

*Research Article***Effects of different dietary levels of vitamin E supplementation on the growth and ovarian development of Climbing Perch, *Anabas testudineus*****Hasan, M.M.¹, Zahangir, M.M.¹, Akhter, F¹ and M.M. Islam^{1*}**¹ Department of Fish Biology and Biotechnology, Chattogram Veterinary and Animal Sciences University, Khulshi, Chattogram-4225, Bangladesh**ARTICLE INFO***Article history :*

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*Keywords :**Anabus testudineus*, Vitamin E
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Vitamin E is one of the most important micronutrients that influence the performance of fish reproduction. The experiment was carried out for 3 months in aquarium with recirculation facilities in the wet laboratory adjacent to the Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University to observe the effects of different dietary level of vitamin E on growth and gonadal development of *Anabus testudineus*. One and half months aged 300 *A. testudineus* were stocked and divided into five treatments each having three replicates. The fish were fed with feed having different levels of vitamin E viz. 0 mg (as control), 50 mg, 100mg, 200 mg, and 400 mg vitamin E/kg feed as treatment-1 to treatment-5, respectively. Fish fed with 50 mg vitamin E/kg feed (under T₂) showed highest (9.12±0.23 g) weight gain in terms of body growth while fishes treated with 0 mg vitamin E kg⁻¹ feed (under T₁) gave the poorest result (6.85±1.32 g). There was no significant difference (p>0.005) between the treatment groups. Gonado-somatic index (%) was highest (0.696±0.44) in fish treated with 100 mg vitamin E/kg feed (under T₃) while 400 mg vitamin E/kg (under T₅) showed the poorest effect (0.311±0.03). No significant difference (p>0.005) was found between the treatment groups. The results of this experiment suggest that inclusion of 100 mg vitamin E/kg feed is the best dose for enhancing gonadal development of *A. testudineus*. The findings indicate that vitamin E content may have a positive impact on gonadal development of this species. The present results also imply that inclusion of higher level of vitamin E exerts an antagonistic effect in terms of growth and gonadal development of this species.

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1. INTRODUCTION

Bangladesh has the third greatest aquatic fish biodiversity in Asia, due to the contributions of the three main rivers systems that flow from the Himalayas into the Bay of Bengal along with the world's largest

flooded wetland, the Bengal Delta (Hussain, 2010). Small Indigenous Species (SIS) have high vitamin A content and serve as an important source of dietary calcium, as the fish are usually cooked and consumed whole, including bones (Ahmed, 2012). Among the small indigenous fishes, climbing perch (*A.*

testudineus) locally known as koi is considered to be the most economic and important fishes. The climbing perch (*A. testudineus*, Bloch, 1792) is a well-known member of the Anabantoidae family which derived their name for bearing labyrinth like accessory breathing organ. Among the small indigenous fishes, climbing perch (*A. testudineus*) locally known as koi and is considered to be the most economic and important fish. It is regarded as a highly esteemed food fish for its fine flavour, restorative value and prolonged freshness out of water. Considering the importance of this species in nutritional, economics and biodiversity the cultivation of *A. testudineus* is becoming increasingly popular among the aquaculturists of Bangladesh. In spite of having many qualities such as high digestibility of protein, presence of vitamin, iodine and fat in muscle, very little attempt has been made to promote its breeding and culture of Koi. For the supply of quality seeds in sufficient numbers the brood fish must be of good quality. So in case of broodstock management, there should be regular supply of balanced food for their growth and development. For the initiation of study on the nutrition it is necessary to determine whether spawning and egg quality are influenced by nutritional quality of broodstock diets or not.

Nutrition in the diet of brood fish is known to have a profound effect on gonad development, fecundity, quality of eggs and larvae. Although precise information on the nutritional requirements of broodstock for gonadal maturation is scanty, it has been found that quantity and quality of feed as well as the feeding regime is important for maintenance of egg quality and successful spawning. Vitamins are one of the most effective additives to nutritionally complete diets for fish production (Gaylord *et al.*, 1998). Vitamin E is recognized as an essential vitamin required for all classes of animals functioning predominantly as an intracellular antioxidant in maintaining the integrity of biological cell membranes (Hidiroglou *et al.*, 1992). As a fat-soluble vitamin, it is the most effective chain-breaking, lipid-soluble antioxidant in biological membranes, where it contributes to membrane stability. In a study of freshwater fish, *Cyprinus carpio* it was found that vitamin E caused higher gonadosomatic index, larger ova and more eggs than a control (Gupta *et al.*, 1987). The importance of vitamin E in fish reproduction has been reported by many scientists (Watanabe *et al.*, 1970; Halver, 2002 and Paul *et al.*, 2004)

