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Effects of dietary vitamin E on growth performance, body composition, antioxidant capacity, and some immune responses in Caspian trout (*Salmo caspius*)

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ABSTRACT

This study was conducted to evaluate the effect of dietary vitamin E on growth performance, feed utilization, biochemical properties and some immune responses in Caspian trout, *Salmo caspius*. Six experimental diets were formulated with semi-purified ingredients supplemented with vitamin E in the form of DL-α-tocopherol acetate to provide the actual vitamin E concentrations of 4.9 (the basal diet), 8.6, 17.4, 35.4, 78.8 and 137.0 mg kg^{-1} diet, respectively. Each diet was assigned to three replicate groups of Caspian trout (initial average weight of 9.73 ± 0.34 g) for eight weeks. Weight gain ratio (WGR), specific growth rate (SGR), and protein efficiency ratio (PER) were found to be significantly enhanced by increasing dietary vitamin E level and reached to the highest values in fish fed with 78.8 mg kg^{-1} vitamin E supplemented diet ($P < 0.05$). A significantly linear increasing trend was recorded in crude protein, fat content, superoxide dismutase (SOD) activity, liver vitamin E concentration, immunoglobulin M (IgM), and alternative complement activity (ACH50), while glutathione peroxidase (GPX) activity was linearly and quadratically enhanced in response to increasing dietary vitamin E supplementations ($P < 0.05$). There were also linearly and quadratically decreasing trend in alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) activities as well as serum glucose (GLC) and malondialdehyde (MDA) levels by supplementing dietary vitamin E ($P < 0.05$). Analysis by the polynomial regression of SGR, GLC, and SOD activity against varying levels of dietary vitamin E revealed that the optimum dietary vitamin E requirements in Caspian trout were 79.44, 78.73, and 82.16 mg kg^{-1}, respectively.

1. Introduction

Vitamin E is a generic descriptor for eight naturally occurring lipophilic compounds with the highest biological activity in the form of α-tocopherol that plays fundamental roles in normal metabolic functions and physiological processes (Combs et al., 2017). As an antioxidant, it protects macromolecules such as nucleic acids, lipoproteins, and unsaturated fatty acids from being oxidized by free radicals produced during normal metabolism or unfavorable conditions such as pollution, infection, and stress (Atkinson et al., 2013; Chen et al., 2004). Dietary supplementation of vitamin E has been also proved to enhance the growth performance (Lu et al., 2016; Bae et al., 2013; Amlashi et al., 2011), increase the survival (Zhou et al., 2013), improve the immunological responses (Montero et al., 1998; Kiron et al., 2004), maintain the flesh quality (Ruff et al., 2003), and reduce the thiobarbituric acid (TBARS) as a secondary product of lipid oxidation (Peng et al., 2009; Zhao et al., 2018).

Fish cannot synthesize all biologically active forms of vitamin E and rely on the exogenous dietary sources for its supply (Peng and Gatlin Iii, 2009). The quantitative requirements of dietary vitamin E have been

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documented in various farmed fish including Atlantic salmon, *Salmo salar* (Hamre and Lie, 1995), rainbow trout, *Oncorhynchus mykiss* (Cowey et al., 1983; Hung et al., 1980), grass carp, *Ctenopharyngodon idellus* (Li et al., 2014), cobia, *Rachycentron canadum* (Zhou et al., 2013), grouper, *Epinephelus malabaricus* (Lin and Shiau, 2005), Rohu, *Labeo rohita* (Sau et al., 2004), mrigal, *Cirrhinus mrigala* (Paul et al., 2004), hybrid striped bass, *Morone chrysops* × *M. saxatilis* (Kocabas and Gatlin Iii, 1999), yellow catfish, *Pelteobagrus fulvidraco* (Lu et al., 2016), hybrid snakehead, *Channa argus* × *Channa maculata* (Zhao et al., 2018) and Japanese eel, *Anguilla japonica* (Shahkar et al., 2018), which ranged from 6 to 200 mg kg^{-1} α-tocopherol. Diets deficient or lacking in vitamin E may lead to reduced growth, impaired erythropoiesis, muscular dystrophy, darkened skin, exudative diathesis, skin depigmentation, liver fat degeneration, and even death (NRC, 2011). On the other hand, excess levels of vitamin E could induce lipid peroxidation in rainbow trout (Tokuda and Takeuchi, 1999), grass carp (Li et al., 2014), and spotted snakehead, *Channa punctatus* (Abdel-Hameid et al., 2012) by acting as a prooxidant in the generation of hydrogen peroxide. Therefore, an appropriate level of vitamin E should be provided to fulfill the fish requirements especially under cultural conditions.

Caspian trout, *Salmo caspius* Kessler, 1877, is a member of the salmon family with the largest size and weight among the other brown trout species that natively distributed in the southern part of the Caspian Sea (Dorafshan et al., 2008). Proper growth rate, high economic value, and appropriate flesh quality made this species a suitable candidate for intensive aquaculture (Arab and Rajabi Islami, 2015; Mohammadian et al., 2020). Despite of numerous researches conducted to consider the nutritional requirement of Caspian trout under cultural conditions (Jami et al., 2019; Jenabi Haghparast et al., 2019; Mohammadian et al., 2020), no information was available concerning the vitamin E requirement of this species. Accordingly, the present study was aimed to evaluate the effect of dietary vitamin E on growth performance, body composition, antioxidant capacity, and immune responses in Caspian trout. The optimal dietary vitamin E level was also determined based on the specific growth rate, serum glucose content and superoxide dismutase activity.

