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Effect of Stocking Density on Growth Performance, Proximate Composition and Hematology of *Anabas testudineus* in Biofloc Culture System

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Authors' contributions

This work was carried out in collaboration among all authors. Author Md.MR did the carried out the research, analyzed the data, and wrote the manuscript. Author MK did the directed technical guidelines and study design, with enhanced the manuscript writing. Author Md. KS did the carried the data collection and took part in authoring the article. Author AHAIR did the designed and guide the research work. All authors read and approved the final manuscript.

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ABSTRACT

In a biofloc fish culture system, this experiment assessed the effects of stocking densities of 200, 250, and 300 fish/m^3 at treatments T_1, T_2, and T_3, respectively, on the growth and production performances of *Anabas testudineus*. With a mean final weight of 121.64 ± 1.354 g, T_1 produced the largest weight, followed by T_2 (111.63 ± 0.552 g) and T_3 (104.65 ± 0.602 g). In addition, the feed conversion ratio in T_1 treatment (0.97 ± 0.011) was noticeably lower than T_2 (1.36 ± 0.020) and T_3 treatment (1.67 ± 0.116). Survival rate in T_1 treatment (86.66%) was substantially greater than T_2 (80.71%) and T_3 treatments (74.81%). The comparative analysis of proximate composition has

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shown lower total microbial colony count, higher protein and fat content, and lower moisture and ash content in biofloc system. There was no significant difference ($p > 0.05$) found in water quality parameter. Significant difference found ($p < 0.05$) in DO among three treatments. Comparing T_1 (1.77 ± 0.017) to T_2 (1.46 ± 0.005) and T_3 (1.16 ± 0.047), BCR in T_1 were higher. There was no significant differences found in HDL, LDL, Triglycerides and cortisol among three treatments but significantly found in control of *A. testudineus*. The higher level of HDL found in T_1 and lower in control, LDL level lower in T_1 and higher in control, triglycerides were lower in T_1 and higher in control, cortisol higher in control and lower in T_1. It might be concluded that the comparatively lower stocking density provides enhanced production and also be suitable in order to maintain better water quality parameters in biofloc culture system. This density is appropriate for the nation's biofloc culture of *A. testudineus*, as evidenced by the lowest production costs and maximum income seen in T_1.

Keywords: Anabas testudineus; growth performance; hematology; probiotic.

1. INTRODUCTION

The introduction of the Thai Koi to the Mymensingh region of the country opened up new possibilities for pond fish production [74]. The fish's unique nutritional properties and delicious flavor have helped it gain popularity throughout time [3], [1,2]. According to [70] as cited in [74], the Thai Koi has exceptionally high levels of physiologically available iron, copper, readily digested fat, and several beneficial necessary amino acids. As a result, the fish is regarded as a valuable component of the diet for the ill and recovering [10]. Presently, the Thai koi has gained commercial value in Bangladesh due to the availability of fry and fingerling, a higher growth rate, and ease of marketing [47]. We assume that the increased adoption of Thai Koi culture and the growth of the fish could be a potential option to contribute to fulfilling nutritional needs and creating opportunities for livelihood for the people of Bangladesh; however, there is little to no information about the current adoption rate of Thai fish culture. However, the actual rate of adoption of Thai koi by the fishers in this region remained unexplored. As an alternative to open pond fish farming, biofloc fish farming has gained enormous popularity worldwide. Moreover, the recently developed biofloc technology is completely new in the aquaculture production of Bangladesh and a large portion of culturists are very interested in learning the techniques and production systems. Surprisingly, the available learning materials and the culture systems are highly lacking as the research based on biofloc technology is in its preliminary stage. Sylhet division is situated in the northeastern part of Bangladesh and is blessed with vast fisheries resources, such as *haor*, rivers, *beels*, flood plains, canals, etc. [48]. The primary limiting considerations in aquaculture are the cost of feed, which makes up 60–70% of the entire production cost, and the availability of land or water. This method's primary goal is to produce a wide variety of microorganisms in situ while boosting the nitrogen cycle and maintaining a higher C:N ratio by encouraging the growth of heterotrophic microbes, which assimilate nitrogenous waste and produce feed that cultivated species can use [22,23]. According to [25], the addition of carbohydrates usually maintains the required C: N through the source (molasses), and the creation of high-quality single cell microbial protein improves the water quality. The concept of flocculation within the system is also the foundation of this technology. In this environment, dense microorganisms grow and primarily serve as a food supply for protein and as a bioreactor that regulates the quality of the water. Because heterotrophs develop at a pace and produce microbial products per unit substrate that is ten times greater than that of autotrophic nitrifying bacteria, the management of harmful nitrogen species happens more quickly in a biofloc environment [67,68], [8]. This study assessed on stocking density of the productivity and financial sustainability of Thai koi in biofloc system.

2. MATERIALS AND METHODS

2.1 Experimental Site

The growth and production performance of *Anabas testudineus* along with proximate composition and microbial content measured in a biofloc system in the wet lab of the Department of Aquatic Resource Management of Sylhet Agricultural University, Sylhet, Bangladesh (Fig. 1).

Fig. 1. (a) Map of Bangladesh showing the location of the study area, (b) Location of the lab, and (c) set-up of the treatment tanks and air pump

2.2 Experimental Design

Anabas testudineus was evaluated to check its viability in the biofloc fish culture system in three treatments with three replications each. Three treatments, namely T_1, T_2, and T_3 were stocked at stocking densities of 200 fish/m^3, 250 fish/m^3 and 300 fish/m^3 respectively. At the end of the culture period from March 2024 to May 2024 (3 months) similar size pond-cultured *Anabas testudineus* were collected to compare the microbial colony count, proximate composition and hematology and considered as a control treatment.

2.3 Fish fry Collection and Transport

Anabas testudineus fries were collected from a private nursery operator (Jhalak hatchery) in the Mymensingh district. Oxygenated polythene bags were used to transport the fries to the experimental site by a pickup van. After transportation fries were acclimatized with the biofloc culture tank water temperature before releasing in those tanks.

2.4 Biofloc set-up

The system consisted of nine culture tanks and one reservoir tank made of an iron rod and PVC

sheet. Each culture tank was cylindrical with 2,000 liters capacity; however, the water volume at each tank were kept at 1,500 liters. An adequate inlet and outlet system was ensured for each of the tanks. At each of the tanks, six air stones (16.5 cm diameter) were used at the end of aerator pipes. The aerators were set up in such a way so that they cover the total area of the tank equally to ensure a continuously homogenous oxygen supply by using a two electro-magnetic air pump motor set. One Emergency Power Supply unit was set up to back up the emergency power supply during the power interruption.

2.5 Floc Development

In order to develop floc 0.8 kg of probiotics was soaked in 18 liters of water for 72 hours and then was applied to each of the tanks at the rate of 2 liter per 1.5 m^3. During the next three days, 50 g of molasses mixed with water was applied to each of the tanks for floc growth and in the following days, 8 g of molasses was mixed with water and applied until the desired floc was observed. After 10 to 15 days, when the floc volume reached 6-10 ml/liter of water, the fish fry was released in the biofloc tanks.

2.6 Stocking of Fish

In T_1, T_2, and T_3, *Anabas testudineus* was stocked at densities of 200 fish/m^3, 250 fish/m^3, and 300 fish/m^3, respectively. The size of the koi fry was 25 to 27gm. Each of the three stocking densities had three replicates. The fries used in experiment T_1 were 9.16 ± 0.008cm in length and 27.58 ± 0.433g in weight (means ± standard error), T_2 was 9.36 ± 0.046 cm in length and 25.60 ± 0.131g in weight, and T_3 was 9.41 ± 0.074 cm in length and 25.21 ± 0.052g in weight. Fish were acclimated to rearing for 7 days in a 1500-l water tank with constant aeration and 30% water was exchanged daily. The fish must be acclimatized before being released into the biofloc tank [66].

2.7 Feeding and Other Management

The system was checked at least four times a day, with feeding taking place twice a day. The fry was fed with a commercially available feed (starter) containing crude protein 33%. The total daily intake was divided into two portions, one in the early morning around 6.00 am, and one in the evening around 7.00 pm. The daily rate of feeding was 6% in the first 2 weeks, 4% for the next 2 weeks then decreased to 3% body weight at the rest of the culture period. To prevent ammonia from spiring at the start of the fish culture cycle, sufficient availability of carbohydrates such as molasses was used for the development of biofloc. The length and weight of 20 fish samples from each of the culture tanks were measured monthly and feeding rates were adjusted accordingly.

