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Original Research Article

The role of vitamin E in polyunsaturated fatty acid synthesis and alleviating endoplasmic reticulum stress in sub-adult grass carp (*Ctenopharyngodon idella*)

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ABSTRACT

Vitamin E (VE) is an essential lipid-soluble vitamin that improves the fish flesh quality. However, the underlying molecular mechanisms remain unclear. This study aimed to investigate the effects of VE on growth performance and flesh quality in sub-adult grass carp (*Ctenopharyngodon idella*). A total of 450 fish (713.53 ± 1.50 g) were randomly divided into six treatment groups (three replicates per treatment) and fed for nine weeks with different experimental diets (dietary lipid 47.8 g/kg) that contained different levels of VE (5.44, 52.07, 96.85, 141.71, 185.66, and 230.12 mg/kg diet, supplemented as dl-α-tocopherol acetate). Notably, the treatment groups that were fed with dietary VE ranging from 52.07 to 230.12 mg/kg diet showed improvement in the percent weight gain, special growth rate, and feed efficiency of grass carp. Moreover, the treatment groups supplemented with dietary VE level of 141.71, 185.66, and 230.12 mg/kg diet showed enhancement in crude protein, lipid, and α-tocopherol contents in the muscle, and the dietary levels of VE ranging from 52.07 to 141.71 mg/kg diet improved muscle pH_{24h} and shear force but reduced muscle cooking loss in grass carp. Furthermore, appropriate levels of VE (52.07 to 96.85 mg/kg diet) increased the muscle polyunsaturated fatty acid content in grass carp. Dietary VE also increased the mRNA levels of fatty acid synthesis-related genes, including *fas*, *scd-1*, *fad*, *elovl*, *srebp1*, *pparγ*, and *lxrα*, and up-regulated the expression of SREBP-1 protein. However, dietary VE decreased the expression of fatty acid decomposition-related genes, including *hsl*, *cpt1*, *acox1*, and *pparα*, and endoplasmic reticulum stress-related genes, including *perk*, *ire1*, *atf6*, *eif2α*, *atf4*, *xbp1*, *chop*, and *grp78*, and down-regulated the expression of p-PERK, p-IRE1, ATF6, and GRP78 proteins. In conclusion, dietary VE increased muscle fatty acid synthesis, which may be partly associated with the alleviation of endoplasmic reticulum stress, and ultimately improves fish flesh quality. Moreover, the VE requirements for sub-adult grass carp (713.53 to 1590.40 g) were estimated to be 124.9 and 122.73 mg/kg diet based on percentage weight gain and muscle shear force, respectively.

1. Introduction

Fish are an important source of animal protein and unsaturated fatty acids (UFA), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) for human beings. Both EPA and DHA have been found to prevent cardiovascular diseases and hypertension (Ruyter et al., 2022; Xie et al., 2022). The composition of muscle UFA in fish is affected by dietary factors. Previous studies

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have shown that different sources of dietary lipids alter the composition of polyunsaturated fatty acids (PUFA) in the muscles of hybrid striped bass (*Morone saxatilis* × *M. chrysops*), rainbow trout (*Oncorhynchus mykiss*), and yellow perch (*Perca flavescens*) (Baron et al., 2013; Bharadwaj et al., 2010; Twibell et al., 2001). Vitamin E (VE), which includes tocopherols and tocotrienols, is a fat-soluble nutrient (Peh et al., 2016). Dietary VE has been shown to notably increase the PUFA content in raw and cooked golden pompano (*Trachinotus ovatus*) (Zhang et al., 2021). A previous study has reported that long-chain polyunsaturated fatty acid (LC-PUFA) synthesis is greater in freshwater fish than in marine fish (Sun et al., 2020). However, there have been limited studies on the effect of VE on PUFA content in the muscles of freshwater fish, and this requires further investigation.

Fatty acid composition is closely related to its synthesis, transport, and catabolism. Transporter proteins (such as fatty acid transporter [CD36] and fatty acid binding protein [FABP]), lipogenic enzymes (such as fatty acid synthase [FAS] and acetyl-CoA carboxylase [ACC]), and lipolytic enzymes (such as adipose triglyceride lipase [ATGL], hormone-sensitive lipase [HSL], and acyl-coenzyme A oxidase [ACOX]) are involved in fatty acid metabolism (Febbraio et al., 2001; Lampidonis et al., 2011; Yao et al., 2023). Moreover, fatty acid desaturase (FAD) and fatty acid elongase (ELOVL) also play vital roles in LC-PUFA synthesis (Xie et al., 2021). In addition, peroxisome proliferator-activated receptor (PPAR), liver X receptor (LXR), and sterol regulatory element-binding protein 1 (SREBP1) are important transcription factors that regulate the expression of fatty acid metabolism-related genes (Kim and Spiegelman, 1996; Lee et al., 2003; Xiao et al., 2010). Despite several reports published on studying the effect of dietary VE on fatty acid metabolism in fish liver, to date, there has been no study reporting the effect of VE on fatty acid metabolism in fish muscle. Dietary VE increased the mRNA levels of *HSL* and decreased the mRNA levels of carnitine palmitoyl transferase-1 (*CPT1*), *PPARγ*, lipoprotein lipase, and *FAS* in the liver of hybrid grouper (♀ *Epinephelus fuscoguttatus* × ♂ *E. lanceolatus*) (Liang et al., 2021). In golden pompano, dietary VE decreased the gene expression of *FAS*, *CPT1*, and *PPARα*, but increased the expression of *FABP*, *FAD*, and *ELOVL* in the liver (Zhang et al., 2021). However, there may be some differences between the fatty acid metabolism in muscle and liver tissues. Lipases are most highly expressed in the liver, whereas FAD and ELOVL are most highly expressed in the skeletal muscle of gilthead sea bream (*Sparus aurata* L.) (Benedito-Palos et al., 2014). Therefore, the effect of VE on muscle fatty acid metabolism may differ from that on the liver, which requires further investigation.

The endoplasmic reticulum (ER) plays a major role in fatty acid synthesis, particularly PUFA. ER stress occurs when unfolded proteins accumulate inside the ER, leading to abnormal fatty acid synthesis (Basseri and Austin, 2012; Scriven et al., 2007). A previous study found that ER stress induced by copper leads to fatty acid metabolism disorders in the liver of yellow catfish (*Pelteobagrus fulvidraco*) (Song et al., 2016). During ER stress, the body triggers an unfolded protein response (UPR) signaling pathway to relieve the stress. UPR is predominantly controlled by the activating transcription factor 6 (ATF6), protein kinase R-like ER kinase (PERK), and inositol-requiring enzyme 1 (IRE1) (Almanza et al., 2019). Previous studies reported that apoptin-induced ER stress increased the expression of IRE1α protein and decreased the mRNA levels of *ACC*, *FAS*, and stearoyl-coenzyme A desaturase-1 (*SCD-1*) in HepG2 cells (Zhu et al., 2021). However, whether VE regulates muscle fatty acid synthesis by relieving ER stress has not yet been reported. Reactive oxygen species (ROS) are important factors that induce ER stress (Almanza et al., 2019). VE is a potent lipid-soluble antioxidant (Peh et al., 2016) that has been reported to reduce ROS content in the muscle of zebrafish (*Danio rerio*) (Liu et al., 2020). In human melanoma cell lines, δ-vitamin E tocotrienols are known to activate UPR (Montagnani Marelli et al., 2016). Thus, VE may regulate ER stress; however, this requires further investigation.

In the current study, we investigated the effects of VE on the growth performance and flesh quality (especially fatty acid composition) in sub-adult grass carp and the possible underlying mechanisms. Furthermore, we investigated, for the first time, the relationship between VE and muscle ER to provide theoretical support for the intrinsic mechanisms by which VE regulates fatty acid synthesis (especially PUFA) in fish. Finally, we estimated the VE requirements of the sub-adult grass carp based on different indicators that could be used as production guidance to improve the development of grass carp culture.

2. Materials and methods

2.1. Animal ethics statement

The Animal Care Committee of Sichuan Agricultural University approved the experimental procedures. The guidelines for laboratory animal care and use of the Animal Nutrition Institute, Sichuan Agricultural University (permit no. YK-2020214011) were strictly followed.

2.2. Animal management and experimental diets

The basal diet, referred to as the semi-purified diet used by Jiang et al. (2020) and Pan et al. (2017), is shown in Table 1. The fatty acid composition of the basic diet is shown in Table 2. Six experimental diets were formulated by supplementing the basal diet with graded

Table 1
Ingredients and nutrient composition of the basal diet (as-fed basis, g/kg).

Ingredients	Content	Nutrients content	Content
Fish meal	20.00	Crude protein [5]	260.00
Casein	100.00	Crude lipid [5]	47.80
gelatin	80.00	Moisture [5]	112.42
Soybean protein concentrate	140.70	Ash [5]	24.00
Fish oil	26.90	Crude fiber [6]	71.70
Soybean oil	16.90	Nitrogen free extract [7]	484.08
α-Starch	280.00		
Corn starch	206.15		
Cellulose	65.00		
Vitamin E free vitamin premix [1]	10.00		
Mineral premix [2]	10.00		
Choline chloride premix [3]	20.00		
$Ca(H_2PO_4)_2$	11.20		
DL-Met	0.40		
L-Trp	0.90		
L-Thr	1.70		
Butylated hydroxyanisole	0.15		
Vitamin E premix [4]	10.00		

[1] Per kilogram of vitamin premix: retinyl acetate (1,000,000 IU/g), 0.193 g; vitamin D_3 (500,000 IU/g), 0.204 g; vitamin K_3 (50%), 0.38 g; thiamine nitrate (98%), 0.1137 g; riboflavin (80%), 0.731 g; vitamin B_6 (98%), 0.452 g; calcium-D-pantothenate (98%), 4.203 g; niacin (99%), 3.44 g; meso-inositol (97%), 28.5 g; vitamin B_{12} (1%), 0.94 g; D-biotin (2%), 1.05 g; folic acid (95%), 0.168 g; vitamin C acetate (95%), 9.77 g. All ingredients were diluted with corn starch to 1 kg.

