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Effects of different dietary levels of vitamin E on the ovarian development and breeding performances of *Clarias batrachus* (Linnaeus)

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Abstract

To observe the effects of vitamin E on the ovarian development and breeding performances of *Clarias batrachus* an experiment was conducted in two phases. The first phase concentrated on studying the effects of vitamin E on ovarian development and the second phase on breeding performances. Eighty female *C. batrachus* broodfish were stocked in 8 cisterns (2.38 × 1.45m^2 each) divided into 4 treatments each having two replicates. Each of the cistern was stocked with 10 females. The fish were fed with feed having different levels of vitamin E viz. 0 mg (served as control), 50 mg, 100 mg and 200 mg vitamin E/kg feed for 3 months to study the effects of vitamin E on the growth and ovarian development of *C. batrachus* broodfish. It was found that growth in terms of body weight of fish fed with 200 mg vitamin E/kg feed (under T_4) was higher, while 50 mg vitamin E/kg feed (under T_2) gave the poorest result. There was no significant difference among different treatments. Gonadosomatic index and fecundity were highest in fish treated with 100 mg vitamin E/kg feed (under T_3), but there was no significant difference among the treatments. After rearing for 3 months fish were used for induction of breeding to study breeding performance. A pituitary gland (PG) dose of 100 mg/kg body weight was used in all treatments. Ovulation rate was 100% in females of all treatments. In case of fertilization and hatching rate the highest result (88.33±2.51 and 82.33±3.05 respectively) was obtained in T_2 (50 mg vitamin E / kg feed), but there was no significant difference between T_2 and T_3. The overall result of this experiment indicates that 50 mg vitamin E/kg feed is the best dose for the breeding performance of female *C. batrachus* broods.

Keywords: *Clarias batrachus*, Vitamin E, Ovarian development, Breeding performance, Larval growth

Introduction

Air-breathing fishes such as shing (*Heteropneustes fossilis*), magur (*Clarias batrachus*), koi (*Anabas testudineus*) etc.are highly popular and have great commercial importance. In spite of higher market demand, little progress has been made in culture due to lack of breeding and culture technologies.

The species *C. batrachus* together with other catfishes comprise a handsome percentage of the total catch in Bangladesh. As bulk of the catch of this fish comes from the wild sources, overfishing together with environmental degradation have posed threat to the existence of many of these fishes.

Among the live fishes, magur has got many qualities such as it grows at faster rate, survives in wide range of environment, fetches high price, possesses medicinal value which make it a perfect candidate for pond culture. Beside this magur is a highly nutritive fish having 78.5% water, 15% protein, 1.3% mineral, 1.0% lipid, 4.8% carbohydrate 0.21% calcium, 0.0007% iron, and 0.011% vitamin. It also gives us an estimated energy of 86kcal in its 100g flesh. In spite of many advantages very little attempt has been made in Bangladesh to promote its breeding and culture.

Nutrition is known to have a profound effect upon gonadal development, fecundity, egg and larval quality of fish. For the initiation of study on the nutrition of broodstock, it is necessary to determine whether spawning and egg quality are influenced by nutritional quality of broodstock diets or not. Vitamin E plays an important role in reproductive physiology in fish as it does in birds and mammals (Watanabe, 1985). As a fat soluble antioxidant, a major function of vitamin E is to prevent peroxidation of polyunsaturated fatty acid of phospholipids and cholesterol in celluler and subcelluler membrane.

Observed effects of deficiency of vitamin E include delay in ovarian development in carp (Watanabe and Takashima, 1977) and decreased egg hatchability and fry survival in ayu (Takeuchi *et al*., 1981). Most deficiency signs observed in fish such as nutritional muscular dystrophy, fatty liver degeneration, anemia, exudative diathesis, erythrocyte hemolysis, hemorrhages, depigmentation and reduction of fertility are related to the peroxidative damage to celluler membrane (National Research Council, 1983).

Considering the above realities, the present investigation was undertaken to observe the effect of different dietary levels of vitamin E on growth and ovarian development and breeding performances such as ovulation rate, fertilization rate and hatching rate of the eggs of *C. batrachus*.