2.8 Measurement of Water Quality Parameters

Water quality parameters namely temperature, dissolved oxygen (DO), p^H, floc volume, salinity, total dissolved solids (TDS), ammonia, nitrite, and electrical conductivity (EC) were measured once daily from 08:00 to 09:00 am. A digital multi-sensor was used to measure the water quality parameters (YSI Multi- Sensor, model: Professional Plus, Brand: YSI, Origin: USA). Ammonia were measured by ammonia test kit (API Ammonia test kit, Made in the USA) and nitrite were measured by nitrite test kit (API Nitrite test kit, Made in the USA).

2.9 Floc Volume Measurement

Imhoff cones were used to determine the volume of floc as the concentration of solids that settled after 10 to 20 minutes [7]. The molasses was supplied as a source of carbon to manage the balance of carbon and nitrogen after the fish were placed in the tanks and the amount of ammonia was calculated.

$$\boldsymbol{ml\ /\ L} = \frac{ml}{L}$$

2.10 Growth Parameters

Total length: A measuring scale was used to determine the total length of the fish (from the tip of the snout to the edge of the caudal fin).

Weight gain: The weight of sample fishes was taken by using a weighing balance (model: EK600i, origin: USA) and weight gain was calculated as, $W = W_2 - W_1$, where W_1 is the initial weight and W_2 is the final weight.

Specific growth rate (SGR):

$$SGR\ (\%/day) = \frac{ln\ wt - ln\ w0}{t} \times 100 \quad (1)$$

The final weight of the fish (w_t) and the initial weight (w_o) were both represented in g, and the experiment period was expressed as the day (t).

Survival rate (%): The survival rate of fish was calculated as follows:

$$Survival\ rate\ (\%) = \frac{Final\ total\ number\ of\ fish}{Initial\ total\ number\ of\ fish} \times 100 \quad (2)$$

Average Daily Gain (ADG): This parameter was calculated using the weight of the fish after 15 days, which was done using the equation:

$$ADG\ (g/day) = \frac{wt - w0}{t} \quad (3)$$

The weight of the fish on the day of closing the measurement denoted as w_t and initial weight denoted as w_0 expressed in g, and the DGR was expressed as g/day (t).

Feed Conversion Ratio (FCR): The FCR is the amount of feed consumed by fish (g) to increase 1 g of live fish weight, calculated to measure fish growth rate and consumed feed.

$$FCR = \frac{Total\ feed\ consumed}{Total\ weight\ of\ fish\ produced} \quad (4)$$

where, Total weight of fish produced = Final weight of the fish - Initial weight of the fish

2.11 Determination of Proximate Composition

The methods used by the Association of Official Analytical Chemists were used to determine the whole-body proximate composition (Moisture, Protein, Lipid and Ash) of sampled fish corpses at the end of the experiment.

2.12 Microbial Colony Count

Microbial load determination of microorganisms was carried by using nutrient agar through using serial dilution and pour plate method. Bacteria colonies that developed after incubation were expressed in the Colony Forming Unit (CFU/g) for the intestine coliform count. An incubator, laminar airflow, autoclave, Erlenmeyer tube, vortex, Bunsen burner, petri dish, test tube, tube rack, needle, dropper, glass cover, digital scale, mortar and pestle, beaker glasses, and micropipettes were employed in this study.

2.13 Hematology Determination of *A. testudineus*

Red blood cells (RBCs) and white blood cells (WBCs) were assessed in blood samples placed in EDTA tubes using an automated hematology counter and a flow cytometer, all within five hours of collection. After being placed in tubes without an anticoagulant agent and allowed to stand at room temperature for 20 minutes, the blood was centrifuged at room temperature for 10 minutes at 1300× g. The resulting serum was then kept at -20 ◦C until it was examined. For the determination of hematological parameters (white blood cells, WBCs; red blood cells, RBCs; hemoglobin concentration, Hb; mean corpuscular volume, MCV; mean corpuscular hemoglobin content, MCH; mean corpuscular hemoglobin concentration, MCHC; and thrombocyte cells, TCs), an electronic blood cell counter (HecoVet, SEAC, Florence, Italy) was used.

For the hematological analysis, whole blood was collected using a heparinized capillary tube following tail ablation. Each sample of whole blood consisted of a pool of whole blood collected from 10 fish. Three replicas were prepared. The whole blood pool sample was then divided and mixed with Natt and Herrick's solution (at a ratio of 1:200) to allow for the subsequent determination of representative total RBC and WBC counts. Using a hemocytometer and an Olympus CX22 compound microscope (magnification of 40X), repeated counts were used to calculate the blood counts.

The samples were subjected to further centrifugation at 4,000 × g for 15 min at 12°C to separate the blood cells. The plasma was then collected and stored at −20°C until the cortisol was determined. An additional aliquot of 40 µL from each replicate was taken to determine plasma cortisol levels using the General Cortisol ELISA kit (MyBioSource, United States), with measurements conducted within the range of 0.5–300 ng/mL. The optical density (OD value) of the samples thereafter was then determined at 490 nm using a BK-EL10C ELISA microplate reader (Biobase, China).

2.14 Analysis of Data

Microsoft Excel and the Statistical Program for Social Science (SPSS 25) were the statistical applications utilized. One-way analysis of variance (ANOVA) was used to examine all parameters for intergroup differences. Post hoc comparisons, LSD (least significant difference), and DMRT (Duncan's multiple range test) were then performed at the $p \leq 0.05$ level.

3. RESULTS

3.1 Water Quality Parameters

The mean temperature of the various treatments (Table 1) was found to be between 25.99 ± 0.066 and 25.80 ± 0.053. In the study, water temperature (°C) in T_1, T_2, and T_3 was 25.99 ± 0.066, 25.86 ± 0.057, and 25.80 ± 0.053, respectively and there were no significant differences found among the treatments. There was a significant change observed ($p<0.05$) in dissolved oxygen levels across the treatments (Table 1). In the study, average dissolved oxygen (mg/l) in T_1, T_2, and T_3 were observed 5.33 ± 0.067, 5.03 ± 0.077, and 4.70 ± 0.086, respectively. In biofloc aquaculture system's p^H has been recognized as a key productivity measure. The culture tanks were found more or less suitable for aquaculture having p^H values between 6.5 and 8.5. For fish culture, a slightly alkaline p^H is better. In this study, the mean p^H value ranged from 7.72 ± 0.018 to 7.75 ± 0.025 under various stocking densities. The current study's ammonia results varied from 0.21 ± 0.028 to 0.36 ± 0.055 (Table 1). Average ammonia concentration did not show any significant variation among the treatments. But sometimes abruptly high ammonia concentration noticed in few tanks and continued for 2-3 days. The mean value of nitrite found biofloc fish culture of *A. testudineus* varied from 1.32 ± 0.103 to 1.36 ± 0.111 (Table 1) without any significant differences among the treatments. Inorganic salts and dissolved compounds make up TDS. The results ranged from 75.5 to 149.07 ppm, with a mean value of 143.49 ± 2.063 ppm in T_1, 147.67 ± 2.307 ppm in T_2, and 149.07 ± 2.412 ppm in T_3 (Table 1). There were no significant levels found in T_1, T_2 and T_3 treatments, respectively. Water conductivity was determined by ionic concentration and temperature. The conductivity of water samples taken from the biofloc aquaculture system ranged from 145.1 to 325.98 µs/cm, with a mean value of 293.73 ± 4.112 µs/cm in T_1, 301.03 ± 4.631 µs/cm in T_2, and 306.11 ± 4.964 µs/cm in T_3 treatments. There was no significant difference found in T_1, T_2 and T_3 treatments, respectively. The value of salinity collected from the biofloc aquaculture system varied from 0.05 to 0.14 ppt with mean of 0.13 ± 0.001 ppt in T_1, 0.13 ± 0.002 ppt in T_2 and 0.14 ± 0.002 ppt in T_3 treatment. There were no significant differences found among the treatments.

Floc volume: Floc volume depicted in Fig. 2. T_1 treatment had the significantly highest floc volume, while T_3 treatment showed the lowest (Table 1) and there were significant differences found in T_1 (10.83 ± 0.116), T_2 (8.70 ± 0.061) and T_3 (7.93 ± 0.078) treatments, respectively.

