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Water quality, fish production, economics and greenhouse gas emission under different dietary protein levels in pond reared with tilapia (*Oreochromis mossambicus*)

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ABSTRACT

The present experiment was carried out to study the water quality, production, economics and greenhouse gas emissions under different dietary protein levels in tilapia (*Oreochromis mossambicus*) farming pond. The experiment was carried out using three treatments with varying dietary protein levels viz. T₁: 27%; T₂: 30% and T₃: 33%, each with two replications. Tilapia stocking density (49400 individuals/ha) was similar across the treatments. Water quality was monitored fortnightly and calculated values of temperature, transparency, DO, CO₂, pH, alkalinity, ammonia-nitrogen and TDS were recorded as 29.30±0.28 to 29.65±0.49°C, 29.37±1.22 to 34.70±2.54 cm, 4.98±0.17 to 6.17±0.03 mg/L, 4.54±0.02 to 5.64±0.24 mg/L, 5.35±0.28 to 6.55±0.07, 86.25±3.81 to 97.77±2.51 mg/L, 0.10±0.01 to 0.35±0.07 mg/L and 522.82±10.85 to 563.43±2.92 mg/L, respectively. The average measurement of weight gain, SGR, survival rate, FCR and yield of tilapia varied from 172.34±2.81 to 227.55±2.84 g, 1.25±0.01 to 1.34±0.11 % bwd⁻¹, 78.00±5.35 to 87.00±7.07 %, and 1.68±0.04 to 1.89±0.05 and 8062.08±70.72 to 9517.40±63.58 Kg/ha/6months, respectively. The mean values of net profit (BDT/ha), CBR and greenhouse gas emission (CO₂e Kg/ha/year) were 320892.91±2474.68 to 484099.20±565.60, 0.49±0.00 to 0.71±0.00 and 7.20±0.12 to 8.52±0.04. Fish yield varied from 16124.16±49.41 (T₁) to 19034.80±116.88 (T₃) kg/ha/year. Greenhouse gas emission was found significantly ($p<0.05$) highest in the treatment T₃, while net benefit and CBR were observed significantly ($p<0.05$) highest in the treatment T₂. Thus, dietary protein level significantly influences water quality, fish growth, net profit and greenhouse gas emission in pond raised with tilapia (*O. mossambicus*).

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INTRODUCTION

Global aquaculture has become one of the world's fastest-growing food production sectors, playing a vital role in promoting food security and promoting sustainable environmental management. In 2022, worldwide aquaculture production achieved a record level of 94.4 million tonnes, surpassing capture fisheries and marking a historic turning point (FAO, 2024). This growth underscores aquaculture's potential to address the rising worldwide demand for aquatic foods. Asia

dominates global aquaculture, contributing over 90% of the total production. Bangladesh has solidified its position as a significant contributor, ranking among the top five Asian countries in aquaculture production (FAO, 2024). Currently, 58.03% of Bangladesh's fish production originates from aquaculture, while 28.15% comes from capture fisheries (DoF, 2024). Tilapia, particularly Mozambique tilapia (*Oreochromis mossambicus*), stands as the second most valuable aquaculture species in Bangladesh. Its cultivation plays a crucial role in improving livelihoods and offering employment opportunities

for millions. Despite its benefits, aquaculture contributes to environmental challenges, notably GHG emissions. The Food and Agriculture Organization in 2014 reported that GHG emissions from fisheries have nearly doubled over the past five decades and are projected to increase by 30% by 2025 (FAO, 2014). Freshwater aquaculture, especially pond systems prevalent in Bangladesh, serves as an important source of methane (CH₄) and nitrous oxide (N₂O) release (Kais & Islam, 2018). Several practices in tilapia farming contribute to GHG emissions. The use of large quantities of feed, organic manure (e.g., cow dung), and inorganic fertilizers enhances productivity but also increases emissions of CO₂, CH₄, and N₂O (Zhang et al., 2024 and Ren et al., 2017). Moreover, the decomposition of organic matter accumulated in pond sediments under anaerobic environments leads to significant CH₄ emissions (Datta et al., 2009). Dietary protein levels in fish feed is crucial for optimal growth but can influence environmental outcomes. Juvenile tilapias require protein levels 32% to 50%, while growing stages need 25% to 30% (Ali et al., 2008). Excessive protein not utilized for growth is metabolized, leading to increased ammonia production, which can deteriorate water quality and elevate GHG emissions (Cho & Kaushik, 1985). Protein constitutes the most costly component of formulated fish diets. Over-supplementation not only escalates production costs but also contributes to environmental degradation (Robb et al., 2017). Conversely, inadequate protein levels can impair fish growth and health (Carneiro et al., 2020). Therefore, it is necessary to optimize protein level for economic viability and climate-smart fish farming. To mitigate GHG emissions from tilapia aquaculture, it is imperative to adopt sustainable practices, including the optimization of dietary protein levels. Such measures can enhance production efficiency, improve water quality, and reduce environmental impacts. Implementing these strategies will not only bolster Bangladesh's aquaculture sector but also contribute to global efforts in combating climate change. Keeping in view, this study was assessed the water

