

Effect of Vitamin E supplemented fish feed on the Reproductive Performance of Gangetic *Mystus* (*Mystus cavasius*)

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Abstract

Vitamins are one of the most effective organic additives nutritional substances that are necessary for growth, reproduction and decrease mortality rate in fish. This study aimed to evaluate the effect of vitamin E supplemented fish feed on reproductive responses of Gangetic *Mystus* (*Mystus cavasius*). Vitamin E is one of the micronutrients that functions as an antioxidant and can accelerate the secretion of reproductive hormones in fishes. Fish cannot synthesize vitamin E and therefore must be supplemented in fish feed especially maternal and paternal diet prior to oogenesis and spermatogenesis to improve reproductive fitness. The completely randomized design method (CRD) was used with five treatments and three replications of *M. cavasius* for 45 days. The doses of vitamin E that were mixed with the feed were 0, 50, 100, 150 and 200 mg/kg of dry fish feed respectively. Fish feed without vitamin E was used as a control experiment. Different doses of vitamin E supplementation into fish feed positively influenced the reproductive frequency, volume of eggs produced, hatching rate, average production of larvae, and survival rate. Highest ovulation rate (90 ± 1.87), fertilization rate (85.63 ± 7.23), hatching rate (70.9 ± 1.02) and survival rate (82.5 ± 1.09) at T₂ and poorest ovulation rate (60.0 ± 5.7), fertilization rate (69.82 ± 7.07), hatching rate (54.13 ± 2.34) and survival rate (62.5 ± 1.34) at T₄, respectively. This study showed that at treatment T₂ (100 mg vitamin E kg⁻¹) in the fish diet during the reproductive period of female *M. cavasius* are sufficient to ensure the best reproductive performance.

Keywords: *Mystus cavasius*, α -tocopherol, supplemented nutrition, reproductive parameters.

Introduction

Optimal fish hatchery results obtain by improving reproductive performance, which can be improve by supplementing the nutritional quality of brood-stock feed. Feed additives added in very little quantity to improve the feeds, which in returns improve the growth performance and decrease mortality rate in fish (Dada, 2015)^[1]. Vitamins are one of the most effective organic additives nutritional substances that are necessary for health, growth, maintenance and also spermatogenesis and oogenesis in animals (Gaylord et al. 1998)^[2]. The most active form of vitamin E is α -tocopherol, which has a very important role in improving fish reproduction. Vitamin E functions as an antioxidant that can maintain the presence of fatty acids and prevent fat oxidation in cell membranes and can accelerate the secretion of reproductive hormones (Hasani et al., 2008; Napitu et al, 2013)^[3,4]. Fish cannot synthesize vitamin E and therefore must be supplemented in fish feed especially maternal and paternal diet prior to oogenesis and spermatogenesis as it is one of the determinant factors for reproductive fitness (Canyurt and Akhan, 2008)^[5]. The addition of vitamin E to comet fish feed (*Carassius auratus*) can increase the reproductive performance of the fish, such as increasing egg diameter, gonad maturity index, and fecundity (Arfah et al. 2013)^[6]. Hence, Vitamin E has always been add in aqua-feeds, for almost all studied fish and crustacean species (Izquierdo and Betancor. 2015)^[7]. Maximum small indigenous species fish (SIS) are catch from floodplains and natural water bodies are still the main source of fish eaten by Bangladeshi rural people. Gangetic *Mystus* (*M. cavasius*) is a cat fish locally known as Gulsha, of the family Bagridae, belong to the small indigenous species (SIS) which is now consider as endangered species due to overfishing and the deterioration of natural habitats (IUCN, 2000)^[8]. In recent years, breeding grounds of this SIS species have severely degraded, because of environmental perturbations and man-induced hazards in the aquatic ecosystems as a result decreases genetic resources (Akhteruzzaman et al. 1991, Kohinoor et al.2009, Rahman M.A et al.2013)^[9,10,11]. Small Indigenous Species have high vitamin A content and serve as an important source of dietary calcium, as the fish are usually cooked and consumed completely, including bones. (Ahmed et al. 2012)^[12]. Unlike large fish, *M. cavasius* are consumed whole with head and bones, provide a significant source of protein and bio-available calcium, zinc, iron and vitamin A (Bogard et al. 2015; Roos et al. 2007a)^[13,14]. *M. cavasius* (*gulsha*) fetching high market value due to its good test, popularity and high nutrients value. Considering these importance and endangered condition of *M. cavasius*, current strategies to increase this fish species production and marketing in Bangladesh. The role of vitamin E supplemented fish feed on the reproductive performance of gulsha not well known until today. This study was conduct to evaluate the effect of different doses of vitamin E supplemented fish feed on the ovulation, fertilization rate, hatching rate and survival rate of larvae and to determine the best dose of vitamin E to accelerate the reproductive parameters of *M. cavasius*(gulsha fish).

