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Effects of dietary vitamin E on the growth and breeding performance of *Ompok pabda*

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Abstract
A 90 day feeding trail was conducted to investigate the effect of vitamin E on the growth and breeding performance of *Ompok pabda*. A total of 84 healthy female broodfish (41.10±0.44g) were divided into 4 treatments *i.e.* treatment T_1, T_2, T_3 and T_4 having three replications each. The fish were fed twice a day with a standard feed (40% protein) having 4 doses of vitamin E *viz.* 0 (served as control), 50, 100 and 150mg vitamin E/kg feed. At the end of the feeding trial, the broodfish were induced to breed with PG extract to observe the effect of vitamin E on feed. After rearing for 90 days with the experimental feeds, it was found that weight gain and specific growth rate of broodfish fed with 100mg vitamin E/kg feed (treatment T_3) was the highest (14.78±0.38g and 2.99±0.11) while 150mg vitamin E/kg feed (treatment T_4) fed fish gave the poorest result (2.97±0.89g and 1.21±0.32). There was no significant difference in terms of length gain of broodfish among the different treatments. The broodfish were induced to breed with equal dose of PG extract (18 and 12mg PG/kg body weight for female and male respectively) to observe the dietary effect of vitamin E on breeding performance. The highest ovulation, fertilization and hatching rate of eggs were found to be 81.48±6.41%, 84.04±3.53% and 68.59±5.03% respectively in the broodfish of treatment T_3 while the poorest (33.33±00%, 52.35±5.02% and 45.70±7.24% respectively) were found in the broodfish under treatment T_4. The results suggest that inclusion of 100mg vitamin E/kg feed is best for enhancing the breeding performance of *O. pabda* broodfish indicating that vitamin E content has a positive impact on reproduction of fish. The present results also imply that inclusion of higher level of vitamin E exerts an antagonistic effect in terms of growth and breeding performance of this species.

Key words: Growth rate, Breeding, Vitamin E, *Ompok pabda*

Introduction

Ompok pabda commonly known as pabda, is an indigenous, small freshwater catfish belonging to the family Siluridae of the order Siluriformes (Siddiqua *et al.* 2000). In Bangladesh, it inhabits in all types of freshwater habitats, especially in rivers, canals, beels, swamps and ponds. In spite of many advantages very little attempt has been made in Bangladesh to promote breeding and culture of *O. pabda*. The total production of *O. pabda* is only 144T from different waterbodies of Bangladesh (FRSS 2008). Its

production can be increased through culture practice but the availability of a large number of fry and fingerlings is a pre-requisite to flourish the culture of this species.

Nutrition in the diet of broodfish 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 gonad 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 an essential nutrient for all species of animals (McDowell 1989). As a fat-soluble vitamin, it is the most effective chain-breaking, lipid-soluble antioxidant in biological membranes, where it contributes to membrane stability. It protects critical cellular structures against damage from oxygen free radicals and reactive products of lipid peroxidation. Aquatic animals have high levels of unsaturated fatty acids to maintain cell membrane fluidity especially at low temperatures; it is assumed that vitamin E plays an important role in this context (Blazer 1992). The importance of vitamin E in fish reproduction has been reported by many scientists (Watanabe *et al.* 1970, Hamre and Lie 1995, Halver 2002 and Paul *et al.* 2004). For example, vitamin E caused higher gonadosomatic index, larger ova and more eggs than a control in a study of freshwater fish, *Cyprinus carpio* (Gupta *et al.* 1987).

Sufficient number of fry and fingerlings of this catfish is, however, quite difficult to obtain from natural waters for stocking in the ponds. Proper techniques of mass production of fry in commercial scale seem to be the most crucial factors in expanding culture practice for this species because market price of fish bears the special preference in aquaculture. Considering the above realities, the present research work was undertaken to observe the effect of vitamin E on growth and breeding performance of *O. pabda* broodfish.

Materials and methods

The research work was conducted in 12 cisterns ($120\times70\times40cm^3$) of the mini hatchery cum breeding complex, Faculty of Fisheries, Bangladesh Agricultural University (BAU), Mymensingh. The breeding trials on the other hand, were carried out in the hapas of $150\times100\times100cm^3$ affixed in the pond beside the hatchery complex with the help of bamboo poles. Prior to stocking of broodfish, each of the cisterns was installed with all the facilities necessary to run the experiment efficiently. An inlet and an outlet were provided with each of the cisterns to facilitate renewal and removal of water concomitantly. Water hyacinths were kept floating at a corner of each cistern with the help of bamboo frame attached with float to provide shelter to the experimental fish. Water hyacinth was used to keep the water cool and clean.

