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Dietary sodium butyrate improves growth performance of Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) reared in biofloc system

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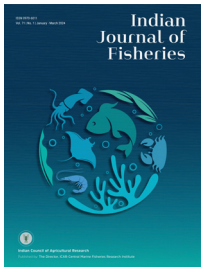

Abstract

This study evaluated the effects of dietary sodium butyrate (SB) supplementation on the growth performance and intestinal morphology of Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) cultured in a biofloc system. A 120 day experiment was conducted using a completely randomised design with five treatments: control (biofloc only) and four SB supplemented diets (0.5%, 1%, 1.5% and 2%), each in triplicate. Fish (Initial mean weight 4.9±0.2 g) was stocked at a density of 40 fish per 0.4 m³ in each tank. The results indicated no significant differences ($p>0.05$) in survival, daily weight gain and feed conversion ratio (FCR) among the treatments. However, final mean weight, total harvest weight and net fish yield were significantly higher ($p<0.05$) in treatments fed 1.5% and 2% SB supplemented diets. Water quality improved in SB supplemented treatments, with significantly lower ammonia and nitrate levels and higher floc volume. Bacterial and phytoplankton abundance increased significantly with higher SB inclusion, whereas zooplankton abundance remained unaffected. Histological analysis revealed enhanced intestinal villi length in SB fed treatments, indicating the improved nutrient absorption. Overall, the integration of sodium butyrate with biofloc technology demonstrated the synergistic effects by improving bacterial abundance, phytoplankton abundance, zooplankton abundance, water quality and fish growth. The study suggests that 1.5–2% dietary SB is optimal for enhancing productivity of Nile tilapia in biofloc system.

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Introduction

Aquaculture has become one of the fastest growing food production sectors worldwide and currently accounts for a major share of the aquatic food supply for human consumption. Global aquaculture production reached about 94.4 million t in 2022, surpassing capture fisheries production and highlighting its increasing importance for food security and nutrition (FAO, 2024). With the ongoing rise in demand for aquatic products, there is an increasing need to develop sustainable, efficient production systems that enhance productivity while minimising environmental impacts. Among the emerging technologies, biofloc technology (BFT) and recirculating aquaculture systems (RAS) have gained considerable attention for their ability to recycle nutrients, improve water-use efficiency, and reduce environmental discharge. Biofloc technology is based on the principle of microbial nutrient recycling, in which heterotrophic bacteria assimilate nitrogenous wastes, such as ammonia, and convert them into microbial biomass that can be utilised as an additional protein source by cultured organisms (Avnimelech, 2012; Crab *et al.*, 2012). In such systems, maintaining an optimal carbon-to-nitrogen (C:N) ratio is essential for promoting heterotrophic bacterial growth, thereby helping control ammonia levels and

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improving water quality. Previous studies have demonstrated that maintaining higher C:N ratios (*e.g.*, 20:1–25:1) enhances floc formation, improves nutrient recycling, and supports better growth performance of Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) in intensive culture systems (Azim and Little, 2008; Hargreaves, 2013). In addition, biofloc systems provide a continuous supply of natural food in the form of microbial aggregates, which improves feed utilisation, growth performance, and survival of cultured species (De Schryver *et al.*, 2008; Emerenciano *et al.*, 2013).

Apart from improving water quality and natural productivity, the use of functional feed additives has recently attracted attention as a strategy to enhance fish health and performance in intensive aquaculture systems. Sodium butyrate (SB), a short-chain fatty acid salt, has been widely investigated for its beneficial effects on intestinal health, nutrient absorption, and immune responses in aquatic animals. Sodium butyrate acts as an energy source for intestinal epithelial cells and promotes intestinal development by increasing villi length and surface area, thereby enhancing nutrient absorption (Bedford and Gong, 2018; Dawood *et al.*, 2018). In addition, SB has been reported to modulate the gut microbiota by promoting beneficial bacterial populations while suppressing pathogenic microorganisms, such as *Vibrio* spp., thereby improving digestive efficiency and disease resistance (Urach *et al.*, 2020; Ge *et al.*, 2023). It also enhances immune and antioxidant responses, increasing resistance to pathogens and environmental stress (Moustafa *et al.*, 2025). Several studies have reported that dietary supplementation of sodium butyrate at levels ranging from 0.5 to 2% can significantly improve growth performance, feed utilisation, intestinal morphology, and immune responses in Nile tilapia (Ali *et al.*, 2018; Resende *et al.*, 2023). However, the effectiveness of such additives may depend on the culture environment and microbial dynamics within the system. In biofloc based aquaculture, the presence of diverse microbial communities, plankton, and organic aggregates creates a complex ecological environment that can interact with dietary supplements and influence fish growth and health. Previous studies have shown that biofloc systems can increase microbial abundance and enhance the natural productivity of phytoplankton and zooplankton communities, thereby improving nutrient availability for cultured fish (Khanjani *et al.*, 2022).

Furthermore, biofloc environments have been reported to influence intestinal histology and immune responses in tilapia positively. Improvements in intestinal villi length, goblet cell abundance, and mucosal structure have been observed in fish cultured in biofloc systems, suggesting enhanced nutrient absorption and disease resistance (Lal *et al.*, 2024). The combination of biofloc technology and dietary functional additives such as sodium butyrate may therefore produce synergistic effects by improving both the external culture environment and the internal physiological condition of fish. Despite increasing interest in biofloc systems and functional feed additives, information on the combined effects of sodium butyrate supplementation and biofloc technology on microbial dynamics, plankton productivity, and intestinal histology in Nile tilapia remains limited. Therefore, the present study was conducted to evaluate the effects of dietary sodium butyrate supplementation on growth performance, intestinal morphology, gut microbial abundance, as well as water quality, in mono-sex Nile tilapia cultured in a biofloc system.

Materials and methods

The study was conducted at the Institute of Agriculture and Animal Science (IAAS), Paklihawa Campus, Bhairahawa, for 120 days, from 22 September 2024 to 23 January 2025. The experimental site is located approximately 4 km south-west of Bhairahawa in the southern plains region of Nepal.

