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Effect of vitamin E supplemented fish feed on growth performance of brood gangetic *Mystus (Mystus cavasius)*

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Abstract

Nutrition is the major crucial factor determining the potential of cultured fish to exhibit its genetic capability for growth. In this study, the impact of dietary vitamin E supplementation of fish feed on growth performance, feed conversion ratio and protein efficiency ratio of Gangetic *Mystus (Mystus cavasius)*, Hamilton, 1822) were investigated. Total 150 number of young *M. cavasius* (weight 33.0±10.0 g) were used the CRD (completely randomized design) method with five treatments and three replications and fed with one of five diets for 45 days. Five different treatments (T₀, T₁, T₂, T₃ and T₄) with three replication having different level of vitamin E such as 0 mg vitamin E/kg (Control-T₀), 50 mg vitamin E/kg (T₁), 100 mg vitamin E/kg (T₂), 150 mg vitamin E/kg (T₃) and 200 mg vitamin E/kg (T₄). Feeds supplied at the rate of 5% for first two weeks, 4% for following two weeks and 3% of the body weight for the rest of the experimental period twice daily throughout the study period. At the end of the (15+30) 45 days study period, the highest mean final weight gain 15.65±0.10 in T₂, whereas, the minimum final weight gain was 7.725±0.49 in T₄. The body length of *M. cavasius* was not increased significantly ($p>0.05$) at different doses of vitamin E supplementation. The food conversion ratio in treatment 0, 1, 2, 3 and 4 were 2.72±0.12, 2.41±0.14, 1.53±0.12, 2.68±0.09, and 3.11±0.10, respectively. Protein efficiency ratio (PER) were found highest (1.96±0.08) at T₂ compared to T₁ (1.25±0.09), T₃ (1.12±0.11) T₀ (1.11±0.10), and T₄ (0.96±0.10), respectively. The specific growth rate (SGR%/day) in treatment 0, 1, 2, 3 and 4 were 0.79±0.05, 0.91±0.07, 1.37±0.10, 0.87±0.08 and 0.79±0.06, respectively. The best performance found for 100 mg vitamin E/kg containing feed could be the best for growth performance of Gangetic *Mystus (Mystus cavasius)*.

Keywords: *Mystus cavasius*, vitamin E (α -Tocopherol), growth parameters, PER, FCR

1. Introduction

Aquaculture is considered as one of the fastest growing protein food producing industries, which supplies over 50% of global fish production (FAO, 2021) [2]. Bangladesh is one of the world's leading inland fish producing countries, contributing about 3.50% to 25.71% GDP (Gross Domestic Product), to agricultural production. Fish is the major protein source contributing about 60% of total animal protein intake (DoF, 2019) [1]. Floodplain fisheries are still the main source of fish eaten by rural people, with SIS contributing the most. Much of the small indigenous fish (SIS) of Bangladesh are caught in floodplains and natural water bodies. Gangetic *Mystus (Mystus cavasius)* is a catfish of the family Bagridae locally known as Gulsha; belong to the small indigenous species (SIS) are in decline production and endangered, due to overfishing and the deterioration of natural habitats (IUCN 2000, Rahman *et al.* 2013) [3, 4]. Now, this species of fish has high market demand and high price due to its preferred good taste, good sources of proteins, contains many trace elements such as, sodium, potassium, calcium, Iron, Iodine, Zinc, magnesium, phosphorus and vitamin A, (Roy and Hossain, 2006, Bogard *et al.*, 2015; Roos *et al.*, 2007a) [5, 6, 7]. Considering the above importance and endangered condition of *M. cavasius*, there should be a focus on production of small fish so that greater quantities are accessible for consumption by the rural poor. Most important, supplements like vitamins often added to manufacture fish feed that enhances the growth and health of cultured fish (Wong *et al.* 2016, Gouda *et al.*, 2020) [8, 9]. In aquaculture, vitamin E is used for the fortification of feed to improve the growth, resistance to stress and

