EPA/DHA, n3/n6 ratios were increased in muscle of fish fed vitamin E supplemented diets. However, differences between adequate and high doses of vitamin E were not significant for most of the fatty acids. In conclusion, dietary vitamin E supplementation reduced the lipid peroxidation which lead to increased PUFA contents in *L. rohita*. However, high doses of vitamin E showed the pro-oxidative effect which promoted lipid peroxidation.

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Authors' Contribution
MF performed the experiment, collected and analyzed the data and wrote the manuscript. MA supervised the experiment and manuscript writing. SZHS helped in experiment performance, data analysis and manuscript writing.

Key words
Proximate composition, TBARS, Antioxidant enzyme activities, α-tocopherol, Fatty acid profile.

INTRODUCTION

Lipid peroxidation is a process in which free radicals which are generated during metabolic breakdown of food attack on the lipid molecules especially membranous poly unsaturated fatty acid (PUFA) to take their free electrons. It is the oxidative degradation of lipids which results in changed membrane fluidity (Mates *et al*., 1999), DNA damage and changed enzymatic activities leading to cell damage (Kanazawa, 1993). The quality of food particularly of those which include high amount of unsaturated fatty acids are more vulnerable to deterioration by lipid oxidation. It causes various broad and long term consequences to food including loss of nutritional values (*e.g.* loss of proteins and PUFAs), production of inedible flavour and smell by unhealthy molecules and reduction of shelf life (Secci and Parisi, 2016). As fishes are poikilotherm, they possess highly unsaturated lipids than that of homeotherms which makes them susceptible to lipid peroxidation (Abele and Puntarulo, 2004). The presence of any potent biological antioxidant reduces the chances of lipid peroxidation in fish (Peng *et al*., 2009; Zhou *et al*., 2013).

Vitamin E, α-tocopherol, is considered as an important fat soluble vitamin since long and performs a vital function in lipid peroxidation termination. It is found between the lipid component of cell membranes and lipoproteins. Animals acquire vitamin E like compounds (tocotrienols and tocopherols) from the algal sources and higher plants present in their diets as they are unable to synthesize them inside their body (Hess, 1993). This fat soluble vitamin functions as chain breaking, free radical scavenging antioxidant, which protects the proteins, lipids and cell membranes from oxidative damage and maintains the tissues integrity of fish including gilthead sea bream (Tocher *et al*., 2002), turbot (Tocher *et al*., 2002) and

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black sea bream (Peng *et al*., 2009). Bai and Gatlin (1993) also reported that lipid peroxidation in fish fillets can be prevented by increasing their anti-oxidation capability through dietary addition of vitamin E.

Table I.- Ingredient and chemical composition of experimental diets.

Ingredients	VE_0	VE_{100}	VE_{150}	VE_{1000}	VE_{1500}
Fishmeal	40	40	40	40	40
Soybean meal	25	25	25	25	25
Wheat flour	13	13	13	13	13
Rice polish	11.7	11.7	11.7	11.7	11.7
Cod liver oil[1]	8	8	8	8	8
Mineral mixture[2]	1	1	1	1	1
Vitamin premix (E free)[3]	1	1	1	1	1
Choline chloride	0.3	0.3	0.3	0.3	0.3
Vitamin E (mg/kg)[4]	0	100	150	1000	1500
Chemical composition					
Dry matter (%)	91.1	90.9	91	91	90.9
Crude protein (%)	34.1	34	34.4	34	34.3
Crude fat (%)	10.9	10.8	11.1	11.1	11
Gross energy (kcal/kg)	4081	4102	4079	4083.3	4087.3
α-tocopherol (mg/kg)	17.90	109.13	160.10	943.53	1401.43

