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Research Article

Growth performance, antioxidant enzymes status, muscle fatty acid composition, and thiobarbituric acid reactive substances of *Cirrhinus mrigala* fed graded levels of vitamin E supplementation

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Abstract

Nowadays, inclusion of antioxidants such as, vitamin E (vit E) in aquafeeds is imperative for optimum yield and improved product quality. Therefore, the present study was designed to evaluate the growth performance, antioxidant enzymes status, thiobarbuteric acid reactive substances (TBARS) and fatty acid composition of muscles of *Cirrhinus mrigala* fed with vit E. Five semi-purified diets designed containing vit E at 0.0, 30.0, 60.0, 90.0, and 120.0 mg kg^{-1} and fed to fish (4.30±0.21 g) in triplicates for 60-days. The growth performance, liver and muscles vit E contents were significantly higher in group D-V (p<0.05). FCR, body indices, muscles TBARS and antioxidant enzymes [catalase (CAT), peroxidase (POX), and superoxide dismutase (SOD)] activity in intestine, liver, kidney and whole body of fish was significantly reduced in group D-V compared to other groups (p<0.05). Moreover, increased muscles polyunsaturated fatty acids (PUFAs) such as, ω-3, ω-6 and decrease unsaturated fatty acids were measured in group D-V (p<0.05). After 15 and 30 days of storage, significantly reduced muscles PUFAs and increased $TBAR_S$ content were observed in all groups except D-V (p<0.05). In conclusion, dietary vit E supplementation (at 120 mg kg^{-1}) could be a useful approach to improve growth performance and oxidative stability by reducing oxidative stress in *C. mrigala*.

Keywords: Antioxidant enzymes, *Cirrhinus mrigala*, Malondialdehyde, Oxidative stability, Polyunsaturated fatty acids

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Introduction

Fish is a rich source of high-quality protein and polyunsaturated fatty acids (PUFAs) which are crucial to maintaining human health. The essential fatty acids (EFA), such as docosahexaenoic acid (DHA) and eicosatetraenoic acid (EPA), present in fish meat, are highly susceptible to oxidation (Murray *et al*., 2000). Thus, oxidation of PUFAs and protein may negatively impact the stability of fish products during their processing and storage (Farris *et al*., 2020). There are a number of factors that can induce oxidative stress in fish during culture, thus consequently deteriorating fish health and muscle quality as well as consumer health (Larsson *et al*., 2014). In aquaculture operations, nutritionally balanced feeds are considered as the major contributor to maintaining the quality of the final product and the health status of animals in captivity. Moreover, the production cost is directly dependent on feed cost; therefore, dietary applications of micronutrients such as antioxidants could be a suitable strategy to conquer the optimum quality of end product at affordable prices (Farris *et al*., 2020).

Vitamin E (vit E) is a generic term, used for eight different isoforms, including four (α, β, γ, Δ) tocopherols and four tocotrienols available in different plant oils. Among these, alpha-tocopherol (AT), also known as vitamin E, is a lipid-soluble vitamin that has the highest biopotency (Iqbal *et al*., 2018). It works as a scavenger of reactive oxygen species (ROS) and free radicals, produced by autoxidation of lipids and protein in cell organelles and biological membranes (Frigg *et al*., 1990). The properties of vit E as an antioxidant have already been investigated in various teleost species (Iqbal *et al*., 2018; Mahmood and Mateen, 2020; Farris *et al*., 2020). The growth performance is generally known as an important index in nutritional studies. Thus, numerous investigations have been made to determine the optimum dietary vit E requirement for different commercial species mainly based on the growth performance such as yellowtail, *Seriola quinque radiata* (119 mg kg^{-1}) (Shimeno, 1991), blue tilapia, *Oreochromis aureus* (25 mg kg^{-1}) (Satoh *et al*., 1987), beluga, *Huso huso* (45 mg kg^{-1}) (Bai and Lee, 1998), rohu, *Labeo rohita* (131.9 mg kg^{-1}) (Sau *et al*., 2004) and grass carp, *Ctenopharyngodon idella* (100.36 mg kg^{-1}) (Li *et al*., 2014).

Numbers of factors are believed to be responsible the proper regulation of antioxidant defense mechanisms in the animal body. Like other animals, fish has a complex antioxidant defense system which has been divided into two main categories; one is enzymatic (superoxide dismutase, catalase, and peroxidase) and the other is non-enzymatic system (free amino acids, peptides, carotenoids, and phenolic compounds). Both systems are equally crucial for health maintenance, cell signaling, development, and reproduction in fish (Undeland, 1997). Previous studies found improved antioxidant capacity with dietary vit E supplementation to turbot,

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Scophthalmus maximus (Tocher *et al*., 2002), grass carp, *C. idella* (Li *et al*., 2014), cobia, *Rachycentron canadum* (Zhou *et al*., 2012), and juvenile rainbow trout, *Oncorhynchus mykiss* (Puangkaew *et al*., 2005).

Indian major carps such as, rohu, *L. rohita*, mrigal, *C. mrigala*, and thaila, *C. catla* have highest share by volume in global aquaculture production. *C. mrigala* is the second most important carp species, widely consumed and cultured in Pakistan under different culturing systems (Iqbal *et al*., 2018). Since, it has rapid growth, good taste, and high consumer demand in local and international markets (Mahmood and Mateen, 2020). As meat quality is the main focus for fish farmers and consumers nowadays. Thus, improved muscle quality with dietary vit E supplementation has been reported in sea bass, *Dicentrarchus labrax* (Prini *et al*., 2000), channel catfish, *Ictalurus punctatus* (Gatlin, 2003), turbot, *S. maximus* (Stephan *et al*., 1995), rohu (Sau *et al*., 2004), grouper, *Epinephelus malabaricus* (Lin and Shiau, 2005), juvenile hybrid tilapia, *Oreochromis niloticus* × *O. aureus* (Huang *et al*., 2003; 2004), and mrigal (Iqbal *et al*., 2018). However, the effects of dietary vit E supplementation on the quality of stored fish muscle quality is still need to be investigated. Therefore, the present study was carried out to evaluate the effect of vit E supplementation on the growth performance, oxidative stability and muscle quality of *C. mrigala*.

Materials and methods

Experimental diets

Feed ingredients were procured from a local feed company (KHUSI FEEDS®) Lahore, ground and, sieved to the required 500 µm particle size. Five isonitrogenous (crude protein; 353 g kg^{-1}), isolipidic (lipid; 150 g kg^{-1}), and isocaloric (energy; 18 MJ kg^{-1}) diets designated as D-I, D-II, D-III, D-IV and D-V were supplemented with vit E as DL-all-rac-α-tocopherol acetate (Manufacturer details: Sigma Aldrich Chemie GmbH Canada: catalogue no. T3376) at 0.0, 30.0, 60.0, 90.0 and 120.0 mg kg^{-1}, respectively (Table 1). All dry ingredients were blended using an electrical mixer (Cambridge CG 502, China) and 15-20 % distilled water was gradually added to acquired suitable texture of dough. The dough was passed through electrical extruder (Model no. SYSLG30-IV, HS code: 8438800000 - China). After spraying the fish oil, the wet noodles were air dried for three days to reduce the excessive moisture up to 10%, after those diets were converted into pellets of 1.5-2.0 mm. All pellets were kept in airtight, labelled polythene bags and refrigerated at -20C until used. Each diet was randomly assigned to three replicated tanks.

Fish rearing

C. mrigala fingerlings were obtained from Government Fisheries Research, and Training Center Manawa, Lahore and transported to the fish rearing facility, Animal House Government College University (GCU), Lahore, Pakistan.

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Table 1: Ingredients and nutrient composition (g kg^{-1} diet) of experimental diets fed to *C. mrigala* for 60 days (on dry basis).

Ingredient (g kg^{-1})	D-I	D-II	D-III	D-IV	D-V
Anchovy fish meal [a]	150	150	150	150	150
Soybean meal	150	150	150	150	150
Sunflower meal	300	300	300	300	300
Corn gluten meal [b]	100	99.970	99.940	99.910	99.880
Rice bran	80	60	60	60	60
Wheat flour	60	60	60	60	60
Cod liver oil	100	100	100	100	100
Toxin binder	20	20	20	20	20
Vitamin premix [c] (Vitamin E free)	20	20	20	20	20
Mineral mixture [d]	20	20	20	20	20
Vitamin E level [e] (supplemented)	0	0.03	0.06	0.09	0.12
Proximate analysis (g kg^{-1} on dry basis)					
Moisture	9.88	10.30	11.24	10.61	10.90
Crude protein	354.16	356.18	354.03	353.17	351.03
Lipid	150.87	148.27	151.99	155.24	152.83
Ash	62.90	62.77	63.15	63.20	63.29
Fiber	72.10	70.70	71.55	70.65	70.53
Energy (MJ kg^{-1})	19.44	18.48	18.77	19.09	18.42
NFE [f]	350.09	352.05	352.03	351.13	350.42
Analyzed vitamin E (mg kg^{-1})	3.57	36.24	62.00	94.18	125.04

[a] Anchovy fish meal purchased from Pakistan Fish Meal; Plot-19 Sector, 16 Korangi Industrial Area, Karachi, Pakistan. Proximate composition of anchovy fish meal was as follows: crude protein = 58.28%, lipid = 7.5%, moisture = 23.15%, ash = 2.78%.

[b] Corn gluten meal (CGM): 30 % CGM was used, and vitamin E was supplemented at the expense of CGM.