[2] Per kilogram of mineral premix: $MnSO_4 \cdot H_2O$ (31.8% Mn), 2.66 g; $MgSO_4 \cdot H_2O$ (15.0% Mg), 256.79 g; $FeSO_4 \cdot H_2O$ (30.0% Fe), 12.61 g; $ZnSO_4 \cdot H_2O$ (34.5% Zn), 8.87 g; $CuSO_4 \cdot 5H_2O$ (25.1% Cu), 0.95 g; Ca $(IO_3)_2$ (3.2% I), 1.56 g; yeast selenium (0.2% Se), 13.65 g. All ingredients were diluted with corn starch to 1 kg.

[3] Choline chloride was diluted with corn starch to 1 kg.

[4] The added form of vitamin E is dl-α-tocopherol acetate. Vitamin E premix was diluted with cellulose to 1 kg.

[5] Crude protein, crude lipid, moisture, and ash contents were measured value.

[6] Crude fiber was calculated by NRC (2011) contents.

[7] Nitrogen free extract was calculated by the formula: 1000 − (crude protein + crude lipid + moisture + ash + crude fiber) (g/kg).

Table 2
Fatty acid composition of the basic diet (% total fatty acids).

Fatty acids	Content
C14:0	5.26
C15:0	0.13
C16:0	11.76
C17:0	0.25
C18:0	4.97
C20:0	0.14
C22:0	0.12
C23:0	0.11
C14:1	0.04
C16:1	1.34
C17:1	0.05
C18:1n9t	0.28
C18:1n9c	13.31
C20:1n-9	0.02
C22:1n-9	0.03
C18:3n-3	19.47
C20:3n-3	0.03
C20:5n-3	3.56
C22:6n-3	2.98
C18:2n6c	21.38
C18:3n-6	1.46
C20:3n-6	0.01
∑SFA	22.74
∑UFA	77.26
∑MUFA	28.37
∑PUFA	48.89
∑n3 (PUFA)	26.04
∑n6 (PUFA)	22.85

SFA = saturated fatty acids; UFA = unsaturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.
∑n3 (PUFA) = C18:3n-3 + C20:3n-3 + C20:5n-3 + C22:6n-3.
∑n6 (PUFA) = C18:2n6c + C18:3n-6 + C20:3n-6.

doses of VE (dl-α-tocopherol acetate): 0 (unsupplemented control), 45, 90, 135, 180, and 225 mg/kg. The final VE contents in the diets were determined by the Guangzhou Huibiao Testing Technology Center (Guangzhou, China) according to *Determination of vitamin E in feeds* (China National Standard, 2008). The dietary VE in different experimental diets were found to be 5.44 (un-supplemented control), 52.07, 96.85, 141.71, 185.66, and 230.12 mg/kg (named VE5.44, VE52.07, VE96.85, VE141.71, VE185.66, and VE230.12), respectively. The diets were prepared using the method described by Mai et al. (2009). Briefly, the ingredients were ground into a fine powder and collected after passing through a 300 μm sieve. Oil and water were added to the premixed dry material and mixed evenly. The mixture was squeezed through an extruder with a mold and air-dried at room temperature (25 ± 5 °C). According to Wang et al. (2016), the diets were broken up, sifted into pellets, and stored at −20 °C.

2.3. Feeding trial

All fish were purchased from Deyang, China. They were acclimated for four weeks under experimental conditions, and then fed with 5.44 mg VE/kg diet for two weeks. Thereafter, a total of 450 fish (initial average weight of 713.53 ± 1.50 g) were randomly assigned into 18 experimental net cages (1.4 m × 1.4 m × 1.4 m) (25 fish in each cage). The experiment was divided into six treatment groups (25 fish per group, three repetitions per treatment). All net cages were located in outdoor freshwater ponds, and microporous aeration was used throughout the experiment. Each net cage was allocated a 100-cm diameter disc with 1 mm gauze at the bottom to collect the uneaten feed. The average water temperature, pH, dissolved oxygen, and total ammonia were 28 ± 3 °C, 7.0 ± 0.3, ≥6.0 mg/L, and < 0.05 mg/L, respectively. During the experiment, 20% of the water was exchanged daily. Water quality was measured every three days using a multiparameter water quality sonde (YSI Inc., Yellow Springs, OH, USA). Fish were fed with the experimental diets four times daily at 07:00, 11:00, 15:00, and 19:00. After 30 min of feeding, the uneaten feed was collected, dried, and weighed to calculate feed intake (FI) as described by Xue et al. (2004). All fish were subjected to natural light conditions, which consisted of approximately 12 h/12 h light and darkness.

2.4. Sample collection

After the nine-week feeding trial, the fish in each net cage were fasted for 24 h and anesthetized with benzocaine (50 mg/L) (Sigma-Aldrich, USA). The fish were then counted and weighed to calculate final body weight (FBW), percent weight gain (PWG), special growth rate (SGR), and feed efficiency (FE). Three fish per cage were randomly chosen and used to calculate the condition factor (CF), viscerosomatic index (VSI), hepatosomatic index (HSI), and slaughter rate. Blood samples were taken from the caudal vein using syringes and then centrifuged at 1700 × *g* at 4 °C for 10 min, and the serum samples were obtained and stored at −20 °C for subsequent analysis of biochemical parameters. The fish were dissected, weighed after removing the head, tail, and fins, and then the left side muscle without skin (the trunk musculature cross-section at the level before and after the dorsal fin) was collected, immediately stored in liquid nitrogen, and then stored at −80 °C. The remaining carcasses were cooked until the remaining muscle could be removed from the fish bone, and the bone was weighed to calculate meat yield (Wu et al., 2022). In addition, three fish per treatment were randomly selected, 1 cm^3 of left side muscle was cut and preserved in 4% paraformaldehyde for muscle histological analysis.

2.5. Sample analysis

2.5.1. Muscle physicochemical indices

Muscle lactate (Kit No. A019-2-1) and hydroxyproline contents (Kit No. A030-2-1) were analyzed using kits obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). For cooking loss analysis, the meat samples were weighed, packaged in sealed plastic bags, and cooked in a water bath at 70 °C for 20 min. After cooking, samples were cooled to room temperature (25 ± 5 °C) with tap water and weighed again. The cooking loss was determined by comparing the percentage of weight of meat samples before and after cooking. The samples (cut into 1.5 cm wide and 1.0 cm deep) were then placed in a shear box (Instron, model 4411, Kramer shear box) to measure the relative shear force (Brinker and Reiter, 2011). The cutting direction of the blade was kept perpendicular to the muscle fiber. The shear force value was calculated as the average of the peak force measurements on each sample in newton (N). Muscle pH was measured at 24 h postmortem using a calibrated pH probe (Testo 205 pH meter; Testo AG, Lenzkirch, Germany).

2.5.2. Muscle nutrition composition

Moisture content was measured by drying the samples at 105 °C to a constant weight in a ventilation drying oven. The crude protein and lipid content were measured using the Kjeldahl method (N × 6.25) and petroleum ether extraction, respectively. The fatty acid composition of the muscle was measured using gas chromatography. Muscle lipids were extracted using the chloroform-methanol method (Folch et al., 1957). After saponification with 0.5 mol/L methanolic potassium hydroxide, fatty acids were methylated using a 14% boron trifluoride-methanol solution, as described by Zengin et al. (2013). The samples were loaded into GC-2010 Plus gas chromatography system (Shimadzu, Co., Ltd., Kyoto, Japan), which was equipped with an SP-2560 (100 m × 0.25 mm inside diameter and 0.2 μm film thickness) capillary column and

flame ionization detector. The detector and injector temperatures were set at 250 °C. The column temperature program was as follows: 5 min at 140 °C, followed by a temperature increase of 8 °C/min to 200 °C, from 200 to 220 °C at 2 °C/min, and from 220 to 240 °C at 5 °C/min. Nitrogen was used as the carrier gas and was maintained at a 1.8 mL/min rate. The injected sample volume was 1 μL. A 37-fatty acid methyl ester mix (Sigma-Aldrich, St. Louis, MO, USA) was used as an external standard, and the results were expressed as percentages of total fatty acids. The VE content in the muscle was determined by the Guangzhou Huibiao Testing Technology Center (Guangzhou, China) according to *National Standard for Safety Determination of Vitamin A, D, and E in food* (China National Standard, 2016).

2.5.3. Serum biochemical parameters, muscle fatty acid metabolism indices, and antioxidant status

Low-density lipoprotein cholesterol (LDL-C) (Kit No. A113-1-1), high-density lipoprotein cholesterol (HDL-C) (Kit No. A112-1-1), total cholesterol (TC) (Kit No. A111-1-1), and triglyceride (TG) (Kit No. A110-1-1) levels in the serum were analyzed using kits from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The SCD-1 (Kit No. YJ660716) and ACC (Kit No. YJ503918) in the muscle were analyzed using enzyme linked immunosorbent assay kits from Shanghai Enzyme-Linked Biotechnology Co., Ltd. (Shanghai, China).

The muscle samples were homogenized with saline at a ratio of 1:9 (wt/vol), the supernatant was then collected by centrifugation at 1700 × *g* at 4 °C for 10 min and used for parameters analysis. Malondialdehyde (MDA) (Kit No. A003-1) and glutathione S-transferase (GST) (Kit No. A004-1), total superoxide dismutase (T-SOD) (Kit No. A001-1), glutathione peroxidase (GPx) (Kit No. A005-1), and protein carbonyl (PC) (Kit No. A087-1) were analyzed using kits from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). ROS (Kit No. S0033S) were analyzed using a kit from Beyotime Biotechnology (Shanghai, China).

2.6. Histopathological analysis

The muscle samples were fixed with paraformaldehyde (4% concentration), then embedded in paraffin, dehydrated with ethanol, and sectioned (5 μm thin). Sections were stained with Oil Red O and light microscopy images were captured using Nikon TS100 microscope (Tokyo, Japan). Percentage areas were analyzed using ImageJ (National Institute of Health, Bethesda, MD, USA).

