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Digestive Enzyme Activity as a Biological Indicator for Assessing the Sustainability of Biofloc and Non-Biofloc Systems in the Nile Tilapia (*Oreochromis niloticus*) Culture

Ayu Winna Ramadhani [1], Diana Aisyah [1]*, Lutfi Syahira[1], Trisnadia Kurniawati[1], Rahadian Tindra Putra[1], Abd. Rahem Faqih[2], Mohamad Fadjar[2], Supriyadi Supriyadi[3]
[1]Departement of PSDKU Aquaculture, Faculty of Fisheries and Marine Science, Brawijaya University
[2]Departement of Aquaculture, Faculty of Fisheries and Marine Science, Brawijaya University
[3]Departement of PSDKU Socio Economic, Faculty of Fisheries and Marine Science, Brawijaya University

Corresponding Author: dianaaisyah@ub.ac.id

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ABSTRACT
Digestive enzyme activity represents a valuable biological indicator for assessing the sustainability of aquaculture systems since it reflects nutrient utilization efficiency, metabolic adaptation, and the ecological performance of cultured species. This study compared the Nile tilapia (*Oreochromis niloticus*) reared in biofloc and conventional (non-biofloc) systems over 3–5 months by analyzing protease, cellulase, and lipase activities, growth performance, and water quality. Protease activity was significantly higher in biofloc fish at 3 months (1.688 ± 0.03 vs. 1.437 ± 0.10 U/mL/min, $P< 0.05$), while cellulase activity showed no statistical differences between systems. Lipase activity was consistently higher in non-biofloc fish (4.8–5.3 vs. 2.8–3.8 U/mL/min, $P< 0.05$), suggesting reduced reliance on endogenous lipid digestion under biofloc conditions due to microbial enzyme contributions. Growth performance revealed higher specific growth rates (SGR) in biofloc fish (2.06% vs. 1.83% per day at 3 months; 1.53% vs. 1.45% at 5 months), though both remained below the optimal range. Water quality parameters were within acceptable limits, with biofloc systems showing stable pH (6.1–8.0) and sufficient dissolved oxygen (5.1–6.9 ppm), while conventional systems maintained higher DO (8.0–10.7 ppm) but greater alkalinity fluctuations. Overall, the findings highlight that biofloc technology enhances early digestive efficiency, supports sustainable growth, and reduces environmental discharge through nutrient recycling, confirming its potential as a more eco-efficient system for the Nile tilapia culture.

INTRODUCTION

The Nile tilapia (*Oreochromis niloticus*) is one of the most widely cultivated freshwater fish species globally, valued for its adaptability to a broad range of aquatic environments including freshwater, brackish, and marine systems its rapid growth, and strong market demand, both domestically and internationally **(Amrullah *et al.*, 2018)**. To meet the growing demand, aquaculture practices have become increasingly intensive, often using conventional systems such as earthen or concrete ponds. These systems rely on the natural ecological balance of the pond environment and periodic water exchange to maintain water quality **(Wicaksana *et al.*, 2015)**. While widely practiced, such systems are often inefficient in nutrient recycling and may contribute to water pollution due to uneaten feed and fish waste. Tilapia culture methods are also faced with challenges, one of which is the problem of pollution due to the use of waste water containing ammonia and nitrogen from the remains of feed and fish feces **(Sunaryo & Kristianto, 2022; Aisyah *et al.*, 2025)**. Biofloc technology (BFT) has emerged as a sustainable aquaculture approach that addresses these limitations. By introducing organic carbon sources to the culture water, biofloc systems increase the carbon-to-nitrogen (C/N) ratio, stimulating the growth of heterotrophic bacteria that assimilate toxic inorganic nitrogen (e.g., ammonia) and convert it into microbial biomass **(Avnimelech, 2009; Sukardi *et al.*, 2018)**. These microbial aggregates—known as flocs—not only improve water quality but also serve as a supplementary protein source when consumed by the fish. The application of bioflocs also provides a source of single-cell protein to support fish growth **(Aisyah *et al.*, 2025)**.

