

Effects of dietary vitamin E supplementation on growth performance, fatty acid composition, lipid peroxidation and peroxisome proliferator-activated receptors (PPAR) expressions in juvenile blunt snout bream *Megalobrama amblycephala*

Yin Zhang · Yang Li · Xiao Liang · Jian Gao

Received: 28 September 2015 / Accepted: 28 March 2016 / Published online: 8 May 2017
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Abstract A 9-week feeding experiment was conducted to evaluate the effects of dietary vitamin E (VE) supplementation on growth performance, liver fatty acid composition, lipid peroxidation and peroxisome proliferator-activated receptors (PPAR) genes expressions in blunt snout bream juveniles. Fish (average initial weight: 0.59 g) were fed diet supplemented with 0, 50, 100, 300 and 500 mg α -tocopherol acetate/kg in triplicates, which were found to, respectively, contain 11.2, 56.3, 114.6, 306.5 and 588.4 mg α -tocopherol/kg diet. Results showed that final weight, body weight gain and specific growth rate significantly increased with increasing dietary VE supplemented level from 11.2 to 56.3 mg/kg. When the broken-line model was employed to estimate the adequate requirement of vitamin E based on body weight gain, the optimal level was 55.5 mg/kg in diet. Hepatosomatic index value significantly decreased with incremental dietary VE levels. However, liver VE concentration showed a direct relationship with the dietary VE level. The percentages of 20:5n-3, 22:6n-3 and total n-3 long chain polyunsaturated fatty acids in liver increased with increasing dietary VE

supplementation. Meanwhile, the expressions of *PPAR- α* , *PPAR- β* and *PPAR- γ* in liver were down-regulated by supplementation of dietary VE level from 56.3 to 588.4 mg/kg. In conclusion, supplementation of more than 55.5 mg/kg vitamin E may improve growth and increase n-3 LC-PUFA content in blunt snout bream, which is beneficial to human consumer.

Keywords *Megalobrama amblycephala* · Vitamin E · Fatty acid composition · Lipid oxidation · PPAR expressions

Introduction

Vitamin E (α -tocopherol) is an effective lipid-soluble, chain-breaking antioxidant that could protect cell membranes from oxidative damage (Mourete et al. 2007). Fish have no capacity to synthesize vitamin E and must rely on a dietary supply. A dietary requirement for vitamin E in fish has been reported in a number of studies including channel catfish (*Ictalurus punctatus*) (Bai and Gatlin 1993), rohu (*Labeo rohita*) fry (Sau et al. 2004), grouper (*Epinephelus malabaricus*) (Lin and Shiau 2005), eel (*Anguilla japonica*) (Bae et al. 2013) and cobia (*Rachycentron canadum*) (Zhou et al. 2013c).

Long chain (LC) n-3 polyunsaturated fatty acids (PUFA) are most commonly defined as PUFA with

Y. Zhang · Y. Li · X. Liang · J. Gao (✉)
College of Fisheries, Key Lab of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education/Key Lab of Freshwater Animal Breeding, Ministry of Agriculture, Huazhong Agricultural University, No. 1 Shizishan Stress, Hongshan District, Wuhan 430070, Hubei Province, China
e-mail: gaojian@mail.hzau.edu.cn

$\geq C20$ and ≥ 3 double bonds, particularly eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). Fish naturally contains high levels of the n-3 LC-PUFA. Recent studies have shown that n-3 LC-PUFAs play a vital role in prevention and treatment of cardiovascular disease, inflammation, aggression, depression, hypertension, autoimmune disorders and cancer in human. However, LC-PUFAs are potentially susceptible to lipid peroxidation (Hamre 2011), which leads to development of undesirable influence on fish metabolism in several ways like decreased growth, increased mortality and lost vitamins concentrations of fish. It has been shown that vitamin E can protect biological membranes containing LC-PUFA against free radicals formation and effectively minimize lipid peroxidation in biological systems in fish (Tocher et al. 2003; Mourente et al. 2000; Gao et al. 2012a). Moreover, Gao et al. (2012b) reported that the contents of n-3 LC-PUFA such as EPA, DHA in fish tissues were increased by supplementation of dietary vitamin E. Therefore, the vitamin E not only prevents increases in lipid peroxidation as antioxidant, but also plays a major role in lipid metabolism in fish.

Functions of the lipid metabolism of peroxisome proliferator-activated receptors (PPARs) have caused a great deal of attention. PPARs have three isotypes, termed *PPAR- α* , *PPAR- β* and *PPAR- γ* . These three isotypes exhibit distinct patterns of tissue expression, differ in their ligand-binding domains and are involved in multiple, distinctive but often complementary physiological pathways and functions (Kuenzli and Saurat 2003). Ruyter et al. (1997) reported PPARs in fish for the first time and determined their responses to peroxisome proliferators and fatty acids. It has been proved that PPARs are ligand-dependent transcription factors which regulate several of the genes involved in lipid metabolism (Qi et al. 2000; Kliwer et al. 2001; Lee et al. 2003). Although the effects of vitamin E on expressions of PPARs in mammals have been reported (Fang et al. 2010), there is no information regarding the effects of vitamin E on the expression levels of PPARs in fish.