2.7. Real-time quantitative PCR

Total RNA was isolated from muscle tissues using RNAios Plus (TaKaRa, Japan), as previously described (Deng et al., 2016). The integrity and purity of the total RNA were evaluated by 1.5% agarose gel electrophoresis and spectrophotometric analysis (A260/280). Subsequently, the RNA of each sample was reverse transcribed using PrimeScript RT reagent Kit with gDNA Eraser (Takara, Japan). Real-time qPCR was performed using SYBR (Aidlab Biotechnologies Co., Ltd.). Primers for the genes were designed according to sequences in GenBank. The real-time quantitative PCR primer sequences are shown in Table S1. β-Actin was used as the reference gene. The relative gene expression levels were calculated by using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

2.8. Western blot

Primary antibodies against PPARα (Cat. No. A18252, 1:1000), PPARγ (Cat. No. A11183, 1:1000), SREBP1 (Cat. No. A15586, 1:1000), and ATF6 (Cat. No. A0202, 1:1000) were purchased from AB clonal Technology (Wuhan, China). The glucose regulatory protein 78 (GRP78) (Cat. No. AF5366, 1:1000), p-PERK (Cat. No. AF4499, 1:1000; Ser1096), and p-IRE1 (Cat. No. AF7150, 1:1000; Ser724) were purchased from Affinity BioReagents (Golden, Colo, USA). HRP Goat Anti-Rabbit IgG (Cat. No. AS014, 1:2000) from ABclonal Technology (Wuhan, China) was used as the secondary antibody. β-Actin (Cat. No. AF7018, 1:1000) was used as the control protein and was purchased from Affinity BioReagents (Golden, Colo, USA). These antibodies were checked and cross-reacted with grass carp proteins of interest.

Western blot was performed as described by Yang et al. (2014). Tissue protein extraction was performed using radio immunoprecipitation assay lysis buffer. The proteins were separated on a 10% sodium dodecyl sulfate-glycine polyacrylamide gel, transferred to methanol-activated polyvinylidene fluoride membranes, blocked with blocking solution for 1 h, and washed with Tris-buffered saline containing 0.05% Tween-20 (TBST). Membranes were incubated with primary antibodies overnight at 4 °C, washed with TBST, and then incubated with secondary antibodies for 1 h at room temperature (25 ± 5 °C). The intensities of the protein bands were quantified using ImageJ software (National Institute of Health, Bethesda, MD, USA). The amount of target protein was normalized by β-actin.

2.9. Calculation of growth performance and statistical analysis

PWG, FE, CF, and VSI were computed using standard formulas based on our previous study (Wu et al., 2022).

$$\text{FI (\%/day)} = [(\text{Feed intake for 9 weeks experiment, g/fish})/63\ \text{days}]/\{[(\text{FBW, g/fish}) + (\text{Initial body weight IBW, g/fish})] \times 2\},$$

$$\text{PWG (\%)} = \{[(\text{FBW, g/fish}) - (\text{IBW, g/fish})]/(\text{IBW, g/fish})\} \times 100,$$

$$\text{FE (\%)} = [(\text{FBW, g/fish}) - (\text{IBW, g/fish})]/(\text{FI, g/fish}),$$

$$\text{SGR (\%)} = \{[\ln(\text{FBW, g/fish}) - \ln(\text{IBW, g/fish})]/63\ \text{days}\} \times 100,$$

$$\text{CF (g/cm}^3\text{)} = [(\text{FBW, g/fish})/(\text{Body length}^3\text{, cm}^3\text{/fish})] \times 100,$$

$$\text{VSI (\%)} = [(\text{Viscera weight, g/fish})/(\text{FBW, g/fish})] \times 100,$$

$$\text{HSI (\%)} = [(\text{Hepatopancreas weight, g/fish})/(\text{FBW, g/fish})] \times 100,$$

$$\text{Slaughter rate (\%)} = [(\text{Carcass weight, g/fish})/(\text{FBW, g/fish})] \times 100,$$

$$\text{Meat yield (\%)} = \{[(\text{Carcass weight without head, tail, and fins, g/fish}) - (\text{fishbone weight, g/fish})]/(\text{FBW, g/fish})\} \times 100,$$

where IBW means initial body weight.

Results are presented as the mean ± SD. One-way analysis of variance (ANOVA) was performed using SPSS 27.0, with Duncan's multiple-range test. Significant differences among treatments at $P < 0.05$ have been indicated using different letters. In addition, the VE requirement of sub-adult grass carp was evaluated using quadratic regression analysis.

3. Results

3.1. Growth performance of sub-adult grass carp

As shown in Table 3, FBW, PWG, FE, FI, SGR, VSI, HSI, and CF initially increased and then decreased with increasing dietary VE

Table 3
Effects of vitamin E on growth performance, visceral index, and slaughter rate of sub-adult grass carp.

Item	Dietary vitamin E levels, mg/kg						*P*-value
	5.44	52.07	96.85	141.71	185.66	230.12	
IBW, g/fish [1]	713.33 ± 0.46	712.80 ± 2.12	713.60 ± 0.80	714.13 ± 1.85	713.60 ± 1.60	713.73 ± 2.54	0.956
FBW, g/fish [1]	1243.47 ± 3.78^{a}	1391.20 ± 8.43^{c}	1590.40 ± 15.98^{f}	1510.93 ± 7.89^{e}	1424.27 ± 13.91^{d}	1329.87 ± 19.67^{b}	<0.01
FI, %/day [1]	1.88 ± 0.002^{a}	2.03 ± 0.007^{c}	2.08 ± 0.015^{d}	2.07 ± 0.010^{d}	2.01 ± 0.014^{c}	1.92 ± 0.023^{b}	<0.01
FE, % [1]	0.46 ± 0.003^{a}	0.50 ± 0.004^{b}	0.58 ± 0.01^{e}	0.55 ± 0.01^{d}	0.52 ± 0.01^{c}	0.50 ± 0.01^{b}	<0.01
PWG, % [1]	74.32 ± 0.57^{a}	95.17 ± 0.79^{c}	122.87 ± 2.23^{f}	111.58 ± 1.01^{e}	99.59 ± 1.53^{d}	86.32 ± 2.24^{b}	<0.01
SGR, %/day [1]	0.88 ± 0.01^{a}	1.06 ± 0.01^{c}	1.27 ± 0.02^{f}	1.19 ± 0.01^{e}	1.10 ± 0.01^{d}	0.99 ± 0.02^{b}	<0.01
CF, g/cm^3 [2]	1.77 ± 0.03^{a}	1.86 ± 0.05^{bc}	1.88 ± 0.02^{bc}	1.91 ± 0.02^{c}	1.89 ± 0.03^{bc}	1.80 ± 0.07^{ab}	0.020
VSI, % [2]	8.03 ± 0.48^{a}	9.12 ± 0.36^{b}	9.59 ± 0.70^{b}	11.05 ± 0.84^{c}	9.43 ± 0.54^{b}	9.35 ± 0.46^{b}	<0.01
HSI, % [2]	1.73 ± 0.10^{a}	1.82 ± 0.08^{a}	1.89 ± 0.08^{ab}	2.08 ± 0.15^{b}	1.93 ± 0.12^{ab}	1.93 ± 0.17^{ab}	0.033
Slaughter rate, % [2]	65.17 ± 0.83	65.06 ± 0.43	64.94 ± 0.70	64.27 ± 1.17	65.05 ± 0.96	64.43 ± 0.27	0.471

IBW = initial body weight; FBW = final body weight; FI = feed intake; FE = feed efficiency; PWG = percent weight gain; SGR = special growth rat; CF = condition factor; VSI = viscerosomatic index; HSI = hepatosomatic index.
Means without a common letter significantly differ ($P < 0.05$).
[1] Results are represented as the mean ± SD. The data are the means of three replicates of 25 fish in each group ($n = 3$).
[2] Results are represented as the mean ± SD. The data are the means of three replicates of three fishes per replicate ($n = 3$).

levels. The highest FBW, PWG, FE, FI, and SGR were found in grass carp fed with 96.85 mg VE/kg diet ($P < 0.05$). The FI in the VE96.85 and VE141.71 groups was higher than that in the other groups ($P < 0.05$). The HSI, VSI, and CF of grass carp reached the maximum at 141.71 mg VE/kg diet ($P < 0.05$). The slaughter rate did not differ among different VE levels ($P > 0.05$).

3.2. Muscle physicochemical indices

As shown in Table 4, the meat yield was the highest for fish fed with 96.85 mg VE/kg diet ($P < 0.05$). The shear force in the muscle showed maximum values in the VE96.85 and VE141.71 groups ($P < 0.05$). The highest pH_{24h} in the muscle was found in the group fed with 96.85 mg VE/kg diet ($P < 0.05$). Moisture and cooking loss in the muscle were the highest in fish fed with the control diet ($P < 0.05$). The crude protein content in VE141.71, VE185.66, and VE230.12 groups were higher than that in the VE5.44 group ($P < 0.05$). Muscle crude lipid content was the highest in the VE141.71 group ($P < 0.05$). The hydroxyproline and lactate contents in the muscle did not differ among the treatment groups ($P > 0.05$).

3.3. Serum biochemical indices and enzyme activities

As shown in Table 5, the highest TG, TC, and LDL-C levels in the serum of grass carp were observed in the VE96.85 group ($P < 0.05$). Serum HDL-C content was the lowest in the VE5.44 group ($P < 0.05$). Muscle ACC activity in the VE96.85 and VE52.07 groups was higher than that in the other groups ($P < 0.05$). Muscle SCD-1 activity was the highest in the VE96.85 group ($P < 0.05$).

3.4. Muscle fatty acid composition and its metabolism-related

The muscle fatty acid composition is shown in Table 6. In the VE52.07 group, the contents of EPA, DHA, SFA, PUFA, n3PUFA, and PUFA/SFA in the muscles of grass carp were the highest, whereas the contents of MUFA and n6PUFA were the lowest ($P < 0.05$). Muscle Oil Red O staining is shown in Fig. 1. The largest area of Oil Red O staining in the muscle was observed in the VE96.85 group ($P < 0.05$).