Feed formulation

All diets were formulated to contain approximately 25% crude protein suitable for tilapia culture. Feed ingredients included rice bran, wheat flour, mustard oil cake, soybean meal, vegetable oil and vitamin-mineral premix. Feed formulation was carried out using the hit-and-trial method in an MS Excel sheet. Ingredients were ground in a mixer grinder (BALTRA Model BMG-153), sieved and thoroughly mixed with vegetable oil, vitamin-mineral premix and water to a reasonable amount homogenously. Then mixed ingredients were poured in an automatic feed machine (Model YL100L-4, Shanghai Jiesu Motor Co. Ltd.) to produce 1 mm pellet size feed. The prepared

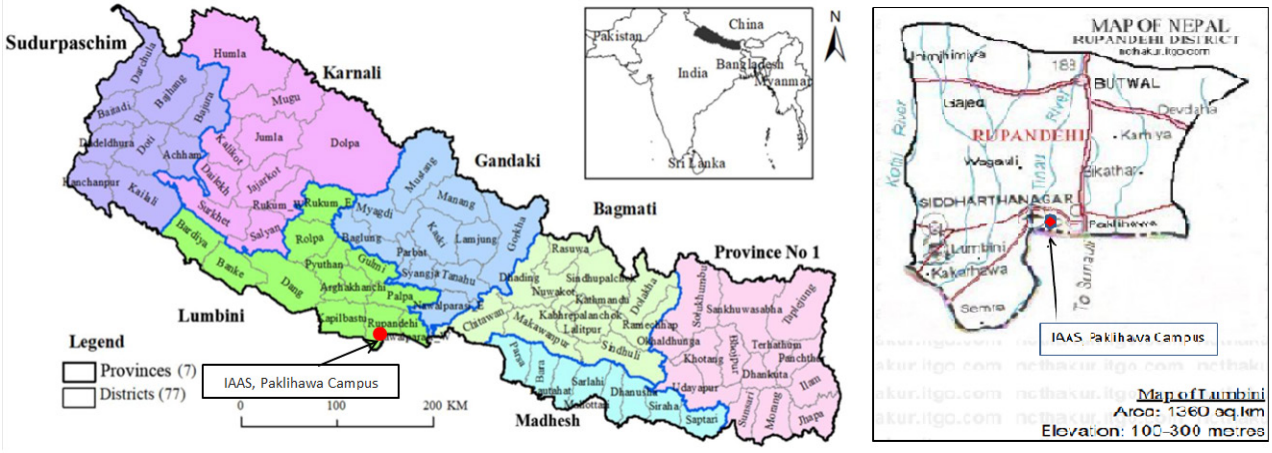

Fig. 1. Map of Nepal showing the experimental site

pellets were sun dried for a week and stored in airtight plastic containers. Then, sodium butyrate was mixed in finished pellet feed based on the treatments. For this, sodium butyrate was dissolved in a small amount of distilled water and sprayed thoroughly over the finished pellet feed, followed by air drying to secure the coating. Post-pelleting application method was selected because sodium butyrate is highly volatile and heat sensitive.

Feed samples were analysed for proximate composition according to Vargas-Rodríguez *et al.* (2016) standard procedures at the Central Fisheries Promotion and Conservation Centre, Balaju, Kathmandu. Final harvesting was carried out after 120 days, with each tank completely drained. Harvested fish were counted and weighed using a PHOENIX electronic balance (Model WT60001X).

Experimental design

The experiment was conducted in 15 circular polytanks in semi-controlled indoor facility, each having 500 l capacity. Water level was maintained at 400 l (0.4 m^3) in each tank during the experiment. A completely randomised design (CRD) with five treatments and three replications per treatment was adopted. Molasses was used as the carbon source and different levels of sodium butyrate (SB) were incorporated to the diet except in control diet. The treatments comprised: T1-biofloc system (C: N 25:1) (control), T2-biofloc system (C: N 25:1) with 0.5% SB, T3-biofloc system (C: N 25:1) with 1% SB, T4-biofloc system (C: N 25:1) with 1.5% SB and T5-biofloc system (C: N 25:1) with 2% SB (Ebeling *et al.,* 2006). Mono-sex Nile tilapia fry with an initial mean weight of 4.9±0.2 g were stocked at a density of 40 fish per 0.4 m^3 in each tank (Avnimelech, 1999). The fry were procured from the Centre for Aquaculture Agriculture Research and Production (CAARP), Chitwan, Nepal. Prior to stocking, tanks were thoroughly cleaned and disinfected with potassium permanganate at 10 mg l^{-1}. The following day, tanks were filled with water from shallow tubewell and aerated continuously for 24 h using Aeroxy tubes connected to a 1 HP ring blower.

Biofloc inoculum was prepared by mixing 10 g probiotic (Provet AQUABAC), 50 g molasses, 2.5 g calcium carbonate and 500 g raw salt in a plastic container with vigorous aeration for 24-36 h depending on ambient temperature to produce fermented carbon organics (FCO) (Gurung *et al.,* 2025). On the basis of favourable environment, the colour of FCO turns into reddish brown which symbolises its readiness and ensures the presence of mature and highly active heterotrophic microbial community in the water (Crab *et al.,* 2012; Avnimelech *et al.*, 2014; Emerenciano *et al.,* 2017). Then, the prepared FCO was added equally to all the tanks. On initiation of the experiment, molasses was added as a carbon source frequently on a daily basis or with feeding according to nitrogen input from feed, with an objective to increase microbial assimilation of ammonia. The dose depends on nitrogen load in the water (Avnimelech, 1999; Crab *et al.,* 2012; Avnimelech *et al.*, 2014). In order to maintain the desired C: N ratio of 25:1 with the applied 25% protein feed, 0.75 to 1.05 ml molasses per day was added based on the fish biomass in the respective tanks with vigorous aeration, for one week in the initial phase after FCO application in the culture water, to develop a stable microbial community {Avnimelech, 1999; Ebeling *et al.,* 2006; De Schryver *et al.,* 2008; Avnimelech *et al.*, 2014; Emerenciano *et al.,* 2017). However, molasses dose was calculated according to ammonia concentration in biofloc system on a periodic basis (Avnimelech, 1999; Ebeling *et al.,* 2006; Crab *et al.,* 2012; Avnimelech *et al.*, 2014).

Molasses dose was calculated as per Table 1 , based on the condition of fermented carbon organics (FCO) and level of ammonia in the culture tanks (Avnimelech, 1999; Ebeling *et al.,* 2006; De Schryver *et al.,* 2008; Avnimelech *et al.*, 2014; Emerenciano *et al*., 2017).

Table 1. Molasses dosage based on the condition of FCO and level of ammonia

Condition	Molasses dose
After FCO (Stable FCO/floc, ammonia normal)	0 ml day^{-1}
Slight ammonia increase	0.4-0.7 ml day^{-1}
Upper safe limit (NH3 >1 mg l^{-1})	1 ml day^{-1} (up to 1.1 ml day^{-1})
In FCO system: Start with zero and add only if ammonia rises	

Total ammonia nitrogen (TAN) consists of toxic unionised ammonia (NH_3) and ammonium ions (NH_4^+) and their proportion depends on pH and temperature. Management guidelines and interpretation of TAN levels for molasses application are provided in Table 2. When NH_3 concentration was below 0.5 mg l^{-1}, no carbon source was added, whereas when ammonia concentration exceeded from 0.5-1.0 mg l^{-1}, molasses was added as per C:N ratio (Avnimelech, 1999; Ebeling *et al.*, 2006; Crab *et al.,* 2012; M. Emerenciano *et al*., 2013; Avnimelech *et al.*, 2014). The carbon content of molasses used in the present study was analysed at Lumbini Agro Environment Lab Pvt. Ltd., Sunawal, Nawalparasi, Nepal. Furthermore, nitrogen (%) in feed was calculated through proximate analysis in IAAS soil lab, Bhairahawa, Nepal.