2. Materials and methods

2.1. Experimental diets and preparation

Dietary composition of the basal diet and its approximate analysis is given in Table 1. Casein (United States Biochemical, Cleveland, OH, USA.) and defatted fish meal (Khazar Kilka Industrial Co., Anzali, Iran) were used as protein sources, while fish oil (refined fish oil of *Clupeonella cultriventris*; Khazar Kilka Industrial Co., Anzali, Iran) and corn oil (Khorasan cotton and oil Seeds Co., Mashhad, Iran) were utilized as lipid sources. Dextrin (United States Biochemical, Cleveland, OH, USA.) and corn starch (United States Biochemical, Cleveland, OH, USA.) were also supplied as the carbohydrate sources. The supplemented fish meal was initially extracted twice in a boiling 2:1 ethanol (ethanol /fishmeal = 2:1, w/v) to eliminate vitamin E from this ingredient before incorporating into the diets (Kosutarak et al., 1995). The utilized oils did not contain synthetic antioxidants. The basal diet was prepared without adding α-tocopherol supplementation and considered as the control diet. Other experimental diets were prepared by adding 10, 20, 40, 80 and 160 mg kg^{-1} tocopherol acetate (TA) in the form of DL-α-tocopherol acetate (Sigma Chemical Co., Steinheim, Germany) at the expense of alpha cellulose to the basal diet. The actual vitamin E concentration in the experimental diets was determined by high pressure liquid chromatography (HPLC) method of Tangney et al. (1981) to be 4.9 (the basal diet), 8.6, 17.4, 35.4, 78.8 and 137.0 mg kg^{-1} diet, respectively. For diet preparation, all feed ingredients were carefully mixed and pelleted as described by Arab and Rajabi Islami (2015). The prepared diets were then sealed in separate vacuum-packed bags and stored in a freezer at −20 °C until used.

Table 1
Feed formulation and proximate composition of basal diet for Caspian trout, *Salmo caspius*.

Ingredients	**g kg^{-1}**
Casein	300.00
fish meal (defatted)	200.00
Dextrin	150.00
Corn starch	100.00
Fish oil	50.00
Corn oil	50.00
Mineral premix	50.00
Vitamin premix (vitamin E free)	20.00
Alpha-cellulose	80.00
Proximate composition	**g kg^{-1}**
Crude protein	462.03
Crude lipid	114.21
Moisture	109.48
Ash	95.05

[2] Vitamin premix (mg or g/kg diet): thiamin, 25 mg; riboflavin, 45 mg; pyridoxine HCl, 20 mg; vitamin B12, 0.1 mg; vitamin K3, 10 mg; inositol, 800 mg; pantothenic acid, 60 mg; niacin acid, 200 mg; folic acid, 20 mg; biotin, 1.20 mg; retinol acetate, 32 mg; cholecalciferol, 5 mg; choline chloride, 2500 mg; ethoxyquin 150 mg; wheat middling 14.012 g. Alpha tocopherol was removed from vitamin premix and added separately for different treatments. Vitamin C in the amount of 177.24 L-ascorbyl-2-monophosphate (AMP). All ingredients were diluted with alpha-cellulose to 1 kg.

[1] Mineral premix (mg kg^{-1} premix): NaF, 2 mg; KI, 0.8 mg; $CoCl_2{\cdot}6\ H_2O$ (1%), 50 mg; $CuSO_4{\cdot}5\ H_2O$, 10 mg; $FeSO_4{\cdot}H_2O$, 80 mg; $ZnSO_4{\cdot}H_2O$, 50 mg; $MnSO_4{\cdot}H_2O$, 60 mg; $MgSO_4{\cdot}7\ H_2O$, 1200 mg; $Ca(H_2PO_3)_2{\cdot}H_2O$, 3000 mg; NaCl, 100 mg. All ingredients were diluted with alpha-cellulose to 1 kg.

2.2. Experimental fish and feeding trial

Caspian trout fingerlings (n = 600) were obtained from coldwater fish research center (CFRC), Tonekabon, Iran. Prior to the experiment, all fish were acclimated to the experimental condition for 3 weeks and fed with the basal diet to deplete possible body reserves of vitamin E. A total of 360 uniform-sized fish (initial average weight of 9.73 ± 0.34 g) were randomly distributed into eighteen 900-L cylindrical fiberglass tanks, resulting in 20 fish in each tank. Each tank was then randomly assigned to one of the three replicates of the six diets. All tanks were provided with a continuous flow of water (approximately 1.0 L min^{-1}) continuously aerated to keep the dissolved oxygen level near saturation (11.3 ± 0.1 mg L^{-1}). During the feeding trial and acclimation period, the water temperature (9.9 ± 0.1 °C) and photoperiod (approximately 14-h light/10-h dark) were maintained at ambient condition. The water pH (7.20 ± 0.28), alkalinity (241.5 ± 6.7 mg L^{-1}), and total ammonia nitrogen (less than 0.05 mg L^{-1}) were also monitored twice a week using a portable multimeter (TPS 90Fl-T, TPS, Brisbane, Australia), except for ammonia which was measured using a portable spectrophotometer (DR 2800, Hach Chemical Co., Loveland, USA). Fish were fed by hand to apparent satiation three times (08:30, 12:30, and 18:30) daily for eight weeks. The amount of diet consumed in each tank was recorded. Care was taken to ensure that no uneaten feed pellets remained in the tanks during feeding.

2.3. Growth measurement and sample collection

At the end of the feeding trial, fish were fasted for 24 h to avoid the inclusion of ingested feed in the results and anesthetized by 200 ppm ground clove, *Syzygium aromaticum*, solution to reduce stress during the measurements. Total weight and body length of all fish were then measured individually to determine growth and nutritional performance indices including survival rate (SR), weight gain ratio (WGR), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), and condition factor (CF). After biometrics, five fish from each

tank were randomly selected for blood collection from the caudal peduncle using 5 mL plastic syringe after body drying with a clean towel from water and mucus. The fish blood obtained from each tank was divided into two groups; the first portion (1 mL) was straightaway transferred to heparin-containing Eppendorf tubes for hematological analysis. The second portion (1.5 mL) was centrifuged at 3000g for 15 min at 4 °C using a Sigma centrifuge (3−30k, Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) and the supernatant was frozen in non-heparinized Eppendorf tubes by liquid nitrogen for further biochemical and immunological analysis. All sera were then kept at −80 °C until use. After obtaining the blood samples, liver was dissected out from the sampled fish, weighed individually, frozen immediately at liquid nitrogen, and stored at −80 °C to calculate the hepatosomatic index (HSI) and vitamin E concentration in the liver. Three additional fish were randomly sampled from each tank and frozen at −80 °C for determining the proximate composition.