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disease as well as for survival of fish and shrimp (Vismara *et al.*, 2003) [10]. A large number of studies have reported that vitamin E deficiency impairs aquatic animal performance, including a reduced weight gain, protein efficiency ratio as well as feed coefficient (Sau *et al.*, 2004; Lee and Dabrowski, 2003; Bai and Lee, 1998; Paul *et al.*, 2004; Hung and Lin, 2004) [11, 12, 13, 14, 15]. The role of vitamin E supplemented fish feed on the growth parameters, feed coefficient ratio and protein efficiency ratio of *M. cavasius* not well known until now. Therefore, the present study designed to evaluate the effect of different levels of vitamin E on the growth performances of genetically improved farmed *M. cavasius* (gulsha).

Material and Method

Experimental fish and feeding regime

Total 150 healthy young *Mystus cavasius* (weight 23.0-42.0 g) were collected from Sharnalata Agro Fisheries Firm, Trisal, and Mymensingh, Bangladesh, where they originally hatched and reared (Figure-1). They were randomly divided into five groups (30 fish per group) according to the vitamin E content in the experimental diet (0 mg, 50 mg, 100 mg, 150 mg and 200 mg/kg) and placed into 15 hapa. For each experimental group, fish were equally divided among three rectangular hapa, leading to a density of 10 fish per net hapa aquarium size (7 ft. x 5.0 ft. x 3.5 ft.), placed in a pond where the incoming water was filtered with aeration. Prior to starting the feeding trial, fish were acclimatized for two weeks on a basal diet that was not supplemented with vitamin E and then fed one of the five experimental diets two times daily (8.0 am and 18.0 pm) for 30 days based on 3-5% percentage body weight. Fish from each aquarium were weighed and length measured at 10 day intervals to monitor growth parameter. The tools used in this study were buckets, aerators, stationery, ruler, section set, digital scales, DO meter, pH meter, and thermometer.

Collection and preparation of experimental feed samples

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) [16]. 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 carried 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.

Determination of growth and nutrient utilization

Total 10.8 kg basal fish feed were supplemented with different doses of vitamin E (50, 100, 150, 200 mg/kg dry fish feed) for this experiment and each treatment contained 2.16 kg basal feed with supplemented vitamin E for 30 days. Therefore, experimental fish fed with 0.8 g feed/fish/day, which contains

0.265 g net protein/day/fish. The biweekly weights of fish and feed supplied recorded. The growth and nutrient utilization parameters such as weight gain (g), percentage weight gain (%), specific growth rate (SGR, %/day), and protein efficiency ratio (PER), feed conversion ratio (FCR) were calculated.

The following variables were calculated

Body weight gain (BWG) = $W_t - W_0$ (Tacon 1990) [17]

Percent body weight gain (PBWG) = $[(W_t - W_0)/W_0] \times 100$ (Bekcan *et al.* 2006) [18]

Specific growth rate (SGR) = $(\ln W_t - \ln W_0) \times 100 t^{-1}$ (Hevroy *et al.* 2005) [19]

Daily growth rate (DGR) = $[(W_t - W_0)/t] \times 100$ (De Silva & Anderson 1995) [20]

Feed conversion ratio (FCR) = total feed intake (g)/total weight gain (g) (Stickeney and Hardy, 1989) [21]

PER = total gain (g)/protein intake (g) (Zeitoun *et al.* (1976). [22]

Where: W_t and W_0 were final and initial fish weights (g), respectively; N_t and N_0 were final and initial numbers of fish in each replicate, respectively; L (cm) was final length; and t is the experimental period in days. The data obtained from the trial were subjected 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% ($p < 0.05$).