Blunt snout bream *Megalobrama amblycephala* is an herbivorous freshwater fish native to China. In China, aquaculture of this fish has expanded rapidly during the last decade because of its fast growth, tender flesh and high diseases resistance. Although few previous studies (Zhou et al. 2013a, b; Liu et al. 2014) have shown the effects of vitamin E

supplementation in diets on immune functions, fillet quality and disease resistance in this fish, limit studies regarded the effects of vitamin E supplementation on growth performance, fatty acid composition, lipid peroxidation and PPAR genes expression in the fish juveniles has been reported. Therefore, the purpose of this study was to evaluate the effects of vitamin E supplementation on the growth performance, fatty acid composition, lipid peroxidation and expressions of PPAR genes in blunt snout bream juveniles.

Materials and methods

Diet formulation and preparation

Five iso-nitrogenous (43.1 %) and iso-lipidic (9.1 %) semi-purified experimental diets were used in this study. The composition of the semi-purified basal diet is presented in Table 1. Graded levels of dl- α -tocopheryl acetate were added to the basal diet at the expense of cellulose to provide the desired levels of 0, 50, 100, 300 and 500 mg/kg diet, which were found to, respectively, contain 11.2 (E11.2), 56.3 (E56.3), 114.6 (E114.6), 306.5 (E306.5) and 588.4 (E588.4) mg α -tocopherol/kg diet, and E11.2 was regarded as the control. The diets were made as follows: all dry ingredients were thoroughly mixed in a blender (SM-168; Muren Corp, Shenzhen, China) for 15 min; the liquid-soluble vitamins which contained different levels of vitamin E were first completely mixed with oil before being poured into the blender and stirred with the dry ingredients for 15 min; Then, 25 % water was added into the mixture which was stirred for 15 min to give a stiff dough. The wet dough was extruded through a 3.0 mm die to form pellets. The moist pellets were dried in a dry-air mechanical convection oven at 60 °C for 2 h and then stored at -20 °C until use. At the time of feeding, the diets were broken into appropriate size. Proximate composition, vitamin E concentrations and thiobarbituric acid reactive substances (TBARS) of the experimental diets are presented in Table 2, and fatty acid compositions are shown in Table 3.

Fish and feeding experiment

Blunt snout bream (initial body weight: 0.59 ± 0.01 g, mean \pm SE) juveniles were obtained

Table 1 Formulation (%) of basal diets

Ingredient	
Defatted fish meal ^a	10
Soybean concentrate	50
CMC ^b	5
α -Starch	8
Dextrin	8
Fish oil	2
Soybean oil	6
Soybean lecithin	6
Vitamins (vitamin E free) ^c	2
Minerals ^d	2
Vitamin E ^e + α -cellulose	1

^a Defatted by 100 % ether

^b Carboxy methyl cellulose

^c Vitamin mixture (mg/kg diet): β -carotene, 32.12 mg; vitamin C, 230 mg; vitamin D₃, 3.24 mg; menadione NaHSO₃ · 3H₂O (K₃), 15.28 mg; thiamine nitrate (B₁), 19.24 mg; riboflavin (B₂), 64.12 mg; pyridoxine-HCl (B₆), 15.28 mg; cyanocobalamin (B₁₂), 0.04 mg; d-biotin, 1.92 mg; inositol, 1283.04 mg; niacin (nicotinic acid), 256.56 mg; Ca pantothenate, 89.34 mg; folic acid, 4.8 mg; choline chloride, 2623.12 mg; *p*-aminobenzoic acid, 127.76 mg

^d Mineral mixture (mg/kg diet): MgSO₄, 3380 mg; Na₂HPO₄, 2153.33 mg; K₂HPO₄, 5913.33 mg; Fe citrate, 733.33 mg; Ca lactate, 8060 mg; Al(OH)₃, 6.67 mg; ZnSO₄, 86.67 mg; CuSO₄, 2.67 mg; MnSO₄, 20 mg; Ca(IO₃)₂, 6.67 mg; CoSO₄, 26.67 mg

