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Effects of different dietary levels of vitamin E on the breeding performance of *Heteropneustes fossilis* (Bloch)

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Abstract
Experiments on the study of different dietary levels of vitamin E on the growth and breeding performance of *Heteropneustes fossilis* brood fish were carried out in two phases. The first phase consisted of studying its ovarian development and the second phase on breeding performance. Sixty female fishes were stocked in twelve experimental chambers of a raceway. The effects of four dietary vitamin E levels *viz.* 0 (served as control), 50, 100 and 200 mg/kg feed, on the somatic growth, ovarian development of brood fish and on their breeding performance were studied. Each treatment had three replications. It was observed that body growth in terms of length and weight was best with 0 mg vitamin E/kg feed and 200 mg vitamin E/kg of feed gave poorest result. The gonado-somatic index and fecundity, however, was highest in the fish fed with 100 mg vitamin E/kg of feed. In case of breeding performance such as ovulation rate, fertilization rate, hatching rate and survival rate, the best result was obtained with 200 mg vitamin E/kg of feed. The overall result of this experiment indicates that 200 mg vitamin E/kg of feed is the best vitamin E dose for *H. fossilis* brood and vitamin E content has a positive impact on ovarian development.

Key words : *H. fossilis*, Vitamin E, Breeding performance

Introduction

Air breathing catfishes such as shingi (*Heteropneustes fossilis*) and magur (*Clarias batrachus*) are potentially important culture species and need to be taken under aquaculture system. These catfishes together comprise a handsome percentage of total fish catch and bulk of the catch of these fishes comes from the wild population. As major part of their production depends on natural source, over fishing together with environmental degradation have posed threat to their very existence.

Among these live fishes, shingi is a popular indigenous air-breathing catfish. This species has got many qualities that make it a perfect candidate for pond culture. It grows rapidly and attains marketable size within one growing season (Islam 1989). It breeds in natural shallow waters during monsoon season usually after heavy shower when the adjoining area of ponds and other depressions get inundated.

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 shingi. In our country farmers are dependent on natural sources for collection of fry. Therefore, proper techniques of induced breeding and larval rearing for production of fry in commercial scale are needed to be developed. For the supply of quality seeds in sufficient numbers the brood fish must be of good quality. So in case of brood stock management, there should be regular supply of balanced food for their growth and development. Although some works have previously been conducted on its induced breeding and fry rearing but the techniques available have not been standardized to recommend at farmer's level. More comprehensive works need to be carried out if its culture has to be popularized. In the present work, some attempts have been made to focus on the brood stock nutrition of shingi 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 brood stock diets or not. Vitamin E plays an important role in reproductive physiology in fish as it does in birds and mammals (Watanabe 1985). Considering the above realities, the present investigation was undertaken to achieve the objectives such as i) to study the effects of different dietary levels of vitamin E on growth of fish and ovarian development and ii) to study the effects of different dietary levels of vitamin E on breeding performance such as ovulation rate, gonado-somatic index, fertilization and hatching rates of eggs and survival of larvae.

Materials and methods

In order to observe the effect of different dietary levels of vitamin E on growth, gonadal development and breeding performance of *H. fossilis,* two experiments were carried out in two phases. In the first phase, brood fish were reared and maintained by providing different dietary levels of vitamin E and in the second phase, breeding performance of the reared broods were investigated.

Experimental sites

The first experiment was carried out in a raceway. The raceway was divided into thirty equal sized chambers where each of the chambers was 183x102x100 cm^3 in size and separated from other by netted frames. The raceway was facilitated with inlet and outlet system which allowed the renewal and removal of water when needed. Since *H. fossilis* is bottom dwelling and prefers shade, raceway bottom was filled with 4 cm mud and some water hyacinths were kept suspended in the chamber. The second experiment was conducted in the Wet Laboratory of Faculty of Fisheries.

Collection and stocking of broods

About 100 females of *H. fossilis* were locally collected and kept in three fiberglass tanks for acclimatization. After three days of conditioning, five similar sized fishes were

stocked in each raceway chamber. Initial length and weight of the fish were recorded. The physico-chemical parameters such as temperature, dissolved oxygen and pH of the raceway water were monitored on regular basis to ensure that the water quality remained suitable for the broods.