Results

Physio-chemical Parameter of Water

Experimental pond water temperature, pH, dissolved oxygen and dissolved ammonia during the experimental period in all the hapa were found within the desirable range for the fish rearing according to Zafar *et al.*, (2015) [23]. Average Temperature, pH, dissolved oxygen and dissolved ammonia of water in all hapa ranged from 26.8-29.8 °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 Genetic *Mystus* (*M. cavasius*)

At the end of 30 days vitamin E supplemented feeding experiment, the body weight gain (BWG), percent of body weight gain (PBWG), daily growth rate (DGR) and specific growth rate (SGR) of *M. cavasius* (gulsha) were determined and presented in Table-1. Average highest body weight gain (15.65±0.10 g) of *M. cavasius* on treatment T₂ (supplemented 100 mg vitamin E/kg feed) was significantly different ($p < 0.05$) from other treatments T₀ (8.825±0.05), T₁ (9.95±0.49), T₃ (8.95±0.05) and T₄ (7.725±0.49), respectively. The lowest body weight gain (7.725±0.49 g) at T₄ (supplemented 200 mg vitamin E/kg feed) [Table-1; Figure-2]. The percentage of body weight gain and specific growth rate was highest in treatment T₂, it is then followed by the fish's in treatment T₁, T₀, T₃, and T₄, respectively (Table-1; Figure-3, 4).

Table 1: Growth Performance Parameters BWG, % BWG, SGR, FCR and PER of *Mystus cavasius* Brood-fish after 30 days fed with experimental vitamin E supplemented fish feed (Mean \pm SD n=10)

Parameters	Treatment (T ₀) without vitamin E supplemented	T ₁ (50 mg vitamin-E/ kg feed)	T ₂ (100 mg vitamin-E/kg feed)	T ₃ (150 mg vitamin E/kg feed)	T ₄ (200 mg vitamin E/kg feed)
Initial Body Weight (g)	33.15 \pm 0.15	36.6 \pm 0.19	29.1 \pm 0.14	28.7 \pm 0.10	27.9 \pm 0.12
Final Body Weight (g)	41.975 \pm 0.14	46.55 \pm 0.18	44.75 \pm 0.17	37.65 \pm 0.19	35.625 \pm 0.11
Body Weight Gain (g)	8.825 \pm 0.05	9.95 \pm 0.49	15.65 \pm 0.10	8.95 \pm 0.05	7.725 \pm 0.49
% Body Weight Gain	26.62 \pm 0.449	27.19 \pm 0.325	53.78 \pm 0.500	31.18 \pm 0.50	27.69 \pm 0.50
Daily Growth rate (g)	0.29 \pm 0.07	0.33 \pm 0.05	0.52 \pm 0.06	0.30 \pm 0.09	0.26 \pm 0.05
Specific Growth Rate	0.79 \pm 0.05	0.91 \pm 0.07	1.37 \pm 0.10	0.87 \pm 0.08	0.79 \pm 0.06

The daily growth rate of *M. cavasius* obtain from this study indicates that treatment T₂ with mean value of 0.53 \pm 0.06 was significantly different ($p < 0.05$) from other treatments and lowest at T₄ with mean values of 0.26 \pm 0.05, respectively (Figure-5).

Effect of vitamin E supplemented fish feed on Protein efficiency ratio (PER) and feed conversion ratio (FCR) of Gangetic *Mystus (M. cavasius)*

Protein efficiency ratio (PER) and feed conversion ratio (FCR) showed in Table 2. The dietary supplementation with

100 mg vitamin E kg⁻¹ had an obviously lower feed coefficient ratio of *M. cavasius* (1.53 \pm 0.08) than that with higher dose 200 mg vitamin E kg⁻¹ (3.11 \pm 0.010) (Figure-6). Protein efficiency ratio (PER) were found highest (1.96 \pm 0.08 g) at T₂ treatment followed by T₀ (1.11 \pm 0.10 g), T₁ (1.25 \pm 0.09 g), T₃ (1.12 \pm 0.11 g) and T₄ (0.96 \pm 0.10 g) treatment respectively, (Figure-7). At the end of 30 days fed with supplementary vitamin E experiment, the variation in the mean average body length of gulsha fish among the different treatments were not significantly (very little) difference ($p > 0.05$) [Table-3]

Table 2: Body weight gain (BWG), Food conversion ratio (FCR) and Protein efficiency ratio (PER) of *M. cavasius* Brood-fish after 30 days fed with experimental vitamin E supplemented fish feed (Mean \pm SD n=10).

