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EFFECT OF DIFFERENT STOCKING DENSITIES IN BIOFLOC-BASED CATFISH (*Clarias batrachus*) CULTIVATION ON WATER QUALITY AND PRODUCTION PERFORMANCE

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ABSTRACT

Biofloc Technology (BFT) is an innovation in fish farming aimed at improving feed efficiency and water quality by assisting heterotrophic bacteria. This study aims to improve water quality (ammonia, nitrite, nitrate, sulfate, phosphate) and fish production performance (average daily growth/ADG, average body weight/ABW, production, specific growth rate/SGR, feed conversion ratio/FCR, survival rate/SR). Catfish with an initial weight of 18.7±0.1 g and length of 11.48±0.02 cm were reared for 40 days with feed amounting to 2-4% of body weight. Parameters were measured every 10 days and analyzed using a Completely Randomized Design (CRD) with 4 treatments (A: without biofloc; B, C, D: using biofloc with densities of 100, 125, and 175 fish/m³). The results showed that biofloc improved water quality (ammonia 0.2165-0.5322 mg/L, nitrite 0.0044-0.0616 mg/L, nitrate 0.2863-19.6422 mg/L, phosphate 0.0169-0.8865 mg/L, and sulfate 2.6097-34.0627 mg/L), and enhanced fish production performance (ADG 1.625-3.85 g/fish/day, ABW 65-154 g/fish, production 5.251-16.176 kg, SGR 3.633-4.642%, FCR 0.849-1.007, and SR 65-89%). Biofloc technology effectively supports catfish survival up to a density of 175 fish/m³, demonstrating that BFT is a cost-effective, sustainable, and environmentally friendly aquaculture technology.

Keywords: Aquaculture Technology, Cost-Effective, Environmentally Friendly, Feed Efficiency, Heterotrophic Bacteria, Sustainable.

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INTRODUCTION

Catfish (*Clarias batrachus*) is Indonesia's second largest freshwater fish commodity after tilapia.[1] The farming of this fish is proliferating due to its ease of cultivation in areas with limited space and water, as well as its vast consumer market. However, technological innovations such as intensive farming with increased stocking density are necessary to meet production targets.[2] Increased stocking density can lead to the accumulation of feed and toxic waste materials for the fish, resulting in stress and disease susceptibility. Biofloc technology allows higher stocking densities without compromising water quality and fish health, enhancing productivity and production efficiency.[3] Biofloc is a fish farming technology that utilizes heterotrophic bacteria to convert organic waste into microbial biomass that the fish can consume. This technology aims to improve fish production efficiency through several fundamental mechanisms, one of which is increasing fish stocking density.[4] The following are some connections between biofloc technology and fish stocking density: (1) Biofloc allows for increased stocking density because this system better controls water quality. The bacteria in biofloc decompose organic waste, reducing the accumulation of harmful ammonia and nitrite; (2) Fish obtain nutrition not only from the provided feed but also from biofloc, which is rich in protein and other nutrients, improving feed conversion and efficiency; (3) Biofloc effectively reduces the concentration of toxic nitrogen compounds such as ammonia and nitrite, enabling higher stocking densities with stable and healthy water conditions; (4) By reducing organic waste and improving water quality, biofloc reduces the risk of disease and stress in fish, creating a more balanced and healthy microbiological environment; (5) Biofloc reduces waste in fish farming by converting organic waste into floc that can be reused as feed, reducing water needs and environmental impact.[5,6] Based on the above, it is necessary to develop biofloc-based catfish farming with high stocking density, the addition of probiotics in feed and water, and without water replacement. Probiotics are expected to function as single-cell protein production machines by utilizing ammonia, which harms fish.[7,8] This single-cell protein is expected to be harvested by the fish, increasing feed efficiency and improving water quality. Research is needed to test the application of biofloc technology in catfish farming with different stocking densities and its impact on water quality and production performance.