[1]Cod liver oil was purchased from Poultry-vet Co, Nazimabad, Karachi, Pakistan. [2] Each kg of mineral mixture contains: $CaCO_3$, 316; KH_2PO_4, 479; $MgSO_4.7H_2O$, 153; NaCl, 51; $CoCl.6H_2O$, 0.0816; Ammonium molybdate, 0.061; $AlCl_3.6H_2O$, 0.255; $ZnSO_4.7H_2O$, 121.33; $CuSO_4.5H_2O$, 210.67; $MnSO_4.5H_2O$, 116.67; $FeSO_4.H_2O$, 100.67. [3]Each kg of Vitamin premix contains; Vitamin A (retinoic acid) 5.0 g, Vitamin B1 (thiamine) 0.5 g, Vitamin B2 (riboflavin) 3.0 g, Vitamin B3 (niacin) 5.0 g, Vitamin B6 (pyridoxine) 1.0 g, Vitamin B7 (biotin) 0.05 g, Vitamin B9 (folic acid) 0.18 g, Vitamin B12 (cobalamin) 0.002 g, Vitamin C (ascorbic acid) 5.0 g, Vitamin D3 (cholecalciferol) 0.002 g, Choline 100 g, Cellulose 815.26 g. [4]Vitmain E (VE) was supplemented in the form of DL-α-toopherol acetat (Sigma-Aldrich).

Vitamin E deficiency has been known to adversely affect the lymphocyte activity and immune responses of neutrophils and macrophages in animals (Tengerdy, 1990). In fish too, dietary vitamin E deficiency has resulted in reduced phagocyte activity (Blazer and Wolke, 1984). Supplementation of high levels of dietary α-tocopherol has been reported to improve the tissue lipids stability from oxidation in sea bass (Gatta *et al*., 2000), turbot, halibut and sea bream (Tocher *et al*., 2002). High dietary vitamin E levels not only preserve fish tissues from rancidity during culture but also during post-harvest storage (Onibi *et al*., 1996). Nevertheless, some previous reports have evidenced the induced lipid peroxidation in the tissues of yellowtail (Ito *et al*., 1999) and rainbow trout (Tokuda and Takeuchi, 1999) by feeding excess dosage levels of α-tocopherol. The dietary vitamin E requirement of a number of fish species has been established and for *Labeo rohita* it is 131.91 mg/kg of dry diet (Sau *et al*., 2004). Research studies have demonstrated that the vitamin E behaves entirely different at high supplementation levels beyond its requirement in certain fish species. Such behavior of vitamin E has been studied for some fish species but very little information is available regarding carp species. Thus, the objective of the present study was to determine the effect of vitamin E at adequate and high supplemental levels on proximate composition, TBARS, antioxidant enzyme activities and fatty acid profile of *L. rohita* fingerlings.

MATERIALS AND METHODS

The present research work was carried out in the Fish Nutrition Laboratory, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Pakistan.

Experimental diets and design

Dietary feed ingredients (fishmeal, soybean meal, wheat flour and rice polish) were purchased from local market and analyzed chemically by following AOAC (1995) before the preparation of experimental diets. The ingredient composition of experimental diets is given in Table I. Experimental diets were made from same basal diet which differed only in the level of α-tocopherol supplementation. Five experimental diets were made containing α-tocopherol at adequate (100 mg/kg and 150 mg/kg) and high (1000 mg/kg and 1500 mg/kg) levels, while, the experimental diet without vitamin E (0 mg/kg) served as control diet (completely randomized design, CRD). The adequate levels covered the concentration that was recommended as the optimal requirement (*i.e.* 131.91 mg/kg) of α-tocopherol for *L. rohita* (Sau *et al*., 2004). Vitamin E was supplemented in the form of DL-α-tocopherol acetate and was obtained from Sigma-Aldrich. All of the added vitamin E, α-tocopherol acetate was mixed with the cod liver oil prior to addition of experimental diets. Cod liver oil was obtained from Poultry-vet Co., Nazimabad, Karachi, Pakistan. For feed manufacturing dry ingredients were ground and sieved (0.05 mm) in cereal grinding machine (FFC-45, JIMO, China). Feed ingredients, vitamin E free vitamin premix, mineral mixture and choline chloride (Unichem Laboratories) were thoroughly mixed in an electric mixer before the oil was added. After the addition of Cod liver oil, approximately 150 ml of distilled water/kg of diet was added. The moist mixture was extruded through hand pelletizer with a 3-mm diameter die. The resulting moist pellets were dried at room temperature using an electric fan to a moisture content of approximately 10%. The proximate composition of experimental diets was determined using standard methods

(AOAC, 1995). All of the five experimental diets were isonitrogenous, isolipidic and isocaloric (Table I). Pellets were ground into small pieces, sieved to obtain appropriate sizes, and stored frozen in self-sealing plastic bags at −18°C before and throughout the feeding trial. The fatty acid profile of experimental diets is presented in Table II.