Adequate number of mature and healthy *O. pabda* were collected from the wild source during the month of February and March 2009 and acclimatized in the cisterns. A total of 84 healthy, strong, and similar sized females were selected for rearing as broodfish for the research work. The brood rearing experiment was started on 1 April 2009 and

continued up to 30 June 2009. There were four treatments (*i.e.* T_1, T_2, T_3 and T_4) each with 3 replications stocked with 7 females. The broodfish of treatment T_1 served as control (i.e. fed vitamin E free diet) while that of treatment T_2, T_3 and T_4 were fed with a feed having 50, 100 and 150mg vitamin E/kg feed respectively.

The four different feeds containing 40% protein were prepared keeping all the ingredients (fish meal, soybean meal, mustard oil cake, rice bran, wheat bran, vitamin mineral premix) in equal amount except for vitamin E. The vitamin E used was in the form of α-tocopherol acitate, marketed as *E-vet* powder manufactured by the *ACME* Laboratories Ltd. The proposed amounts of vitamin E were weighed using a sensitive electric balance and were mixed thoroughly with the different experimental feeds. The feeds were prepared in the form of pellet (1.5mm) by using a pelleting machine and stored in refrigerator. The compositions of experimental feeds are shown in the Table 1.

Table 1. Formation and composition of experimental feed

Ingredient	Inclusion level (%) in treatments			
	T_1	T_2	T_3	T_4
Fish meal (%)	40.00	40.00	40.00	40.00
Soybean meal (%)	29.69	29.69	29.69	29.69
Mustard oil cake (%)	15.00	15.00	15.00	15.00
Rice bran (%)	5.15	5.15	5.15	5.15
Wheat bran (%)	5.15	5.15	5.15	5.15
Flour (%)	4	4	4	4
Vitamin mineral premix (%)	1	1	1	1
Vitamin E (mg/kg feed)	0	50	100	150

The experimental feeds were administered directly into the corresponding cisterns twice daily *ad libitum*. Each of the cisterns was siphoned every morning to remove faeces and uneaten feed particles. During sampling at 15 days interval, all the fish from each replication of a treatment were caught and weight (g) and length (cm) of each fish were measured by using a sensitive electric balance and a measuring scale respectively and recorded. During the experimental period temperature, dissolved oxygen and pH were recorded weekly.

For breeding trial, a total of 36 broodfish from four treatments were selected and kept in 4 separate cisterns for about 6 hours under gentle but continuous shower for conditioning prior to injection with PG extract at a dose of 12 and 18mg/kg body weight of male and female respectively. Mature and healthy males of *O. pabda* collected from the broodstock pond were kept in fiberglass tank.

After injecting PG extract, both females and males were kept together treatment wise in the hapa set in a pond for spawning. Most of the broodfish were found to oviposit within 9 to 10h post injection. The broods were removed from the hapas after 10.5h of injection when

the spawning was completed. Continuous water flow was maintained in the hapa with porous PVC pipes for aeration. When the breeding was completed the fertilized eggs were removed from the hapas and placed in separate trays ($101.6\times40.6\times12.7cm^3$) treatment wise for incubation. The trays were previously filled with filtered pond water to reduce the temperature difference and environmental shock. Gentle shower was maintained through porous PVC pipes for aeration of eggs.

For calculation of fertilization and hatching rates of eggs produced by the females of each treatment, a portion of the eggs were taken and incubated in 12 bowls of 40cm diameter corresponding to the treatments, *i.e.* three replications for each treatment. The remaining eggs were incubated in separate trays. Soon after fertilization, the embryonic development started and the fertilized eggs looked watery and slightly transparent. Within 6 hours of incubation, the numbers of fertilized and unfertilized eggs from each bowl for respective treatment were counted based on the appearance of the eggs. The unfertilized eggs turned opaque and whitish in colour few hours post fertilization. After completion of hatching, the number of larvae of each bowl was counted by siphoning them out. The hatching completed within 22h at 24-25°C.