Material and Method:*Experimental diets.*

The experimental diet samples were analyzed for moisture (%), ash (%), crude protein (%), crude fiber (%), lipids (%) and NFE (%). These analyses were carried out at the Fish Nutrition Laboratory of the Bangladesh Agricultural University, Mymensingh using following AOAC (2000) method (AOAC, 2000)^[15]. Proximate compositions of the feed components are found: Protein-33.17%, Carbohydrate-35.55%, Lipid-5.5%, Fiber-5.65%, Ash-7.77% and Moisture-12.38%. Five different graded levels of vitamin E (α -tocopherol) at 0, 50, 100, 150 and 200 mg Kg⁻¹ diets were included with the basal diet as treatment T₀, T₁, T₂, T₃ and T₄ respectively. T₀ treated as control. Feed preparation with vitamin E supplementation was carry-out bi-weekly to prevent long storage that degrades vitamin content. The feeds were air dried and put in an airtight container and stored at -20 °C until fed.

Experimental fish and feeding regime:

Total 100 numbers of brood *Mystus cavasius* (weight: 23.0-42.7g) were obtained from Shornolota Agro Fish Hatchery in Trisal, Mymensingh and were transferred to the place of experiment and acclimated for 2 weeks. Young *M. cavasius* (gulsha) fed a supplementary vitamin E free diet (basal diet) for 2 weeks while acclimating to experimental conditions. Total ninety (90) uniform male and female fish randomly selected and stocked into fifteen (15) net hapa aquarium (7 ft. x 5.0 ft. x 3.5 ft.), placed in a pond which in turn randomly assigned to each treatment. Controlled temperature was (28±2°C) and three replicate aquarium were assign to each dietary treatment. Pond was half-filled with deep tube-well water and water quality was maintain by continuous aeration. Fishes supply fed approximately 5% of their body weight daily, two equal feedings time (8.0 am, and 18.0pm) for 30 days. Feed preparation was carry out bi-weekly to prevent long storage.

Selection of female brood fish:

After rearing 30 days with the experimental diets, all fishes were remove from the hapa and kept in several cisterns treatment wise under shower. Good and healthy mature broods were select for breeding. Identification of male and female broods done based on some external features. Their swollen abdomen and round and swollen urogenital papillae could easily recognize the females (Figure-1). On the other hand, the mature males were identifies by their flat abdomens and long protruded genital papillae.



Figure 1. *Mystus cavasius* (Gulsha fish)

Effect of vitamin E on the breeding performance:

Forty five (45) females were divided into five doses of supplemented vitamin E and marked as T₀, T₁, T₂, T₃ and T₄ having three females in each treatment in such a way that the average weight of 3 females under each treatment remained approximately similar. The females under each treatment were kept separately in different cisterns. Similarly forty-five (45) males were divided into five treatments T₀, T₁, T₂, T₃ and T₄ having three males in each treatment in such a way that the average weight of three males under each treatment remained approximately similar. The weight of selected female broods ranged from 43 – 56 g, whereas the weight of the males varied from 28-34 g. For induced breeding both male and female *M. cavasius* (gulsha) fish was injected on dorsal region of the brood fish for stimulation at a single dose of OVUHOME hormone, 0.125 µg/g and 0.25 µg/g body weight of male and female respectively. After injecting, both females and males were kept together at a ratio of 1:1 treatment wise in the respective cisterns (6.5 ft. × 4.0 ft. × 3.5 ft.) for spawning. Most of the brood fishes were found to ovulate within 6 to 10 h post injection. The broods were removed from the tank after 10.5h of injection when the spawning was complete. Continuous water flow was maintained in the tank with porous PVC pipes for aeration. When the breeding was complete, the fertilized eggs were removed from the tanks and placed in separate trays (40 cm × 30 cm × 10 cm) 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.