Among the beneficial microorganisms in biofloc systems, *Bacillus subtilis* is commonly used for its dual role in improving water quality and acting as a probiotic that supports fish health and digestion **(Febriyanti *et al.*, 2018; Munaeni *et al.*, 2022)**. These microbial communities produce exoenzymes such as protease, amylase, cellulase, and lipase, which break down organic matter into simpler, absorbable nutrients **(Marlida, 2020)**. When ingested, these exoenzymes may complement the fish's endogenous digestive enzymes and enhance nutrient assimilation **(Zokaeifar *et al.*, 2012; Hoseinifar *et al*., 2015)**. The Nile tilapia is classified as an omnivore but tends to exhibit herbivorous feeding behavior, particularly in early life stages and in natural or semi-natural pond conditions. Its diet often includes algae, detritus, and plant matter, making carbohydrate- and fiber-degrading enzymes like amylase and cellulase especially important **(Gómez-Marquez *et al.*, 2003; Krogdahl *et al.*, 2005)**. Therefore, digestive efficiency in tilapia is strongly associated with enzyme activity, which can serve as a biological indicator of the fish's ability to utilize available nutrients effectively. Digestive enzymes serve as significant biological indicators in fish, providing insights into their physiological and nutritional states. These enzymes, which include proteases, lipases, and amylases, are produced in the exocrine pancreas and play crucial roles in the hydrolysis of consumed macronutrients, allowing fish to absorb the nutrients essential for growth and reproduction **(Kalhoro *et al*., 2018; Cao *et al.*, 2024)**. The relationship between digestive enzyme activity and fish growth performance has been extensively studied. For instance, changes in enzyme activity are associated with variations in diet composition and nutritional intake, highlighting the enzymes' role in nutrient utilization **(Lazzari *et al.*, 2010; Özel *et al.*, 2022)**.

Manipulating the C/N ratio in biofloc systems has been shown to enhance microbial community structure, nitrogen removal efficiency, and protein recycling **(Hargreaves, 2013; Emerenciano *et al.*, 2017)**. This microbial activity can directly influence fish digestion and growth by improving feed conversion and reducing environmental load. Measuring digestive enzyme activity, therefore, provides insight into how aquaculture systems impact nutrient utilization and fish health. This study aimed to compare the biofloc and conventional aquaculture systems in the Nile tilapia culture by analyzing digestive enzyme activity, growth performance, and water quality. The findings will contribute to the understanding of sustainable aquaculture practices that enhance biological efficiency while reducing environmental impact.

MATERIALS AND METHODS

Research objects and experimental site

This study employed a stratified random sampling design. Stratified random sampling involves dividing the population into homogeneous groups, where each group consists of subjects with similar characteristics. Samples were then randomly selected from each group. The research was conducted to evaluate the efficiency of conventional and biofloc aquaculture systems across different age groups of the Nile tilapia (*Oreochromis niloticus*)—specifically at 3 and 4 months old: approximately 15– 20cm in length and 100–150 grams in weight; 5 months old: approximately 20– 25cm in length and 200–250 grams in weight. The experimental sites included Khawi Fish Farm and the Sumberpasir Laboratory of Universitas Brawijaya, Malang, Indonesia. The Nile tilapia cultured using the biofloc system were sourced from Khawi Fish Farm, where they were reared in 4-meter diameter HDPE round tanks equipped with bottom aeration at a stocking density of 100 fish/m³. Meanwhile, fish raised under the conventional (non-biofloc) system were obtained from the Sumberpasir Laboratory, where they were maintained in 2.5-meter diameter semi-concrete tanks with aeration and continuous water flow at a stocking density of 75 fish/m³. These two systems provided comparative samples to assess the impact of biofloc versus conventional culture methods on fish performance and digestive enzyme activity at different growth stages.

Protease enzyme activity

Protease enzyme activity was analyzed following the method described by **Bergmeyer and Grassi (1983)**. In this study, casein was used as the substrate, while tyrosine served as the standard. The analysis measured the enzyme's ability to hydrolyze protein, resulting in the release of tyrosine. The absorbance was measured using a spectrophotometer at a wavelength of 550nm. The enzyme activity was calculated using the following formula.

Enzyme activity (u/mL) = (Act-abl/ ast- abl)* P/T

Description:
U = Unit of protease enzyme activity
Act = Absorbance value of the sample
Abl = Absorbance value of the blank
Ast = Absorbance value of the standard
P = Dilution factor
T = Incubation time (minutes)

Cellulase enzyme activity

The cellulase enzyme activity assay began with the collection of samples from the fish digestive tract, which were then homogenized in a buffer solution. Carboxymethyl cellulose (CMC) was added as a substrate to the sample to measure cellulase activity, and the mixture was incubated at a specific temperature in a water bath. After incubation, the reaction was stopped, and the amount of glucose produced was measured using a spectrophotometer at a wavelength of 540nm. The absorbance values were then applied to a standard curve equation, and the final enzyme activity was calculated using the following formula **(Fitriliyani, 2018)**.