3.2 Growth and Production Performances

The final length of *A. testudineus* varied with the change of stocking density. The effect of stocking densities on the total length of A. *testudineus* is presented in Fig. 3. The results revealed that stocking density has a significant ($p<0.05$) effect on the total length of *A. testudineus*. The maximum length (18.26 cm) and minimum length (15.17 cm) were also recorded in T_1 and T_3, respectively. It was observed that the highest total length for each sampling was found in T_1 followed by T_2 and T_3. In response to the ultimate weight of *A. testudineus* shown in Fig. 4, the effect of stocking density remained considerable. A significant difference ($p<0.05$) was found in final weight among the three treatments (Table 2). Highest final weight (121.64 g) recorded in T_1, followed by T_2 (111.63 g), and T_3 (104.65 g) at the end of the trial (Fig. 4). Different parameters of *A. testudineus* were observed for 90 days (Table 2). The statistical analysis showed that the value of specific growth rate (SGR) of *A. testudineus* cultured in biofloc was significantly different ($p<0.05$) among the treatments T_1 (1.40 ± 0.020%), T_2 (1.38 ± 0.001%), and T_3 (1.34± 0.007%) respectively (Table 2). T_1 showed significantly ($p<0.05$) higher weight gain (94.05 ± 1.556) than T_2 (86.02 ± 0.426) and T_3 (79.44 ± 0.652) (Table 2). Weight gain of T_2 also showed significantly higher than T_3. The study found a stronger increasing trend of weight gain in the third and last months of the culture period and growth was observed much slower in the first month (Fig. 4). During the current study's inquiry, a significant difference was found in T_1 (1.04 ± 0.020), T_2 (0.95 ± 0.005), and T_3 (0.88 ±0.005) among the three treatments respectively (Table 2). Diet efficiency is measured by FCR. The lower the value, the better for the culture, as it requires less feed to produce one unit weight of fish. In the present study, significantly lower FCR was obtained in T_1 (0.97 ± 0.011), than T_2 (1.36 ± 0.020), and T_3 (1.67 ± 0.116) (Table 2). The lowest survival percentages (%) was found in T_3 (74.81 ± 1.959 percent) and T_1 had significantly higher (86.66 ± 3.892) FCR where the stocking density was lower (200 fish/m^3). However, there was a significant difference ($p<0.05$) found in survival percentages (%) among the three treatments (Table 2).

Table 1. Water quality parameter of *A. testudineus* (mean ± standard error) (n=270)

Parameters	Treatments		
	T_1	T_2	T_3
Temperature (°C)	25.99 ± 0.066[a]	25.86 ± 0.057[ab]	25.80 ± 0.053[b]
DO (mg/L)	5.33 ± 0.067[a]	5.03 ± 0.077[b]	4.70 ± 0.086[c]
Electrical conductivity (µs/cm)	293.73 ± 4.112	301.03 ± 4.631	306.11 ± 4.964
TDS (mg/L)	143.49 ± 2.063	147.67 ± 2.307	149.07 ± 2.412
Salinity (ppt)	0.13 ± 0.001	0.13 ± 0.002	0.14 ± 0.002
Ammonia (mg/L)	0.21 ± 0.028[a]	0.26 ± 0.033[ab]	0.36 ± 0.055[b]
Nitrite (mg/L)	1.36 ± 0.111	1.32 ± 0.103	1.33 ± 0.102
pH	7.75 ± 0.025	7.77 ± 0.021	7.72 ± 0.018
Floc volume (ml/L)	10.83 ± 0.116[a]	8.70 ± 0.061[b]	7.93 ± 0.079[c]

Significant differences were detected (p<0.05) among treatments with the superscript letter

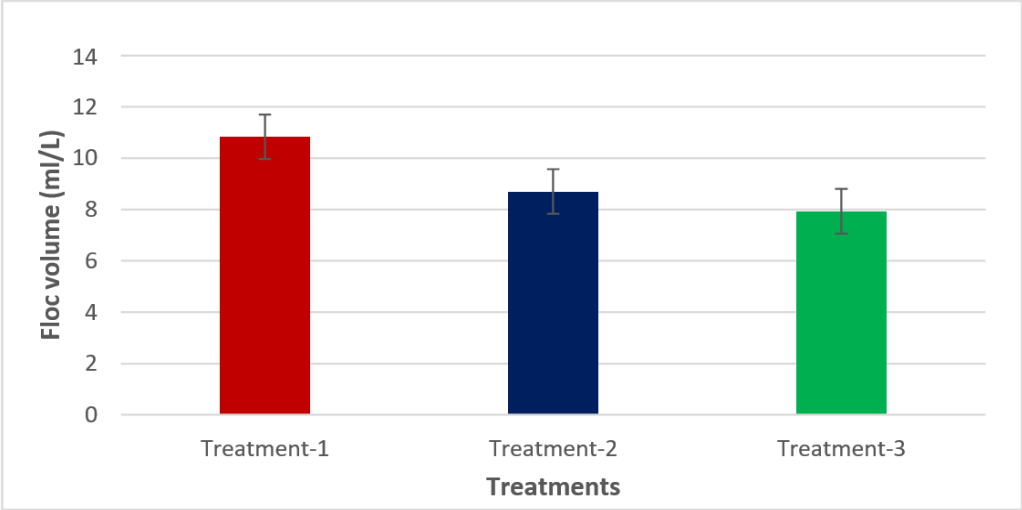

Fig. 2. Floc volume of *A. testudineus* in different treatments throughout the experimental period

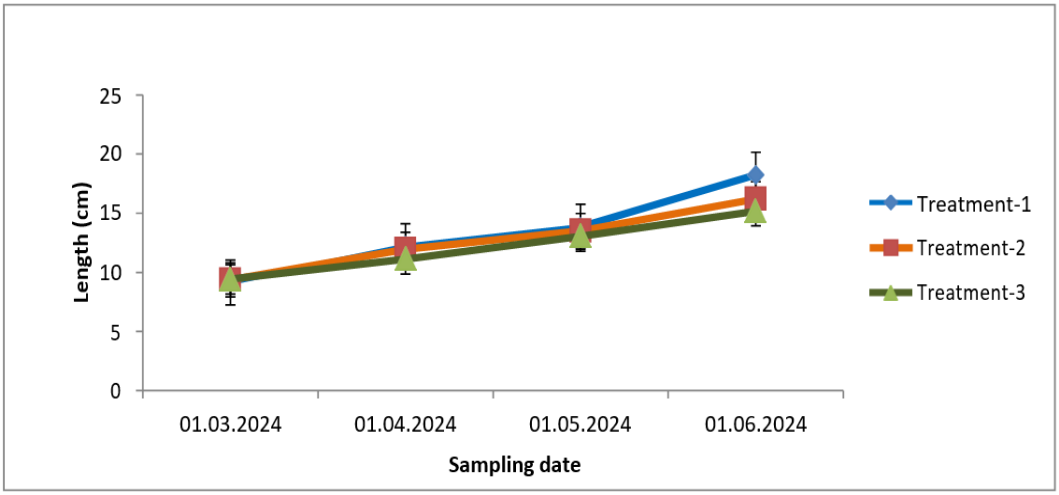

Fig. 3. Length (cm) variation of *A. testudineus* among three different treatments in the study period

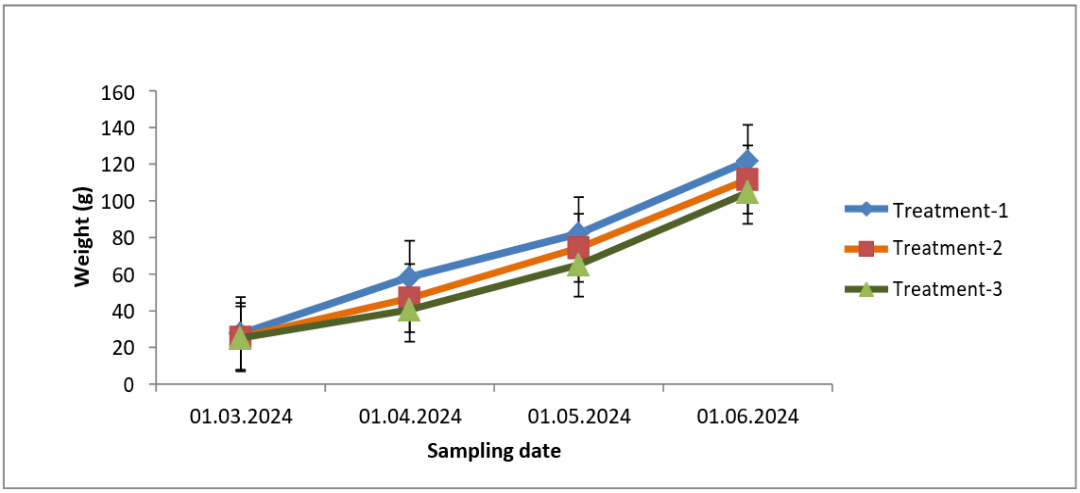

Fig. 4. Weight (g) variation of *A. testudineus* among three different treatments in the study period