Table 2. Management guidelines and interpretation of TAN levels for molasses application

TAN (mg l^{-1})	Interpretation	Molasses action
0–0.5	Good/controlled	No molasses addition
0.5–1.0	Slight increase	Add small carbon dose
>1.0	High ammonia load	Add calculated molasses dose + checking of aeration/floc
>2 mg l^{-1}	Risk zone	Immediate management required

Raw salt was added to adjust total dissolved solids (TDS) to levels suitable for biofloc development (Emerenciano *et al*., 2013; Avnimelech *et al.*, 2014). Prior to application, the salt was washed two to three times to remove impurities and contaminants. TDS was measured 3 h after addition using a TDS meter (Techtonics) to confirm that the desired range was attained. Floc formation was monitored using an Imhoff cone throughout the culture period. When floc volume reached 15–30 ml l^{-1}, additional carbon sources were temporarily discontinued and resumed based on floc density and ammonia concentration in each tank. Total dissolved solids represent dissolved salts, minerals, and trace elements in water. Freshwater typically contains 400–500 ppm TDS, whereas biofloc systems require 1000–1500 ppm. To increase TDS, raw non-iodised salt was added, with approximately 1 g l^{-1} salt, increasing TDS by about 500–800 ppm. In this study, TDS was maintained at approximately 1000 ppm, which is considered suitable for tilapia culture and also helps reduce nitrite toxicity. In the biofloc system, sludge was removed at weekly intervals by draining it through the

outlet. Depending on the amount of sludge removed, tanks were partially refilled with freshwater. The aeration system was regularly monitored to ensure proper airflow, and adjustments were made as needed. In addition, Aeroxy tube pores were cleaned periodically to remove excess floc accumulation and debris. In the control treatment without biofloc, partial water exchange was performed weekly.

Experimental feeding

Fish fry were fed at 3% of their body weight throughout the experimental period. Feeding was carried out once daily between 09:00 and 10:00 hrs. For growth monitoring, approximately 20% of fish from each tank were randomly sampled monthly. Individual fish weight was measured using a portable electronic balance (PHOENIX Model WT150001XJ; precision 0.1 g). At the end of the experiment, all fish were harvested and counted to determine survival and production. Growth parameters and survival were calculated using the following formulae:

Total initial weight (g) = No. of fish stocked x Initial mean weight

Total final weight (g) = No. of fish harvested x Final mean weight

$$\text{Daily weight gain (g fish day}^{-1}) = \frac{\text{Mean final weight} - \text{Mean initial weight}}{\text{Culture period}}$$

Total weight gain (g) =Total harvest weight (g) -Total initial weight (g)

Total harvest weight (g) = Final harvest weight (g) - Initial stock weight (g)

$$\text{Gross fish yield (GFY) (kg m}^{-3}\text{ y}^{-1}) = \frac{\text{Total harvest weight (kg)} \times 365}{\text{Culture period (days)} \times \text{Culture unit (m}^3) \times 1000}$$

$$\text{Net fish yield (NFY) (kg m}^{-3}\text{ y}^{-1}) = \frac{\text{Total harvest wt. (g)} - \text{Total stocked wt. (g)} \times 365}{\text{Culture period (days)} \times \text{Culture area (m}^3) \times 1000}$$

$$\text{Apparent feed conversion ratio (AFCR)} = \frac{\text{Total weight gain}}{\text{Total feed given}}$$

$$\text{Survival rate \%} = \frac{\text{Total no, of fish harvested}}{\text{Total no. of fish stocked}} \times 100$$

Analysis of water quality parameters

Water quality parameters, including temperature, dissolved oxygen, pH, and total dissolved solids (TSS), were recorded daily between 08:00 and 10:00 hrs. Whereas, ammonia, nitrite, nitrate, total suspended solids, and floc volume were measured weekly. Temperature and dissolved oxygen were measured using a Lutron PDO-519 m, pH was measured using a Hanna HI meter 98107 and TDS was using a TDS-3 meter. Ammonia, nitrite, and nitrate concentrations were analysed using BIONIX freshwater master test kits. Total suspended solids were determined using the gravimetric method, where water samples were filtered through pre-weighed glass fibre filters, dried in an oven, and reweighed.

Analysis of bacterial load in the rearing water

To monitor the abundance of heterotrophic bacteria in the experimental tanks, bacterial counts were estimated using the standard plate count technique. For this, a 100 µl water sample was spread onto the Nutrient agar plates and incubated at 37°C for 24 h. Following incubation, the resulting bacterial colonies were counted, and the abundance of heterotrophic bacteria was expressed as colony-forming units per millilitre (cfu ml^{-1}).

Estimation of phytoplankton and zooplankton abundance in the experimental tanks

Phytoplankton and zooplankton density in the rearing water were estimated following standard plankton enumeration methods. One litre of water sample was collected from the experimental tank at each sampling point and filtered through an appropriate plankton net to concentrate the planktonic organisms. The concentrated samples were preserved in 5% formalin solution in labelled bottles and allowed to settle for 48 h before analysis. For phytoplankton enumeration, a well-mixed aliquot of the concentrated sample was transferred to a Sedgwick–Rafter counting chamber and examined under a compound microscope. Phytoplankton cells were identified to the lowest possible taxonomic level and counted following the method described by Guillard and Sieracki (2005). Cell density was expressed as cells l^{-1}. Zooplankton abundance was estimated using the same concentrated samples. After thorough mixing, an aliquot was transferred to a Sedgwick–Rafter counting chamber and examined under a stereomicroscope or compound microscope, depending on the size of the organisms. Zooplankton individuals were identified to the lowest practicable taxonomic level and counted. The abundance of zooplankton was expressed as individuals per litre of water.

Histological analysis of fish intestine

At the end of the experiment, 15 Nile tilapia fishes were randomly selected from each experimental tank for histological analysis. Fishes were dissected and intestinal tissues were carefully excised. The tissues were fixed in 10% neutral buffered formalin for 18–24 h, followed by washing in 70% ethanol, dehydration through a graded ethanol series and clearing with xylene. The tissues were embedded in paraffin wax at 56–58°C using a vacuum embedding bath. Sections of 4 µm thickness were prepared using a rotary microtome (Medimeas MRM-RM) and stained with haematoxylin and eosin following standard histological procedures (Ferguson, 1989; Prophet, 1992; Suvarna *et al.*, 2013; Wendt Campos *et al.*, 2017). The stained sections were examined under a microscope at x200 magnification, and intestinal villi length was measured using QuPath image analysis software.

Statistical analyses

Data obtained during the experiment were analysed using analysis of variance (ANOVA) to determine differences among treatments. Statistical analysis was performed using SPSS version 3.6.3 and GraphPad Prism 5. Mean values and standard errors were calculated for each treatment, and differences were considered significant at $p<0.05$.

Results

The ingredient composition and proximate nutritional composition of the experimental diets formulated with different levels of sodium

butyrate supplementation are presented in Table 3. All diets were formulated to be isonitrogenous and isoenergetic, differing only in the level of sodium butyrate inclusion.

Table 3. Ingredient (%) and proximate composition of different diets (% on dry matter basis).