2.4. Proximate composition analyses

The moisture, crude protein, crude lipid and ash content in the experimental diets and fish were measured using the official methods of analysis (AOAC, 2000). Moisture content was analyzed by drying the samples in an oven (ED 53, Binder, Tuttlingen, Germany) at 105 °C until the weight became constant. Crude protein was determined by measuring the total nitrogen (N × 6.25) based on the Kjeldahl method after an acid digestion using an auto-Kjeldahl System (FP-528, Leco Instruments, St. Joseph, MI, USA). Crude lipid was estimated following solvent (petroleum ether) extraction for 2–4 h using a Soxhlet system (64826 Supelco, Soxhlet Extraction Apparatus, Sigma-Aldrich, St Louis, MO, USA). Ash content was evaluated following mass loss after combustion of dried samples for 12 h at 550 °C in a muffle furnace (Isuzu, Tokyo, Japan).

For determination of vitamin E concentrations, aliquots of test diets and liver samples (2.00 g) were homogenized for 2 min in 5 mL ethanol and centrifuged at 3000g for 5 min. The supernatant (20 μL) was then passed through a 0.22 syringe filter and subjected to vitamin E analysis by reverse-phase HPLC (LC-40, Shimadzu, Kyoto, Japan) equipped with an octadecyl column (4.6 × 205 mm and 5 μm particle size; GIST C18, Shimadzu, Kyoto, Japan) following the method described by Tangney et al. (1981) with some modification. The mobile phase was methanol which was delivered at a flow rate of 1 mL min^{-1}. The absorbance was recorded by an ultraviolet detector at a wave length of 290 nm.

2.5. Biochemical analyses

Serum superoxide dismutase (SOD), glutathione peroxidase (GPX), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) activities were determined using commercial diagnostic kits (Pars Azmun Co. Ltd., Karaj, Iran) on an automatic biochemistry analyzer (Prestige 24i, Tokyo Boeki Group, Tokyo, Japan) based on the protocols recommended by the manufacturer. One activity unit of SOD was specified as the enzyme quantity necessary to induce a 50% inhibition of nitro blue tetrazolium reduction rate (Beauchamp and Fridovich, 1971). One activity unit of GPX was expressed as the enzyme quantity required to catalyze the oxidation of one μmol min^{-1} NADPH (Flohé and Günzler, 1984). One activity unit of ALP was defined as the enzyme quantity needed to hydrolyze one μmol min^{-1} 4-nitrophenyl phosphate (Ghodrati et al., 2021). One activity unit of AST and ALT was presented as the enzyme quantity that generate 1.0 μmole min^{-1} glutamate and pyruvate at pH 8.0 at 37 °C, respectively (Reitman and Frankel, 1957).

Serum total cholesterol was determined using the method as described by Wybenga et al. (1970). Serum glucose level was spectrophotometrically determined according to the method proposed by Slein (1965). Serum malondialdehyde (MDA) concentration was also measured as thiobarbituric acid reacting substance based on the method of Ohkawa et al. (1979) by using a commercial kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.6. Innate Immune analyses

Serum lysozyme activity (LA) was determined using a turbidimetric method developed by Ellis (1990). Briefly, 175 μL of the *Micrococcus lysodeikticus* solution (0.375 mg mL^{-1} sodium phosphate buffer 0.05 M and pH 2.6) was assorted with 25 μL of the serum sample into the wells of a 96-well plate in triplicate. After rapid mixing, the light absorption was read after 180 s at 670 nm by a spectrophotometer (Biophotometer D30, Eppendorf, Hamburg, Germany). Sodium phosphate buffer was used as blank and results were expressed as the amount of lysozyme causing a decrease in absorbance of 0.001 min^{-1}.

Serum total immunoglobulin (Ig) level was determined based on the method of Siwicki and Anderson (1993). An aliquot of serum sample (0.1 mL) was incubated for 2 h after mixing with an equal volume of 12.0% polyethylene glycol solution (Sigma Chemical Co., St. Louis, MO, USA). The mixture was then centrifuged at 5000g for 15 min at 4 °C using a centrifuge (3−30k, Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) and the supernatant was diluted 30 times with 0.85% NaCl. The difference in protein content of the serum samples before and after polyethylene glycol precipitating treatment was evaluated based on a rapid microprotein determination method (Bradford, 1976) and considered as the Ig content.

Serum alternative complement pathway (ACH50) was evaluated following the method reported by Yano (1992). Rabbit red blood cells (RaRBC) were washed four times in ethylene glycol tetraacetic acid-magnesium-gelatin veronal buffer (EGTA-Mg-GVB) and resuspended to 2 × 10^8 cells mL^{-1} in the same buffer. Individual 100 μL aliquots of a serially diluted serum with EGTA-Mg-GVB buffer was incubated for 120 min at 20 °C after mixing with 50 μL of RaRBC suspension. The hemolytic reaction was stopped by adding 1.575 mL EDTA–GVB buffer containing 10 mM EDTA. The mixture was centrifuged at 1600g for 5 min to assess the relative hemoglobin content in the supernatant by measuring the optical density at 414 nm. A lysis curve was obtained for each sample by plotting percent hemolysis against the amount of serum added (mL) on a log-log scaled graph. The volume of serum complement giving 50% hemolysis (ACH50) was determined and the number of ACP50 U mL^{-1} was calculated for each group.

2.7. Calculations and Statistical analysis

The growth performance and feed utilization were evaluated by calculating WGR, SGR, FCR, PER, CF, SR, and HSI using the following equations:

Weight gain ration (WGR, %) = 100 × [(final weight (g) - initial weight (g)] / initial weight (g)]

Specific growth rate (SGR, % day^{-1}) = 100 × [Ln final weight (g) - Ln initial weight (g)] / days

Feed conversion ratio (FCR) = dry feed intake (g) / wet weight gain (g)

Protein efficiency ratio (PER, %) = 100 × weight gain (g) / protein intake (g)

Condition factor (CF, g cm^{-3}) = 100 × final weight (g) / body length (cm^3)

Survival rate (SR, %) = 100 × (final fish number) / (initial fish number)

Hepatosomatic index (HSI, %)=100×liver weight (g) / body weight (g)

Data analysis was performed by SPSS-19 software. The normality of the data was initially assessed using the Kolmogorov-Smirnov test. Data that were found to be normally distributed were then tested for homogeneity of variance by Levene's test. Orthogonal polynomial contrasts were used to assay the linear and quadratic effect of vitamin E supplementation in Caspian trout at a level of $P < 0.05$ significance. The

quadratic regression model was used for estimating the optimum requirement of vitamin E according to Zeitoun et al. (1976). Results were expressed as mean±SEM.

