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Effects of vitamin E on growth and maturation in the Asian catfish (*Clarias macrocephalus*) at puberty

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Abstract

The maturation of male fish is a problematic aspect of *Clarias macrocephalus* and probably one of the causes of the population's disappearance in the usual places where they once abound. The present study aimed to explore feed additives to enhance its maturation from puberty. Vitamin E (VE) is essential in the reproduction of animals, including fish. A growth trial was conducted in which groups of *C. macrocephalus* were fed experimental diets containing graded levels of VE in the form of α-tocopheryl acetate (α-TA), namely, 0, 100, 200, 300 and 400 mg VE·kg^{-1} diet. After 90 days, growth and maturation were assessed, and reproductive performance was tested by conducting induced spawning and artificial fertilization. Results showed growth and nutrient utilization indices were better in groups fed diets containing VE than in the control group. Maturation indices such as GSI, gonad weight, lengths, egg diameter, and fecundity were enhanced in the VE-treated groups. Reproductive performance, such as fertilization, hatching, and larval survival rate, was enhanced in *C. macrocephalus* broodstock in VE-treated groups. Quantitative estimates for optimal VE dose that elicited maximal response values ranged from 202.1 to 230.0 mg VE·kg^{-1} diet.

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Introduction

Vitamin E (VE) is a fat-soluble antioxidant that play important role in fish growth, reproduction, and fertility. It has eight different forms; the most important one is α-tocopherol which has the most significant effect on fish health and growth (Ahmed et al., 2021). VE plays a leading role in reproductive fitness, and it is considered necessary for maternal and paternal fish diets, especially before spermatogenesis (Canyurt and Akhan, 2008).

VE is known to be important in the reproduction of fish species. Increasing VE in the diet increases spawning success, egg survival, hatchability, and larval survival of Ayu (*Plecoglossus altivelis*), red sea bream (*Pagrus australis*), increases the gonadosomatic index and vitellogenesis of common carp (*Cyprinus carpio*) (Watanabe and Takashima 1977; Kanazawa, 1985) and bighead carp (*Aristichthys nobilis*) (Santiago and Gonzal 2000). Watanabe (1985) showed that the VE content is high in eggs and low in the tissues of brood fish after spawning, suggesting some physiological functions of this vitamin in spawning, fertilization, and hatching. Deficiency symptoms in fish include delayed ovarian development in carp (Watanabe and Takashima, 1977) and decreased egg hatchability and fry survival in ayu (Takeuchi et al., 1981). Most VE deficiency signs observed in fish are related to the peroxidative damage to the cellular membrane such as nutritional muscular dystrophy, fatty liver degeneration, anemia, exudative diathesis, erythrocyte hemolysis, hemorrhages, depigmentation and reduction of fertility (National Research Council, 1983).

Currently, there is no known area in the Philippines where *Clarias macrocephalus*, a native catfish in the country, are being cultured because of their gradual disappearance from the usual places where they used to abound. A few freshwater fish farmers try to maintain the broodstock of this catfish for use in their production of hybrids. These farmers maintain the broodstock of *C*. *macrocephalus, using* only the female broodstock to be crossed with male African catfish. This is done because maturation in male *C. macrocephalus* is very problematic, and also hybrids exhibit hybrid vigor for fast growth and less vulnerability to diseases. Some researchers have thought about propagating pure *C. macrocephalus* and intended to do some reintroduction of this species. Artificial fertilization in pure species of *C. macrocephalus* involves sacrificing two to three male native catfish for every female catfish, which could result in a skewed sex distribution in the long run. This could be detrimental to the remaining *C. macrocephalus* population that they may end up extinct faster than by leaving it alone. What may be needed is to explore the possibility of making the male *C. macrocephalus* healthy and making maturation conditions better and timelier through nutritional strategies. We have demonstrated previously that we could enhance both growth and maturation by incorporating dietary benfotiamine (Bautista et al., 2022a) or dietary 17α-methyltestosterone into the diet of male catfish (Bautista et al., 2022c) at puberty. We also demonstrated the effect of VE on fry and juvenile *C. macrocephalus* to enhance growth performance, feed utilization, and survival, as well as elevated its resistance to abrupt shift to higher salinity exposure (Bautista et al., 2022b). The minimum requirement ranges for dietary α-TA to elicit optimum growth and survival under salinity stress were 170.23 - 233.3 mg·kg^{-1} α-TA and 200 mg·kg^{-1} α-TA to 276.75 mg·kg^{-1} α-TA, respectively. In the present study, our objective was to assess VE on its effects on the growth and maturation of both male and female C. *macrocephalus* during puberty.