Enzyme activity (u/mL) = Reducing sugar concentration/(reaction time*enzyme volume)

Lipase enzyme activity

Lipase enzyme activity was measured using the titrimetric method. The procedure involved adding 2mL of olive oil, 1mL of phosphate buffer (pH 7), and 1mL of enzyme extract. The mixture was incubated at 35°C for 30 minutes. After incubation, 1mL of acetone and 1mL of ethanol were added, followed by 5 drops of phenolphthalein indicator. The solution was then titrated with 0.05 N NaOH until a pink color appeared. For the blank, ethanol and acetone were added first, followed by incubation.

Specific growth rate (SGR)

Specific growth rate (SGR) refers to the change in individual body weight over time. The SGR value is obtained through sampling. Specific growth rate can be calculated using the formula provided by **Ihsan *et al*. (2021)**:

$$\mathbf{SGR = (LnWt - LnWo/t) * 100\%}$$

Description:

Wt = Final weight at the end of the rearing period (grams)

Wo = Initial weight at the beginning of the rearing period (grams)

t = Duration of the rearing period (days)

Water quality

Water quality was assessed using several parameters, including pH (acidity), dissolved oxygen (DO), and temperature. A pH meter was used to measure acidity, a DO meter to measure dissolved oxygen levels, and a thermometer to measure temperature. These parameters were recorded regularly throughout the duration of the study.

Statistical analysis

The analysis was conducted after data collection to examine differences between groups using an unpaired t-test, which specifically compares two distinct groups—in this case, fish reared in biofloc and non-biofloc systems at different ages. Prior to performing the unpaired t-test, data were subjected to normality and homogeneity tests to ensure the assumptions of the t-test were met. A *P*-value less than 0.05 ($P< 0.05$) from the t-test indicates a statistically significant difference in digestive enzyme activity between the fish cultured in biofloc and non-biofloc systems at a specific age group. Conversely, a p-value greater than 0.05 ($P > 0.05$) suggests that the difference between the two groups is not statistically significant.

RESULTS

Protease enzyme activity

The protease activity in the digestive tract of the Nile tilapia (*Oreochromis niloticus*) showed consistently higher mean values in fish reared under biofloc systems compared to those under conventional (non-biofloc) systems across all age groups, as shown in Fig. (2). At 3 months, the protease activity in biofloc fish was significantly higher (1.688 ± 0.03 u/mL/min) than in non-biofloc fish (1.437 ± 0.10 u/mL/min) with a *P*-value of 0.019, indicating a significant ($P< 0.05$) difference. At 4 months, the values were 2.513 ± 0.47 (biofloc) and 2.157 ± 0.07 (non-biofloc), but the difference was not statistically significant ($P= 0.264 > 0.05$). Similarly, at 5 months, the values were 1.407 ± 0.302 (biofloc) and 1.171 ± 0.697 (non-biofloc), with no significant difference observed ($P= 0.619 > 0.05$).

The larger fish (5 months) exhibits more developed body size compared to the smaller fish (3 months). These differences reflect distinct growth stages that influence digestive enzyme activity. Older fish typically have more mature digestive systems, resulting in higher or more efficient enzyme production, especially protease and lipase. In contrast, younger fish rely more on external sources or have lower endogenous enzyme activity, affecting nutrient absorption and growth performance.

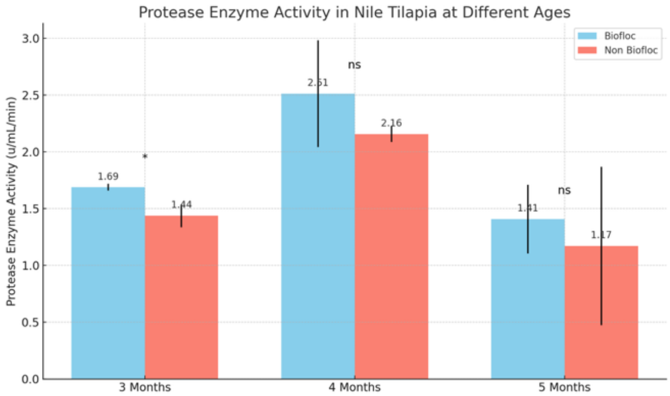

Fig. 1. Protease enzyme activity in the Nile tilapia at ages 3, 4, and 5 months under biofloc and non-biofloc systems

