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Full Length Article

Effect of Dietary Vitamin E and Citric Acid Supplementation on the Antioxidation, Metabolism of Nucleic Acids and Growth of Juvenile Cobia

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Abstract

This experiment was conducted to investigate the effect of various dietary supplementation of VE or/and CA on the antioxidation, metabolism of nucleic acids, and growth performance of cobia (*Rachycentron canadum*) juveniles. Seven groups of cobia juveniles with 3 replicates per group were cultured in tanks in laboratory using filtered and aerated seawater and fed using 7 specific diets. The 7 diets were: one control diet, D0, with no VE and CA supplementation, and 6 experimental diets, D1 to D6, to which were added VE 100, 0,100, 75, 50, 25 IU and CA 0,12, 12, 6, 3, 1.5 g per kg of dried feed, respectively. The juveniles were fed for 12-week and sampled randomly for analysis at the initial and end of the experiment. The final average body length (BL), body weight (BW), specific growth rate (SGR), protein efficiency ratio (PER), total antioxidant capacity (T-AOC) and inhibiting hydroxyl radical capability (IHRC) in the muscle, livers, and serum of the fish fed the diets with both VE and CA supplementation were greater significantly (P<0.05) than the fish fed the diets with only VE or CA supplementation. These parameters of the fish fed D5 were the highest and greater significantly (P<0.05) than the fish fed other diets and D0. The average ratio of RNA:DNA in the muscle of the fish fed the diets with VE plus CA supplementation was higher significantly (P<0.05) than the fish fed D0 and the diets with only VE or CA supplementation, of which the RNA/DNA in the fish fed D5 was the highest. We concluded that dietary VE and/or CA supplementation could promote considerably the antioxidation, RNA/DNA, and growth performance of cobia juveniles, in which the optimum diet was D5, with 50 IU of VE and 3 g of CA supplements in our experiment. © 2018 Friends Science Publishers

Keywords: Supplement; Growth performance; Antioxidation; Metabolism; Cobia

Introduction

A new seawater species, cobia, is developed rapidly in subtropical and tropical areas (Holt *et al*., 2007; Ding *et al.*, 2017). However, it is a necessary to study further the nutritional biochemistry and physiology of cobia for its suitable artificial feed (Zhou *et al*., 2012; Ding *et al*., 2017).

The feed conversion rate, protein efficiency rate, growth, and survival rate of fish were reported to be promoted by dietary citric acid (CA) supplement (Sarker *et al*., 2007; Khajepour and Hosseini, 2010; Nagai *et al*., 2010; Sarker *et al*., 2012), which is defined as an environment-friendly feed additive (Su *et al*., 2014; Shah *et al*., 2015). Citric acid affects the metabolism, growth, and development of fish through the CA cycle since CA is an organic acid with multiple physiological functions and high nutritional value (Ng and Koh, 2016).

Dietary VE supplement was proved to promote the feed conversion rate, protein efficiency rate (Li and Gatlin III, 2009; Zhou *et al*., 2013), growth and survival rate of fish (Montero *et al*., 1999; Sealey and Gatlin III, 2002). Vitamin E could considerably promote the biochemical and physiological processes as well as status of antioxidation in fish (Kocabas and Gatlin III, 1999; Brigelius-Flohé *et al*., 2002; Khan and Thomas, 2004; Zhong *et al*., 2007). For fish, VE is an essential nutrient and have to be obtained from diet (Li *et al*., 2008; Li and Gatlin III, 2009; Zhou *et al*., 2013).

No report was on the effect of the supplementation of dietary VE plus CA in cobia (Zhou *et al.*, 2013). However, the combined VE and CA supplements were reported to promote synergistically the biochemical and physiological processes of animals by regenerating each other to amplify their functions (Xu *et al*., 2012).

In this experiment, the dietary supplementation of VE plus CA was supposed to function in the antioxidant capacity, metabolism, and growth performance of juvenile cobia. The study reports the effect of various dietary supplementation of VE or/and CA on the antioxidant capacity, metabolism of nucleic acids, feed utilization,

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growth performance, composition, and survival of cobia juveniles fed for 12 weeks, and discusses the working mechanism of dietary supplementation.

Materials and Methods

Materials

Fish oil, north pacific white fishmeal, containing protein > 65%, and VE, (±)-α-tocopherol from vegetable oil, type V, 1000 IUg^{-1} (Sigma T3634), were sourced from USA. The other materials were experimental grade from different companies in China.