Table II.- Fatty acid[1] profile of experimental diets.

Fatty acids	VE_0	VE_{100}	VE_{150}	VE_{1000}	VE_{1500}
14:0 n-0	4.20	4.15	4.21	4.20	4.11
16:0 n-0	9.74	9.72	9.71	9.70	9.72
18:0 n-0	2.70	2.69	2.71	2.72	2.70
16:1 n-7	11.70	11.84	11.66	11.77	11.72
18:1 n-7	12.40	12.37	12.44	12.36	12.43
18:1 n-9	16.50	16.58	16.56	16.55	16.45
18:2 n-6	4.10	4.11	4.03	4.11	4.17
20:4 n-6	2.20	2.26	2.30	2.29	2.27
18:3 n-3	4.40	4.20	4.30	4.38	4.42
20:5 n-3	10.00	10.80	10.40	11.02	10.65
22:5 n-3	2.30	2.36	2.39	2.40	2.41
22:6 n-3	12.10	12.20	12.15	12.21	12.28
Others[2]	7.66	6.72	7.14	6.29	6.67
Total	100.00	100.00	100.00	100.00	100.00
Saturated	16.64	16.56	16.63	16.62	16.53
Monounsaturated	40.60	40.79	40.66	40.68	40.60
n-3	28.80	29.56	29.24	30.01	29.76
n-6	6.30	6.37	6.33	6.40	6.44
n-9	16.50	16.58	16.56	16.55	16.45
ARA/EPA	0.22	0.21	0.22	0.21	0.21
EPA/DHA	0.83	0.89	0.86	0.90	0.87
n-3/n-6	1.75	1.78	1.77	1.81	1.81
Monoenes/Polyenes	0.79	0.78	0.78	0.77	0.77

[1]Fatty acid = % total fatty acid detected. [2]Others= Sum of 15:0, 15:1, 16:1 n-9, 16:2 n-7, 17:0, 17:1 n-7, 18:2 n-3, 20:1 n-9, 21:5 n-3, 22:1 n-9, 22:2 n-6, 22:4 n-6.

Experimental fish and rearing conditions

Physically healthy *L. rohita* fingerlings were obtained from Government Fish Seed Hatchery, Faisalabad, Pakistan, and transported alive to the Fish Nutrition Laboratory. Prior to the feeding trial, all fish were acclimated to the indoor rearing conditions for 2 weeks in cemented tanks (1000 L) while feeding a basal diet. At the onset of feeding trial twenty-five fish were stocked randomly into triplicate tanks (70-l) for each dietary treatment with near uniform biomass (initial body weight 3.58±0.04 g). Fish were fed the test diets to satiation, 6 days a week for 8 weeks. The daily ration was divided into two, and fed to the fish at 08:00 and 16:00 h. The fish were weighed every 2 weeks and their ration adjusted accordingly. After three hours of feeding the water was exchanged with filtered fresh water (Habib *et al*., 2018). Round the clock aeration was provided through capillary system to all the experimental tanks. During experimental period the dissolved oxygen and pH were monitored constant throughout the experimental duration with the help of HANNA DO meter (model HI 9147) and AMPROBE pH meter (model WT-80), which were regulated between 5.8-7.3 mg/L and 7.4-8.6, respectively. The water temperature ranged from 24.9-28.7°C throughout the trial.

Sample collection and analysis

At the termination of feeding trail, fish were starved for 24 h, and anesthetized using MS-222 (Sigma-Aldrich) and sacrificed. Five fish from each tank were randomly collected, homogenized in meat mincer and used for whole body proximate, antioxidant enzymes and TBARS analysis. From the other five fish, muscles were dissected out pooled, homogenized and utilized for analysis of fatty acid profile.