Cellulase enzyme activity

Cellulase activity in the Nile tilapia (*Oreochromis niloticus*) showed variable trends across different age groups and culture systems. At 3 months of age, fish reared in the biofloc system exhibited cellulase activity ranging from 0.055 to 0.083 µ/mL/min, whereas those in the non-biofloc system ranged from 0.049 to 0.064 µ/mL/min. Although the average activity was higher in the biofloc group, statistical analysis revealed no significant difference between the two systems (P = 0.346 > 0.05). A similar pattern was observed at 4 months, where the biofloc system showed slightly higher cellulase activity (0.053–0.060 µ/mL/min) compared to the non-biofloc system (0.048–0.054 µ/mL/min), again with no significant difference. Interestingly, by the 5th month, the non-biofloc system showed marginally higher activity (0.052–0.058 µ/mL/min) than the biofloc group (0.051–0.056 µ/mL/min), but the difference remained statistically insignificant, as shown in Fig. (2).

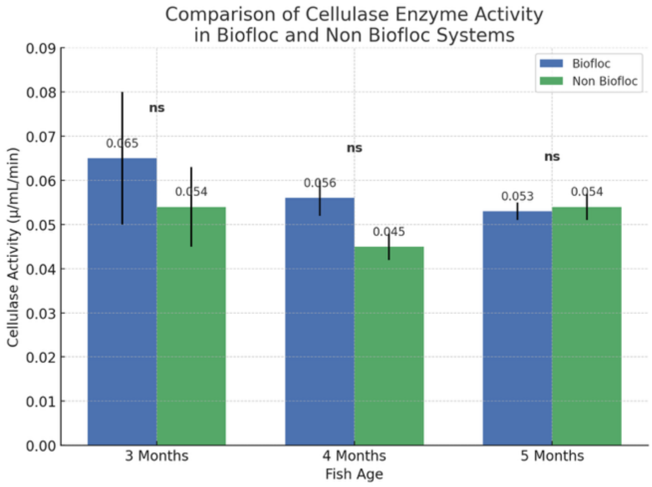

Fig. 2. Cellulose enzyme activity in the Nile tilapia at ages 3, 4, and 5 months under biofloc and non-biofloc systems

Lipase enzyme activity

The lipase enzyme activity in the Nile tilapia (*Oreochromis niloticus*) showed significant differences between biofloc and non-biofloc (conventional) systems across all age groups. At 3 months of age, fish raised in biofloc systems exhibited lipase activity ranging from 2.883 to 3.833 U/mL/min, whereas fish in non-biofloc systems had higher activity ranging from 4.833 to 5.333 U/mL/min. This difference was statistically significant (P = 0.010 < 0.05). Similar trends were observed at 4 and 5 months of age, where lipase activity remained consistently higher in the non-biofloc systems, with significant differences (P= 0.006 and P = 0.015, respectively), as shown in Fig. (3).

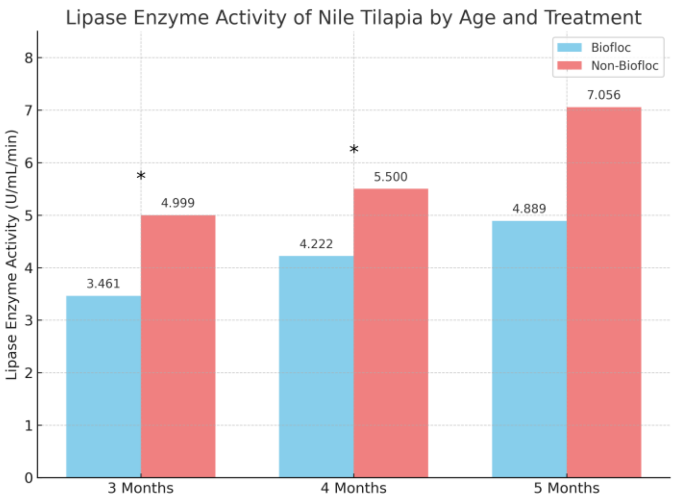

Fig. 3. Lipase enzyme activity in the Nile tilapia at ages 3, 4, and 5 months under biofloc and non-biofloc systems

Specific growth rate (SGR)