Acclimatization and Culture of Experimental Fish

Cobia juveniles was approved for the experiment by an accredited Ethics Committee, Guangxi University, Nanning, China.

Two thousands (2000) cobia juveniles were from Seed-breeding Farm of Liusa, Zhanjiang, China and they were 22-d of age, with a body length (BL) ~3.68 cm and body weight (BW) ~2.50 g. The juveniles were firstly acclimatized for 2-week in tanks in laboratory as Ding *et al.* (2017).

After being acclimatized for 2-week, 457 juveniles, with BW 7.32 ± 0.26 g and BL8.02 ± 0.32 cm, were selected for formal experiment. Thirty seven (37) juveniles were sampled for 0 week and other 420 were assigned randomly into 7 groups in triplicate. Twenty (20) juveniles were put in a 400 L volume tank and total of 21 tanks. The juveniles were reared in laboratory using the same filtered and aerated seawater as acclimatization (Ding *et al.*, 2017). The seawater, with dissolved oxygen content ≥ 6 mg L^{-1}, salinity 27 – 30 gL^{-1}, pH 7.8 – 8.0, 26 – 32°C, was from Beibu-gulf, Fangcheng, China. The juveniles were reared and treated as Ding *et al.* (2017). The juveniles were fed one of 7 diets, including one control diet, D0, which had no VE and CA added, and 6 experimental diets, D1 to D6, included VE 100, 0,100, 75, 50, 25 IU and CA 0,12, 12, 6, 3, 1.5 g supplementation per kg of dried feed, respectively (Table 1). This experimental design was according to Ding *et al.* (2009, 2017), Cheng *et al*. (2012) and Pohlenz *et al*. (2012), in which about 11% of lipid and 47% of protein in the diets (Table 1). The additional quantity of VE was referenced the experiments of Li *et al*. (2014), Ding *et al.* (2017), in which fish requirement of VE was 6.25—200 IU per kg of dried feed. The additional quantity of CA was based on the experiment of Sarker *et al.* (2007), in which 10 g CA per kg of dried feed or less was for an efficient supplement level in alternative plant protein source diets.

The juveniles were fed twice daily at about 5% of the total wet weight of fish at the feeding time using pellets made freshly as the method of Ding *et al.* (2017). The feeding experiment was lasted for 12-week.

Sampling and Chemical Analysis

The juveniles were sampled at the initial and end of the experiment. At 0 week, 9 fish were randomly sampled for whole fish and 28 fish for organs/tissues. At the end of week 12, 3 juveniles each tank were freely sampled for whole fish and another 6 juveniles for tissues/organs. The whole fish, muscle, liver, and blood were sampled and stored as the methods of Ding *et al.* (2017).

The methods of Peng *et al*. (2009) and Weikle (2012) were respectively applied to analyze the concentration of VE and CA in the diets, using a high-performance liquid chromatography (HPLC, Agilent 1200, Agilent Company, USA).

The method of Xu *et al.* (2017) was employed to analyze the fatty acids of the feed using a gas chromatograph (GC, Agilent 6890 N, USA).

The methods of the Association of Official Analytical Chemists (AOAC, 1995) were used to analyze the moisture content, ash, crude protein, and fat of the feed and the whole fish.

Extraction and Determination of DNA and RNA

The method of Zheng *et al*. (2009) was applied to extract and quantify DNA and RNA in the tissues/organs of the juveniles.

Determination of Protein and Antioxidant Index

A sample, 0.3 g of liver or muscle sample of the juveniles, was grounded into powder in a mortar under liquid nitrogen and homogenized in 1.5 mL of 0.65% sodium chloride in a glass homogenizer at 4℃. The homogenate was centrifuged for 10 min at 12000 g at 4℃ to obtain supernatant for assay.

The method of Bradford (1976) was used to analyze the protein in the tissues/organs of fish.

The IHRC, T-AOC, and MDA in above supernatant and serum were determined respectively using a spectrophotometer and assay kits produced by Nanjing Jiancheng biological company, China according to the User's Manual (Tian *et al*., 2011; Yu *et al*., 2011).