^e DL- α -tocopheryl acetate, Nacalai Tesque, Inc. Tokyo, Japan

from Haida Co., Ltd., Tuanfeng County, Hubei Province, China and acclimated to laboratory conditions for 2 weeks by feeding a commercial feed (35 % crude protein and 10 % crude lipid, Haida Co., Ltd.). The growth experiment for 9 weeks was conducted

indoors in a system with flow-through freshwater at 1 l/min. Three tanks (50 l water/tank) were randomly assigned to each dietary treatment with 15 fish per tank. All fish were hand-fed to apparent satiation with the designated diets thrice daily (8:00, 14:00 and 20:00 h). Uneaten diets were collected, freeze dried and weighed for corrected feed intake calculation. Dead fish were removed and weighed daily, and the number of dead fish was recorded to calculate the mortality (%) at the end of the feeding trial. A diurnal cycle of 12 h/12 h light/dark was maintained with fluorescent lights. Water temperature ranged from 20 to 24 °C, pH fluctuated between 7.0 and 7.5 and dissolved oxygen was maintained at approximately 6.5 mg/l during the feeding trial.

Sample collection and analyses

At the end of the feeding trial, all fish were fasted for 24 h prior to final sampling and were killed in an ice-slurry. Two fish from each tank were randomly collected and stored at -20 °C for whole body composition analysis. Three fish from each tank were randomly chosen to dissect the liver without any excreta and were stored at -80 °C after having been stored in liquid nitrogen for 8 h for the PPAR genes expression level determinations. Livers of the other fish from each tank were dissected out and weighed individually to calculate the hepatosomatic index (HSI). The liver samples were collected, pooled and stored at -80 °C for the determination of the vitamin E concentration, fatty acid composition and TBARS value.

The diets and whole fish body were analyzed for moisture, crude protein, total lipid and ash, in triplicate, using standard AOAC methods (A.O.A.C 1990).

Table 2 Proximate composition (%), analyzed α -tocopherol content and thiobarbituric acid reactive substances (TBARS) of test diets

	Diet group				
	E11.2	E56.3	E114.6	E306.5	E588.4
<i>Proximate composition (%)</i>					
Moisture	12.6	13.2	13.3	13.9	13.4
Crude fat (dry mass)	7.0	7.1	7.4	7.3	7.2
Crude ash (dry mass)	6.6	6.8	7.0	7.2	7.1
Crude protein (dry mass)	42.5	41.7	42.1	42.1	42.0
Analyzed vitamin E (mg/kg)	11.2	56.3	114.6	306.5	588.4
TBARS (μ mol MDA ¹ /mg dry mass)	19.8	18.0	19.8	20.0	19.4

Values are means of triplicate measurements

¹ MDA malondialdehyde

Table 3 Fatty acid composition (%) of test diets

	Diet group				
	E11.2	E56.3	E114.6	E306.5	E588.4
<i>Fatty acid composition (% of total fatty acids)</i>					
14:0	6.8 ± 0.3	6.9 ± 0.4	7.0 ± 0.8	6.9 ± 0.8	6.7 ± 0.7
16:0	12.7 ± 0.4	12.6 ± 0.5	12.9 ± 0.4	12.9 ± 0.8	13.1 ± 0.8
18:0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Σ saturated	19.6 ± 0.6	19.6 ± 0.3	20.0 ± 1.2	19.9 ± 2.0	19.9 ± 1.0
16:1n-7	0.6 ± 0.1	0.5 ± 0.1	0.4 ± 0.0	0.5 ± 0.0	0.5 ± 0.0
18:1n-9	15.0 ± 0.9	15.5 ± 1.3	15.7 ± 0.7	14.3 ± 0.4	15.3 ± 0.6
18:1n-7	3.6 ± 0.5	3.5 ± 0.4	3.5 ± 0.1	3.1 ± 0.1	3.5 ± 0.1
20:1n-9	0.3 ± 0.2	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
22:1n-9	0.3 ± 0.1	0.4 ± 0.2	0.3 ± 0.0	0.3 ± 0.1	0.3 ± 0.1
Σ monoenes	19.8 ± 1.6	20.0 ± 1.8	20.0 ± 0.1	18.2 ± 0.5	19.8 ± 2.3
18:2n-6	54.4 ± 1.3	53.6 ± 1.3	54.1 ± 1.7	55.3 ± 1.1	54.4 ± 1.2
20:4n-6	0.5 ± 0.1	0.5 ± 0.3	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.1
Σ n-6 fatty acids	54.5 ± 1.5	54.2 ± 1.3	54.7 ± 1.7	55.9 ± 1.1	55.0 ± 1.2
18:3n-3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
18:4n-3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1
20:4n-3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
20:5n-3	1.1 ± 0.0	1.2 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.2 ± 0.1
22:5n-3	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0
22:6n-3	1.4 ± 0.2	1.1 ± 0.8	1.5 ± 0.1	1.0 ± 0.6	1.5 ± 0.1
Σ n-3 fatty acids	2.9 ± 0.2	3.0 ± 0.8	2.8 ± 0.1	2.5 ± 0.6	3.1 ± 0.4