In order to study the effect of different dietary levels of vitamin E on the growth and breeding performance of the broodfish, the following parameters were studied:

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

$$\text{Weight gain (\%)} = \frac{\text{Mean final weight - mean initial weight}}{\text{mean initial wieght}} \times 100$$

$$\text{Specific growth rate (SGR, \% day)} = \frac{\text{Log}_e W_2 - \text{Log}_e W_1}{T_2 - T_1} \times 100$$

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)

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

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

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

The gain in weight and length, specific growth rate of broods, ovulation rate, fertilization rate and hatching rate of eggs etc. were tested using one-way analysis of variance (ANOVA). Significant results ($p<0.05$) were further tested using Duncan's Multiples Range Test (DMRT) to identify significant difference between means. The statistical analysis was performed with the aid of the computer software SPSS programme.

Results

The growth performance in terms of weight gain, % weight gain, specific growth rate (SGR) during the experimental period of broodfish of *O. pabda* fed with different dietary levels of vitamin E under four feeding treatments is presented in Fig. 1. The average initial weights of the broodfish in four treatments were 41.17±0.78g, 41.08±0.49g, 41.62±0.76g and 40.55±1.14g in T_1, T_2, T_3 and T_4 respectively. At the end of the experimental period (90 days), the final weight of the broodfish of four treatments were found to be 45.09±0.62g, 48.88±0.78g, 56.39±0.59g and 43.51±0.54g in treatment T_1, T_2, T_3 and T_4 respectively. The highest weight gain was observed to be 14.78±0.38g in the broodfish of treatment T_3 (fed with feed having 100mg vitamin E/kg of feed) followed by 7.80±0.96g in treatment T_2 (50mg vitamin E/kg of feed), 3.93±1.10g in T_1 (0mg vitamin E/kg of feed) and 2.97±0.89g in T_4 (150mg vitamin E/kg of feed) (Table 2). Statistical analysis showed that both weight gain and percent weight gain of broodfish were significantly higher ($P<0.05$) in treatment T_3 compared to the other treatments. The highest specific growth rate (% SGR) of the broodfish of treatment T_3 (fed with 100mg vitamin E/kg of feed) was found to be 2.99±0.11% which was significantly higher ($p<0.05$) than those of other three treatments. Treatment T_4 yielded poorest result in terms of weight gain (2.97±0.89g), percent weight gain (7.32±1.41%) and specific growth rate (1.21±0.32) after the completion of the experimental period (Table 2).

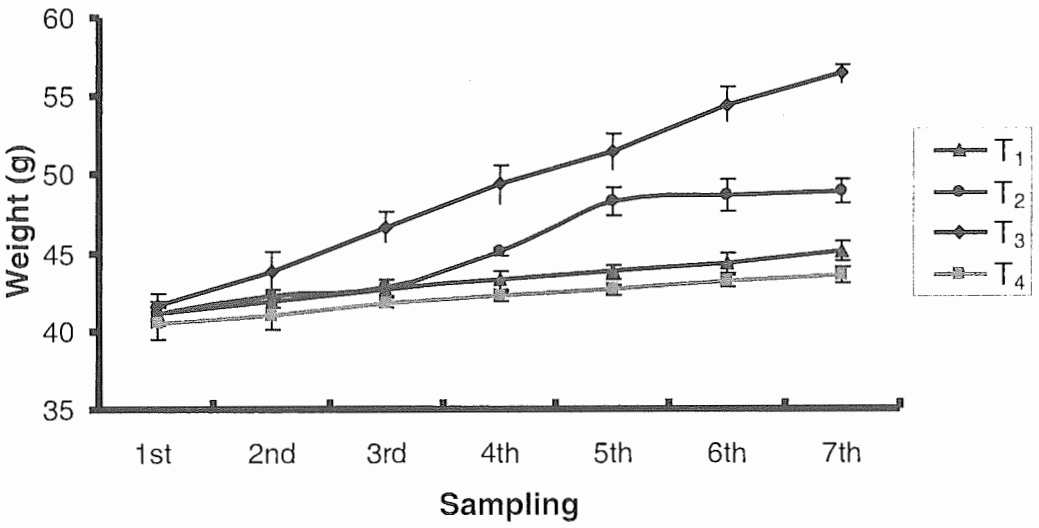

Fig. 1. The fortnightly growth response (weight) of *O. pabda* broodfish reared under different dietary levels of dietary vitamin E for a period of 90 days

Table 2. Weight gain, percent weight gain and specific growth rate of *O. pabda* broodfish under different doses of vitamin E (± SD)