Experimental design and feed formulation

Twelve raceway chambers were divided into four groups, which corresponded to four experimental treatments and each of the treatment had three replications. Feeds were formulated for different treatments using four different levels of vitamin E such as 0 (served as control), 50, 100 and 200 mg/kg feed. For preparing feed, finely ground and sieved fish meal, sesame meal, soybean meal, mustard oil cake, rice bran and wheat flour, and vitamin mineral premix (Evit tablet) were used. The proximate composition of the ingredients was determined following the standard methods given by Association of Official Analytical Chemists (AOAC 1980) (Table 1). The formulation of the experimental diets is shown in Table 2. To maintain an approximately 40% protein level in the feed, required amount of ingredients were mixed and converted into pellets by using a hand machine. These pellets were dried and stored in plastic bag with heat sealing and kept in a refrigerator. The formulation of all the experimental diets was same and they differed from each other only by the amount of vitamin E added, hence there was no variation in protein percentage among the diets.

Table 1. Proximate composition of dietary ingredients (% dry matter basis)

Ingredients	Dry matter	Protein	Lipid	Ash	Nitrogen free extract (NFE)[1]
Fish meal	91.66	66.50	8.91	15.58	9.01
Mustard oil cake	91.28	34.43	6.99	10.10	48.48
Soybean meal	90.14	49.53	1.52	8.33	40.62
Sesame meal	91.82	22.77	8.09	18.17	50.97
Rice bran	91.35	14.92	4.38	12.31	68.39
Wheat bran	89.83	14.0	3.97	4.98	77.05

[1]Nitrogen free extract calculated as 100 - % (moisture+ crude protein + lipid + ash)

Table 2. Formulation (%) of experimental diets

Ingredients	Inclusion level (%)			
	Feed –1	Feed-2	Feed-3	Feed-4
Fish meal	40.0	40.0	40.0	40.0
Sesame meal	15.0	15.0	15.0	15.0
Soybean meal	13.88	13.88	13.88	13.88
Mustard oil cake	13.88	13.88	13.88	13.88
Rice bran	6.12	6.12	6.12	6.12
Wheat bran	6.12	6.12	6.12	6.12
Wheat flour	4.0	4.0	4.0	4.0
Vitamin mineral premix	1.0	1.0	1.0	1.0
Vitamin E	0 mg	50 mg	100 mg	200 mg

Feeding and sampling

The brood fishes were fed two times a day up to satiation. The unused foodstuffs, debris and faeces were removed from the chambers by draining out water. Sampling of fish was done fortnightly. During sampling all the fishes from each chamber were caught by scoop net and their lengths and weights were measured. Growth of the fish were determined by following ways:

Length gain (cm) = Mean final length – mean initial length

Weight gain (g) = Mean final weight – mean initial weight

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

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)

Estimation of gonado-somatic index and fecundity of brood fish

One fish from each chamber was collected and total length and weight was taken separately. The fish were dissected and ovaries were removed and weighed. The gonado-somatic index was calculated by using the following formula:

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

Fecundity of fish was determined by following gravimetric methods (Lagler 1952). The dissected ovaries were preserved at 5% formalin and three samples (0.1 to 0.5 g) were taken from the anterior, central and posterior regions of each ovary. The samples were weighed and the eggs were counted from each sample. Fecundity was determined by applying following formula:

$$\text{Fecundity (F)} = \frac{\text{N x Gonad weight (g)}}{\text{Sample weight (g)}}$$

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

Induced breeding

Two females from each replication of each treatment were selected and induced with PG extract injection. Mature males of *H. fossilis* were collected from ponds and kept in separate fiberglass tanks. The amount of PG to be required was determined by

$$\text{Weight of required amount of PG (mg)} = \frac{W_{tb} \times 7.0}{100}$$

where, W_{tb} represents total body weight of the fish to be injected and 7.0 represents the rate of PG in mg to be injected/100 g body weight of females.

The total volume of the PG 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 PG extract in ml to be injected/100 g body weight.