Muscle mRNA and protein levels related to fatty acid metabolism are shown in Figs. 2 and 3, respectively. The *fabp* mRNA level in the VE52.07 group was higher than that in the other groups ($P < 0.05$). The *cd36* mRNA levels in the VE141.71 and VE185.66 groups were lower than those in the other groups, except for the VE96.85 group ($P < 0.05$). PPARα protein levels did not differ among the groups ($P > 0.05$). The *hsl*, *acox1a*, and *pparα* mRNA levels in the VE52.07 and VE96.85 groups were lower than those in the other groups ($P < 0.05$). The *acox1b* mRNA level of the VE96.85 group was the lowest ($P < 0.05$). The *cpt1b* mRNA levels in the VE52.07, VE96.85, and VE141.71 groups were lower than those in the other groups ($P < 0.05$). The *cpt1a* mRNA levels in the VE141.71, VE185.66, and VE230.12 groups were lower than those in the other groups ($P < 0.05$). The *atgl* mRNA levels did not differ among the groups ($P > 0.05$). The VE230.12 group had higher SREBP1 levels than the

Table 4
Effects of vitamin E on physicochemical indices in the muscle of sub-adult grass carp.

Item	Dietary vitamin E levels, mg/kg						*P*-value
	5.44	52.07	96.85	141.71	185.66	230.12	
Meat yield, % [1]	61.18 ± 0.88^{a}	62.29 ± 0.40^{ab}	62.53 ± 0.59^{b}	62.33 ± 0.99^{ab}	62.34 ± 0.88^{ab}	61.52 ± 0.77^{ab}	0.090
Shear force, N [1]	11.69 ± 1.13^{a}	15.76 ± 1.71^{bc}	17.35 ± 1.15^{c}	16.76 ± 1.16^{c}	15.45 ± 1.63^{bc}	13.12 ± 1.02^{ab}	<0.01
Cooking loss, % [1]	19.08 ± 0.68^{b}	16.89 ± 1.83^{a}	16.70 ± 1.02^{a}	16.81 ± 1.18^{a}	18.28 ± 1.49^{ab}	18.58 ± 1.33^{ab}	0.067
pH_{24h} [1]	6.27 ± 0.01^{a}	6.35 ± 0.01^{bc}	6.42 ± 0.01^{d}	6.37 ± 0.01^{c}	6.34 ± 0.01^{b}	6.27 ± 0.01^{a}	<0.01
Hydroxyproline, μg/mg tissue [2]	1.03 ± 0.10	1.00 ± 0.10	1.01 ± 0.05	1.03 ± 0.07	1.05 ± 0.07	1.00 ± 0.13	0.532
Lactate content, mmol/g protein [2]	0.61 ± 0.02	0.56 ± 0.03	0.59 ± 0.02	0.60 ± 0.06	0.60 ± 0.05	0.58 ± 0.07	0.610
Moisture, % [2]	77.53 ± 0.38^{b}	76.56 ± 2.16^{ab}	76.56 ± 0.52^{ab}	75.43 ± 0.16^{a}	75.70 ± 1.40^{a}	75.84 ± 0.55^{a}	0.010
Crude protein, % [2]	18.20 ± 0.37^{a}	19.25 ± 0.77^{ab}	19.36 ± 0.37^{ab}	19.91 ± 0.97^{b}	19.87 ± 1.79^{b}	19.85 ± 0.63^{b}	0.011
Crude lipid, % [2]	3.06 ± 0.16^{a}	2.98 ± 0.27^{a}	4.23 ± 0.24^{c}	4.48 ± 0.26^{d}	4.06 ± 0.14^{c}	3.75 ± 0.13^{b}	<0.01
α-Tocopherol, mg/100 g [3]	0.21 ± 0.06^{a}	0.42 ± 0.05^{ab}	0.52 ± 0.11^{bc}	0.60 ± 0.07^{bc}	0.92 ± 0.28^{d}	0.74 ± 0.18^{cd}	<0.01

Means without a common letter significantly differ ($P < 0.05$).
[1] Results are represented as the mean ± SD. The data are the means of three replicates of three fishes per replicate ($n = 3$).
[2] Results are represented as the mean ± SD. The data are the means of three replicates of two fishes per replicate ($n = 3$).
[3] Results are represented as the mean ± SD. The data are the means of three replicates of one fish per replicate ($n = 3$).

Table 5
Effects of vitamin E on serum lipids profile and muscle fatty acid synthase activities of sub-adult grass carp.

Item	Dietary vitamin E levels, mg/kg						*P*-value
	5.44	52.07	96.85	141.71	185.66	230.12	
Serum							
TG, mmol/L	2.98 ± 0.21^{a}	3.38 ± 0.30^{a}	5.65 ± 0.77^{d}	5.43 ± 0.36^{cd}	5.10 ± 0.41^{c}	4.48 ± 0.37^{b}	<0.01
TC, mmol/L	8.33 ± 0.63^{a}	10.21 ± 0.85^{b}	11.65 ± 0.80^{c}	10.56 ± 1.16^{b}	10.29 ± 0.58^{b}	10.12 ± 0.54^{b}	<0.01
HDL-C, mmol/L	4.06 ± 0.28^{a}	4.76 ± 0.50^{b}	5.17 ± 0.30^{b}	5.14 ± 0.56^{b}	5.12 ± 0.18^{b}	5.13 ± 0.49^{b}	<0.01
LDL-C, mmol/L	3.18 ± 0.10^{a}	3.56 ± 0.33^{a}	5.93 ± 0.45^{d}	5.02 ± 0.25^{c}	5.00 ± 0.34^{c}	4.16 ± 0.49^{b}	<0.01
Muscle							
ACC, U/g tissue	3.97 ± 0.29^{a}	4.96 ± 0.45^{b}	5.10 ± 0.92^{b}	3.88 ± 0.34^{a}	3.92 ± 0.29^{a}	3.94 ± 0.36^{a}	<0.01
SCD-1, U/g tissue	5.59 ± 0.44^{a}	6.04 ± 0.50^{ab}	6.30 ± 0.86^{b}	6.11 ± 0.43^{ab}	6.07 ± 0.31^{ab}	5.59 ± 0.14^{a}	<0.01

TG = triglyceride; TC = total cholesterol; HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol; ACC = Acetyl CoA carboxylase; SCD-1 = stearoyl-coenzyme A desaturase.
Results are represented as the mean ± SD. The data are the means of three replicates of two fishes per replicate ($n = 3$). Means without a common letter significantly differ ($P < 0.05$).

Table 6
Effects of vitamin E on fatty acid composition in the muscle of sub-adult grass carp (% total fatty acids).