quality, fish production, economics and greenhouse gas emission under different dietary protein levels in pond reared with tilapia (*Oreochromis mossambicus*).

MATERIALS AND METHODS

Location and duration

The experimental trail was undertaken over six months, spanning July to December, 2024 in six farmer managed ponds (average area of 0.04±0.01 ha and water depth 1.51±0.15m) located at Harian of Paba, Rajshahi, Bangladesh (Figure 1).

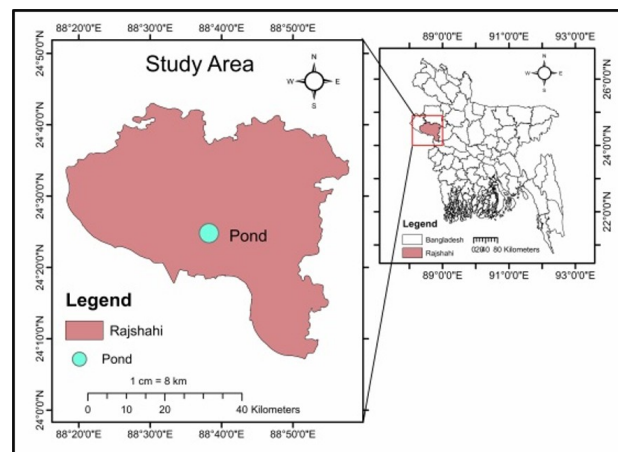


Figure 1. Map showing the study area.

Experimental design

The experiment was implemented with three different treatments of dietary protein levels (T₁: 27%, T₂: 30% and T₃: 33%) each treatment was replicated twice. Stocking density, initial stocking weight, and fish growing period were same for all the treatments. Experiment layout is shown in Table 1.

Table 1. Experimental layout for tilapia (*Oreochromis mossambicus*) farming in ponds under three different treatments.

Water quality	Treatments			F-value	p-value
	T ₁	T ₂	T ₃		
Pond area (ha)	0.04±0.01 ^a	0.04±0.01 ^a	0.04±0.00 ^a	0.005	0.995
Pond depth (m)	1.51±0.15 ^a	1.52±0.00 ^a	1.51±0.15 ^a	0.005	0.995
Stocked <i>O. mossambicus</i> (no./pond)	2000.00±282.84 ^a	2000.00±565.68 ^a	2000.00±309.83 ^a	0.000	1.000

Figures bearing common letter(s) in a row as superscript do not differ significantly (p > 0.05)

Pond management

Aquatic vegetation was manually removed, while undesirable fish species were eliminated through repeated netting. Subsequently, the ponds were treated with agricultural lime at an application rate of 250 kg/ha, after seven days of liming, all the research ponds were fertilized with inorganic fertilizer (Urea: 25kg/ha, TSP: 15 kg/ha) to increase natural productivity of the ponds. All the research ponds were stocked at morning with tilapia (*Oreochromis mossambicus*) after three days following basal fertilization. The fish were fed daily at a feeding rate of 5% of their total body weight. The feeding rate was modified based on the recorded body weight of the fish, as measured during monthly sampling.