Materials and Methods

In order to observe the effect of different dietary levels of vitamin E on growth, gonadal development and breeding performances of *C. batrachus*, an experiment was carried out in two phases. At first, broodfish were reared and maintained in the cisterns for 90 days providing different dietary levels of vitamin E to observe the growth and gonadal development of broodfish, and in the second phase, breeding performances of the reared broods were investigated.

Description of the experimental sites

The first phase of the experiment was conducted in 8 cisterns located at the Mini Hatchery and Breeding Complex of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. Each of the cistern was 4′×8′×1.5′ in size. The water depth of 30 cm was maintained in each cistern. Each cistern was provided with gentle shower throughout the experimental period. Water hyacinth was kept suspended at the corner of each cistern with the help of bamboo attached with float as a shelter for the fish. Water hyacinth also helped to keep the water cool and clean. Two small pieces of PVC pipe of 10.6 cm diameter were also placed at the bottom of each cistern as a shelter for the fish. Outlet of each cistern was provided with net with the help of a metal structure. Each cistern was provided with inlet and outlet system which allowed the renewal and removal of water. The breeding trials on the other hand, were carried out at Backyard Hatchery and Wet Laboratory of the Department of Fisheries Biology and Genetics.

Effect of vitamin E on the growth and ovarian development

Collection of broodfish and stocking: Magur for present work was collected from the Reliance Aqua Farms, Bailor, Trishal, Mymensingh. One hundred and fifty females of *C. batrachus* were collected for this experiment and kept in the cistern for 15 days for conditioning. Then 80 healthy, strong and more or less equal sized fish were used for the experiment. Weight of the individual fish was recorded in gram.

Experimental design: For the study of the effect of vitamin E on the growth and ovarian development, 8 cisterns were divided into four groups containing 2 cisterns in each group. These four groups corresponded to four experimental treatments. Each cistern was stocked with 10 female. Feed with four different levels of vitamin E viz. 0 mg (served as control), 50 mg, 100 mg and 200 mg vitamin E/kg feed were administered for studying the growth and ovarian development of fish.

Feed formulation: Fish meal, soybean meal, mustard oil cake, rice bran, wheat bran, wheat flour, vitamin mineral premix were used for the preparation of feed. The source of vitamin E was E-vet powder manufactured by ACI Company. A feed containing 37 % protein was 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 sensitive electric balance and the required amount of vitamin E was added and mixed thoroughly. The composition of experimental feed is shown in Table 1. After mixing all the ingredients, adequate amount of water was added and converted into pellets by pelleting machine. Then the pellets were dried and stored in the plastic bag in air tight condition and kept in refrigerator. After formulation of feed proximate composition of the formulated feeds were determined following the standard methods given by Association of Official Analytical Chemists (AOAC, 1980) in the Nutrition Laboratory of Faculty of Fisheries, BAU, Mymensingh. Proximate composition of the different feed is shown in Table 2.

Table 1. Composition of experimental feed

Ingredients	Inclusion level (%)			
	Feed 1 (T_1)	Feed 2 (T_2)	Feed 3 (T_3)	Feed 4 (T_4)
Fish meal	40	40	40	40
Soybean meal	19.58	19.58	19.58	19.58
Mustard oil cake	15	15	15	15
Rice bran	10	10	10	10
Wheat bran	10	10	10	10
Wheat flour	4	4	4	4
Vitamin mineral premix	1	1	1	1
Vitamin E	0 mg	50 mg	100 mg	200 mg

Table 2. Proximate composition of different feeds (Dry matter basis)

Treatment	% dry matter	% lipid	% protein	% ash	% crude fiber	% carbohydrate
T_1	88.92	10.07	37.27	14.21	6.13	32.32
T_2	88.75	9.99	37.11	14.37	6.09	32.44
T_3	88.74	10.11	37.13	14.51	5.99	32.26
T_4	88.23	10.17	37.04	14.23	6.20	32.36

Feeding and sampling of the experimental fish: The broodfish were fed two times a day at 9am and 6pm. In first few days, feeds were applied at the rate of 2-3 % of the body weight and increased gradually according to the demand of the fish.