Item	Dietary vitamin E levels, mg/kg						*P*-value
	5.44	52.07	96.85	141.71	185.66	230.12	
C14:0	2.11 ± 0.10^{c}	1.51 ± 0.08^{a}	1.85 ± 0.03^{b}	1.88 ± 0.04^{b}	1.94 ± 0.09^{b}	2.16 ± 0.05^{c}	<0.01
C15:0	0.15 ± 0.003^{d}	0.12 ± 0.002^{a}	0.12 ± 0.005^{ab}	0.13 ± 0.001^{bc}	0.13 ± 0.004^{c}	0.14 ± 0.005^{c}	<0.01
C16:0	22.60 ± 0.28^{b}	22.33 ± 0.26^{b}	20.88 ± 0.08^{a}	21.07 ± 0.62^{a}	21.43 ± 0.79^{a}	20.65 ± 0.03^{a}	<0.01
C17:0	0.24 ± 0.02^{b}	0.23 ± 0.01^{b}	0.24 ± 0.01^{b}	0.24 ± 0.01^{b}	0.16 ± 0.01^{a}	0.15 ± 0.01^{a}	<0.01
C18:0	4.79 ± 0.23^{c}	6.01 ± 0.18^{d}	4.44 ± 0.21^{b}	4.28 ± 0.21^{b}	4.32 ± 0.21^{b}	3.82 ± 0.003^{a}	<0.01
C20:0	0.07 ± 0.01^{b}	0.18 ± 0.01^{d}	0.09 ± 0.01^{c}	0.10 ± 0.01^{c}	0.10 ± 0.01^{c}	0.04 ± 0.0005^{a}	<0.01
C22:0	0.97 ± 0.07^{a}	1.30 ± 0.05^{c}	1.30 ± 0.04^{c}	1.21 ± 0.02^{bc}	1.16 ± 0.08^{b}	0.95 ± 0.02^{a}	<0.01
C23:0	2.03 ± 0.08^{cd}	2.60 ± 0.05^{e}	2.11 ± 0.06^{d}	1.81 ± 0.14^{b}	1.86 ± 0.15^{bc}	1.41 ± 0.08^{a}	<0.01
C14:1	0.12 ± 0.01^{ab}	0.11 ± 0.001^{a}	0.11 ± 0.001^{a}	0.12 ± 0.002^{b}	0.12 ± 0.01^{b}	0.14 ± 0.004^{c}	<0.01
C16:1	9.77 ± 0.24^{b}	8.03 ± 0.07^{a}	10.17 ± 0.16^{bc}	10.62 ± 0.28^{c}	10.35 ± 0.48^{c}	11.59 ± 0.11^{d}	<0.01
C17:1	0.16 ± 0.01^{a}	0.15 ± 0.01^{a}	0.20 ± 0.003^{b}	0.20 ± 0.01^{b}	0.20 ± 0.02^{b}	0.20 ± 0.02^{b}	<0.01
C18:1n9t	0.26 ± 0.01^{a}	0.25 ± 0.002^{a}	0.29 ± 0.01^{b}	0.30 ± 0.01^{b}	0.24 ± 0.01^{a}	0.24 ± 0.02^{a}	<0.01
C18:1n9c	35.01 ± 1.06^{bc}	27.81 ± 0.97^{a}	34.55 ± 0.09^{b}	35.36 ± 0.16^{bc}	35.97 ± 0.30^{c}	39.34 ± 0.39^{d}	<0.01
C20:1n-9	0.12 ± 0.01^{d}	0.09 ± 0.003^{a}	0.09 ± 0.002^{ab}	0.10 ± 0.002^{b}	0.11 ± 0.002^{c}	0.11 ± 0.002^{cd}	<0.01
C22:1n-9	0.14 ± 0.001^{c}	0.08 ± 0.003^{a}	0.08 ± 0.001^{a}	0.11 ± 0.01^{b}	0.10 ± 0.002^{b}	0.10 ± 0.002^{b}	<0.01
C18:3n-3	0.06 ± 0.01^{a}	0.06 ± 0.004^{a}	0.06 ± 0.01^{a}	0.06 ± 0.004^{a}	0.07 ± 0.002^{a}	0.10 ± 0.002^{b}	<0.01
C20:3n-3	0.11 ± 0.002^{c}	0.10 ± 0.004^{b}	0.10 ± 0.01^{ab}	0.09 ± 0.005^{ab}	0.10 ± 0.003^{ab}	0.09 ± 0.002^{a}	<0.01
C20:5n-3 (EPA)	1.95 ± 0.14^{a}	2.69 ± 0.04^{d}	2.43 ± 0.03^{c}	2.15 ± 0.10^{b}	2.14 ± 0.13^{b}	1.85 ± 0.05^{a}	<0.01
C22:6n-3 (DHA)	7.75 ± 0.62^{a}	16.29 ± 0.27^{d}	10.95 ± 0.17^{c}	9.37 ± 0.70^{b}	9.29 ± 0.45^{b}	7.05 ± 0.24^{a}	<0.01
C18:2n6c	9.14 ± 0.33^{d}	7.11 ± 0.25^{a}	7.40 ± 0.11^{a}	7.86 ± 0.12^{b}	8.41 ± 0.11^{c}	8.38 ± 0.10^{c}	<0.01
C18:3n-6	0.16 ± 0.01^{d}	0.14 ± 0.01^{b}	0.13 ± 0.002^{a}	0.15 ± 0.01^{cd}	0.15 ± 0.003^{c}	0.15 ± 0.001^{c}	<0.01
C20:3n-6	0.77 ± 0.06^{b}	0.98 ± 0.02^{d}	0.84 ± 0.01^{c}	0.81 ± 0.03^{bc}	0.81 ± 0.01^{bc}	0.68 ± 0.02^{a}	<0.01
∑SFA	32.95 ± 0.46^{c}	34.28 ± 0.40^{d}	31.04 ± 0.10^{b}	30.72 ± 0.94^{b}	31.11 ± 0.82^{b}	29.30 ± 0.06^{a}	<0.01
∑UFA	67.05 ± 0.46^{b}	65.72 ± 0.40^{a}	68.96 ± 0.10^{c}	69.28 ± 0.94^{c}	68.89 ± 0.82^{c}	70.70 ± 0.06^{d}	<0.01
∑MUFA	47.09 ± 1.15^{b}	38.36 ± 0.44^{a}	47.06 ± 0.10^{c}	48.77 ± 1.60^{c}	47.91 ± 0.38^{bc}	52.40 ± 0.39^{d}	<0.01
∑PUFA	19.96 ± 0.98^{b}	27.36 ± 0.13^{d}	21.91 ± 0.06^{c}	20.51 ± 0.72^{b}	20.97 ± 0.54^{bc}	18.30 ± 0.33^{a}	<0.01
∑n3 (PUFA)	9.88 ± 0.73^{a}	19.13 ± 0.27^{d}	13.54 ± 0.16^{c}	11.68 ± 0.79^{b}	11.60 ± 0.46^{b}	9.09 ± 0.28^{a}	<0.01
∑n6 (PUFA)	10.08 ± 0.36^{d}	8.23 ± 0.24^{a}	8.37 ± 0.10^{a}	8.83 ± 0.13^{b}	9.37 ± 0.12^{c}	9.21 ± 0.11^{c}	<0.01
∑n3/∑n6	0.98 ± 0.06^{a}	2.33 ± 0.10^{d}	1.62 ± 0.04^{c}	1.32 ± 0.10^{b}	1.24 ± 0.04^{b}	0.99 ± 0.03^{a}	<0.01
PUFA/SFA	0.61 ± 0.03^{a}	0.80 ± 0.01^{d}	0.71 ± 0.00^{c}	0.67 ± 0.01^{b}	0.67 ± 0.03^{b}	0.62 ± 0.01^{a}	<0.01

SFA = saturated fatty acids; UFA = unsaturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.
∑n3 (PUFA) = C18:3n-3 + C20:3n-3 + C20:5n-3 + C22:6n-3.
∑n6 (PUFA) = C18:2n6c + C18:3n-6 + C20:3n-6.
The un-identified peak for the fatty acid composition analysis was 2.7%.
Results are represented as the mean ± SD. The data are the means of three replicates of one fish per replicate ($n = 3$). Means without a common letter significantly differ ($P < 0.05$).

control group ($P < 0.05$). The PPARγ protein levels showed no differences among all groups ($P > 0.05$). The mRNA levels of *fas*, *scd-1*, *fad*, *elovl2*, *srebp1*, and *pparγ* were the lowest in the control diet ($P < 0.05$). The *lxrα* mRNA levels in the VE52.07 and VE96.85 groups were higher than those in the other groups ($P < 0.05$). The *elovl5* mRNA levels in the control and VE230.12 groups were lower than those in the other groups ($P < 0.05$).

3.5. Antioxidant-related parameters

As shown in Table 7, the highest MDA, PC, and ROS contents in the muscle were found in the VE5.44 (control) group ($P < 0.05$). The VE5.44, VE52.07, and VE96.85 groups had lower muscle T-SOD activities than the other groups ($P < 0.05$). Muscle GST activity was highest in the VE96.85 group ($P < 0.05$). However, GPx activity in the muscle did not differ among the groups ($P > 0.05$).

3.6. ER stress-related parameters

As shown in Figs. 4 and 5, the levels of p-PERK, p-IRE1, ATF6, and GRP78 proteins were the lowest in the VE141.71 group ($P < 0.05$). The mRNA levels of *perk*, *ire1*, *atf6*, eukaryotic initiation factor 2 (*eIF2α*), X box-binding protein-1 (*XBP1*), and *grp78* were highest in the control group ($P < 0.05$). The muscle C/EBP homologous protein

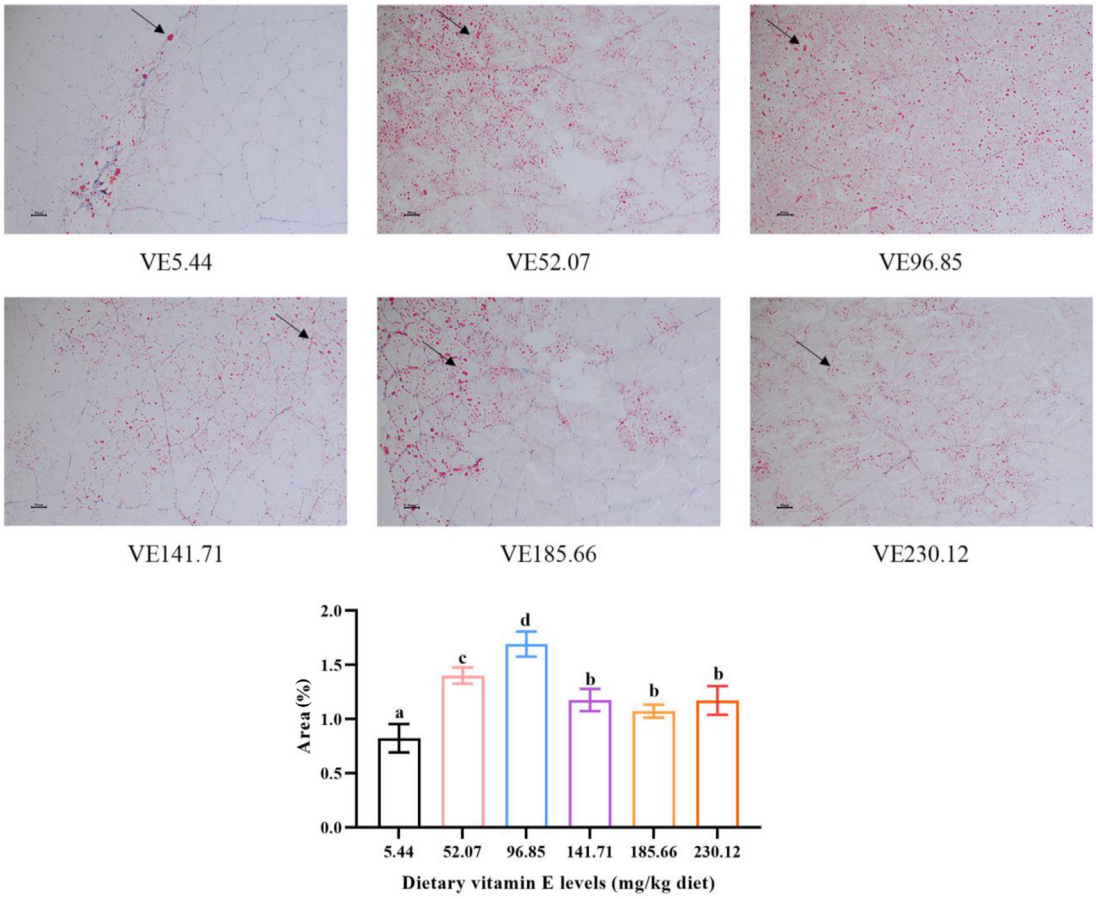

Fig. 1. Oil red O staining in the muscle of sub-adult grass carp (200× magnification). The red part is the lipid droplet (black arrows). Results are represented as the mean ± SD. The data are the means of three replicates. Means without a common letter significantly differ ($P < 0.05$).

(*CHOP*) in the VE96.85 group was lower than that in the other groups, except for the VE52.07 group ($P < 0.05$). The activating transcription factor 4 (*ATF4*) mRNA levels in the VE185.66 and VE230.12 groups were lower than those in the other groups ($P < 0.05$).

