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Original Article

The effect of different dietary protein levels on the water quality and reproductive performance of Nile tilapia (*Oreochromis niloticus*) broodstock in biofloc system

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Abstract

This study investigated the effect of protein levels on the water quality and reproductive performance of Nile tilapia (*Oreochromis niloticus*) in the biofloc system. Three isocaloric diets with different protein levels (32%, 38%, and 44%) were formulated. A total of nine one-tone tanks with a water holding capacity of 0.9 tons were prepared and equipped with the necessary materials to form biofloc. Seventy-two female and thirty-six male fish were tagged, numbered, and randomly released into the tanks at a female: male ratio of 2:1. The results showed that the lowest amount of TAN (0.21 mgL^{-1}), NO_2, and NO_3 were observed in the treatment of 32% protein. Mean final weight in the 32% and 38% treatments was 350 and 356 g, respectively, with no significant difference. The highest absolute fecundity (654) was observed in the 32% group, but no significant difference was found between the absolute fecundity of the 38% and 44% groups. The longest interval (17.8 days) was observed in the 44% protein group. The percentage of fertilization and hatching did not significantly differ between the groups. The highest percentage of fertilization (93%) and hatching (89%) were observed in the 32% protein group. The total number of eggs per female fish did not differ between the 32% (12840) and 38% (12670) groups, while the 44% group had the lowest number of eggs (12040). The present study showed that a dietary protein level of 32% is optimal for water quality and reproductive performance of Nile tilapia in the biofloc system.

Keywords: biofloc technology; crude protein; fecundity; Nile tilapia; reproductive performance

1 | INTRODUCTION

Nutritional quality is crucial for broodstock fish, significantly influencing the quality and quantity of their offspring (Khanjani *et al.* 2024a). Research indicates that the diet of broodstock affects various reproductive parameters, including maturation, fecundity, and egg quality (Cardona *et al.* 2016; Khanjani *et al.* 2024a). For instance, diets rich in protein and essential fatty acids (EFAs) lead to higher egg production and better hatchability than low-protein or EFA-deficient diets (Watanabe *et al.* 1984). Moreover, the nutritional composition of broodstock diets can induce nutritional programing, which affects off-spring's growth and metabolic performance even into later life stages (Turkmen *et al.* 2017). Proteins are essential for vitellogenesis and the composition of yolk in oocytes, significantly impacting reproductive performance and off-spring quality in tilapia broodstock (Engdaw and Geremew 2024). The yolk, primarily composed of proteins, lipids, and other nutrients, is critical for the developing embryos, as it provides the necessary sustenance during early development (Engdaw and Geremew 2024). Previous studies have summarized the protein requirements for tilapia broodstock, emphasizing the need for a balanced diet rich in essential nutrients to optimize reproductive outcomes (Sousa *et al.* 2013; Santiago and Laron 2002).

Numerous studies have discovered that providing optimal protein to broodstock fish results in improved body weight, growth, and egg quality (Ribeiro *et al.* 2017). For instance, in tilapia broodstock, fish fed 40%, 35%, and 30% crude protein, maximum body size, and egg quality were observed in the 35% and 40% groups. There is a strong association between body size with prior maturation of gonads, as many investigations have reported on the earlier development of eggs in large-sized broodstock species (de Oliveira *et al.* 2014). Many authors elucidated that crude protein content of 30 – 40% is optimal for tilapia broodstocks' maximum growth and reproductive performance. For instance, Marty (2003) suggested 35% crude protein is sufficient for tilapia broodstocks, while Khattab *et al.* (2001) reported the crude protein in the range of 27 – 37% for maximum efficiency. In contrast, El-Sayed and Kawanna (2008) and de Oliveira *et al.* (2014) reported that increasing the crude protein level from 30% up to 40% would significantly improve tilapia's growth and reproductive performance. Numerous studies indicate that optimal protein levels in broodstock diets significantly enhance body weight, growth, and egg quality in fish. For instance, tilapia broodstock-fed diets containing 35% to 40% crude protein exhibited maximum body size and egg quality, while those on a 30% protein diet showed inferior results (El-Sayed *et al.* 2003; Ribeiro *et al.* 2017). A strong correlation exists between body size and gonadal maturation, with larger broodstock often developing eggs earlier (Ghaedi *et al.* 2019).

