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Uttam Kumar Sarker
Department of Fish Health Management, Sylhet Agricultural University, Sylhet, Bangladesh

Md. Istiaque Hossain
Department of Fisheries, Faculty of Fisheries, University of Rajshahi, Rajshahi, Bangladesh

Md. Mokter Hossain
Department of Fish Health Management, Sylhet Agricultural University, Sylhet, Bangladesh

Rikta Sarkar
Department of Fish Health Management, Sylhet Agricultural University, Sylhet, Bangladesh

Md. Moshiur Rahman
Department of Fish Health Management, Sylhet Agricultural University, Sylhet, Bangladesh

Md. Abdullah-Al-Mamun
Department of Fish Health Management, Sylhet Agricultural University, Sylhet, Bangladesh

MM Mahbub Alam
Department of Fish Health Management, Sylhet Agricultural University, Sylhet, Bangladesh

Corresponding Author:
MM Mahbub Alam
Department of Fish Health Management, Sylhet Agricultural University, Sylhet, Bangladesh

Effects of major immunostimulant (Betamune) on health and production of Nile tilapia *Oreochromis Niloticus*

Uttam Kumar Sarker, Md. Istiaque Hossain, Md. Mokter Hossain, Rikta Sarkar, Md. Moshiur Rahman, Md. Abdullah-Al-Mamun and MM Mahbub Alam

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Abstract
Immunostimulants are often use as dietary supplements as these can improve the innate defense of animals providing resistance to pathogens during periods of high stress, such as grading, reproduction, sea transfer and vaccination. The study was conducted to determine the performance of a commonly used immunostimulant, "Betamune" from ACI animal Health Limited on the growth of Nile Tilapia *Oreochromis niloticus.* The experiment was performed in the wet laboratory of the Department of fish Health Management, Sylhet Agricultural University, Sylhet, with 10 aquaria and 110 Tilapia fry for 28 days. Average initial body weight of the Tilapia fry was 2.75 g. The immunostimulant was used in separate nine aquaria at lower dose, recommended dose and the higher dose respectively and designated as T_1, T_2 and T_3. Each treatment had three replications. One aquarium was used as control. Tilapia fingerlings were fed with "Misha feed" (commercial feed) at the rate of 10% of the body weight for 28 days. Betamune was given at 1ml, 2ml and 3ml/kg feed for T_1, T_2 and T_3 respectively. The fish in control was fed with feed without any immunostimulant. Mean final weights of T_1, T_2, T_3 and control were found 9.63, 11.12, 8.69 and 7.40 g respectively. Mean weight gain were 6.88, 8.37, 5.94 and 4.65 g respectively. Percent weight gain were 250.07%, 304.50%, 215.84% and 168.91%. Specific growth rate (%/day) were 4.47, 4.99, 4.12 and 3.53. The FCR of T_1, T_2, T_3 and control were 1.35, 1.10, 1.56 and 1.99 respectively. Survival rate were 90.91%, 100%, 81.82% and 54.55% respectively. Among the treatments T_2 showed the best result and control showed the least growth. Thus Betamune can be used as an immunostimulant at dose 2ml/kg feed to get profitable growth of tilapia (*Oreochromis niloticus*) in Bangladesh aquaculture. However, the results may be cross-checked in pond condition before its implementation.

Keywords: immunostimulant, mean weight gain, Nile tilapia, percent weight gain, specific growth rate, aquaculture, food conversion ration

1. Introduction

The world's biggest overwhelmed river delta, Bangladesh, and the three principal river systems (The Padma, the Meghna and the Jamuna) that originate from the Himalayas and falls into the Bay of Bengal. Bangladesh is one of the biodiversity hotpot in Asia following China and India. In open water capture aquaculture production, Bangladesh obtained the 3rd position and the 5th position in the World, respectively (FAO, 2018) [9]. Bangladesh's fisheries speak to practically 7% of the world's inland fish creation. The fisheries sector makes significant commitments to the nation's food security, nourishing status furthermore, financial development through generation, employment, and foreign exchange. Bangladesh fisheries has been playing a vital role in poverty alleviation, contributing to GDP (4.39%), export earnings (2.79%) and animal protein supply (60%) with a self-sufficient fish production and per capita consumption about 63 g per day against 60 g per day (DoF, 2018) [7].

Though Bangladesh has plentiful fisheries assets yet they have never been used appropriately and consistently. The creation of fish per hectare in our area is lower than a portion of the fish producing countries of the world.

This is because of absence of legitimate information on the scientific fish culture and management practices used by the countries like China, Vietnam, and Indonesia. For proficient aquaculture production and sustainable management of open water fisheries resources, a comprehension of different management components for sustainable production in the water bodies is fundamental.