3. Result

3.1. Fish performance and nutrient utilization

Growth performance and feed efficiency of fish fed diets containing incremental vitamin E levels are given in Table 2. After 8 weeks of feeding trial, the WGR, SGR, and PER were linearly ($P < 0.05$) and quadratically ($P < 0.05$) increased by increasing dietary vitamin E level and reached to the highest values in fish fed with 78.8 mg kg^{-1} vitamin E supplemented diet. Conversely, FCR was linearly ($P < 0.05$) and quadratically ($P < 0.05$) decreased by increasing dietary vitamin E level with the lowest value in fish fed diet supplemented with vitamin E at 78.8 mg kg^{-1}. Fish fed diets supplemented with vitamin E showed a significant linear trend in CF by increment of dietary vitamin E inclusion with the highest value in fish fed diet supplemented with 78.8 mg kg^{-1} vitamin E. SR and HSI were increased linearly ($P < 0.05$) and quadratically ($P < 0.05$) with increase in dietary vitamin E supplementation up to the above level.

3.2. Body composition

The changes in whole body proximate composition of Caspian trout after feeding with different levels of vitamin E diets are presented in Table 3. There was a significantly linear decreasing trend in moisture content of fish fed vitamin E-supplemented diets ($P < 0.05$). In contrast, crude protein and fat level of fish show a significant increasing trend in response to different dietary vitamin E supplementations ($P < 0.05$). However, no significant difference was recorded in ash content of fish fed diets supplemented with different levels of vitamin E at the end of feeding trial ($P > 0.05$). With increasing levels of vitamin E in the diet, the amount of vitamin E in liver tissue linearly and quadratically increased ($P < 0.05$) and reached to the highest value of 56.80 ± 3.19 in fish fed diet supplemented with 137.0 mg kg^{-1} vitamin E (Fig. 1).

3.3. Serum biochemical changes

The effect of graded levels of dietary vitamin E on biochemical characteristics of Caspian trout was shown in Table 4. Vitamin E linearly affected the SOD and ALP activities of fish after an 8-week feeding trial ($p < 0.05$), while GPX activity was affected linearly and quadratically by dietary vitamin E inclusion ($p < 0.05$). The AST and ALT activities were both linearly and quadratically declined by enhancing dietary vitamin E concentration ($P < 0.05$) and reached to the least of 399.00 ± 17.62 U mL^{-1} and 9.67 ± 0.86 U mL^{-1} in fish fed the highest vitamin E concentration, respectively. However, no significant difference was recorded in serum TC of fish among the experimental treatments ($P < 0.05$). The serum concentration of GLC linearly and quadratically reduced as the vitamin E supplementation increased to 35.4 mg kg^{-1} diet ($P < 0.05$), where it enhanced gradually thereafter. Similarly, the MDA content in the serum linearly declined by increasing dietary vitamin E supplementation and reached to the lowest value of 137.67 ± 1.76 μmol L^{-1} in fish fed with the highest dietary vitamin E supplementation ($P < 0.05$).

3.4. Non-specific immune responses

There was a significantly linear ($P < 0.05$) and quadratic ($P < 0.05$) increase in LA activity by feeding the fish with graded level of dietary vitamin E, with the highest activity in fish fed with 137.0 mg kg^{-1} vitamin E supplemented diet (Table 5). The IgM also showed a linear increasing trend by enhancing dietary vitamin S level with the highest value of 13.40 ± 0.53 mg dL^{-1} in fish fed diet supplemented with 137.0 mg kg^{-1} vitamin E. The ACH50 had a similar pattern as IgM content with the highest value of 142.33 ± 2.60 U mL^{-1} in fish fed diet supplemented with 78.8 mg kg^{-1} vitamin E.

3.5. Dietary vitamin E requirement

Based on the curvilinear regression analyses, the best SGR, GLC content, and SOD activity were obtained at dietary vitamin E levels of 79.44, 78.73, and 82.16 mg kg^{-1} diet, respectively (Fig. 2). Accordingly, the optimum dietary vitamin E required for Caspian trout lies in a range of 78.73–82.16 mg kg^{-1} diet.

4. Discussion

Considering the role of nutrition in aquaculture costs (more than 50%), it should be acknowledged that successful fish farming requires the use of complete, efficient and optimal feed to provide all nutritional requirement such as proteins, carbohydrates, lipids, minerals and vitamins for quick and healthy growth of fish (Cho et al., 2005; Mohseni et al., 2013). Results of the present study illustrates that supplementation of vitamin E in the diet is essential for higher survival and better growth of Caspian trout. Fish fed diet unsupplemented with the vitamin E had the lower WGR, SGR, PER, as well as the lower FCR than those fed diets supplemented with different levels of vitamin E. These findings are in accordance to some earlier reports on yellow catfish (Lu et al., 2016), hybrid snakehead (Zhao et al., 2018), great sturgeon (Amlashi et al., 2011), and Japanese eel (Shahkar et al., 2018). The highest mortality of fish fed the basal diet also suggest that dietary vitamin E improves the survival of Caspian trout.

Based on the second-order polynomial regression analysis of SGR, the minimum dietary vitamin E content in Caspian trout is estimated to be 79.44 mg kg^{-1}. This value was close to those reported for some other fish species, such as 78–111 mg kg^{-1} for cobia (Zhou et al., 2013), 61–115 for grouper (Lin and Shiau, 2005), and 100 mg kg^{-1} for common carp (Watanabe et al., 1970), 100–200 mg kg^{-1} for grass carp (Li et al., 2014), and 80.5 mg kg^{-1} for hybrid snakehead (Zhao et al., 2018). However, it is higher than those documented for the other fish species such as 28 mg kg^{-1} for hybrid striped bass (Kocabas and Gatlin Iii,

Table 2
Growth performance and feed utilization of Caspian trout, *Salmo caspius*, fed diet supplemented with different levels of vitamin E for eight weeks*.