Due to over fishing and climatic change sufficient number of fry and fingerlings of *A. testudineus* is, however, quite difficult to obtain from natural waters for stocking in the ponds. So the dependency of this fish fry and fingerling is increasing on hatchery production. So manipulation of breeding performance of *A. testudineus* can increase the seed production to fulfill the requirement of fish farmer. Considering the above realities, the present research work was undertaken to observe the effect of vitamin E on growth and gonadal development of Koi, *A. testudineus*. The aim of the research was to find the effect of different dietary levels of vitamin E on growth and ovarian development of *A. testudineus*.

2. MATERIALS AND METHODS

Description of experimental site and system

The experiment was conducted in aquarium with recirculation facilities in the Wet Laboratory of the Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University, Chattogram for a period of 3 months. The experimental system consists of 15 rectangular glass aquaria each of size (60×30×45 cm) containing about 75L of water. Two big sized glass aquariums were used for conditioning and stocking of fish. All the aquaria were placed in a metal frame for easier handling and for better observation and accessibility. Underground water from deep tube well was used in the aquaria during experimental period. An adequate level of oxygen in each aquarium was maintained through artificial aeration using aerators.

Preparation of recirculatory system

The entire recirculatory system was consisted of two identical unit. Each unit had 8 glass aquaria. Water was supplied from 250L water tank by 1 HP water pump (RFL, model: RSJ 10M) through recirculatory pipe. In the recirculatory system to filtrate the water a biological filter was used. The biological filter drums contained filter sponge that promoted settling of wastes by increasing retention time and provided a substrate for attachment of nitrifying bacteria. Two different sized and two different layer gravel stones were used in biological filter for filtration of water. A charcole layer was also used to remove dissolved organic molecules, chemicals, chlorine and chloramine and certain heavy metals. In that system the water passed the entire filter layer by water pressure from bottom anti-gravitationally. About 10% of the total water in the system was flashed out and replenished with tap water daily.

Collection of fish and stocking

Two months aged experimental species *A. testudineus* were collected from Hazrat Owlish Fokir Matsho Unnoyon Hatchery (Pvt.) Ltd., Potiya, Chattogram. The collected fish were acclimatized in conditioning tank for 10 minutes. Then fish were released gently in conditioning tank for 6 days before stocking in the treatment aquaria. During conditioning sufficient oxygen supply was maintained through artificial aeration. Then healthy, strong and more or less equal sized fishes were used for the experiment. The fishes were then randomly released into different treatment groups after proper conditioning. Twenty fishes were stocked in each aquarium. Before stocking, weight of every individual fish was taken by digital precision electric balance.

Experimental design

To observe the effect of vitamin E on the growth and ovarian development, 15 aquariums were divided into five groups containing 3 aquariums in each group. These five groups corresponded to five experimental treatments (T₁, T₂, T₃, T₄, and T₅) and each treatment group had three replicates. Each aquarium stocked with 20 fishes. Feed with five different levels of vitamin E viz. 0 mg (served as control), 50 mg, 100 mg, 200 mg and 400 mg vitamin E/kg feed were administered for studying the growth and gonadal development of fish.

Feed formulation

Fish meal, soybean meal, mustard oil cake, rice bran, corn meal, fish oil, wheat flour, vitamin and mineral premix were used for the preparation of feed. The source of vitamin E was Esco Sel-E Plus from Escopharma. A feed containing around 35% protein were prepared keeping all the ingredients same except vitamin E. To do this, required amount of finely ground and sieved ingredients were weighed as per formulae with a digital precision electric balance and the required amount of vitamin E were added and mixed thoroughly. Feed was formulated by Pearson square method (Table 1). After mixing all the ingredients, adequate amount of water was added and converted into pellets by pelleting machine. During preparing feed with different vitamin E doses, the pellete machine and related equipments were washed thoroughly to avoid any cross contamination. Then the pellets were dried under sunlight and stored in the plastic bag in air tight condition and kept in refrigerator. After formulation of feed, proximate composition of the formulated feeds was analyzed according to standard procedures given by Association of Official Analytical Chemists (AOAC, 1980). Proximate composition of feed showed in Table 2.