Indian major carps, Chinese carps, other carps, tilapia and catfishes are considered as the main cultured freshwater species in Bangladesh. Among the freshwater fishes, tilapia production is gradually increasing, indicating a progressive future for commercial production in Bangladesh. Tilapia is such a species which is regular to our purchasers and farmer also and farming practices are accessible at variable scale.

In the 21st Century, the culture of tilapia has increased throughout the World considering its production and international trading (Hernandez *et al.*, 2014). The production of tilapia in Bangladesh is increasing very fast ranking 4th in the World and 3rd in Asia (DoF, 2018) [7]. Tilapia culture is getting popularity as it is resistant to many diseases and can survive in diverse environmental conditions and culture systems (Jatoba *et al.*, 2011) [29]. A more efficient and health management could lead to reduced cost and stability in the production system and improve the economics of aquaculture operations (John *et al.*, 2007) [11].

Different products i.e. Beta-Glucans, probiotics, plant constituents, etc. used in feed as dietary supplements to improve the immune mechanism in fish providing resistance to pathogens (Rodrigues *et al.*, 2020; Vidal *et al.* 2016; Wang *et al*., 2016; Sirimanapong *et al*., 2015; Syahidah *et al*., 2015; Barman *et al*., 2013; Awaad *et al.*, 2012; Bricknell and Dalmo 2005; Dugenci *et al.*, 2003; Rodriguez *et al.*, 2003; Smith *et al.*, 2003; Sahoo and Mukherjee, 2002) [19, 24, 22, 23, 2, 1, 3, 25, 26, 27, 28]. These products are often originated from different sources i.e., bacteria, brown algae or red algae, terrestrial fungi (Kader *et al*., 2018; Elkamel and Mosaad, 2012) [12, 8]. β-glucan is one of the vital immunostimulants used in aquaculture (Meena *et al.*, 2013) [16]. Betamune is a widely used immunostimulant which decreases mortality rate, improves the general animal's health status, and also increases the animal's resistance to any form of stress (Awaad *et al.*, 2012) [1]. The study was carriedout to evaluate the effects of immunostimulant (betamune) on growth performances and survivality of tilapia (*O. niloticus*).

2. Materials and Methods

2.1 Study area

The study was done in 10 aquaria in the wet lab of Fish Health Management Department under the Faculty of Fisheries at Sylhet Agricultural University, Sylhet, Bangladesh. Underground water was used for rearing the fish.

2.2 Collection and Acclimatization of Fish

Tilapia fingerlings (*Oreochromis niloticus*) were collected from BRAC Fish Hatchery clocated at Sreemangal in Moulvibazar of Bangladesh, having no clinical signs for diseases before stocking. Oxygen bag was used to carry tilapia fingerlings which helped to reduce injury and stress. For acclimatization, tilapia fingerlings were released in the aquarium after submerged the poly bag with fishes for some time to adjust the temperature. Then the fishes came out according their own will. Throughout the rearing period oxygen supply was provided with continuous aeration.

2.3 Selection of the Immunostimulant

The immunostimulant (betamune) was selected through personal contact with the representative of ACI Limited, Animal Health division.

The description of the selected immunostimulant with its composition, company name, manufacturer's name (Table 1), price, presenting form, mixing rate and benefits as written on the packet are presented below:

Trade name: Betamune (Figure 1), Volume: 50 ml, Company name: ACI Limited, Animal Health division, Presenting form: Liquid, Price: 420 Tk., Mixing rate:1-3 ml/ kg of feed.

Table 1: Product details of Immunostimulant Betamine

Product name and type		Composition	Company name	Country of origin
Betamune Feed additive)	Beta 1, 3 D- Glucan	1500 mg	ACI	Canada
	Propionibacterium granulosum	8.5 mg		
	Propylene Glycol	500 mg		
	Water	Upto 50 ml		

Benefits of Betamune are: To Increase the efficiency and the response of animal's during the vaccination, to decrease the mortality rate and improve the general animal's health status, to increase the animal's resistance to any form of stress, and broadly use to help eliminating all kinds of pathogen.