Table 2. Growth performance (mean ± standard error) of *A. testudineus* for 90 days

Parameters	Treatments		
T_1		T_2	T_3
Initial length (cm)	9.16 ± 0.008	9.36 ± 0.046	9.41 ± 0.074
Final length (cm)	18.26 ± 0.094^a	16.21 ± 0.066^b	15.17 ± 0.060^c
Initial weight (g)	27.58 ± 0.433	25.60 ± 0.131	25.21 ± 0.052
Final weight (g)	121.64 ± 1.354^a	111.63 ± 0.552^b	104.65 ± 0.602^c
ADG (g/day)	1.04 ± 0.015^a	0.95 ± 0.005^b	0.88 ± 0.005^c
Specific growth rate (%)	1.40 ± 0.020^b	1.38 ± 0.001^b	1.34 ± 0.007^a
FCR	0.97 ± 0.011^a	1.36 ± 0.020^b	1.67 ± 0.116^c
Survival rate (%)	86.66 ± 3.892	80.71 ± 0.989	74.81 ± 1.959
Weight gain (g)	94.05 ± 1.556^a	86.02 ± 0.426^b	79.44 ± 0.652^c

Significant differences were detected ($p<0.05$) among treatments with the superscript letter.

3.3 Proximate Composition

In the present study, the amount of moisture content varied from 16.10 ± 0.512 to 21.99 ± 0.286% (Table 3). The moisture content was obtained 17.15 ± 0.480, 16.10 ± 0.512, 17.00 ± 0.491 and 21.99 ± 0.286% in T_1, T_2, T_3, and control, respectively. No significant changes ($p>0.05$) were found in moisture percent among the three treatments, but significant differences ($p<0.05$) found in the control treatment. There was no significant variation ($p>0.05$) observed in protein percent among the three treatments, but significant differences ($p<0.05$) were found with the control treatment. There was a significant variation ($p<0.05$) found in lipid percentage across the four treatments respectively (Table 3). There were no significant changes ($p>0.05$) in ash content among the three treatments, but significant differences ($p <0.05$) were identified with the control treatment (Table 3).

Table 3. Proximate composition (Mean ± SE) of *A. testudineus* cultured in biofloc

Parameter	Treatments			
	T_1	T_2	T_3	Control
Moisture (%)	17.15 ± 0.480^a	16.10 ± 0.512^a	17.00 ± 0.491^a	21.99 ± 0.286^b
Protein (%)	47.45 ± 0.448^c	46.49 ± 0.097^c	45.12 ± 0.294^b	39.32 ± 0.630^a
Lipid (%)	25.14 ± 0.187^b	26.17 ± 0.357^c	28.04 ± 0.078^d	20.66 ± 0.286^a
Ash (%)	10.26 ± 0.497^a	11.24 ± 0.595^a	9.84 ± 0.211^a	18.03 ± 0.363^b

Significant differences were detected ($p<0.05$) among treatments under the same superscript letter reared for 90 days

3.4 Microbial Colony Count

The microbial colony count differs significantly ($p<0.05$) between T_1, T_2, T_3 and control treatments, respectively. (Fig. 5). In the biofloc aquaculture system, the colony count of *A. testudineus* was lower than in the natural source (control) *A. testudineus*.

3.5 Benefit-Cost Ratio (BCR)

Economies of different stocking densities on *A. testudineus* for compensatory growth under different treatments are summarized in Table 4. Variations in the mean values of the total cost (BDT/m^3), and net revenue (BDT/m^3) among the treatments are shown in Table 4. Variations in the mean values of CBR among the treatments are shown in Table 4. A simple economic analysis was performed to estimate the Cost-Benefit Ratio (CBR) of this biofloc aquaculture system. The total cost, and cost-benefit ratio were significantly different ($p<0.05$) among the treatments. The highest total cost (3420.00 ± 35.276 BDT/m^3) was found with treatment T_3 whereas the lowest cost (2293.33 ± 23.094 BDT/m^3) was found with treatment T_1. The highest total revenue was found with treatment T_2 whereas the lowest revenue was found with treatment T_3 (Table 4). The best result for net revenue (1780 ± 24.037 BDT/m^3) was found with treatment T_1. Cost-benefit ratio (CBR) was found significantly higher (1.77 ± 0.017) in treatments T_1, than T_2 (1.46 ± 0.005), and T_3 (1.16 ± 0.047).

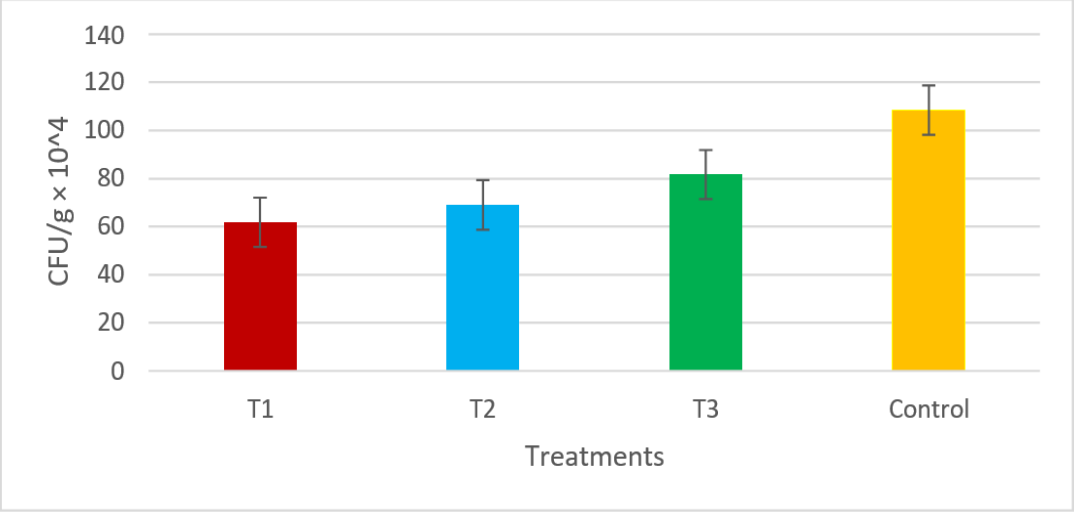

Fig. 5. Microbial colony count of *A. testudineus* throughout the culture period

Table 4. Economics of different stocking levels on *A. testudineus* for compensatory growth under different treatments (mean± standard error) (Currency in BDT)

Particulars	Treatments		
	T_1	T_2	T_3
Tank cost	500.00 ± 0.000	500.00 ± 0.000	500.00 ± 0.000
Fry cost	1500.00 ± 0.000	1875.00 ± 0.000	2250.00 ± 0.000
Feed cost	1440.00 ± 34.641^a	1980.00 ± 34.641^b	2380.00 ± 52.915^c
Total cost/m^3	2293.33 ± 23.094^a	2903.33 ± 23.094^b	3420.00 ± 35.276^c
Total Yield (kg)	20.36 ± 0.088^a	25.56 ± 0.290^b	29.86 ± 0.920^c
Net Yield (kg/m^3)	6.45 ± 0.216	16.12 ± 0.230	15.93 ± 0.757
Total Revenue/m^3	4073.33 ± 17.638^{ab}	4261.11 ± 48.432^b	3982.22 ± 122.76^a
Net Revenue/m^3	1780.00 ± 24.037^a	1357.77 ± 25.627^b	562.22 ± 155.55^c
BCR	1.77 ± 0.017^a	1.46 ± 0.005^b	1.16 ± 0.047^c

Significant differences were detected ($p<0.05$) among treatments under the same superscript letter reared for 90 days

3.6 Hematology of *A. testudineus*

There was no significant difference found ($p<0.05$) in haemoglobin, white blood cell, lymphocyte, monocyte, eosinophil, basophil in the blood of *A. testudineus* (Table 5). Significant difference found in red blood cell, neutrophils, platelet count, hematocrit, mcv, mchc and cortisol in the blood of *A. testudineus* (Table 5). Red blood cells were higher in control treatment (4.84 ± 0.005) than T_1 (4.39 ± 0.005), T_2 (4.90 ± 0.005) and T_3 (4.62 ± 0.005) treatments respectively. Cortisol was lower in T_2 (568.20 ± 1.331) and higher in control (602.00 ± 1.527) treatment (Fig. 6).