EXPERIMENTAL

Material and Methods

The equipment used in the cultivation of *C. batrachus* seedlings includes: (1) fiber tanks measuring 1x1x1 m^3, (2) Resun LP 100 aerators, (3) hoses, (4) air stones, and (5) nets. For observation and data measurement, the equipment includes (1) trays, (2) digital scales, (3) digital cameras, (4) writing tools, (5) tools for checking biofloc density such as Imhoff cones, and (6) water quality measurement tools such as pH meters, water quality checkers, measuring cups, bottles of various sizes, filter paper, drop pipettes, test tubes, hotplates, ovens, Spectrophotometers UV-Vis, and analytical balances (Shimadzu). The materials used in this study include: (1) 1500 *C. batrachus* seedlings with an average weight of 18.7±0.1 g and length of 11.48±0.02 cm, (2) feed with a protein content of 31–33%, minimum fat of 3–5%, and maximum fiber of 4–6%, given at 2-3% of body weight, (3) *Biolakto lactobacillus* probiotics, (4) preparation media with a dose of *Biolakto lactobacillus* probiotics at 10 g/m^3, dolomite lime at 50 g/m^3, non-iodized salt at 1 kg/m^3, and molasses at 100 mL/m^3, and (5) chemicals for water quality and metal parameter analysis.

General Procedure

Preparing the media for biofloc probiotics involves several essential steps to ensure optimal conditions for the growth and development of the probiotic bacteria used in the biofloc system. The selected probiotic bacteria for this process are *Biolakto lactobacillus*, and the cultivated fish species is *Clarias batrachus*. The pond water is sourced from filtered well water, which is aerated to maintain good water quality. Aeration is critical in sustaining a high dissolved oxygen level in the pond. In a biofloc system, intensive turbulent mixing is necessary to prevent solid particle sedimentation and maintain oxygen levels, with up to 6 mg/L of oxygen required for optimal conditions. The aerator layout should be strategically planned and regularly adjusted to avoid solid particle build-up in areas with little or no current. To apply biofloc in the pond, non-iodized salt is dissolved at 1 kg/m^3, and dolomite lime at 50 g/m^3 is added to the pond water. Once the water clears, molasses is added at 100 mL/m^3, followed by probiotics at 10 g/m^3, and the mixture is allowed to sit for 6-8 days. Throughout this period, monitoring the pond water quality regularly is important, paying particular attention to parameters such as ammonia, nitrite, nitrate, pH, and dissolved oxygen. When stocking the pond, quarantined *C. batrachus* seedlings are introduced based on specific treatment plans: Treatment A (100 fish/m^3, without biofloc), Treatment B (100 fish/m^3, with biofloc), Treatment C (125 fish/m^3, with biofloc) and Treatment D (175 fish/m^3, with biofloc). Feeding should be adjusted to meet the needs of the fish and biofloc development, with care taken to avoid overfeeding, which could lead to water quality deterioration. To measure biofloc density, 1000 mL of pond water is collected and placed in an Imhoff cone. After allowing the water to settle for 20 minutes, the floc sediment at the bottom of the cone is measured. Biofloc density is then determined in mL/L based on the volume of the floc sediment.

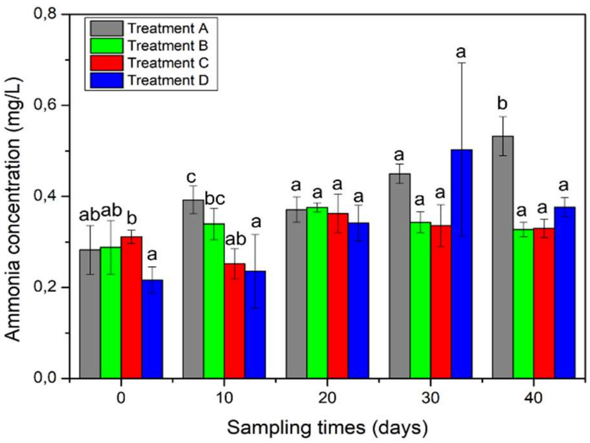
Statistical Analysis

This study used a completely randomized design (CRD) consisting of four treatments and three replicates. The treatments used were: A (100 fish/m^3, control), B (100 fish/m^3, biofloc), C (125 fish/m^3, biofloc), and D (175 fish/m^3, biofloc). The data obtained were presented in tables and graphs and analyzed using analysis of variance (ANOVA) with SPSS software. If the ANOVA showed a significant difference (F calculated > F table), further analysis was conducted using Duncan's multiple range test.