The major problem for tilapia seed production is the low egg production during spawning and lack of spawning synchrony (Bhujel 2000). To overcome this issue and besides management strategies, the choice of production system may improve reproductive performance. Ekasari *et al.* (2015) showed that the biofloc technology (BFT) improved tilapia's reproductive performance and increased the number of larvae produced. The BFT is an aquaculture system based on limited water exchange, where microbial conversion of nutrient waste into microbial biomass is maximized, and the resulting bioflocs can be utilized back by the fish as a food source (Avnimelech 2009; Khanjani *et al.* 2022). Additionally, the microbial protein generated within the floc can provide an adequate protein source to meet the nutritional requirements of broodstock (Gamboa-Delgado and Márquez-Reyes 2018). This dual role of the biofloc system acting as both a waste management structure and a source of nutrition highlights its importance in broodstock development (Khanjani *et al.* 2024a). Several studies investigated the BFT on fish and shrimp reproduction. For instance, the relative fecundity of African catfish broodstock in BFT was 26% higher than that of the control group (Ekasari *et al.* 2016). The shrimp *Farfantepenaeus duorarum*, raised in BFT, achieved better reproductive performance than the clear-water system (Emerenciano *et al.* 2014).

Water quality, growth performance, and reproductive performance of aquatic animal broodstock are affected by the rearing system (Khanjani *et al.* 2024a; Khanjani 2025). Despite the result of these studies, a more comprehensive view of the effect of dietary protein levels and BFT on the reproductive performance of tilapia has not been conducted to date. In the present study, the effect of different dietary protein levels on the water quality and reproductive performance of Nile tilapia (*Oreochromis niloticus*) broodstock was investigated.

2 | METHODOLOGY

2.1 Fish and tanks

This experiment was conducted over three months at the National Research Center for Inland Saline Aquatics, located in Yazd, Iran. The fish were manually sexed by examining the genital papillae, weighed individually, and tagged for individual recognition before being stocked. In the present experiment, three different protein levels in the Nile tilapia diet were investigated in three replicates in a biofloc system. Three isocaloric diets with different protein levels (32%, 38%, and 44%) were formulated. A total of 9 one-tone tanks with a water holding capacity of 0.9 tons were prepared and equipped with the necessary materials to form biofloc. Seventy-two female (221 ± 6 g) and 36 male fish (185 ± 5 g) were tagged, numbered, and randomly released into the tanks at a female: male ratio of 2:1. The culture system was provided with continuous aeration using a 3.5 HP air compressor, venturis, and heater with a thermostat to keep the water temperature at 28.0 – 29.0°C. Three isocaloric floating tilapia feed (3 mm) were formulated and produced in a local fish feed company, with 32%, 38%, and 44% crude protein levels, and randomly assigned to nine tanks with three replicates for each feed. The fish were fed at 2 – 3% body weight daily for 90 days. The ingredients used to prepare feed and their proximate compositions are given in Table 1. The feed chemical composition was determined by the standard method described by AOAC (2023).

TABLE 1 Raw materials and proximate analysis of experimental diets with varying protein levels.

Ingredients	Feeds (containing % peotein)		
	32	**38**	**44**
Oats	80	40	0
Corn meal	80	40	0
Wheat flour	232	192	152
Corn gluten	100	120	170
Soybean meal	345	375	396
Cow gelatin	8	8	8
Fish meal (60%)	100	170	220
Soybean oil	40	40	40
Premix complex	10	10	10
Additives complex	5	5	5
Proximate composition (% of DM)			
Moisture	9.8	10.2	9.7
Crude protein	31.89	38.3	43.9
Ether extract	6.8	7.1	6.9
Crude fiber	5.9	5.7	4.8
Ash	5.2	5.6	6.1
NFE	40.41	33.1	28.46
GE (Mj kg^{-1})	18.34	18.66	18.96
GP:GE ratio	17.4	20.5	23.15
Feed C:N ratio	7.89	5.40	4.05

NFE = nitrogen free extract, GE = gross energy.

2.2 Biofloc preparation

For the preparation of biofloc, the tanks were disinfected using hypochrin (20 ppm) and then washed. At the beginning of the experiment, a carbon-to-nitrogen ratio of 20 was provided, and over time, this ratio decreased to 15 and then 12 based on the water and fish conditions. To create the carbon to nitrogen (C/N) ratio, the amount of nitrogen present in the water and then the nitrogen and carbon present in the feed were calculated according to (Ghaedi *et al.* 2022). Then the following materials were added to each tank's water to get a C/N ratio of 20 and form the floc. Urea (44%) 24 g, NPK fertilizer 5 gr, 270 g fish feed with 35% crude protein, dolomite 56 g, molasses 900 g, probiotic 10 g, kaolin 37 g, clay 15 g, and wheat bran 100 g. At the beginning, 70% of these materials were added, and the remaining was added two days later. After a week, the water ammonia content was checked to readjust the C/N ratio. According to the data and over time, sufficient amounts of nitrogen and molasses as carbon sources were added to each tank to get C/N equal to 15. After three weeks, the floc volume reached 5-10 ml/L, and the fish were stocked. The biofloc system in each tank was well-managed and kept steady.