Parameters	0 mg vitamin E+ 24 gm basal fish fed/30 days/fish	50 mg vitamin E+ 24 gm basal fish fed/30 days/fish	100 mg vitamin E+ 24 gm basal fish fed/30 day/fish	150 mg vitamin E+ 24 gm basal fish fed/30day/fish	200 mg vitamin E+ 24 gm basal fish fed/30 day/fish
Body Weight Gain (g)	8.825 \pm 0.05	9.95 \pm 0.49	15.65 \pm 0.10	8.95 \pm 0.05	7.725 \pm 0.49
Feed conversion ratio (FCR)	2.72 \pm 0.12	2.41 \pm 0.14	1.53 \pm 0.12	2.68 \pm 0.09	3.11 \pm 0.10
Net protein in supplemented fish feed (33.2%)	0 mg vitamin E+ 7.97 g net protein in basal feed/30 days/fish	50 mg vitamin E+ 7.97 g net protein in basal feed/30 days/fish	100 mg vitamin E+ 7.97g net protein in basal feed/30 days/fish	150 mg vitamin E+ 7.97g net protein in basal feed/30 days/fish	200 mg vitamin E+ 7.97g net protein in basal feed/30 days/fish
Protein efficiency ratio (PER)	1.11 \pm 0.10	1.25 \pm 0.09	1.96 \pm 0.08	1.12 \pm 0.11	0.96 \pm 0.10

[Total 24.0 g basal fish feed/fish/30 days and 7.97 g protein/fish/30 days used for this experiment]

Table 3: Average Initial length, Final length, Increase in length and percentage of length increase of *M. cavasius* (gulsha fish) for experiments. n= (10)

Treatment	Mg of vitamin E/ Kg fish feed	Average Initial length (cm)	Average final length (cm)	Average length increase in cm	Average length increase (%)
T ₀	0mg	14.3 \pm 0.168	15.46 \pm 0.145	1.162 \pm 0.017	8.12 \pm 0.062
T ₁	50mg	14.85 \pm 0.25	16.05 \pm 0.18	1.20 \pm 0.028	8.08 \pm 0.093
T ₂	100mg	14.75 \pm 0.35	16.27 \pm 0.41	1.52 \pm 0.017	10.30 \pm 0.52
T ₃	150mg	16.10 \pm 0.027	17.05 \pm 0.095	0.95 \pm 0.054	5.90 \pm 0.045
T ₄	200mg	15.2 \pm 0.040	16.05 \pm 0.075	0.85 \pm 0.017	5.74 \pm 0.082

Discussion

All water quality parameters and density of fishes in each hapa are most important for the growth of brood *M. cavasius* fish. Water temperature fluctuation affects the fish's metabolism activity, physiology and growth rate. During the experimental period, the temperature ranges recorded from 26.8 °C - 29.8 °C, which support the normal growth temperature range 14.0-38.0 °C for tilapia and gulsha (Khairuman and khairul Amri, 2005) [24]. The water pH values recorded for *M. cavasius* growth, was 6.7-7.5, within the recommended rang (Santhosh and Singh, 2007) [25]. During this study, the dissolved oxygen concentration recorded was slightly fluctuated in the range of 5.2-6.1 mgL⁻¹, however the mean recorded values falls within the recommended range (Bhatnagar and Sangwan, 2009) [26]. The concentration of NH₄-N was 0.25 mg L⁻¹ -0.5 mg L⁻¹, which was also acceptable.

Effect of vitamin E supplementation on the growth performance

Research works indicated that vitamin E at a certain dose

range promoted the growth of aquatic animals (Taveekijakarn P *et al.*, 1996, Falahatkar B *et al.*, 2012) [27, 28]. Min He *et al.* (2017) [29] evaluate the impact of dietary vitamin E supplementation on growth performance, of channel catfish (*Ictalurus punctatus*, Rafinesque 1818).