Calculation and Statistics

Weight gain (WG, g) = FBW − IBW;

Specific growth rate (SGR, % day^{-1}) = 100 × ln (FBW − IBW)/day;

Feed conversion ratio (FCR) = feed intake (g)/WG (g);

Crude protein intake = [feed intake (g) × crude protein contained in the feed (%)]/day;

Protein efficiency ratio (PER) = WG (g)/crude protein intake (g);

Hepatopancreas somatic indice (HSI, %) = liver weight (g)/BW (g) × 100;

Table 1: Ingredient of experimental diets for cobia juveniles

Ingredient[a]	Diet (g kg^{-1} of dried feed)						
	D0	D1	D2	D3	D4	D5	D6
White fish meal	560.00	560.00	560.00	560.00	560.00	560.00	560.00
Soybean	200.00	200.00	200.00	200.00	200.00	200.00	200.00
Casein	50.00	50.00	50.00	50.00	50.00	50.00	50.00
Granulesten	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Fish oil	50.00	50.00	50.00	50.00	50.00	50.00	50.00
Vitamin mixture (VE-free)[b]	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Mineral mixture[c]	15.00	15.00	15.00	15.00	15.00	15.00	15.00
α-starch	100.00	100.00	100.00	100.00	100.00	100.00	100.00
VE (IU kg^{-1} of dried feed)	0	100.00	0	100.00	75.00	50.00	25.00
CA (g kg^{-1} of dried feed)	0	0	12.00	12.00	6.00	3.00	1.50
pH and proximate composition (%)							
pH	6.98	6.98	1.83	1.83	2.18	2.41	2.57
Moisture	7.62	7.10	7.27	7.22	7.63	7.17	7.92
Ash	11.23	11.69	11. 91	11.74	11.95	11.99	11.76
Crude protein	47.08	47.06	47.12	46.97	47.04	46.95	47.00
Crude lipid	11.52	11.55	11.35	11.38	11.60	11.32	11.48
VE (IUkg^{-1} of dried feed)	25.70	125.25	25.74	125.43	100.30	75.15	50.30
CA (g kg^{-1} of dried feed)	0	0	11.87	11.89	5.95	2.97	1.48
Composition (%) of fatty acids[d]							
∑SFAs	27.10	27.61	27.56	27.65	27.58	27.74	27.57
∑MUFAs	35.05	34.88	35.10	34.86	34.93	34.90	35.02
∑n-6PUFAs	9.16	9.13	9.14	9.15	9.08	9.07	9.17
∑n-3 PUFAs	28.41	28.02	27.94	28.09	28.06	27.96	27.91
∑PUFAs	37.89	37.53	37.42	37.59	37.50	37.37	37.43
∑n-3HUFAs	26.59	26.31	26.17	26.33	26.32	26.21	26.19
N-3/n-6	3.10	3.07	3.06	3.07	3.09	3.08	3.04
DHA+EPA	24.17	23.98	23.82	24.01	24.02	23.87	23.87
DHA/EPA	0.56	0.57	0.56	0.57	0.56	0.57	0.57

[a]The ingredient of D0, the abbreviation of control diet, was basic composition of feed, which had no VE and CA added. D1 to D6 are abbreviation of diets 1 to 6, to which were added VE 100, 0, 100, 75, 50, 25 IU and CA 0, 12, 12, 6, 3, 1.5 g per kg of dried feed, respectively. The same below

[b]Vitamin mixture: vitamin A, 80 000 IU; vitamin D_3, 40 000 IU; vitamin K_3, 120 g; vitamin B_1, 150 mg; vitamin B_2, 320 mg; vitamin B_6, 300 mg; vitamin B_{12}, 2 mg; niacin nicotinic acid, 15 mg; potassium pantothenic, 720 mg; folic acid, 40 mg; biotin, 2 mg; inositol, 2 000 mg; vitamin C, 1000 mg; choline chloride, 10 000 mg (Ding *et al.*, 2017)

[c]Mineral mixture: iron, 160 mg; zinc, 600 mg; manganese, 40 mg; copper, 200 mg; iodine, 10 mg; magnesium, 200 mg; cobalt, 20 mg; molybdenum, 20 mg (Ding *et al.*, 2017)

[d]MUFAs is the abbreviation of monounsaturated fatty acids. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; PUFAs, polyunsaturated fatty acids; HUFAs, highly unsaturated fatty acids

In which FBW is the final body weight (g) and IBW is the initial body weight, measured in grams (Ding *et al*., 2009, 2017).

All data were done analysis of two-way ANOVA and variance (ANOVA) using SPSS 18.0 for windows. The data were further compared using Duncan's multiple range test. The analysis of monodic linear regression was performed among the mean ratio of RNA:DNA in the muscle, liver, and serum of the juveniles and their mean BW and SGR, respectively, using Excel 2010 for windows.