2.3 Water quality

Water quality parameters, including salinity, temperature, dissolved oxygen (DO), and pH, were measured daily before feeding practice using standard devices including a portable conductivity meter (Hach HQ30d, USA), waterproof pen digital thermometer (TP 300, China), oxygen meter (Hach HQ30d, USA), and pH meter (Hach HQ30d, USA). Total ammonia nitrogen (TAN), nitrite (NO_2-N), and nitrate (NO_3-N) were monitored weekly by using Spectrophotometer (Perklin Elmer Lambda 25, USA) according to the method described by APHA (2005). Biofloc volume (BV), total dissolved solids (TDS), and total suspended solids (TSS) were measured weekly according to the method described by Hargreaves (2013).

2.4 Fish reproductive performance

Nile tilapia females in each tank were checked weekly for eggs. Females carrying eggs in the buccal cavity were identified from the tag number, and the eggs were gently removed and counted and a 1 ml sample was preserved in 10% formalin solution for measuring egg diameter, while the rest were labeled and frozen for chemical analysis. Of the fixed samples, 100 eggs were weighed. The eggs from the females in each experimental tank were incubated in PVC hatcheries with a blind and rounded bottom with a net volume of 2.5 L. The reproductive parameters and offspring characteristics were as follows: average number of eggs per spawning: total number of eggs per tank/number of spawning; average number of spawning per female: total number of spawning / total female number; spawning interval: time elapsed from one spawning to the next of repeated spawning; average absolute fecundity: total number of eggs per tank / total female numbers; average relative fecundity: total number of eggs/gr in female numbers; total number of eggs per tank throughout the study; and the mean weight of the eggs.

At the end of the experimental period, the mean weight of the females, apparent feed conversion, specific growth rate, survival, feed efficiency ratio, and protein efficiency were calculated. Females in each experimental unit were anesthetized by immersion in 150 ppm clove oil solution. The fish were then weighed and dissected to measure the liver and gonads weight. Finally, the gonadosomatic (GSI) was calculated based on the method described by Ghaedi *et al.* (2013).

2.5 Data analysis

Statistical analysis was performed using a one-way analysis of variance (ANOVA) to assess differences among treatment groups, utilizing the JAMOVI statistical software. Post hoc comparisons were conducted using Tukey's Honest Significant Difference (HSD) test to identify specific differences between group means. The normality (Kolmogorov-Smirnov test was used) and homogeneity (Levene's test was used) of variances were verified before analysis to ensure the assumptions of ANOVA were met (n = 9). All results were reported with a significance level of $p < 0.05$, indicating statistically significant differences.

3 | RESULTS

3.1 Water quality

The data of water indices is shown in Table 2. The data of biofloc parameters showed that increasing the dietary protein level significantly affects the water quality parameters in the biofloc system so that total ammonium nitrogen (TAN), nitrite-N (NO_2), nitrate-N (NO_3), total dissolved solids (TDS), total suspended solids (TSS), and biofloc volume (BV) were considerably highest and lowest in the 44% and 32% groups, respectively.

The highest amount of TAN (0.49 mg L^{-1}), nitrite (0.31 mg L^{-1}), and nitrate (3.1 mg L^{-1}) was observed in the treatment with 44% protein, which showed a significant difference from other treatments ($p < 0.05$). Contrary to these indices, the 44% group dissolved oxygen was significantly lower than in other groups ($p < 0.05$). pH and alkalinity did not differ significantly between groups. The temperature was constant between 27 and 29°C throughout the experimental period for all treatments. The correlation data between different dietary protein levels and floc indices indicate a direct and strong effect of increasing dietary protein on NO_2, NO_3, TAN, TDS, TSS, and BV. These data show that increasing the dietary protein level leads to an increase in BV and ultimately, an increase in TAN in the experimental tanks (Figure 1).

TABLE 2 Physicochemical water quality indices in tanks fed diets with varying protein levels (values are expressed as mean ± SD; *n* = 13).