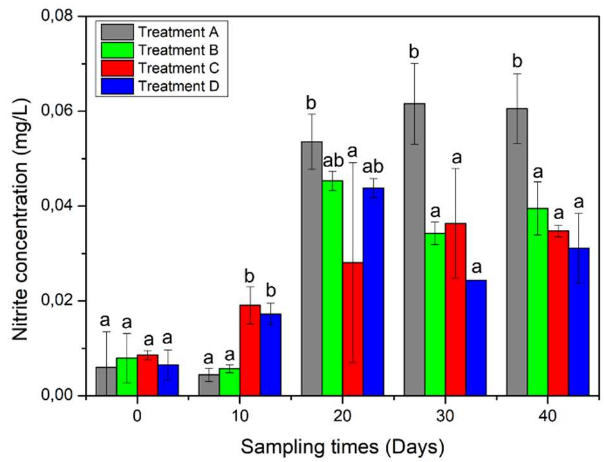
RESULTS AND DISCUSSION

Dynamics of Water Quality

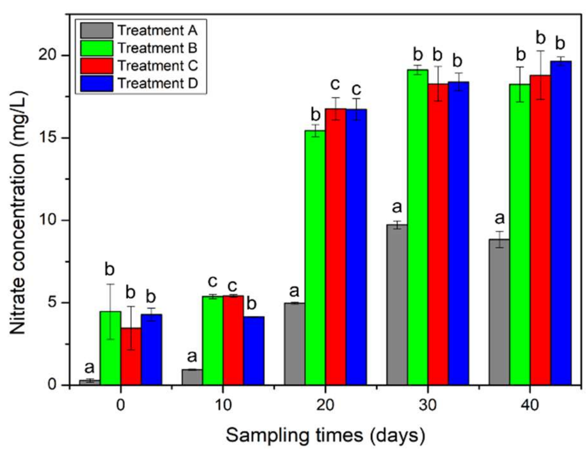
The water quality data (ammonia, nitrite, nitrate, phosphate, and sulfate) for *C. batrachus* farming based on biofloc technology with different stocking densities are shown in Fig.-1.



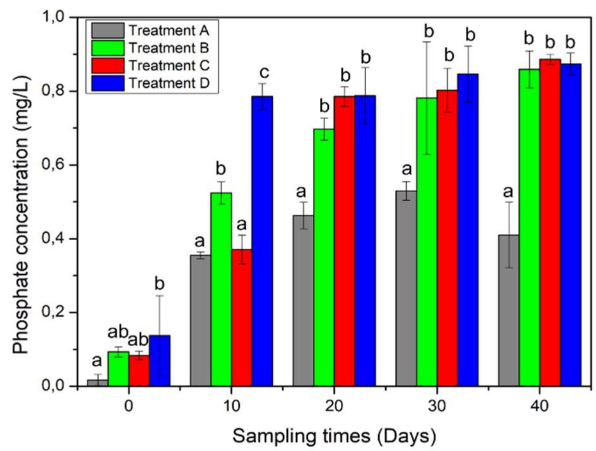
(a)



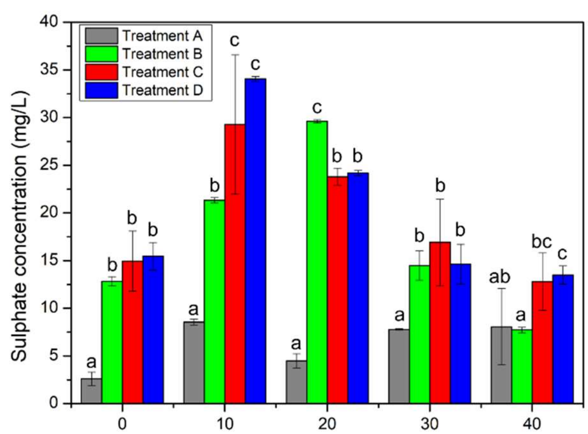
(b)



(c)



(d)



(e)

Fig.-1: Water Quality in *C. batrachus* Farming Based on Biofloc Technology with Different Stocking Densities at Different Sampling Times (days): a) Ammonia, b) Nitrite, c) Nitrate, d) Phosphate, e) Sulfate
Source: Authors' Elaboration Based on Analysis Results

Information: Average ± SD (n=3) with different superscript letters at the exact sampling times (days) indicating significant differences ($p<0.05$). A (100 fish/m³, without biofloc), B (100 fish/m³, with biofloc), C (125 fish/m³, with biofloc), D (175 fish/m³, with biofloc).