Sampling of fish was done fortnightly. During sampling all fish from each cistern were caught with the help of scoop net after lowering the water level. Then the weight of each fish was taken with a sensitive electric balance. At the time of each sampling all the cisterns were washed with potassium permanganate and the cisterns were refilled with new water.

Estimation of gonadosomatic index and fecundity of broodfish: For the estimation of gonadosomatic index 2 fish from each replication were taken. The total length and weight of each fish was recorded separately to the nearest centimeter and gram, respectively. The fish were dissected and ovaries removed and weighed and gonadosomatic index was calculated.
Fecundity of each fish was determined following gravimetric method (Lagler, 1952). In this method, three samples (0.1-0.5 g) were taken from the anterior, central and posterior regions of each preserved ovary. Weights of the samples were taken by using an electric balance.

After weighing, the eggs of each sample were counted and the average was obtained. Then the fecundity was calculated.

Parameters studied for growth and ovarian development of broodfish: In order to study the effect of vitamin E level on the growth and ovarian development following parameters were studied.

i. Weight gain (g) = Mean final weight- mean initial weight.
ii. Specific growth rate:

$$\text{SGR (\% day)} = \frac{\text{Log}_e W_2 - \text{Log}_e W_1}{T_2 - T_1} \times 100 \quad \text{(after Brown, 1957)}$$

Where, W_1= The initial live body weight (g) at time T_1 (day)
W_2= The final live body weight (g) at time T_2 (day)

iii. Gonadosomatic index (GSI), $\text{GSI (\%)} = \frac{\text{Gonad weight (g)}}{\text{Body weight (g)}} \times 100$

iv. Fecundity, $F = \frac{N \times \text{Gonad weight (g)}}{\text{Sample weight (g)}}$

Where, F is the fecundity and N is the number of eggs in the sample

Effect of vitamin E on the breeding performance

After rearing for three months broodfish of 2 replicates of each treatment were kept in one cistern. Then 3 females from each treatment were selected to perform induced breeding to determine the effect of vitamin E on the breeding performance.

Inducing agent and preparation of injection

The amount of PG to be weighed was calculated by using the following formula:

$$\text{Weight of required amount of PG (mg)} = \frac{W_{tb} \times 10}{100}$$

Where, W_{tb} represents total body weight of all the fish to be injected and 10 represents the rate in mg of PG to be injected/100 g body weight of females.
The total volume of extract to be prepared was calculated by the following formula:

$$\text{Volume of extract (ml)} = \frac{W_{tb} \times 0.5}{100}$$

Where, 0.5 represents the volume of the extract in ml to be injected/100 g body weight. The weighed PG was homogenized with a small volume of distilled water and the homogenate was centrifuged. The clear supernatant was transferred to a vial and made to pre-determined volume with distilled water.

Based on the body weight of the gravid female required volume of extract was taken in a graduated 1.0 ml hypodermic syringe. The extract was injected intramuscularly behind the pectoral fin above the lateral line.

The females were checked for ovulation after 12 hours of injection. As soon as the females ovulated the eggs were collected by stripping the fish. Percent ovulation of the females was calculated treatment-wise. The milt was collected from male by dissecting out the testes and macerated them in 0.85 % NaCl solution. To ensure fertilization the sperm suspension was mixed with eggs by gentle stirring with a feather and then a little water was added to the egg-sperm mixture to activate the sperms to fertilize the eggs.

Incubation and hatching of the fertilized eggs: For calculation of fertilization and hatching rate of eggs produced by the females of each treatment a portion of eggs was taken and incubated in 3 separate bowls of 32 cm diameter. The remaining eggs of the females under each treatment were incubated in separate trays (101.6×40.6×12.7 cm^3). All the incubation bowls and trays were provided with gentle shower to ensure adequate aeration. Soon after fertilization, the embryonic development started and the fertilized eggs looked blackish or watery and transparent while unfertilized eggs looked whitish and opaque. Within 6 hours of incubation, the numbers of fertilized and unfertilized eggs from each bowl for the respective treatment were counted based on colour. After completion of hatching, the number of newly hatched larvae of each bowl was counted by siphoning them out.