Parameters (%)	Dietary crude protein levels (%)		
	32	38	44
Temperature (°C)	28.5 ± 0.8^{a}	28.2 ± 0.5^{a}	28.6 ± 0.4^{a}
pH	6.84 ± 0.01^{a}	6.56 ± 0.01^{a}	6.95 ± 0.01^{a}
DO (mg L^{-1})	5.98 ± 0.6^{a}	5.11 ± 0.4^{b}	4.71 ± 0.5$^{b, c}$
Alkalinity (mg L^{-1})	110 ± 6^{a}	112 ± 5^{a}	109 ± 7^{a}
TAN (mg L^{-1})	0.21 ± 0.02^{c}	0.35 ± 0.08^{b}	0.49 ± 0.09^{a}
NO_2 (mg L^{-1})	0.21 ± 0.07^{c}	0.27 ± 0.03^{b}	0.31 ± 0.05^{a}
NO_3 (mg L^{-1})	2.3 ± 0.7^{c}	2.6 ± 0.5^{b}	3.1 ± 0.4^{a}
BV (ml L^{-1})	35 ± 7^{c}	38 ± 5^{b}	47 ± 3^{a}
TDS (mg L^{-1})	17.3 ± 0.5^{c}	19.7 ± 0.4^{b}	24.1 ± 0.7^{a}
TSS (mg L^{-1})	590 ± 12^{c}	610 ± 18^{b}	672 ± 15^{a}

Abbreviation: dissolved oxygen (DO), total ammonium nitrogen (TAN), nitrite-N (NO_2), nitrate-N (NO_3), biofloc volume (BV), total suspended solids (TSS), total dissolved solids (TDS); values in the same row with different letters are significantly different ($p < 0.05$).

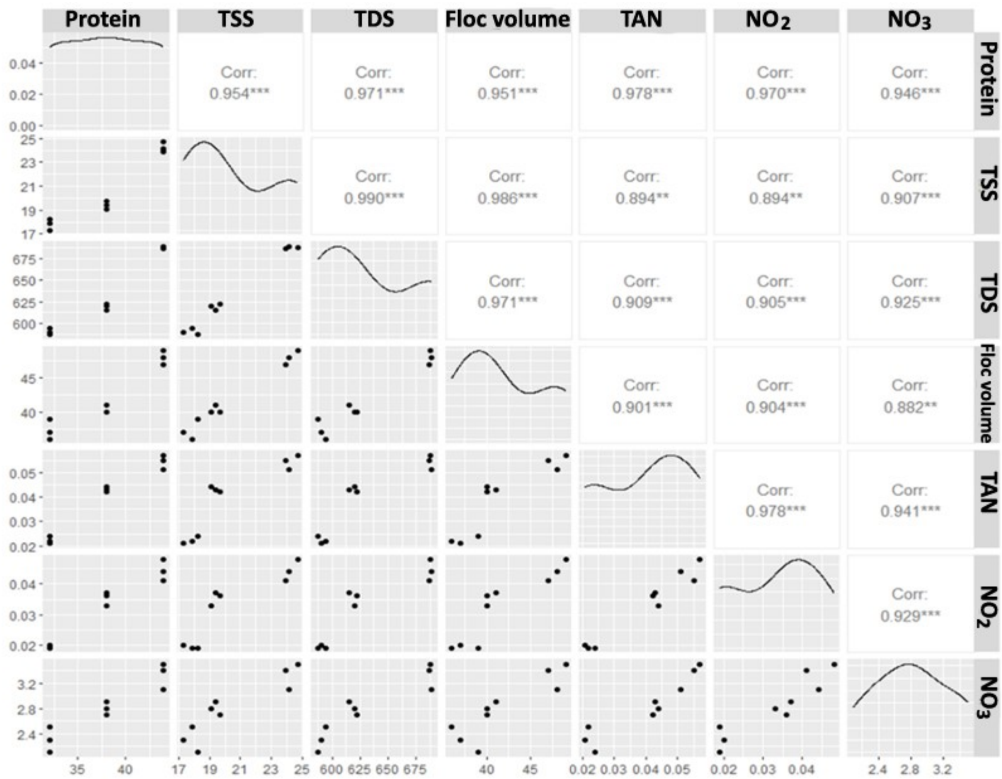

FIGURE 1 Correlations between dietary protein levels and biofloc indices.