Ammonia Concentration

Ammonia in *C. batrachus* farming comes from fish waste and decomposing organic material. High ammonia levels are toxic, causing fish stress and even death. Treatment A (100 fish/m³ without biofloc) tended to have higher ammonia content compared to other treatments (Fig.-1a), and this difference was statistically significant ($p<0.05$). This phenomenon leads to symptoms like the surface to breathe oxygen, reduced appetite, and foul-smelling water.[9] High ammonia results from high-protein feed, fish density, and low water exchange.[10] Water was changed to prevent mass fish mortality in Treatment A, and salt was added at 20-30 g after the 10^{th}, 20^{th}, and 30^{th} sampling days. In treatments with biofloc (B, C, D), microorganisms converted ammonia into less harmful compounds, improving water quality and keeping ammonia levels low. Higher stocking densities (Treatment D) produced high ammonia levels due to increased waste, especially on the 30^{th} and 40^{th} sampling days. Ammonia levels fluctuated in Treatments B, C, and D (Fig.-1a), likely due to the microorganism's ability to manage the excess ammonia. The biofloc system supports high-density farming by reducing the need for water exchange through effective waste processing. The significance of the research shows that proper ammonia management is important to maintain an optimal environment for C. *batrachus* growth, with all treatments staying within acceptable ammonia limits (0.2165-0.5322 mg/L).

Nitrite Concentration

The relationship between nitrite and fish stocking density in the biofloc system is crucial for fish health. Initially, nitrite levels were low and insignificant ($p>0.05$), but they rose and fluctuated significantly ($p<0.05$) from day 10 to 40. At high stocking densities, more ammonia is produced, which nitrifies bacteria and converts them to nitrite and nitrate higher.[8] Fig.1-b shows that treatment A had higher nitrite levels due to incomplete conversion to nitrate, unlike Treatments B, C, and D, which had better microbial activity. In high-density treatments, nitrite levels fluctuated as the biofloc system rapidly converted nitrite to nitrate.[11] High nitrite levels (Treatment A) are toxic, stressing fish and increasing disease risk. Proper feeding and aeration are necessary to manage nitrite levels and ensure fish health.[12,13] In Treatments B, C, and D, routine monitoring of ammonia, nitrite, and nitrate is essential to ensure effective and safe nitrite levels in the biofloc system. Adding carbon sources like molasses supports heterotrophic bacteria that convert nitrite into safer nitrogen forms.[14] The biofloc system lowers nitrite by breaking down waste into secondary food, preventing toxicity. Nitrite levels fluctuated (0.0044-0.0616 mg/L) but mostly stayed within safe limits (<0.06 mg/L).[15] Effective nitrite management reduces fish stress and boosts growth.[16]

Nitrate Concentration

From day 10 to 40, nitrate levels increased (Fig.-1c), especially in higher-density treatments, due to the conversion of waste into nitrate, with the biofloc system maintaining water quality. Ammonia waste is processed through nitrification, where bacteria convert ammonia to nitrite, then to nitrate, and denitrification, where nitrate is converted back to nitrogen for reuse by biofloc.[17] Nitrate levels (0.2863 to 19.6422 mg/L) fluctuated but remained within the 20 mg/L limit. [15]. Although nitrate is not as harmful as ammonia or nitrite, high nitrate can affect fish health, growth, and reproduction. [18]. The primary source of nitrate originates from fish feces, influenced by the type of feed consumed by the fish.[19] So, monitoring and managing nitrate in biofloc systems is crucial. Proper nitrate management, partial water changes, and aquaponics can help maintain water quality, supporting healthy fish production and reducing feed costs.