Parameters studied for breeding performance

Percent ovulation: $$\%\ \text{ovulation} = \frac{\text{No. of fish ovulated}}{\text{Total no. of fish injected}} \times 100$$

Percent fertilization: $$\%\ \text{fertilization} = \frac{\text{No. of fertilized eggs} \times 100}{\text{Total no. of eggs (fertilized} + \text{unfertilized)}}$$

Percent hatching: $$\%\ \text{hatching} = \frac{\text{No. of eggs hatched}}{\text{Total no. of eggs}} \times 100$$

Physico-chemieal condition of water

Temperature, dissolved oxygen (DO) and pH of water in each cistern under each treatment were recorded on sampling date. Temperature was recorded by using a celcius thermometer, DO was measured by a digital DO meter (multi 340 i/set, Germany) and pH was measured by a portable digital pH meter (MICRO-TEMP, pH 500)

Statistical analysis

The gain in weight, specific growth rate, gonadsomatic index, fecundity, ovulation rate, fertilization rate, hatching success were all tested using one-way analysis of variance (ANOVA). Significant results (P<0.05) were further tested using Duncan's Multiple Range Test (DMRT) to identify significant difference between means. All statistical analyses were performed with the aid of the computer software SPSS programme.

Results and Discussion

Water temperature, dissolved oxygen and pH during the brood rearing period in the cisterns 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 *C. batrachus* broodfish (Table 3). The goal of the present work was to find out if there is any positive impact of vitamin E on the growth, gonadal development and breeding performances of the female of *C. bactrachus*. The results presented here indicated that there was a positive correlation between dietary vitamin E level and breeding performance.

Table 3. Physico- chemical parameters of the cisterns during the brood rearing period

Sampling No	Parameters	T_1	T_2	T_3	T_4
Initial	Temperature (°C)	27.5± 0.3	27.7±0.4	27.7±0.5	27.5±0.3
	pH	7	7.2	7.1	7.3
	Dissolved Oxygen (DO) (mg/l)	5.9±0	6.0±0.1	5.8±0.2	6.1±0.1
1st	Temperature (°C)	28.0±.7	28.0±0.5	28.0±0.8	28.0±0.5
	pH	7.1	6.9	6.9	7.2
	Dissolved Oxygen (DO) (mg/l)	5.5±0.1	5.4±0.2	5.6±0.3	5.5±0.2
2nd	Temperature (°C)	28.0±0.6	28.0±0.5	28.0±0.7	28.0±0.5
	pH	7.2	7.4	7.2	7.3
	Dissolved Oxygen (DO) (mg/l)	5.7±0	5.6±0.1	5.5±0.3	5.4±0.2
3rd	Temperature (°C)	27.5±0.3	27.8±0.6	27.8±0.5	27.6±0.3
	pH	7.1	7.3	7.1	7.5
	Dissolved Oxygen (DO) (mg/l)	5.9±0.1	6.0±0.3	5.8±0.1	5.8±0.2
4th	Temperature (°C)	28.0±0.5	28.0±0.4	28.0±0.5	28.0±0.6
	pH	7.3	7.5	7.1	7.4
	Dissolved Oxygen (DO) (mg/l)	5.4±0.2	5.6±0.4	5.4±0.3	5.2±0.2
5th	Temperature (°C)	27.9±0.7	28.0±0.5	28.3±0.8	27.8±0.5
	pH	6.8	6.9	7.1	7.2
	Dissolved Oxygen (DO) (mg/l)	5.9±0.2	6.0±0.2	5.8±0.1	5.8±0.2
6th	Temperature (°C)	28.0±0.5	28.0±0.7	27.8±0.5	27.9±0.6
	pH	7.1	7.2	7.4	7.2
	Dissolved Oxygen (DO) (mg/l)	5.5±0.1	5.7±0.1	5.3±0.2	5.6±0.1