Chemical Analysis of Fish feed

Chemical analysis of the raw fish estimated according to different standard analytical methods developed for proximate analysis:

Determination of moisture of raw fish paste were conducted following the (AOAC, 2003)^[16] method.

$$\text{Moisture content (\%)} = \frac{\text{Loss of weight}}{\text{Weight of sample taken}} \times 100$$

The crude protein of the fish pest powders were determined by Micro-Kjeldhal method (Pearson, 1999, AOAC 2003)^[17, 16]. Briefly, the percentage of nitrogen of fish samples was calculated and the percentage of protein in the samples was calculated by multiplying the percentage of N with an empirical factor 6.25.

Percentage of Nitrogen (%)

$$= \frac{\text{Mili – equivalent of N}_2(0.014) \times \text{N HCl} \times \text{Titration value (ml)}}{\text{Weight of sample (gm)}} \times 100$$

Total lipid content of fish powders were determined by method as described in Bligh and Dyer method (Bligh, and Dyer, 1959)^[18] and calculated using following equation.

$$\text{Total lipid content (\%)} = \frac{\text{weight of fat residue}}{\text{weight of sample taken}} \times 100$$

The ash content of the fish samples was determined as the inorganic residues oxides, sulphates, silicates and chlorides in the dry muscle. The samples heated to the temperatures of 500°C - 600°C in a muffle furnace for about 3 hours. Afterwards, the percentage of ash content was calculated according to (AOAC, 2003, Farzanaand Mohajan., 2015)^[16,19].

$$\text{Ash content (\%)} = \frac{\text{Weight of ash}}{\text{weight of sample taken}} \times 100$$

Ovulation, fertilization hatching and survival rates were calculate using the following formula:

$$\text{Ovulation rate} = \frac{\text{No of fish ovulated}}{\text{Total no.of fish injected}} \times 100 \quad (\text{Ali F. et al., 2014})^{[20]}$$

$$\text{Fertilization rate} = \frac{\text{No.of fertilized eggs}}{\text{Total no.of eggs (fertilized+unfertilized)}} \times 100 \quad (\text{Ali M. et al., 2016})^{[21]}$$

$$\text{Hatching rate} = \frac{\text{No.of eggs hatched}}{\text{Total no.of fertilized eggs}} \times 100 \quad (\text{Rahman M.M et al., 2013})^{[22]}$$

$$\text{Survival rate} = \frac{\text{No.of hatchlings survived}}{\text{Total no.of hatchling}} \times 100$$

RESULTS

Experimental pond water temperature, pH, dissolved oxygen and dissolve ammonia during the brood rearing period in all the hapa were found with the desirable range for the fish rearing according to Boyd (1979)^[23] and Zafar *et al.* (2015)^[24]. Average Temperature, pH, dissolved oxygen and dissolve ammonia of water in all hapa under different treatments ranged from 26.8 - 28.3°C, 6.7 - 7.6, 5.2 - 6.1 mg/L and 0.25 - 0.5 mg/L, respectively.