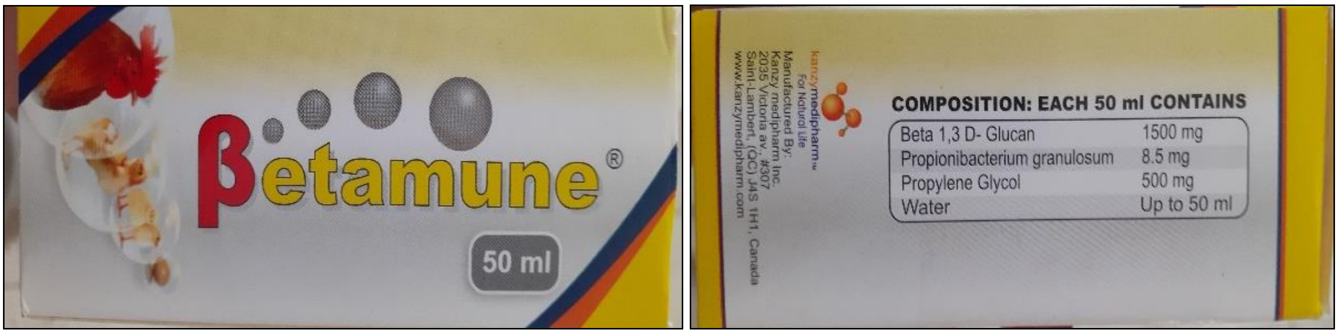

Fig 1: Betamune used as immunostimulant in the present study

2.4 Experimental design

Ten aquarium were used in the experiment of which 9 were used for 3 treatments (T_1, T_2 and T_3) with 3 replications and 1 for control (figure 2). In this experiment, fishes in the control tank were fed with diet-1 containing no betamune. Fishes in the T_1, T_2 and T_3 were fed with diet-2 containing 1 ml betamune, diet-3 containing 2 ml betamune, and diet-3 containing3 ml betamune, respectively. Randomly selected 11 tilapia fingerlings were stocked in each aquarium under the treatments and in the control. The fishes were fed with immunostimulant treated feed for 28 days. The water (50%) of the aquarium was changed for 2 days interval.

Fig 2: Experiment setup

2.5 Stocking of fish fry

The experiment was conducted in 10 glass aquaria (Figure 2). The size of each aquaria was 63.5×30.5 ×38.1 cm^3. Total water holding capacity of individual aquarium was 50 liters. In total 110 Tilapia fingerlings were stocked into 10 glass aquarium at rate of 11 per aquarium. The mean initial weight of an individual tilapia fingerling was 2.75 g. All aquaria were provided with continuous aeration.

2.6 Preparation of Immunostimulant supplemented feed

Commercial diet named Misha Feed (marketed by ACI Godrej limited) were utilized as feed for tilapia (Figure 3). Commercially available betamule liquid (50ml) marketed by the ACI Animal Health was used as feed additives. To prepare the immunostimulant supplemented feed 1ml, 2ml and 3ml of betamune liquid was sprayed well separately with per kg feed and dried in room temperature (Figures 4, 5). After drying, crumbled feeds were separated by gentle pressure. The experimental feeds were kept in air tight plastic pots and used for daily feeding (Figure 6). The basal diet was mixed with betamune 1ml/kg for T_1, 2ml/kg for T_2 and 3ml/kg for T_3. Basal diet without betamune was served as control. Three replications for each treatment were used during the experiment.

Fig 3: Misha feed: Commercial feed used in present study

Fig 4: Spraying betamune

Table 2: Experimental design: stocking of tilapia fingerlings in 10 aquaria of three treatments and control

Treatments with different doses of Immunostimulant "Betamune"	Aquarium No.	Replications (R) (Aquarium No.)	Stocking Density/ aquarium	Mean initial weight (g/fish)
T_1 (1ml/kg feed)	1	R_1	11	2.75 g
	2	R_2		
	3	R_3		
T_2 (2ml/kg feed)	4	R_1		
	5	R_2		
	6	R_3		
T_3 (3 ml/kg feed)	7	R_1		
	8	R_2		
	9	R_3		
Control (No betamune used)	10			

Fig 5: Drying of feed in room temperature

2.7 Rearing of fingerlings with feed containing immunostimulant

The duration of the experiment was 28 adys (from 07 October 2019 to 03 November, 2019). Nine aquarium containing 11 tilapia fingerlings were fed with commercial feed contained immunostimulant (Betamune) @ 10% of the body weight. The fishes in control aquarium was fed with commercial feed without immunostimulant. Feed was delivered two times in a day in the morning (at 9:00 am) and in the afternoon (at 4:00 pm) as showed in Figure 7. The status of the feed utilization was monitored whether the feed was consumed or deposited on the bottom of the aquarium. About 50% water was changed every week (Figure 8).

Fig 6: Feeding

Fig 7: Changing of water

2.8 Sampling procedure of fish

The fishes of each were weighed at every seven days interval and data were recorded for further use. A fine meshed scoop net was used to catch fishes from the aquarium, a blotting was used to remove excess water from the fish body and a digital balance was used to weigh the fishes (Figures 9 and10). The fishes were released in the respective aquarium after weighing.

Fig 8: Captured fish for sampling

Fig 9: Weighing of fish sample

2.9 Growth parameters

Different parameters i.e., Weight Gain (g), Percent Weight Gain (%), Specific Growth Rate (%), Food Conversion Ratio and Survival Rate (%) were analyzed to investigate the effects of immunostimulant on the growth of the experimental fishes.