Values are expressed as mean ± SE from triplicate groups

The diet moisture level was determined by drying the sample at 105 °C to a constant weight. The fish samples were freeze dried (Freezone 2.5, Labconco, Co., Ltd., USA) prior to proximate composition analyses. Loss in weight represented whole body moisture content. Total lipid was determined following the method of Bligh and Dyer (1959). The measurement of TBARS was carried out using a method adapted from Yagi (1987). The vitamin E contents in fish and diets were determined by high-performance liquid chromatography (HPLC) with a UV detector (FLD) according to Sau et al. (2004). The fatty acid composition of test diets and livers was determined by a gas chromatography (Agilent Technologies Inc., Santa Clara, California, USA; column: OmegawaxTM 320) according to the method of Gao et al. (2012a). The temperatures of the injector and flame ionizing detector (FID) were set at 200 and 240 °C, respectively. High-purity helium was used as the carrier gas at a flow rate of 1 ml/min. The samples (1.0 µl) were manually injected into an injection port, and the identified fatty acids were presented as area percentage of total fatty acids.

Total RNA was extracted from the liver using RNAiso Plus (Takara Co. Ltd., Japan). RNA samples were treated by RQ1 RNase-Free DNase prior to RT-PCR (Takara Co. Ltd., Japan) to avoid genomic DNA amplification. cDNA was generated from 500 ng DNase-treated RNA using ExScriptTM RT-PCR kit (Takara Co. Ltd., Japan). Analyses of gene transcript levels were conducted by the real-time PCR method. Real-time PCR was employed to determine mRNA levels based on the SYBR Green I fluorescence kit. Primers for the real-time PCR analysis were designed using Primer5 Software, based on the cDNA sequences available in GenBank (*PPAR-α*, HM140628; *PPAR-β*, HM140629; *PPAR-γ*, HM140627). Real-time PCR was performed in a Mini Option real-time detector (Bio-Rad, USA). The fluorescent quantitative PCR solution consisted of 5 µl SYBR[®] premix Ex TaqTM (2×), 0.4 µl PCR forward primer (20 µM), 0.4 µl PCR reverse primer (10 µM) (primers sequences are given in Table 4), 1.0 µl RT reaction (cDNA solution) and 3.2 µl dH₂O. The cDNA solution was previously diluted to 20 %. The reaction conditions were as follows:

Table 4 Nucleotide sequences of the primers used to assay gene expression by real-time PCR

Target gene	Forward (5′–3′)	Reverse (5′–3′)	Annealing temperature (°C)
<i>PPAR-α</i>	GTGCCAATACTGTCGCTTTCAG	CCGCCTTTAACCTCAGCTTCT	60
<i>PPAR-β</i>	CATCCTCACGGGCAAGAC	TGGCAGCGGTAGAAAGACA	60
<i>PPAR-γ</i>	AGCTTCAAGCGAATGGTTCTG	AGGCCTCGGGCTTCCA	60
<i>β-actin</i>	CGGACAGGTCATCACCATTG	CGCAAGACTCCATACCCAAGA	60
<i>Rpl3</i>	TCTGGAGGACTGTAAGAGGTATGC	AGACGCACAATCTTGAGAGCAG	60

PPAR peroxisome proliferator-activated receptors

Rpl13a ribosomal protein L13a

95 °C for 30 s followed by 40 cycles consisting of 95 °C for 5 s and 57 °C for 30 s. The fluorescent flux was then recorded, and the reaction continued at 72 °C for 6 s and 95 °C for 5 s. All amplicons were initially separated by agarose gel electrophoresis to ensure that they were of correct sizes. The gene expression levels were normalized toward the mean of the two reference genes (*β-actin* and *Rpl13a*). Normalized gene expressions of the control group were set to 1, and the expression of each target gene for the groups with added vitamin E was expressed relative to the control group. The optimized comparative Ct ($2^{-\Delta\Delta C_t}$) value method was used here to estimate gene expression levels.

Statistical analysis

All data were subjected to Levene's test of equality of error variances and one-way ANOVA followed by Tukey's test using SPSS 16.0 (SPSS 16.0, Michigan Avenue, Chicago, IL, USA). Probability values of $P < 0.05$ were considered statistically significant. Dietary vitamin E requirement of blunt snout bream juveniles was estimated by the broken-line regression (Robbins 1986).

Results

Growth performances of the fish fed diets contained different levels of vitamin E for 9 weeks are given in Table 5. Final weight, body weight gain and specific growth rate significantly increased with incremental dietary vitamin E levels from 11.2 to 56.3 mg/kg, but no significant differences were observed among

E56.3, E114.6, E306.5 and E588.4 groups. There were no significant differences in survival and condition factor (CF) among treatments. HSI significantly decreased with incremental dietary vitamin E concentrations. The broken-line model was employed to determine the optimal point for the maximal body weight gain (Fig. 1). The result showed that the breakpoint at 55.5 mg/kg based on body weight gain, giving the least mean square error.