3.2 Fish growth and reproductive performance

The protein levels in the diets influenced the final mean weight of the females (Table 3). By increasing the dietary protein level, the mean weight showed a decreasing trend, with the highest in the 38% group, followed by 32% and ultimately 44% group ($p < 0.05$). Reproductive performance indices of Nile tilapia broodstock are presented in Table 2. The data shows a direct and strong correlation between floc protein and egg diameter and hatch rate. In all reproductive indices, the effect of floc protein is direct and strong. The interesting thing is that the correlation of dietary protein with reproductive indicators in the biofloc system has been reversed so that with the increase in the level of dietary protein, the reproductive parameters have not improved, while the biofloc protein has been able to have a very positive, strong and direct effect on improving reproductive efficiency in biofloc system (Figure 2).

While the highest absolute fecundity (654) was observed in the 32% group (ANOVA: $p < 0.05$), there was no significant difference in this indicator between the 38% and 44% groups ($p < 0.05$). There was significant difference between the total egg weight and egg diameter among the groups, while the highest total egg weight (4.81 g) and lowest egg diameter (2.70 mm) were observed in the 32% group ($p < 0.05$) (Table 3). The spawning interval in the 44% group (17.8 days) was higher than others ($p < 0.05$). The lowest spawning interval was observed in the 32% group, followed by the 38%. Afterward, the highest time was recorded in the 44% group. The average number of eggs per spawning was the lowest in the 44% group (671) and showed a significant difference from other groups ($p < 0.05$). The total number of eggs per female fish in the 44% group was lowest ($p < 0.05$). The total number of eggs per female fish did not significantly differ between the 32% (12840) and 38% (12670) groups, while the 44% group had the lowest number of eggs (12040). The highest level of the GSI was observed in the 32% (4.42), and the lowest in the 44% group (3.22). The fertilization and hatching rate percentage did not vary across treatments. The relative fecundity (number of eggs per gram of female fish weight) was highest in the 32% and lowest in the 44% group ($p < 0.05$). The highest production volume was observed in the 44% group (5.72 ml), which showed a significant difference with the other two groups ($p < 0.05$) (Table 3).

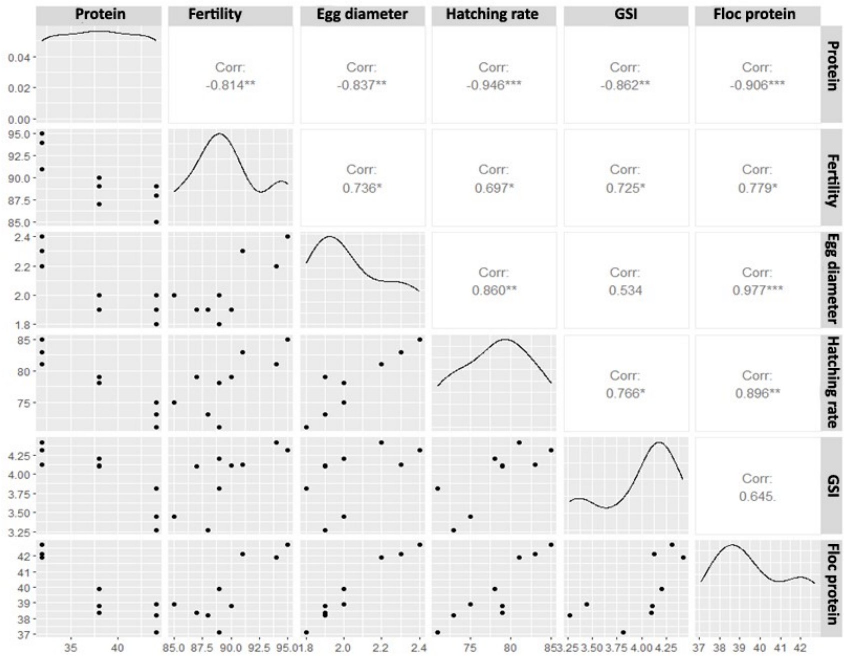

FIGURE 2 Correlation between dietary protein levels and reproductive indices.

Table 3: Reproductive performance indices in female Nile tilapia fed varying protein levels.