Phosphate Concentration

During the sampling period (days 0-40), phosphate levels increased, especially in biofloc-based ponds, due to high stocking densities (Fig.-1d). Phosphate is produced from decomposing organic matter, such as unconsumed feed and fish waste, which rises with higher fish density, leading to more organic matter and excretion. If unmanaged, elevated phosphate can harm water quality and fish health. Microorganisms in bioflocs help reduce phosphate by consuming it for metabolism. However, phosphate can also settle in sediment and later release back into the water. Proper management is needed to control phosphate levels and maintain fish health.[20] Phosphate levels (0.0169 to 0.8865 mg/L) across all treatments stayed within the safe limit of $\leq$ 1.0 mg/L.[15] Good management involves regularly monitoring phosphate levels and implementing measures such as partial water replacement or using aquatic plants to absorb phosphate. This will help maintain balanced phosphate levels, supporting biofloc microorganisms and fish health. Excessive phosphate can harm water quality, lead to eutrophication, and reduce production efficiency. In biofloc systems, aquaponics can help reduce phosphate levels while providing plant growth benefits. Careful phosphate management is essential, especially at high stocking densities.[21]

Sulphate Concentration

Sulphate (SO_4^{2-}) originates from the decomposition of organic matter, particularly proteins containing sulfur, such as fish feed. Microorganisms convert these compounds into sulfate, which remains stable in water. From day 0 to day 20, sulfate levels generally increase, except in treatment A (Fig.-1e), due to high fish stocking density, leading to more sulfur-rich waste. Biofloc helps decompose this matter, but under anaerobic conditions, sulfate can turn into toxic hydrogen sulfide (H_2S).[22,23] From day 20 to day 40, higher stocking densities do not always increase sulfate, as microorganisms can effectively degrade it under aerobic conditions. Sulfate levels (2.6097-34.0627 mg/L) stay within safe limits (<300 mg/L).[15] Maintaining oxygen levels prevents harmful sulfide formation. Sulphate typically does not directly affect fish growth if maintained within normal levels. However, sulfide formation can lead to stress, poisoning, and even fish death.[24] Thus, ensuring well-oxygenated water conditions and effective organic matter management helps maintain a healthy environment for fish, especially under high stocking densities. Effective biofloc systems improve water quality by helping microorganisms degrade waste, reducing risks even in high stocking densities.

Production Performance

Production performance data (average daily growth, average body weight, production, specific growth rate, feed conversion ratio, survival rate) for biofloc-based catfish *(C. batrachus*) farming at different stocking densities are presented in Table-1.

Table-1: Production Performance of *C. batrachus* in Biofloc-Based Systems at Different Stocking Densities

Parameters	A	B	C	D
Production (kg)	5.289±0.044^a	10.470±0.170^b	12.165±0.387^c	15.654±0.586^d
ADG ($\frac{g}{fish}/day$)	2.022±0.012^a	2.97±0.02^d	2.84±0.096^c	2.56±0.103^b
ABW($\frac{g}{fish}$)	80.55±0.488^a	118.973±0.692^d	111.097±3.856^c	102.323±4.125^b
SGR (%)	3.651±0.012^a	4.626±0.014^d	4.454±0.088^c	4.248±0.1^b
FCR	1.327±0.017^b	0.953±0.015^a	0.925±0.026^a	0.980±0.063^a
SR (%)	65.67±0.577^a	88.0±1^b	87.33±1.155^b	8743±0.57^b
Production (mL/L)	-	39.047±2.18^a	40.95±2.18^a	42.38±0.83^a

Source: authors elaboration based on analysis results

Information: Average ± SD (n=3) with different superscript letters horizontally indicating significant differences ($p<0.05$). A (100 fish/m³, without biofloc), B (100 fish/m³, with biofloc), C (125 fish/m³, with biofloc), D (175 fish/m³, with biofloc).

Average Daily Growth (Adg), Average Body Weight (Abw), Specific Growth Rate (Sgr), and Production

Based on Table-1, the production performance (ADG, ABW, SGR, and production) of *C. batrachus* under various treatments shows varied outcomes. Growth values tend to be fluctuating and dynamic, with ADG (2.022-2.97 g/unit/day), ABW (80.55-118.973 g/unit), and SGR (3.651-4.626%) indicating the fastest growth trend in B > C > D > A. For production (5.289-15.654 kg), the highest production is observed in D > C > B > A. Despite differing trends in growth (ADG, ABW, SGR) versus production, stocking density significantly influences growth (ADG, ABW, SGR) and production ($p < 0.05$). Biofloc-based catfish farming (B, C, D) exhibits faster growth and better performance than non-biofloc farming (A) due to several key factors related to water quality management, nutrient provision, and fish health. Studies on the growth performance of *C. batrachus* similar to biofloc-based Tilapia (*Oreochromis niloticus*) farming[25] Indicate that a 20% reduction in feed dosage leads to increased fish growth comparable to treatment A (100 fish/m³ water) fed 100% of the feed. This finding suggests that reducing feed by 10-20% can be substituted with biofloc consumption, evidenced by the presence of plankton in the fish gut. Research evaluating the nutritional abundance of biofloc by Anand *et al.* (2014) Reports that the primary nutrients in dry biofloc material are protein (24.30%) and essential fatty acids such as palmitate (46.54%), cis-vaccinate (15.37%), linoleate (10.67%), and oleate (9.19%).[26]