Effect of vitamin E supplementation on the growth performance of M. cavasius(gulsha fish)

At the end of the 30 days feeding trial with different doses of vitamin E supplemented fish feed, the total weight gain (TWG), Percentage of body weight gain (PBWG), Specific growth rate (SGR) of male and female gulsha fish were determined and presented in Table-1 and 2. The highest body weight gain of male and female brood gulsha fish on treatment T₂ (supplemented 100mg vitamin E/kg feed) were 33.90±1.00g; 55.60±1.60 g significantly different (P<0.05) from other treatments T₀, T₁, T₃ and T₄ respectively ((Figure-2). The lowest weight gain obtained at T₄ (supplemented 200mg vitamin E/kg feed) (28.5±1.04g; 43.2±0.20 g). The specific growth rate of male and female gulsha fish were the highest in treatment T₂ (1.12, 1.62), it is then followed by that of the fish's in treatment T₁, T₀, T₃, T₄ (Figure-3). The lowest SGR of male and female were obtained at treatment T₀ (without vitamin E

0mg/kg, 0.79, 0.79) and T₄ (high dose 200 mg/kg of vitamin E, 0.68, 0.90) respectively. Brood fish fed under higher doses of vitamin E supplementation at treatment T₃ (supplemented 150mg vitamin E/kg feed) and T₄(supplemented 200mg vitamin E/kg feed) had the least body weight gain, percentage of body weight gain and Specific growth rate (Table 1, 2).

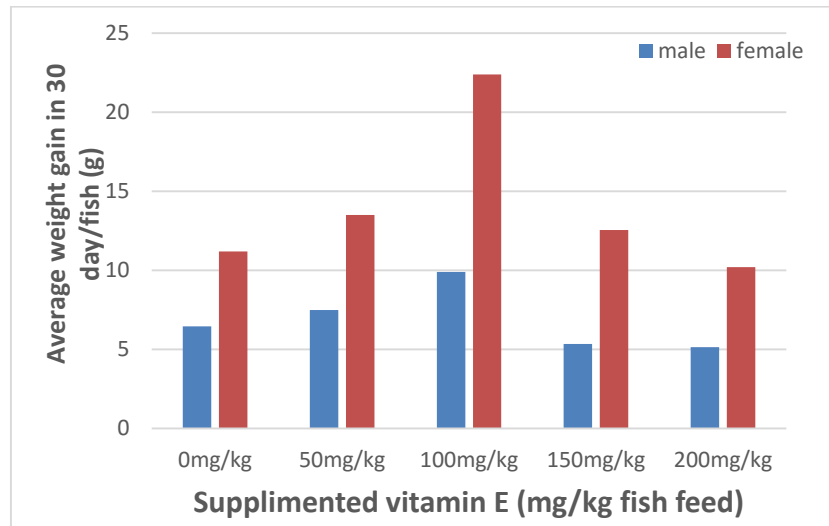


Figure 2. Average weight gain of *M. cavasius*(male and female) by feeding 30 days with different doses of supplemented vitamin E fish feed.

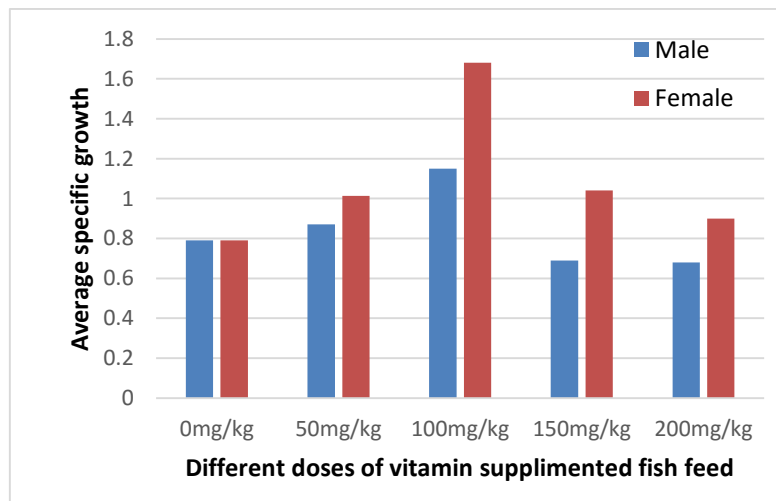


Figure 3. Average specific growth of *M. cavasius*(male and female) by feeding 30 days different doses of supplemented vitamin E fish feed.