Parameters	Dietary crude protein levels (%)		
	32	38	44
Initial weight (g)	221.7 ± 5.8^{a}	222.2 ± 4.6^{a}	221 ± 5.1^{a}
Final weight (g)	350 ± 3.5^{a}	356 ± 2.1^{a}	345 ± 3.2^{b}
Total egg weight (g)	4.81 ± 0.42^{a}	4.43 ± 0.64^{b}	4.02 ± 0.81^{c}
Absolut fecundity	654 ± 85^{a}	612 ± 64^{b}	609 ± 91$^{b, c}$
Relative fecundity	6.05 ± 1.27^{a}	5.70 ± 1.19^{b}	4.51 ± 1.01^{c}
Egg diameter (mm)	2.70 ± 0.41^{c}	2.78 ± 0.71$^{a, b}$	2.80 ± 0.57^{a}
Spawning intervals (days)	14.7 ± 1.1^{c}	16.6 ± 1.2^{b}	17.8 ± 1.7^{a}
Egg per spawning	890 ± 47^{a}	773 ± 39^{a}	671 ± 54^{c}
Egg number per female	12840 ± 1250^{a}	12670 ± 1310^{a}	12040 ± 1250^{b}
Gonadosomatic index (GSI)	4.42 ± 0.8^{a}	4.2 ± 0.9^{b}	3.22 ± 0.9^{c}
Egg volume (ml)	5.38 ± 0.92$^{b, c}$	5.43 ± 0.65^{b}	5.72 ± 0.86^{a}
Fertilization rate (%)	93.0 ± 0.18^{a}	92.7 ± 0.15^{a}	92.1 ± 0.5^{a}
Hatching rate (%)	89.0 ± 3.1^{a}	88.1 ± 2.1^{a}	89.2 ± 3.6^{a}

3.3 Biofloc composition

Biofloc biochemical composition data are shown in Table 4. According to the results, no significant difference was observed in the biochemical compositions of biofloc (protein, lipid, ash, fiber, and NFE) ($p < 0.05$). Crude protein levels ranged from 37.93 – 39.11%, crude lipid from 3.99 – 4.12%, and ash from 12.91 – 13.8% of dry weight. The overall results of the present study showed that increasing the dietary protein level in Nile tilapia farming in biofloc system reduces water quality and reproductive performance, and has no significant effect on the biochemical composition of biofloc.

TABLE 4 Proximate analysis of floc derived from different tanks fed diets with varying protein levels.

Parameters (%)	Dietary crude protein levels (%)		
	32	38	44
Moisture	8.2 ± 1.1^{a}	8.5 ± 1.2^{a}	8.4 ± 1.3^{a}
Crude protein	37.93 ± 2.38^{a}	38.41 ± 3.62^{a}	39.11 ± 1.2^{a}
Crude lipid	4.12 ± 0.71^{a}	3.99 ± 0.98^{a}	4.12 ± 0.71^{a}
Ash	13.8 ± 0.56^{a}	12.91 ± 0.82^{a}	13.21 ± 0.47^{a}
Fiber	6.27 ± 0.54^{a}	5.72 ± 0.97^{a}	5.90 ± 0.98^{a}
NFE	27.68 ± 1.1^{a}	30.47 ± 1.5^{a}	29.26 ± 1.1^{a}

4 | DISCUSSION

4.1 Water quality

In this study, the water quality parameters were appropriate for Nile tilapia fish cultivation (Khanjani *et al.* 2024b). Some treatments showed significant differences in the DO, TAN, NO_2, NO_3, TSS, and BV parameters. In the present study, the highest amount of DO was observed in the treatment of 32% protein, probably due to the lower activity of bacteria and the lower volume of BV in this treatment. Dietary protein levels directly influence BV, as higher protein increases nitrogen loading in the water, requiring additional carbon sources for control. This promotes microbial biomass development, increasing BV. A protein level of 32% appears to maintain BV within an optimal range, supporting favorable physicochemical water conditions (Ochieng Ogello *et al.* 2014). In the present study, the lowest amount of TAN, NO_2, and NO_3 in water was observed in the treatment with 32% protein due to feeding with rations containing less protein. A lower protein diet, 32%, can enhance water quality by reducing nitrogenous substance release, allowing beneficial heterotrophic bacteria to thrive, which is crucial for maintaining a healthy aquaculture environment (Arshad *et al.* 2024).

Biofloc systems with optimal C/N ratios enhance microbial activity, reducing nitrogenous waste such as TAN, which is known to be toxic at elevated levels (Jamal *et al.* 2020). Improved water quality, characterized by lower TAN levels and balanced DO, supports better hormonal regulation and gamete development, enhancing reproductive outcomes (Ghimire *et al.* 2023). Cleaner water reduces physiological stress, directing energy toward processes critical for reproduction, such as gametogenesis, spawning, and larval survival. Moreover, optimal water conditions reduce the likelihood of reproductive disruptions caused by stress or exposure to toxic nitrogenous compounds (Johari *et al.* 2024). Research indicates that dietary protein levels significantly influence water quality in biofloc systems, affecting fish health and reproductive outcomes (Khanjani *et al.* 2024b). In the current study, A 32% crude protein diet optimizes nitrogen utilization, and better water conditions. This improvement in water quality positively impacts reproductive performance, contributing to a higher GSI (Binalshikh-Abubkr *et al.* 2021; Linh *et al.* 2024).