[c] One kilogram of vitamin premix contains: Vitamin A 15 I.U, Vitamin B1 5000 mg, Vitamin B2 6000 mg, Vitamin B6 4000 mg, Vitamin B12 9000 mg, Vitamin C 15000 mg, Vitamin D3 3 I.U, Vitamin K3 4000 mg, Folic acid 750 mg, Calcium pantothenate 10000 mg, Nicotinic acid 25000 mg

[d] One kilogram of mineral granules contains: Calcium 155 gr, Phosphorus 135gm, Magnesium sulphate 55 gr, Iron 1000 mg, Zinc sulphate 3000 mg, Sodium 45 gr, Manganese 2000 mg, Copper sulphate 600 mg, Cobalt 40 mg, Iodine 40 mg, Selenium 3 mg Manufactured by Nawan Laboratories (PVT) LTD, Animal Health Division Karachi- Pakistan).

[e] Analytical grade vitamin E was used as alpha-tocopherol acetate (Sigma Aldrich).

[f] Nitrogen free extract (NFE) was calculated applying the official method of AOAC (1995), (AOAC-942.05): NFE = 100 – (crude protein + lipid + ash+ fiber + moisture).

D-I represents control diet (vit E 0 mg kg-1) whereas, D-II, D-III, D-IV and D-V indicates vit E at 30, 60, 90, and 120 mg kg^{-1}, respectively.

The experiment was approved by the Animal Ethics Committee GCU. Fish were fed to apparent satiation on a commercial diet (ORYZA ORGANICS® crude protein; 300 gkg^{-1}) for two weeks during acclimatization. Before starting the experiment, a prophylactic dip (5 g NaCl L^{-1} distilled water) was provided to the fish to avoid infections (Rowland and Ingram, 1991). Fish of similar age and size, having the mean initial weight 4.30±0.21 g, were stocked in glass tanks (120 L water holding capacity) at a stocking density of 20 fish per tank. Initially, all the fish groups were hand-fed at the rate of 2% of live wet body weight. Later on, the feeding rate was adjusted according to the apparent satiation of fish into two (morning 9:00 and evening 17:00 h)

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feeding times for 60-days. After the feeding event of three hours, the uneaten diet was collected from each tank oven-dried at 105C for 6 h; and stored for further calculations. After that, tanks were washed and refilled with new water. The quality of water was monitored by measuring the physico-chemical variables, including dissolved oxygen; mean±SD; 5.9±1.3 mg L^{-1} (AMPROBE DO meter), temperature; mean±SD; 27.9±1.5ºC (Model HI 9147 temperature meter; HANNA USA), pH; mean±SD; 7.5±1.3 (Model HI 9147 pH meter; HANNA USA), salinity; mean±SD; 27±2‰, and ammonia; mean±SD; 0.06±0.03 mg L^{-1} on daily basis. Continuous aeration was maintained through the capillary system, and natural photoperiod (light 12: dark 12 h) was provided throughout the experiment.

Calculations and sampling

The initial mean weight of each replicated tank was recorded at the start of the experiment, and fish per each tank were weighed after every two weeks. At termination, fish fasted for 24 h; counted, and the final weight was recorded individually for each replicated tank. The calculations were done by applying standard equations as follows:

$$WG\ (g) = Wf\ (g) - Wi\ (g)$$

$$WGR = 100 \times \frac{Wf\ (g) - Wi\ (g)}{Wi\ (g)}$$

$$Survival\ (\%) = \frac{Final\ number\ of\ fish}{Initial\ number\ of\ fish} \times 100$$

$$FCR = \frac{Dry\ feed\ intake\ (g)}{Wet\ weight\ gain\ (g)}$$

$$VSI\ (\%) = \frac{Weight\ of\ viscera\ (g)}{Total\ body\ weight\ (g)} \times 100$$

$$HSI\ (\%) = \frac{Weight\ of\ liver\ (g)}{Total\ body\ weight\ (g)} \times 100$$

Where, WG, WGR, Wf, Wi, FCR, VSI, and HSI represents weight gain, weight gain rate, final weight, initial weight, feed conversion ratio, hepatosomatic index, and viscerosomatic index, respectively.

Five fish (*n*=5) were randomly selected from each tank (a total of 15 fish per diet), anesthetized with the (50 mg L^{-1}) solution of tricaine methanesulfonate (TMS-222 Sigma Aldrich catalogue no. E 10521®): liver, whole muscle, kidney, intestine, and whole-body samples were taken, immediately frozen in liquid nitrogen and kept at -80 ºC to analyze the vit E content and antioxidant enzymes activity. Another batch of five fish were randomly separated from each replicated tank (*n*=5; a total of 15 fish per diet) and stored at -20ºC to determine the whole-body composition.

Storage conditions

Ten fish was randomly collected (*n*=10) from each replicated tank (a total of 30 fish per diet): fins, scales, skin, and viscera were removed, and whole muscles were extracted. The obtained mass of muscles was equally distributed into three batches named as batch 1 (B1), batch 2 (B2), and batch 3 (B3). The B1 was immediately used to measure the muscles malondialdehyde (MDA) content and fatty acid profile on the day of harvesting (0 day). However, batch 2 and 3 of muscles samples were packed separately in airtight plastic polythene bags and stored at -80ºC to analyze the MDA and fatty acid profile after the

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storage time of 15 and 30 days, respectively.

Biochemical analyses

The proximate composition of experimental diets (Table 1) and whole-body of fish was analyzed according to the guidelines of Association of Official Analytical Chemist (1995). The crude protein content was determined by N percentage × 6.25 (Company name: Hanon Advanced Technology Group Co., Ltd. Model no. K1100F micro-Kjeldhal Autoanalyzer-China), lipid was evaluated through ether extraction method (Soxtec System HT2 1045), moisture was assessed by the oven (Manufacturer name: Memmert, Model no. MMUN30-Germany) drying at 105 ºC for 6 h. The nitrogen free extract (NFE) was calculated using the formula as; NFE=100–(crude protein+ lipid+ ash+ fiber + moisture).

Antioxidant enzyme assays

The enzyme extract was prepared by homogenizing one gram of tissue (liver, kidney, intestine and whole body) sample in phosphate buffer pH 7.4 (tissue 1 g and phosphate buffer 3 mL) for the antioxidant enzyme assay. Tissue mass was passed through muslin cloth and filtered with Whatman filter paper No.1. The filtrate was centrifuged (CenLee 18R Benchtop) at 10,000 rpm for 10 minutes; and supernatant was separated and used to determine the activity of antioxidant enzymes. Catalase (CAT) activity (mU mg^{-1} tissue) was determined by evaluating its ability to decompose hydrogen peroxide (H_2O_2) at 240 nm (Iqbal *et al*., 2018). The superoxide dismutase (SOD) activity was measured through the reduction of nitro blue tetrazole (NBT) at 560 nm (Giannopolitis and Ries, 1977) and peroxidase (POX) activity was determined by measuring its ability of breakdown of hydrogen peroxide (H_2O_2) at 470 nm (Civello *et al*., 1995).

Analysis of malondialdehyde (MDA) content

Muscles MDA content was determined by measuring thiobarbituric reactive substances (TBARs) following Iqbal *et al*. (2018) with slight modifications. One gram of muscles sample was homogenized in potassium chloride (KCl 11.5 g L^{-1}) with 3 mL of tris-maleate (80 mM, pH 7.4) solution. TBARs were induced by adding 1 mL of (2 mM) ascorbic acid and samples were incubated at 37ºC for 25-30 minutes. The colorimetric reaction was produced by adding 5 mL of 0.7 M hydrochloric acid (HCl) and 5 mL of 0.05 M thiobarbituric acid (TBA) and then glass tubes were boiled for half an hour. Five mL of trichloroacetic acid (TCA) solution (200 gL^{-1}) was added and samples were refrigerated, and centrifuged at 495 *g* for 7 minutes. The commercially available standard of MDA was used to acquire a calibration curve. The TBA values were determined photometrically, and maximum absorbance was measured at 530 nm.

Determination of vitamin E content

The lipid was extracted from liver and muscle samples through the ether

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extraction method (Soxtec System HT2 1045) and vit E content was determined (Lee *et al*., 2003) through high performance liquid chromatography (HPLC) system (Perkin-Elmer Co., Seri 200, USA). The mobile phase contained 2-propanol and hexane (0.5:100) and the flow rates of a pump (LC-10AT) were 1 $mLmin^{-1}$ at 30C. Ten µL of prepared sample was injected into HPLC system equipped with reverse phase column (Kinetex 5u EVO C18 150x4.6mm; Phenomenex Co, USA), and the response was recorded from 292 nm (excitation) to 325 nm (emission).

Fatty acid profiling

Muscle fatty acid content was analyzed by applying the International Union of Pure and Applied Chemistry (IUPAC, 1987). Lipid was extracted from muscle samples, and fatty acid methyl esters (FAMEs) were prepared using methanol. A 500 mg lipid sample was mixed with 25 mL of 0.5 N potassium hydroxide (KOH) solution and refluxed for 10 minutes. After heating, 15 mL of ammonium chloride-methanol-H_2SO_4 solution was added and again refluxed for 30 minutes. Thereafter, the solution was cooled down, separated into layers using a separator burette and residues were dissolved in petroleum ether (20 mL). The prepared samples of FAME were used to determine the fatty acid content by gas chromatography system (SHIMADZU, GC, 17A, Japan), using a flame ionized detector (FID). The fatty acids content was measured as % of total fatty acids detected and identified by comparing the retention times of commercially available standards.