Table 2: Growth Performance Parameters of *M. cavasius* (Female) Brood-fish after 30 days fed with experimental vitamin E supplemented fish feed (Mean \pm SD).

| Parameters | Treatment (T ₀) No vitamin E supplemented | T ₁ (50mg vitamin-E/ kg feed) | T ₂ (100mg vitamin- E/kg feed) | T ₃ (150mg vitamin E/kg feed) | T ₄ (200mg vitamin E/kg feed) |
|--------------------------------------|---|--|---|--|--|
| Female Initial Body Weight (g) | 42.2 \pm 1.05 | 38.2 \pm 0.95 | 34.2 \pm 0.90 | 34.0 \pm 1.01 | 33.0 \pm 1.00 |
| Female Final Body Weight (g) | 53.4 \pm 1.15 | 50.6 \pm 1.05 | 55.6 \pm 1.00 | 46.55 \pm 1.06 | 43.2 \pm 1.04 |
| Body Weight Gain (g) | 11.2 \pm 0.05 | 12.4 \pm 0.49 | 21.4 \pm 0.10 | 12.55 \pm 0.05 | 10.2 \pm 0.49 |
| % Body Weight Gain | 41.5 \pm 0.449 | 42.2 \pm 0.325 | 62.77 \pm 0.500 | 37.51 \pm 0.50 | 30.75 \pm 0.50 |
| Specific Growth Rate | 0.79 | 0.94 | 1.62 | 1.04 | 0.90 |

Table 1: Growth Performance Parameters of *M. cavasius* (Male) Brood-fish after 30 days fed with experimental vitamin E supplemented fish feed (Mean \pm SD).

| Parameters | Treatment (T ₀) No vitamin E supplemented | T ₁ (50mg vitamin-E/ kg feed) | T ₂ (100mg vitamin- E/kg feed) | T ₃ (150mg vitamin E/kg feed) | T ₄ (200mg vitamin E/kg feed) |
|------------------------------------|---|--|---|--|--|
| Male Initial Body Weight (g) | 24.1 \pm 1.05 | 25.0 \pm 0.95 | 24.0 \pm 0.90 | 23.4 \pm 1.01 | 22.9 \pm 1.00 |
| Male Final Body Weight (g) | 30.55 \pm 1.15 | 32.50 \pm 1.05 | 33.9 \pm 1.00 | 28.75 \pm 1.06 | 28.05 \pm 1.04 |
| Body Weight Gain (g) | 6.45 \pm 0.05 | 7.50 \pm 0.49 | 9.90 \pm 0.10 | 5.35 \pm 0.05 | 5.15 \pm 0.49 |
| % Body Weight Gain | 25.5 \pm 0.449 | 30.0 \pm 0.325 | 47.5 \pm 0.500 | 23.5 \pm 0.50 | 21.5 \pm 0.50 |
| Specific growth rate | 0.79 | 0.88 | 1.12 | 0.69 | 0.68 |

Effect of vitamin E on the breeding performance

Induced breeding:

Brood-fish *M. cavasius* (gulsha fish) were induced to breed using ovuhom (Compound S-GnRha) at a dose of 0.25 $\mu\text{g/gm}$ for female and 0.125 $\mu\text{g/gm}$ for male respectively. The breeding performance of female brood fish fed with different levels of vitamin E in terms of ovulation percentages, fertilization rate, hatching rates of eggs and survival of larvae were shown in Figure-4, 5, 6 and 7. Brood fish fed with 100mg vitamin E kg^{-1} in T₂ demonstrated significantly higher ($p < 0.05$) ovulation rate (90.0 \pm 1.87) than that of fish fed with 0mg, 50mg, 150mg, and 200 mg vitamin E kg^{-1} feed. The poorest ovulation rate (60.0 \pm 5.70) was obtained from fish fed with higher dose of vitamin E supplemented feed (200mg/kg) at treatment T₄. No significant differences were observed between the ovulation rate of fishes T₀ and T₁ and between T₃ and T₄ (Fig-4). The fertilization rate of eggs was found significantly higher ($P < 0.05$) in T₂ followed by T₃, T₁, T₀ and T₄ (Fig-5). The best fertilization rate (85.63 \pm 7.23) was observed in T₂ and poorest (69.82 \pm 7.09) was obtained in T₄. Similar results were obtained in hatching of eggs produced by females in different treatments and T₂ showed significantly ($p < 0.05$) higher hatching than that of T₁, T₃, T₀ and T₄ (Figure-6). The highest hatching rate (70.9 \pm 1.02) was observed in eggs produced from female brood fish of T₂ and poorest (54.13 \pm 2.34) was observed with the brood fish of T₄ (Fig-7). After three days of rearing, highest survival rate of the larvae (82.5 \pm 1.09) was observed in the larvae produced from *M.cavasius* brood fish reared under T₂ treatment while poorest (62.5 \pm 1.34) was obtained with the brood fish of T₄ treatment (Fig-8).