Monitoring of water quality parameters

Water quality indicators were measured fortnightly within 9:00am to 10:00 am. A Celsius thermometer with a range of

0°C to 120°C was used to measure the water temperature. The transparency of water (cm) was recorded with the help of a secchi disk. The water pH and dissolved oxygen (mg/l) were determined by the help of a multimeter (HQ40d, HACH, USA). Ammonia-nitrogen (mg/l), carbon dioxide (mg/l) and alkalinity (mg/l) of water were estimated using a HACH kit (FF-2, USA).

Quantitative analysis of CH₄ and N₂O

CH₄ emission was determined after Lesschen et al. (2011) as follows:

$$\text{CH}_4 \text{ emission from culture system} = \text{EF} \times \text{T} \times \text{A} \times 10^{-6} \text{ Gg CH}_4 \text{ year}^{-1}$$

EF stands for methane emission factor = 0.351 kg CH₄ ha⁻¹ day⁻¹

N₂O-N emission was determined after Hu et al. (2012) as follows:

$\text{N}_2\text{O-N}$ emission from culture system = $\text{EF} \times \text{P} \times 10^{-3} \text{ Gg } \text{N}_2\text{O-N year}^{-1}$

EF stands for $\text{N}_2\text{O-N}$ (Nitrous oxide) emission factor = $0.00169 \text{ kg } \text{N}_2\text{O-N metricton}^{-1} \text{ year}^{-1}$

Greenhouse gas emissions were calculated in terms of CO_2 equivalents based on relative global warming potentials (GWP) values, where the 100-year global warming potentials of CH_4 and $\text{N}_2\text{O-N}$ were estimated as 25 and 265 times those of CO_2 , respectively (Pearce et al., 2014).

Thus,

Global warming potential = $25 \times \text{CH}_4 + 265 \times \text{N}_2\text{O-N}$

Monitoring of fish growth

The fish will be sampled fortnightly to assess the growth and calculation of feed ration. At least 10% of the cultured fishes will be assessed during each sampling occasion using a cast net in each treatment pond. The subsequent parameters will be used for the study of fish growth and yield:

Initial weight (g) = Average body weight of fish at stocking

Final weight (g) = Body weight of fish at the time of harvest

Weight gain (g) = Average final body weight - average initial body weight

Specific growth rate (SGR, $\% \text{ bwd}^{-1}$) = $(\text{Ln final weight} - \text{Ln initial weight}) / \text{culture period} \times 100$

Survival rate (%) = Number of fish harvested / number of fish stocked $\times 100$

Yield (kg/ha/yr) = Fish biomass at harvest - Fish biomass at stocking

Feed performance tested through calculating the feed conversion ratio (FCR) as follows:

$\text{FCR} = \text{Feed fed in dry weight} / \text{live weight gain (g)}$

Economics

The economic viability of the different treatments was evaluated using a simple cost-benefit analysis. Cost Benefit Ratio (CBR = Net benefit / total investment) was calculated for this study.

Statistical analysis

The recorded data were subjected to analysis of variance (ANOVA), and significant differences among treatment means were determined using SPSS statistical software.

RESULTS AND DISCUSSION

Water quality parameters

The average values of the water quality indicators among various treatments have been shown in Table 2. The average range of water temperature of this study varied from 29.300.28 to 29.650.49C in the T_2 and T_1 treatment respectively. Ahsan et al. (2013) noted the value of temperature spanned from 28 to 31C. Boyd (1998) reported 25 to 32C is suitable for warm water aquaculture species, which strongly support the water temperature recorded throughout the experimental period. The average water transparency level ranged from 29.371.22 to 34.702.54 cm in the treatment T_3 and T_1 , respectively. Kohinoor et al. (2001) reported that transparency value ranging from 15-58 cm in culture pond. The mean transparency value