The whole body composition such as total lipid, moisture, ash and crude protein was not influenced by increasing levels of dietary vitamin E. Contents of total lipid content (dry mass), moisture, crude ash (dry mass) and crude protein (dry mass) were 25.7 ± 2.5 , 58.9 ± 1.2 , 9.8 ± 0.5 and 62.6 ± 1.5 %, respectively.

Liver fatty acid compositions of the fish fed test diets are shown in Table 6. The levels of 18:2n-6 and total n-6 fatty acids in liver decreased with increasing levels of dietary vitamin E. In contrast, the levels of EPA and DHA increased with increasing levels of vitamin E supplementation. Liver total n-3 fatty acid level in the fish fed the E588.4 diet was the highest, while the lowest in the fish fed the E11.2 diet.

Liver vitamin E concentrations increased with incremental levels of dietary vitamin E (Table 7). The liver vitamin E concentration in fish fed E306.5 diet increased significantly compared with the E11.2 group. The highest liver vitamin E concentration was detected in fish fed the E588.4 diet, which was significantly higher than that in E11.2, E56.3 and E114.6 group. Liver TBARS values are presented in Table 7. No significant differences were detected on the liver TBARS value, although the value trended a slight decrease with incremental dietary vitamin E levels.

Table 5 Growth performance of blunt snout bream juvenile fed test diets for 9 weeks

	Diet group				
	E11.2	E56.3	E114.6	E306.5	E588.4
Initial weight	0.59 ± 0.01	0.59 ± 0.02	0.59 ± 0.01	0.59 ± 0.01	0.59 ± 0.02
Final weight (g)	1.3 ± 0.2 ^a	1.7 ± 0.1 ^b	1.7 ± 0.1 ^b	1.7 ± 0.0 ^b	1.7 ± 0.2 ^b
BWG ¹ (%)	126.0 ± 27.1 ^a	192.1 ± 16.5 ^b	181.9 ± 15.2 ^b	194.4 ± 5.4 ^b	195.5 ± 39.7 ^b
SGR ² (%)	1.3 ± 0.2 ^a	1.7 ± 0.1 ^b	1.6 ± 0.1 ^b	1.7 ± 0.1 ^b	1.7 ± 0.2 ^b
Survival (%)	91.7 ± 3.6	97.9 ± 3.6	98.0 ± 3.4	95.9 ± 3.6	100.0 ± 0.0
Mortality (%)	8.3 ± 3.6	2.1 ± 3.6	2.0 ± 3.4	4.1 ± 3.6	0.0 ± 0.0
FCR ³	1.9 ± 0.3	1.5 ± 0.3	1.5 ± 0.3	1.5 ± 0.1	1.6 ± 0.3
CF ⁴	0.75 ± 0.09	1.04 ± 0.12	0.97 ± 0.07	0.94 ± 0.04	0.97 ± 0.08
HSI ⁵	3.06 ± 0.19 ^b	2.91 ± 0.29 ^{ab}	2.77 ± 0.20 ^a	2.63 ± 0.09 ^a	2.46 ± 0.10 ^a

Values are mean ± SEM from triplicate groups (15 fish/tank). Means in each row with different letters are significantly different ($P < 0.05$). Absence of letters indicates no significant difference between treatments

¹ BWG, body weight gain (%) = $100 \times (\text{final weight} - \text{initial weight}) / (\text{initial weight})$

² SGR, specific growth rate = $100 \times (\ln \text{final weight} - \ln \text{initial weight}) / (\text{duration})$

³ FCR, feed conversion ratio = dry feed intake (g)/weight gain (g)

⁴ CF, condition factor = body weight × 100 (g)/total length (cm)³

⁵ HSI, hepatosomatic index = $100 \times (\text{liver weight}/\text{body weight})$

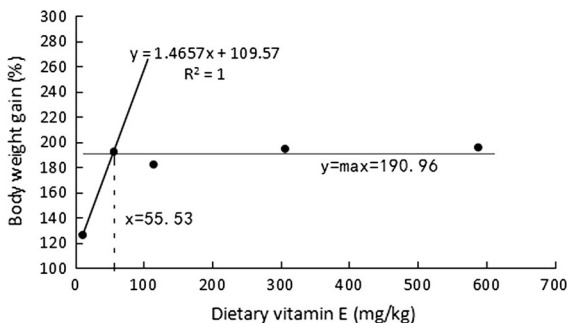


Fig. 1 The effect of dietary vitamin E on weight gain (%) of blunt snout bream juveniles fed test diets for 9 weeks. Each point represents the mean of three tanks of fish with 15 fish per aquarium ($n = 3$). Dietary vitamin E requirement derived from the broken-line regression model is 55.53 mg/kg

The expression levels of *PPAR-α*, *PPAR-β* and *PPAR-γ* in liver are shown in Fig. 2. The expressions of *PPAR-α* in E56.3, E114.6, E306.5 and E588.4 groups were significantly lower than that in E11.2 group. The highest expression of *PPAR-β* was found in fish fed E56.3 diet, and the expression of *PPAR-β* decreased with increasing dietary vitamin E levels from 56.3 to 588.4 mg/kg. Similarly, *PPAR-γ* expression in liver significantly decreased with increasing dietary vitamin E levels from 56.3 to 588.4 mg/kg.