Statistical analyses

All data were collected as the mean of three replicates (n=3) ± standard deviation (SD). After verifying the homogeneity of variance, data were subjected to one-way analysis of variance (ANOVA). Tukey's Honesty Significant (THS) test was used as the post hoc test to determine the difference among means of different dietary groups by adjusting the level of significance at p<0.05. Regression model was applied to determine the effects of vit E levels on final body weight, FCR, and vit E contents in liver and muscles of *C. mrigala*. All statistical analyses were performed using SPSS (Version IBM 22.0) for Windows.

Results

Growth performance and FCR

The growth performance in terms of FBW, WG and WGR of *C. mrigala* fed on graded levels of vit E supplementation for 60 days were significantly affected by the dietary vit E supplementation. The increased growth performance in terms of final body weight (FBW), weight gain (WG), and weight gain rate (WGR) was found in group fed with D-V compared to the control and other vit E supplemented groups (p<0.05). The positive linear regression was observed between the dietary vit E level and FBW of *C. mrigala* with the following equation: Y=1.811x + 6.123; R^2 = 0.9644; p-value=0.0001 (Fig. 1). Similarly, vit E

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supplementation level showed a significant effect on FCR of *C. mrigala*. Lowest value of FCR was found in group D-V, whereas highest was observed in the control group (p<0.05). The negative linear relation was found between dietary vit E level and FCR with following equation: Y=- 0.368x+2.958; R^2=0.970; p-value=0.0001 (Fig. 2). Furthermore, no mortalities were observed throughout the feeding experiment among different treatment groups (p>0.05) (Table 2).

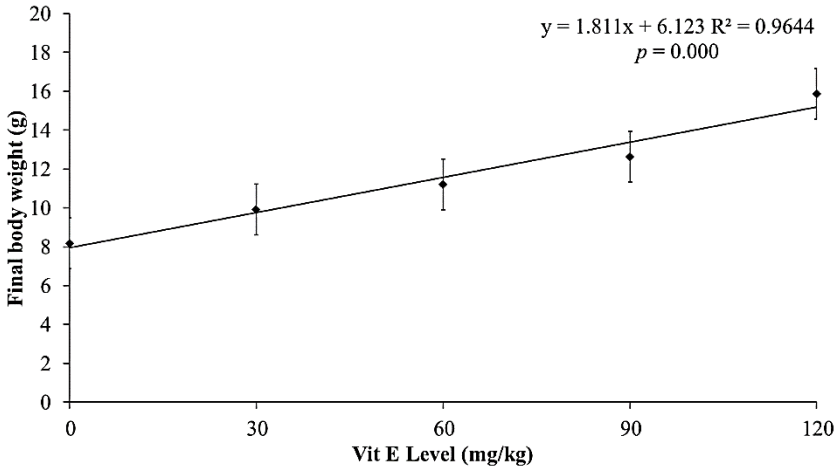

Figure 1: Linear regression analysis for the final body weight (FBW) of *C. mrigala* fed with graded levels of vit E supplementation for 60 days. The different points in graph shows the mean values of three replicates (n=3) ±standard deviateion (SD) that are significantly different at p<0.05.

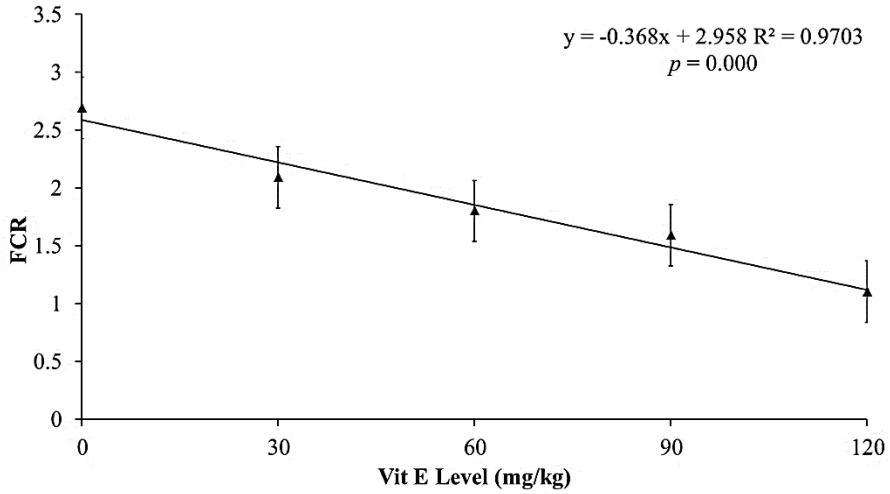

Figure 2: Linear regression analysis for feed conversion ratio (FCR) of *C. mrigala* and vit E level (mg kg^{-1}) in diets. The different points in the graph shows the mean values of three replicates (n=3) ±standard deviation (SD) that are significantly different at p<0.05.

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Table 2: The growth performance of *C. mrigala* fed graded levels of vitamin E supplementation for 60 days.

Parameters	D-I	D-II	D-III	D-IV	D-V
IBW (g)	4.18±0.04	4.54±0.24	4.18±0.05	4.53±0.03	4.10±0.02
FBW (g)	8.16 ±0.02^{e}	9.92±0.01^{d}	11.21±0.09^{c}	12.63±0.16^{b}	15.86±0.16^{a}
WG (g)	3.98±0.01^{e}	5.38±0.22^{d}	7.03±0.03^{bc}	8.09±0.13^{b}	11.75±0.19^{a}
WGR	92.21±1.30^{e}	118.79±1.27^{d}	168.30±1.43^{c}	178.49±1.56^{b}	286.36±6.13^{a}
FCR	2.69±0.02^{a}	2.09±0.12^{b}	1.80±0.04^{c}	1.59±0.03^{d}	1.10±0.01^{e}
SR (%)	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00

Note: The data were presented as the mean values of three replicate (n=3) ±standard deviation (SD). The values in the same row with different superscript are significantly different at p<0.05. D-I represents control diet (vit E 0 mg kg^{-1}) whereas, D-II, D-III, D-IV and D-V supplemented with with vit E at 30, 60, 90 and 120 mg kg^{-1}, respectively. IBW=initial body weight, FBW=final body weight, WG=weight gain, WGR=weight gain rate, FCR=feed conversion ratio, SR=survival rate.

Body indices

The body indices of *C. mrigala* including, viscerosomatic index (VSI) and hepatosomatic index (HSI) were significantly affected by the dietary vit E supplementation levels (Fig. 3). The lowest value of VSI was calculated in the groups fed with D-IV, and D-V, whereas, HSI was significantly decreased in group fed with D-V compared to the control and other vit E supplemented groups (p<0.05).

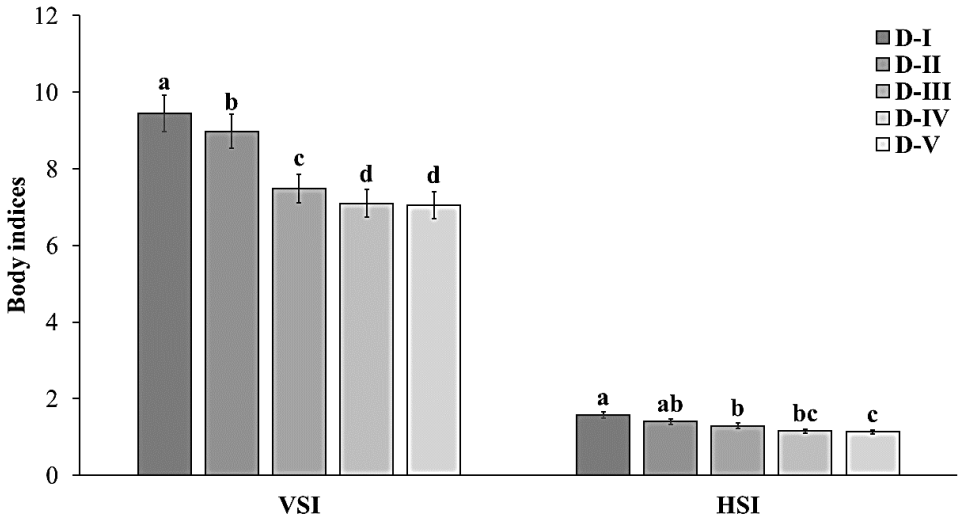

Figure 3: One-way analysis of variance (ANOVA) for the viscerosomatic index (VSI) and hepatosomatic index (HSI) of *C. mrigala* fed with graded levels of vit E supplementation for 60 days. The graph bar are showing the mean values of three replicates (n=3) ±standard deviateion (SD) having different superscripts are significantly different at p<0.05. D-I represents control diet (0 mg kg^{-1}) whereas, D-II, D-III, D-IV and D-V supplemented with vit E at 30, 60, 90 and 120 mg kg^{-1}, respectively.

Whole-body composition

Figure 4 shows the whole-body composition of *C. mrigala* fed with graded levels of vit E supplementation for 60 days. There was no significant difference in dry matter, protein, and lipid contents among the different dietary groups (p>0.05).