Proximate composition (Estimated crude protein %)	Ingredients	Percentage
11	Rice bran	40
12	Wheat flour	3
38	Mustard oil cake	20
49	Soybean meal	35
-	Vegetable oil	1
-	Vitamin and mineral premix *(Agrim Fort)	1

*Vitamin mineral premix per kg contains the following: Vitamin A -7,00,000 IU, Vitamin D3-70,000 IU, Vitamin E-250 mg, Cobalt - 250 mg, Copper-1200 mg, Iodine-325 mg, Iron-1500 mg, Magnesium-6000 mg, Potassium-100 mg, Sodium-5.9 mg, Manganese-1500 mg, Sulphur-0.72%, Zinc- 9600 mg, DL-Methionine-1000 mg, Calcium-25.5%, Phosphorus-12.75% (Gurung *et al.*, 2024)

Growth parameters

The growth performance of Nile tilapia cultured in different biofloc treatments with sodium butyrate supplementation during the 120 day's experimental period are presented in Table 4. The initial stocking number, initial mean weight, initial weight and survival did not differ significantly among the treatments ($p>0.05$), indicating uniform distribution of experimental fish in the beginning of the experiment. The initial mean weight ranged from 4.6±0.1 to 5.2±0.2 g fish^{-1}. After 120 days of culture, the final mean weight differed significantly among the treatments ($p<0.05$). The lowest final mean weight was found in control group (T1: 15.8±5.1g fish^{-1}) which was significantly lower ($p<0.05$) than all other treatments except T2 (25.3±3.5 g fish^{-1}). Supplementation of sodium butyrate at 2% (T5) yielded the maximum final mean weight of 35.7±1.8 g fish^{-1}, representing a substantial increment over the control treatment. Although, Duncan's multiple range test showed that the growth performance of fish in T5 treatment did not differ significantly ($p>0.05$) from those in the T3 (27.5±1.3 g fish^{-1}) and T4 (33.9±2.2 g fish^{-1}) treatments. Similarly, no significant variations ($p>0.05$) were found between T2 and T3 or between T3 and T4, indicating a transitional growth gradient between the intermediate supplementation levels. The final harvest weight also varied significantly among the treatments ($p<0.05$). The highest harvest weight was recorded in T5 (1359.3±85.8 g), followed by T4 (1355.1±87.0 g), whereas the lowest value was recorded in T1 (932.0±46.1 g). Similarly, gross fish yield showed significant differences among the treatments ($p<0.05$) with maximum yield recorded in T4 and T5 (10.3±0.7 kg m^{-3} year^{-1}) and minimum was recorded in T1 (2.7±0.4 kg m^{-3} year^{-1}). The final harvest number was significantly influenced by the treatments ($p<0.05$). The highest survival rate was observed in T2, T3, T4 and T5 with 100%, 98.3%, 97.5% and 97.5% survival respectively, whereas the control treatment found the lowest survival rate (90.0±5.2%). Moreover, apparent feed conversion ratio (AFCR) was found ranging from 0.5 to 0.9 but the difference among the treatments was not statistically significant ($p>0.05$). Similarly, daily weight gain also did not show significant difference among the treatments ($p>0.05$)

Water quality parameters

The mean values and monthly variations of water quality parameters among the different treatments during the 120 day experimental period are presented in Table 5. The water quality parameters showed variations among the treatments depending on the level of sodium butyrate supplementation, although most of the parameters remained within suitable ranges for Nile tilapia culture throughout the experimental period. Water temperature did not vary significantly among the treatments ($p>0.05$). The mean temperature values were found ranging from 20.7±1.20°C in T1 to 20.9±1.25°C in T3. The monthly temperature trend showed gradual changes during the culture period with comparatively lower temperature recorded during the winter months and higher values during the initial experimental months. Similar temperature conditions among the treatments indicated that all the experimental units were exposed to comparable environmental conditions. The pH value differed significantly among the treatments ($p<0.05$). The pH values in sodium butyrate supplemented treatments remained slightly lower as compared to the control treatment. Monthly variation of pH showed gradual fluctuation throughout the experimental period, with all the treatments maintained alkaline

Table 4. Growth and yield of Nile tilapia in different treatments in a 120 day experimental period (Mean±SE)

Growth parameters	Treatments				
	T1 (Control)	T2	T3	T4	T5
Initial stocking No.	40±0.0	40±0.0	40±0.0	40±0.0	40±0.0
Initial mean weight (g fish^{-1})	4.6±0.2	4.9±0.1	4.6±0.1	5.2±0.2	5.1±0.2
Total initial weight (g)	182.2±6.7	197.0±4.8	183.4±4.6	209.3±9.3	204.2±6.4
Final harvest no.	35.3±1.5^{b}	40.0±0.0^{a}	40.0±0.0^{a}	40.0±0.0^{a}	38.0±1.0^{a}
Final mean weight (g)	15.8±5.1^{c}	25.3±3.5^{bc}	27.5±1.3^{ab}	33.9±2.2^{ab}	35.7±1.8^{a}
Final harvest weight (g)	932.0±46.1^{b}	1011.8±53.0^{b}	1098.9±51.2^{b}	1355.1±87.0^{a}	1359.3±85.8^{a}
Daily weight gain (g fish^{-1} day^{-1})	0.1±0.1	0.4±0.2	0.2±0.0	0.2±0.0	0.3±0.0
Gross fish yield (kg m^{-3} y^{-1})	2.7±0.4^{c}	7.7±0.4^{b}	8.4±0.4^{b}	10.3±0.7^{a}	10.3±0.7^{a}
Net fish yield (kg m^{-3} y^{-1})	3.1±2.0^{b}	6.2±0.4^{ab}	7.0±0.4^{a}	8.7±0.6^{a}	8.8±0.6^{a}
Apparent feed conversion ratio (AFCR)	0.9±0.15	0.5±0.03	0.5±0.16	0.6±0.08	0.6±0.19
Survival rate (%)	90.0±5.2	100±0.0	98.3±1.7	97.5±2.5	97.5±2.5

Mean values with different superscript letters within the same row are significantly different ($p<0.05$)

condition suitable for fish growth. Accordingly, dissolved oxygen (DO) concentration also showed significant differences among the treatments ($p<0.05$). The highest DO concentration was recorded in T1 (9.75±0.47 mg l^{-1}), whereas comparatively lower values were observed in sodium butyrate supplemented treatments where the lowest value was recorded in T4 (8.65±0.57 mg l^{-1}). The monthly DO variation showed a decreasing trend during certain periods of the culture cycle, however, DO values remained within the acceptable level for Nile tilapia culture. Furthermore, Ammonia concentration varied significantly among the treatments ($p<0.05$). The highest ammonia concentration was recorded in control treatment T1 (0.53±0.04 mg l^{-1}), whereas sodium butyrate supplemented treatments recorded much lower ammonia concentrations (approximately 0.10 mg l^{-1}). Monthly ammonia fluctuations showed that there was a greater variation in the control treatment as compared with sodium butyrate supplemented treatments. Nitrite concentration also differed significantly among the treatments ($p<0.05$). The lowest nitrite concentration was observed in control treatment (0.15±0.00 mg l^{-1}) and the highest value was recorded in T4 (0.40±0.03 mg l^{-1}). Monthly changes in nitrite concentration showed fluctuations during the culture period that reflected the variation in microbial transformation processes within the biofloc system. Similarly, nitrate concentration showed significant variation among the treatments ($p<0.05$). The highest nitrate level was observed in T1 (1.33±0.16 mg l^{-1}), whereas comparatively lower concentrations were recorded in sodium butyrate supplemented treatments, especially in T3 and T5 (0.23±0.01 mg l^{-1}). Monthly nitrate trends indicated differences in nutrient cycling among the treatments.