		WGR	SGR	FCR	PER	CF	SR	HSI
4.9		130.14 ± 1.71	1.49 ± 0.02	1.81 ± 0.16	0.97 ± 0.02	0.97 ± 0.03	73.33 ± 1.67	1.35 ± 0.01
8.6		142.03 ± 3.27	1.58 ± 0.03	1.55 ± 0.14	1.05 ± 0.01	1.00 ± 0.05	76.67 ± 1.67	1.38 ± 0.02
17.4		162.27 ± 3.30	1.72 ± 0.02	1.31 ± 0.08	1.20 ± 0.01	1.02 ± 0.04	78.33 ± 0.33	1.42 ± 0.02
35.4		201.21 ± 1.73	1.97 ± 0.01	1.13 ± 0.02	1.48 ± 0.02	1.04 ± 0.04	80.00 ± 1.67	1.59 ± 0.03
78.8		204.92 ± 1.01	1.99 ± 0.01	1.05 ± 0.08	1.52 ± 0.01	1.06 ± 0.05	83.33 ± 1.67	1.63 ± 0.01
137.0		180.62 ± 4.98	1.84 ± 0.03	1.28 ± 0.09	1.33 ± 0.01	1.01 ± 0.05	78.33 ± 1.67	1.56 ± 0.04
P-values	Linear	0.000	0.000	0.000	0.000	0.024	0.003	0.000
	Quadratic	0.000	0.000	0.000	0.000	0.061	0.027	0.026

* Values are given as means ± SEM (n = 9); WGR (%), weight gain ration; SGR (% d^{-1}), specific growth rate; FCR, feed conversion ratio; PER (%), protein efficiency ratio; CF (g cm^{-3}), condition factor; SR (%), survival rate; HSI (%), hepatosomatic index.

Table 3
Whole-body proximate composition (g kg^{-1} wet weight) of Caspian trout, *Salmo caspius*, fed diet supplemented with different levels of vitamin E for eight weeks*.

		Moisture	Protein	Lipid	Ash
4.9		699.67 ± 2.03	180.04 ± 1.16	68.43 ± 0.26	19.67 ± 0.88
8.6		697.33 ± 2.91	180.41 ± 1.79	68.67 ± 0.12	19.17 ± 0.49
17.4		694.67 ± 2.20	180.35 ± 0.057	70.60 ± 0.35	19.67 ± 1.20
35.4		694.00 ± 1.53	182.79 ± 1.26	74.27 ± 0.23	19.73 ± 0.85
78.8		691.33 ± 3.53	189.80 ± 0.76	74.90 ± 0.26	21.53 ± 0.66
137.0		686.67 ± 1.20	190.64 ± 0.34	76.50 ± 0.44	21.00 ± 1.15
P-values	Linear	0.001	0.000	0.000	0.094
	Quadratic	0.600	0.825	0.555	0.557

* Values are presented as means ± SEM (n = 9).

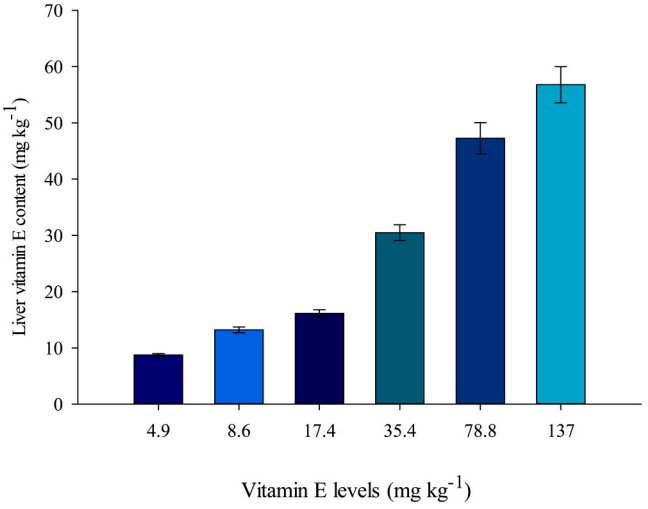

Fig. 1. Liver vitamin E content of Caspian trout, *Salmo caspius*, fed diets supplemented with different levels of vitamin E (mg kg^{-1}) for eight weeks. Error bars represent means ± SEM of three replicates.

1999), 33 mg kg^{-1} for yellow catfish (Lu et al., 2016), 31 mg kg^{-1} for red drum, *Sciaenops ocellatus* (Peng and Gatlin Iii, 2009), and 30 mg kg^{-1} for rainbow trout (Woodall et al., 1964). Some other previous researches has been indicated that weight gain or feed efficiency were not responsive to dietary vitamin E at least in a dose-dependent manner (Chen et al., 2004; Gaylord and Rawles, 1998; Hamre et al., 1997). The discrepancy of minimum vitamin E requirement may be attributed to the fish species, life stage, feeding regime, rearing environment, diet composition, and even method of sample analysis (Hamre, 2011; Lu et al., 2016). It has been reported that elevated dietary lipid content specially in the form of unsaturated fatty acids or low levels of other antioxidants like vitamins C, selenium and astaxanthin can provoke the dietary vitamin E requirement to protect the cell against lipid peroxidation (Hamre, 2011; Puangkaew et al., 2004).

On the other hand, fish fed the diet supplemented with 137.0 mg kg^{-1} vitamin E in the present study possessed lower WGR, SGR, and PER, as well as higher FCR compared to those fed with 78.8 mg kg^{-1} vitamin E. Similarly, high levels of vitamin E depressed growth performance and impaired feed utilization in rainbow trout (Kiron et al., 2004), spotted snakehead (Abdel-Hameid et al., 2012), and yellow catfish (Lu et al., 2016). The growth reduction by feeding with dietary vitamin E at super-optimum levels is most likely explained by the imbalance and accumulation of vitamin E radicals, which may act as prooxidants (Li et al., 2013).