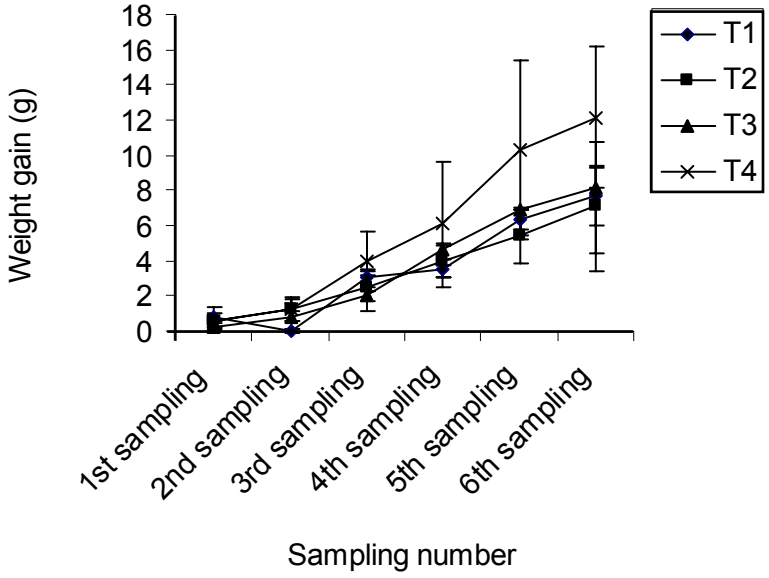
Effects of vitamin E on growth

The growth pattern of female broods of *C. batrachus* fed with different dietary levels of vitamin E content in terms of gain in weight during the experimental period is presented in Fig. 1(a). The average initial weights in four treatments were 82.76±.63 g, 82.56±.51 g, 80.41±1.33 g and 80.27±1.20 g, respectively in T_1, T_2, T_3 and T_4. At the end of the 90 days experimental period, final weight of the broodfish of four treatments were 90.46±2.51 g, 89.64±5.12 g, 88.57±2.97 g and 92.57±2.46 g, respectively in treatments T_1, T_2, T_3 and T_4. At the end of the 3 months experimental

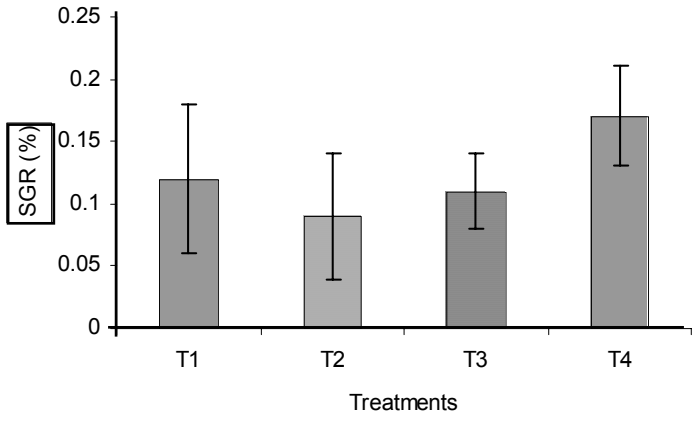
period gain in weights were7.7±1.73, 7.09±3.70, 8.14 ±1.10 and 12.16±4.05, respectively in T_1, T_2, T_3 and T_4. So in case of weight gain higher result was obtained in T_4 followed by T_1, T_3 and T_2. From the Fig. 1(a) it is seen that at the 6th sampling there was almost no weight gain in T_1, T_2 and T_3 except T_4. Statistical analysis by ANOVA showed that there was no significant difference among the weight gains of T_1, T_2, T_3 and T_4 at the end of 3 months experimental period.

Results on the specific growth rate (% day) of female *C. batrachus* broodfish fed on feeds containing different levels of vitamin E has been shown in Fig.1 (b). Results of specific growth rate were similar to that of weight increase. Statistical analysis by ANOVA showed that there was no significant (P>0.05) difference among the four different treatments.

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



(a)



(b)

Fig. 1. Weight gain (a) and specific growth rate (b) of female *C. batrachus* broodfish reared under different dietary levels of vitamin E. Vertical bars= ± SD.