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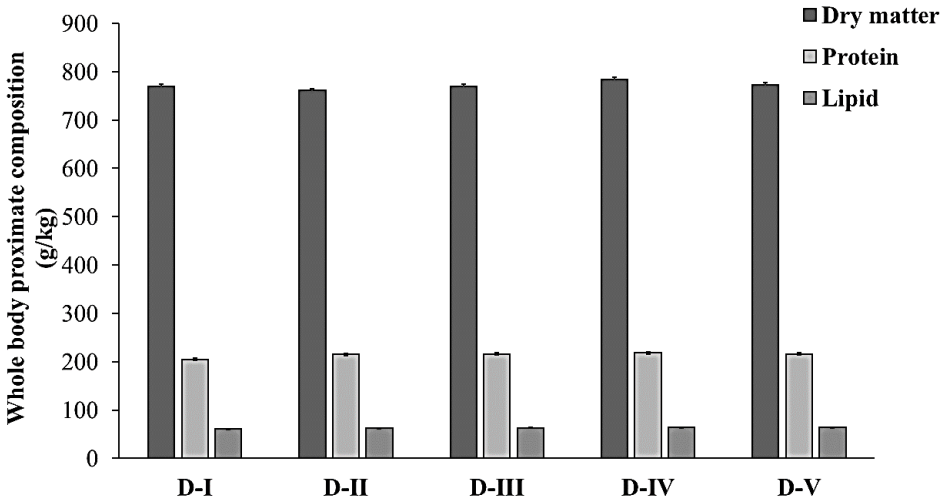

Figure 4: The whole-body proximate composition including dry matter, protein and lipid of *C. mrigala* fed with graded levels of vit E supplementation for 60 days. The different points in graph are showing the mean values of three replicates (*n*=3) ±standard deviateion (*p*>0.05). D-I respresents control diet (0 mg kg^{-1}) whereas, D-II, D-III, D-IV and D-V supplemented with vit E at 30, 60, 90 and 120 mg kg^{-1}, respectively.

Liver and muscles vitamin E content

The dietary vit E supplementation showed a positive effect on liver and muscle vit E contents in *C. mrigala*. The significantly increased vit E content was exhibited by the group fed with D-V; however, decreased vit E content was observed in the control group (p<0.05). The following regression equations were acquired with the positive linear relationship between the dietary vit E supplementation level and liver and muscles vit E content in *C. mrigala* as follows: Y=27.448x-12.774; R^2=0.9877; p-value=0.0001 and Y=2.857x-1.259; R^2=0.8779; p-value=0.01, respectively (Fig. 5).

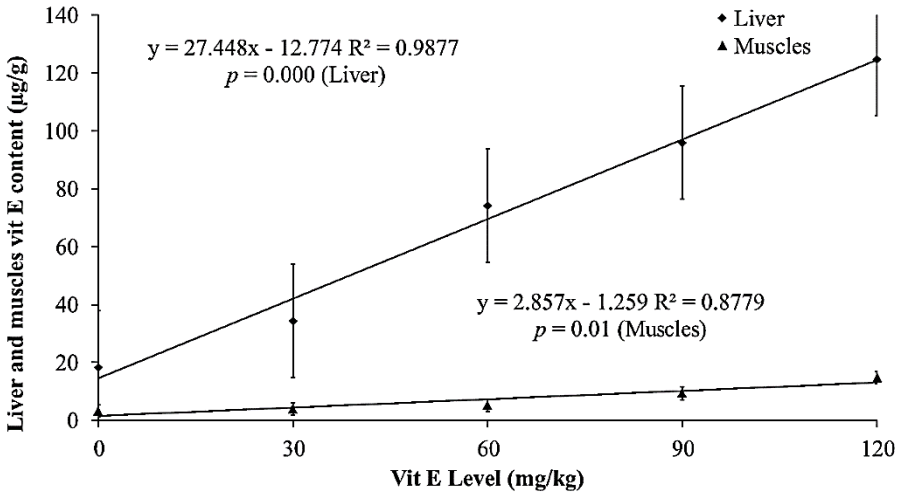

Figure 5: The linear regression analysis between the vit E supplementation level (mg kg^{-1}) and liver and muscles vit E content (µg g^{-1}) of *C. mrigala*. The different points in the graph are the mean values of three replicates (*n*=3) ±standard deviateion (SD) showing significant difference among the different dietary groups (*p*<0.05).

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Antioxidant enzymes status

The antioxidant capacity of *C. mrigala* was significantly influenced by dietary vit E supplementation (Fig. 6). The significantly decreased activity of CAT (Fig. 6a), POx (Fig. 6b) and SOD (Fig. 6c) was measured in intestine, kidney, liver and whole-body of *C. mrigala* fed with vit E supplementation as compared to the control group (p<0.05). However, among vit E supplemented groups significantly lowest values of CAT, POX and SOD were exhibited by the group fed with D-V treatment (p<0.05).

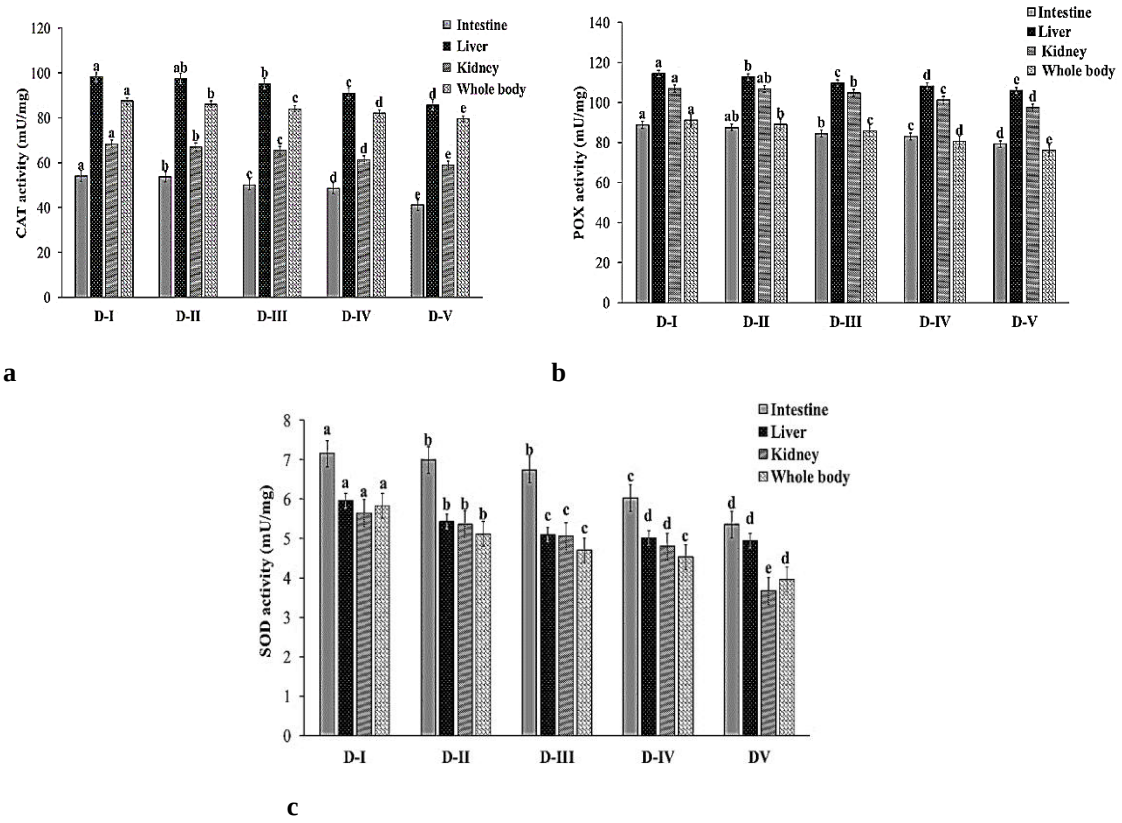

Figure 6: The activity (mU mg^{-1}) of (a) catalase (CAT), (b) peroxidase (POX) and (c) superoxide dismutase (SOD) in the intestine, liver, kidney, and whole body of *C. mrigala* fed with graded levels of vit E supplementation for 60 days. The graph bar are the mean values of three replicates (n=3) ±standard deviateion (SD) showing the significant difference among different treatment groups (p<0.05). D-I represents control diet (0 mg kg^{-1}) whereas, D-II, D-III, D-IV and D-V supplemented with vitE at 30, 60, 90 and 120 mg kg^{-1}, respectively.

Muscle fatty acid composition (At 0 day)

The muscle fatty acids (FAs) composition (% of the total detected fatty acids) of *C. mrigala* fed with graded levels of vit E supplementation is presented in Table 3. The various categories of FAs were observed, i. e., saturated fatty acids (SFAs), monoene or monounsaturated fatty acids (MUFAs), highly unsaturated fatty acids (HUFAs), and polyunsaturated fatty acids (PUFAs) in *C. mrigala* muscles. The results obtained showed that muscle FAs composition was significantly influenced by dietary vit E supplementation. The levels of SFAs

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and MUFAs were significantly increased; however, PUFAs (omega-3 and omega-6) were decreased in the control group compared to vit E supplemented groups (p<0.05). MUFAs and omega-6 fatty acids were not significantly different among vit E supplemented groups (p>0.05). The highest contents of omega-3 FAs were measured in group D-V with significant difference from D-II, D-III and D-IV treatment groups (p<0.05).

Table 3: Fatty acid profile of *C. mrigala* fillet fed graded levels of vitamin E supplementation for 60 days.