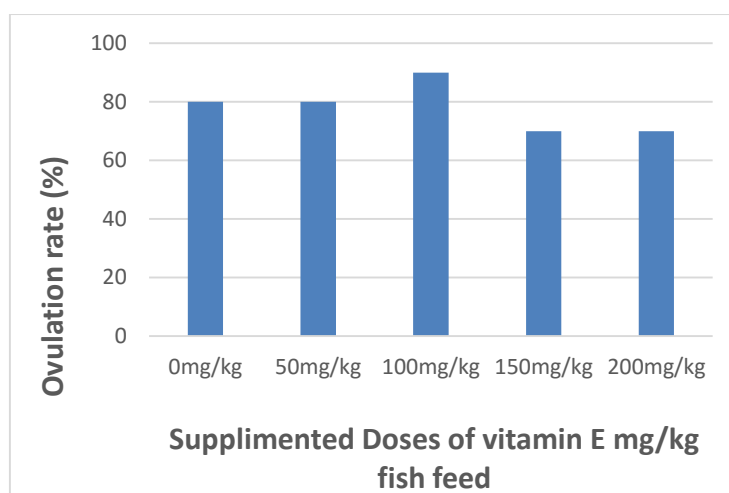


Figure 4. Effect of Experimental Diets on Ovulation rate of *M. cavasius*

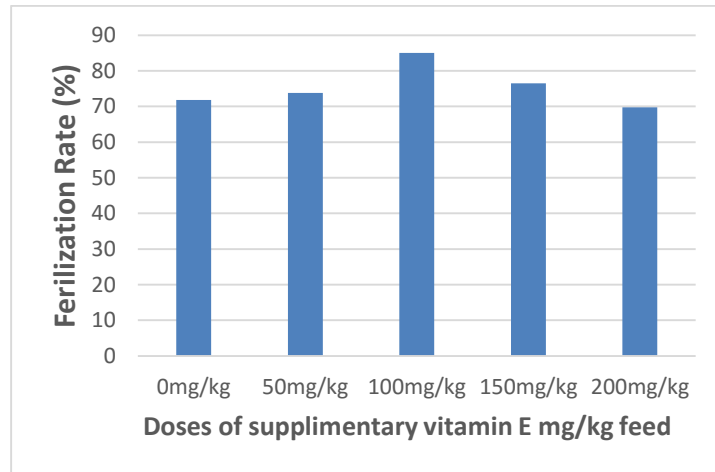


Figure 5. Effect of Experimental Diets on Fertilization rate of *M. cavasius*



Figure 6. Fertilized egg and hatchling of *M. cavasius*.