4.2 Fish growth and reproductive performance

In this study, higher protein diets (32% to 38%) generally resulted in increased final weights, but excessive protein (44%) led to lower weight gains due to increased nitrogenous waste, which can deteriorate water quality (Khalafalla *et al.* 2020). Diets with lower protein but higher carbon can promote biofloc formation, improving growth and water quality. This balance is essential for optimizing fish growth and aquaculture systems' sustainability (Soliman and Abdel-Tawwab 2022).

The total weight of eggs produced did significantly differ among groups, the 32% protein group showed higher egg weight. This may be attributed to a favorable C/N ratio, which supports better water quality and biofloc system performance, enhancing reproductive outcomes despite lower protein content (Freato *et al.* 2012). The reason for this may be the effect of high carbon in the diet on the positive performance of the biofloc system as they reduce nitrogenous waste and maintain water quality, which is crucial for tilapia broodstock maintenance (Fülber *et al.* 2010). Likewise, it is important to note that the optimal protein level in the diet for egg production may vary depending on factors such as the fish's age, size, and environmental conditions. Excessive protein levels can lead to increased nitrogen waste, which negatively affects water quality and may not be cost-effective for aquaculture operations (Teodósio *et al.* 2020). When formulating diets, balancing protein levels to enhance egg production and quality while minimizing environmental impacts is crucial. Studies suggest that diets with a protein content of around 30% can optimize growth and reproductive outcomes without excessive nitrogen waste (Lara-Flores *et al.* 2010). Tilapia are omnivorous and require a balanced protein diet to support growth and reproduction (de Alba *et al.* 2021). Research indicates that increasing dietary protein levels can enhance reproductive performance, with optimal levels often around 30-35% for maximum egg production and quality (Mabroke *et al.* 2013). Higher protein diets (up to 45%) can improve growth rates, but excessive protein may lead to increased nitrogen waste, negatively affecting water quality and overall fish health. This balance is crucial as poor water quality can impair reproductive success (Hafedh 1999). While higher protein levels can enhance reproductive outcomes, they may not always be cost-effective. Diets with lower protein levels (around 30%) can still support good reproductive performance while minimizing environmental impacts and costs (Teodósio *et al.* 2020).

Increasing dietary protein levels can significantly influence the reproductive performance of Nile tilapia. Studies indicate that protein levels around 35% to 40% enhance egg production and larval survival rates. For instance, a study found that a 35% protein diet improved reproductive metrics compared to lower levels, while a 40% protein diet also yielded positive results regarding egg maturation and survival rates (Siddiqui *et al.* 1998; Mabroke *et al.* 2013). However, excessively high protein levels, such as 50%, can lead to decreased egg production and quality, as research by de Oliveira *et al.* (2014) demonstrated. The optimal protein level may vary based on species, age, and environmental conditions (Engdaw and Geremew 2024). Thus, while moderate increases in protein can enhance reproductive outcomes, excessively high levels may be detrimental.

There was significant difference in absolute and relative fecundity between the groups. However, the group with 32% protein was higher than the others. This is directly related to the improvement in water quality due to the low protein content of the diet and the provision of a significant portion of nutrients by the floc. The relationship between dietary protein levels and reproductive performance in fish is complex. While some studies indicate that lower protein levels can lead to improved fecundity due to better water quality and nutrient availability from floc, others suggest that moderate protein levels enhance egg production and reproductive efficiency (Mechaly *et al.* 2024; Meurer *et al.* 2024). Research by Mansour *et al.* (2022) and Bombardelli *et al.* (2017) supports the idea that increased dietary protein correlates with higher egg production. However, Meurer *et al.* (2024) reported that reproductive efficiency may be greater in tilapia-fed low to moderate protein diets than those on high protein diets. This suggests that while protein is essential for reproduction, excessively high levels may not yield the best outcomes, and optimal protein levels can vary based on specific conditions and species.

Ekasari *et al.* (2015) observed that absolute fecundity in tilapia cultured within a biofloc practice was significantly higher than in conventional systems, highlighting the substantial influence of protein levels on reproductive performance. This study observed the highest egg production in the group receiving the diet containing 32% protein. Although previous studies have demonstrated a strong and direct relationship between dietary protein levels and egg production in fish, it appears that the protein available in the biofloc, which was consumed by the fish and met their protein requirements, played a significant role. A possible explanation for the reduced egg production in other groups is the high protein intake, which likely led to greater metabolic energy allocation for protein breakdown (Watanabe *et al.* 1984).