Sampling was carried out to measure the specific growth rate (SGR) of the Nile tilapia (*Oreochromis niloticus*) reared under biofloc and non-biofloc systems. Fish were sampled at 3, 4, and 5 months of age using standardized procedures to ensure consistency and reliability in growth data collection. The SGR was calculated based on changes in body weight over time, which reflects the efficiency of nutrient assimilation and overall health status of fish under different environmental conditions. A clear distinction between the two culture systems was observed in terms of water color and turbidity, which visually indicates the differing microbial and organic content. The biofloc system exhibited turbid, brownish water due to the presence of suspended microbial aggregates (flocs), composed of bacteria, organic matter, and residual feed (Fig. 4a). This environment fosters continuous nutrient recycling and microbial activity that can influence fish metabolism and growth performance.

a b

Fig. 4. The distinction between the two culture systems, in terms of water color and turbidity: **a.** Biofloc and **b.** Non-biofloc

Conversely, the non-biofloc system displayed clearer or green-tinted water, commonly associated with phytoplankton-dominated environments (Fig. 4b). This system lacks additional microbial inoculants and carbon sources, resulting in minimal floc formation. These differences in water quality parameters are critical in interpreting the variations in SGR observed between the two systems, providing insight into the ecological and physiological impacts of biofloc technology on fish growth dynamics. The specific growth rate (SGR) comparison reveals that the Nile tilapia grown in biofloc systems start with a higher SGR (2.06% per day at 3 months) compared to non-biofloc (1.83%). Over time, the SGR declines in both groups, but remains consistently higher in biofloc fish (1.53% at 5 months) versus non-biofloc (1.45%). Despite this advantage, the SGR values in both conditions fall below the optimal range (2.3–4.4%), as shown in Fig. (5).

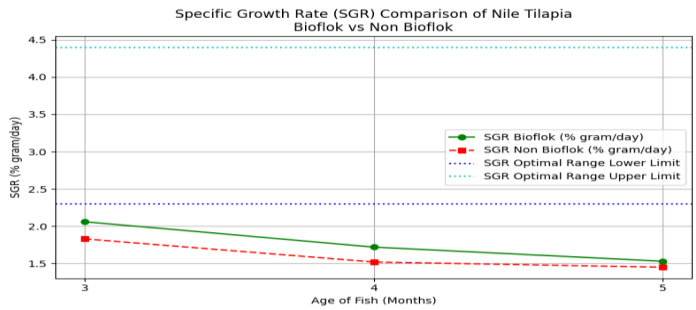

Fig. 5. Specific growth rate (SGR) of the Nile tilapia under biofloc and non-biofloc conditions for ages 3, 4, and 5 m

Water quality

The temperature ranges in both biofloc (~26.2– 29.9°C) and non-biofloc (~29.1–31.7°C) ponds fall within the SNI (Indonesian National Standard) recommended range of 25– 32°C. Optimal temperature supports metabolic and growth processes in the Nile tilapia **(El-Sayed, 2006)**. pH in biofloc ponds (6.1–8.0) remains within the recommended range (6.5–8.5), while non-biofloc ponds exhibited slightly higher pH (8.1–9.4) especially at 4 months, which may indicate alkalinity fluctuations. DO concentrations in biofloc systems (5.11–6.92 ppm) generally meet or exceed the SNI standard minimum of 3 ppm, supporting aerobic respiration. DO levels in non bioflok systems were notably higher (8.03–10.79 ppm), likely due to lesser microbial oxygen demand (Fig. 6).