2.9.1 Weight Gain

Weight Gain (g)=Mean Final Weight (g)-Mean Initial Weight (g)

2.9.2 Percent Weight Gain (%)

$$\text{Percent weight gain (\%)} = \frac{\text{Mean Initial Weight} - \text{Mean Final Weight}}{\text{Mean Initial Weight}} \times 100$$

2.9.3 Specific growth rate (SGR %/day)

$$\text{Specific growth rate (\%)} = \frac{\text{LnW2} - \text{LnW1}}{\text{T 2} - \text{T1}} \times 100$$

Where, W1 is the initial live body weight (g) at time T1; W2 is the initial live body weight (g) at time T2; T2-T1 is the duration of the experiment in day

2.9.4 Food Conversion Ratio (FCR)

$$Food\ Conversion\ Ratio = \frac{Amount\ of\ Dry\ feed\ Fed\ (g)}{Live\ Weight\ Gain\ (g)}$$

2.9.5 Survival Rate (%)

$$Survival\ Rate\ (\%) = \frac{Total\ number\ of\ fish\ harvested \times 100}{Total\ number\ of\ fish\ stocked}$$

2.10 Water quality parameters:

The water quality parameters such as water temperature, dissolved oxygen (DO), pH were monitored weekly throughout the experimental period (figure 11).

Fig 11: Monitoring water quality parameters (temperature, dissolved oxygen, pH)

2.10.1 Temperature

Water temperature (℃) of the aquarium was measured weekly with the help of YSI Professional Plus Multi-Parameter Water Quality Measuring Instrument (figure 11).

2.10.2 Dissolved oxygen
Dissolved oxygen (mg/L) of the water was measured weekly by using YSI Professional Plus Multi-Parameter Water Quality Measuring Instrument (figure 11).

2.10.3 pH
YSI Professional Plus Multi-Parameter Water Quality Measuring Instrument was used to measure the pH of water in every 7 days (figure 11).

2.11 Statistical analysis
Microsoft excel and SPSS (Statistical Package for The Social Sciences) analytical tool was used to perform statistical analysis like ANOVA, average and significance level was determined at $p<0.05$ to see whether the influence of different treatments on these parameters were significant or not. The graphs were made using MS Excel 2016.

3. Results

3.1 Physicochemical parameters of the aquarium water
The results of the water quality parameters such as temperature (°C), dissolved oxygen (mg/l), pH are shown in Table 3. Temperature ranged from 26-29.60(°C), Dissolved O_2 from 5.03- 8.0 and pH from 7.32- 8.10.

Table 3: Water quality parameters in three different treatments during the study period

Water quality parameters	Range of values
Temperature(°C)	26-29.60
Dissolved oxygen (mg/l)	5.03-8.0
pH	7.32-8.10

3.2 Acceptability of the diet
The fish fingerlings were observed to consume readily the feed given and their acceptance was satisfactory. After application of the feed, the fish were seen to come near the feed and consume most of the feed within 3 minutes.

3.3 Growth performance of fish
Growth performance of the tilapia fingerlings (*Oreochromis niloticus*) in terms of weight (g) gain under different treatments for a period of 28 days is presented in Table 4.

Table 4: Growth performances of the tilapia fingerlings (*Oreochromis niloticus*) observed in T_1, T_2, T_3 and Control during the study period

Treatment	Initial Body Weight Per fish (g)		Final Body Weight Per fish (g)		Weight gain (g)		%Weight gain		SGR (%/day)		FCR		Survival rate (%)	
	Mean	SD[1](±)	Mean	SD(±)	Mean	SD(±)	Mean	SD(±)	Mean	SD(±)	Mean	SD(±)	Mean	SD(±)
T_1	2.75	0.00	9.63^a	0.046	6.88^a	0.046	250.07^a	1.68	4.47^a	0.017	1.35^a	0.009	90.91	0.00
T_2	2.75	0.00	11.12^b	0.042	8.37^b	0.042	304.50^b	1.54	4.99^b	0.014	1.10^b	0.029	100	0.00
T_3	2.75	0.00	8.69^c	0.036	5.94^c	0.036	215.84^c	1.32	4.12^c	0.015	1.56^c	0.010	81.82	0.00
Control	2.75	0.00	7.40^d	0.051	4.65^d	0.051	168.91^d	1.87	3.53^d	0.025	1.99^d	0.022	54.55	0.00
LSD		_	.001		.003		.002		.0015		.0015			-
Level of significance		ND	*		*		*		*		*			ND

[1] Values given with (±) are standard deviation.
ND = Not-determined
LSD = Least significant difference
*= Significant ($p<0.05$)

3.3.1: Initial Body weight /fish
The mean initial body weight of fish at start of the experiment was 2.75 in T_1, T_2, T_3 and control respectively.

3.3.2 Final Body weight
The mean final body weight of fish at start of the experiment were 9.63±0.046, 11.12±0.042, 8.69±0.036 and 7.40±0.051 in T_1, T_2, T_3 and control respectively. The highest value was found at T_2 (Figure 12). Final body weight in the three treatments were significantly different from each other (Table 4).