Treatments	Initial weight (g)	Final weight (g)	weight gain (g)	weight gain%	SGR%
T_1	41.17±0.78	45.09±0.62	3.93±1.10^c	9.54±1.85^c	1.45±0.31^c
T_2	41.08±0.49	48.88±0.78	7.80±0.96^b	18.98±1.06^b	2.05±0.25^b
T_3	41.62±0.76	56.39±0.59	14.78±0.38^a	35.50±1.05^a	2.99±0.11^a
T_4	40.55±1.14	43.51±0.54	2.97±0.89^c	7.32±1.41^c	1.21±0.32^c

Values in the column with different superscripts are significantly different. SGR= Specific Growth rate

The average initial lengths of the broodfish of four treatments were 21.03±0.13cm, 20.88±0.57cm, 21.14±0.11cm and 20.63±0.28cm in T_1, T_2, T_3 and T_4 respectively (Table 3). The final lengths of the broodfish of four treatments were found to be 21.63±0.64cm, 21.69±0.83cm, 22.39±1.83cm and 21.16±0.28cm in treatment T_1, T_2, T_3 and T_4 respectively. The highest length gain was observed to be 1.25±0.11cm in the broodfish of treatment T_3 followed by 0.81±0.21cm in treatment T_2, 0.60±0.18cm in T_1 and 0.53±0.16cm in T_4. The highest percent length gain was observed to be 5.92±1.20% in treatment T_1 while the lowest was found to be 2.58±.78% in treatment T_4. Statistical analysis showed that there were no significant differences of means in terms of length gain and percent length gain among the treatments (Table 3).

Table 3. Length and percent length gain of *O. pabda* under different feeding treatments (± SD)

Treatments	Initial length (cm)	Final length (cm)	Length gain (cm)	% Length gain
T_1	21.03±0.13	21.63±0.64	0.60±0.18	2.83±0.76
T_2	20.88±0.57	21.69±0.83	0.81±0.21	3.87±0.55
T_3	21.14±0.11	22.39±1.83	1.25±0.11	5.92±1.20
T_4	20.63±0.28	21.16±0.28	0.53±0.16	2.58±0.78

The highest ovulation, fertilization and hatching rate of eggs were observed in the broodfish of treatment T_3 while the lowest was observed in treatment T_4 (Table 4). Statistical analysis showed that there was a significant difference ($p<0.01$) among the treatments. Duncan's Multiple Range Test (DMRT) showed that breeding performance of the broodfish of treatment T_3 was significantly higher ($p<0.01$) compared to the treatment T_1, T_2 and T_4. Treatment T_1 and T_2 were also significantly different ($p<0.01$) compared to treatment T_4. There were no significant difference between treatment T_1 and T_2.

Water temperature, dissolved oxygen and pH during the brood rearing period in the cistern 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 *O. pabda* broodfish. Temperature, pH and dissolved oxygen of water in bowls under different treatments ranged between 27.3 to 28.3°C, 6.8 to 7.5 and 5.3 to 6mg/l respectively.

Table 4. Breeding performance of *O. pabda* female broods reared under different doses of vitamin E when treated with equal dose (18mg PG/kg fish) of PG extract (±SD)

Treatment	Ovulation (%)	Fertilization (%)	Hatching (%)
T_1	59.26±6.4^b	71.56±3.41^b	57.95±2.34^b
T_2	62.97±6.41^b	68.16±3.12^b	58.53±4.72^b
T_3	81.48±6.41^a	84.04±3.53^a	68.59±5.03^a
T_4	33.33±0.00^c	52.35±5.02^c	45.70±7.24^c

Values in the column with different superscripts are significantly different

Discussion

The need of dietary vitamin E to maximize the breeding performance of *O. pabda* is clearly demonstrated in the present study. Weight of fish of different treatment increased with increase in dietary incorporation of vitamin E up to requirement level. The present result in terms of growth of the broodfish shows that the diet containing 100mg vitamin E/kg of feed (treatment T_3) is sufficient to support optimal growth of *O. pabda* broodfish. The result of the present study is in agreement with the reports of the earlier workers on *Cirrhinus cirrhosus* requiring 99mg vitamin E/kg feed (Paul *et al.* 2004), *Cyprinus carpio* requiring 100mg vitamin E/kg feed (Watanabe *et al.* 1970) and 80-100mg/kg (Halver 2002) and *Salmo salar* requiring 120mg vitamin E/kg feed (Hamre and Lie 1995).