Results

Changes in Cobia Juveniles

Changes of the growth performance and feed utilization: The final average body length (BL) of the fish fed the diets with both VE plus CA supplementation was longer than the fish fed the diets with only VE or CA supplementation. The final average BL of the D5 fish was the longest and differed significantly (P<0.05) from the fish fed D0 and other diets, as shown in Table 2.

The final mean BW of the fish fed the diets with VE plus CA supplementation was heavier than the fish fed the diets with VE or CA supplementation alone. The final mean BW of the D5 fish was the heaviest and differed significantly (P<0.05) from the fish fed D0 and other diets (Table 2).

The average specific growth rate (SGR) of the fish fed the diets with VE plus CA supplementation was greater than the fish fed the diets with only VE or only CA added. The average SGR of the D5 fish was the greatest and differed significantly (P<0.05) from the fish fed D0 and other diets (Table 2).

The average hepatopancreas somatic indice (HSI) of the fish fed the diets with VE plus CA supplementation was lower than the fish fed the diets with only VE or CA supplementation. The average HSI of the D5 fish was the lowest and differed significantly (P<0.05) from the fish fed D0 and other diets (Table 2).

The average feed conversion ratio (FCR) of the fish fed the diets with VE plus CA supplementation was lower than the fish fed the diets with a single VE or CA

Table 2: Effect of different dietary VE and/or CA supplement(s) on the body length (BL), body weight (BW), specific growth rate (SGR), hepatopancreas somatic indice (HSI), feed conversion ratio (FCR), and protein efficiency ratio (PER) of cobia juveniles fed for 12 weeks

Diet	D0	D1	D2	D3	D4	D5	D6
BL(cm)	26.92 ± 1.16^{d}	28.02 ± 2.17^{d}	27.83 ± 1.21^{d}	30.27 ± 1.20^{c}	32.36 ± 1.22^{b}	34.82 ± 1.17^{a}	32.04 ± 2.19^{b}
BW(g)	163.66 ± 5.56^{d}	171.05 ± 4.06^{d}	167.27 ± 6.20^{d}	214.75 ± 7.75^{c}	294.66 ± 5.47^{b}	418.84 ± 7.18^{a}	308.70 ± 8.02^{b}
SGR (%day^{-1})	3.70 ± 0.16^{d}	3.75 ± 0.18^{d}	3.72 ± 0.20^{d}	4.02 ± 0.23^{c}	4.40 ± 0.10^{b}	4.82 ± 0.12^{a}	4.45 ± 0.20^{b}
HSI (%)	3.33 ± 0.21^{a}	2.90 ± 0.16^{b}	2.87 ± 0.19^{b}	2.15 ± 0.13^{c}	1.71 ± 0.13^{d}	1.38 ± 0.12^{e}	1.87 ± 0.14^{d}
FCR	2.03 ± 0.16^{a}	1.94 ± 0.12^{a}	1.98 ± 0.09^{a}	1.53 ± 0.12^{b}	1.10 ± 0.10^{c}	0.77 ± 0.08^{d}	1.05 ± 0.13^{c}
PER	1.05 ± 0.14^{d}	1.10 ± 0.10^{d}	1.07 ± 0.08^{d}	1.39 ± 0.12^{c}	1.93 ± 0.09^{b}	2.76 ± 0.12^{a}	2.02 ± 0.15^{b}

All above data are means ± SE (n = 3 × 3 × 3). Different superscript letters in the same row indicate significant differences among groups (P<0.05)

T**able 3:** Effect of different dietary VE and/or CA supplement(s) on the composition (% wet weight) of whole cobia juveniles fed for 12 weeks

Diet	D0	D1	D2	D3	D4	D5	D6
Crude protein	17.97± 0.48	17.81 ± 0.58	18.49 ± 0.99	18.42 ± 0.50	18.19 ± 0.60	18.12 ± 1.03	18.26 ± 1.26
Ash	3.75 ± 0.73	3.72 ± 0.46	3.92 ± 0.67	3.80 ± 1.01	3.76 ± 0.79	3.71 ± 0.73	3.66 ± 0.45
Crude fat	8.27 ± 0.91	7.97 ± 0.62	8.34 ± 0.78	8.15 ± 1.07	8.07 ± 0.62	8.33 ± 0.86	8.27 ± 0.58
Moisture	71.06 ± 2.54	71.59 ± 2.85	70.62 ± 3.86	71.04 ± 2.56	71.12 ± 3.66	71.29 ± 4.92	69.98 ± 2.76

All data are mean ± SE (n = 3 × 3). The data with different superscript letters in the same row indicate significant difference (P<0.05) among the groups