Materials and Methods

Experimental fish and set up

A total of 120 immature native catfish (ABW=103.94±1.01g) were randomly distributed into 15 rectangular tanks (61 cm x 122 cm x 61 cm) filled with shallow

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water (about 250 L) in a static culture and well-aerated system. Eight catfish were stocked in each tank consisting of 4 males and 4 females with an average body weight of 103.94 ± 1.01 g. Fish were acclimatized to controlled conditions and commercial diets for one week. Siphoning off of waste was done daily, and water was replaced 3 times a week.

Experimental diets

Five experimental diets were prepared: a basal diet with no α-tocopheryl acetate (TA) and diets containing 100 mg, 200 mg, 300 mg, and 400 mg α-TA. All diets contained ~44.0% crude protein and ~2.6% crude fat. Feed composition is shown in **Table 1.**

Table 1 Composition of experimental diets.

Ingredients	*α TA (mg)*				
	0	*100*	*200*	*300*	*400*
Fish meal (sardines)	496.00	496.00	496.00	496.00	496.00
Soybean meal	267.00	267.00	267.00	267.00	267.00
Corn starch	125.00	125.00	125.00	125.00	125.00
Cod liver oil	22.00	22.00	22.00	22.00	22.00
Vitamin mix	25.00	25.00	25.00	25.00	25.00
Mineral mix	25.00	25.00	25.00	25.00	25.00
CMC	40.00	39.90	39.80	39.70	39.60
Alpha Tocopherol	0.00	0.10	0.20	0.30	0.40
Total	1000.00	1000	1000	1000	1000
	Proximate analysis (% dry weight)				
Moisture	7.70				
Crude Protein	43.68				
Crude Fat	2.62				
Crude Fiber	1.61				
NFE	23.41				
Ash	2.07				

[a] Vitamin mix (estimated amount in 25 g mix per kg diet): Vitamin A, 9 mg; Vitamin D3, 1.25 mg; Vitamin B1, 200 mg; Vitamin B2, 200 mg; Vitamin B6, 125 mg; Vitamin B12 50 mcg/kg; Niacin, 1000 mg; Calcium Pantothenate, 500 mg; Biotin, 1 mg; Folic Acid, 45 mg; Ethoxyquin, 12.5 mg; a-TA at 0, 100, 200, 300, 400 mg·kg-1 diet.
[b] Mineral mix (estimated amount in 25 per kg diet): Fe, 1000 mg; Mn, 250 mg; Zn, 1000 mg; Cu, 100 mg; Cu, 100 mg; I, 45 mg, Co, 0.5 mg; Se, 1 mg.