The weighed PG was homogenized with distilled water and the homogenate was centrifuged. The supernatant (PG) was taken in a 1.0 ml graduated hypodermic syringe and injected intramuscularly to the fish near dorsal fin. After injection each female was kept separately for ovulation. The males did not receive any inducing agent.

The females were checked for ovulation hourly beginning from 6 hrs post injection and continued up to 12 hrs of injection. As soon as the females ovulated the eggs were collected by stripping and placed in a clean fertilization tray. The milt was collected from the male by dissecting out the testes and macerating them in 0.85% sodium chloride solution. The fertilization was done by mixing the sperm suspension with eggs using a feather and then a little water was added to the egg-sperm mixture.

Incubation and hatching of the fertilized eggs

A portion of fertilized eggs from an individual female of each treatment was homogenously spread on plastic bowls (15 cm diameter). All the incubation bowls received gentle shower and adequate aeration. Soon after fertilization when embryonic development started, the fertilized eggs looking blackish or greenish in colour were counted for respective females. After completion of hatching, the number of newly hatched larvae of each bowl was counted by siphoning them out.

Larval rearing

From the second day of hatching, the larvae were provided with live feed. They were reared for seven days to observe the effect of vitamin E on their survivability as the larvae produced from the broods were maintained under different dietary levels of vitamin E. Twelve plastic bowls each of 10l capacity were divided into four groups corresponding to four treatments and each of the bowls was stocked with 80 larvae as a stocking rate of 8 larvae/l. Continuous water flow of nearly equal rate was maintained in all the bow. Tubificid worms were used as feed and administered twice a day *ad libitum*. At the end of the experiment, the total number of larvae was counted and the length and weight were measured by random sampling of 10 fry in each bowl.

Statistical analysis

The specific growth rate, gonado-somatic index, fecundity, ovulation rate, fertilization rate, hatching success and survival rate of larvae up to eight days from first feeding were tested using one-way analysis of variance (ANOVA). Duncan's Multiple Range Test (DMRT) was also applied to identify significant difference ($P<0.05$) between means.

Results

Effects of vitamin E on growth and ovarian development

The growth, in terms of length and weight gain of the brood fish of *H. fossilis* fed with different dietary vitamin E content have been found satisfactory in all the treatments but the highest growth was observed in T1 whereas the lowest performance was in T4. Fish in treatment 2 showed better performance than those in T3. The specific growth rate (SGR) is presented in Fig. 1 where SGR was highest in T1 followed by T2, T3 and T4. There was no significant difference ($P>0.05$) between the SGRs of fish in T1 and T2, and between T3 and T4, respectively. However, SGR of fish in T1 and T2 were significantly ($P<0.05$) better than those of T3 and T4.

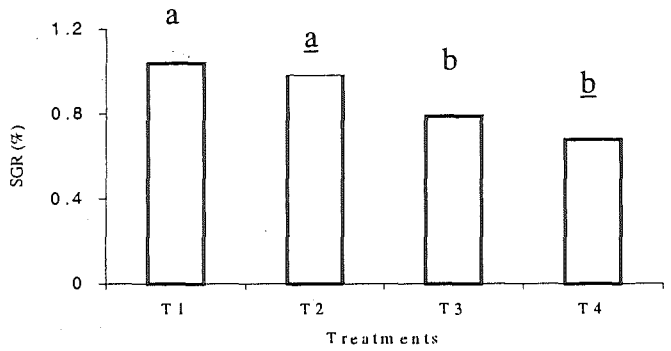

Fig. 1. Specific growth rate (SGR %) of *H. fossilis* brood fish under different treatments. Bars with different letters indicate significant difference ($P<0.05$).

Gonado-somatic index is very important parameter for understanding gonadal development. Results of GSI presented in Fig. 2 showed that fish in T3 produced highest GSI followed by T1, T2 and T4. However, there was no significant difference ($P>0.05$) among the GSI values in different treatments. In case of fecundity, it was found that the number of ovum/g body weight of fish was highest in T3 and lowest in T4 (Fig. 2). No significant difference was observed among the treatments for fecundity. The water quality of the raceway was in suitable range during the rearing period of broods (Table 3).