Table 4: Effect of different dietary VE and/or CA supplement(s) on the ratio and amount amount (μg mg^{-1}, but μg mL^{-1} for serum) of RNA and DNA in the muscle, liver, and serum of cobia juveniles fed for 12 weeks

	Diet	D0	D1	D2	D3	D4	D5	D6
Muscle	RNA	8.62 ± 0.25^{d}	8.93 ± 0.19^{d}	8.78 ± 0.31^{d}	11.52 ± 0.12^{c}	13.48 ± 0.22^{b}	15.86 ± 0.15^{a}	13.62 ± 0.28^{b}
	DNA	3.78 ± 0.18^{a}	3.75 ± 0.15^{a}	3.80 ± 0.13^{a}	3.78 ± 0.09^{a}	3.82 ± 0.19^{a}	3.83 ± 0.20^{a}	3.75 ± 0.16^{a}
	RNA/DNA	2.28 ± 0.16^{d}	2.38 ± 0.09^{d}	2.31 ± 0.13^{d}	3.05 ± 0.08^{c}	3.53 ± 0.16^{b}	4.14 ± 0.16^{a}	3.63 ± 0.12^{b}
Liver	RNA	19.98 ± 0.78^{c}	23.82 ± 0.45^{b}	24.83 ± 0.36^{ab}	24.94 ± 0.69^{ab}	25.24 ± 0.65^{a}	23.54 ± 0.58^{b}	22.65 ± 0.63^{b}
	DNA	4.83 ± 0.23^{a}	4.53 ± 0.40^{a}	4.74 ± 0.26^{a}	4.83 ± 0.50^{a}	4.62 ± 0.24^{a}	4.58 ± 0.33^{a}	4.39 ± 0.18^{a}
	RNA/DNA	4.14 ± 0.36^{b}	5.26 ± 0.38^{a}	5.24 ± 0.42^{a}	5.16 ± 0.80^{a}	5.46 ± 0.34^{a}	5.14 ± 0.52^{a}	5.16 ± 0.23^{a}
Serum	RNA	4.95 ± 0.12^{b}	4.61 ± 0.19^{c}	4.81 ± 0.22^{bc}	5.03 ± 0.30^{ab}	5.08 ± 0.16^{ab}	5.26 ± 0.26^{a}	4.97 ± 0.32^{b}
	DNA	0.38 ± 0.01^{a}	0.37 ± 0.02^{a}	0.36 ± 0.02^{a}	0.43 ± 0.02^{a}	0.38 ± 0.01^{a}	0.41 ± 0.02^{a}	0.35 ± 0.02^{a}
	RNA/DNA	13.03 ± 0.75^{bc}	12.46 ± 0.83^{c}	13.36 ± 0.58^{b}	11.70 ± 0.63^{d}	13.37 ± 0.90^{b}	12.83 ± 0.66^{bc}	14.20 ± 0.71^{a}

All above data are mean ± SE (n = 3 × 3 × 3). The data with different superscript letters in the same row indicate significant difference among data (P<0.05)

supplementation. The average FCR of the D5 fish was the lowest and differed significantly (P<0.05) from the fish fed D0 and other diets (Table 2).

The average protein efficiency ratio (PER) of the fish fed the diets with VE plus CA supplementation was greater than the fish fed the diets with only VE or CA supplementation. The average PER of the D5 fish was the greatest and differed significantly (P<0.05) from the fish fed D0 and other diets (Table 2).

Changes of the survival and composition: The survival rate of the fish fed the diets with various supplementation of VE or/and CA for 12 weeks was 100%, which was the same as the D0 fish.

The moisture content, ash, crude fat, and protein of whole fish were not different significantly (P>0.05) among the fish fed various supplementation of VE or/and CA and D0 (Table 3).

Changes of the metabolism of nucleic acids: The metabolism of nucleic acids in the juveniles was considerably promoted by dietary VE or/and CA supplement (s). The average ratio of RNA:DNA in the muscle of the juveniles fed the diets with VE plus CA supplementation was higher than the fish fed the diets with VE or CA supplementation alone. When the mean ratio of RNA:DNA in the muscle of the fish fed the diets with various dietary VE or/and CA supplementation was regressed against their mean BW and SGR, $R^2_{BW} = 0.95$ and $R^2_{SGR} = 0.99$, showing linear positive correlation. However, no positive linear correlation was found between the mean ratio of RNA:DNA in the livers and serum of the fish and their mean BW,or SGR. The highest average ratio of RNA:DNA was in the muscle of the D5 fish, the livers of the D4 fish, and the serum of the D6 fish, in which their average ratio of RNA:DNA differed significantly (P<0.05) from the D0 fish, respectively (Table 4).