Table 1: Composition of experimental feed (Pearson square method)

Ingredients	Inclusion level (%)	Protein content in ingredients (%)	Protein contents in diets CP (%)
Fish meal	35	60	21
Mustard oil cake	23	28	6.44
Rice bran	9	12	1.08
Wheat flour	9	14	1.26
Soybean meal	10	45	4.5
Corn meal	9	8	0.72
Fish oil	3	-	-
Vitamin premix	3	-	-
Binder	1	-	-
Vitamin E	***	-	-
Total	100.00		35.00
***Vitamin E inclusion level= (T ₁ =0, T ₂ =50, T ₃ =100, T ₄ =200, T ₅ =400) mg/kg feed			

Table 2: Proximate composition of feed (% dry matter basis)

Treatment	Dry matter (%)	Lipid (%)	Protein (%)	Ash (%)	Crude fibre (%)	Moisture (%)	NFE*
T ₁	91.98	7.41	34.13	10.51	5.49	8.02	34.44
T ₂	88.32	6.74	33.16	11.17	5.34	11.68	31.91
T ₃	92.62	9.17	33.69	10.64	5.26	7.38	33.86
T ₄	90.50	9.13	33.25	11.22	5.32	9.50	31.58
T ₅	90.85	8.72	33.00	10.99	5.08	9.15	33.06

*NFE=Nitrogen-free extract

Feeding and sampling of the experimental fish

Fishes were fed two times in a day at around 9.00 a.m and 5.00 p.m. Feeding rate was 4% of total fish body weight basis. Sampling was done at every 15 days interval. During first sampling bulk weight was taken from all treatments. Then 6 fishes from each treatment (two fishes from each replicates) were taken randomly. Prior to weighing, fish were caught with a fine mesh scoop net and excess water was then removed from fish body by gently blotting on a soft tissue paper. Weighing was done by using digital precision electric balance.

Estimation of gonado-somatic index

For the estimation of gonado-somatic index two fishes from each replication were taken. The total length and weight of each fish was recorded separately. Then fishes were killed by pithing on head. After that fishes were dissected and carefully removed the upper fat layer in side the body cavity by forcep and brush. Then carefully gonad was taken out from the fish. Weighing of gonad was done by using digital precision electric balance and gonado-somatic index was calculated. After that gonad was preserved in a plastic airtight vial with 10% formalin. During sampling fish were handled very carefully. The aquaria were washed and cleaned during sampling time.

Calculations

The study parameters were calculated as per following formulae:

i. Weight gain (g) = Mean final weight- Mean initial weight

ii. Specific growth rate, SGR (%) = $\frac{\log_e W_2 - \log_e W_1}{T_2 - T_1} \times 100$ (after Brown, 1957)

Where, W₁= the initial live body weight (g) at time T₁ (day)

W₂= the final live body weight (g) at time T₂ (day)

iii. Gonado-somatic index (GSI),

$$GSI (\%) = \frac{\text{Gonad weight (g)}}{\text{Body weight (g)}} \times 100$$

Physico-chemical parameter of water

The water quality parameters such as water temperature, dissolved oxygen (DO), and pH were monitored weekly throughout the experimental period (**Table 3**). Water temperature of the aquaria was measured with the help of an oxygen meter (Waterproof Tester, model: 7031). Dissolved oxygen of the water was measured by using an oxygen meter (Waterproof Tester, model: 7031). Electronic pH meter (ORP, model 6011) was used to measure the pH of water.

Statistical analysis

All statistical analyses were performed with the aid of the computer software SPSS programme. The gain in weight, specific growth rate, gonado-somatic index were all tested using one-way analysis of variance (ANOVA). The level of significance was set when the P value is ≤ 0.05 .

3. RESULTS AND DISCUSSIONS

Water quality perspective

Water temperature, dissolved oxygen and pH during the brood rearing period in the aquariums were found to be in the desirable range according to Boyd (1979), Jhingran and Pullin (1985) and Rahman *et al.* (1982). There was no indication of the adverse effect of water quality parameters on the survival and growth of *A. testudineus*. Data on physico-chemical parameters of water i.e. temperature, pH and dissolved oxygen during the experimental period are presented in were 27.7 to 28.2°C; 6.8 to 7.6 ppt; and 5.3 to 6.2 ppm, respectively.