Discussion

In the present study, body weight gain of blunt snout bream markedly increased with increasing supplemented vitamin E levels from 11.2 to 56.3 mg/kg. Results of the broken-line model indicated that the vitamin E requirement of blunt snout bream juveniles for optimum growth was considered to be 55.53 mg/kg under the present experimental conditions, which was similar to most fish species, e.g., 50 mg/kg for rainbow trout (*Salmo gairdneri*) (Cowey et al. 1983), 50 mg/kg for channel catfish (*I. punctatus*) (Wilson et al. 1984) and 31 mg/kg for red drum (*Sciaenops ocellatus*) (Li and Gatlin 2009). However, Zhou et al. (2013b) found that dietary supplementation of 100–150 mg/kg vitamin E promoted *M. amblycephala* growth, improved meat quality and strengthened the immune system of the fish. It is well known that the dietary vitamin E requirement of fish is affected by several factors such as fish size, cultured condition, the dietary lipid level, the degree of unsaturation of the dietary lipid source and the oxidation of the dietary lipid. There were no significant differences in growth performance when supplementations of vitamin E exceed 56.3 mg/kg (even the vitamin E content in E588.4 group was about 10 times higher than that in

Table 6 Liver fatty acid composition (%) of blunt snout bream juvenile fed test diets for 9 weeks

	Diet group				
	E11.2	E56.3	E114.6	E306.5	E588.4
14:0	0.9 ± 0.2	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.2	0.6 ± 0.1
16:0	15.0 ± 0.9	15.0 ± 0.3	15.5 ± 0.5	15.8 ± 0.3	16.2 ± 0.9
17:0	0.4 ± 0.2	0.2 ± 0.0	0.3 ± 0.1	0.7 ± 0.2	0.7 ± 0.1
18:0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.1
Σ saturated	16.7 ± 0.5	16.1 ± 0.2	16.6 ± 0.5	17.6 ± 0.6	17.6 ± 0.9
16:1n-7	2.8 ± 0.0 ^b	2.5 ± 0.1 ^{ab}	2.4 ± 0.1 ^{ab}	2.1 ± 0.6 ^{ab}	1.9 ± 0.3 ^a
18:1n-9	27.7 ± 1.1	27.9 ± 1.0	29.5 ± 1.3	30.8 ± 2.2	29.3 ± 1.7
18:1n-7	7.1 ± 1.1 ^a	8.5 ± 0.5 ^a	9.0 ± 0.5 ^{ab}	9.0 ± 0.4 ^{ab}	9.4 ± 0.2 ^b
20:1n-9	1.0 ± 0.1	0.7 ± 0.4	0.8 ± 0.1	1.0 ± 0.2	1.3 ± 0.1
22:1n-11	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.4 ± 0.2	0.3 ± 0.1
Σ monoenes	39.0 ± 2.2	39.7 ± 1.4	41.9 ± 0.8	42.2 ± 2.9	42.2 ± 1.9
18:2n-6	33.9 ± 3.0 ^b	33.3 ± 1.6 ^b	29.9 ± 1.0 ^b	27.2 ± 4.4 ^{ab}	22.6 ± 2.1 ^a
20:2n-6	0.3 ± 0.0	0.4 ± 0.3	0.2 ± 0.1	0.2 ± 0.1	0.4 ± 0.3
20:4n-6	1.0 ± 0.1 ^a	1.1 ± 0.1 ^a	1.1 ± 0.1 ^a	1.2 ± 0.1 ^{ab}	1.3 ± 0.1 ^b
Σ n-6 fatty acids	35.2 ± 3.0 ^b	34.8 ± 1.9 ^b	31.2 ± 0.9 ^{ab}	28.6 ± 4.5 ^{ab}	24.3 ± 1.8 ^a
18:3n-3	0.2 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	0.4 ± 0.3	0.2 ± 0.1
18:4n-3	0.3 ± 0.2	0.1 ± 0.0	0.4 ± 0.3	0.3 ± 0.2	0.4 ± 0.2
20:4n-3	0.6 ± 0.0	0.5 ± 0.0	0.5 ± 0.1	0.6 ± 0.1	0.7 ± 0.1
20:5n-3	2.0 ± 0.2 ^a	2.5 ± 0.3 ^{ab}	2.7 ± 0.3 ^{ab}	3.0 ± 0.6 ^{bc}	3.6 ± 0.4 ^c
22:5n-3	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.2	0.3 ± 0.0
22:6n-3	3.4 ± 0.6 ^a	4.0 ± 0.3 ^a	4.6 ± 0.5 ^a	5.4 ± 1.4 ^a	8.7 ± 1.4 ^b
Σ n-3 fatty acids	6.6 ± 0.9 ^a	7.5 ± 0.6 ^{ab}	8.6 ± 1.1 ^{ab}	10.0 ± 2.4 ^{abc}	13.8 ± 2.0 ^c