Changes of the IHRC, T-AOC, and MDA: The mean inhibiting hydroxyl radical capability (IHRC) level in the serum, muscle, and liver of the fish fed the diets with VE plus CA supplementation was greater significantly (P<0.05) than the fish fed the diets with only VE or CA supplementation, respectively. The greatest mean IHRC was found in the muscle and liver of the D5 fish and the serum

Table 5: Effect of different dietary VE and/or CA supplement (s) on the total antioxidant capacity (T-AOC, $Umgprot^{-1}$, but UmL^{-1} for serum), inhibiting hydroxyl radicalcapability (IHRC, $Umgprot^{-1}$, but UmL^{-1} for serum), and malondialdehyde (MDA, $nmolmgprot^{-1}$, but $nmolmL^{-1}$for serum) in the muscle, liver, and serum of cobia juvenilesfed for 12 weeks

	Diet	D0	D1	D2	D3	D4	D5	D6
Muscle	T-AOC	1.53 ± 0.12^{e}	1.94 ± 0.09^{d}	1.99 ± 0.14^{d}	2.38 ± 0.16^{c}	2.96 ± 0.08^{b}	3.62 ± 0.13^{a}	2.90 ± 0.14^{b}
	IHRC	18.98 ± 0.52^{e}	25.12 ± 0.49^{d}	26.02 ± 0.18^{d}	32.68 ± 0.65^{c}	41.82 ± 0.26^{b}	50.78 ± 0.52^{a}	40.65 ± 0.72^{b}
	MDA	1.55 ± 0.05^{a}	1.18 ± 0.04^{b}	1.13 ± 0.04^{b}	0.92 ± 0.05^{c}	0.71 ± 0.03^{d}	0.42 ± 0.03^{e}	0.73 ± 0.02^{d}
Liver	T-AOC	6.05 ± 0.12^{e}	7.53 ± 0.63^{d}	7.26 ± 0.15^{d}	8.93 ± 0.73^{c}	10.87 ± 0.48^{b}	13.63 ± 0.42^{a}	10.52 ± 0.23^{b}
	IHRC	130.33 ± 5.46^{e}	162.72 ± 3.89^{d}	156.34 ± 4.68^{d}	190.95 ± 5.32^{c}	223.53 ± 7.02^{b}	278.89 ± 8.24^{a}	231.76 ± 8.14^{b}
	MDA	0.83 ± 0.02^{a}	0.49 ± 0.02^{b}	0.53 ± 0.01^{b}	0.33 ± 0.01^{c}	0.20 ± 0.01^{d}	0.14 ± 0.02^{e}	0.19 ± 0.01^{d}
Serum	T-AOC	6.86 ± 0.52^{e}	9.45 ± 0.42^{c}	8.03 ± 0.26^{d}	10.94 ± 0.56^{b}	12.98 ± 0.18^{a}	11.12 ± 0.36^{b}	10.72 ± 0.48^{b}
	IHRC	1005.86 ± 42.03^{d}	1002.03 ± 20.56^{d}	998.96 ± 28.98^{d}	1158.98 ± 32.68^{c}	1392.87 ± 32.88^{a}	1231.56 ± 25.32^{b}	1112.62 ± 35.64^{c}
	MDA	0.30 ± 0.02^{a}	0.19 ± 0.02^{b}	0.20 ± 0.01^{b}	0.15 ± 0.02^{c}	0.10 ± 0.01^{d}	0.15 ± 0.02^{c}	0.14 ± 0.02^{c}

All above data are mean ± SE (n = 3 × 3 × 3). The data with different superscript letters in the same row indicate significant difference among data (P<0.05)