On the other hand, higher amount of vitamin E in the diet of the broodfish (fed with 150mg vitamin E/kg of feed) resulted in poor growth. This finding is also in line with that of vitamin E requirement of broodfish of shing (*Heteropneustes fossilis*) and magur (*Clarias batrachus*) where higher doses (200mg vitamin E/kg of feed) showed an antagonistic effect on growth (Mollah *et al.* 2003, Roy and Mollah 2009). Studies with rainbow trout (*Salmo gairdneri*) (Cowey *et al.*, 1981, 1983) and channel catfish (*Ictalurus punctatus*) (Wilson *et al.* 1984) showed that weight gain did not respond to dietary vitamin E supplementation. Kiron *et al.* (2004) reported poor growth and feed utilization by incorporating 1000mg vitamin E/kg of feed in the diet of rainbow trout (*Oncorhynchus mykiss*). However, no significant difference was observed in terms of length gain of the broodfish of *O. pabda* in different feeding treatments. This seems to coincide with the result of that of Roy and Mollah (2009) and Jarboe and Robintte (1989). It is reported that vitamin E deficiency can lead to immunological malfunctions and reduce disease resistance in Salmonid fish (Lygren *et al.* 2000, Lygren *et al.* 2001) but there have been some discrepancies in literature regarding effects of higher dietary levels of vitamin E than normally used (Waagbø 1994, Wahli *et al.* 1998). Excess α-tocopherol inhibits the action of protein kinase C (PKC) in vascular smooth muscle cells leading to growth arrest (Boscoboinik *et al.* 1991).

Nevertheless, the beneficial effect of dietary vitamin E supplementation on fish reproduction was not found in many studies (Mollah, *et al.* 2003, Roy and Mollah 2009).

The result of the present study showed a positive impact of inclusion of vitamin E in the diet on the breeding performance of female *O. pabda*. The best ovulation rate of broodfish, fertilization rate and hatching percentage of the fertilized eggs produced, growth rate and survival rate of the larvae were obtained with the fish fed 100mg vitamin E/kg of feed i.e. treatment T_3. Other doses also showed positive result except treatment T_4 where the broodfish were fed with 150mg vitamin E/kg of feed. Takeuchi *et al.* (1981) conducted an experiment on the broodfish of 'ayu' *Plecoglossus altivelis* and observed better hatching percentage and survival of larvae with 3.4mg vitamin E/100g diet. Mollah *et al.* (2003) found better fertilization rate, hatching rate and survival rate of the larvae of *Heteropneustes fossilis* fed 200mg vitamin E/kg feed. Better fertilization and hatching rate and survival of larvae of *Clarias batrachus* was also observed when the broodfish were fed with feed having 50mg and 100mg vitamin E/kg of feed, however, Roy and Mollah (2009) recommended 50mg vitamin E/kg of feed for *Clarias batrachus* based on economics of brood rearing and larvae production.

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 diet. Similarly Sutjaritvongsanon (1987) reported that a mixture of 35% fish meal, 30% soybean meal, 20% corn meal, 15% rice bran and 10mg/kg BHT together with 100mg vitamin E/kg of feed was suitable for stimulating gonad development and spawning in goldfish (*Carassius auratus*). 100mg vitamin E/kg of feed is also known to increase the number of pleopodal eggs significantly in freshwater crayfish, *Astacus leptodactylus* (Harlıoğlu and Barım 2004). Therefore, it seems that vitamin E requirement of fish is species specific so for as its requirement is concerned in gonad development and breeding performance of fish.

Inclusion of higher levels of vitamin E (150mg vitamin E/kg of feed) in the diet of broodfish drastically reduced the ovulation rate of broods, fertilization and hatching rate of the fertilized eggs. The result coincides with that of Roy and Mollah (2009), where higher doses of vitamin E in the diet of broodfish of *Clarias batrachus* also affected the fertilization and hatching percentage. Eskelinen (1989) found that a high dietary α-týocopherol level failed to increase the survival of eggs and fry in a study on the different diets on eggs production and egg quality of Atlantic salmon (*Salmo salar*). Generally higher levels of vitamin E can cause a condition of hypervitaminosis that is evidenced by retardation in growth (Harlıoğlu and Barım 2004). Excess vitamin might have deterred the usual maturation of the gonad leading to poor breeding performance.

Vitamin E has been found to have positive impact on breeding performances of some other species as well. It seems important to conduct experiments of similar nature to investigate the quantitative retention of vitamin E in the gonad and eggs and its (vitamin E) mode of action on gonad to understand the function and characterization. The success obtained through this work can serve as an important base for future research on this topic.

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