Biofloc concentration differed significantly among the treatments ($p<0.05$). The highest floc concentration was recorded in T5 (33.15±2.22 mg l^{-1}), followed by T2, T3 and T4, but the lowest concentration was recorded in control treatment T1 (5.99±2.95 mg l^{-1}). Monthly observations showed that there was gradual build up of biofloc biomass with the progress of the experiment, especially in sodium butyrate supplemented treatments. Total dissolved solids (TDS) also showed significant differences among the treatments ($p<0.05$). The highest TDS value was recorded in T4 (2251.1±112.25 mg l^{-1}) followed by T2 and T3, and the lowest value was observed in T1 (408.1±39.92 mg l^{-1}). Monthly fluctuations of TDS indicated that there was increasing dissolved nutrient accumulation in biofloc treatments during the culture period. Total suspended solids (TSS) also differed significantly among the treatments ($p<0.05$). The highest TSS concentration was recorded in T5 (207.1±2.34 mg l^{-1}) followed by T4 (199.2±0.41 mg l^{-1}) and the lowest concentration was recorded in T1 (187.3±4.08 mg l^{-1}). Monthly variation of TSS showed that there was a gradual change associated with biofloc development and microbial biomass formation.

Bacterial load in the rearing water

Bacterial abundance showed that there was significant variation among the treatments ($p<0.05$) (Table 6) . The highest bacterial count was recorded in T5 (1938.9±126.0 ×10^5 cfu ml^{-1}) followed by T4 (1391.1±138.6 ×10^5 cfu ml^{-1}). The lowest bacterial abundance was found in control treatment T1 (409.9±59.9 ×10^5 cfu ml^{-1}). Increase in sodium butyrate supplementation levels resulted in significantly higher bacterial abundance as compared to the control treatment ($p<0.05$).

Phytoplankton and zooplankton abundance in the experimental tanks

The abundance of phytoplankton differed significantly among the treatments during the experimental period ($p<0.05$) (Table 6). The highest phytoplankton density was recorded in T5 (2% sodium butyrate supplementation) with a concentration of 110±2.3 ×10^2 cells l^{-1} followed

Table 5. Mean and range of water quality parameters recorded in different treatments during the experimental period of 120 days (Mean±SE)

Parameters	T1(Control)	Treatments			
		T2	T3	T4	T5
Temp (^{0}C)	20.7±1.20 (14.14-29.4)	20.8±1.19 (14.46-29.6)	20.9±1.25 (14.38-29.8)	20.8±1.22 (14.4-29.7)	20.8±1.19 (14.4-29.6)
pH	8.38±0.02^a (8-8.6)	8.11±0.04^b (7.2-8.5)	8.08±0.04^{bc} (7.1-8.51)	8.06±0.05^{bc} (7.3-8.45)	8.09±0.06^c (7.4-8.44)
DO (mg l^{-1})	9.75±0.47^a (7.5-12.95)	8.92±0.64^b (6.1-11.97)	8.80±0.61^b (5.6-12.14)	8.65±0.57^b (5.2-11.92)	8.94±0.66^b (5.6-12.81)
NH_3 (mg l^{-1})	0.53±0.04^a (0-1)	0.10±0.0^b (0-0.25)	0.9±0.01^b (0-0.25)	0.9±0.01^b (0-0.25)	0.10±0.02^b (0-0.25)
NO2 (mg l^{-1})	0.15±0.00^a (0-1)	0.29±0.00^b (0-0.5)	0.32±0.01^b (0-0.2)	0.40±0.03^b (0-0.25)	0.32±0.00^b (0-0.5)
NO3 (mg l^{-1})	1.33±0.16^a (0-1)	0.31±0.07^b (0-1)	0.23±0.01^b (0-0.5)	0.25±0.02^b (0-0.5)	0.23±0.01^b (0-0.5)
Floc (mg l^{-1})	5.99±2.95^c (0-17)	29.20±2.99^b (0-50)	26.76±2.67^b (0-50)	28.48±1.89^b (0-50)	33.15±2.22^a (0-50)
TDS (mg l^{-1})	408.1±39.92^d (0-724)	2142.9±110.04^b (155-3390)	2123.4±82.53^b (132-3600)	2251.1±112.25^a (134-3600)	2029.2±167.17^c (123-3390)
TSS (mg l^{-1})	187.3±4.08^c (31.7-381.8)	193.9±1.86^{bc} (25.7-390.3)	193.0±1.39^{bc} (24.8-389.9)	199.2±0.41^b (31.1-403.2)	207.1±2.34^a (31.5-412.3)

Mean values with different superscript letters within the same row are significantly different ($p < 0.05$).

by T4 (1.5% sodium butyrate) with 105±3.0 ×10² cells l⁻¹ and T2 (0.5% sodium butyrate) with 103.3±0.7×10² cells l⁻¹. The lowest phytoplankton abundance was recorded in control treatment T1 (73.3±6.7×10² cells l⁻¹). Although, the abundance recorded in T2 and T4 was statistically similar with T5. The increased phytoplankton abundance in supplemented treatments corresponded with the improved microbial activity and higher floc development observed in the treatments supplemented with sodium butyrate. Among the sodium butyrate supplemented treatments, T3 showed comparatively lower phytoplankton abundance (93.7±3.7×10² cells l⁻¹), which was found significantly higher than the control but lower than the treatments with higher sodium butyrate supplementation levels. The results indicate that sodium butyrate supplementation had influenced the plankton productivity in the biofloc systems. The percentage composition of phytoplankton groups during the experimental period showed variation among the treatments (Fig. 2). Different phytoplankton groups contributed to the total phytoplankton community that suggests changes in the aquatic microbial food web under different dietary treatments. Higher phytoplankton abundance in T5 and T4 treatments indicated the better environmental conditions for phytoplankton proliferation as compared to the control treatment.

In contrast, zooplankton abundance did not differ significantly among the treatments (p>0.05) during the experimental period (Table 6). The highest zooplankton abundance was observed in T4 (58±3.5×10² individuals l⁻¹), followed by T5 (55.7±1.7×10² individuals l⁻¹), T2 (53.0±5.3×10² individuals l⁻¹) and T3 (49.7±6.7×10² individuals l⁻¹). The lowest abundance was recorded in control treatment T1 (47.3±3.5×10² individuals l⁻¹). Though, numerically higher zooplankton density was observed in sodium butyrate supplemented treatments, the differences among the treatments were statistically non-significant (p>0.05). The percentage composition of zooplankton groups (Fig. 3) showed variation in community structure among the treatments; although, the overall abundance was found comparable across all the treatments. The absence of significant differences in zooplankton abundance suggests that sodium butyrate supplementation in biofloc system had a stronger influence on phytoplankton and bacterial populations than zooplankton development.