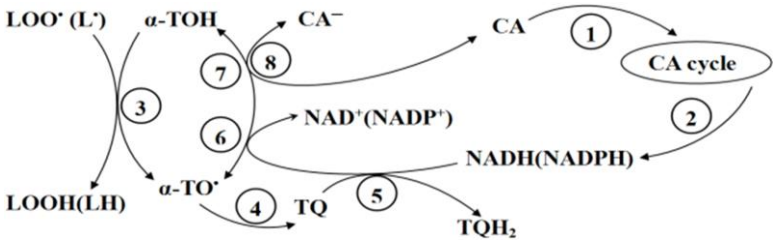

Fig. 1: Hypothesis on the regeneration or transformation of VE and CA, in which LH is the abbreviation of polyunsaturated lipid; L·, lipid radical; LOO·, lipid peroxyl radical; LOOH, lipidhydroperoxide; *α-TOH,* α-tocopherol;α-TO·, α-tocopheroxyl radical; TQ, α-tocopherolquinone;TQH_2, α-tocopherol hydroquinone. In the figure, ①indicates CA to participate in CA cycle and ②to produce NADH (NADPH) (Mailloux *et al.*, 2007; Kiliaan and Hageman, 2010); ③*α-TOH* becomes α-TO·after providing H^+ to reduce LOO·as LOOH (Brigelius-Flohé, 2009); ④α-TO·is further oxidated to beTQ (Siegel *et al*., 2004); ⑤TQ is reduced to be TQH_2 by NADH (NADPH) (Siegel *et al.*, 2004) ;⑥α-TO· is reduced to beα-TOH by NADH (NADPH) (Sikka, 2004);⑦CA provides H^+ to reduce α-TO·as α-TOH (Choe and Min, 2009); ⑧α-TOH provides H^+ to reduce CA^- to be CA (Selvakumar *et al.*, 2011)

of the D4 fish, in which their mean IHRC differed significantly (P<0.05) from the fish fed D0 and other diets, respectively (Table 5).

The mean total antioxidant capacity (T-AOC) in the serum, muscle, and liver of the fish fed the diets with VE plus CA supplementation was greater significantly (P<0.05) than the fish fed the diets with a single VE or CA supplementation, respectively. The greatest mean T-AOC was found in the muscle andliverof the D5 fish and the serum of the D4 fish, in which their mean T-AOC differed significantly (P<0.05) from the fish fed D0 and other diets, respectively (Table 5).

The mean malondialdehyde (MDA) in the serum, muscle, and liver of the fish fed the diets with VE plus CA supplementation was lower significantly (P<0.05) than the fish fed the diets with only VE or CA supplementation, respectively. The lowest mean MDA was found in the muscle and liver of the D5 fish and the serum of the D4 fish, in which their mean MDA differed significantly (P<0.05) from the fish fed D0 and other diets, respectively (Table 5).

Discussion

The effect of VE plus CA supplementation (D3 to D6) on the growth performance, feed utilization, metabolism of nucleic acids, and antioxidant capacity of cobia juveniles was found to be greater significantly than VE (D1) or CA supplementation alone (D2). The facts show that the supplementation of VE plus CA functioned cooperately and promoted synergistically the utilization of feed, growth performance, metabolism of nucleic acids, and anti-oxidation in the juveniles. The reasons are supposed to be: both VE and CA could cooperate to promote the digestion, absorption, utilization of nutrients, and to protect the growth performance from oxidation and disease. Vitamin E and CA could cooperately inhibit lipid peroxidation by removing effectively superoxide anion (O_2^-) (Vareltzis and Hultin, 2007; Vareltzis *et al*., 2008; Brigelius-Flohé, 2009). Both VE and CA decompose and remove reactive oxygen free radicals produced in the process of catabolism and interrupting lipid peroxidation (Vareltzis and Hultin, 2007; Vareltzis *et al*., 2008; Brigelius-Flohé, 2009). If both CA and VE were supplied, CA might inhibit LOOH produced by VE breaking off the free radical chain reaction in lipid peroxidation, in which LOOH is an active substance with very strong oxidation (Pokorny *et al*., 2000; Brigelius-Flohé, 2009; Xu *et al*., 2012). Usually, CA does not work as a main antioxidant, but it functions synergistically as a good antioxidant. Additionally, VE and CA could regenerate each

other to amplify their functions. Moreover, VE (α-TOH) and NADH (NADPH) produced by CA cycle (Mailloux *et al*., 2007; Kiliaan and Hageman, 2010) could form a new compound, α-tocopherol hydroquinone (TQH_2), of which antioxidant capacity is 5 times of α-TOH (Siegel *et al*., 2004). Therefore, VE and CA could function with increasing and complementary effects in the metabolism of nucleic acids, utilization of feed, antioxidation, and growth performance of cobia juveniles (Fig. 1). The effects of juvenile cobia fed D5 were the greatest and significantly greater than the fish fed other diets with VE plus CA supplementation. The results imply that higher or lower VE and CA simultaneously in the diets **(**D3, D4 and D6**)** were not ideal for cooperately promoting the growth performance, feed utilization, metabolism of nucleic acids, and antioxidant capacity of the juveniles. Citric acid could provide a suitable acidic environment for VE function (Table 1). Therefore, it can be concluded that the optimum VE and CA supplements in fish feed should be beneficial. In the experiment, D5, with the supplementation of VE 50 IU and CA 3 g per kg of dried feed, was the best beneficial, and correlates well with the report of Cai *et al*. (2013), in which CA supplement was generally limited to not more than 0.5% in the fish feed. The CA supplementation was 2.0-3.0 g per kg of dried feed for carp (*Carassius auratusgibelio)* (Leng *et al*., 2006) and white shrimp (*Litopenaeus vannamei)* (Su *et al*., 2014).