Results of the present study illustrate that supplementation of 100 mg vitamin E kg⁻¹ in the diet is essential for better growth parameters of *M. cavasius*. Although several studies have investigated the effects of vitamin E on weight gain, as well as stress responses (Gatlin *et al.*, 1992; Bai and Gatlin 1993) [30, 31]. Fish fed diet with un-supplemented or higher doses of vitamin E had the lower BWG, SGR, PER, as well as the higher FCR than those fed diets supplemented with 100 mg kg⁻¹ levels of vitamin E (Figure-2, 4, 7, 6). These findings are in accordance to some earlier reports on yellow catfish (Lu *et al.*, 2016) [32], hybrid snakehead (Zhao *et al.*, 2018) [33], great sturgeon (Amlashi *et al.*, 2011) [34], and Japanese eel (Shahkar *et al.*, 2018) [35]. Channel catfish require relatively high levels of vitamin E and its deficiency significantly inhibits fish growth (Jobling M. NRC, 2012) [36]. Raja James *et al.*, (2008) [37] reported that fish fed with 300 mg vitamin E/kg diet had

the best feeding rate, weight gain, and specific growth rate in goldfish (*Carassius auratus*). A high levels of vitamin E depressed growth performance and impaired feed utilization in rainbow trout (Kiron *et al.*, 2004) [38] and spotted snakehead (Abdel-Hameid *et al.*, 2012) [39]. The growth reduction by feeding with excess dietary vitamin E levels is most likely explain by the imbalance and accumulation of vitamin E radicals, which may act as pro-oxidants (Li *et al.*, 2013) [40]. Vitamin E deficiency induces excessive production of toxic lipid peroxides, leading to a decrease of weight gain and feed efficiency in animals. The addition of vitamin E in diet prevents variable unsaturated fatty acids in diets and tissues of aquatic animals from oxidative rancidity and maintains a normal metabolism, thus increasing the weight gain as well as feed efficiency of animals (Wassef E. *et al.*, 2001) [41]. One of the first signs of vitamin E deficiency is erythrocyte fragility closely followed by anemia, poor growth, poor food conversion, pericarditis, and ceroid deposits in spleen and liver (Halver and Hardly, 2002) [42]. These results suggested that dietary supplementation with vitamin E is beneficial for the growth of catfish.

Effect of vitamin E supplementation on the Food conversion ratio (FCR) and Protein efficiency ratio (PER)

In addition, dietary vitamin E supplementation decreased the feed coefficient in the present study, which has been an important and visual index to evaluate the feed quality. A low FCR is a good indication of a high quality feed. Adding an

appropriate dose of vitamin E reduced the feed coefficient, increased the feed quality, and feed efficiency (Bai and Lee, 1998, Huang and Lin, 2004) [43, 44], and the results of the present study were consistent with this. In our findings, the lowest FCR of *M. cavasius* (1.53 ± 0.49) at the treatment T₂ (100 mg vitamin E kg⁻¹ feed) and the highest FCR at T₄ (3.11 ± 0.49 , Figure-6). A very good feeds that have a low FCR allow more fish to be grow in a pond because there is less waste polluting the water. The FCR in T₂ was within expected range but in case of T₄, it was higher than the accepted value. Protein efficiency ratio (PER) were found of gulsha fish (1.96 ± 0.12) at T₂ treatment followed by T₁ (1.25 ± 0.08), T₀ (1.11 ± 0.10), T₃ (1.12 ± 0.11) and T₄ (0.96 ± 0.10) treatment respectively (Figure-7). This can be important, because while feed with higher levels of protein might be more expensive per kilogram, because it is possible to use less feed, it may actually be the cheapest way to feed fish.

In conclusion, stocking density is an important factor that can affect fish growth. Stressful conditions can cause the normal function of the fish to be disrupt and growth to slow down. However, fulfill the above condition with dietary vitamin E supplementation at 100 mg/kg fish feed promote the growth of channel *M. cavasius* (gulsha fish) so, the optimal dietary vitamin E supplementation should be 100 mg/kg feed. Due to the feasibility and efficacy of oral vitamin E supplementation for channel gulsha fish, the present study provided an experimental foundation for the use of vitamin E for growth of gulsha fish in the aquaculture industry.