Growth performance parameters

To assess the growth performance of native catfish, the weight gain (WG), specific growth rate (SGR), feed intake (FI), feed conversion ratio (FCR), and survival rate (SR) were computed using the following formula:

Weight Gain (WG, g) = Final Average Body Weight (FABW) – Initial Average Body Weight (IABW)

Specific Growth Rate (SGR, % day^{-1}) = 100*[Ln (FABW)–Ln (IABW)]/[(No. of days)]

Feed Intake (FI, g) = sum of daily feed offered for the whole trial period (g)

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Feed Conversion ratio (FCR) = Total Feed Intake (g)/Weight Gain (g)

Survival Rate (%) = 100*[(Total no. of fish survived)/(Total no. of fish stocked)]

Indices of first maturation

Dissection of the liver and testis of the fish was done by cutting the ventral part of the fish's body using a scalpel and scissors. Dissected liver and pair of testes were placed in a petri dish in which length and weight (g) were measured. The formula used to compute gonadosomatic and hepatosomatic indices was as follows:

Gonadosomatic Index (GSI, Testes/Ovary) (%) = 100*(Testes/Ovary weight (g) / Body weight (g))

Hepatosomatic Index (HSI, %) = 100*(Liver weight (g) / Body weight (g))

The liver and gonads (i.e., ovary or testis) were excised, and their length and weight were measured. Batch fecundity was estimated for each female fish by taking samples consisting of three portions from one lobe of the ovary (~0.50 g), and ripe eggs were counted. This procedure was done assuming that the left and the right ovaries have the same egg density, such that extrapolation could be made for the total weight of the ovary. The ovulation rate was calculated using the percentage of ovulated catfish females among all injected females.

Induced Spawning and Artificial Fertilization

C. macrocephalus females were injected intramuscularly with a commercial solution of gonadotropin and dopamine inhibitor (0.5 $ml \cdot kg^{-1}$; Ovaprim, Syndel Lab. Ltd., USA) into the dorsal muscle above the lateral line and below the anterior part of the dorsal fin. Stripping of eggs was performed the following morning. Male catfish were sacrificed, the testis excised, cut into small pieces, and squeezed through a gauze onto the stripped eggs contained in a small bowl; the remaining portion of the testis on the gauze was also used by washing with a small amount of distilled water. The mixture of macerated testis and ovulated eggs was then gently stirred with disinfected chicken feathers for about 5 min, and the fertilized eggs were laid out onto improvised egg mats (30 cm x 30 cm soft nylon mosquito net firmly held on by a PVC tube frame) for incubation on a well-aerated tank.

Reproductive performance

Counting of fertilized eggs was done at least 12 h after incubation. The fertilization rate was calculated as the percentage of fertilized eggs (i.e., the total number of eggs minus the number of nonviable eggs that appeared opaque and whitish) over the total number. Hatching rate was calculated as the percentage of viable eggs on the mosquito net over the total number of fertilized eggs. Survival rate of offspring was calculated as the percentage of eggs that produced live larvae plus those larvae that survived in 7 days after hatching over the total number of hatched larvae. The formulae for the indices described above are given as follows:

Fecundity, F = (No. of eggs in the sample / Sample weight) x Gonad weight

Ovulation (%) = 100*(No. of fish ovulated / Total no. of fish injected)

Fertilization Rate (%) = 100*(No. of fertilized eggs / Total no. of eggs in a batch)

Hatching Rate (%) = 100*(No. of eggs hatched / Total no. of fertilized eggs)

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Larval survival Rate (%) = 100*(Total no. of survived larvae until day 7 / Total no. of larvae at day 1)

Estimation of optimum hormone dosage and statistical analysis

Data were analyzed by fitting a quadratic regression equation used in fish to estimate protein and amino acids (Chiu et al., 1988; Zeitoun et al., 1976). This model was deemed appropriate for treating almost all hyperbolic data in which the response parameters peaked and declined past the highest level of the independent variable. In this method, a quadratic equation is used to fit the response data obtained from feeding a dietary series:

$$R = a + bI + cI^2$$

Where R is the measured response; I is the dietary nutrient concentration; and a, b, and c are constants that are calculated to provide the best fit of the data. The value of I that produces the maximum response I_{max} is calculated as follows:

$$I_{max} = -0.5\ (b/c)$$

The standard error of the mean (SEM) was calculated for all mean values. Data were subjected to analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT) to determine differences in means ($p<0.05$). All statistical analyses were done with the Statistical Package for the Social Sciences (SPSS) Version 24 software (Chicago, Illinois, USA).