Values are expressed as mean ± SE from triplicate groups (pooled livers of three fish per tank). Data with same alphabets are not significantly different ($P > 0.05$)

Table 7 Vitamin E concentration, TBARS value of blunt snout bream juvenile fed test diets for 9 weeks

	Diet group				
	E0	E50	E100	E300	E500
Vitamin E (mg/kg wet weight)	31.8 ± 10.4 ^a	43.0 ± 14.7 ^a	53.1 ± 2.8 ^{ab}	68.5 ± 6.6 ^{bc}	89.2 ± 1.9 ^c
TBARS (μmol MDA ¹ /mg wet weight)	40.5 ± 6.4	33.1 ± 10.0	30.9 ± 0.6	28.5 ± 8.9	28.0 ± 8.6

Values are expressed as mean ± SE from triplicate groups (pooled livers of five fish per tank). Data with same alphabets are not significantly different ($P > 0.05$)

¹ MDA malondialdehyde

E56.3 group). It indicated that an excess in vitamin E supplement did not further improve the growth performance of the fish. The similar cases also appeared in several previous studies (Chen et al. 2004; Yong et al. 2014; Hua et al. 2014). This suggested that 56.3 mg/kg vitamin E level reached a saturated requirement of blunt snout bream juvenile. In this study, the body weight gains in all groups were lower compared to our previous study using same fish species with similar initial weight (Li et al. 2015). This

could be because the feeding trial in this study was conducted under a relatively low water temperature (20–24 °C).

Hepatosomatic index (HSI) value has been reported to be correlated with the amount of fat deposition (Gao et al. 2012b), who reported that supplementation of vitamin E significantly reduced whole body fat content and HSI value in red sea bream (*Pagrus major*). Similarly, in this study, the highest HSI value was found in fish fed the E11.2 diet. But the value

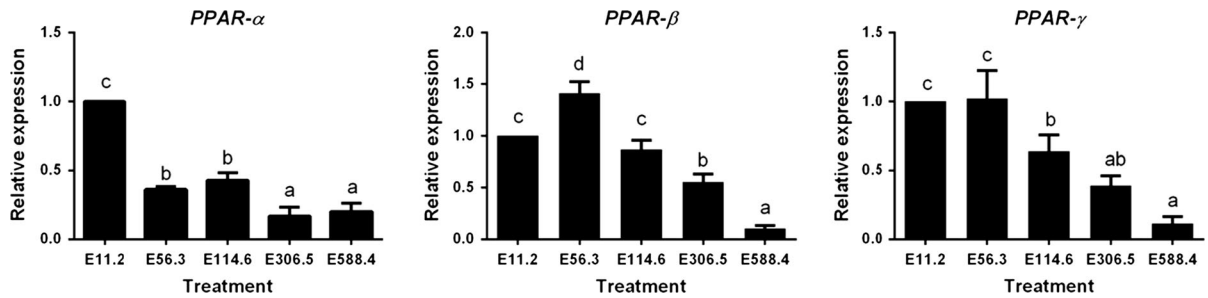


Fig. 2 Relative mRNA expression levels of PPARs (*PPAR-α*, *PPAR-β*, *PPAR-γ*) genes in liver (pooled livers of three fish per tank) of blunt snout bream fed different levels of vitamin E.

Different letters above the bars showed significant difference among different treatments ($P < 0.05$), data with same alpha-bets are not significantly different ($P > 0.05$)

decreased with incremental dietary supplementation of vitamin level, indicating that dietary vitamin E promoted liver fat metabolism. Vitamin E functions as a lipid-soluble antioxidant, protecting biological membranes and lipoproteins against oxidation. Baker and Davies (1996) reported that when fish were fed a diet without a vitamin E supplement, the lipid radicals could not be digested, which probably resulted in fatty infiltration into liver cells and enlarging the live size. Similar results were also found in halibut (*Hippoglossus hippoglossus*) (Tocher et al. 2003) and gilthead sea bream (*Sparus aurata*) (Mourente et al. 2002).