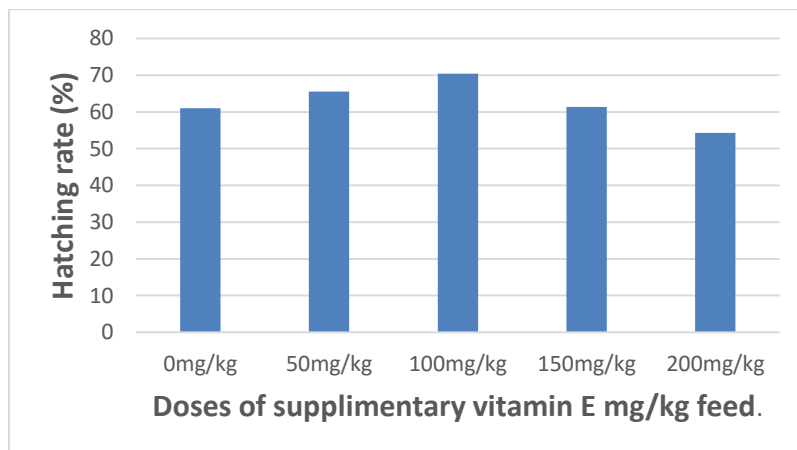


Figure 7. Effect of Experimental Diets on hatching rates of *M. cavasius*

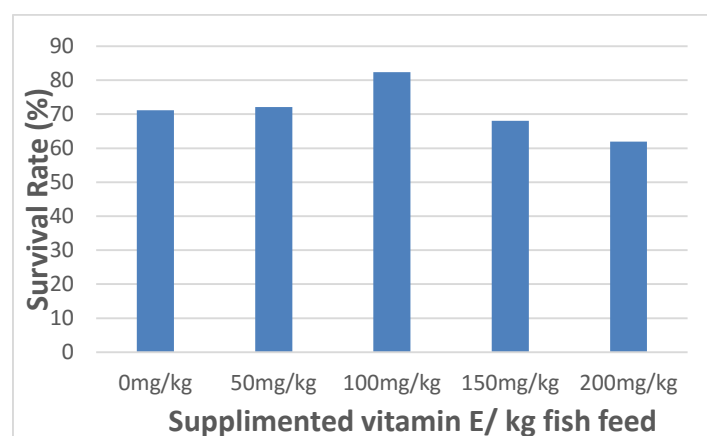


Figure 8. Effect of vitamin E supplemented Experimental Diets on Survival rate of *M. cavasius*

DISCUSSION

Monitoring water quality parameters:

All water quality parameters are important for the growth development of the fry and fingerlings. Water temperature fluctuation affects the fish's metabolism activity, physiology and growth rate. The temperature ranges recorded from 26.8°C – 29.3°C which support the normal growth temperature range 14°C – 38°C for tilapia and gulsha (Khairuman and Khairul., 2005)^[25]. The water pH values recorded for *M. cavasius* growth, egg incubation and fry rearing was 6.7 – 7.5. According to Santhosh and Singh (2007)^[26], the suitable pH range for fish cultivation is 6.5 – 8.5, a water pH level above and below this range will be stressful to the fish strain. Dissolved oxygen in the range of 4.5 – 8 mg L⁻¹ is generally consider suitable for fresh water fish cultivation (Bhatnagar and Sangwan,2009)^[27]. During this study, the dissolved oxygen concentration recorded was slightly fluctuated in the range of (5.2 – 6.1 mg L⁻¹), but the mean recorded values falls within the recommended range. If the oxygen levels drop below 4mg/l, the fish may stop feeding, become stressed and suffer electrolyte loss. All of these issues can cause the fish to die and ultimately affect the yield. Un-ionized ammonia concentrations could be quite toxic to catfish fry. The maximum limit of ammonia concentration in the aquatic system is 0.5 mg L⁻¹ (Meade 1985; Santhosh and Singh 2007)^[28, 26]. In the present study, the concentration of NH₄-N was 0.25 mg L⁻¹ -0.5 mg L⁻¹. The concentration of NH₄-N was within the recommended range, which was a not cause for fry mortality.