3.7 Lipid profile in the blood of *A. testudineus*

There were significant differences found in cholesterol between the two treatments and control. Significant differences ($p < 0.05$) were found in HDL among the three treatments and control (Fig. 7). HDL was higher in T_2 (129.00 ± 0.577) and lower in control (32.00 ± 0.577) treatments. LDL was higher in control (141.00 ± 0.577) and lower in T_3 (34.33 ± 1.202) treatments. There was no significant difference ($p> 0.05$) found in triglycerides among the three treatments but significant difference found in control treatment (Table 6). There was no significant difference ($p > 0.05$) found in total protein, AST and ALT among three treatments but significant difference found in control treatments (Table 6).

3.8 Proximate Composition of Floc during *A. testudineus* culture

There was no significant difference found in lipid and ash among three treatments (Table 7). Significant differences were found in T_1 and T_2 treatments of moisture and protein content during *A. testudineus* culture.

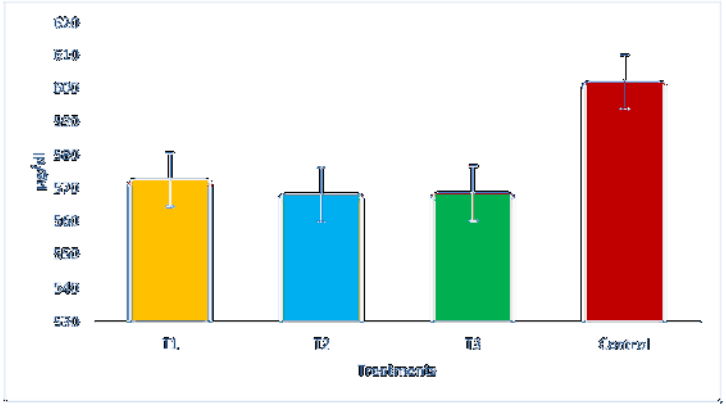

Fig. 6. Cortisol in the blood of *A. testudineus* during culture period

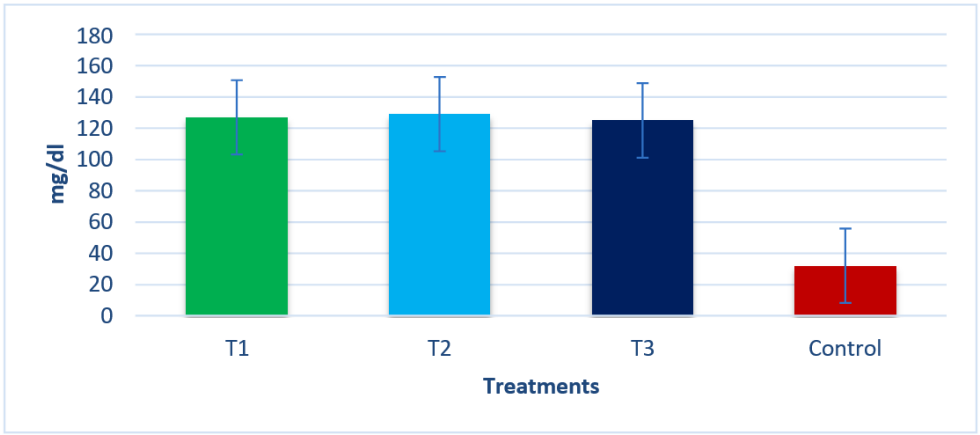

Fig. 7. HDL in the blood of *A. testudineus* during culture period

Table 5. Different blood parameters in the blood of *A. testudineus* in different treatments (Mean ± SE)

Blood parameters	Treatments			
	T_1	T_2	T_3	Control
HB (%)	14.10 ± 0.577	15.20 ± 0.838	14.30 ± 0.360	13.56 ± 0.606
WBC (cmm)	3000.00 ± 577.35	8000.00 ± 577.35	6533.33 ± 548.73	2000.00 ± 577.35
RBC	4.39 ± 0.005^{a}	4.90 ± 0.005^{b}	4.62 ± 0.005^{c}	4.84 ± 0.005^{d}
Neutrophils (%)	34.00 ± 0.577^{a}	35.00 ± 0.577^{a}	29.00 ± 0.577^{b}	15.00 ± 0.577^{c}
Lymphocyte (%)	60.67 ± 0.882	61.67 ± 1.202	78.33 ± 0.333	80.00 ± 0.577
Monocyte (%)	2.00 ± 0.577	2.33 ± 0.333	2.00 ± 0.577	2.00 ± 0.00
Eosinophil (%)	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	2.00 ± 0.00
Basophil (%)	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
Platelet count (cmm)	120000.00 ± 5773.503^{a}	116666.67 ± 8819.171^{a}	110000.00 ± 10000.00^{ab}	90333.33 ± 333.333^{b}
HCT (%)	51.00 ± 0.577^{a}	52.00 ± 0.577^{a}	51.00 ± 0.577^{a}	48.00 ± 0.577^{b}
MCV (fL)	117.00 ± 0.577^{a}	117.00 ± 0.577^{a}	116.00 ± 0.577^{a}	98.00 ± 0.577^{b}
MCH (Pg)	32.00 ± 0.577	31.00 ± 0.577	31.33 ± 1.202	27.00 ± 0.577
MCHC (g/dl)	28.33 ± 0.333^{ab}	30.00 ± 0.577^{a}	27.00 ± 0.577^{b}	28.00 ± 0.577^{b}
RDW-CV (%)	11.00 ± 0.577^{a}	11.00 ± 0.577^{a}	11.00 ± 0.577^{a}	8.00 ± 0.577^{b}
Cortisol (µg/dl)	572.56 ± 0.606^{b}	568.20 ± 1.331^{a}	568.46 ± 0.611^{a}	602.00 ± 1.527^{c}

Significant differences were detected (p<0.05) among treatments under the same superscript letter reared for 90 days

Table 6. Lipid profile in the blood of *A. testudineus* in different treatments (Mean ± SE)

Blood parameters	Treatments			
	T_1	T_2	T_3	Control
Cholesterol (mg/dl)	193.00 ± 0.577^{a}	199.00 ± 0.577^{b}	200.00 ± 0.577^{b}	153.00 ± 0.577^{c}
HDL (mg/dl)	127.00 ± 0.577^{a}	129.00 ± 0.577^{b}	125.00 ± 0.577^{c}	32.00 ± 0.577^{d}
LDL (mg/dl)	37.00 ± 0.577^{a}	35.00 ± 0.577^{ab}	34.33 ± 1.202^{b}	141.00 ± 0.577^{c}
Triglycerides(mg/dl)	145.67±0.577^{a}	146.67±0.822^{a}	145.33±0.882^{a}	227.00±0.577^{a}
AST (SGOT) U/L	51.00 ± 0.577^{a}	52.00 ± 0.577^{a}	50.67 ± 1.202^{a}	45.00 ± 0.577^{b}
ALT (SGPT) U/L	26.00 ± 0.577^{a}	25.67 ± 1.202^{a}	27.33 ± 0.882^{a}	80.00 ± 0.577^{b}
ALP (U/L)	8.0 ± 0.577	8.33 ± 0.882	8.33 ± 0.667	10.00 ± 0.577
Total Protein (g/dl)	6.50 ± 0.057^{a}	6.66 ± 0.145^{a}	6.60 ± 0.115^{a}	5.40 ± 0.057^{b}

Significant differences were detected (p<0.05) among treatments under the same superscript letter reared for 90 days

Table 7. Proximate composition of floc in *A. testudineus* culture (Mean ± SE)

Particulars	Treatments		
	T_1	T_2	T_3
Moisture (%)	23.12 ± 0.577^{a}	25.26 ± 0.577^{b}	26.34 ± 0.577^{b}
Protein (%)	25.68 ± 0.577^{b}	24.61± 0.577^{b}	21.76 ± 0.577^{a}
Lipid (%)	4.40 ± 0.577	3.80 ± 0.577	3.5 ± 0.577
Ash (%)	46.80 ± 0.577	47.33 ± 0.577	48.40 ± 0.577

Significant differences were detected (p<0.05) among treatments under the same superscript letter reared for 90 days

3.9 Microbial Contamination in Biofloc Tank Water

Microbial status in biofloc tank before mixing of probiotics: In the case of biofloc fish farming, iron-free water was added to the pre-release fish seed in tank and microbial colony test was performed according to the treatment. There was no significant difference found in colony count of *Klebsiella spp*. and *Staphylococcous aureus* among three treatments, respectively. Significant difference found in the colony count of *Acienobacter spp.* It shows microbial colonization according to treatment, which is presented below.