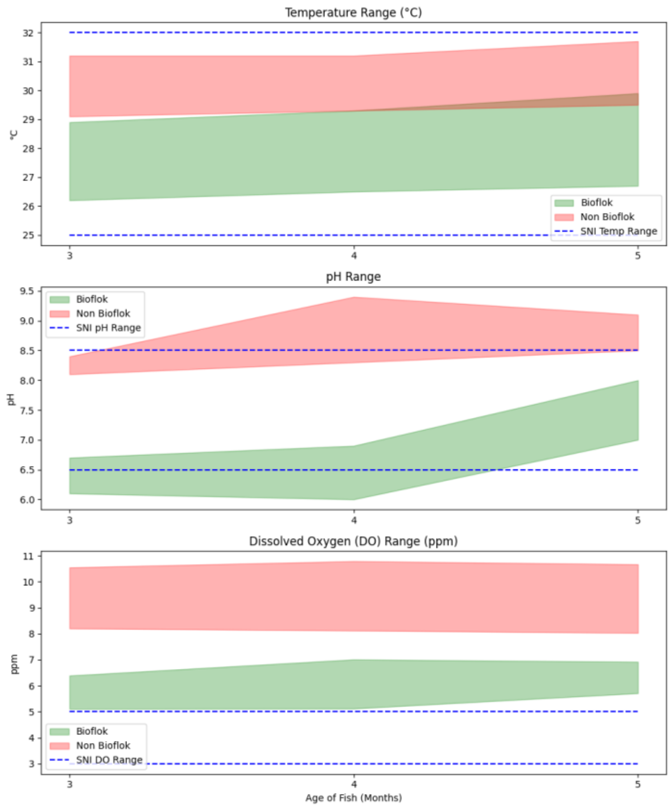

Fig. 6. The ranges of key water quality parameters across 3, 4, and 5 months for both biofloc and non-biofloc ponds

DISCUSSION

Juvenile tilapia experience rapid digestive development in early growth stages. Studies on hybrid juvenile tilapia revealed that protease, amylase, and lipase activities increase quickly as fish grow from 55 to 225g, then stabilize with age **(Jun-sheng *et al*., 2006)**. Thus, the significant difference at 3 months aligns with dynamic enzyme ontogeny, a growth phase marked by heightened digestive capacity. By 4 and 5 months, tilapia reach digestive maturity, and enzyme activity naturally plateaus. As such, the biofloc and non-biofloc groups converge, producing non-significant differences. Importantly, protease activity recorded in both systems remained above physiological thresholds necessary for effective digestion, ensuring sustained growth and health. Only at 3 months did biofloc-raised tilapia exhibit significantly higher protease activity, coinciding with a phase of increased digestive development. As fish matured, both culture systems supported enzyme activity within optimal ranges, leading to similar performance after early growth. The transient enzyme boost in biofloc systems underscores its capacity to optimize early growth while enhancing nutrient recycling and environmental sustainability confirming it's potential as a viable and eco-friendly approach to tilapia farming.

The results of this study indicate that there were no significant differences in cellulase enzyme activity between the Nile tilapia reared in biofloc and conventional (non-biofloc) systems across all age groups (3, 4, and 5 months). Although the biofloc system occasionally demonstrated slightly higher enzymatic activity, particularly at earlier stages, both systems maintained cellulase levels above the minimum functional threshold required for efficient digestion in the Nile tilapia. The comparable performance suggests that tilapia, as herbivorous-leaning omnivores, can utilize dietary fiber effectively regardless of the rearing system, thanks in part to microbial contributions from both biofloc and natural microbial communities in conventional systems **(Gaye-Siesseger *et al*., 2004; Sánchez-Moyano *et al*., 2010)**. From an environmental perspective, the biofloc system offers distinct advantages. While both systems support adequate enzyme activity and fish growth, biofloc systems promote nutrient recycling and reduce organic waste discharge by converting nitrogenous compounds into microbial biomass. This contributes to lower water exchange requirements and a reduced ecological footprint **(Crab *et al*., 2012)**. Moreover, the integration of microbial probiotics in the biofloc system enhances environmental resilience and supports a more sustainable aquaculture practice **(Dawood *et al*., 2020)**. Therefore, even though cellulase activity does not significantly differ between systems, the environmental benefits of biofloc culture make it a favorable option for sustainable tilapia farming.

The lipase enzyme activity in the Nile tilapia (*Oreochromis niloticus*) showed significant differences between biofloc and non-biofloc (conventional) systems across all age groups. The higher lipase activity in conventional systems can be attributed to the lack of microbial supplementation in the water, requiring fish to produce more endogenous enzymes to digest lipids in their feed. In contrast, biofloc systems are rich in microbial communities such as *Bacillus subtilis*, which produce extracellular lipases and contribute to the partial digestion of organic matter. These microbial enzymes reduce the burden on the fish's digestive system, aiding in the lower measured lipase activity in the biofloc group **(Crab *et al*., 2012; Xu & Pan, 2012; Long *et al*., 2015)**. Additionally, biofloc biomass may serve as an alternative nutrient source, particularly for omnivorous and herbivorous fish like the Nile tilapia, altering digestive demands **(Lara *et al*., 2017)**. Although lower endogenous lipase activity is recorded in biofloc systems, growth performance and health are not compromised, suggesting efficient nutrient assimilation supported by the biofloc microbial loop. From an environmental perspective, biofloc systems offer advantages such as reduced water use, better waste recycling, and improved sustainability **(Crab *et al.*, 2012; Emerenciano *et al.*, 2013)**. These findings suggest that biofloc technology can support sustainable aquaculture practices while maintaining optimal digestive efficiency in the Nile tilapia culture.