FA (% of total detected)	D-I	D-II	D-III	D-IV	D-V
14:0	12.17±0.03^{a}	7.23±0.04^{b}	4.10±0.01^{b}	2.85±0.02^{c}	2.18±0.05^{d}
16:0	14.59±0.05^{a}	9.26±0.04^{b}	5.07±0.01^{c}	4.59±0.04^{d}	4.42±0.01^{d}
18:0	2.79±0.02	2.77±0.02	1.82±0.02	1.80±0.02	1.73±0.02
Total SFAs	29.55±0.01^{a}	19.26±0.01^{b}	10.99±0.00^{c}	9.24±0.01^{c}	8.33±0.02^{d}
16:1n-7	12.71±0.20^{a}	6.94±0.03^{b}	6.73±0.01^{b}	6.39±0.02^{b}	6.12±0.02^{b}
18:1n-9	12.83±0.07^{c}	12.98±0.03^{c}	13.09±0.03^{ab}	13.57±0.02^{ab}	13.74±0.04^{a}
18:1n-7	11.74±0.04^{a}	9.53±0.01^{b}	8.13±0.03^{c}	8.11±0.01^{c}	8.06±0.01^{c}
Total Monoene	37.28±0.08^{a}	29.45±0.01^{b}	27.95±0.01^{b}	28.07±0.00^{b}	27.92±0.01^{b}
18:2n-6	3.76±0.04^{c}	6.23±0.01^{a}	6.32±0.05^{a}	5.24±0.10^{b}	6.73±0.11^{a}
20:4n-6	1.17±0.07^{b}	3.76±0.03^{a}	3.77±0.02^{a}	4.59±0.03^{a}	3.65±0.31^{a}
Total ω-6 FAs	4.93±0.02^{b}	9.99±0.01^{a}	10.09±0.02^{a}	10.16±0.04^{a}	10.38±0.01^{a}
18:3n-3	4.50±0.16^{b}	11.77±0.29^{a}	11.50±0.09^{a}	11.43±0.02^{a}	11.37±0.02^{a}
20:5n-3	13.90±0.11^{d}	14.54±0.09^{c}	18.80±0.25^{b}	20.42±0.19^{a}	20.90±0.04^{a}
22:5n-3	6.49±0.02^{e}	10.81±0.81^{d}	14.71±0.20^{bc}	15.20±0.04^{b}	19.25±0.05^{a}
22:6n-3	3.35±0.11^{d}	4.18±0.28^{c}	5.96±0.20^{b}	7.03±0.09^{a}	7.89±0.00^{a}
Total ω-3 FAs	28.24±0.05^{e}	41.30±0.37^{d}	50.97±0.08^{c}	54.08±0.06^{b}	59.41±0.02^{a}

Note: The data are presented as the mean values of three replicates (n=3) ±standard deviation (SD). The values in a row having different superscripts are significantly different at p<0.05. D-I represents control diet (vitE 0 mg kg^{-1}) whereas, D-II, D-III, D-IV and D-V supplemented with vitE at 30, 60, 90 and 120 mg kg^{-1}, respectively. SFAs: saturated fatty acids, Monoene: monounsaturated fatty acids, ω-6 FAs: omega-6 fatty acids, ω-3 FAs: omega-3 fatty acids.

Muscle fatty acid composition (After 15- and 30-day storage)

Data related to the effects of 15 and 30 day of storage on muscles fatty acid composition of *C. mrigala* fed with graded levels of vit E supplementation are summarized in Tables 4 and 5, respectively. The increased SFAs and MUFAs contents were observed in all treatment groups except the D-V group after the storage period of 15 and 30 days, compared to the FA composition of fresh muscles (0 day). Furthermore, a significant decline was observed in omega-3 and omega-6 FAs contents in the control and vit E supplemented groups (p<0.05) after storage. The fish group fed with the D-V showed no significant (p>0.05) decrease in muscle omega-3 and omega-6 FAs contents after the storage of 15 and 30 days, compared to the FAs composition of fresh muscle (0 day).

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Table 4: Fatty acid profile of refrigerated fillet of *C. mrigala* fed graded levels of vitamin E supplementations for 60 days (after the storage period of 15 days).

FAs (% of total detected)	D-I	D-II	D-III	D-IV	D-V
14:0	16.36±0.52^{a}	10.27±0.50^{b}	7.35±0.40^{c}	4.76±0.12^{d}	2.78±0.41^{e}
16:0	19.9 9±0.79^{a}	11.77±1.05^{b}	8.65±0.49^{c}	6.53±0.44^{d}	4.53±0.40^{e}
18:0	9.52±0.48^{a}	4.75±0.53^{b}	3.40±0.33^{c}	2.54±0.27^{d}	1.74±0.12d^{e}
Total SFAs	45.87±0.16^{a}	26.79±0.30^{b}	19.40±0.08^{c}	13.83±0.16^{d}	8.92±0.016^{e}
16:1n-7	14.74±1.00^{a}	8.57±0.08^{b}	8.71±0.11^{b}	7.05±0.00^{bc}	5.94±0.02^{c}
18:1n-9	16.13±0.82^{a}	14.98±0.48^{b}	14.55±0.43^{b}	14.05±0.75^{b}	10.05±0.91^{c}
18:1n-7	13.37±0.53^{a}	11.97±0.90^{b}	10.59±0.33^{bc}	10.15±0.68^{bc}	7.14±0.13^{d}
Total Monoene	44.24±0.23^{a}	35.52±0.41^{b}	34.86±0.16^{b}	31.25±0.04^{c}	23.13±0.44^{d}
18:2n-6	2.07±0.08^{d}	6.20±0.07^{b}	6.25±0.02^{b}	5.12±0.07^{bc}	6.55±0.19^{a}
20:4n-6	0.99±0.00^{c}	2.70±0.01^{ab}	3.74±0.03^{a}	3.28±0.30^{a}	3.60±0.02^{a}
Total ω-6 FAs	3.06±0.04^{d}	8.90±0.04^{c}	9.99±0.00^{b}	8.40±0.16^{c}	10.15±0.05^{a}
18:3n-3	1.51±0.08^{c}	9.42±0.48^{bc}	10.18±0.81^{ab}	11.08±0.04^{a}	11.11±0.43^{a}
20:5n-3	2.05±0.17^{e}	9.13±0.43^{d}	11.68±0.76^{c}	14.35±0.61^{b}	20.11±0.48^{a}
22:5n-3	1.70±0.52^{e}	8.26±0.85^{cd}	9.37±0.64^{c}	15.79±0.53^{b}	19.44±0.51^{a}
22:6n-3	1.57±0.40^{e}	2.48±0.40^{d}	4.52±0.49^{c}	5.30±0.53^{b}	7.14±0.27^{a}
Total ω-3 FAs	6.83±0.20^{e}	29.29±0.37^{d}	35.75±0.14^{c}	46.52±0.26^{b}	57.80±0.10^{a}

Note: The data are presented as mean values of three replicates (n=3) ±standard deviation (SD). The values in a row having different superscripts are significantly different at p<0.05. D-I represents control diet (vitE 0 mg kg^{-1}) whereas, D-II, D-III, D-IV and D-V supplemented vit E at 30, 60, 90 and 120 mg kg^{-1}, respectively. SFAs: saturated fatty acids, Monoene: monounsaturated fatty acids, ω-6 FAs: omega-6 fatty acids, ω-3 FAs: omega-3 fatty acids.

Table 5: Fatty acid profile of refrigerated fillet of *C. mrigala* fed graded levels of vitamin E supplementation for 60 days (after the storage period of 30 days).

FAs (% of total detected)	D-I	D-II	D-III	D-IV	D-V
14:0	17.02±0.81^{a}	11.71±0.41^{b}	9.45±0.83^{c}	5.02±0.01	2.99±0.37
16:0	20.90±0.09 ^{a}	12.96±0.03 ^{b}	10.34±0.33^{c}	7.19±0.31	4.68±0.24
18:0	6.24±0.42 ^{a}	5.45±0.48 ^{b}	4.03±0.05^{c}	3.33±0.49	1.78±0.07
Total SFAs	45.16±0.36 ^{a}	30.12±0.24 ^{b}	23.82±0.39^{c}	15.54±0.24	9.45±0.15
16:1n-7	15.62±0.34 ^{a}	9.83±0.27 ^{b}	9.00±0.12^{b}	8.57±0.37^{b}	6.47±0.37
18:1n-9	17.66±0.36 ^{a}	15.90±0.19 ^{b}	15.84±0.13^{b}	15.14±0.05^{b}	11.00±0.16
18:1n-7	14.82±0.28 ^{a}	12.69±0.28 ^{b}	12.40±0.51^{b}	11.38±0.44	7.33±0.58
Total Monoene	48.10±0.04 ^{a}	37.97±0.04 ^{b}	37.24±0.22^{b}	35.09±0.20	24.08±0.21
18:2n-6	1.25±0.18^{d}	3.53±0.13^{bc}	4.93±0.05^{b}	5.03±0.04^{b}	6.43±0.08 ^{a}
20:4n-6	0.86±0.03^{d}	1.93±0.05^{c}	2.75±0.10^{b}	2.08±0.05^{b}	3.46±0.11^{a}
Total ω-6 FAs	2.11±0.10^{d}	5.46±0.05^{c}	7.68±0.03^{b}	7.11±0.00^{b}	9.89±0.02^{a}
18:3n-3	1.11±0.09^{c}	9.03±0.07^{b}	9.26±0.23^{b}	10.50±0.43^{a}	10.99±0.01^{a}
20:5n-3	1.52±0.05^{e}	8.58±0.19^{d}	10.45±0.39^{c}	13.50±0.35^{b}	19.82±0.08^{a}
22:5n-3	1.04±0.52^{d}	7.50±0.45^{dc}	8.32±0.18^{c}	14.44±0.40^{b}	18.72±0.06^{a}
22:6n-3	0.96±0.40^{d}	1.34±0.27^{c}	3.23±0.23^{bc}	4.44±0.41^{b}	7.05±0.05^{a}
Total ω-3 FAs	4.63±0.23^{e}	26.45±0.15^{d}	31.26±0.09^{c}	42.88±0.03^{b}	56.58±0.02^{a}

Note: The data are presented as the mean values of three replicates (n=3) ±standard deviation (SD). The values in a same row having different superscripts are significantly different at p<0.05. D-I represents control diet (vitE 0 mg kg^{-1}) whereas, D-II, D-III, D-IV and D-V supplemented with vit E 30, 60, 90 and 120 mg kg^{-1}, respectively. SFAs: saturated fatty acids, Monoene: monounsaturated fatty acids, ω-6 FAs: omega-6 fatty acids, ω-3 FAs: omega-3 fatty acids.