Table 8. Bacterial colony in biofloc tank water before mixing probiotics (Mean ± SE)

Particulars	Treatments (cfu/ml) 10^4		
	T_1	T_2	T_3
Klebsiella spp.	70.33 ± 9.387	71.67 ± 10.477	69.00 ± 1.155
Acinobacter spp.	105.00 ± 6.028a	290.00 ± 13.229b	323.33 ± 8.819b
Staphylococcus aureus	26.67 ± 2.603	20.33 ± 1.453	26.33 ± 0.882

Fig. 8. *Klebsiella spp.* On EMB

Fig. 9. *Acinobacter spp.* on Acinetobacter selective agar

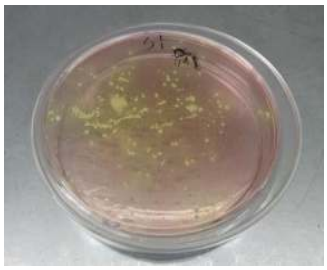

Fig. 10. *S. aureus* on MSA

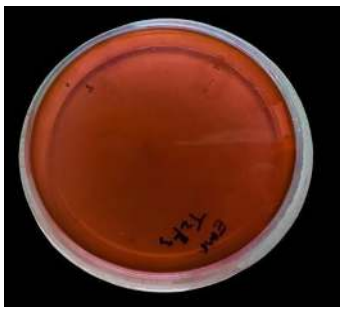

Fig. 11. *Klebsiella spp.* on EMB

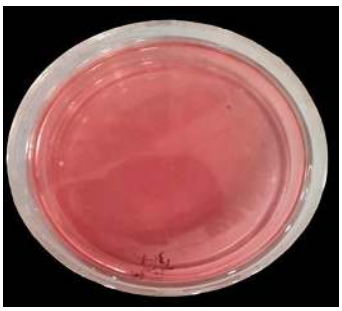

Fig. 12. *S. aureus* on MSA

Fig. 13. *Acinobacter spp.* on Acinetobacter selective agar

Table 9. Bacterial colony in biofloc tank water after mixing of probiotics (Mean ± SE)

Particulars	Treatments (cfu/ml) 10^4		
	T_1	T_2	T_3
Klebsiella spp.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Acinobacter spp.	68.67 ± 1.764[a]	71.00 ± 2.309[a]	81.67 ± 2.028[b]
Staphylococcus aureus	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

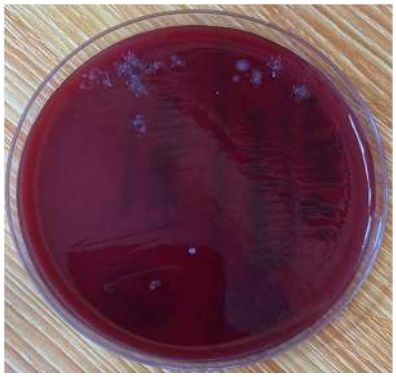

Fig. 14. Colony of *Bacillus sp*

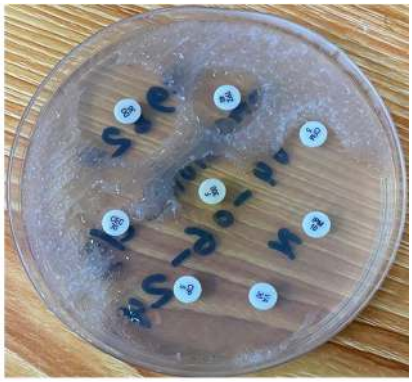

Fig. 15. Colony of *Lactobacillus sp*

Microbial status in biofloc tank after mixing of probiotics: Water treatment wise microbial colony test in biofloc tank after stocking of probiotic mixed water and fish fry. Probiotics in water prevent the growth of harmful bacteria and probiotics promote the growth of beneficial bacteria. There was no microbial colony found in *Klebsiella spp*. *Staphylococcous aureus*. Microbial colony found in *Acienobacter spp*. There were significant differences found in T_3 among three treatments, respectively.

3.10 Colony of *Bacillus sp* and *Lactobacillus sp* in probiotics

The beneficial effects of productive performance noted in this study could have also been influenced by the probiotics added to the water. Probiotic substances from Lactobacillus sp.) and Bacillus subtilis), like those employed in this study, are useful in preventing harmful bacteria from acting, enhancing the body's ability to absorb nutrients from food, assisting the immune system, and hastening waste breakdown and nutrient recycling [26]. [82,83] claim that because these microorganisms are present in the water, the fish's feed conversion rate is improved and their availability of nutrients results in an increase in production. Fish used the food more efficiently in tanks where probiotics were added, according to [46]. This is most likely because microbes aid in nutritional absorption and digestion.

Probiotics include a culture of bacteria. Nearly all strains of Bacillus and Lactobacillus were discovered in probiotics. It's presented Fig. 14 and Fig. 15.

4. DISCUSSION

4.1 Water Parameter

In our investigation, temperature fluctuations occurred normally, with fish able to withstand variations in temperature between 25 and 28 °C and having desirable floc volume. [90] and [24] discovered similar outcomes in their investigations. According to [9], floc stability is best at temperatures between 25 and 32°C. However, because of an increase in fish fecal matter, higher temperatures (>35 °C) may result in a decrease in floc stability. The biofloc fish culture media are impacted by temperature variations as well [9]. As per the findings of [76] and [11], our p^H values were deemed suitable for fish growh in biofloc system, as indicated by Table 1. High oxygen solubility at low temperatures is caused by decreased bacterial activity [36],[78,79]. Fish growth is restricted by a p^H of greater than 9.0 or less than 6.5, according to [7]. In an intense culture such as Biofloc Technology, dissolved oxygen is critical because microorganisms require oxygen to function metabolically in order to break down organic molecules [34]. Higher dissolved oxygen concentrations in the media were discovered to regulate larger and more compact flocsizes into smaller ones by [86]. It will therefore be simpler

for the fish to eat. Treatment-I had a higher DO level than the other treatments, which could have been brought on by higher bacterial activity, lower fish stocking density, and outside oxygen sources. Consequently, Treatment-I had a higher possibility of fish growth and was more compatible with floc formation than the others. Ammonia concentrations in biofloc system also increase as a result of fish waste building up and unfinished feed degrading. Temperature and p^H can have an impact on rising ammonia levels in the culture system, according to [34]. Even though the temperature and DO were ideal, the stocking density only made the ammonia levels higher. The stocking density increases with increased metabolic activity. Consequently, there was more ammonia in the biofloc media. Treatment-3 therefore had a higher ammonia content among three treatments. As per [76] and [11], the ammonia content in the treatment tanks was optimal, as indicated by Table 1. A change in oxygen transport and the oxidation of molecules in the tissue are the primary effects of nitrite compounds on the fish body, according to [61]. More ammonia and nitrite were given by the increased stocking density in Treatment-3 (450 fish/tank) compared to Treatment-1 (300 fish/tank). [76] and [73] found that 3.25 was the ideal FCR value for raising *C. batrachus* fry. Furthermore, the results of the current study differ with those of [65], who computed an FCR value of 1.37 in pond fish farming. It is important to remember that different factors can affect FCR values, and that best results are often obtained in a culture that is fish-friendly [19]. According to earlier findings, biofloc may increase the activity of the digestive enzymes [87]. There were notable variations in SGR (Specific Growth Rate), suggesting that the fish's improved food acceptance was facilitated by the addition of carbohydrates [7],[32]. This is explained by the flocs' role as extra nutrition, which includes essential minerals, amino acids, and exogenous digestive enzymes to improve digestion in the end [7], [52]. According to [21], in tanks that include biofloc, the concentration of total suspended particles is usually much higher than in tanks that have regular water. A significant effect of stocking density on the length of catfish (*Sperata aor*) was noted in the research carried out by [6]. Studies on shrimp [50,51], [18] have also revealed comparable results about the effects of biofloc systems on water quality in aquaculture settings. Although earlier studies have shown that the biofloc system has positive effects on fish production, the precise mechanisms underlying the improved growth performance are yet unknown. One explanation is that biofloc serves as a readily available extra food source that gives the fish a consistent supply of essential nutrients such as protein (including essential amino acids), polyunsaturated fatty acids, vitamins, and minerals. Evidence supporting this hypothesis may be found in the works of [7], [85], [9],[25],[52]. Fish from natural sources are clearly polluted by physical handling [37], [40,41], and a variety of sources, including dust, soils, flies, and animals, contribute to a larger burden of germs than fish raised on farm. Moreover, the biofloc technology flocculated heterotrophic culture system provided the culture species with around 18–29% of their daily feed [14,15]. Additionally, probiotics can contribute to a more stable and healthy microbial population in the biofloc system, which benefits fish raised in aquaculture [15]. One explanation for the declining benefits and overall productivity observed in this study could be a shorter cultural epoch.