The observed decline of SGR over time in both treatments echoes the general growth patterns reported for the Nile tilapia in aquaculture **(El-Sayed, 2006)**. As fish age, growth in proportional terms slows even as absolute weight increases, reflecting physiological maturity and energy partitioning shifting from growth to maintenance and reproduction. The temperature ranges in both biolfoc and non-biofloc ponds fall within the SNI (Indonesian National Standard) recommended range of 25– 32°C. Biofloc systems tend to maintain slightly cooler water, the presence of biofloc particles increases the water's turbidity. Turbid water can absorb and retain less solar radiation at surface compared to clearer water, potentially reducing warming by sunlight. pH in biofloc ponds remains within the recommended range (6.5–8.5), while non-biofloc ponds exhibited slightly higher pH. Consistent pH regulation in bioflok systems has been noted to improve nutrient cycling and reduce stress in fish **(Tacon *et al.*, 2002)**. DO concentrations in bioflok systems generally meet or exceed the SNI standard minimum of 3 ppm, supporting aerobic respiration. DO levels in non bioflok systems were notably higher, likely due to lesser microbial oxygen demand. However, bioflok's stable moderate DO is consistent with previous observations of balanced oxygen consumption and microbial biomass efficiency **(Avnimelech, 2015)**.

CONCLUSION

This study demonstrated that digestive enzyme activity can serve as a biological indicator of sustainability in the Nile tilapia aquaculture systems. Biofloc culture enhanced early protease activity and supported comparable growth and health outcomes while reducing fish reliance on endogenous lipase, thanks to microbial contributions. Although both biofloc and conventional systems maintained adequate water quality and growth, biofloc offered greater environmental benefits through nutrient recycling and reduced waste discharge. The novelty of this work lies in linking digestive enzyme dynamics to sustainability outcomes, highlighting biofloc as an eco-efficient alternative. The results highlight both enzymatic adaptations and the potential of biofloc technology to support sustainable aquaculture.

Protease activity was consistently higher in the biofloc system across all age groups, with a significant difference observed only at 3 months ($P< 0.05$). This suggests that microbial activity and organic matter in the biofloc system stimulate early protease production, but enzyme levels tend to stabilize in older fish regardless of the rearing environment. These results confirm that biofloc systems provide sufficient support for protein digestion.

Cellulase activity showed slight elevation in the biofloc group at 3 and 4 months, though the differences were not statistically significant ($P > 0.05$). The minimal cellulase activity in both systems is consistent with the omnivorous diet of the Nile tilapia, and the presence of microbial consortia in the biofloc system may provide auxiliary enzymatic assistance without substantially altering endogenous production.

In contrast, lipase activity was significantly higher in the conventional system across all age groups ($P < 0.05$). The higher lipase activity in conventional systems can be attributed to the lack of microbial supplementation in the water, requiring fish to produce more endogenous enzymes to digest lipids in their feed. In contrast, biofloc systems are rich in microbial communities such as *Bacillus subtilis*, which produce extracellular lipases and contribute to the partial digestion of organic matter. These microbial enzymes reduce the burden on the fish's digestive system, leading to lower measured lipase activity in the biofloc group.

Despite these differences, both systems supported healthy growth and metabolic function in the Nile tilapia. The microbial communities inherent in biofloc not only enhance protein digestion but also reduce the fish's reliance on endogenous lipid-degrading enzymes by providing extracellular enzymes and alternative nutrient sources. In contrast, fish in conventional systems may compensate for the lack of microbial support by increasing internal enzyme production.

From an environmental and sustainability perspective, biofloc systems offer significant advantages, including reduced water usage, improved nutrient recycling, and lower environmental discharge. Therefore, while both systems are viable for the Nile tilapia culture, the biofloc system presents a more sustainable and efficient option, aligning well with modern aquaculture's ecological and production goals. Biofloc technology should be prioritized in the Nile tilapia farming, especially during early growth phases, to improve digestive efficiency, optimize nutrient utilization, and minimize environmental impacts.

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