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Muscle TBARs content (Before and after storage)

The dietary vit E supplementation significantly showed markedly effects on muscle TBARs level (Fig. 7). The significantly lowest TBARs value was found in the group fed with D-V at the day of harvesting (day 0), compared to the control and vit E supplemented groups (p<0.05). Later on, a significant increase in TBARs values was observed after 15 and 30 day of storage in control and vit E supplemented (D-II, D-III, D-IV and D-V) groups. Though, a non-significant difference was observed in TBARs values in the group fed with D-V treatment after the storage of 15 and 30 days compared to the 0 day (fresh muscles) (p>0.05).

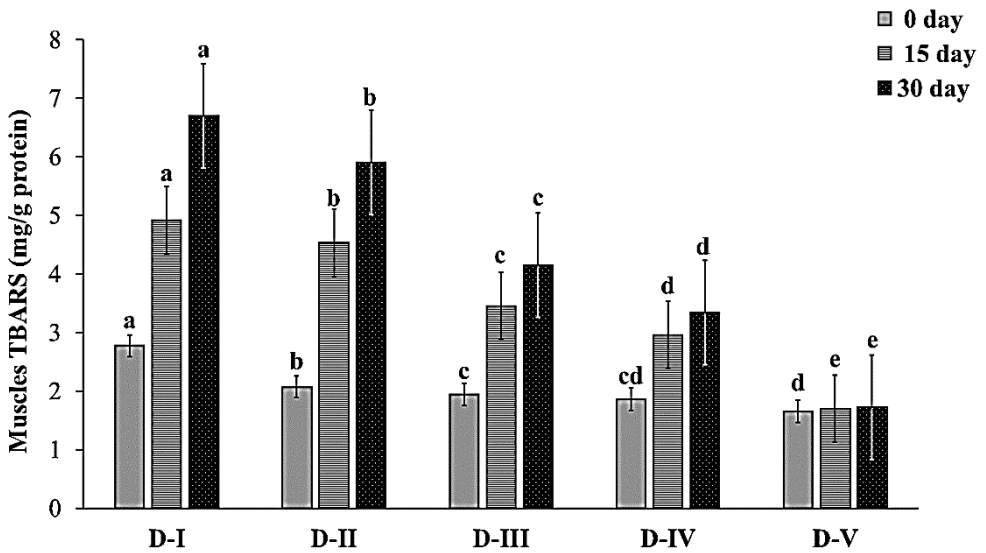

Figure 7: The thiobarbituric acid reactive substances (TBARs) contents of muscles of *C. mrigala* fed with graded levels of vit E supplementation after 0, 15 and 30 days of storage. The graph bar showing the mean values of three replicates (n=3) ±standard deviateion (SD) with the significant difference among different treatment groups (p<0.05). D-I represents control diet (0 mg kg^{-1}) whereas, D-II, D-III, D-IV and D-V are supplemented with vit E at 30, 60, 90, and 120 mg kg^{-1}, respectively.

Discussion

The use of antioxidants in aquafeeds is imperative for optimum yield and improved product quality of aquatic organisms. Since antioxidants plays a vital role in different biochemical and physiological functions as a growth enhancer, immunostimulants, and signal transporter (Li and Gatlin III, 2009). As an antioxidant, vit E scavenges free radicals; thus, providing oxidative stability to biological membranes (Frigg *et al*., 1990). In the present study, improved growth performance and decreased FCR of *C. mrigala* was observed with the dietary vit E supplementation which is comparable to previous findings in teleost species (Paul *et al*., 2004; NRC, 2011; Gao *et al*., 2012a). Similar to our results, improved growth performance and reduced FCR were reported by Gao *et al*. (2012a) and Paul *et al*. (2004) with dietary vit E supplementation at 100 and 200 mg kg^{-1}

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in red sea bream, *Sparus microcephalus*, and rohu, respectively. These findings indicated that vit E enhances growth performance; hence it is an essential micronutrient, that regulate various physiological processes in the fish body. Additionally, vit E has found to be effective dietary element for increased nutrient availability by protecting the diets from oxidation thus, proper nutrient utilization by vit E supplementation might be responsible for the improved growth performance in present study (Wu *et al*., 2017). In contrast, few studies reported non-significant effects of dietary vit E supplementation on the growth performance, FCR and feed efficiency in channel catfish and red sea bream fed with fresh and oxidized fish oil (Wilson *et al*., 1984; Gao *et al*., 2012b). In growth studies, comparison of the effect of different vit E treatments is often difficult due to several variables such as the difference in fish species and size, culture conditions and feeding duration, which may affect growth and feed consumption in cultured fish. The dietary vit E supplementation did not affect the survival rate of *C. mrigala* in the present study, which is similar to the result of Farris *et al*. (2020) in Japanese eel, *Anguilla japonica*.

The organosomatics including viscerosomatic index (VSI) and hepatosomatic index (HSI) of *C. mrigala* in the present study, were significantly reduced by the dietary vit E supplementation. These results are similar to the findings of Baker and Davies (1996a) and Tocher *et al*. (2002) in African catfish (*Clarias gariepinus*), and halibut (*Hippoglossus hippoglossus* L.), respectively. As, vit E is a lipid-soluble antioxidant, it facilitates the transportation and utilization of various nutrients such as, lipids and lipoproteins in the fish body (Iqbal *et al*., 2018). However, dietary vit E deficiency may induce fat deposition, especially in the liver which causes liver enlargement and mal-functioning (Baker and Davies, 1996b). Moreover, the formation of large size vacuoles and ceroid accumulation in hepatocytes might be another plausible explanation for increased HSI due to inadequate supply of vit E in diets (Thorarinsson *et al*., 1994). On the other hand, some studies suggested contrast results in Nile tilapia (Wu *et al*., 2017), Atlantic salmon (Lygren *et al*., 2000), and turbot (Niu *et al*., 2014), which might be attributed to the variation in lipid sources and dietary lipid level (Farris *et al*., 2020).

The present study suggested that whole body composition was not significantly affected by dietary vit E supplementation; however, high moisture and low protein contents were observed in vit E deficient diets. This is comparable to the findings of Gatta *et al*. (2000), Pirini *et al*. (2000), and Wu *et al*. (2017) in sea bass, sea bream and genetically improved farmed tilapia, respectively. Conversely, Sau *et al*. (2004) and Huang *et al*. (2003) reported improved whole-body protein in rohu and hybrid tilapia fed with vit E supplemented diets. It is established that difference in the chemical composition of the fish body is closely associated to

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the nutritional quality of feed, culturing environment, seasonal variations, and physical condition of fish (Fogaca *et al*., 2009).

The antioxidant enzymes are the first line of defense against the oxidative stress induced by reactive oxygen species (ROS) and other free radical and/or non-radical species in body. In the present study, improved antioxidant capacity was observed with vit E supplementation in the form of decreased activity of CAT, SOD, and POX in the intestine, liver, kidney, and the whole body of *C. mrigala*. Similar results were obtained in sea bream, grass carp, Nile tilapia and mrigal (Tocher *et al*., 2002; Li *et al*., 2014; Iqbal *et al*., 2018). The increased activity of antioxidant enzymes in various tissues due to the deficiency of vit E supplementation indicates it decreased oxidative stability by increasing the number of ROS in body tissues. It has already been reported that O_2^- catalyzed by SOD into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2). Afterward, CAT decomposes this H_2O_2 into molecular oxygen (O_2) and water (H_2O) (Labazi *et al*., 2015). Thus, vit E in the cell promotes the scavenging of superoxide anions which are toxic end products of uncontrolled lipid oxidation (Li and Gatlin III, 2009). Additionally, oxidation of lipoprotein and PUFAs is inhibited by vit E by breaking the chain reactions of ROS production, consequently enhancing oxidative stability (Burton and Traber, 1990). In the present study, the highest CAT and POX activity was detected in the liver, whereas highest SOD activity was observed in the intestine of *C. mrigala*. The plausible reason behind this response is that the liver is the main site for various biological and metabolic processes and plays a key role in nutrient transportation within the fish body. Hence, response of antioxidant enzymes to dietary vit E supplementation might be varied in different body organs (Sant'Ana and Mancini-Filho, 1995).

In the present study, liver and muscle vit E contents were significantly increased by increasing the dietary vit E supplementation levels. This is in agreement with previous studies conducted with Korean rockfish, *Sebastes schlegeli*, grouper, channel catfish, and hybrid striped bass, *Morone chrysops* ♀ x *M. saxatilis* ♂ (Bai and Gatlin III, 1993; Bai and Lee, 1998; Sealey and Gatlin III, 2002; Lin and Shiau, 2005). This elevation in liver and muscles vit E contents in fish by increasing the dietary vit E supplementation is indicated that fish is not enabled to synthesize vit E inside the body; hence, fish rely on dietary source for this vital dietary element (Farris *et al*., 2020).