Results

Water Quality Parameters

Ranges of average values for various indices of water quality are presented in **Table 2**. No significant water quality fluctuation was observed during the study, and the indices were optimum for the average growth of *C. macrocephalus.*

Table 2 Means of water quality parameters monitored during the experiment.

	Water Quality Parameters					
α- TA *(mg)*	DO (ppm)	Temp. (°C)	pH	*NH_3/NH_4 (ppm)*	*NO_2 (ppm)*	*NO_3 (ppm)*
0	4.94±0.06	26.20±0.02	6.52±0.02	0.32±0.04	0.07±0.04	4.41±0.54
100	4.92±0.12	26.15±0.06	6.51±0.01	0.33±0.05	0.11±0.03	4.33±0.67
200	5.04±0.05	26.21±0.06	6.54±0.03	0.31±0.09	0.09±0.01	4.39±0.53
300	4.91±0.03	26.27±0.08	6.55±0.01	0.39±0.03	0.08±0.00	4.44±0.56
400	4.98±0.02	26.26±0.07	6.53±0.02	0.36±0.06	0.10±0.02	4.38±0.52

Growth Performance

FABW of fish was significantly higher in all VE groups than those in the control group, with those in 200 mg·kg^{-1} α-TA exhibiting the highest FABW value ($P < 0.05$) (**Table 3**). In this decreasing order, fish in the 100 mg·kg^{-1} group showed significantly higher WG and SGR values, followed by 200, 300, 400, and 0 mg·kg^{-1} groups ($P < 0.05$), in this decreasing order.

Table 3 Growth performance of *Clarias macrocephalus* at puberty, fed with diets containing different levels of vitamin E (α-TA).

α- TA (mg)	*IABW (g)*	*FABW (g)*	*WG (g)*	*SGR (%·day^{-1})*
0	102.46±1.40 ^{a}	133.04±0.80 ^{a}	30.58±0.97 ^{a}	0.29±0.01 ^{a}
100	100.83±2.44 ^{a}	147.96±2.46 ^{cd}	47.13±3.64 ^{d}	0.43±0.04 ^{c}
200	106.54±2.15 ^{a}	151.71±2.98 ^{d}	45.17±1.49 ^{cd}	0.39±0.01 ^{bc}
300	104.79±3.36 ^{a}	144.88±2.05 ^{bc}	40.08±2.04 ^{bc}	0.36±0.03 ^{bc}
400	105.08±1.30 ^{a}	141.33±0.71 ^{b}	36.25±0.81 ^{ab}	0.33±0.01 ^{ab}

Values in the same column with different superscript letters are significantly different ($p<0.05$). Values were expressed as mean + SEM; IABW – initial average body weight; FABW – final average body weight; WG – weight gain; and SGR – specific growth rate.

Feed Utilization and Survival

No significant differences in FI values were observed among the dietary groups. In contrast, catfish in all VE groups exhibited significantly better feed conversion ratio (FCR) than did the control catfish, with the 100 mg·kg^{-1} group exhibiting significantly the lowest (i.e., best) FCR value ($P < 0.05$) (**Table 4**). All groups exhibited a 100% survival rate throughout the feeding trial.

Table 4 Feed intake, feed conversion ratio, and survival of catfish fed the experimental diets for 90 days.

α-TA (mg)	*FI (g)*	*FCR*	*Survival (%)*
0	94.59±1.09 ^{a}	3.10±0.09 ^{c}	100.00±0.0 ^{a}
100	100.10±1.42 ^{a}	2.15±0.13 ^{a}	100.00±0.0 ^{a}
200	103.33±4.65 ^{a}	2.29±0.06 ^{ab}	100.00±0.0 ^{a}
300	99.53±2.69 ^{a}	2.50±0.15 ^{ab}	100.00±0.0 ^{a}
400	95.24±0.89 ^{a}	2.63±0.08 ^{b}	100.00±0.0 ^{a}

Values in the same column with different superscript letters are significantly different ($p<0.05$). Values were expressed as mean + SEM; FI – feed intake; and FCR – feed conversion ratio.