Effects of vitamin E on gonadosomatic index and fecundity

Results of gonadosomatic index give a clear indication about the gonadal development as well as breeding season of a fish. Data on gonadosomatic index are presented in Fig. 2(a). In this case highest gonadosomatic index was found in T_3 followed by T_4, T_1 and T_2. The ANOVA test showed that there was no significant difference among treatments regarding gonadosomatic index. In case of fecundity (Fig. 2b) it was found that number of ova/g body weight of fish was the highest in T_3 and the lowest in T_2. Statistical analysis by ANOVA showed that there was no significant difference among the four different treatments. 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 adding vitamin E in their diets. Similarly Sanchai-Sutjaritvongsanon (1987) reported that a mixture of 35% fish meal, 30% soybean, 20% corn meal, 15% rice bran and 10 mg/kg BHT plus 100 mg vitamin E/kg feed was suitable for stimulating the development of gonad and spawning in goldfish (*Carassius auratus*). Therefore, it seems that vitamin E requirement is species specific as far as its requirement is concerned in gonadal development and breeding performance of fish.

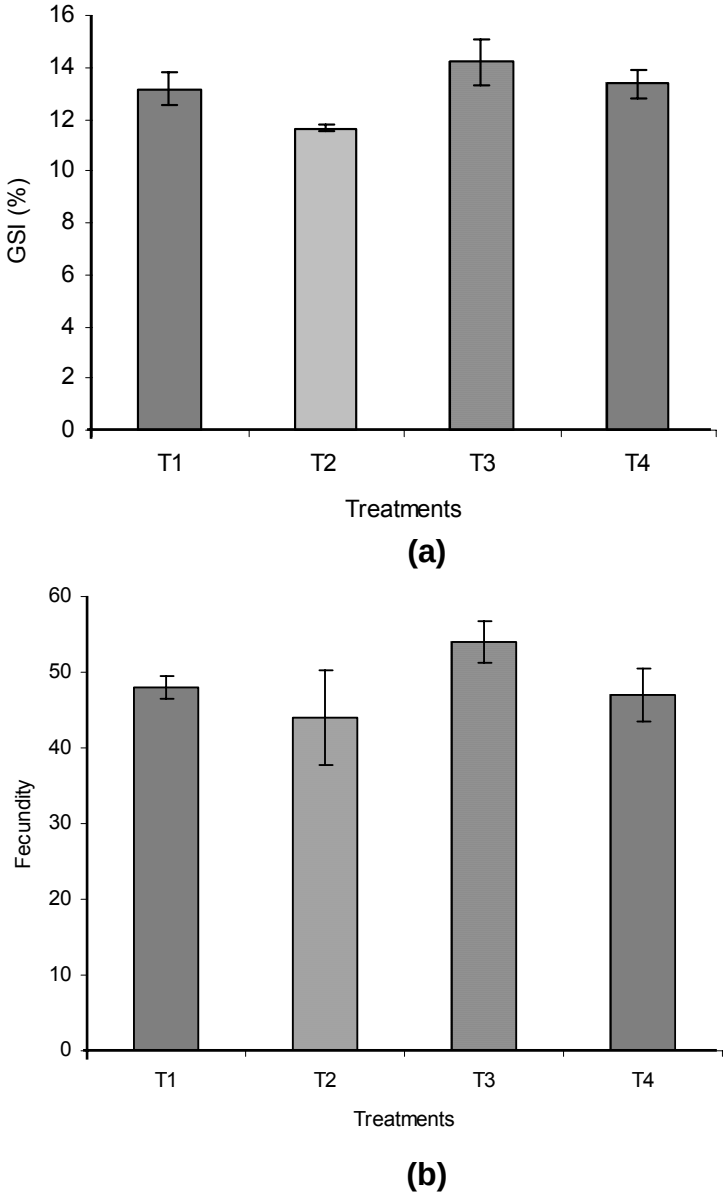


Fig. 2. Comparison of gonadosomatic indices (a) and fecundity (b) of female *C. batrachus* broodfish reared under different dietary levels of vitamin E. Vertical bars= ± SD

Effects of vitamin E on breeding performance

Ovulation of fish: In order to observe the effect of different dietary levels of vitamin E on the breeding performance, a breeding trial was performed at the 2nd phase of the experiment. Ovulation rate was hundred percent in all the treatments.