4.2 Growth Performance

Due to the lower fish stocking density, Treatment-1 is more successful because there is a reduced risk of stress and bodily harm. Probiotics and continuous aeration also aided in *A. testudineus* growth and survival. Numerous recent research [17] have shown the combined impact of enhanced water quality and heterotrophic bacterial load on the biofloc technology to promote fish growth and survival. The best growth rate is found in lower stocking densities [62] because there is less rivalry, more space, and food options in these densities [29]. Additionally, [72] research demonstrated that a reduced stocking density in *Clarias batrachus* results in greater sizes and a higher survival rate. Biofloc technology (BFT), which can lower the cost of additional feed, is an excellent operating system option for contemporary aqua farming. As per earlier projections, aquaculture output had to be maintained at 60% of expenses [49]. [81] discovered that following eight weeks of testing, there was a substantial difference in the ultimate weight of *Heteropneustes fossilis* (23.65 ± 0.02). [43,44] reported a 28–33 g increase in weight. As stocking density rose in this study, *A. testudineus* overall length and weight dropped (Figs. 3 and 4). [6] discovered that stocking density has a substantial effect on the length of catfish (*Sperata aor*). Table 2 of the current investigation shows that there was no significant difference ($p<0.05$) in survival among three treatments, with considerably higher survival reported at the

lowest stocking density of 200 fish/m^3 of water volume. In the treatment T_1 the higher survival rate was found due to low stocking density. The production of *A. testudineus* was calculated by using its corresponding formula. In the T_3 treatment more production was gained then followed by T_2 and T_1. Fish growth metrics and survival suffer when stocking density rises, as reported by [55]. According to [59] and [33], increased stocking density can deteriorate water quality, which will hinder the growth, resistance to illness, and survivability of culture organisms. Higher stocking densities negatively affect fish survival and growth, claims [84]. According to [77], survival and FCR are significant growth indicators in many fish culture systems that indicate successes or failures in the aqua farm. The existence of microbes and particles in culture systems provides the cultured species with an extra food supply and may help to promote their growth [18],[75]. *Mystus gulio* was found to have FCR values ranging from 1.50 to 2.00 by [80]. [64] also found that *H. fossilis* had a similar FCR value of 2.17 in their investigation. Compared to the current study, FCR value of 0.97 to 1.67 it shows that the bifloc system is able to provide better feed efficacy.. That being said, [73] found that 3.25 was the ideal FCR value for raising *C. batrachus* fry. Furthermore, the results of the current study differ with those of [65], who computed an FCR value of 1.37 in pond fish farming. It is important to remember that different factors can affect FCR values, and that best results are often obtained in a culture that is fish-friendly [19]. According to earlier findings, biofloc may increase the activity of the digestive enzymes [87], There were notable variations in SGR (Specific Growth Rate), suggesting that the fish's improved food acceptance was facilitated by the addition of carbohydrates [7], [32]. This is explained by the flocs' role as extra nutrition, which includes essential minerals, amino acids, and exogenous digestive enzymes to improve digestion in the end [7], [52]. [21], in tanks that include biofloc, the concentration of total suspended particles is usually much higher than in tanks that have regular water. A significant effect of stocking density on the length of catfish (*Sperata aor*) was noted in the research carried out by [6]. One explanation is that biofloc serves as a readily available extra food source that gives the fish a consistent supply of essential nutrients such as protein (including essential amino acids), polyunsaturated fatty acids, vitamins, and minerals. Evidence supporting this hypothesis may be found in the works of [7], [85], [9], [25], [52]. Fish from natural sources are clearly polluted by physical handling [37],[40], and a variety of sources, including dust, soils, flies, and animals, contribute to a larger burden of germs than fish raised on farm. Moreover, the biofloc technology flocculated heterotrophic culture system provided the culture species with around 18–29% of their daily feed [15]. Additionally, probiotics can contribute to a more stable and healthy microbial population in the biofloc system, which benefits fish raised in aquaculture [15]. One explanation for the declining benefits and overall productivity observed in this study could be a shorter cultural epoch.

4.3 Proximate Composition

There were no discernible differences in the moisture percentage ($p>0.05$) among three treatments. However, there were significant differences ($p<0.05$) found in the control treatment. [12] observed 6.9% - 14.2% moisture in dried marine fishes. Fish have a moisture content that ranges from 16.97 to 26.19 percent, depending on age, sex, season, and other variables, according to [27]. According to the proximate composition of the whole-body carcass of the *A. testudineus*, as the C/N ratio increased, the contents of protein and ash increased while those of moisture and lipids declined. A higher carbon-nitrogen ratio was linked to an enhanced carcass composition in crucian carp (*Carassius auratus*), according to [88]. Raising the C/N ratio in Nile tilapia (*Oreochromis niloticus*) was discovered by [60] to decrease fat content while boosting protein and ash content. There was no significant variation ($p>0.05$) found in protein (percent) among the three treatments, but there were significant differences ($p<0.05$) found in the control treatment. There was a significant variation ($p<0.05$) found in lipid percentage across the two treatments and control (Table 3). There were no significant changes ($p>0.05$) in ash (percent) across the three treatments, but significant differences ($p <0.05$) were identified in the control treatment (Table 3). The protein content of *A. testudineus* in the biofloc tank was 46.35 ± 0.279 percent, which was higher than the natural source (control) of *A. testudineus* 39.32 ± 0.630 percent. In the biofloc tank, *A. testudineus* had a lipid content of 26.45 ± 0.207 percent, which was higher than the natural source (control) of *A. testudineus*, which had a lipid content of 20.66 ± 0.286 percent. In the biofloc system, the ash content of *A. testudineus* was 10.44 ± 0.434 percent, which was lower than the natural source

(control) of *A. testudineus* 18.03 ± 0.363 percent. According to previous research on SIS [31], [37], [40], the ultimate proximate composition (Protein, fat, and ash) of *A. testudineus* was found to be quite comparable in this study.

4.4 Microbial Colony Count

The microbial colony count was significantly higher in control treatments than in T_1, T_2, and T_3 treatments respectively (Fig. 5). This study enumerated microbial colony count from the gut of the fish species. [58] also identified the microorganisms in the digestive system of fish and also reported that living fish usually harbor some common Gram-negative facultative anaerobic bacteria in their gut throughout the year. However, high bacterial load decreases the quality of fish quickly which spoils the food product and is responsible for different microbial diseases, which was also reported by [39]. They found that the bacterial strain isolated from the intestine is found positive in the proteinase test, and catalase activities. This property indicated that most of the bacteria are associated with spoilage activities thus contributing to the quality deterioration. Contaminated food by different pathogens may harbor virulence genes that are responsible for disease outbreaks [63]. In the present study, microbial colony count of cultured *A. testudineus* in biofloc tank was found lowest in T_1, T_2, and T_3 treatments than control. This may be due to slightly higher temperature in open water than in the biofloc tanks. [28] also reported that the bacterial population grows higher during monsoon due to availability of more nutrients and favorable temperature in open. Since fish are grown in controlled conditions in biofloc tanks and probiotics are used, biofloc fish have less microbial colonies. On the other hand, there are opportunities for microbial contamination in ponds where fish are farmed, such as transfer of parasites by birds, bathing of cows, use of cow dung, use of poultry droppings, therefore the microbial colony count is higher in the control treatment.