Since the liver is an important store of energy reserves, the HSI could be used as an estimate of nutritional and energy status in fish (Chellappa et al., 1995). Lu et al. (2016) found a significant increase in HSI of yellow catfish when dietary vitamin E level increased from 8.9 to 156.9 mg kg^{-1}. Li et al. (2014) also recognized a lower HSI in grass carp fed the sub-optimal levels of vitamin E. The elevated values in HSI of Caspian trout fed vitamin E deficient diets recorded in this study may be associated with the accumulation of vitamin E along with lipid in the liver (Amlashi et al., 2011). Although the liver proximate composition was not evaluated in the present study, Improved carcass lipid as well as hepatic vitamin E concentration in the fish fed diet supplemented with higher vitamin E further supports the result of HSI. These findings clearly indicate that dietary vitamin E protects the cellular lipids storage from uncontrolled oxidation. However, some studies supported not significant influence of dietary vitamin E levels on HSI (Peng and Gatlin

Table 5
Non-specific immune responses of Caspian trout, *Salmo caspius*, fed diet supplemented with different levels of vitamin E for eight weeks*.

		LA	IgM	ACH50
4.9		50.67 ± 2.03	6.07 ± 0.35	131.67 ± 2.03
8.6		49.00 ± 2.31	5.27 ± 0.46	130.33 ± 2.01
17.4		52.33 ± 3.53	7.60 ± 0.32	133.33 ± 1.76
35.4		60.00 ± 2.08	7.23 ± 0.41	137.00 ± 1.73
78.8		65.00 ± 3.21	12.77 ± 0.35	142.33 ± 2.60
137.0		71.67 ± 2.73	13.40 ± 0.53	140.33 ± 3.18
P-values	Linear	0.000	0.00	0.001
	Quadratic	0.000	0.750	0.779

* Values are presented as means ± SEM (n = 9). LA (U mL^{-1}), lysozyme activity; IgM (mg dL^{-1}), immunoglobulin M; ACH50 (U mL^{-1}), Alternative complement activity.

Table 4
Serum biochemical characteristics of Caspian trout, *Salmo caspius*, fed diet supplemented with different levels of vitamin E for eight weeks*.

		SOD	GPX	ALP	AST	ALT	TC	GLC	MDA
4.9		51.00 ± 1.15	123.67 ± 2.73	788.33 ± 43.88	580.00 ± 19.40	18.67 ± 2.03	187.67 ± 2.60	51.00 ± 1.73	173.00 ± 10.12
8.6		52.33 ± 0.88	126.67 ± 3.48	789.67 ± 63.59	534.33 ± 22.00	16.13 ± 0.58	194.33 ± 6.96	49.67 ± 1.76	161.30 ± 3.75
17.4		53.00 ± 1.73	128.67 ± 4.18	721.00 ± 51.03	473.67 ± 21.67	15.33 ± 0.88	192.00 ± 3.21	45.33 ± 1.45	155.32 ± 1.86
35.4		59.00 ± 1.15	189.67 ± 5.17	664.67 ± 60.40	428.33 ± 18.89	13.67 ± 1.45	176.00 ± 7.23	31.67 ± 0.88	142.33 ± 2.03
78.8		60.00 ± 2.08	146.67 ± 3.71	650.33 ± 77.74	436.33 ± 8.41	13.10 ± 0.95	180.31 ± 9.56	37.67 ± 1.76	140.00 ± 4.58
137.0		56.00 ± 1.14	147.33 ± 4.84`	554.33 ± 23.52	399.00 ± 17.62	9.67 ± 0.86	182.00 ± 2.65	42.67 ± 2.33	137.67 ± 1.76
P-values	Linear	0.001	0.000	0.002	0.000	0.001	0.066	0.000	0.000
	Quadratic	0.076	0.004	0.572	0.445	0.756	0.972	0.001	0.360

* Values are presented as means ± SEM (n = 9). SOD (U L^{-1}), superoxide dismutase; GPX (U L^{-1}), Glutathione peroxidase; ALP (U L^{-1}), alkaline phosphatase; AST (U L^{-1}), aspartate aminotransferase; ALT (U L^{-1}), alanine aminotransferase; TC (mg dL^{-1}), total cholesterol; GLC (mg dL^{-1}), glucose; MDA (μmol L^{-1}), malondialdehyde.