of the current study closely resembled those documented by Uddin et al. (2007), Begum et al. (2014), Ali et al. (2018). The average value of DO in this study spanned from 4.980.17 to 6.170.03 mg/L in the treatment T_3 and T_1 , respectively. Kumar et al. (2023), Rahman et al. (2016) and Sharmin et al. (2019) who reported DO ranged from 6.22 to 9.00, 4.78 to 6.82 and 4.90 to 9.00 mg/L, respectively which were strongly agreed with the recorded mean DO value of the current study. The pH value in this study was ranged from 5.350.28 to 6.550.07 in the treatment T_3 and T_1 , respectively. Boyd (1998) noted that the distribution of pH was 7-9 for fish culture. The average value of pH determined during the experiment agreed with the outcomes of Ogola et al. (2020), Nabi et al. (2017) and Talukdar et al. (2017) who recorded the value of pH ranges from 8.52 to 9.23, 7.700.25 to 8.500.25 and 6.880.11 to 6.960.11, respectively. The mean value of free carbon dioxide (CO_2) during the present culture period was 4.540.02 to 5.640.24 mg/L in the T_1 and T_3 treatment respectively. Majumder et al. (2017), Sharmin et al. (2019) who found CO_2 values ranges from 2.00 to 4.00 mg/L and 2.82.87 to 4.061.07 mg/L, respectively which were slightly different with the recorded of the present experiment as well. The average fluctuation of alkalinity during the culture period spanned from 86.253.81 to 97.772.51 mg/L in the treatment T_1 and T_3 , respectively. Ali et al. (2018) noted that the alkalinity value in fish culture pond was ranged from 129.17 to 151.83 mg/L. Mahmud et al. (2021), Kumar et al. (2023) who found alkalinity values ranges from 31.090.25 to 54.100.34 and 74.5 to 132.5 mg/L, respectively. The average value of $\text{NH}_3\text{-N}$ in the present experiment was ranged from 0.100.01 to 0.350.07 mg/L in the T_1 and T_3 treatment, respectively. The desirable value of $\text{NH}_3\text{-N}$ for fish culture was <0.1 mg/L (Boyd, 1998). Milstein et al. (2009) observed that $\text{NH}_3\text{-N}$ as 0.09 to 0.99 mg/L for aquaculture system. The mean value of TDS in the present experiment fluctuated from 522.8110.85 to 563.432.92 mg/L in the treatment T_1 and T_3 , respectively. According to WHO standard, the applicable suitable range of TDS for fish culture is 400 to 500 mg/L (Sinha et al., 2015). Kumar et al. (2023) found TDS values ranges from 319.12 to 468.00 mg/L for diverse fish production.

Greenhouse gas emission

Greenhouse gas emissions of tilapia farming with the findings of the various treatments are shown in Table 3 and total emission of GHGs is presented in Figure 5. In this study the value of GHGs emission ranges from 7.20 ± 0.127 to $8.52 \pm 0.014 \text{ kg } \text{CO}_2\text{e/ha/year}$, where the lowest and highest value was recorded in the treatment T_1 and T_3 , respectively. Poor feed management and poor water quality might lead to increased greenhouse gas emission from culture pond of aquaculture system (Robb et al., 2017). Islam et al. (2020) suggested that GHGs emission from aquaculture sector can be reduced by better feed management and feed conversion ratio, reducing pond nitrous oxide (N_2O) etc. Hu et al. (2012) stated that $\text{N}_2\text{O-N}$ production from aquaculture would be increased to 101.495 Mt CO_2e by 2030 if this sector still continues to grow 7.10%/year around the world. Improvement of culture system can be an effective alternative to reduce the GHGs release from aquaculture sector (Fang et al., 2017, Liu et al., 2014 and Nobre et al., 2010). From the above discussion it may conclude that the recorded value of CO_2e kg/year emission under different treatment was strongly agreed with the foresaid scientists.

Table 2. Water quality parameters in ponds under different treatments.

Water quality	Treatments			F-value	p-value
	T ₁	T ₂	T ₃		
Temperature (°C)	29.65±0.49 ^a	29.30±0.28 ^a	29.35±0.02 ^a	0.581	0.612
Transparency (cm)	34.70±2.54 ^b	32.74±0.33 ^{ab}	29.37±1.22 ^a	5.355	0.102
DO (mg/L)	6.17±0.03 ^c	5.50±0.14 ^b	4.98±0.17 ^a	35.167	0.008
CO ₂ (mg/L)	4.54±0.02 ^a	4.66±0.07 ^a	5.64±0.24 ^b	32.248	0.009
pH	6.55±0.07 ^b	6.30±0.14 ^b	5.35±0.28 ^a	22.905	0.015
Alkalinity (mg/L)	86.25±3.81 ^a	91.02±2.15 ^{ab}	97.77±2.51 ^b	7.880	0.064
NH ₃ -N (mg/L)	0.10±0.01 ^a	0.20±0.00 ^a	0.35±0.07 ^a	1.800	0.306
TDS (mg/L)	522.82±10.85 ^a	538.25±10.39 ^{ab}	563.43±2.92 ^b	10.753	0.043