Effect of sodium butyrate supplementation on histomorphology of intestine

Microscopic examination of histological sections of intestine from the experimental fishes revealed distinct architectural differences in the mucosal lining, especially on the development and elongation of the intestinal villi, among various treatment groups (Fig. 4a–e). The control group (T1) fishes presented the shortest intestinal mucosal villi, with a mean villus length of approximately 210 µm. In contrast, the dietary supplementation with sodium butyrate (T2, T3, T4, and T5) resulted in significant increase (p<0.01/p<0.001)

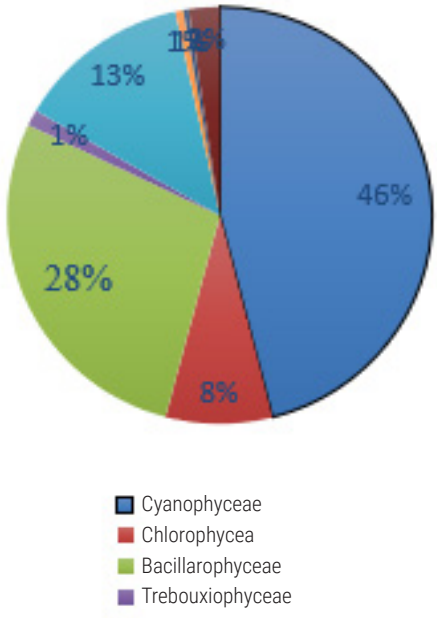

Fig. 2. Percentage composition of different phytoplankton classes in the experimental tanks

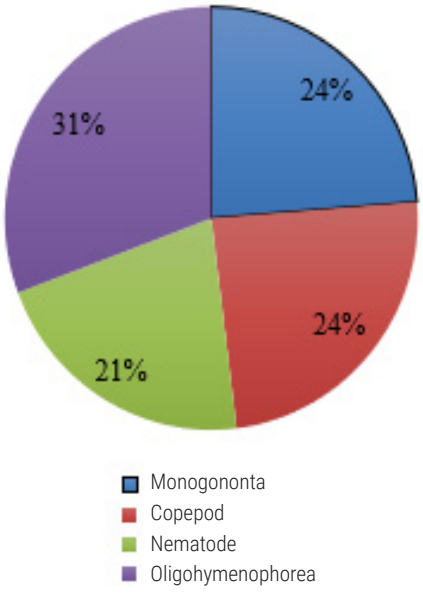

Fig. 3. Percentage composition of different zooplankton classes in the experimental tanks

Table 6. Bacterial counts, phytoplankton and and zooplankton density (×10² cells l⁻¹) in different treatments during the experimental period of 120 days (Mean±SE)

	Treatments				
	T1 (Control)	T2	T3	T4	T5
Bacterial count (×10⁵ cfu ml⁻¹)	409.9±59.9^{c}	1065.0±184.6^{b}	1311.4±152.0^{b}	1391.1±138.6^{b}	1938.9±126.0^{a}
Phytoplankton (cells l⁻¹)	73.3±6.7^{c}	103.3±0.7^{ab}	93.7±3.7^{b}	105±3.0^{ab}	110±2.3^{a}
Zooplankton density (Individuals l⁻¹)	47.3±3.5^{a}	53±5.3^{a}	49.7±6.7^{a}	58±3.5^{a}	55.7±1.7^{a}

Mean values with different superscript letters within the same row are significantly different (p<0.05)

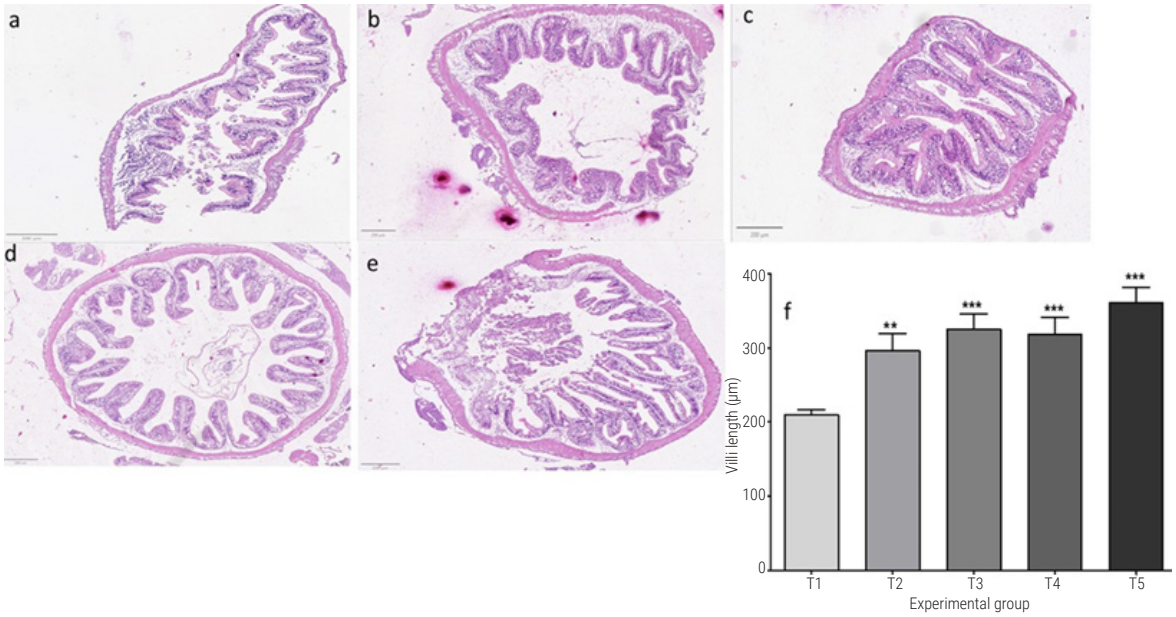

Fig. 4. Representative photomicrographs of histological sections of intestine of experimental fish from different treatment groups. Panels a-e correspond to treatments T1 to T5, respectively, while panel f shows the measurement of intestinal villi length

in intestinal villi length as compared with the control group. Fish in T2 demonstrated a significant increase in villus length over the control ($p<0.01$), with mean value close to 300 µm. The greatest improvement was observed in T3, T4 and T5, where villus lengths exceeded 320 µm and were significantly higher than those of the control group ($p<0.001$). Although no statistically significant differences were observed among the individual sodium butyrate supplemented groups (T2–T5), a clear dose-dependent trend of increasing villus length was evident. T5 experimental group exhibited the greatest physiological response, and the highest mean villus length of approximately 360 µm.