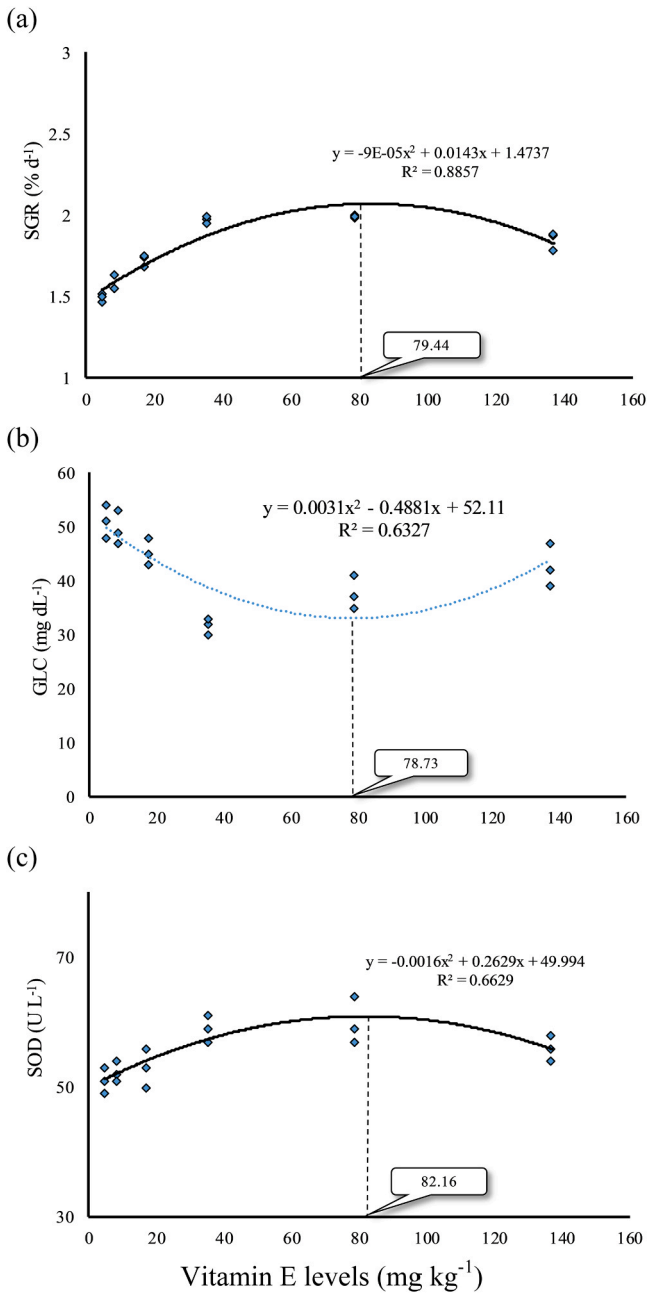

Fig. 2. Second-order polynomial regression analysis for the relationship between specific growth rate (SGR) (a), glucose content (GLC) (b), and superoxide dismutase activity (SOD) (c) against varying levels of dietary vitamin E in Caspian trout, *Salmo caspius*.

Iii, 2009; Zhou et al., 2013).

In the present study, the protein content and crude lipid in whole body were significantly influenced by increasing dietary vitamin E supplementation. Similar results have been stated by Huang et al. (2003) and Sau et al. (2004) who found the reduced whole-body crude protein and lipid in the vitamin E-deficient hybrid tilapia, *Oreochromis niloticus* × *O. aureus*, and rohu fry, respectively. However, diets containing different levels of vitamin E did not significantly affect whole body proximate composition of cobia (Zhou et al., 2013), sea bass, *Dicentrarchus labrax* (Gatta et al., 2000), and Japanese eel (Bae et al., 2013). It has been proven that decreased whole body crude protein followed by increased moisture content in fish fed a vitamin E deficient could be related to the severity of muscular atrophy (Watanabe et al., 1977).

Due to the indispensable and fat-soluble properties of α-tocopherol, liver is the major organ of vitamin E storage in fish (Peng and Gatlin Iii, 2009). Present results showed a linearly and quadratically increase model of α-tocopherol deposition in liver of Caspian trout by increasing the dietary vitamin E supplementation, which is consistent with those reported in other fish species (Bae et al., 2013; Peng et al., 2009; Sau et al., 2004; Zhao et al., 2018). However, no liver saturation of α-tocopherol was obtained in response to dietary vitamin E concentration used in the present study. A number of factor may increase the vitamin E deposition such as dietary levels of oxidized lipid, vitamin C, astaxanthin, selenium, and probably other minerals essential for antioxidant enzymes activity have been shown to influence the dietary vitamin E requirement to a considerable extent (Hamre, 2011; Liu et al., 2019; Peng and Gatlin Iii, 2009; Yi et al., 2018). In grouper, pancreatic α-tocopherol reached to the saturation point by feeding with more than 200 mg kg^{-1} vitamin E (Lin and Shiau, 2005). Accordingly, higher levels of vitamin E supplementation appears to be necessary for liver saturation with vitamin E in Caspian trout.

Dietary vitamin E also demonstrated some relationship with the activities of antioxidant defense system of fish (Li et al., 2014). Under normal physiological conditions, the antioxidant defense of fish prevents the uncontrolled generation of reactive oxygen substances via enzymes like SOD, CAT, and GPX (Trenzado et al., 2009). SOD is a crucial antioxidant enzyme which form the first line defense against the reactive oxygen species by catalyzing the dismutation of superoxide radical into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2) (Rodríguez-Ariza et al., 1993). Fish fed the vitamin E unsppplemented diet in the present study had the lowest SOD activity, while the highest SOD activity was observed in Caspian trout fed diet supplemented with 78.8 mg kg^{-1} vitamin E. Earlier studies have also shown a similar increasing pattern of SOD activity by supplementing the vitamin E in diet of cobia (Zhou et al., 2013), grass carp (Pan et al., 2017), and japenese eel (Shahkar et al., 2018). These results suggest that vitamin E could sufficiently improve the scavenging ability of superoxide onions, partly due to the upregulation in mRNA expression of SOD in the head kidney and spleen of fish (Pan et al., 2017). Based on the regression analysis, the minimum dietary vitamin E requirement for maximizing the SOD activity in Caspian trout was found to be 82.12 mg kg^{-1} diet.

The dismutation product of SOD could be removed by GPx, another pivotal antioxidant enzyme which protect cell membranes by catalyzing the reduction of various hydroperoxides (e.g., H_2O_2) to H_2O via oxidation of reduced glutathione into its disulfide form (Marín-García, 2014). In general, vitamin E prevents the peroxides synthesis, while GPX eliminates the presynthesized peroxides (Zhao et al., 2018). There was a positive correlation between GPX activity in serum and dietary vitamin E in Caspian trout. This phenomenon is also found in grass carp (Li et al., 2014), hybrid snakehead (Zhao et al., 2018), and mirror carp, *Cyprinus carpio* (Wang et al., 2019), which support the hypothesis that vitamin E is involved in the GPX activity, probably by lowering the concentration of lipid peroxides in the tissues, thereby sparing the need for GPX (Yang et al., 1976).