Fig 1: 90 day's old *M. cavasius* (Gulsha fish)

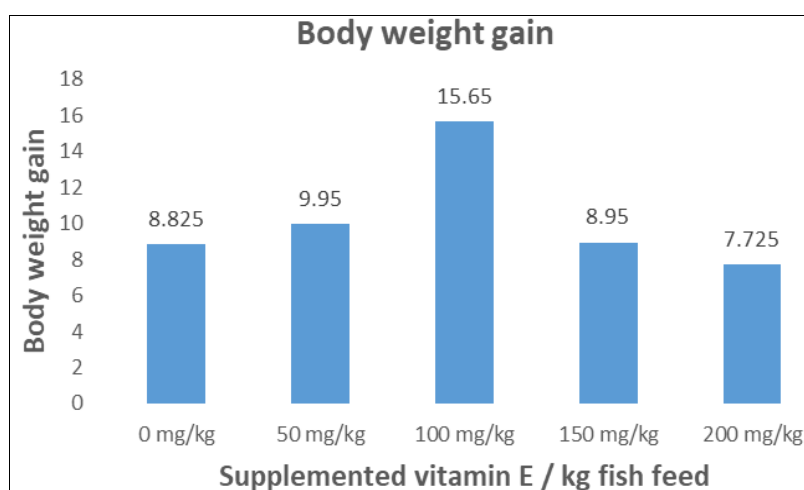


Fig 2: Average weight gain of *M. cavasius* by feeding different doses of supplemented vitamin E fish feed.

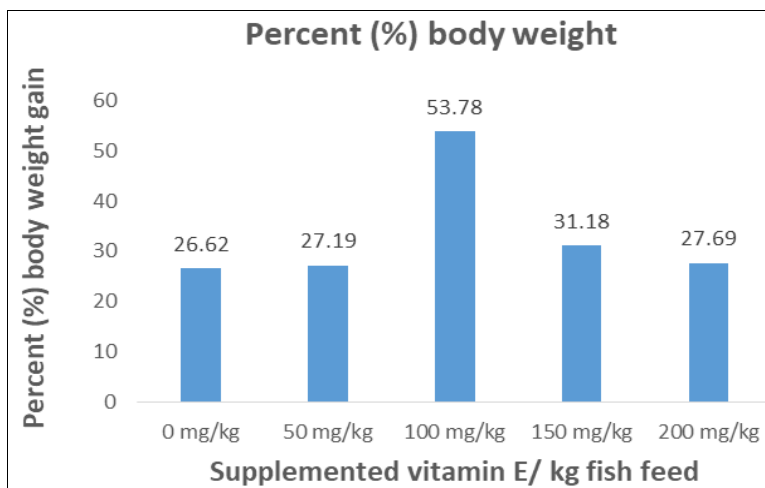


Fig 3: Percent of body weight gain of *M. cavasius* by feeding different doses of supplemented vitamin E fish feed.

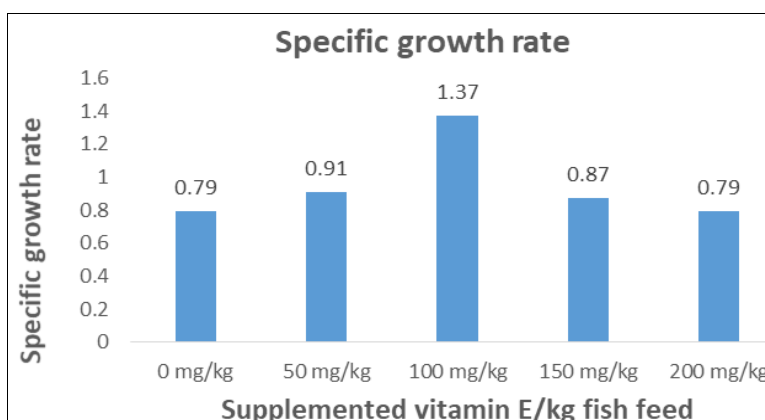


Fig 4: Average specific growth of *M. cavasius* by feeding different doses of supplemented vitamin E fish feed.