4. Discussion

4.1. Dietary VE increased growth performance and meat quality in grass carp

In the present study, dietary VE enhanced the PWG, SGR, and FE in sub-adult grass carp. Similar findings have been reported in juvenile and young grass carp (Li et al., 2014; Pan et al., 2017), red drum (*Sciaenops ocellatus*) (Peng and Gatlin, 2009), and cobia (*Rachycentron canadum*) (Zhou et al., 2013). However, there were differences in PWG, SGR, and FE among grass carp at different growth stages. At different levels of dietary VE supplementation, PWG, SGR, and FE percentage in juvenile grass carp (11.2 to 20.93 g) were found to be 103.28 to 185.90, 1.26 to 1.88, and 0.45 to 0.56, respectively (Li et al., 2014). While PWG, SGR, and FE percent in young grass carp (BW = 266.39 to 1026.63 g) ranged from 222.75 to 285.48, from 1.67 to 1.93, and from 0.62 to 0.73, respectively, at different dietary VE levels (Pan et al., 2017). Previous studies found that the PWG, SGR, and FE in sub-adult grass carp fed with different amounts of dietary proteins were from 62.98 to 91.34, from 0.81 to 1.08, and from 0.46 to 0.61, respectively (Dong et al., 2022). In the present study, the PWG, SGR, and FE in the sub-adult grass carp (713.53 to 1590.40 g) were found to be in the range from 74.32 to 122.87, from 0.88 to 1.27, and from 0.46 to 0.58, respectively, at different VE supplementation, which were similar to those in other studies on sub-adult grass carp with varied dietary profiles. Furthermore, CF and organ weight indices are important indicators of animal growth (Du and Turchini, 2022). Our results showed that dietary VE increased the CF, VSI, and HSI in sub-adult grass carp. However, VE did not affect the CF and HSI in cobia (Zhou et al., 2013), or the VSI and HSI in pufferfish (*Takifugu obscurus*) (Cheng et al., 2018). This difference might be due to the different experimental conditions and fish species. In addition, we found that high levels of VE (exceeding 96.85 mg/kg diet) reduced the growth performance of grass carp. Similar results were found in golden pompanos (Zhang et al., 2021) and tilapia (Qiang et al., 2019). This might be related to the antagonistic effect of VE on vitamin A. It has been found that dietary high levels of vitamin A reduced α-tocopherol levels in pig liver tissue (Ayuso et al., 2015; Olivares et al., 2009). However, there have been limited studies on the effect of dietary high VE levels on vitamin A metabolism in fish, and this requires further investigation. Meat production is an important quality parameter for many cultured animals and the proximate composition of fillets reflects their nutritional value (Grigorakis, 2007; Lv et al., 2021). In the present study, dietary VE increased meat yield, muscle crude protein, and crude lipid content in sub-adult grass carp. Previous studies have shown that VE improved meat quality by increasing antioxidant capacity. The muscle crude protein and lipid contents of tilapia

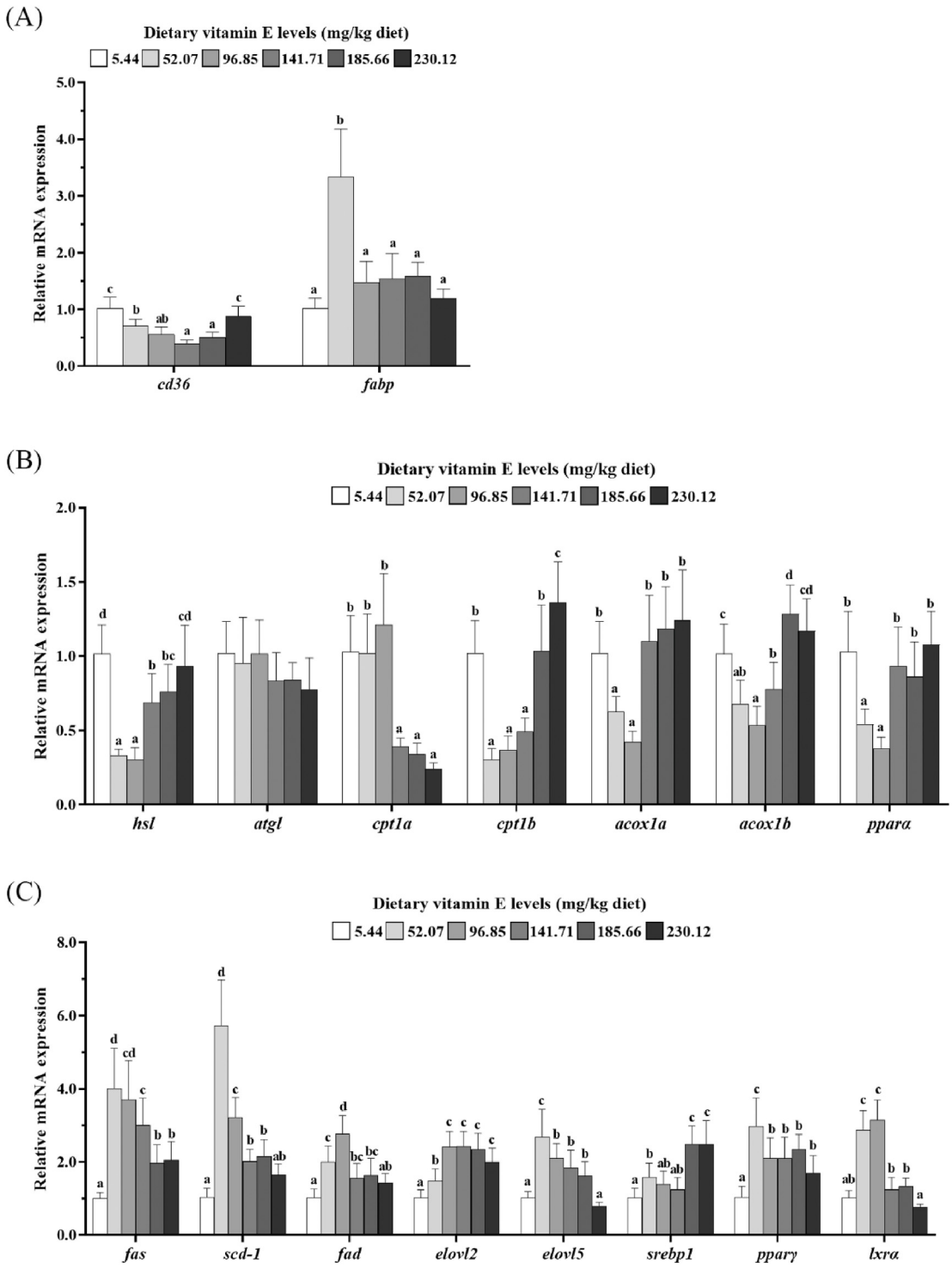

Fig. 2. Effects of vitamin E on the relative mRNA levels of fatty acid metabolism-related genes in the muscle of sub-adult grass carp. (A) Fatty acid transport, (B) fatty acid decomposition, and (C) fatty acid synthesis of sub-adult fish. Results are represented as the mean ± SD. The data are the means of three replicates with two fishes per replicate ($n = 3$). Means without a common letter significantly differ ($P < 0.05$).

(*Oreochromis niloticus*) (Wu et al., 2016) and hybrid groupers (Huang et al., 2022) were increased by dietary supplementation with VE. Physicochemical indices of muscle are useful markers of meat quality. In our experiment, we found that appropriate levels of VE (52.07 to 141.71 mg/kg diet) increased muscle pH_{24h} and hardness but decreased muscle cooking loss in sub-adult grass carp. A study on tilapia reported that dietary VE increases the muscle hardness (Wu et al., 2016). These results indicated that dietary VE could improve the nutritional value and physicochemical properties of fish muscle. VE prevents the oxidation of membrane phospholipids, thereby maintaining the integrity of muscle cell membranes (Descalzo and Sancho, 2008), which could account for the elevated meat quality associated with VE supplementation.

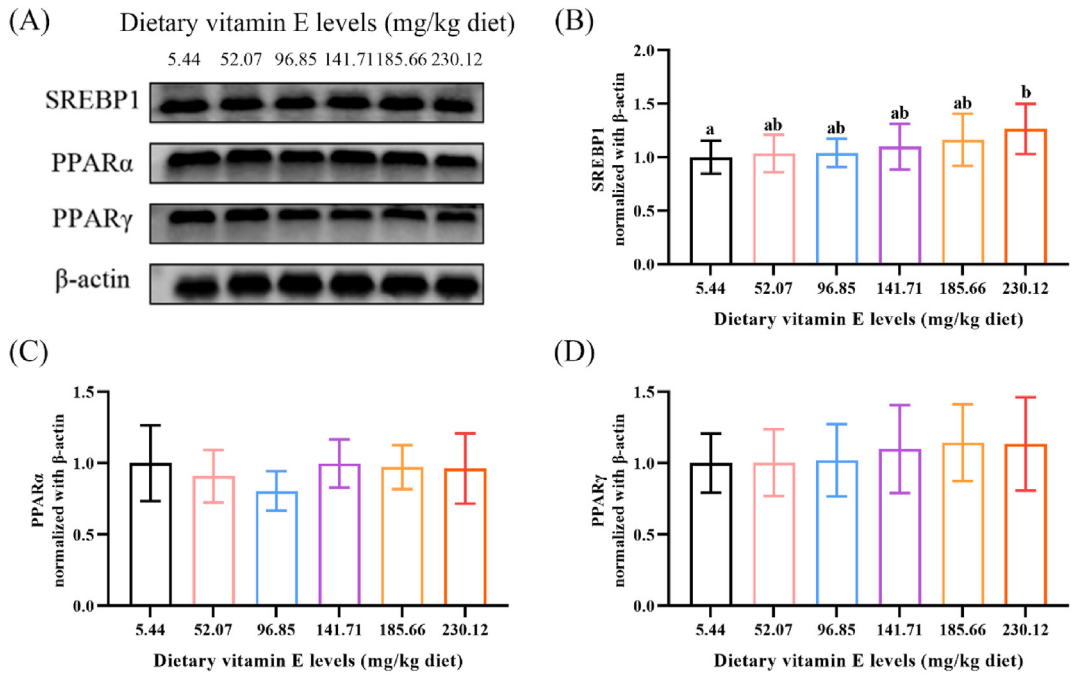

Fig. 3. Effects of vitamin E on the relative protein expressions of fatty acid metabolism-related proteins in the muscle of sub-adult grass carp. (A) Western blots for SREBP1, PPARα, and PPARγ. Quantification of (B) SREBP1, (C) PPARα, and (D) PPARγ normalized to β-actin. Results are represented as the mean ± SD. The data are the means of three replicates with two fishes per replicate ($n = 3$). Means without a common letter significantly differ ($P < 0.05$). SREBP1 = sterol regulatory element-binding protein 1; PPARα = peroxisome proliferator-activated receptor α; PPARγ = peroxisome proliferator-activated receptor γ.