Fertilization rate and hatching rate: Average fertilization rates were recorded as 66.66±9.02, 88.33±2.51, 81.67±3.06 and 59.83±1.53 in T_1, T_2, T_3 and T_4, respectively. The highest fertilization rate was observed in T_2 and lowest fertilization rate was observed in T_4 (Fig. 3a). Statistical analysis by ANOVA showed that there was a significant (P<0.01) difference among different treatments. Duncan Multiple Range Test (DMRT) showed that T_2 and T_3 were significantly (P<0.05) different from T_1 and T_4, but there was no significant difference between T_2 and T_3. T_1 and T_4 were also not significantly different. During the experiment, the average hatching rates were found to be 39.33±7.64, 82.33±3.05, 75.33±4.72 and 49.00±2.02, respectively in T_1, T_2, T_3 and T_4. The highest hatching rate was found in T_2 and lowest in T_1 (Fig. 3b). The ANOVA test showed that there was a significant difference (P<0.01) between different responding treatments. DMRT showed that T_2 and T_3 were significantly different (P<0.05) from T_1 and T_4, but there was no significant difference between T_2 and T_3, and T_1 and T_4.

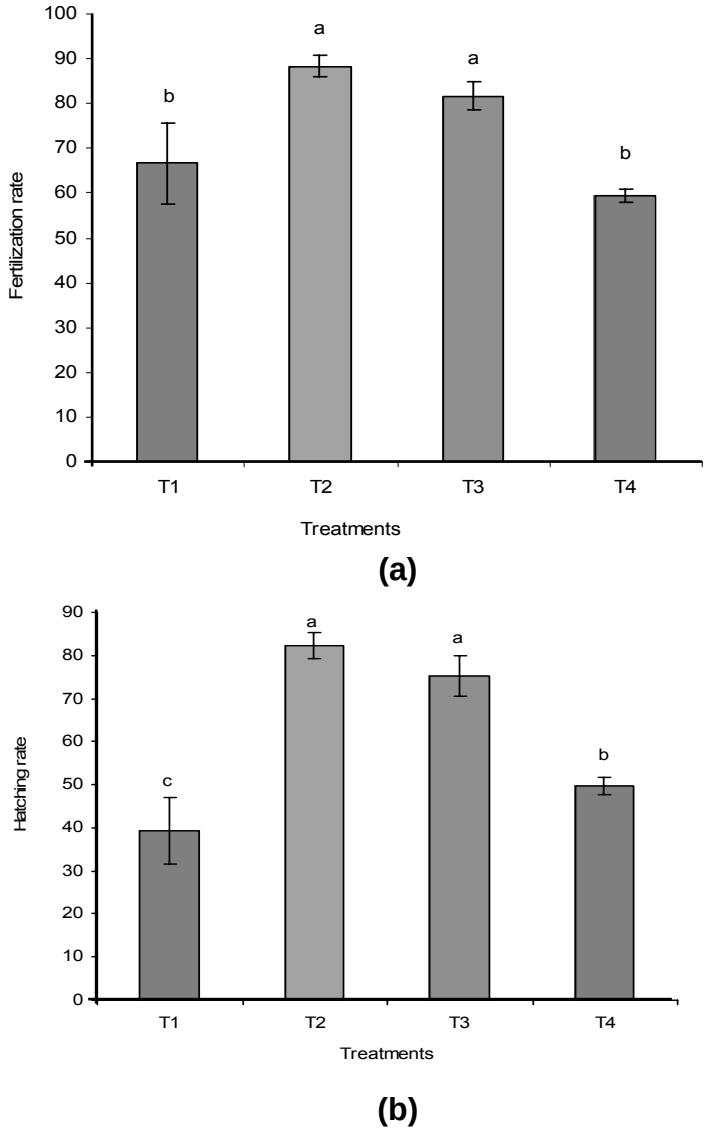


Fig. 3. Comparison of fertilization rate (a) and hatching rate (b) of the eggs produced by the female *C. batrachus* broodfish reared under different dietary levels of vitamin E. Vertical bars= ± SD. Columns marked with the different letter are significantly different.