Discussion

Growth performance and yield

The present study showed that sodium butyrate (SB) supplementation in a biofloc system significantly improved growth and production of Nile tilapia during the experimental period of 120 days. Initial stocking density, mean weight and survivability did not differ significantly ($p>0.05$), indicating uniform starting condition for all the treatments (T1 to T5). Significant improvement was found in the final body weight among the treatment groups compared to control group ($p<0.05$) which indicates the positive effect of SB supplementation under biofloc condition. Specially, sodium butyrate (SB) inclusion within a biofloc system operating at C:N ratio of 25:1 noticeably enhanced the final weight of Nile tilapia. The significant increase in final mean weight from T1 (control) to T5 (2% SB) reveals that SB acts synergistically with the biofloc environment to improve nutrient assimilation. Sodium butyrate acts as a primary energy source for colonocytes and intestinal epithelial cells, promoting villi development, enhancing digestive enzyme activity and optimising microflora proliferation (Silva *et al.*, 2022). The superior growth trend found at higher inclusion levels of SB (1.5% to 2%) closely reflects the findings of Da Silva *et al*. (2020), who reported that dietary organic acid blends substantially improve weight gain and feed utilisation efficiency in tilapia cultured in intensive system. Furthermore, the lack of a significant difference ($p>0.05$) among the higher inclusion levels of SB (T3, T4 and T5) indicates a potential changing effect, suggesting that beyond 1 to 1.5% inclusion threshold, the marginal growth benefit per unit of SB decreases. This threshold behaviour coincides with Akhtar *et al*. (2021), who had observed that while structural intestinal metrics improve with organic acid additives, excessive supplementation yields diminishing returns due to metabolic saturation. The strong performance in the biofloc treatments further strengthens the concept that structural microbial flocs combined with exogenous organic acids create a highly bioavailable nutritional matrix that accelerates somatic growth in teleost fish species (Hostins *et al*., 2019). Final harvest weight followed a similar trend with significantly higher production in SB supplemented treatments ($p<0.05$). The highest harvest weight was recorded in T5, followed by T4, while T1 showed the lowest harvest weight. The greater harvest weight in T4 and T5 indicates that SB supplementation promoted better individual fish growth as well as overall system productivity. According to recent findings in biofloc systems, improved fish growth production is linked with the enhanced microbial protein formation, efficient nitrogen recycling and continuous availability of natural food particles in the culture system (Khanjani *et al.,* 2024). Gross fish yield was also significantly influenced by treatments ($p<0.05$). The highest yield was observed in T4 and T5, followed by T3 and T2, whereas the lowest yield was found in control treatment T1. This substantial improvement in yield indicates that SB supplementation enhanced the productivity of the biofloc system. Biofloc systems depend on the microbial conversion of dissolved organic matter into microbial biomass, which can serve as a supplementary nutrition to the fish and improve feed efficiency (Raza *et al.,* 2024). The net fish yield also showed a similar pattern, with the highest values found in

T5 and T4 and T1 had the lowest value. Higher production in SB treatments may be due to enhanced microbial activity and improved nutrient utilisation. Biofloc system recycles nitrogenous wastes into microbial biomass, reducing nutrient loss and increasing available protein for fish (Rajeev *et al.,* 2024).

Survival rate was significantly higher in SB treatments ($p<0.05$), with highest values in T2 to T4 and T5, whereas T1 showed the lowest survival. Improved survival might be due to better water quality, reduced stress and enhanced immunity, since butyrate is known to modulate inflammation and support beneficial gut microbiota (Liu *et al.,* 2024). Feed conversion ratio (AFCR) did not differ significantly ($p>0.05$), but the values were lower in SB treatments, indicating better feed utilisation, which could be attributed to improved digestion and microbial protein from biofloc. Similarly, daily weight gain showed no significant difference among treatments, but higher values waere observed in SB treatments, possibly due to individual variation and experimental duration, whereas final weight indicated a positive SB effect.sector.

Water quality parameters

The present study showed that supplementation of sodium butyrate in a biofloc culture system influenced the several key water quality parameters during 120 day culture period. Most of the parameters were found within the optimal range for tilapia culture that confirmed the suitability of biofloc technology (BFT) for maintaining water quality stability as reported in previous studies (Avnimelech, 2012; Crab *et al.,* 2012; Emerenciano *et al.,* 2017). Temperature did not differ significantly among the treatments ($p>0.05$) which ranged from 20.7±1.20°C in T1 to 20.9±1.25°C in T3, indicating the uniform environmental exposure across all the experimental groups. Monthly fluctuations in temperature from 14.14 to 29.8°C reflected seasonal variation rather than treatment effects. Similar observations have been reported in biofloc systems where temperature is primarily governed by ambient condition rather than the dietary or microbial manipulations (Azim and Little, 2008; Emerenciano *et al.,* 2017). Values were within the acceptable range for Nile tilapia, athough slightly below the optimal growth temperature, which might have moderately affected metabolism. pH differed significantly ($p<0.05$), with the highest value in T1 (8.38±0.02) and lowest in T4 and T5. The pH decline in SB treatments suggests enhanced microbial activity and organic matter degradation, consistent with pH reduction typically visible in biofloc systems due to nitrification and CO_2 accumulation (De Schryver *et al*., 2008; Kuhn *et al*., 2010). pH in all the treatment tanks were found within the suitable alkaline range favourable for tilapia production. Similarly, dissolved oxygen (DO) levels differed significantly ($p<0.05$), with the highest value found in T1 and comparatively lower values in SB supplemented treatments, especially in T4. The reduction in DO in SB supplemented treatments may be linked with the increased microbial respiration due to enhanced biofloc formation and organic carbon utilisation. Similar findings have been reported in intensive biofloc systems where microbial oxygen demand increases with the higher floc density (Hargreaves, 2013; Ekasari *et al*., 2014). In spite of variations, DO remained above the critical levels for tilapia (>5 mg l^{-1}) that ensures a suitable culture condition. A clear improvement in nitrogen dynamics was seen in SB supplemented treatments. Total ammonia nitrogen (TAN) was highest in T1, whereas markedly lower concentrations (0.10 mg l^{-1}) were found in SB supplemented treatments (T2 to T5). This reduction indicates that enhanced nitrification and microbial assimilation of nitrogen, likely to be stimulated by SB improving heterotrophic bacterial activity. Biofloc system is known to convert toxic nitrogen into microbial biomass efficiently (Avnimelech, 2012; Crab *et al*., 2012). Accordingly, nitrite concentrations also showed significant variation ($p<0.05$), with higher values in SB supplemented treatments as compared to T1. This suggests that an active nitrification pathway where ammonia is rapidly oxidised to nitrite before conversion to nitrate indicates the dynamic nitrogen cycling within the system. Similarly, nitrate levels were highest in T1 , which significantly reduced in SB supplemented treatments. The lower nitrate accumulation in SB supplemented treatments suggests that more efficient assimilation of nitrogen into microbial biomass rather than accumulation in water which is found to be a desirable outcome in biofloc system (Najdegerami *et al.,* 2016; Emerenciano *et al.,* 2017). Biofloc concentration varied significantly among the treatments ($p<0.05$), with the highest value found in T5, followed by T2 to T4 and the lowest was found in T1. This indicates that sodium butyrate has positively influenced the microbial aggregation and floc development, likely by enhancing heterotrophic bacterial proliferation and carbon utilisation efficiency. Similar results in floc density with organic acid supplementation have been reported in recent biofloc studies (Xu *et al.,* 2022). Furthermore, Total dissolved solids (TDS) showed significant variation ($p<0.05$) among the treatments, with the highest value found in T4 and lowest value in T1. The increased TDS in SB supplemented treatments reflects the increased microbial biomass and dissolved organic matter accumulation. High TDS is a common characteristic of biofloc system due to the continuous recycling of nutrients and suspended microbial aggregates (Hargreaves, 2013; Emerenciano *et al.,* 2017). Accordingly, TSS differed significantly ($p<0.05$), increasing from T1 to T5, indicating the enhanced floc formation and particulate accumulation in SB treatments.