Proximate composition of diet and whole body samples were determined following AOAC (1995). Moisture content was determined by drying samples in an oven at 105°C until constant weight. Samples used for crude protein were acid digested and analyzed by Kjeldahl's method (nitrogen×6.25). Crude fat was estimated using Soxhlet apparatus by extraction with petroleum ether. Ash analysis was performed by incineration of sample in muffle furnace at 600°C for 6h. The caloric values of experimental diets were determined using adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline, USA).

Fats of diet samples were extracted by petroleum ether extraction method and used for determination of α-tocopherol. α-tocopherol analysis was performed by high performance liquid chromatography (HPLC) following the method described by Anwar *et al*. (2006). Whole body lipid peroxidation was determined colorimetrically as thiobarbituric acid reactive substances (TBARS), as described by Gatta *et al*. (2000). For antioxidant enzymes assay, enzyme extract was prepared by homogenizing whole body sample in phosphate buffer. The activity of superoxide dismutase (SOD) was determined by measuring its ability to inhibit the photo reduction of nitroblue tetrazole (NBT) following the method of Giannopolitis and Ries (1977). Catalase activity was determined by its ability to decrease the H_2O_2 concentration at 240 nm (Chance and Mehaly, 1977). The activity of peroxidase was determined by measuring its ability to reduce the concentration of H_2O_2 at A_{470} nm (Civello *et al*., 1995).

The extracted fats from diet and muscle samples

(petroleum ether extraction method) were analyzed for their fatty acid composition by gas chromatography. Fatty acid profile was determined from the fatty acid methyl esters (FAME) derivatives of the transesterified fats following IUPAC (1987). Fatty acid methyl esters were analyzed with gas chromatography (GC) (SHIMADZU, model GC-17A FID) and identified by comparing their relative and absolute retention time with those of authentic known standards. Relative concentrations were calculated and expressed as mass-percentages of the total identified fatty acids.

Statistical analysis

Statistical analyses were performed using CoStat computer package (Version 6.303, PMB 320, Monterey, CA 93940, USA). Data are presented as mean and pooled standard error (PSE). The data were subjected to one-way analysis of variance. Multiple comparisons among mean values were made with Student-Newman-Keul's test. Differences among treatments were considered significant at a probability level of $p<0.05$.

RESULTS

Proximate composition

Effect of low and high doses of vitamin E supplementation on proximate composition of *L. rohita* fingerlings is given in Table III. Moisture, crude protein, crude lipid and ash content in fish whole body were not affected by vitamin E supplementation.

Lipid peroxidation

The effect of medium and high doses of vitamin E on TBARS level in whole body is given in Table IV. The minimum value of TBARS was recorded in VE_{150} group, which was increased with high supplementation level of vitamin E. The supplementation below 150 mg/kg di*et al*so resulted in higher TBARS values. However, its maximum value was recorded in the control group (VE_0).

Vitamin E supplementation at different levels provoked statistically significant differences in the activities of antioxidant enzymes among different treatments (Table IV). In the present study, both of the medium levels (VE_{100} and VE_{150}) exerted similar effects on the activities of whole body antioxidant enzymes. Moreover, the highest dose (VE_{1500}) of vitamin E supplementation more profoundly enhanced the activities of these enzymes. The differences between highest vitamin E supplemented diet and control diet (VE_0) were statistically non-significant.

Fatty acid profile

Treatment effects on muscle fatty acid profile are given in Table V. Supplementation of vitamin E decreased the saturated fatty acids (SFA) and monoenes while increased the PUFAs compared to control group. Similarly, eicosapentaenoic acid (EPA, 20:5 n-3), docosapentaenoic acid (DPA, 22:5 n-3), docosahexanoic acid (DHA, 22:6 n-3), total n-3 PUFA and total n-6 PUFA were increased, while arachidonic acid (ARA, 20:4 n-6) was decreased in muscle of fish fed vitamin E supplemented diets.

Table III.- Effect of high doses of vitamin E on whole body proximate composition of *L. rohita* fingerlings.