The result of the present study showed a positive impact of vitamin E on the breeding performance of female *C. batrachus*. The best fertilization rate and hatching rate of the eggs were obtained with fish fed 50 mg vitamin E/kg feed, other doses however also showed positive result. Takeuchi *et al.* (1981) who conducted an experiment on the broodfish of 'ayu' *Plecoglossus altivelis,* also observed better hatchability and survival of larvae with 3.4 mg vitamin E/100 g of diet which is closed to the findings of the present experiment. Mollah *et al.* (2003) found better fertilization rate and hatching rate of the eggs and survival rate of the larvae of *Heteropneustes fossilis* produced from the broodfish fed 200 mg vitamin E/kg feed.

So, considering the results mentioned above it may be concluded that vitamin E had a positive impact on the breeding performance of female *C. batrachus* broodfish and vitamin E content of 50 mg/kg feed was the best treatment to exert such an effect.

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References

AOAC (Association of Official Analytical Chemists). 1980. Official methods of analysis of the Association of Official Analytical Chemists. *In*. W. Hoewitz, ed. 2nd ed., Washington D.C., 78 pp.

Boyd, C.E. 1979. Water quality management for pond fish culture. Elsevier Sci. Publ. Co. Amsterdam-Oxford, New York, 318 pp.

Brown, M.E. 1957. Experimental studies on growth. *In* M.E. Brown, ed. The Physiology of Fishes. Academic Press. New York, 1:361-400.

Gupta, S.O., Khan, H.A. and Bhowmick, R.M. 1987. Observation on the effect of vitamin E and growth hormone on the gonadal maturity of carps. J. Inland Fish. Soc. India., 19(2): 26-31.

Jarboe, H.H and Robinette, H.R. 1989. Effect of selected vitamin E supplementations of channel carfish production feeds. The Progressive Fish-Culturist, 51:91-94.

Jhingran, V.G. and. Pullin R.S.V. 1985. A Hatchery Manual for the Common Chinese and Indian Major carps. ICLARM studies and reviewers, 11: 191 pp.

Lagler, K.F. 1952. Studies in fresh-water fishery biolology. Ann Arbor, Michigan, 199 pp.

Mollah, M.F.A., Sarder, M.R.I and Begum, T. 2003. Effect of different dietary levels of vitamin E on the breeding performance of *Heteropneustes fossilis* (Bloch). Bangladesh J. Fish. Res., 7(1): 11-20.

National Research Council. 1983. Nutrients Requirements of Domestic Animals, Nutrient Requirements of Warmwater Fishes and Shellfishes. National Academy Press, Washington D.C.,102 pp.

Rahman, M.S., Chowdhury, M.Y., Haque, M.A. and. Haq, M.S. 1982. Limnological studies of four ponds. Bangladesh J. Fish., 2-5 (1-2): 25-35.

Sanchai-Sutjaritvongsanon. 1987. Level of vitamin E content suitable for gonad developing and spawning of goldfish, *Carassius auratus* (Linn.). Abstract of Master of Science Thesis. Fisheries science, 12: 2.

Takeuchi, M., Ishii, S. and Ogiso, T. 1981. Effect of dietary vitamin E on growth, vitamin E distribution and mortalities of the fertilized eggs and fry in ayu *(Plecoglossus altivelis)*. Bull. Tokai Reg. Fish. Res. Lab., 104:111-122.

Watanabe, T. 1985. Importance of the study of broodstock nutrition for further development of aquaculture. *In*: N. C.B. Eowey. A.M. Mackie and J.G. Bell (eds), Nutrition and Feeding in Fish. Academic Press, London, pp. 395-413.

Watanabe, T. and Takashima, F. 1977. Effect of α-tocopherol deficiency on carp-VI. Deficiency symptoms and change of fatty acid and triglyceride distribution in adult carp. Bull. Japan. Soc. Sci. Fish., 43: 819-830.