Spawning interval refers to the time between consecutive spawning events in fish. In this study, the group fed a 32% protein diet exhibited shorter spawning intervals, likely due to favorable water quality conditions. Diets with adequate protein levels improve overall fish health and energy availability, enabling more efficient allocation of resources to reproductive processes. However, when dietary protein exceeds optimal levels (e.g. 38% or 44%), the excess is metabolized for energy or excreted, increasing metabolic costs and potentially reducing the energy available for reproduction, thereby negating further improvements in spawning intervals (Siddiqui *et al.* 1998; Ali and Wootton 1999). Additionally, favorable water quality conditions are crucial for successful spawning. Poor water quality can constrain spawning activities and negatively impact fish broodstocks (Luczkovich *et al.* 2024). Therefore, maintaining optimal dietary protein levels and ensuring good water quality is essential for enhancing spawning frequency and intervals in tilapia under biofloc system operation.

The study found that tilapia fed a 32% crude protein diet exhibited the highest GSI, indicating enhanced reproductive investment. Higher GSI values suggest improved reproductive performance (Shourbela *et al.* 2021). The optimal dietary protein level for maximizing GSI and reproductive performance can vary based on tilapia species, age, size, and specific farming conditions (Green *et al.* 2024). It is suggested that future research should investigate the effects of different diet compositions (lipid, and ash), different carbon sources, and different carbon to nitrogen ratios in biofloc systems on the reproductive performance of Nile tilapia.

4.3 Biofloc composition

The composition of biofloc in aquaculture systems is indeed influenced by dietary protein levels. Research indicates that higher dietary protein can enhance the total organic matter, protein, carbohydrate, and lipid content of bioflocs while reducing ash content (El-Sayed 2021). However, the current study suggests that variations in floc protein composition across different treatments were not significant, indicating that system dynamics may play a more critical role than feed composition in determining nutrient levels in bioflocs (Xu and Pan 2014). Biofloc is composed of 12 to 50% protein, 0.5 to 41% lipids, 14 to 59% carbohydrates, and 3 to 61% ash in a dry weight basis in different culture conditions (El-Sayed 2021). Biofloc comprised 34.69% protein, 4.83% lipid, 8.22% fiber, and 19.65% ash in African catfish breeders rearing tanks (Ekasari *et al.* 2016).

Optimal dietary protein levels for maintaining biofloc quality can vary based on specific system conditions, such as the C/N ratio and carbon source type (Mansour *et al.*

2022). This highlights the complexity of biofloc systems, where dietary inputs and environmental factors interact to influence the overall composition and quality of the biofloc produced. Thus, while dietary protein is important, it is not the sole determinant of biofloc nutrient profiles. It is recommended to use biofloc meal in fish diets. Biofloc used as a nutritious feed source to replace protein sources (such as fish meal) and its function in the growth of farmed fish should be investigated. Using biofloc meal in the diet can help sustainable aquaculture (by reducing environmental impacts, and reducing fishing).

5 | CONCLUSIONS

In conclusion, this study has underscored that a dietary protein level of 32% is optimal for the reproductive performance of Nile tilapia in the biofloc system. This protein level resulted in the highest absolute fecundity, shortest spawning interval, and highest fertilization and hatching rates without compromising the final weight or gonadal development index. Increasing dietary protein to 38% or 44% did not provide additional benefits and, in some cases, negatively impacted reproductive parameters such as the spawning interval and the gonadal development index. The chemical composition of the biofloc remained consistent across treatments, suggesting that biofloc could be a stable supplementary nutritional resource. These results provide valuable insights for optimizing dietary protein levels to enhance tilapia reproduction in biofloc-based aquaculture systems.

It is suggested that future studies should examine the effects of different dietary components (lipid and ash), different carbon sources, and the carbon to nitrogen ratio in the biofloc system on the reproductive performance of Nile tilapia. In future research, biofloc meal could also be used as a dietary component or as a replacement for fish meal in Nile tilapia diets.

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ETHICAL APPROVAL

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed in this study.

CONFLICT OF INTEREST

The author declares no conflict of interest.

AUTHORS' CONTRIBUTION

Alireza Ghaedi: conceptualization, data curation, investigation, methodology, project administration, supervision, validation, writing - original draft, review & editing. Mohammad Hossein Khanjani: conceptualization, validation, visualization, writing - review & editing.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on a reasonable request from the corresponding author.

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