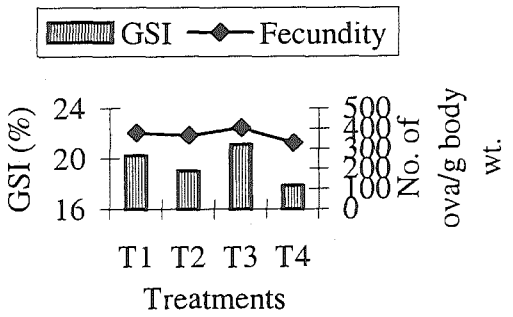

Fig. 2. GSI and fecundity of *H. fossilis* brood fish under different treatments.

Table 3. Physico-chemical parameters of the raceway water where the *H. fossilis* brood fish reared

Sampling number	Parameters		
	Temperature (°C)	Dissolved oxygen (ml/l)	pH
1st	28.5	6.0	7.0
2nd	29.0	6.3	7.2
3rd	28.0	6.0	7.0
4th	30.0	7.0	7.5
5th	29.5	6.5	7.5
6th	30.0	7.0	7.0

Effects of vitamin E on breeding performance

Induced breeding

The breeding performance of female brood fish, fed with different levels of vitamin E, in terms of ovulation percentage, fertilization and hatching rates of eggs are shown in Fig. 3. Brood fish fed with control feed (no vitamin E) in T1 and fish fed with 50 mg vitamin E containing feed in T2 demonstrated higher ovulation than that of fish fed with 100 mg and 200 mg vitamin E containing feed in T3 and T4 respectively. No significant differences were observed between the ovulation rate of fish of T1 and T2, and between T3 and T4. However, ovulation rate of broods in T1 and T2 were significantly better than those in T3 and T4. The fertilization rate of eggs was found significantly ($P<0.05$) higher in T4 followed by T1, T3 and T2 (Fig. 3). Similar results were obtained in hatching of eggs produced by females in different treatments and T4 showed significantly ($P<0.05$) higher hatching rate than that of T1, T3 and T2 (Fig. 3).

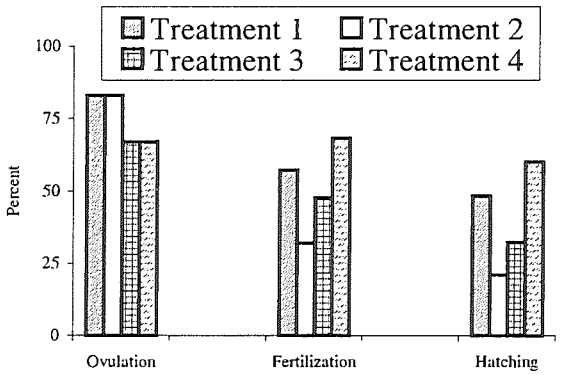

Fig. 3. Ovulation rate of females, fertilization and hatching rates of eggs *of H. fossilis* under different treatments. Bars with different letters indicates significant differences ($P<0.05$) in the respective parameters.

Larval rearing

Average initial length and weight of one day old *H. fossilis* larvae of different treatments were 4.5 ± 0.50 mm and 2.8 ± 0.29 mg respectively. After seven days of feeding with Tubificid worms, the average increment of length and weight of larvae were 4.7 ± 0.46 mm and 2.4 ± 0.35 mg respectively. The larvae of all the treatments showed good survival but T4 had highest survival rate than those of the rest. However, no significant difference was observed between the survival rates of the larvae among different treatments (Fig. 4).

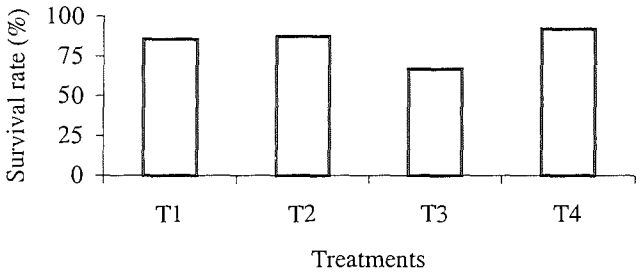

Fig. 4. Survival rate of *H. fossilis* larvae under different treatments.