	Experimental diets					PSE	P-value
	VE_0	VE_{100}	VE_{150}	VE_{1000}	VE_{1500}		
Moisture (%)	72.80	72.31	72.69	72.60	73.00	0.34	NS
Crude protein (%)	16.94	17.17	17.26	17.13	17.18	0.31	NS
Crude Lipids (%)	4.97	4.73	5.17	4.96	5.53	0.28	NS
Ash (%)	2.81	2.86	2.94	2.49	3.21	0.30	NS

The data are mean of three replicates. Mean values within a row sharing different superscript letters are significantly different ($p<0.05$). Pooled standard error (PSE) = √MSE/n (where MSE is mean-squared error). NS, non-significant ($p>0.05$).

Table IV.- Effect of high doses of vitamin E on whole body lipid peroxidation of *L. rohita* fingerlings.

	Experimental diets					PSE	P-value
	VE_0	VE_{100}	VE_{150}	VE_{1000}	VE_{1500}		
TBARS	2.29^{a}	1.86^{c}	1.44^{e}	1.69^{d}	2.07^{b}	0.017	$p<0.05$
SOD	2.89^{a}	1.09^{c}	1.15^{c}	1.99^{b}	2.63^{a}	0.111	$p<0.05$
Catalase	69.27^{a}	42.69^{c}	44.25^{c}	58.49^{b}	68.25^{a}	0.697	$p<0.05$
Peroxidase	87.67^{a}	68.89^{c}	71.86^{c}	82.52^{b}	86.16^{ab}	1.282	$p<0.05$

The data are mean of three replicates. Mean values within a row sharing different superscript letters are significantly different ($p<0.05$). Pooled standard error (PSE) = √MSE/n (where MSE is mean-squared error). TBARS, Thiobarbituric acid-reactive substances (mg/g protein). SOD, superoxide dismutase (units/min/mg protein); Catalase (Units/min/mg protein); Peroxidase (mUnits/min/mg protein).

Table V.- Effect of high doses of vitamin E on muscle fatty acid[1] profile of *L. rohita* fingerlings.

	Experimental diets					PSE	P-value
	VE_0	VE_{100}	VE_{150}	VE_{1000}	VE_{1500}		
14:0 n-0	3.83	3.79	3.79	3.79	3.78	0.017	NS
16:0 n-0	9.13^{a}	8.93^{b}	8.99^{b}	8.93^{b}	8.98^{b}	0.018	p<0.05
18:0 n-0	4.31^{a}	4.04^{b}	4.03^{b}	4.04^{b}	4.05^{b}	0.014	p<0.05
16:1 n-7	9.86^{a}	9.44^{b}	9.44^{b}	9.49^{b}	9.47^{b}	0.027	p<0.05
18:1 n-7	11.74	11.74	11.71	11.73	11.7	0.013	NS
18:1 n-9	15.41	15.37	15.37	15.34	15.38	0.023	NS
18:2 n-6	3.66	3.63	3.6	3.62	3.63	0.014	NS
20:4 n-6	4.74^{b}	5.40^{a}	5.45^{a}	5.45^{a}	5.39^{a}	0.018	p<0.05
18:3 n-3	3.9	3.89	3.89	3.88	3.91	0.012	NS
20:5 n-3	9.72^{b}	9.91^{a}	9.92^{a}	9.88^{a}	9.89^{a}	0.015	p<0.05
22:5 n-3	4.7^{b}	4.88^{a}	4.86^{a}	4.89^{a}	4.91^{a}	0.02	p<0.05
22:6 n-3	14.62^{c}	14.67^{a}	14.66^{ab}	14.64^{abc}	14.63^{bc}	0.008	p<0.05
Others[2]	4.37	4.3	4.28	4.32	4.28		
Total	100	100	100	100	100		
Saturated	17.27^{a}	16.76^{b}	16.81^{b}	16.75^{b}	16.81^{b}	0.024	p<0.05
Monounsaturated	37.01^{a}	36.56^{b}	36.53^{b}	36.56^{b}	36.56^{b}	0.023	p<0.05
n-3	32.95^{b}	33.35^{a}	33.33^{a}	33.30^{a}	33.33^{a}	0.018	p<0.05
n-6	8.41^{b}	9.03^{a}	9.05^{a}	9.07^{a}	9.01^{a}	0.025	p<0.05
n-9	15.41	15.37	15.37	15.34	15.38	0.023	NS
ARA/EPA	0.49^{b}	0.55^{a}	0.55^{a}	0.55^{a}	0.54^{a}	0.002	p<0.05
EPA/DHA	0.66^{b}	0.68^{a}	0.68^{a}	0.67^{a}	0.68^{a}	0.001	p<0.05
n-3/n-6	2.14^{b}	2.17^{a}	2.17^{a}	2.17^{a}	2.17^{a}	0.003	p<0.05
Monoenes/Polyenes	0.65^{a}	0.63^{b}	0.63^{b}	0.63^{b}	0.63^{b}	0.001	p<0.05