Maturation at the termination of the experiment

GSI and HSI values of Male C. macrocephalus

The length and weight measurements of male testes and liver, as well as the gonadosomatic index (GSI) and hepatosomatic index (HIS) at the termination of the experiment, are summarized in **Table 5**. Testis of male catfish fed VE-supplemented diets were significantly heavier than those fed the basal diet ($P < 0.05$). Similarly, the GSI values of the male catfish in all VE-treated groups were significantly higher than those of the control male catfish ($P < 0.05$). Male liver weight (LW), liver length (LW), and HSI values of male catfish were significantly higher in VE groups than of those male catfish fed the basal diet ($P < 0.05$). The male catfish exhibited the highest values in all the indices mentioned in the 200 mg·kg^{-1} group.

Table 5 Length and weight measurements of testes and liver, GSI and HSI of male native catfish after 90 days of feeding.

a- TA (mg)	*Testes weight (g)*	*Testes length (cm)*	*GSI (male)*	*Male LW (g)*	*Male LL (cm)*	*HSI (male)*
0	0.33±0.03 ^{a}	2.20±0.05 ^{a}	0.25±0.03 ^{a}	0.50±0.06 ^{a}	1.40±0.12 ^{a}	0.38±0.04 ^{a}
100	0.57±0.03 ^{b}	2.47±0.03 ^{a}	0.39±0.03 ^{b}	0.82±0.04 ^{b}	2.07±0.18 ^{b}	0.56±0.04 ^{b}
200	0.63±0.03 ^{b}	2.53±0.03 ^{a}	0.42±0.02 ^{b}	1.00±0.12 ^{b}	2.03±0.09 ^{b}	0.66±0.06 ^{b}
300	0.55±0.02 ^{b}	2.48±0.02 ^{a}	0.38±0.02 ^{b}	0.83±0.03 ^{b}	1.83±0.03 ^{b}	0.57±0.01 ^{b}
400	0.52±0.04 ^{b}	2.50±0.03 ^{a}	0.36±0.03 ^{b}	0.85±0.09 ^{b}	2.08±0.14 ^{b}	0.59±0.05 ^{b}

Values in the same column with different superscript letters are significantly different (p<0.05). Values were expressed as mean ± SEM; GSI=gonadosomatic index; HSI=hepatosomatic index; LW=liver weight (g); LL=liver length (cm).

Ovary, GSI, and HSI of Female C. macrocephalus

Ovary weight (OW), ovary length (OL), and GSI values of female catfish in the 200 and 100 mg·kg^{-1} groups fed VE-supplemented diets were significantly higher than those of the control female catfish (P < 0.05); those in the 100, 200 and 300 mg·kg^{-1} groups exhibited that were statistically similar (P > 0.05).

Table 6 Length and weight measurements of ovary and liver, GSI and HSI of female native catfish after 90 days of feeding.

a-TA (mg)	*Female OW (g)*	*Female OL (cm)*	*GSI*	*Female LW (g)*	*Female LL (cm)*	*HSI*
0	7.62±0.78 ^{a}	4.27±0.14 ^{a}	5.88±0.58 ^{a}	0.50±0.06 ^{a}	1.47±0.03 ^{a}	0.39±0.04 ^{a}
100	14.27±2.28 ^{bc}	5.70±0.26 ^{c}	9.57±1.45 ^{c}	0.77±0.03 ^{c}	1.40±0.10 ^{a}	0.52±0.02 ^{a}
200	14.90±0.75 ^{c}	5.57±0.24 ^{c}	9.65±0.38 ^{c}	0.72±0.06 ^{bc}	1.63±0.09 ^{a}	0.47±0.04 ^{a}
300	11.63±0.52 ^{bc}	5.35±0.20 ^{bc}	7.99±0.33 ^{ab}	0.73±0.12 ^{bc}	1.55±0.09 ^{a}	0.50±0.08 ^{a}
400	10.55±0.53 ^{ab}	4.72±0.37 ^{ab}	7.62±0.35 ^{ab}	0.48±0.08 ^{a}	1.38±0.06 ^{a}	0.35±0.06 ^{a}