As a potent antioxidant, the major role of vit E is to maintain the equilibrium between the production and consumption rate of ROS. Since, these ROS could initiate chain reactions that consequently cause oxidation of PUFAs, lipoproteins, glycolipids, glycoproteins, enzymes, DNA, and RNA (Fogaca *et al*., 2009). In the current study, improved lipid stability was observed by analyzing the content of PUFAs in muscles of *C.*

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mrigala fed with vit E supplementation. Similar findings were reported in sturgeon, *Acipenser naccarii* (Agradi *et al*., 1993), turbot, and halibut (Tocher *et al*., 2002). Vit E protects long-chain polyunsaturated fatty acids (C20:4, C20:5 and C22:5) (Diplock *et al*., 1989). Hence, maintains the integrity of biological membranes due to its antioxidant properties, which could be the reason for increased omega-3 and omega-6 fatty acids contents in the muscles of fish fed vit E in the current study (Kagan, 1989). Also, improved lipid stability in *C. mrigala* muscles was observed after 15 and 30 day of storage with the highest level of supplementation (120 mg kg^{-1}). Similar results were suggested for Japanese flounder, gilthead sea bream, and Caspian brown trout, *Salmo trutta caspius* (Wang *et al*., 2006; Sotoudeh *et al*., 2016; Izquierdo *et al*., 2019). These findings demonstrated that dietary vit E supplementation may influence the oxidation rates in processed fish products. It has been established that the rate and extent of oxidation are greatly influenced by pre- and post-slaughter stress factors. When the animal is slaughtered, number of variables are involved, that could affect the quality of the end product during processing and storage, such as; the slaughtering method, change in pH and body temperature (Sant'Ana and Mancini-Filho, 1995; Fogaca *et al*., 2009).

Moreover, decreased TBARs were observed with the increasing dietary vit E supplementation in the present study. Additionally, TBARs content was not evaluated after the storage time of 15 and 30 day in the group that received the highest level of vit E supplementation (120 mg kg^{-1}) in the current study. Gatta *et al*. (2000) and Lin and Shiau (2005) reported similar results in sea bass and grouper, respectively. It has been reported that dietary vit E accumulates in fish muscles, and later, it terminates the oxidation process by scavenging ROS. In addition, vit E slowdowns the process of TBARs formation; therefore, it maintains the quality of fish products during storage (de Zwart *et al*., 1999). The lactic acid content is gradually elevated in the muscles after slaughter, which reduces its pH from neutral to acidic. These changes in pH influence the colour and water-holding capacity of the muscles. Furthermore, an increased rate of metallic autoxidation deteriorates the flavor of fish fillet. However, vit E present in the hydrocarbon part of lipid bilayer of membranes, stabilize its integrity by controlling the movement of ions across the membranes, thus maintaining fillet quality (Wu *et al*., 2017).

Based on the results of the present study, it can be concluded that dietary vit E supplementation improves the growth performance and antioxidant capacity of *C.* mrigala by reducing oxidative stress. Also, it is an effective tool to maintain lipid stability of frozen fish products at the supplementation level of 120 mg kg^{-1}. Hence, the quality and storage time of processed fish products might be improved by dietary vit E supplementation without the risk of rancidity.

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References

Agradi, E., Abrami, G., Serrini, G., Mckenzie, D., Bolis, C. and Bronzi. P., 1993. The role of dietary n-3 fatty acid and vitamin E supplements in growth of sturgeon (*Acipenser naccarii*). *Comparative Biochemistry Physiology A,* 105, 187-195. https://doi.org/10.1016/0300-9629(93)90194-9.

AOAC, 1995. Association of Official Analytical Chemist. *Official methods of analysis of the Association of Official Analytical Chemists*, 12th ed, Washington, DC, USA.

Bai, S.C. and Gatlin III, D.M., 1993. Dietary vitamin E concentration and duration of feeding affect tissue α-Tocopheryl acetate concentrations of channel catfish (*Ictalurus punctatus*). *Aquaculture,* 113, 129-135. https://doi.org/10.1016/0044-8486(93)90346-Z.

Bai, S.C. and Lee, K.J., 1998. Different level of dietary DL-*α* -tocopherol acetate affect the vitamin E status of juvenile Korean rockfish, *Sebastes schlegeli*. *Aquaculture*, 161, 405-414.

Baker, R.T.M. and Davies, S.J., 1996a. Changes in tissue *α* -tocopherol status and degree of lipid peroxidation with varying *α* -tocopherol acetate inclusion in diets for the African catfish. *Aquaculture Nutrition,* 2, 71-79.

Baker, R.T.M. and Davies, S.J., 1996b. Increased production of docosahexaenoic acid (22: 6 n–3, DHA) in catfish nutritionally stressed by the feeding of oxidized oils and the modulatory effect of dietary α-tocopheryl acetate. *Journal of Fish Biology,* 49, 748-752. https://doi.org/10.1111/j.1095-8649.1996.tb00073.x

Burton, G.W. and Traber, M.G., 1990. Vitamin E: antioxidant activity, biokinetics and bioavaibility. *Annual Reviews of Nutrition,* 10, 357-382. https://doi.org/10.1146/annurev.nu.10.070190.002041.

Civello, P.M., Marting, G.A., Chaves, A.R. and Anan, M.C., 1995. Peroxidase from strawberry fruit by partial purification and determination of some properties. *Journal of Agriculture Food & Chemistry*, 43, 2596-2601. https://doi.org/10.1021/JF00058A008.

de Zwart, L.L., Meerman, J.H., Commandeur, J.N. and Vermeulen, N.P., 1999. Biomarkers of free radical damage applications in experimental animals and in humans. *Free Radical Biology & Medicine*, 26, 202-226. https://doi.org/10.1016/s0891-5849(98)00196-8.

Diplock, A.T., Guang, L.X., Yeow, C.L. and Okikiola, M., 1989. Relationship of tocopherol structure to biological activity, tissue uptake, and prostaglandin biosynthesis. *Annuals of New York Academy of Sciences,* 570, 72-84.

https://doi.org/10.1111/j.1749-6632.1989.tb14909.x.

Farris, N.W., Kim, D., Hamidoghli, A., Won, S., Lee, S., Bae, J. and Bai, S.C., 2020. Dietary α-Tocopheryl acetate and arachidonic acid synergistically improves superoxide dismutase activity in female Japanese eel brood stock, *Anguilla japonica*. *Aquaculture*, 522, 735-100. https://doi.org/10.1016/j.aquaculture.2020.735100.

Fogaça, F.H.S., Sant'Ana, L.S. and Carneiro, D.J., 2009. Dietary α-tocopheryl acetate on muscles quality of tilapia. *Acta Science Animal Science*, 31, 339-445. https://doi.org/10.4025/actascianimsci.v31i4.6929.

Frigg, M., Prabucki, A.L. and Ruhdel, E.U., 1990. Effect of dietary vitamin E levels of oxidative stability of trout muscles. *Aquaculture*, 84, 145-158. https://doi.org/10.1016/0044-8486(90)90344-M.

Gao, J., Koshio, S., Ishikawa, M., Yokoyama, S., Kader, M.A. and Laining, A., 2012a. Interaction between vitamin C and E on oxidative stress in tissues and blood of red sea bream (*Pagrus major*). *Aquatic Sciences*, 60, 119-126. https://doi.org/10.1111/jwas.12048.

Gao, J., Koshio, S., Ishikawa, M., Yokoyama, S., Maung, R.E.P. and Han, Y., 2012b. Effect of dietary oxidized fish oil with vitamin E supplementation on growth performance and reduction of lipid peroxidation in tissues and blood of red sea bream *Pagrus major*. *Aquaculture*, 356-357, 73-79. https://doi.org/10.1016/j.aquaculture.2012.05.034.

Gatlin, D.M., 2003. Nutrition and Fish Health. In book: Fish Nutrition. pp. 671-702. https://doi.10.1016/B978-012319652-1/50013-6.

Gatta, P.P., Pirini, M., Testi, S., Vignola, G. and Monetti, P.G., 2000. The influence of different levels of dietary vitamin E on sea bass *Dicentrarchus labrax* flesh quality. *Aquaculture Nutrition*, 6, 47-52. https://www.cabdirect.org/20001411285.

Giannopolitis, C.N. and Ries, S.K., 1997. Superoxide dismutase I occurrence in higher plants. *Plant Physiology*, 59, 309-314. https://doi.org/10.1104/pp.59.2.309.

Huang, C.H., Chang, R.J., Huang, S.L. and Chen, W., 2003. Dietary vitamin E supplementation affects tissue lipid peroxidation of hybrid tilapia, *Oreochromis niloticus* × *O. aureus*. *Comparative Biochemistry & Physiology B*, 134, 265-270. https://doi.org/10.1016/s1096-4959(02)00256-7

Huang, C.H., Higgs, D.A., Balfry, S.K. and Devlin, R.H., 2004. Effect of dietary vitamin E level on growth, tissue lipid peroxidation and erythrocyte fragility of transgenic Coho salmon, *Oncorhynchus kisutch*. *Comparative Biochemistry & Physiology*, 139, 199-204. https://doi.org/10.1016/j.cbpb.2004.09.001.

Iqbal, M., Afzal, M., Anjum, K.M., Yaqub, A., Shah, S.Z.H. and

[DOR: 20.1001.1.15622916.2023.22.1.2.5]

Fatima, M., 2018. Growth response, antioxidant status and fatty acid profile of *Cirrhinus mrigala* fingerlings fed on practical diets after treated with vitamin E supplementation. *Pakistan Journal of Zoology*, 13, 120-128.

IUPAC, 1987. International Union of Pure and Applied Chemistry. Standard methods for the analysis of oils, fats and derivatives, 7th rev. enlarged ed., edited by Paquot, C., and A. Hautfenne. Blackwell Scientific, London.

Izquierdo, M., Domínguez, D., Jiménez, J.I., Saleh, R., Hernández-Cruz, C.M., Zamorano, M.J. and Hamre, K., 2019. Interaction between taurine, vitamin E and vitamin C in micro diets for gilthead seabream (*Sparus aurata*) larvae. *Aquaculture*, 498, 246-253. https://doi.org/10.1016/j.aquaculture.2018.07.010.