Discussion

Breeding performance of fish depends on the quality of brood maintenance and the gonadal development of fish. The ultimate goal of the present research was to find out if there was any positive impact of vitamin E on the gonadal development and breeding performance of the brood fish of *H. fossilis*. The results obtained in the experiment provided a clear indication that there was a positive correlation between dietary vitamin E level and breeding performance of the fish.

It is generally agreed that for producing quality brood, they should be maintained in a good environment with proper diet. Here all the experimental brood fishes were maintained in a raceway where water temperature, dissolved oxygen and pH were found to be in the desirable range as reported by Boyd (1979) and Rahman *et al.* (1982). Therefore, there was no evidence of adverse effect of water quality on the existence and growth of female broods. However, it was observed that fish in the treatment 1 which was provided feed with no vitamin E had best growth rate, whereas fish in T4 fed highest level of vitamin E had poorest growth performance. This result was coincided with Dube and Trung (1993) who reported the best growth in terms of length and weight increment of gold fish with a vitamin E content of 50 mg/100 g of diet and the least growth with 200 mg vitamin/100g of diet. The somatic and gonadal growths of *H. fossilis* were antagonistic in nature, that is, with the increased rate of gonadal development the rate of somatic growth slows down. Similar phenomena were also reported in other fish species (Purdm 1976, Utter *et al.* 1983, Malison 1985).

In the events of fertilization rate, hatching rate and survivability of larvae of *H. fossilis*, a positive impact of vitamin E was observed and the highest level of vitamin E containing feed (T4) yielded best results. Takeuchi *et al.* (1981) conducted an experiment with brood fish of Ayu, *Plecoglossus altivelis* and observed better hatchability and survivability of larvae with 3.4 mg vitamin E/ 100 g of diet. Sanchai-Sutjaritvongsanon (1987) reported that a mixture of 35% of fishmeal, 30% soybean, 20% corn meal, 15% rice bran and 10 mg/kg BHT plus 100 mg/kg vitamin E was suitable for stimulating gonad development and spawning of gold fish. Although larvae in all the treatments were fed with Tubificid worms highest survival was found in T4. It could be resulted from the influence of vitamin E as the mother of larvae fed with high-level vitamin E diet and vitamin E could be incorporated into the eggs during oogenesis. King (1985) reported that the presence of vitamin E in the diet of rainbow trout had a significant effect on the final levels of alpha-tocopherol in eggs than fish deprived of vitamin E. During egg development, alpha-tocopherol was slowly, but efficiently transferred from the yolk to the developing embryo. Mortalities during egg development were inversely related to the alpha-tocopherol content of the eggs.

Stocking density is recognized as an important factor which directly affects the growth, survival and production of fish (Backiel and Le Cren 1978). Generally highest stocking density results in the reduction of growth and survival (Sarder *et al.* 1991) and increase food conversion ratio, together with severe competition of food and space. During the larvae rearing, 8 larvae/l was stocked and good survival was found in all the treatments. This is an agreement with the work of Mollah (2001) where optimum stocking density of *H. fossilis* larvae was between 10 and 20 per/l. Tubificid worms were used for larvae rearing which was recommended by Haque and Barua (1989) as best live feed for first feeding of *H. fossilis* larvae. Tubificid worms were also reported suitable live feed for nursing the *H. fossilis* larvae (Gheyas 1998) and for other indigenous and exotic catfish of similar nature (Yasmin and Mollah 1997, Mollah *et al.* 1998).

In the current research work, mainly six parameters were assigned to study the gonadal development and breeding performance such as gonadosomatic index, fecundity, ovulation percent, fertilization rate, hatching rate and survivability of larvae. The gonado-somatic index and fecundity were highest in the fish provided with 100 mg vitamin E/kg feed, whereas for the rest of above mentioned parameters a dose of 200 mg/kg feed proved to be best. Though vitamin E has a positive impact on breeding performance of *H. fossilis* and other fish species, the present research is probably the first ever work of its nature in Bangladesh. Therefore, the preliminary success obtained through this work can serve as an important base for future research on this topic.

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