Values in the same column with different superscript letters are significantly different (p<0.05). Values were expressed as mean ± SEM; GSI=gonadosomatic index; HSI= hepatosomatic index; OW = ovary weight; OL = ovary length; Female LW=liver weight of female catfish; Female LL=liver length of female(cm).

Fecundity, Egg Diameter, and Ovulation Rate

The fecundity and egg diameter of female C. *macrocephalus* in all VE groups were significantly higher than those of the control female catfish (P < 0.05), indicating maturation (**Table 7**). However, there were no significant differences among the groups (P < 0.05) concerning ovulation rate.

Table 7 Fecundity, egg diameter, and ovulation rate of native catfish fed the experimental diets for 90 days.

a- TA (mg)	*Fecundity (x10^{3})*	*Egg Diameter (mm)*	*Ovulation Rate (%)*
0	3.55±0.38 ^{a}	1.00±0.06 ^{a}	55.56±11.11 ^{a}
100	6.87±1.15 ^{b}	1.20±0.00 ^{b}	100.00±0.0 ^{a}
200	6.95±0.38 ^{b}	1.20±0.00 ^{b}	88.89±11.11 ^{a}
300	5.67±0.31 ^{b}	1.17±0.03 ^{b}	77.78±11.11 ^{a}
400	5.01±0.25 ^{ab}	1.10±0.06 ^{ab}	88.89±11.11 ^{a}

Values in the same column with different superscript letters are significantly different (p<0.05). Values were expressed as mean ± SEM

Reproductive Performance at First Maturation

Fertilization Rate, Hatching Rate, and Larval Survival Rate

The overall reproductive performance of *C. macrocephalus* in the VE groups was significantly better than that of the control catfish (P < 0.05) (**Table 8**). Significantly highest FR values were exhibited by the 100, 200, and 300 mg·kg^{-1} groups which were statistically similar (P < 0.05). HR and LSR values were statistically similar in all VE groups and higher than the control catfish.

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Table 8 Effects of vitamin E (α -TA) on the reproductive performance of native catfish (*Clarias macrocephalus*).

α- TA (mg)	*Fertilization rate (%)*	*Hatching rate (%)*	*Larval survival rate (%)*
0	51.60±2.21 ^{a}	40.14±2.37 ^{a}	40.36±1.42 ^{a}
100	64.68±0.69 ^{c}	54.18±0.98 ^{bc}	53.87±1.35 ^{bc}
200	68.74±1.58 ^{c}	59.38±2.59 ^{c}	56.11±0.62 ^{c}
300	66.45±0.90 ^{c}	53.90±0.55 ^{bc}	52.58±1.09 ^{bc}
400	58.80±1.76 ^{b}	50.99±1.26 ^{b}	51.01±1.41 ^{b}

Values in the same column with different superscript letters are significantly different ($p<0.05$). Values were expressed as mean ± SEM.

Optimum values that elicited the highest values for somatic growth, maturation, and reproductive response indices of the *C. macrocephalus* at puberty were calculated employing the quadratic regression equation (**Table 9**). The range of the optimum values for all responses was in the range of 202.1 to 230.0 mg·kg^{-1} α-TA.

Table 9 Response parameters fitted into a quadratic regression equation to estimate I_{max} (i.e., optimum dietary vitamin E requirement).