Table 3: Range of temperature, pH and dissolved oxygen in aquariums under different treatments

Sampling No.	Parameters	T ₁	T ₂	T ₃	T ₄	T ₅
1 st	Temperature (°C)	27.7±0.2	27.7±0.1	27.9±0.2	27.7±0.4	27.8±0.4
	pH	7.1	7.00	7.3	7.2	6.9
	Dissolved oxygen (DO) (mg/l)	5.9±0.1	6.0±0.3	5.7±0.2	6.1±0	6.2±0.2
2 nd	Temperature (°C)	28±0.4	28±0.3	28.1±0.3	28±0.2	28.2±0.1
	pH	7.1	7.1	7.0	7.0	7.0
	Dissolved oxygen (DO) (mg/l)	5.7±0.3	5.5±0.2	5.6±0	5.5±0	5.3±0.3
3 rd	Temperature (°C)	27.9±0.2	28±0.1	28±0.2	28.1±0.1	28±0.4
	pH	6.8	6.9	7.0	7.0	7.1
	Dissolved oxygen (DO) (mg/l)	6.0±0.2	5.9±0.1	6.1±0.02	5.8±0.2	5.8±0.4
4 th	Temperature (°C)	28±0.3	27.9±0.4	28.1±0.3	28±0.3	28±0.1
	pH	7.0	7.1	6.9	7.2	7.3
	Dissolved oxygen (DO) (mg/l)	5.6±0.4	5.8±0.3	5.4±0.2	5.7±0.3	5.9±0.4
5 th	Temperature (°C)	27.7±0.1	28.1±0.2	28±0	27.8±0.3	27.6±0.1
	pH	7.2	7.2	7.1	7.2	7.0
	Dissolved oxygen (DO) (mg/l)	5.7±0.2	5.5±0.1	5.6±0	5.7±0.1	5.6±0.4
6 th	Temperature (°C)	27.6±0.1	27.3±0	27.7±0.2	27.4±0.1	27.7±0.3
	pH	7.2	7.3	7.5	7.6	7.6
	Dissolved oxygen (DO) (mg/l)	5.6±0.2	5.7±0.1	5.9±0.3	5.6±0.2	5.9±0.2

Effect of vitamin E on growth and gonadal development

Effect on growth performance

The growth pattern of *A. testudineus* fed with different dietary levels of vitamin E content in terms of weight gain during the experimental period is presented in Figure 1. The average initial weights in five treatments were 34.51±0.87g, 34.45±1.22g, 34.25±0.75g, 34.48±0.32g, and 33.76±0.41g in T₁, T₂, T₃, T₄ and T₅, respectively. At the end of the three months experimental period,

final weight of the fishes of five treatments were 41.37±2.19g, 43.57±0.99g, 41.42±0.79g, 43.04±1.56g, and 42.93±0.87g in treatments T₁, T₂, T₃, T₄ and T₅, respectively. In case of weight gain higher result was found in T₂ (9.12±0.23g) followed by T₁, T₃, T₄ and T₅. Statistical analysis by ANOVA showed that there was no significant difference (P>0.05) among the weight gain of T₁, T₂, T₃, T₄ and T₅ at the end of three months experimental period.

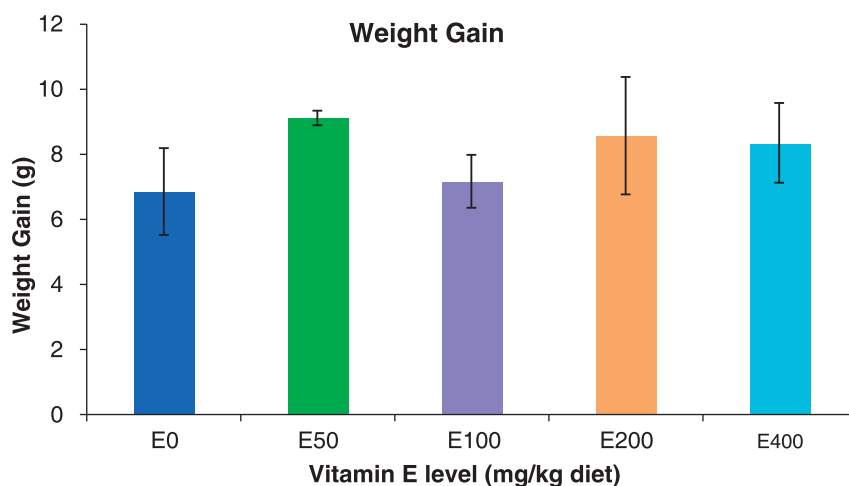


Figure 1: Weight gain of *A. testudineus* reared under different dietary levels of vitamin E. (Vertical bars= ±S.D.)