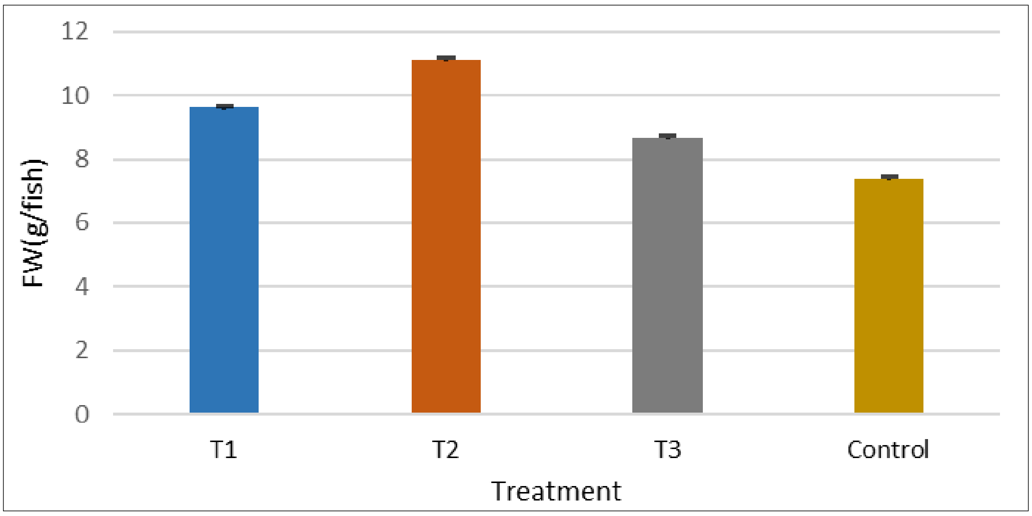

Fig 12: Mean Final body weight (g/fish) of tilapia (*Oreochromis niloticus*) in different treatments at the end of the experimental period

3.3.3 Weight gain
The mean weight gain of fish at start of the experiment were 6.88±0.046, 8.37±0.042, 5.94±0.036 and 4.65±0.051 in T_1, T_2, T_3 and control respectively. The highest value was found at T_2 (Figure 14). Weight gain in the three treatments were significantly different from each other (Table 4).

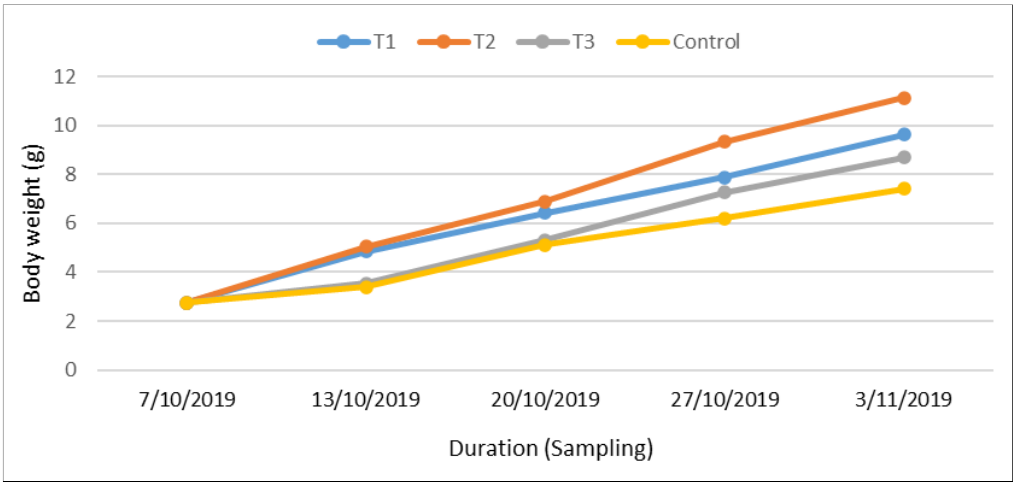

Fig 13: Weight gain of tilapia (*Oreochromis niloticus*) in different sampling date in different treatments