Fish growth and yield

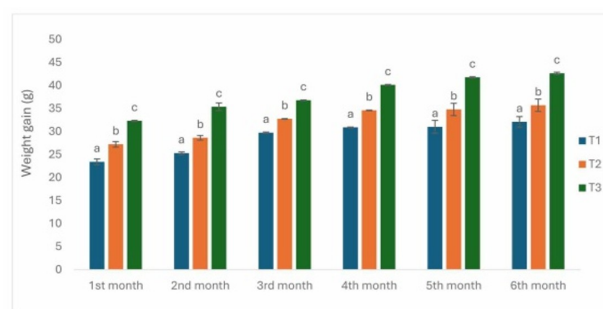
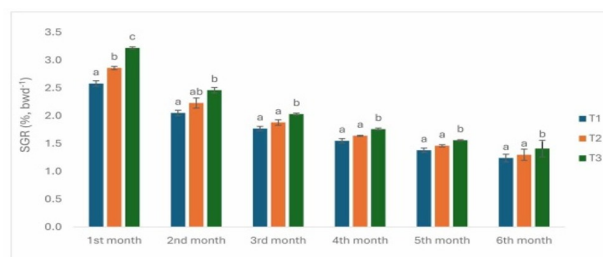
Monthly changes in weight gain and specific growth rate (SGR, %) was measured over the culture period under various treatments are shown in Figures 2 and 3 and the mean variation of fish growth and yield of fishes is presented in Table 4. Average final weight of tilapia (*Oreochromis niloticus*) in 150 days culture period was ranged from 1911.00 to 2131.00 reported by Begum et al. (2014). The average final weight of *O. mossambicus* was measured within the range of 192.342.90 to 247.552.57g in the treatment T₁ and T₃ respectively. Satter et al. (2019) observed that final average weight of tilapia ranged from 354.5 to 455.2g. The average weight gain of tilapia in the current study was as 172.342.81 to 227.552.84g.

The minimum weight gain was noted in the treatment T₁ and the uppermost weight gain was measured in the treatment T₃. The average value recorded during the experiment agreed with the outcomes of Satter et al. (2019) and Begum et al. (2014) who recorded the mean weight gain of tilapia ranges from 306.1 to 406.7 and 1911.00 to 2131.00g, respectively. The mean specific growth rate of tilapia in this study spanned from 1.250.01 to 1.340.11 where the least and maximum value was obtained in the treatment T₁ and T₃, respectively. Sharmin et al. (2019) noted that the value of SGR in tilapia monoculture varied from 4.61 to 5.49. Kohinoor et al. (1997) reported the survival rate of GIFT strain as 95.75% in fish pond. The average survival rate was recorded in the present study ranged from 78.005.35 to 87.007.07% where the lowest and highest value was observed in the treatment T₁ and T₂ respectively. Nabi et al. (2017) suggested that survival rates were 900.20 to 920.45 of monosex tilapia.

The average value of yield in the current study extended from 16124.1649.41 to 19034.80116.88 kg/ha/year, where the least and peak value was assessed in the T₁ and T₃ treatment, respectively. Satter et al. (2019) noted that yield ranged from 10937.58 to 13809.55 kg/ha/year for tilapia and Indian major carp culture. Majumder et al. (2017), Nabi et al. (2017) and Green et al. (2002) recorded mean value of yield ranged from 6750 to 12006 kg/ha/year 1356.03 to 999.856 kg/ha/4 month and 3206.97 to 7373 kg/ha/year, respectively. From the above discussion it can be claimed that the total mean yield values in the current study is more or less similar to the foresaid scientists.

Feed Conversion Ratio (FCR)

The mean FCR spanned from 1.680.04 to 1.890.05 where the least and peak value was found in the treatment category T₃ and T₁, respectively. Nabi et al. (2017) found the mean value of FCR ranges from 1.30.14 to 1.950.45 which are highly consistent with the recorded mean FCR of the present investigation.