Feed Conversion Ratio (FCR)

In this study, flock volume ranged from 39.047 to 42.38 mL/L, within the 20-50 mL/L range.[27] Higher flock volumes indicate better quality as flocs serve as an additional nutrient source for *C. batrachus*. Statistically, stocking density did not significantly influence flock volume formation ($p > 0.05$) (Table-1). In biofloc systems, flock volume is closely related to the amount of available and processed nutrients, where microorganisms convert nitrogen into protein for fish. Effective flock volume management is necessary to prevent water quality issues and fish stress while ensuring microorganisms can work effectively. Flocs also serve as an additional protein source for fish, reducing the need for supplementary feed and enhancing production efficiency. The FCR values fluctuate, with the best performance in treatments C > D > B > A. Stocking density significantly impacted FCR in treatment A but not in B, C, or D ($p > 0.05$). Biofloc-based farming (B, C, D) shows better FCR than non-biofloc (A) due to (1) Microorganisms converting waste into consumable nutrients; (2) Improved water quality from ammonia and nitrite conversion; (3) Reduced fish stress leading to better digestion; (4) Continuous nutrient supply from microorganisms; (5) Fewer disease issues; and (6) Fish utilizing both feed and biofloc, reducing external feed needs and improving FCR.[8,28] Using microorganisms as an additional feed source, improving water quality, and reducing stress and diseases make biofloc systems more efficient in feed utilization than non-biofloc farming. The FCR in this study (0.925 to 1.327) (Table-1) is better than previous findings of 1.48. Differences in FCR among fish species arise from factors like metabolic rate and growth pattern, nutritional needs, digestive efficiency, adaptation to farming, feeding behavior, energy use for growth, growth patterns, and immune strength.[29,30] To achieve optimal FCR, it is crucial to consider the specific needs of each fish species and adjust feeding strategies and farming conditions accordingly based on their biological and ecological characteristics.[31]

Survival Rate (SR)

Table-1 indicates that the highest survival rate is observed in treatments B > D > C > A, where biofloc-based catfish farming (treatments B, C, and D) shows higher survival rates compared to non-biofloc farming (treatment A). Statistically, stocking density significantly influences ($p < 0.05$) the survival of catfish in non-biofloc farming compared to biofloc-based farming. At the same time, there is no significant difference ($p > 0.05$) among biofloc-based ponds (B, C, and D). Factors contributing to better survival in biofloc systems include: (1) Microorganisms degrade waste and ammonia, maintaining stable water quality; (2) Biofloc provides additional food source, enhancing growth and health; (3) Microorganisms reduce disease risk by competing with pathogens; (4) Uneaten feed is converted into biofloc, improving feed efficiency; (5) Biofloc helps control pH, temperature, and oxygen levels; (6)) Biofloc larger than 100 µm contains 27.8% protein and 7.5% lipids, while biofloc smaller than 48 µm is rich in essential amino acids. Catfish can utilize biofloc as a protein source, similar to shrimp, tilapia, and shellfish.[32,33] These factors create a healthier environment, boosting survival and productivity.

CONCLUSION

The results of this study indicate that the application of the biofloc system in catfish farming with high stocking densities provides numerous benefits, including improved water quality (ammonia, nitrite, nitrate, phosphate, sulfate) and fish production performance (average daily growth, average body weight, production, specific growth rate, feed conversion ratio, and survival rate). Reducing ammonia, nitrite, nitrate, phosphate, and sulfate concentrations contributes to better growth, feed efficiency, and survival rates. The biofloc system is a sustainable and effective solution for achieving optimal and environmentally friendly catfish production. With biofloc technology, catfish can be supported up to a 175 fish/m³ density. This demonstrates that biofloc technology enhances production efficiency and reduces negative impacts on the aquatic environment.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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