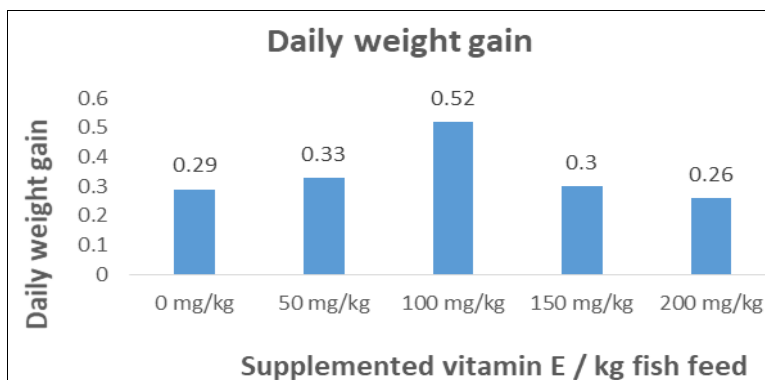


Fig 5: Weight gain/day of *M. cavasius* by feeding different doses of supplemented vitamin E basal fish feed.

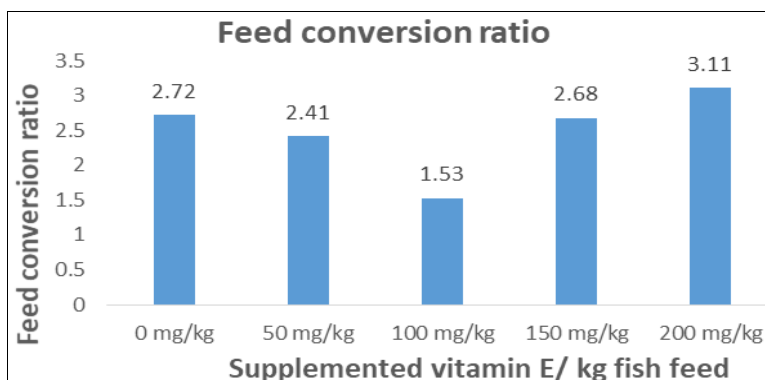


Fig 6: Feed conversion ratio of *M. cavasius* feeding with different doses of supplemented vitamin E basal fish feed.

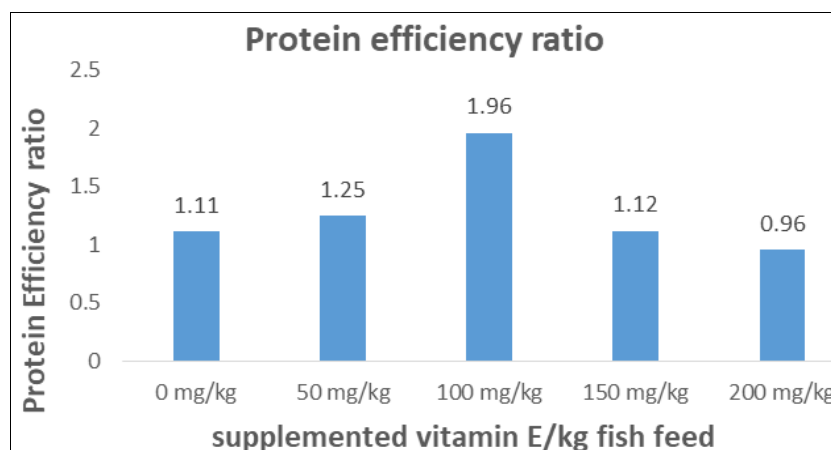


Fig 7: Protein efficiency ratio of *M. cavasius* by feeding different doses of supplemented vitamin E basal fish feed.

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Authors' Contributions

This research includes MS thesis work of first author. However, the last author will be treated as team leader and principal supervisor.

Conflict of interest

The authors declare that there is no conflict of interest.

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