Kagan, V.E., 1989. Tocopherol stabilizes membrane against phospholipase A., free fatty acid, and lyso phospholipids. *Annuals of New York Academy of Sciences*, 570, 121-135. https://doi.org/ 10.1111/j.1749-6632. 1989.tb14913.x.

Labazi, M., McNeil, A.K., Kurtz, T., Lee, T.C., Pegg, R.B., Angeli, J.P.F., Conrad, M. and McNeil, P.L., 2015. The antioxidant requirement for plasma membrane repair in skeletal muscle. *Free Radical Biology & Medicine*, 84, 246-253. https://doi.org/10.1016/j.freeradbiomed.2015.03.016

Larsson, T., Koppang, E.O., Espe, M., Terjesen, B.F., Krasnov, A., Moreno, H.M. and Mørkøre, T., 2014. Fillet quality and health of Atlantic salmon (*Salmo salar* L.) fed a diet supplemented with glutamate. *Aquaculture*, 426, 288-295. https://doi.org/10.1016.j.aquaculture.2014.01.034.

Lee, B.L., New, A.L. and Ong, C.N., 2003. Simultaneous determination of tocotrienols, tocopherol, retinol and major carotenoids in human plasma. *Clinical Chemistry*, 49, 2056-2066. https://doi.org/10.1373/clinchem.2003.022681.

Li, J., Liang, X.F., Tan, Q.S., Yuan, X.C., Liu, W.L., Zhou, Y. and Li. B., 2014. Effects of vitamin E on growth performance and antioxidant status in juvenile grass carp *Ctenopharyngodon idellus*. *Aquaculture*, 430, 21-27. https://doi.org/10.1016/j.aquaculture.2014.03.019.

Li, P. and Gatlin III, D.M., 2009. Dietary vitamin E requirement of the red drum *Sciaenops ocellatus*. *Aquaculture Nutrition*, 15, 313-319. https://doi.org/10.1111/j.1365-2095.2008.00596.x.

Lin, Y.H. and Shiau, S.Y., 2005. Dietary vitamin E requirement of grouper, *Epinephelus malabaricus*, at two lipid levels, and their effects on immune responses. *Aquaculture*, 248, 235-244. https://doi.org/10.1016/j.aquaculture.2005.04.020.

[DOR: 20.1001.1.15622916.2023.22.1.2.5]

Lygren, B., Hamre, K. and Waagbø, R., 2000. Effect of induced hyperoxia on the antioxidant status of Atlantic salmon *Salmo salar* L. fed three different levels of dietary vitamin E. *Aquaculture Research*, 31, 401-407. https://doi.org/10.1046/j.1365-2109.2000.00459.x.

Mahmood, T. and Mateen, A., 2020. Effect of dietary omega-3 and micronutrients on growth, meat quality and health status of polycultured cyprinid species. *Pakistan Journal of Agricultural Sciences*, 57, 509-516. https://doi.org/Corpos ID 218868917.

Murray, R.K., Granner, D.K., Mayes, P.A. and Rodwell, V.W., 2000. Harper´s Biochemistry. 25th ed. Stamford, Connecticut: Appleton and Lange.

Niu, H.X., Jia, Y.D., Hu, P., Meng, Z. and Lei, J.L., 2014. Effect of dietary vitamin E on the growth performance and nonspecific immunity in sub-adult turbot (*Scophthalmus maximus*). *Fish & Shellfish Immunology*, 41, 501-506. https://doi.org/10.1016/j.fsi.2014.09.033.

NRC, 2011. National Research Council. Nutrient requirements of fish and shrimp. The National Academy Press, Washington, pp. 190.

Paul, B.N., Sarkar, S. and Mohanty, S.N., 2004. Dietary vitamin E requirement of mrigal, *Cirrhinus mrigala* fry. *Aquaculture*, 242, 529-536. https://doi.org/10.1016/j.aquaculture.2004.08.037.

Pirini, M., Gatta, P.P., Testi, S., Trigari, G. and Monetti, P.G., 2000. Effect of refrigerated storage on muscle lipid quality of sea bass (*Dicentrarchus labrax*) fed on diets containing different levels of vitamin E. *Food Chemistry*, 68, 289-293. https://doi.org/10.1016/S0308-8146(99)00190-9.

Puangkaew, J., Kiron, V., Satoh, S. and Watanabe, T., 2005. Antioxidant defense of rainbow trout (*Oncorhynchus mykiss*) in relation to dietary n-3 highly unsaturated fatty acids and vitamin E contents. *Comparative Biochemistry & Physiology*, 140, 187-196. https://doi.org/10.1016/j.cca.2005.01.016.

Rowland, S. J. and Ingram, B. A., 1991. Diseases of Australian native fishes with particular emphasis on the ectoparasitic and fungal diseases of Murray cod (*Maccullochella peeli*), golden perch (*Macquaria ambigua*) and silver perch (*Bidyanus bidyanus).* Fisheries Bulletin 4 NSW Fisheries, Sydney, NSW, Australia.

Sant'Ana, L.S. and Mancini-Filho, J.M., 1995. Oxidative protection in the use of antioxidants in vivo in animal muscles. *Nutrition Notebooks*, 10, 48-63.

Satoh, S., Takeuchi, T. and Watanabe, T., 1987. Requirement of tilapia for alpha-tocopherol. *Nippon Suisan Gakkaishi*, 53, 119-124.

Sau, S.K., Paul, B.N., Mohanta, K.N. and Mohanty, S.N., 2004. Dietary

[DOR: 20.1001.1.15622916.2023.22.1.2.5]

vitamin E requirement, fish performance and carcass composition of rohu (*Labeo rohita*) fry. *Aquaculture*, 240, 359-368. https://doi.org/10.1016/j.aquaculture.2004.02.008.

Sealey, W.M. and Gatlin III, D.M., 2002. Dietary vitamin C and vitamin E interact to influence growth and tissue composition of juvenile hybrid striped bass (*Morone chrysops* ♀ x *M. saxatilis* ♂) but have limited effects on immune responses. *The Journal of Nutrition*, 132, 748-755. https://doi.org/10.1093/jn/132.4.748.

Shimeno, S., 1991. Yellowtail, *Seriola quinque radiata*. Handbook of nutrient requirements of finfish. Wilson RP. CRC Press, Boca Raton, FL. pp.181-191.

Sotoudeh, E., Kenari, A.A., Khodabandeh, S. and Khajeh, K., 2016. Combination effects of dietary EPA and DHA plus alpha-tocopherol: effects on performance and physiological status of Caspian brown trout (*Salmo trutta caspius*) fry. *Aquaculture Nutrition*, 22, 1101-1115. https://doi.org/10.1111/anu.12361.

Stephan, G., Guillaume, J. and Lamour, F., 1995. Lipid peroxidation in turbot (*Scophthalmus maximus*) tissue: effect of dietary vitamin E and dietary *n*-6 or *n*-3 polyunsaturated fatty acids. *Aquaculture*, 130, 251-268. https://agris.fao.org/record ID NL9503238.

Thorarinsson, R., Landolt, M.L., Elliott, D.G., Pascho, R.J. and Hardy, R.W., 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.

Tocher, D.R., Mourente, G., Eecken, A.V.D., Evjemo, J.O., Diaz, E., Bell, J.G., Geurden, I., Lavens, P. and Olsen, Y., 2002. Effects of dietary vitamin E on antioxidant defence mechanisms of juvenile turbot (*Scophthalmus maximus* L.), halibut (*Hippoglossus hippoglossus* L.) and sea bream (*Sparus aurata* L.). *Aquaculture Nutrition*, 8, 195-207. https://doi.org/10.1046/j.1365-2095.2002.00205.x.

Undeland, I., 1997. Lipid oxidation in fish - Causes, changes and measurements. In: Methods to Determine the Freshness of Fish in Research and Industry. (Eds.). International Institute of Refrigeration. Paris, France, pp. 241-257.

Wang, Z., Mai, K., Liufu, Z., Ma, H., Xu, W., Ai, Q., Zhang, W., Tan, B. and Wang, X., 2006. Effect of high dietary intakes of vitamin E and n-3 HUFA on immune responses and resistance to *Edwardsiella tarda* challenge in Japanese flounder (*Paralichthys olivaceus*, Temminck and Schlegel) *Aquaculture Research*, 37, 681-692. https://doi.org/10.1111/j.1365-2109.2006. 01481.x.

Wilson, R.P., Bowser, P.R. and Poe, W.E., 1984. Dietary vitamin E

[DOR: 20.1001.1.15622916.2023.22.1.2.5]

requirement of fingerling channel catfish. *The Journal of Nutrition*, 114, 2053-2058.

Wu, F., Jiang, M., Wen, H., Liu, W., Tian, J., Yang, C. and Huang, F., 2017. Dietary vitamin E effects on growth, fillet textural parameters, and antioxidant capacity of genetically improved farmed tilapia (GIFT), *Oreochromis niloticus*. *Aquaculture International*, 25, 991-1003. https://doi.org/10.1007/s10499-016-0089-7.

Zhou, Q.C., Wang, L.G., Wang, H.L., Wang, T., Elmada, C.Z. and Xie, F.J., 2012. Dietary vitamin E could improve growth performance, lipid peroxidation and non-specific immune responses for juvenile cobia (*Rachycentron canadum*). *Aquaculture Nutrition*, 19, 421-429. https://doi.org/10.1111/j.1365-2095.2012.00977.x.