Response parameter	*Quadratic regression eqn.*	R^2	*(I_{max})*
Weight gain (g)	$y = -0.0003x^2 + 0.1297x + 32.714$	0.7736	216.2
Testis weight (g)	$y = -5E\text{-}06x^2 + 0.0023x + 0.3509$	0.8913	230.0
GSI male (%)	$y = -3E\text{-}06x^2 + 0.0013x + 0.2623$	0.8985	216.2
Ovary weight (g)	$y = -0.0001x^2 + 0.0585x + 8.3843$	0.7997	220.0
GSI female (%)	$y = -7E\text{-}05x^2 + 0.0301x + 6.3534$	0.7511	215.0
Egg diameter (mm)	$y = -4E\text{-}06x^2 + 0.0018x + 1.0186$	0.8962	225.0
Fecundity (X10^3)	$y = -7E\text{-}05x^2 + 0.0283x + 3.9346$	0.8135	202.1
Hatching rate (%)	y = -0.0003x2 + 0.1488x + 41.065	0.9203	248.0
Fertilization rate (%)	y = -0.0003x2 + 0.1528x + 51.99	0.9928	254.7
Larval survival rate (%)	y = -0.0003x2 + 0.1227x + 41.651	0.8827	204.5

Discussion

This was the first study ever conducted on the dietary VE requirement of *Clarias macrocephalus* at puberty. The study lasted 90 days, and no disease incidence or mortality occurred. All growth and feed utilization indices of juvenile fish were better in the VE groups than in the control group. These growth and feed utilization indices revealed that the optimum VE dose was calculated at 216.2 mg·kg^{-1} α-TA using the quadratic regression equation.

α-Tocopheryl acetate (α-TA) is the primary VE source used for the fortification of feed in aquaculture, intending to improve the growth, resistance to stress and disease as well as the survival of fish and shrimp (Cowey et al., 1983; Boggio et al., 1985; Shakar et al., 2018). Qualitative improvement of somatic growth observed in the present study in *C. macrocephalus* at puberty when VE was supplemented may be similar to other growth studies in their positive effects. However, the high quantitative VE optimum dose in the present study was not comparable with most growth studies. But when the comparison is made with those involving maturing fish species, our quantitative results are in line with their observations. In the Nile tilapia, the developmental stage affects the dietary VE requirement for optimal growth and the diet's total lipid and fish oil content. Almost three times higher VE level (300 mg α-TA·kg^{-1} diet) than the juvenile stage was reported to be necessary for optimal growth of Nile tilapia adults fed with a diet prepared by soybean oil and 10% total lipid content (Nascimento et al., 2014). Also, twice this amount in the Nile tilapia was found insufficient with a diet prepared by only fish oil and 9.2% total lipid content (Gammanpila et al., 2007). Survival in the eying stage of the fertilized eggs was reduced when ayu (*Plecoglossus altivelis*)

were fed diets with low α-tocopherol levels (Takeuchi et al. 1981). Gupta et al. (1991) demonstrated that feeding *Cyprinus carpio* with VE-supplemented diets showed higher GSI, bigger ova, and more eggs. The histological studies demonstrated that when α-tocopherol-deficient ovaries did not accumulate yolk granules or vesicles in oocytes, oocyte development was retarded (Watanabe and Takashima 1977).

Some studies reported no effects of VE-supplemented diets. For example, after feeding Nile tilapia broodstocks with diets containing VE for 5 months, GSI and egg diameter were not enhanced (Gammanpila et al., 2007). King et al. (1983) showed no differences in rainbow trout growth, pre-spawning mortality rate, egg development, or egg hatchability between the two diets, one containing α-tocopherol and one lacking it. High concentrations of α-tocopherol in the Atlantic salmon broodstock diet did not increase the survival of eggs and fry (Eskelinen, 1989). In pandani *Pseudotropheus socolofi*, a freshwater ornamental fish, the developmental stage did not affect the dietary VE requirement (Erdogan & Arslan, 2019).