**Figure 2.** Monthly variation in weight gain (g).**Figure 3.** Monthly variation in SGR (%/bwd -1).

Economics of fish farming

Feed performances of tilapia farming with different treatments are presented in Figures 4 and economics of tilapia farming with various treatments are shown in Table 5. Total cost (BDT/ha) fluctuated from 153009.09 to 781759.94 and benefit cost analysis varied from 1.02 to 1.41 reported by Nabi et al. (2017). The mean total cost and CBR recorded from the present study was 646548.543535.61 to 772750.384808.55 and 0.490.00 to 0.710.00. The lowest and highest value of CBR was observed with the treatment category T₁ and T₂ respectively. Rahman et al. (2016) noted that the net monetary return from monosex tilapia culture as 180186.50 to 208301.28 BDT/ha and benefit cost analysis as 1.44 to 1.79. The mean net profit (BDT/ha) of the present investigation was spanned from 320892.912474.68 to 484099.20565.60, where the least and highest value was observed in the treatment category T₁ and T₂, respectively. Ali et al. (2018) estimated that the net profit as 151771 to 636779 BDT/180 days. Overall findings indicated that tilapia fed with 30% dietary protein level performed better than the other treatments in terms of survival rate, net benefit, CBR and reduced GHGs emission. Findings clearly indicated that 30% protein in feed increased the production, benefit and reduced greenhouse gas emission in tilapia monoculture pond. Almost 23.82% fish production increased from the current fish production achieved with treatment T₂ as compared to the production achieved with tilapia monoculture as reported by Majumder et al. (2017).

Assumption almost agreed with Rahman et al. (2016), Green et al. (2002), Hu et al. (2012), Robb et al. (2017) and Islam et al. (2020) who worked on growth, production of tilapia, pond

management strategies for tilapia and GHGs emission from aquaculture respectively.

Table 3. Annual greenhouse gases (GHGs) emission of tilapia (*Oreochromis mossambicus*) farming in ponds under different treatments.

Items (GHGs) (Kg/ha/year)	Treatments		
	T ₁	T ₂	T ₃
CH ₄	0.000029±0.00004 ^a	0.000029±0.000028 ^a	0.000029±0.00004 ^a
N ₂ O-N	0.0136±0.000 ^a	0.0152±0.007 ^b	0.0160±0.007 ^b
Total emission (CO ₂ e, Kg/ha/6month)	3.62±0.636 ^a	4.04±0.028 ^b	4.26±0.007 ^c
Total emission (CO ₂ e, Kg/ha/year)	7.20±0.127 ^a	8.05±0.056 ^b	8.52±0.014 ^c

Table 4. Fish growth and yield under different treatments.

Parameters	Treatments			F-value	p-value
	T ₁	T ₂	T ₃		
Initial weight (g)	20.00±0.35 ^a	20.00±0.88 ^a	20.00±0.70 ^a	0.000	1.000
Final weight (g)	192.34±2.90 ^a	210.53±5.59 ^b	247.55±2.57 ^c	70.152	0.003
Weight gain (g)	172.34±2.81 ^a	190.53±5.67 ^b	227.55±2.84 ^c	63.642	0.003
SGR(% bwd ⁻¹)	1.25±0.01 ^a	1.30±0.03 ^{ab}	1.34±0.11 ^b	11.589	0.039
Survival rate (%)	85.00±5.65 ^a	87.00±7.07 ^a	78.00±5.35 ^a	2.175	0.261
Yield(kg/ha/6 months)	8062.08±70.72 ^a	9025.38±91.95 ^b	9517.40±63.58 ^b	379.231	0.000
Yield (kg/ha/Yr)	16124.16±49.41 ^a	18050.76±91.99 ^b	19034.80±116.88 ^c	1203.528	0.000

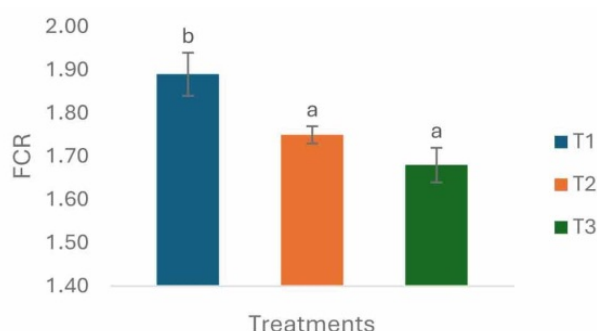


Figure 4. Mean value of FCR.