4.5 Hematology of *A. testudineus*

Hematological investigations in culture fish, like all animals used in the production sector, represent the basic tool for the assessment of the health status and well-being of the animals [57]. In order to assess fish health status and interpret hematological parameter fluctuations most effectively, one must have a thorough understanding of environmental elements as well as the physical and chemical characteristics of water [13]. In this regard, temperature, salinity, and p^H of water, the photoperiod, and the farming system affect the hematological parameters in culture fish. Moreover, it has been demonstrated that the availability of food, as well as its type, may even influence erythropoiesis in fish [16], therefore, this aspect must be considered during a hematological assessment. In the last few years, the development of new methods for the differentiation and quantification of blood cells in fish species has acquired great interest. In order to expedite hematological examinations and ensure dependable findings, it is now necessary to determine a reference range for the species of farmed fish and implement an automated assessment method.

There was no significant difference found ($p<0.05$) in haemoglobin, white blood cell, lymphocyte, monocyte, eosinophil, basophil in the blood of *A. testudineus* (Table 5). Significant difference found in red blood cell, neutrophils, platelet count, hematocrit, mcv, mchc and cortisol in the blood of *A. testudineus* (Table 5). Red blood cells were higher in control treatment (4.84 ± 0.005) than T_1 (4.39 ± 0.005), T_2 (4.90 ± 0.005) and T_3 (4.62 ± 0.005) treatments respectively. Cortisol was lower in T_2 (568.20 ± 1.331) and higher in control (602.00 ± 1.527) treatment. Platelet count in blood of *A. testudineus* was higher in T_1 and lower in control. A fish's hematological characteristics define its physiological state and stress tolerance, as well as its overall health [35]. Increased haemoglobin, RBC and HCT levels in the present study indicate a good health status, which helps protect fish from stressful environments [89].The major causes of somewhat damaging alterations in aquatic animals' blood parameters are environmental stresses and contaminants [30]. Higher levels of hemoglobin, red blood cells, and hematocrit (HCT) in this study suggest that fish are in high condition, which helps shield them from stressful situations [89]. The current study's findings on African catfish (*Clarias gariepinus*) fingerlings cultivated in a biofloc-based system were comparable to those of [24], where they found better hematological status of *A. testudineus* in T_1, T_2 and T_3 treatments rather than control. A better WBC count in T_1 treatment indicated a better immune action of the fish body in response to the presence of stressors or infection [45]. The WBC count in the control group was considerably lower than in the biofloc treatment groups in research by [38] to assess the effect of biofloc technology on the growth

performance and biochemical parameters of *Oreochromis niloticus*. Anaemia in stinging catfish was not brought on by alteration of the C/N ratio, as evidenced by the lack of change in mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin (MCHC) among the treatments. Similarly, [89] observed in a biofloc culture that various blood parameters including lymphocytes, MCV (Mean corpuscular volume), and MCH (Mean corpuscular haemoglobin) and MCHC (Mean corpuscular haemoglobin concentration) were at favourable levels for Stinging catfish.The platelet count in *A. testudineus* revealed significant differences under different experimental conditions [69].

4.6 Lipid profile of *A. testudineus*

There were significant differences found in cholesterol between the two treatments and control. Significant differences were found in HDL among the three treatments and control. HDL was higher in T_2 (129.00 ± 0.577) and lower in control (32.00 ± 0.577) treatments. LDL were higher in control (141.00 ± 0.577) and lower in T_3 (34.33 ± 1.202) treatments. No significant difference was found in triglycerides among the three treatments but significant difference was found in control treatment (Table 6). There was no significant difference ($p > 0.05$) found in total protein, AST and ALT among three treatments but significant difference found in control treatments (Table 5).

[56] reported that decreased total protein content may cause chronic liver diseases, malnutrition under starvation. It was depicted that the measurement of globulin, albumin and total protein may be considered to detect the state of diseases like immune disorders, impaired kidney activity and liver dysfunction [53]. Generally, in fish albumin and total protein content are decreased due to exposure of various pollutants and pesticides [54]. Enhanced activity of ALP might be related to dysfunction and hepatic damage; elevation of ALP activity may cause an increased Trans phosphorylation in *C. punctatus* exposed to monocrotophos. These changing patterns in ALP activity in blood plasma were also noted in Tilapia [42] and common carp [53].

Energetic metabolites level like cholesterol and triglycerides in fish are considered as an important diagnostic characteristic. Triglycerides as a major health status index. Triglyceride content in blood plasma was more in pond than tank condition because after natural uptake and absorption through gut as lipid, finally, it is transported to liver and enhanced the level.

HDL cholesterol is normally described as the capacity of transferring excess cholesterol from non-hepatic cells to the liver [20]. Therefore, various subtractions of high-density lipoprotein play various roles in this transport and during this transfer some minor subtractions may remain present in individuals who have HDL deficiency. In this study higher HDL in T_1 treatment and lower in control. The level of HDL produced from non-hepatic cells into the liver and other organs of fish is correlated with the LDL and cholesterol. The present findings of LDL higher in control and lower in T_1 treatment. LDL also plays a vital role to maintain HDL and cholesterol level of fish organs and also helps to smooth function of HDL of fish body.

4.7 Proximate Composition of Floc in *A. testudineus* culture

The relatively high ash content of flocs of this study resulted in an ingredient with a relatively low crude lipid, low crude protein content to bio-flocs (Table 7). The crude lipid and protein content of the dried flocs decreased with increasing ash content, while the crude lipid and protein content of the ash free dry weight of the flocs remained unaltered. This indicates that the crude lipid and protein content of the micro-organisms did not vary in time. This is an interesting property for valorization of extracts of proteins or lipids of flocs in aquaculture feed. In most studies in which bio-flocs are included in fish diets, they usually replace fishmeal because of its high cost in fish diets and limited resource [85].

4.8 Benefit-Cost Ratio (BCR)

The net profit was 1780.00 BDT/m^3 in T_1, followed by T_2 and T_3 treatment. The present study showed that the highest growth rate was found in T_1 in which production was less, although their stocking density was also less than T_2 and T_3. But the experiment emphasized that using high stoking density lead to maximize output. For considering that view in mind, Thai koi which can tolerate crowding condition can be cultured intensively in the tank to raise the production higher. To assess the BCR produced by the biofloc fish culture system, a cost-benefit analysis of *A. testudineus* monoculture was conducted. For the total cost, there was a significant difference ($p< 0.05$) found in three

treatments. Treatment T_3 had the highest production costs, followed by treatments T_1 and T_2. This might be because the treatment T_3 increased the price of *A. testudineus* fry and feed. This result corroborated the assertion made by [4,5] that the cost of feed in fish production was very high. The total revenue was higher in treatment T_2 whereas the lowest revenue was found in treatment T_3. The significantly highest net revenue (1780.00 ± 24.037 BDT/m^3) was obtained from treatment T_1 where the stocking density was 200 fish/m^3. In addition, the lowest net revenue (562.22 ± 155.55 BDT/m^3) was found with treatment T_3 where stocking density was 300 fish/m^3. These findings were not very encouraging for the production and economic return of *A. testudineus* in the biofloc aquaculture system. It might be due to the variation in stocking density and feed cost. Samad *et al.* (2016) found more or less similar best BCR (1:1.78) in the experiment on the nursery of *Labeo bata* and this finding was more or less similar to the present study. However, the best finding of [71] in the case of *C. batrachus* was 1:1.24 which was significantly different from the present findings. Less total production and less benefit might be due to a short cultural period.

5. CONCLUSION

Biofloc is an alternative technology to fish farming. It provides a boost to doubling fish production and encourages small landholders and entrepreneurs to take up fish farming. In the present study the biofloc technique was used for Thai Koi (*A. testudineus*) fish production in a small scale. The growth and benefit cost ratio were encouraging. This technique is also most suitable for the areas where the land and water availability is the major constrain. Lower stocking densities (200 fish/m^3) in Biofloc Technology demonstrated superior *A. testudineus* growth performance with a lower FCR and improved water quality metrics than high stocking densities. There is a need of more research on water quality, microorganism profile and growth related parameters due to consumption of floc and conversion of ammonia to protein with reference to Thai Koi culture with higher stocking density.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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