To further understand the effect of dietary vitamin E on antioxidant ability of Caspian trout, MDA content was also evaluated in this study. MDA is the final product of lipid peroxidation which has been suggested as a biomarker of endogenous oxidative damage in fish (Faizan et al., 2014). In the present study, the serum MDA content was found to be highest in fish fed the basal diet and declined by further inoculation of dietary vitamin E to the lowest value of 137.67 ± 1.76 μmol L^{-1} in fish fed the highest dietary vitamin E level. In accordance, Zhou et al. (2013) illustrated that hepatic MDA values of cobia was inversely related to the dietary vitamin E levels. decreasing MDA content and increasing SOD activity has been also reported in muscle of tilapia (Wu et al., 2017). These findings all demonstrated that vitamin E deficiency causes lipid oxidation in fish mainly due to the formation of considerable amounts of oxidative radicals, while dietary vitamin E supplementation could reduce fish lipid oxidation, probably as a direct consequence of reactive oxygen species scavenging by α-tocopherol or indirect effect driven by

the increase in the activity of antioxidant enzymes such as SOD and GPX.

The functional role of vitamin E is also known to be associated with the serum concentration of hepatic enzymes such as ALP, AST, and ALT (Abdulazeez et al., 2019). ALP is a leading biomarker of hepatobiliary injury that catalyze the hydrolysis of organic phosphate esters (Aulbach and Amuzie, 2017), while levels of plasma AST and ALT activities could also give information about liver condition (Ghodrati et al., 2021). Li et al. (2014) reported decreased serum ALT activity of grass carp with the supplementation of vitamin E. In addition, serum AST and ALT activities were both decreased in hybrid snakehead with the supplementation of vitamin E (Zhao et al., 2018). Findings of the present study have been also demonstrated that serum ALP, AST, and ALT activities were decreased by increment of dietary vitamin E supplementation, representing that vitamin E is required for a better function in the liver of Caspian trout due to the antioxidant function of α-tocopherol which stabilizes biological membranes and avoids hepatic cell damage (Shahkar et al., 2018; Xiaoyuan, 2000).

The absorption of nutrients like glucose has been shown to be associated with the sodium uptake via the activity of Na^+/K^+-ATPase, which its activity indirectly reflect the cell energy metabolism (He et al., 2017). In agreement with the results obtained in Japanese eel (Shahkar et al., 2018), dietary supplementation of vitamin E in the present study decreased the serum glucose concentration of Caspian trout. This may be contributed to the higher activity of the Na^+/K^+-ATPase, which promotes the somatic cells to absorb more glucose and lower its serum concentration. However, further investigation is required to fully understand the causal mechanisms. However, curvilinear regression analysis estimated the minimum dietary requirement for vitamin E to be 78.73 mg kg^{-1} diet for Caspian trout based on the serum glucose level.

The innate immune system is the first line of defense mechanism against a broad spectrum of pathogens and is more vital for fish compared to the other vertebrates (Rebl and Goldammer, 2018). Lysozyme is a mucolytic enzyme of innate immune system with leukocytic origin that involved in the hydrolysis of the β-(1, 4) linked glycoside bonds of bacterial cell wall peptidoglyscans causing its protective roles against wide range of microbial invasions (Saurabh and Sahoo, 2008). In the current research, lysozyme activity of Caspian trout was amplified by increasing dietary vitamin E concentration. Dietary supplementation of vitamin E has been also shown to reinforce serum lysozyme activities in grass carp (Pan et al., 2017) and cobia (Zhou et al., 2013). Yildirim-Aksoy et al. (2009) presented higher serum lysozyme activity of channel catfish fed the diet supplemented with highly unsaturated fatty acids. Accordingly, increased lysozyme activity of Caspian trout can be attributed to the protective effect of vitamin E against oxidation of unsaturated fatty acids that, in turn, strengthened the immune system of fish.

The ACH50 is another constitutively active part of the innate immune system with a type of cascade reaction that plays an essential role in alerting the host of the presence of potential pathogens (Merle et al., 2015). Despite the essentiality of vitamin E for certain serum protein level, its role in complement synthesis and function is not fully understood. In this study, ACH50 of fish was enhanced by elevating the dietary vitamin E level. Lin and Shiau (2005) delineate lower ACH50 activity in grouper fed the basal diet, and highest in those fed diet supplemented with the maximum dietary vitamin E supplementation. Pan et al. (2017) found declined ACP in grass carp fed diet deficient in α-tocopherol. Any reduction in n-3 highly unsaturated fatty acid may lead to inhibition in complement protein synthesis by disturbing the membrane of immune cells like leukocytes (Montero et al., 1998), which may explain the higher innate immunity responses of Caspian trout fed higher vitamin E compared to those fed the deficient diet. Further research in this area can clarify the dimensions of the issue.

IgM is a high molecular weight protein secreted by B lymphocytes that function as the most important antibody in teleost fish by activating complement system after binding to the antigens (Vallejos-Vidal et al., 2016). Vitamin E is clearly necessary for optimum immune function but its role in complement synthesis and function is not fully understood. There is currently limited research about the effect of vitamin E on immunoglobulin synthesis in fish. Dietary supplementation of vitamin E has been ameliorated primary and secondary antibody responses in broiler chicken (Dalia et al., 2018). A significant increase in IgM content was also found with the addition of 95 mg kg^{-1} vitamin E in northern whiting, *Sillago sihama* (Dalia et al., 2018). In close agreement with the above results, findings of the present study displayed that supplemental dietary vitamin E enhanced the IgM content of Caspian trout. Although the effect of vitamin E on IgM synthesis is somewhat unclear, vitamin E may act as a immunostimulant agent because of its up-regulating capacity on interleukin-1 production with subsequent effects on activation of T and B cells (Stabel et al., 1992). Systematic attempts are required to further examine possible correlation between dietary vitamin E and the immune functions in fish.

In conclusion, findings of the current study revealed that dietary vitamin E inclusion significantly thrives growth performance, improves feed utilization, changes proximate composition, enhances liver vitamin E concentration, improves some immune responses, and induce some antioxidant responses in Caspian trout. Analysis by the polynomial regression of SGR, GLC content, and SOD activity against varying levels of dietary vitamin E revealed that the optimum dietary vitamin E requirements in Caspian trout were 79.44, 78.73, and 82.16 mg kg^{-1}, respectively.

CRediT authorship contribution statement

Morteza Saheli: Formal analysis, Investigation. **Houman Rajabi Islami**: Conceptualization, Supervision. **Mahmoud Mohseni**: Project administration, Data curation. **Mehdi Soltani**: Validation, Data curation, Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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