Table 7
Effects of vitamin E on the antioxidant related parameters in the muscle of sub-adult grass carp.

Item	Dietary vitamin E levels, mg/kg						*P*-value
	5.44	52.07	96.85	141.71	185.66	230.12	
MDA, nmol/g tissue	12.53 ± 1.02^{d}	5.06 ± 0.39^{c}	3.50 ± 0.36^{a}	3.44 ± 0.19^{a}	4.43 ± 0.25^{b}	4.96 ± 0.18^{bc}	<0.01
PC, nmol/mg protein	7.41 ± 0.51^{c}	6.46 ± 0.62^{b}	4.43 ± 0.42^{a}	4.37 ± 0.43^{a}	4.30 ± 0.40^{a}	4.43 ± 0.39^{a}	<0.01
ROS, % DCF fluorescence	100.00 ± 2.22^{d}	83.64 ± 4.12^{c}	69.56 ± 5.35^{b}	74.86 ± 3.92^{b}	69.82 ± 6.58^{b}	51.85 ± 4.95^{a}	<0.01
T-SOD, U/mg protein	119.71 ± 3.91^{a}	118.57 ± 3.69^{a}	118.78 ± 5.33^{a}	129.89 ± 6.66^{b}	137.42 ± 8.09^{c}	137.70 ± 5.06^{c}	<0.01
GPx, U/mg protein	68.21 ± 4.33	71.08 ± 4.74	73.58 ± 5.55	70.98 ± 5.89	73.70 ± 5.56	70.57 ± 4.19	0.653
GST, U/mg protein	86.45 ± 11.58^{a}	154.13 ± 14.98^{c}	187.99 ± 9.95^{d}	138.11 ± 7.71^{b}	97.72 ± 7.36^{a}	89.54 ± 9.60^{a}	<0.01

MDA = malondialdehyde; PC = protein carbonyl; ROS = reactive oxygen species; DCF = dichlorofluorescein; T-SOD = total superoxide dismutase; GPx = glutathione peroxidase; GST = glutathione S-transferase.
Results are represented as the mean ± SD. The data are the means of three replicates of two fishes per replicate ($n = 3$). Means without a common letter significantly differ ($P < 0.05$).

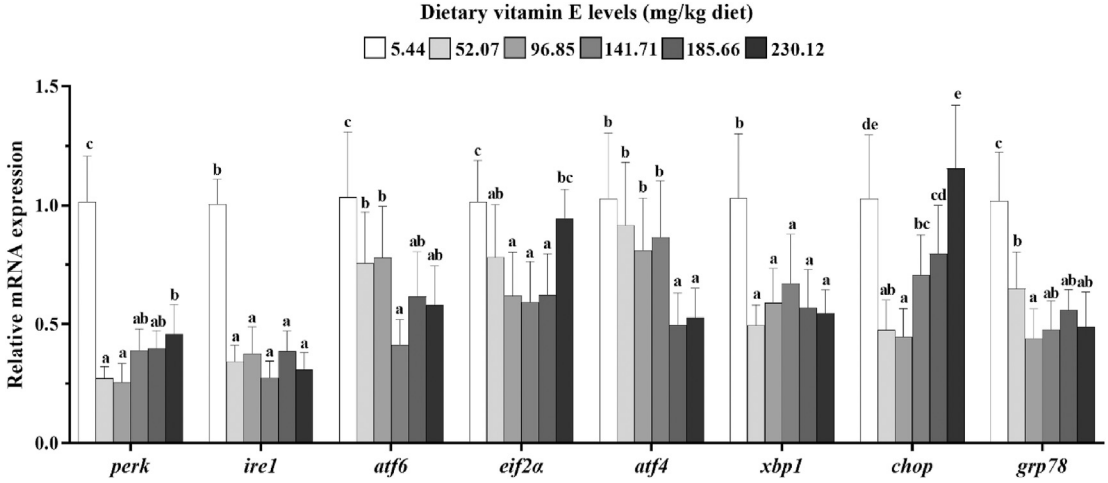

Fig. 4. Effects of vitamin E on the relative mRNA expressions of endoplasmic reticulum stress-related genes in the muscle of sub-adult grass carp. Results are represented as the mean ± SD. The data are the means of three replicates with two fishes per replicate ($n = 3$). Means without a common letter significantly differ ($P < 0.05$).

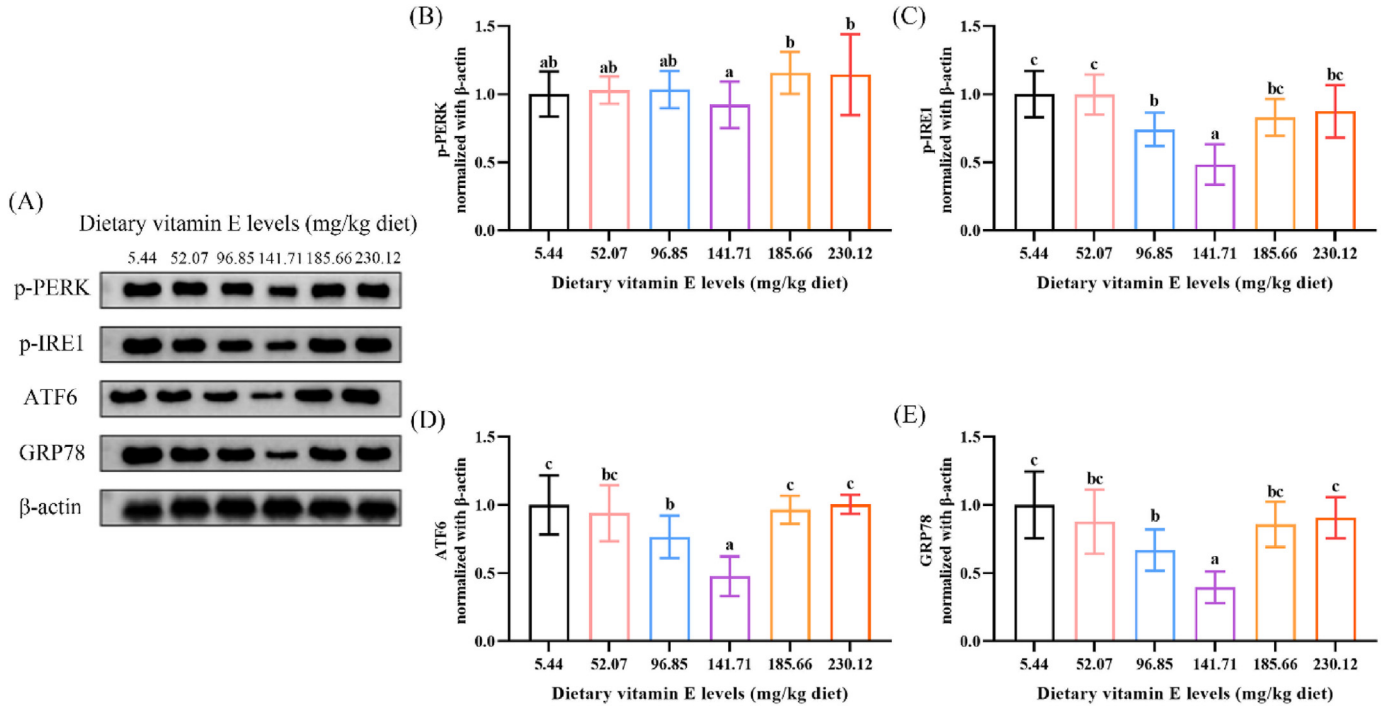

Fig. 5. Effects of vitamin E on the relative protein expressions of endoplasmic reticulum stress-related proteins in the muscle of sub-adult grass carp. (A) Western blots for p-PERK (Ser1096), p-IRE1 (Ser724), ATF6 and GRP78. Quantification of (B) p-PERK (Ser1096), (C) p-IRE1 (Ser724), (D) ATF6, and (E) GRP78 normalized to β-actin. Results are represented as the mean ± SD. The data are the means of three replicates with two fishes per replicate ($n = 3$). Mean values within the same row with different superscripts are significantly different ($P < 0.05$). p-PERK (Ser1096) = protein kinase R-like endoplasmic reticulum kinase (phospho-Ser1096); p-IRE1 (Ser724) = inositol-requiring enzyme 1 (phospho-Ser724); ATF6 = activating transcription factor 6; GRP78 = glucose regulatory protein 78.

4.2. Dietary VE improved muscle fatty acid metabolism in grass carp

The PUFA in muscle could also reflect the nutritional value of muscle (Xie et al., 2021). In this study, dietary VE increased EPA, DHA, and PUFA contents in the muscles of sub-adult grass carp. Similar results have been reported for Nile tilapia (Navarro et al., 2012) and golden pompano (Zhang et al., 2021). Therefore, we investigated how VE increased PUFA content in the muscle of grass carp.