The data are mean of three replicates. Mean values within a row sharing different superscript letters are significantly different (p<0.05). Pooled standard error (PSE) = √MSE/n (where MSE is mean-squared error). NS, non-significant (p>0.05). [1]Fatty acid = % total fatty acid detected. [2]Others= Sum of 15:0, 15:1, 16:1 n-9, 16:2 n-7, 17:0, 17:1 n-7, 18:2 n-3, 20:1 n-9, 21:5 n-3, 22:1 n-9, 22:2 n-6, 22:4 n-6.

However, linoleic acid (18:2 n-6) and alpha linolenic acid (18:3 n-3) did not respond to the vitamin E supplementation. The ARA/EPA, EPA/DHA, n3/n6 ratios in muscle increased with dietary addition of vitamin E, though, monoene/polyene ratio decreased. Nevertheless, differences between adequate and mega doses of vitamin E were not significant for most of the parameters.

DISCUSSION

Lipid peroxidation is a well-known free radical chain reaction phenomenon involving lipid peroxyl radical as chain carrier. Vitamin E takes hydrogen atom from hydroxyl group of peroxyl radical and changes itself into α-tocopheroxyl radical, hence, preventing lipid peroxidation (Tappel, 1992). Therefore, basic role of vitamin E is to scavenge the hydrogen atom and make lipid peroxyl radical inactive before its attack on other lipid substrate. Normally, in tissues, α-tocopheroxyl radical reacts with another lipid peroxyl radical and form a stable non-radical product or it is reduced by vitamin C and glutathione (Sato *et al*., 1990).

In this study, whole body proximate analysis remained un-affected by dietary manipulation. Similar observations were recorded by Gatta *et al*. (2000) in *Dicentrarchus labrax*, Amlashi *et al*. (2011) in *Huso huso*, Bae *et al*. (2013) in *Anguilla japonica*, and Li *et al*. (2014) in grass carp. However, Abdel-Hameid *et al*. (2011) observed increased body protein contents in *Channa punctatus* up to 140 mg/kg vitamin E diet and then decrease at higher levels. They also observed decreased fat level with increase in vitamin E level, which showed promoted fat

assimilation and transportation tendency of vitamin E resulting in reduced fat accumulation in storage sites in fish body (Mourente *et al*., 2007).

Thiobarbituric acid reactive substances (TBARS) assay is one of the most common assays for the measurement of lipid peroxidation product which is malondialdehyde (MDA). In the present study, minimum TBARS values were recorded in the whole body of fish group having 100 mg/kg vitamin E in diet as compare to fish feeding on other supplementation levels. However, maximum TBARS values were recorded in control group. This is consistent with the findings of Tocher *et al*. (2002) and Wang *et al*. (2015) who reported higher levels of TBARS at higher level of vitamin E supplementation (1000 mg/kg) compared to other lower levels (100 mg/kg) in sea bream (*Sparus aurata*) and sea cucumber (*Apostichopus japonicas*), respectively. The TBARS concentrations were lower at adequate vitamin supplementation levels and increased when vitamin E was supplemented at super high levels in the present study. In such conditions, vitamin C may become scarce to reduce the α-tocopheroxyl radical which is produced in normal physiological mechanisms. Hence, this α-tocopheroxyl radical attacks on lipid substrate to start a free radical chain reaction (Terao and Matsushita, 1986; Mukai and Okauchi, 1989). This prooxidative effect may produce more lipid peroxyl radicals, increasing the TBARS contents in tissues.