Effect of vitamin E supplemented fish feed on Reproductive parameters

Vitamins are organic compounds that are essential for life; as they are required in trace amounts for normal growth, reproduction and health (Gasco et al., 2018; Gouda et al., 2020)^[29,30]. However, supplementation of vitamins improved the fertilizing capacity of sperm of yellow perch (Lee and Dabrowski, 2004)^[31] and fertilization rates of Japanese quail (*Coturnixjaponica*) (Biswas et al., 2006)^[32].The relationship

of vitamin E on the breeding performances of *M. cavasius* was clearly demonstrate in this experiment. The result of the present study showed a positive impact of inclusion of vitamin E in the diet on the breeding performances of *M. cavasius*. The highest result of ovulation rate of brood fish, fertilization rate, hatching rate of the fertilize eggs produced and survival of the larvae were observed in T₂ (fish fed supplemented with 100 mg vitamin E/kg feed) and poor result was observed in T₄ (brood fish fed supplemented with higher doses of 200 mg vitamin E/kg feed). This variation in respect of different breeding parameters has occurred because vitamin E is an essential nutrient for all species of animals (McDowell, 1989)^[33]. As in higher vertebrates, vitamin E deficiency affects reproductive performance, causing immature gonads and lower hatching rate and survival of offspring (Izquierdo *et al.*, 2001)^[34]. The dietary requirement of vitamin E has been demonstrated in a number of fish, such as Sarowar and Mollah, 2009^[35] in case of *O. pabda* where they recommended 100mg vitamin E/kg of fed for nutritional requirement of *O. pabda* for better reproductive performances. Better fertilization, hatching rate and survival rate of larvae of *C. batrachus* was also observed when the brood fish were fed with supplemented 59 □ 100 mg vitamin E/kg of fish feed, however, Roy A., 2008^[36] recommended 50 mg vitamin E/kg of fish feed for *C. batrachus* based on economics of brood rearing and larvae production. Harlioglu *et al.* (2002)^[37] found that the use of vitamin E at a rate of 80 mg/kg of feed could effectively increase the number of pleopodal eggs of fresh water crayfish (*A.leptodectylus*). Harlioğlu and Barim (2004)^[38] reported an increase in egg production of crayfish (*Astaculeptodactylus*) when added 100 mg vitamin E kg⁻¹ in the diet. It may likewise affect embryo membrane permeability and hatchability of fish eggs. These results has similarity with the result of our findings. The better performance of larvae produced from vitamin E treated female broods compared to the control may be due to the fact that vitamin E is known to have a profound effect on the immune response of the younger fishes (Lygren *et al.*, 2001; Lygren *et al.*, 2000)^[39,40] which may enable them to grow faster. Watanabe *et al.* (1985)^[41] and Harlioğlu *et al.* (2002)^[37] recommend 100 mg kg⁻¹ of α -tocopherol to increase the number of larvae produced by red sea bream snapper (*Pagrus major*) and crayfish, respectively. Larvae growth depends on factors of feed quality, water quality, and stocking density. Stocking density is an important factor that can affect fish growth. Certain stocking density will have a positive and negative effect on the growth rate (Niazie *et.al*, 2013)^[42]. High stocking density will cause competition in gaining space, feed and oxygen which can cause stress to fish (Hermawan *et. al.*, 2015)^[43]. Fish that have stress occur because of environmental conditions that are not suitable for the survival of larvae (Kadarini T *et al.*, 2010)^[44]. Stressful conditions can cause the normal function of the fish to be disrupt and growth to slow down. In the present experiment, best reproductive performance (Ovulation rate, Fertilization rate, Hatching rate and Survival rate) were observe at the supplementation dose of vitamin E (100 mg/kg) and decreased sharply with the increase in supplementation rate 150 and 200 mg/kg dry fish feed.

CONCLUSION

Based on the research results, giving vitamin E with different doses in fish feed had an influence on the development of gonad maturity, fecundity and reproductive parameters of gulsha (*M. cavasius*). Giving vitamin E as much as 100 mg, kg⁻¹ is the best dose for ripening *M. cavasius* (gulsha) gonads with maximum rate of ovulation, fertilization, hatching and survival respectively.

Data Analysis: The data obtained from the trial were subject to one-way analysis of variance (ANOVA) (using SPSS 16.0 programme) to test for effects of dietary treatments. When ANOVA identified significant difference among groups, multiple comparison tests among means were performed using Duncan's new multiple range test. For each comparison, statistically significant differences were determined by setting the aggregate type I error at 5% (P<0.05).

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Authors' Contributions:

This research includes MSc thesis work of first author. However, the last author treated as team leader and principal supervisor. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

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