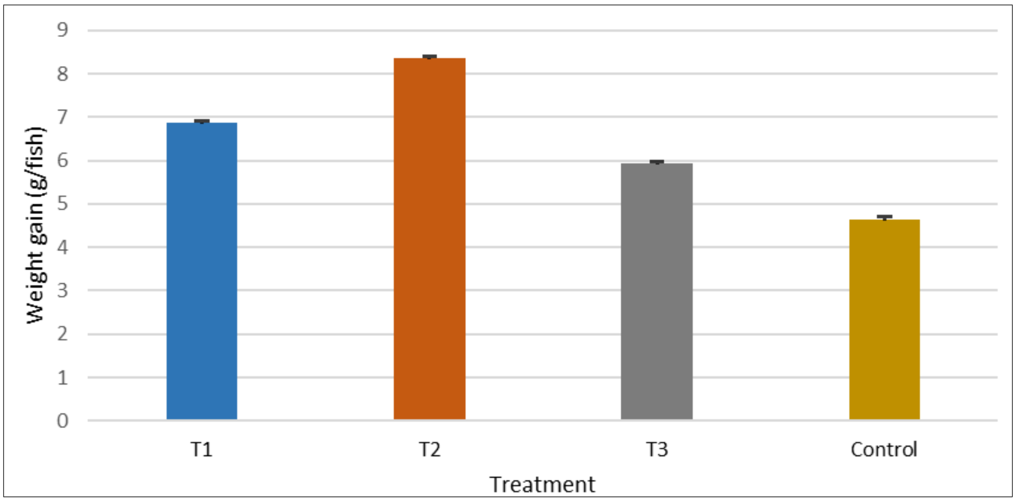

Fig 14: Mean body weight gain (g/fish) tilapia (*Oreochromis niloticus*) in different treatments at the end of the experimental period

3.3.4 Percent weight gain
The mean percent weight gain of fish at start of the experiment were 250.07±1.68, 304.50±1.54, 215.84±1.32 and 168.91±1.87 in T_1, T_2, T_3 and control respectively. The significantly highest percent weight gain was recorded at T_2 (figure 15).

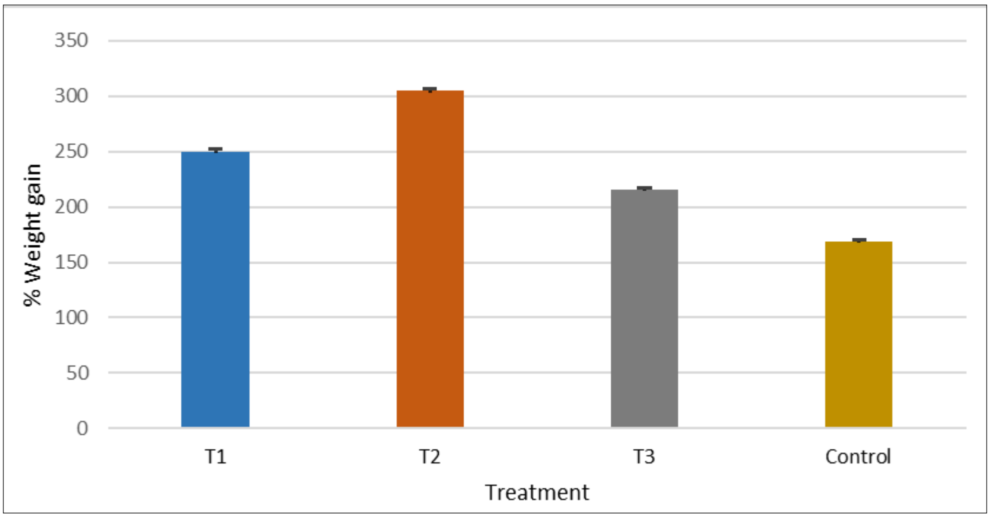

Fig 15: Mean percent weight gain (g ± SD) of tilapia (*Oreochromis niloticus*) in different treatments at the end of the experimental period

3.3.5 Specific Growth Rate (SGR%)

The mean specific growth rate of fish at start of the experiment were 4.47±0.017, 4.99±0.014, 4.12±0.015 and 3.53±0.025 in T_1, T_2, T_3 and control respectively. The significantly highest SGR value was recorded at T_2 (figure 16).

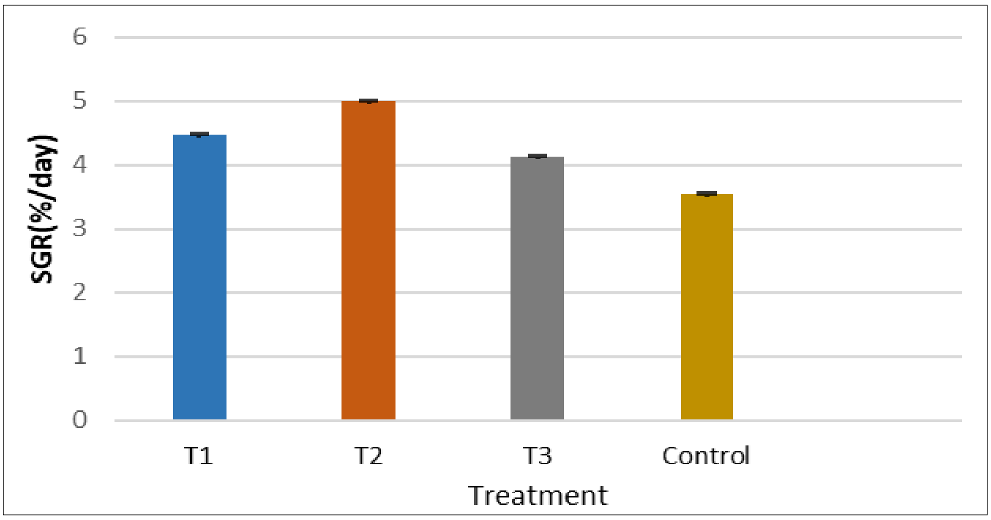

Fig 16: Specific growth rate (%/day ± SD) of tilapia (*Oreochromis niloticus*) in different treatments at the end of the experimental period