Superoxide dismutase and catalase are scavenger of superoxide (O^{-2}) and hydrogen peroxide (H_2O_2), respectively, while peroxidases are also acting on hydrogen peroxide (H_2O_2) and lipid hydroperoxides. In the present study, lowest activities of SOD, catalase and peroxidase were recorded at 100 mg/kg vitamin E supplementation. Vitamin E reduced the production of O^{-2} which is a substrate of SOD and non-availability of substrate may act as a reason for decreased SOD activities. Similar to our results, decreased activities of these enzymes were also observed in rainbow trout by feeding a vitamin E supplemented diet (Palace *et al*., 1993). Puangkaew *et al*. (2005) also described reduced activities of these enzymes in plasma and kidney of rainbow trout in response to dietary supplementation of vitamin E. Furthermore, a slight increase in the activities of these enzymes were also recorded in the present study by increasing the vitamin E supplementation from 100 mg/kg to 150 mg/kg diet.

Moreover, a more profound increase was also observed in the activities of these enzymes at mega doses of vitamin E supplementation. These results may owe to the hypothesis that supplementation of α-tocopherol above its requirements accelerates the lipid peroxidation in a biological system (Kaewsrithong *et al*., 2001). Thus, production of more antioxidant enzymes is a physiological adaption of animal to compensate the induced oxidative stress (Dandapat *et al*., 2000). This is in accordance with the observations of studies of Wang *et al*. (2015) in sea cucumber and Puangkaew *et al*. (2004) in rainbow trout.

In the present study, decreased level of SFA and monoenes while increased levels of PUFA were observed in fish fed vitamin E supplemented diets. However, increasing the dietary supplementation of vitamin E did not cause any further increase or decrease in the percentage of these fatty acids. It is evident from present results that vitamin E deficiency affects the fatty acid composition and its adequate levels are sufficient to satisfy the minimum requirement of fish to maintain the fatty acid profile. Similarity in fatty acid profile at all vitamin E supplemented levels can also be attributed to same dietary lipid source which was cod liver oil in this case. Pirini *et al*. (2000) also reported the direct reflection of dietary composition of fatty acids in fish muscle. Watanabe *et al*. (1977) also suggested that supplementation of vitamin E influences the fatty acid profile, however, a supplementation level greater than its requirement show little effects on fatty acid profile. Presence of higher level of highly unsaturated fatty acid (HUFA, 20:5 and 22:6), in the current study, may also be attribute to the capability of freshwater species to bioconvert linolenic acid (18:3 n-3) to 20:5 n-3 (EPA) and 22:6 n-3 (DHA) HUFA by using elongases and desaturases (Sargent *et al*., 1989). The n-3 HUFA percentages are oftenly lower in farmed fish as compare to their wild relatives because the lipids oftenly used in manufactured feeds are deficient of n-3 fatty acids and contain large amount of SFA and monounsaturated fatty acids (Ackman and Takeuchi, 1986). Nevertheless, the percentages of fatty acid found in the present study confirm that diets with marine fish (cod) liver oil and appropriate amount of vitamin E are capable of providing farmed fish, a quite similar fatty acid pattern as the wild specimens, particularly rich in n-3 HUFA (Sharma *et al*., 2010). Bai and Lee (1998) reported lower PUFA levels and PUFA/SFA ratio in Korean rockfish fed low levels of vitamin E. Lower n-3 fatty acids percentages in the muscle of Atlantic halibut were observed by feeding vitamin E deficient diet (Lewis-McCrea and Lall, 2007).

CONCLUSION

The present study showed no effect of dietary supplementation of vitamin E on whole body proximate composition of *L. rohita* fingerlings. The study also demonstrate reduced lipid peroxidation by dietary supplementation which lead to increased PUFA contents in fish muscles. However, high doses of vitamin E showed the pro-oxidative effect which promoted lipid peroxidation.

Statement of conflict of interest

The authors declare no conflict of interest.

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