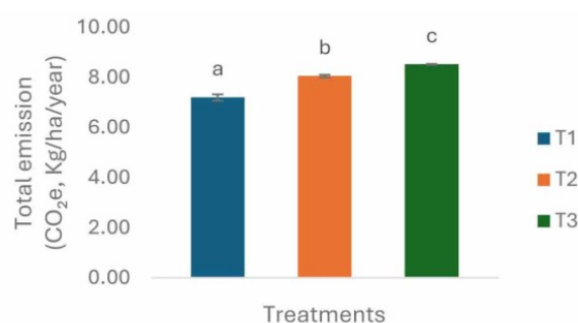


Figure 5. Mean value of CO₂e (Kg/ha/year).

Table 5. Economics of tilapia (*Oreochromis mossambicus*) farming in ponds under different treatments.

Items	Treatment		
	T ₁	T ₂	T ₃
Variable cost (BDT/ha)			
Seed (including transportation)	98800.00±91.92 ^a	98800.00±212.13 ^a	98800.00±141.42 ^a
Feed	507712.56±474.16 ^a	5488351.23±212.43 ^b	631950.30±742.10 ^c
Fixed cost (BDT/ha)			
Lime	10000.00±707.10 ^a	10000.00±282.84 ^a	10000.00±565.68 ^a
Fertilizer	5000.00±565.68 ^a	5000.00±573.58 ^a	5000.00±212.13 ^a
Labour	12000.00±1414.21 ^a	12000.00±707.10 ^a	12000.00±424.26 ^a
Sampling & harvesting	15000.00±707.10 ^a	15000.00±424.26 ^a	15000.00±282.84 ^a
Total cost (BDT/ha)	646548.54±3535.61 ^a	689151.49±3111.48 ^b	772750.38±4808.55 ^c
Total return (BDT/ha)	967440.25±1060.93 ^a	1173250.70±3677.09 ^b	1207049.54±1979.98 ^c
Net Profit (BDT/ha)	320892.91±2474.68 ^a	484099.20±565.60 ^c	434299.15±2828.57 ^b
CBR	0.49±0.00 ^a	0.71±0.00 ^c	0.56±0.00 ^b

Conclusion and recommendation

This experiment investigated that dietary protein level significantly influences water quality, fish growth, net profit and also greenhouse gas emission in pond raised with tilapia (*O. mossambicus*). Significantly ($p < 0.05$) highest net economic return and CBR were found with treatment T₂ (i.e., dietary protein level 30%) while lower dietary protein level T₁ (i.e., dietary protein level 27%) reduced greenhouse gas emission but compromised with growth and net returns. This study focused only on testing the emission of GHGs emission with dietary protein levels. Further research can be conducted with focus on feeding frequency, waste management and culture system towards the development of sustainable tilapia farming in farmer managed ponds.

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DECLARATIONS

Author contribution statement: Conceptualization: M. A. H. and M. M. A. S.; Methodology: N.K.; Software and validation: M. M. K and T.P.; Formal analysis and investigation: M. M. A. S. and N. K.; Resources: M.A.H.; Data curation: N. K. and M. M. K; Writing—original draft preparation: M. M. A. S; Writing—review and editing: M. M. A. S.; Visualization: T. P. and M. M. K.; Supervision: M. A. H; Project administration: M. A. H.; Funding acquisition: M. M. A. S. and M. A. H. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest: The authors certify that no conflicts of interest exist regarding the preparation and publication of this manuscript.

Ethics approval: This study was conducted in view of the institutional ethical guidelines and does not harm the human participants.

Consent for publication: All co-authors have reviewed the manuscript and agreed to its submission for publication in AAES.

Data availability: The data that support the findings of this study are available on request from the corresponding author.

Supplementary data: No supplementary data is available for the paper.

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Additional information: No additional information is available for this paper.

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