3.3.6 Food Conversion Ratio (FCR)

The mean food conversion ratio (FCR) of fish were 1.35±0.009, 1.10±0.029, 1.56±0.010 and 1.56±0.022 in T_1, T_2, T_3 and control respectively. The highest FCR was obtained at T_2 and lowest FCR was obtained in Control (figure 17).

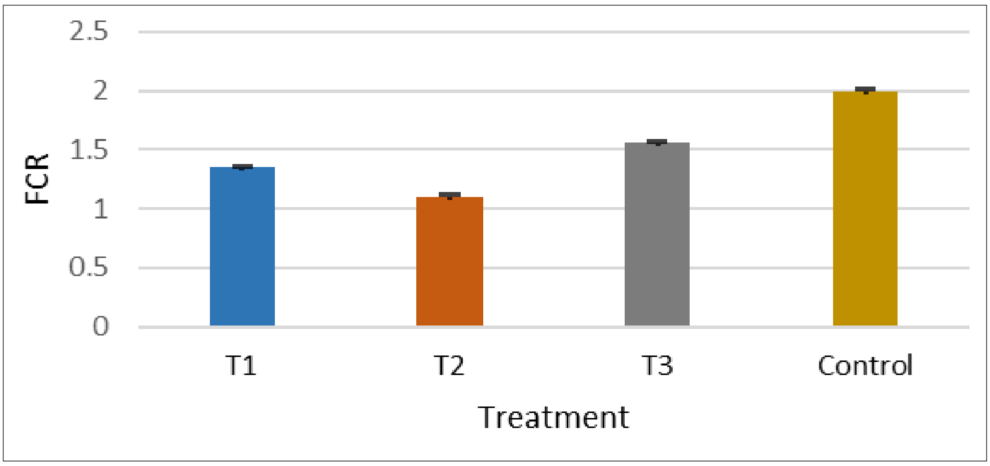

Fig 17: Food conversion ratio (Mean ± SD) of tilapia (*Oreochromis niloticus*) in different treatments during the experimental period

3.3.7 Survival rate

The highest survival rate was recorded at T_2 and lowest survival rate was recorded at Control (figure 18).

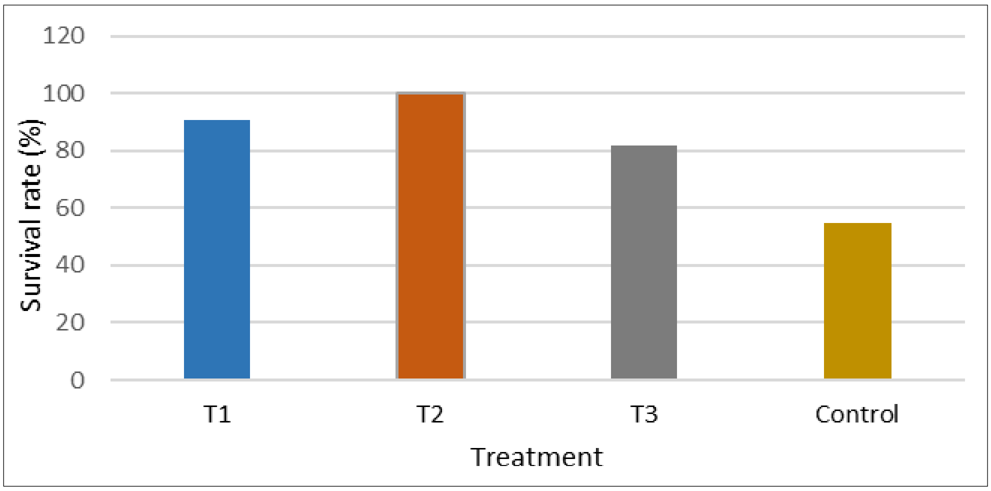

Fig 18: Survival rate of tilapia (*Oreochromis niloticus*) in different treatments at the end of the experimental period