Bacterial abundance

Bacterial abundance varied significantly among the treatments ($p<0.05$), showing a clear dose dependent response to sodium butyrate supplementation. The highest bacterial population was found in T5 followed by T4, T3 and T2, whereas the lowest abundance was observed in the control T1. The gradual increase in bacterial load with higher SB inclusion indicates that sodium butyrate worked as a metabolic enhancer for heterotrophic bacterial proliferation. This trend was found to be consistent with the previous results that organic acids, including butyrate stimulated the microbial growth and improved carbon utilisation efficiency in biofloc systems by enhancing heterotrophic pathways (De Schryver and Verstraete, 2009; Crab *et al.,* 2012). Increased bacterial abundance in SB supplemented treatments also match with the improved nitrogen assimilation and floc formation observed in the present study, suggesting stronger microbial loop activity and enhanced nutrient recycling.

Phytoplankton and zooplankton abundance

Phytoplankton abundance also differed significantly among the treatments ($p<0.05$). The highest phytoplankton abundance was recorded in T5, followed by T4, T2 and T3, whereas the lowest value was found in T1. The enhanced phytoplankton density in SB

supplemented treatments suggest improved nutrient availability and favourable water quality conditions, driven by microbial activity. In biofloc systems, phytoplankton growth is often stimulated by recycled nitrogen compounds and stable pH conditions which will enhance the primary productivity (Avnimelech, 2012; Emerenciano *et al*., 2017). Higher phytoplankton abundance in T5 and T4 could be attributed to increased TSS and floc associated nutrient recycling, which helps algal growth. Lower values in T1 indicate weaker nutrient recycling and microbial activity without SB supplementation. Hence, SB indirectly enhances primary productivity by improving microbial nutrient interaction.

Zooplankton abundance did not differ significantly among the treatments ($p > 0.05$), with numerically higher values found in SB supplemented treatments. The highest abundance was found in T4, followed by T5, T2 and T3, with the lowest in T1. Zooplankton showed no significant difference among the treatments, indicating stable populations less influenced by SB, compared to bacterial and phytoplankton abundance. Zooplankton dynamics is primarily driven by grazing pressure and system balance rather than dietary additives (Hargreaves, 2013; Ekasari *et al*., 2015). Slightly higher values in SB treatments might be due to increased food availability contributed by higher microbial and phytoplankton growth.

Integrated microbial interaction

Findings of the study show that sodium butyrate supplemented treatments significantly enhanced the microbial abundance, especially by the stimulation of bacterial proliferation and supporting phytoplankton growth. This is likely due to the improved nutrient recycling and energy transfer within the biofloc system. The stronger response of bacteria as compared to zooplankton suggests that SB mainly affects the lower trophic levels, which will subsequently influence the higher trophic dynamics indirectly. The percentage composition of phytoplankton and zooplankton groups (Figs. 2 and 3) further assists the presence of a dynamic microbial food web, with SB supplementation contributing to a more productive and stable microbial environment. Such kind of microbial stabilisation is critical for intensive aquaculture systems where water quality and nutrient cycling directly affect the fish performance (Crab *et al*., 2012; Emerenciano *et al*., 2017).

Histological impacts of sodium butyrate on intestinal architecture

The intestinal mucosa serves as a primary physiological barrier and as the principal site for nutrient digestion and absorption, while villus height serves as a direct indicator of the functional integrity and absorptive capacity of the intestine. In the present study, qualitative and quantitative histological evaluations (Fig.4) showed that dietary supplementation of sodium butyrate (T2 to T5) affected the mucosal histomorphology, resulting in a distinct, dose dependent elongation of the intestinal villi as compared to the control group (T1) (Lan *et al.,* 2020). Significant increase in villus length was observed in all sodium butyrate supplemented groups ($p<0.01$ for T2; $p<0.001$ for T3-T5) strongly highlights the potent trophic efficacy of sodium butyrate on the gut epithelium. As a key short chain fatty acid (SCFA), butyrate acts as a primary desirable oxidised energy substrate for enterocytes and mucosal epithelial cells. This rapid energetic fueling directly promotes cellular metabolism and division (Kotunia *et al.,* 2004). Histologically, longer villi show increased rate of epithelial cell proliferation changing upward from the intestinal crypts. Recent finding confirms that sodium butyrate influences mucosal mitotic activity *via* epigenetic regulation, especially through the inhibition of histone deacetylases (HDACs) which increases expression of genes driving cellular differentiation and tissue growth. These findings demonstrates a substantial architectural shift from the shorter, stunted villi in the control group (Fig, 4a) to the longer, well-developed villi seen in the treated cohorts (Kotunia *et al.,* 2004; Lan *et al.,* 2020; Chen *et al.,* 2025). Although the morphometric variations among the sodium butyrate supplemented groups (T2, T3, T4, and T5) were not statistically significan, a clear, dose-dependent trend of increasing villus length was evident, with the highest mean value recorded in T5 (360 µm, Fig.4f). This gradual improvement in intestinal morphology is consistent with the observations of Lan *et al.* (2020) and Chen *et al.* (2025), who reported that increasing dietary sodium butyrate supplementation promotes intestinal villus elongation and improves villus integrity (Lan *et al.,* 2020). From a functional viewpoint, the progressive increase in villus length enhances the absorptive surface area of the intestine, thereby improving nutrient uptake and utilisation.. The well-developed and elongated villi observed in the sodium butyrate treated groups, particularly in T5 (Fig. 4e), are also likely to strengthen intestinal barrier function, reduce susceptibility to luminal pathogens, and improve resistance to intestinal stress. The uniform tissue architecture and enhanced villus development observed in the histological sections further support the beneficial role of dietary sodium butyrate in improving intestinal morphology and maintaining gut health in Nile tilapia (Lan *et al.,* 2020; Lin *et al.,* 2023).

Dietary sodium butyrate supplementation in a biofloc system significantly improved the growth performance and intestinal health in Nile tilapia along with improvement in water quality and microbial activity. The most favourable responses were observed at 1.5–2% inclusion levels, indicating the potential of sodium butyrate as an effective feed additive for enhancing fish performance and promoting sustainable intensive aquaculture.

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