The diet with VE supplement alone (D1) promoted considerably the antioxidant capacity in the analytical organs/tissues of the juveniles in the experiment. Vitamin E supplement could considerably promote the antioxidant capacity in the serum, liver, and muscle of cobia by scavenging reactive oxygen species (ROS) since VE is a very effective ROS scavenger in biological systems (Brigelius-Flohé, 2009; Xu *et al*., 2012). Vitamin E could promote antioxidant status (Singh *et al*., 2013) and break off the free radical chain reaction in lipid peroxidation (Brigelius-Flohé, 2009; Xu *et al*., 2012) as well as produce less MDA. However, the growth performance of the juveniles was not significantly improved in the fish fed a single VE supplementation in the diet (D1). Li and Gatlin III (2009) also reported weight gain of juvenile red drum was not responsive to dietary VE.

The effect of CA supplementation alone (D2) in juvenile cobia was similar to that of VE supplementation alone (D1). The reasons for this are supposed to be: the antioxidation and antistress could be promoted by CA in juvenile cobia. As an effective antioxidant, CA effectively remove the superoxide anion and chelate metal ions to break off the free radical chain reaction in lipid hydroperoxide (Choe and Min, 2009; Xu *et al*., 2012). However, the growth performance of juvenile cobia was not significantly promoted in the fish fed the diet with CA supplement alone (D2). It is consistent with the report of Sarker *et al*. (2007), in which the specific growth rate (SGR) of red sea bream (*Chrysophrys major*) was not significantly improved in the fish fed 1 or 2% CA supplementation in the diets.

The effect of different dietary VE and/or CA supplementation on the ratio of RNA:DNA in the muscle of juvenile cobia was found to differ significantly ($P < 0.05$) from that in the serum and liver of fish in the experiment. The mean ratio of RNA:DNA in the muscle was found to have a linear positive correlation to the mean BW, $R^2_{BW} = 0.95$, and SGR, $R^2_{SGR} = 0.99$, when regressed against each other. However, the mean ratio of RNA:DNA in the serum and liver of juvenile cobia was not found to have a linear positive correlation to the mean BW and SGR. When the fish is growing, RNA is continuously synthesized and the RNA/DNA ratio of the fish is also changing in all organs/tissues. However, their RNA/DNA ratios are different from organ/tissue to organ/tissue since their functions are different from each other. The liver of fish is main metabolic organ and blood is an exchanging media, whereas the fish muscle is a main tissue to indicate the growth of the fish. The RNA/DNA ratio of the muscle is correlative to the weight of the fish and indicates the situation of growth and nutritional condition. The present results are similar to our former report (Xu *et al*., 2009), in which the RNA/DNA ratio in the muscle of cobia juveniles fed eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) had a more obvious positive relationship with the mean BW than in the liver. The ratio of RNA:DNA in the muscle of juvenile cobia fed arachidonic acid (AA) was also found to display a more obvious positive relationship with the mean BW than in the liver and serum (Liu, 2008). The serum, liver, and muscle are the representative tissues/organs of fish, which reflect the effect of the feed nutrients on the juvenile by examining the changes of the physiological and biochemical processes in these tissues/organs.

The effects of various dietary VE and/or CA supplements on MDA, IHRC and T-AOC in the serum of juvenile cobia varied and differed from those in the muscle and liver. This was because nutrients are continuously exchanged in serum, which is a medium of nutrients. On the other hand, the enzymes and other contents of serum vary with time, nutrient, physiology, temperature, and other factors (Ding *et al*., 2017). Whereas the muscle and liver are quite stable organs/tissues.

Conclusion

In summary, dietary VE and CA supplementation could promote considerably the growth performance, utilization of feed, metabolism of nucleic acids, and antioxidant capacity of cobia juveniles; the optimum was the D5, with a VE of 50 IU and a CA of 3 g added.

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