LC-PUFA, particularly EPA and DHA, are particularly sensitive to lipid peroxidation. Vitamin E plays an antioxidant role in cell membranes by preventing fatty acids and cholesterol against lipid oxidation. Therefore, it is believed that LC-PUFA must combine with vitamin E to protect against physiological oxidation (Hamre and Lie 1995; Gao et al. 2012b). In the present study, the percentages of liver EPA, DHA and total n-3 LC-PUFA significantly increased with increasing levels of dietary vitamin E from 11.2 to 588.4 mg/kg meanwhile DHA and total n-3 LC-PUFA had no significant differences among E11.2, E56.3, E114.6 and E306.5. In addition, it was interesting that DHA in the liver was 2.5-fold increased when vitamin E level increased from 11.2 to 588.4 mg/kg and hepatic n-3 LC-PUFA was more than doubled between the fish fed the extreme diets. The positive correlation between liver n-3 PUFA and dietary vitamin E level was also observed in channel catfish (*I. punctatus*) (Lim et al. 2010). Moreover, Lebold et al. (2011) reported that feeding zebrafish (*Danio rerio*) a vitamin E-deficient diet resulted in decreasing the percentage of n-6 and n-3 LC-PUFA of

fish viscera. Thus, the protective effect of vitamin E could be a factor contributing to the increase in liver n-3 LC-PUFA content (Lim et al. 2010). Several previous studies have reported that LC-PUFA synthesis is affected by the concentration of vitamin E. Lebold et al. (2011) observed that zebrafish fed a vitamin E-deficient diet had a higher expression of the genes responsible for LC-PUFA synthesis like *fads2* and *elovl2* than those fed a diet with adequate vitamin E.

Results from the present study showed that liver deposition of vitamin E increased directly in response to elevated dietary supplementation of vitamin E in blunt snout bream as reported for other fish species including rainbow trout (*S. gairdneri*) (Jumroensri et al. 2005), coho salmon (*Oncorhynchus kisutch*) (Chen et al. 2004), channel catfish (*I. punctatus*) (Bai and Gatlin 1993) and red sea bream (*Pagrus major*) (Gao et al. 2012b). Hepatic TBARS analysis is one of the most popular and widely used methods for assessing tissue peroxidation. Furthermore, malondialdehyde (MDA), as a secondary oxidation product of polyunsaturated fatty acids, is an important indicator of the oxidative changes of lipid (Mourente et al. 2000). Supplementation of dietary vitamin E could reduce the liver TBARS value in fish has been demonstrated in a number of studies (Gao et al. 2012b; Qi et al. 2000).

PPARs play a critical role in regulating insulin sensitivity and lipid metabolism. *PPAR-α* gene plays an important role in the control of lipid metabolism, by regulating the expression of target genes encoding enzymes involved in the peroxisomal and mitochondrial β -oxidation of fatty acids of blunt snout bream (Lu et al. 2013). *PPAR-β* induced fatty acid oxidation

by regulating genes involved in fatty acid transport, β -oxidation, and mitochondrial respiration in various tissues (Dressel et al. 2003; Yang et al. 2016). *PPAR- γ* plays an important role in lipid accumulation and adipocyte differentiation which are associated with insulin resistance and diabetes in humans (Stumvoll and Haring 2002). Overall, the expressions of *PPAR- α* , *PPAR- β* and *PPAR- γ* were down-regulated by supplementation of dietary vitamin E level from 56.3 to 588.4 mg/kg. The downregulation of three PPAR gene expressions in the fish fed diets with incremental vitamin E level might be associated with antioxidant effects of vitamin E. The n-6 and n-3 LC-PUFA have been shown to promote lipid oxidation by inducing *PPAR- α* (Jump 2002). Therefore, supplementation of dietary vitamin E increased tissues n-3 LC-PUFA against lipid oxidation through suppression expression of PPARs genes. To date, this was the first study to determine the effects of vitamin E supplementation on PPARs expressions in fish, and further studies are needed to investigate the reason for these effects.

The results of this study indicated that supplementation of dietary vitamin E could improve growth performance in blunt snout bream juveniles. When the broken-line model was employed to estimate the adequate requirement of vitamin E based on body weight gain, the optimal level was 55.5 mg/kg in diet. However, increasing the dietary vitamin E increased the percentages of liver EPA, DHA and total n-3 LC-PUFA and decreased the expression levels of PPARs in the liver of blunt snout bream. Reduced expressions of liver PPARs might be due to an increase of n-3 LC-PUFA by antioxidant effects of vitamin E. The mechanism of effects of dietary vitamin E on PPARs expression should be studied in future. In conclusion, supplementation of more than 55.5 mg/kg vitamin E may improve growth and increased n-3 LC-PUFA in blunt snout bream, which is beneficial to human consumer.

Acknowledgments This study was supported by the Fundamental Research Funds for the Central Universities of China (Project 2013PY074).

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