4. Discussion

A more efficient and health management could lead to reduced cost and stability in the production system and improve the economics of aquaculture operations (John *et al.*, 2007) [11]. Growth, health condition and water quality management are the key concern in any kind of fisheries. To cope with the slow growth rate, disease and water quality deterioration various strategies was developed throughout the globe. The use of immunostimulants is one of the promising strategies. Immunostimulants enhance non-specific immune response and thus, increase the resistance to diseases which can contribute to maintain good health of fish in aquaculture (Sakai, 1999) [20]. A more efficient and health management could lead to reduced cost and stability in the production system and improve the economics of aquaculture operations (John *et al.*, 2007) [11]. In this investigation the commercial immunostimulant (Betamune) treated fish diets (T_1, T_2 and T_3) as well as basal diet without immunostimulant (control) were used to feed tilapia fingerlings and showed significant diffrences in growth parameters. Betamune was given at 1ml, 2ml and 3ml/kg feed for T_1, T_2 and T_3 respectively. The fish in control was fed with feed without any immunostimulant. Mean weight gain were 6.88, 8.37, 5.94 and 4.65 g respectively. Mean final weights of T_1, T_2, T_3 and control were found 9.63, 11.12, 8.69 and 7.40 g respectively. Specific growth rate (%/day) were 4.47, 4.99, 4.12 and 3.53. Survival rate (%) were 90.91%, 100%, 81.82% and 54.55%, respectively. Percent weight gain were 250.07%, 304.50%, 215.84% and 168.91%. The FCR of T_1, T_2, T_3 and control were 1.35, 1.10, 1.56 and 1.99 respectively. Among the treatments T_2 showed the best result and control showed the least growth. The efficacy of "Betamune" was investigated and reported 10 points less in cumulative feed conversation ratio and significantly increased in final body weight of broiler chicks (Awaad *et al.*, 2012) [1]. The role turmeric, rosemary and thyme on growth performance of Tilapia were investigated and reported higher weight gain and specific growth rates in comparison to the control (Hasan *et al.*, 2018) [10]. Garlic and Chloramphenicol antibiotic were used as growth promoters and immunostimulants in regards to growth performance and survival rate of tilapia and reported that 30g garlic and 30 mg chloramphenicol showed highest growth performce in tilapia with the significantly increased weight gain, final weight and specific growth rate (Shalaby *et al.*, 2006) [21].

In the present study, the feed conversion ratio (FCR) in treatment 1 was 1.35±0.009, treatment 2 was 1.10±0.029, treatment 3 was 1.56±0.010, and in control was 1.99±0.022. The FCR was significantly better ($P<0.05$) in the treatments group compared to the control group. Hassan *et al.*, (2018) [10] observed that feed conversion ratio was significantly better ($P<0.05$) in the turmeric, rosemary and thyme groups compared to the control group. The survival rate in treatment 1 was 90.91(±0.00), treatment 2 was 100(±0.00), treatment 3 was 81.82(±0.00) and in control was 54.55(±0.00). Survival rate was higher in treatment 2 where the betamune level 2ml/kg feed and lower in control where fish reared without betamune. Laith *et al.*, (2017) [14] reported that the lowest mortality (27%) in tilapia fed with feed containing 5 mg/kg extract of *Excoecaria agallocha* in compare to the control with the highest mortality (97%).

The water quality parameters have significant influences on the reproduction and growth of fish including other biological activities. During the present study, temperature varied from 26°C to 29.60°C; DO concentration in water varied from 5.03 to 8.0 mg/L; pH range varied from 7.32 to 8.10. The recommended ranges of DO should be 5-23 ppm (Makori, 2017; Riche and Garling, 2003) [15 18], temperature should be 25-30°C for higher production of fish (Makori, 2017; Kausar and Salim, 2006; DeWalle *et al.*, 1995) [15, 13, 6] and pH should be 6.0-9.0 (Bryan *et al.*, 2011; Crane, 2006; DeWalle *et al.*, 1995; BEAR, 1992) [4, 5, 6]. But Ngugi *et al.* (2007) [17] reported a wide range of temperature (20 to 35 °C) as ideal for tilapia production.

5. Conclusion

In aquaculture, feed is considered as one of the major constrains against its greatest expansion as it is the single largest cost item in the operating system. In fish culture, feed cost accounts to about 60-80% of the total cost which is very expensive to poor farmers, especially for the farmers of Bangladesh. Ever- rising price of feed ingredient and volatile price of aquaculture products challenge the profitability of all aquaculture operations in Bangladesh.

The growth promoters are mainly used for promoting growth and enhancing fish production. There is a close relationship between growth promoters and physical condition of fishes. It was also found that aquaculture production largely dependent on suitable growth promoters where dosages were in accurate form. If the dosages are not in perfect concentration, it may hamper the production. Based on the findings of the present study, Betamune can be used as an immunostimulant at the rate of 2ml/kg feed to get profitable growth of tilapia (*Oreochromis niloticus*) in Bangladesh aquaculture. As the present study was performed and assessed in aquariums of the wet lab, the immunostimulant (betamune) could be applied in the aquaculture ponds to evaluate their comparative efficacy of two different conditions. The effects of betamune should be evaluated considering the species, quality, source and application methods.

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