Result of specific growth rate was similar to that of weight gain of *A. testudineus* which is shown in Figure 2. Statistical analysis by ANOVA shows that there was no significant difference ($P>0.05$) between the different treatments on SGR (%).

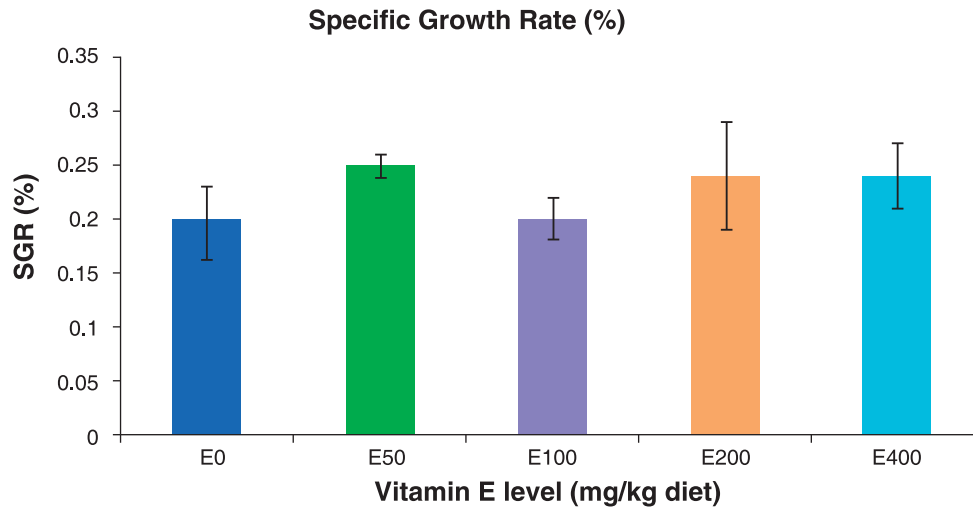


Figure 2: Specific growth rate of *A. testudineus* reared under different dietary levels of vitamin E. Vertical bars= \pm S.D.

Effect on gonado somatic index

It is a tool for measuring the sexual maturity of fish in correlation to ovary development and testes development. Clear indication of gonadal development of experimental fish was found from gonado-somatic index. At the end of this experiment the percent of gonado-somatic index (%) were 0.392 ± 0.04 , 0.356 ± 0.07 , and 0.311 ± 0.03 in

T₁, T₂, T₃, T₄ and T₅, respectively. Data on gonado-somatic index is presented in Figure 3. In this case highest gonado-somatic index (%) was found 0.696 ± 0.44 in T₃ (100 mg vit. E/kg feed) followed by T₁, T₂, T₄ and T₅. The ANOVA test showed that there was no significant ($P>0.05$) difference among treatments regarding gonado-somatic index.