Fatty acid content is related to fatty acid transport. Serum TG, TC, HDL-C, and LDL-C levels may reflect the fatty acid metabolism in animals (Yuan et al., 2016). In this study, we found that the dietary VE increased serum TG, TC, LDL-C, and HDL-C levels. However, dietary VE reduced serum TG and TC content in tilapia (Qiang et al., 2019) and serum TC and LDL content in hybrid groupers (Huang et al., 2022). The reason for this result may be the effect of different species, but there are no other studies on the effect of VE on the serum lipid composition of grass carp, thus, it needs to be further investigated. FABP is an important fatty acid transporter. In this study, dietary VE increased muscle *FABP* mRNA levels. Similarly, dietary VE increased the mRNA levels of liver *FABP* in tilapia (Qiang et al., 2019) and golden pompanos (Zhang et al., 2021). These results suggest that VE could regulate fatty acid transport, but it varies from species to species. CD36 is an important fatty acid transporter on the cell membrane that activates mitochondrial fatty acid oxidation in the muscles (Pepino et al., 2014). In the present study, dietary VE decreased the muscle CD36 mRNA levels. However, limited studies have investigated the effect of VE on CD36 expression in fish muscles. A study in rabbits found that VE inhibited the increase in CD36 mRNA levels in the aorta (Ozer et al., 2006). Therefore, we further studied the effect of VE on fatty acid decomposition in the muscles.

Fatty acid catabolism begins with the breakdown of lipids into fatty acids, followed by β-oxidation of fatty acids, which could be catalyzed by HSL, ATGL, CPT1, and ACOX, respectively (Reubsaet et al., 1988; Schreurs et al., 2010). In the present study, dietary VE decreased *HSL*, *ATGL*, *CPT1a*, *CPT1b*, *ACOX1a*, and *ACOX1b* mRNA levels in fish muscles. However, limited studies have investigated the mechanisms by which VE affects fatty acid catabolism. Dietary oxidized fish oil has been shown to increase the mRNA level of *CPT1* in the mid-intestine of Wuchang bream (*Megalobrama amblycephala*) compared to dietary fish oil (Song et al., 2019). VE can inhibit lipid peroxidation in aquatic feeds (Wang et al., 2015). Therefore, we speculated that dietary VE might inhibit fatty acid decomposition through its antioxidant capacity, which requires further investigation. Similarly, dietary VE reduced the mRNA levels of *HSL* and *CPT1* in the liver of golden pompano (Zhang et al., 2021) and the mRNA levels of liver *CPT1* in hybrid grouper (Liang et al., 2021). These results suggested that dietary VE inhibits fatty acid catabolism. From these results, we speculated that dietary VE might increase the PUFA content in muscle by inhibiting fatty acid oxidation. However, PUFA content is not only related to fatty acid decomposition but also to fatty acid synthesis. Therefore, we studied the effect of VE on fatty acid synthesis in the muscles.

FAS, ACC, SCD-1, FAD, and ELOVL play important roles in the catalysis of fatty acid synthesis (Brownsey et al., 2006; Flowers and Ntambi, 2008; Liu et al., 2010). The present study showed that dietary VE increased the mRNA levels of *FAS*, *SCD-1*, *FAD*, *ELOVL2*, and *ELOVL5* in muscle tissue. Similarly, dietary VE increased *FAD* and *ELOVL* mRNA levels in the liver of golden pompanos (Zhang et al., 2021). However, only a few studies have investigated the mechanisms by which VE affects fatty acid synthesis. The metabolite of VE, tocopherol quinone, is an essential enzyme cofactor of FAD (Infante, 1999). Therefore, we speculated that dietary VE might promote PUFA synthesis through its metabolites. However, dietary VE

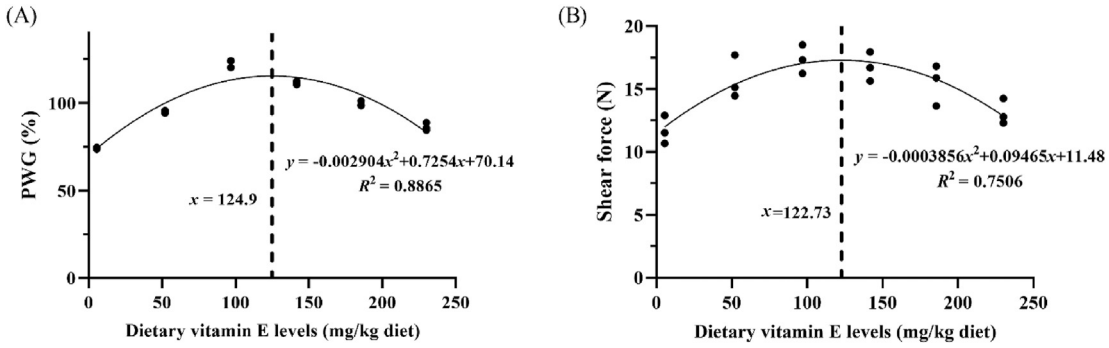

Fig. 6. The quadratic regression analysis of (A) percent weight gain (PWG) and (B) muscle shear force of sub-adult grass carp.

reduced the liver *FAS* mRNA levels in golden pompano (Zhang et al., 2021) and hybrid groupers (♀ *E. fuscoguttatus* × ♂ *E. lanceolatus*) (Liang et al., 2021). Differences in the expression of fatty acid metabolism-related genes in the liver and muscle might be partly responsible for these different results (Benedito-Palos et al., 2014). However, the detailed mechanisms require further investigation. These results suggested that dietary VE promoted fatty acid synthesis. However, further investigation on how VE regulates fatty acid synthesis and fatty acid decomposition is necessary.

Fatty acid metabolism-related gene transcription levels are regulated by multiple signaling molecules such as the SREBP and PPAR families, and LXR (Horton et al., 2002; Reubsaet et al., 1988). In our experiment, we found that dietary VE promoted the mRNA levels of *SREBP1*, *PPARγ*, and *LXRα*, and the protein levels of SREBP1 but reduced the mRNA levels of *PPARα* in muscle tissue of grass carp. Similarly, VE reduced the mRNA level of *PPARα* in the liver of golden pompanos (Zhang et al., 2021). These results suggested that dietary VE might promote fatty acid synthesis and inhibit fatty acid decomposition partly through SREBP1, PPARα, PPARγ, and LXRα. However, there are limited studies on the mechanisms by which VE affects transcription factors related to fatty acid metabolism. Metabolites of VE could bind to the ligand of the transcription factor PPARγ (Arifi et al., 2023). Therefore, we speculated that dietary VE might regulate fatty acid metabolism through its metabolites, which requires further investigation.

4.3. The effect of dietary VE in alleviating muscle ER stress in grass carp is partly associated with its antioxidant capacity

The ER is an important organelle for fatty acid synthesis (Bogdanovic et al., 2015). ER stress is a physiological and pathological process that occurs when the homeostatic state of ER is disrupted. PERK, IRE1, ATF6, and GRP78, and their regulated downstream signaling molecules of eIF2α, CHOP, ATF4, and XBP1 are the main regulator of ER stress (Kimata and Kohno, 2011). In our study, dietary VE reduced p-PERK, p-IRE1, ATF6, and GRP78 protein levels and PERK, IRE1, ATF6, GRP78, eIF2α, CHOP, ATF4, and XBP1 mRNA levels in the muscle, suggesting that dietary VE could alleviate ER stress via the UPR signaling pathway. Similarly, dietary VE reduced p-eIF2 protein levels in the aortas of rabbits that were fed a high-cholesterol diet (Bozaykut et al., 2020). Dietary VE inhibits muscle ER stress, which is likely due to its antioxidant capacity. Oxidative stress is an important trigger for ER stress (Almanza et al., 2019). A study using the human lung epithelial cell line A549 found that oxidative damage increased the levels of UPR-related proteins (Wang et al., 2019). VE is an important antioxidant in fish muscle (Pazos et al., 2005). Our study found that dietary VE increased the α-tocopherol content, and T-SOD and GST activities, and decreased muscle MDA, PC, and ROS levels, suggesting that dietary VE reduced oxidative stress in grass carp muscle. Therefore, dietary VE can alleviate ER stress in grass carp muscles, partly through its antioxidant capacity. In addition, we found that dietary high doses of VE promoted ER stress, which might be related to the fact that excess VE promotes oxidative stress. Notably, high dietary levels of VE supplementation with other antioxidants (vitamin C and selenium) were found to reduce oxidative stress (Hamre et al., 1997; Naderi et al., 2019).

4.4. The VE requirements of sub-adult grass carp

The VE requirements for sub-adult grass carp by quadratic regression analysis of PWG and muscle shear force were 124.9 and 122.73 mg/kg diet, respectively (Fig. 6). These values were slightly higher than that for young grass carp (266.39 to 1026.63 g) (116.2 mg/kg diet) (Pan et al., 2017). This difference might be related to the crude lipid content of the diets. Dietary crude lipid content affects VE requirements. In tilapia, the VE requirement was 76.1 mg/kg diet at high dietary lipid levels (130 g/kg), which was higher than that of 43.2 mg/kg diet requirement at low dietary lipid levels (60 g/kg) (Jiang et al., 2020). The crude lipid of this experimental diet (47.8 g/kg) was higher than that in young grass carp (33.04 g/kg) (Pan et al., 2017).

5. Conclusion

In summary, dietary VE improved growth performance, meat quality, and muscle PUFA content in sub-adult grass carp. Furthermore, the increase in muscle PUFA content in grass carp on supplementation with appropriate levels of dietary VE (52.07 to 96.85 mg/kg diet) might be associated with increased fatty acid synthesis-related genes and proteins, decreased fatty acid decomposition-related genes and proteins, and decreased ER stress, which might be related to the antioxidant properties of VE. This provides a novel possible molecular mechanism for the use of VE to improve the meat quality of grass carp. In addition, the VE requirements for sub-adult grass carp based on the PWG and muscle shear force were estimated to be 124.9 and 122.73 mg/kg diet, respectively.

Author contributions

Ke Yao: Manuscript writing, Formal analysis. **Lin Feng:** Methodology, Supervision. **Wei-Dan Jiang:** Methodology. **Yang Liu:** Methodology. **Lu Zhang:** Resources. **Hai-Feng Mi:** Resources. **Xiao-Qiu Zhou:** Writing - review & editing, Funding acquisition, Project administration, Supervision. **Pei Wu:** Conceptualization, Supervision. **Pei Wu** had primary responsibility for the final content of the manuscript.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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Appendix supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2023.09.008.

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