The high VE requirement in the present study may have stemmed from the requirement being measured at puberty (i.e., a period that covers the immature to mature stage). This period is characterized by processes such as the formation of gonads which accumulate lipids which in turn require VE. E.g., unsaturated fatty acids such as arachidonic acid (ARA) were preferentially accumulated in tongue sole (*Cynoglossus semilaevis*) gonad lipids during maturation (Xu et al., 2017), and its concentrations were highest in gonad liver and muscle lipids of mature male fish and lowest in mature female fish. Compared with female tongue sole, males had higher DHA concentrations in gonad lipids but lower concentrations in liver lipids. It has been suggested that an elevation of unsaturated fatty acids can invoke an increased requirement for VE as intracellular antioxidants to protect the cells (Halliwell and Chirico, 1993).

An early study on the Nile tilapia by Areechon et al. (2003) reported that the higher VE (600 mg·kg^{-1}) in the diet increased the number of spawners. This dose is much higher than the present study's highest dose (400 mg·kg^{-1}). Little information is available on the adverse effects of megadoses of VE on fish growth or reproduction. Excess tocopherol appears harmless because it can be easily stored in lipids (Guillaume et al. 2001). However, Tokuda and Takeuchi (1995) showed that excess doses of α-tocopherol might induce lipid peroxidation in the tissues of rainbow trout. In the Nile tilapia (Gammanpila et al., 2007) and milkfish *Chanos chanos* (Emata et al., 2000), there were no significant effects of supplementation of VE on the spawning percentage, spawning frequency, and total spawns hapa^{-1} at 500 and 600 mg·kg^{-1}, respectively. Emata et al. (2000) pointed out that the total egg production, the mean number of eggs per spawning, and number of spawns were not affected by supplementation of 500 mg·kg^{-1} VE in combination with vitamin C in the diet of milkfish.

GSI values give a clear indication of the gonadal development as well as the readiness of the fish to breed. The maximum GSI values for either male or female C. *macrocephalus* in the present study were elicited at 215-216.2 mg·kg^{-1}. In *Clarias batrachus*, the highest GSI was observed in the 100 mg·kg^{-1} group, followed by 200, 0, and 50 mg·kg^{-1} (Roy and Mollah, 2009). In goldfish *Carassius auratus*, 100 mg·kg^{-1} VE was reported suitable for stimulating the development of gonads and spawning (Sanchai-Sutjaritvongsanon, 1987). VE supplementation of 219.3 mg·kg^{-1} diet) yields twice the number of eggs in ornamental fish pandani, *Pseudotropheus socolofi* (Erdogan and Arslan, 2019). Also, GSI values in *Heteropnestes fossilis* brood fish were highest in fish with 100 mg VE·kg^{-1} feed, whereas in fecundity and ovulation rate, a dose of 200 mg·kg^{-1} feed proved to be best (Mollah et al., 2003).

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For the effects of VE on fertilization, hatching, and larval survival, very few reports have been done. The present study obtained the best values at 254.7, 248.0, and 204.5 mg VE·kg^{-1} diet. In pandani (*P. socolofi*), all reproductive indices were improved, including pre- and post-larval survival rates at 100 mg·kg^{-1} VE (Erdogan and Arslan, 2019). A much earlier report on *C. batrachus* obtained the best fertilization and hatching rate of the eggs at 50 mg·kg^{-1} VE (Roy & Mollah, 2009), while better hatchability and larval survival in 'ayu' *Plecoglossus altivelis* broodfish were obtained at 34 mg VE·kg^{-1}diet. In *Heteropneustes fossilis*, better fertilization rate, hatching rate of the eggs, and larval survival rate were produced by broodfish fed 200 mg VE·kg^{-1}diet.

In conclusion, the present study showed that supplementing VE (α-TA) in the diets of Clarias macrocephalus during puberty enhanced growth, maturation, and reproductive performance. Optimal dietary levels of VE for various response criteria ranged from 202 to 230 mg VE·kg^{-1} diet.

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