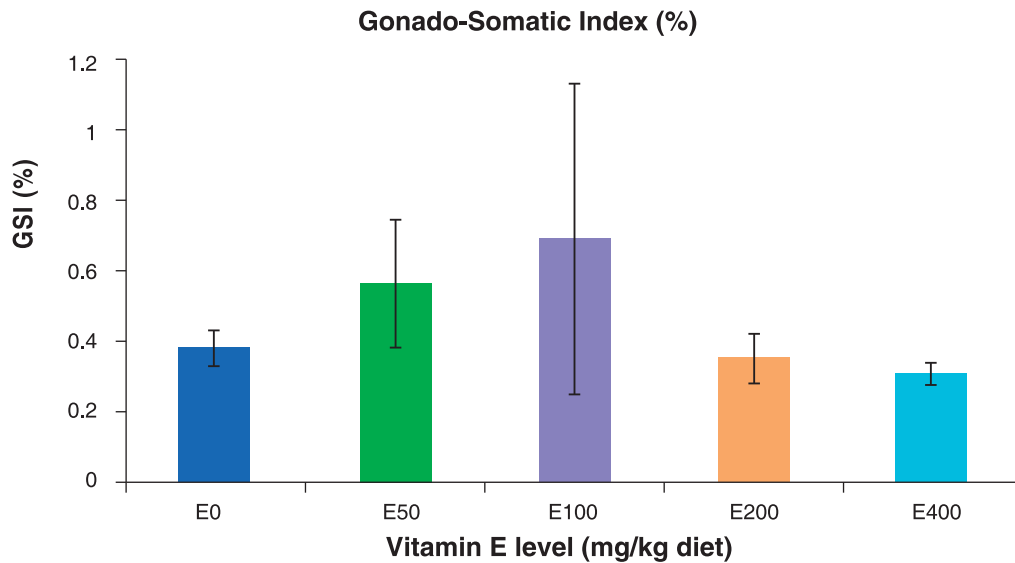


Figure 3: Comparison of gonado somatic indices of *A. testudineus* reared under different dietary levels of vitamin E. Vertical bar= \pm S.D.

The main purpose of the present research was to find out if there is any positive impact of vitamin E on the growth and gonadal development of the female *A. testudineus*. The results presented for *A. testudineus*, indicate that there exists a positive correlation between dietary vitamin E level and gonadal development.

The present results indicate that there was no significant difference among the fishes treated with 0, 50, 100, 200 and 400 mg vitamin E kg⁻¹ feed in terms of weight gain. Similar results were also observed by Jarboe and Robinette (1989) who reported no significant differences in survival conversion or weight gain among the fish fed with three different dietary levels of vitamin E viz. 72 mg, 144 mg and 36 or 66 mg vitamin E/kg feed.

There were positive impact by the implementation of different dietary doses of vitamin E on gonadal development of *A. testudineus* on 3 months experimental period. The higher gonado-somatic index was obtained in treatment T₃ containing 100 mg vitamin E/kg feed. Other doses also showed positive result on the gonadal development of *A. testudineus*. Mollah *et al.* (2003) observed highest gonado-somatic index and fecundity of the larvae of *Heteropneustes fossilis* fed with 100 mg vitamin E/kg of feed. Roy and Mollah (2009) observed heigher ovarian development and effective breeding performance in *Clarias batrachus* fed with 50 mg vitamin E/Kg of feed.

Gupta *et al.* (1987) observed higher gonadosomatic index, bigger ova and complete spawning in three major carps (*Labeo rohita*, *Catla catla* and *Cyprinus carpio*) by feeding feed containg vitamin E in their diet. James *et al.* (2008) observed that 300 mg vitamin E/kg diet is the optimum level for improving reproduction and immune response in *C. auratus*. Therefore it seems that vitamin E requirement is species specific so for as its requirement is concerned in gonadal development and breeding performance of fish.

Water temperature, dissolved oxygen and pH during the brood rearing period in the aquarium were found to be in the desirable range according to Boyd (1979), Jhingran and Pullin (1985) and Rahman *et al.* (1982). There was no indication of the adverse effect of water quality parameter on the existence and growth of *A. testudineus*.

Therefore considering the result mentioned above it is evident that vitamin E had a positive impact on the gonadal development of *A. testudineus* and vitamin E content of 100 mg/kg of feed was the best to exert such effect. In conclusion the present study support the use of vitamin E in the diet for the improvement of reproductive performance of *A. testudineus*.

4. CONCLUSION

Demand of food is increasing day by day with the increase of population throughout the world. But there is a limitation of resources. Only a proper and scientific methods can utilize this limited resources to met the increasing demand. Similarly the want of fish is increasing due to its nutritional value. But destruction of natural breeding, rearing and feeding ground of fish the natural production of fish is decreasing day by day. Commercial culture practice depends on availability of healthy broodstock and quality seeds. A considerable number of studies have been conducted to develop suitable induced breeding and larval rearing techniques for *A. testudineus* so that its culture practice can be popularized. To produce healthy and quality seeds it is necessary to increase gonadosomatic indices which ensure higher number and quality seeds. So it was felt necessary to investigate how the status of seeds production for this species can be improved by improving gonadal development and as such the present attempt was to find out the effect of dietary vitamin E on the growth and gonadal development of *A. testudineus*. At the end of this research it was found that vitamin E has a positive impact on gonadal development of *A. testudineus* and further research is needed to see the effects of vitamin E supplementation on ovulation, breeding performance and quality of eeds. This preliminary success obtained through this research can serve as an important baseline for future research on this topic.

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