Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 28(5): 39 – 57 (2024)
www.ejabf.journals.ekb.eg

Growth, Physiological Responses, and Flesh Quality of the Nile Tilapia, *Oreochromis niloticus*, Cultured at Different Stocking Densities Using the Biofloc System

Mohamed M. Refaey[1*], Marwa M. Zaher[2], Ahmed I. Mehrim[1]

[1]Animal Production Department, Faculty of Agriculture, Mansoura University, PO. 35516, Egypt
[2]National Institute of Oceanography and fishers, (NIoF), Cairo, Egypt

[*]**Corresponding Author: m_moaaz@mans.edu.eg**

ARTICLE INFO

Article History:
Received: July 8, 2024
Accepted: Aug. 7, 2024
Online: Sep. 1st, 2024

Keywords:
Aquaculture systems,
Nile tilapia,
Stress,
Stocking density,
Hemato-biochemical,
Flesh quality

ABSTRACT

In recent years, biofloc technology has grown rapidly for various reasons, including improved water quality, cost-effectiveness, sustainability, and environmental friendliness. Therefore, the purpose of this study was to evaluate the impact of fish culture systems (a traditional system (TRS) and biofloc system (BFS)) on growth, hematological parameters, serum biochemical markers, and flesh quality of *O. niloticus* reared in different stocking densities (SDs). A total of 900 on-grown *O. niloticus*, weighing 53.17±3.32g, as initial body weight, were randomly distributed into continuously aerated tanks in two different culture systems (BFS and TRS) and stocked at densities of 50 and 100 fish/m^3 for 60 days. As the SD increased, all growth performance and feed efficiency indices decreased significantly. Fish reared in the BFS showed superior growth performance and feed conversion ratio (FCR) compared to the TRS group. Fish stocked in the BFS at a density of 100 fish/m^3 showed much better growth performance than TRS. Hematological indicators were enhanced in fish cultured in the BFS, especially at higher SD. Conversely, fish reared in the TRS displayed significant increases in levels of serum cortisol, glucose, total cholesterol, and triglycerides than fish reared in the BFS. Fish stocked at SD of 100 fish/m^3 and reared in the BFS exhibited a significant decrease in cortisol levels and improvements in flesh quality, as indicated by decreased drip loss and frozen leakage rate compared to those in the TRS. Overall, the findings indicate the BFS's ability to mitigate the adverse effects of increasing SD on fish, emphasizing its potential as a promising technology for enhancing fish productivity and obtaining a healthy product.

INTRODUCTION

The aquaculture industry contributes significantly to world food security, as indicated by its production reaching approximately 223.2 million metric tons **(FAO, 2024)**. However, this industry faces various challenges, including water scarcity, increased nitrogenous waste, and environmental impacts **(Khanjani *et al*., 2022, 2023)**. Fish only benefit from 25% of the nitrogen they consume, converting it into meat, while the remaining 75% of nitrogen is released into the water **(Piedrahita, 2003)**. A biofloc system (BFS) has been working for the growth of heterotrophic bacteria and other microorganisms by adding a carbohydrate source into the water, hence regulating the

carbon-to-nitrogen ratio (C:N ratio) **(Avnimelech, 2009; Ahmad *et al*., 2017; Khanjani *et al*., 2023)**. This process converts ammonia and other nitrogenous compounds into microbial proteins, known as flocs **(Azim & Little, 2008; Avnimelech, 2009)**. **Pellegrin *et al*. (2022)** indicated that bioflocs serve as an extra nutritional source for aquaculture organisms, resulting in improved feed conversion rates and optimal dietary protein utilization.

Fish survival, growth, and general health in fish culture are all directly impacted by stocking density (SD), which is crucial in fish farming. Increasing SD leads to heightened aggressiveness, increased competition for food, and increased waste output, ultimately resulting in chronic stress **(Liu *et al*., 2018; Oliveira *et al*., 2022)**. Consequently, researchers and farmers have investigated a variety of approaches to mitigating the problems associated with increased SD. These methods include incorporating immune stimulants into fish feed, such as medicinal herbs **(Dawood *et al*., 2020; Ahmed *et al*., 2021)**, as well as using materials like zeolite to address deterioration in water **(Zenhom *et al*., 2020; Mansour *et al*., 2022)**. Additionally, modern systems, like recycling systems that remove toxic ammonia, have been implemented. However, the expensive installation, operating, and maintenance costs have limited the widespread use of these systems, especially in developing countries **(Schneider *et al*., 2006)**. Moreover, traditional fish farming systems require frequent water changes to maintain acceptable water quality criteria for fish farming. Unfortunately, this leads to the discharge of wastewater containing substances such as ammonia, phosphorus, and carbon, which can pollute the surrounding environment **(Cohen *et al*., 2005)**. Furthermore, wastewater can contribute to the emergence and spread of disease-causing organisms **(Piedrahita, 2003)**. As a result, there is a need for cost-effective technology that allows for higher SDs while minimizing negative environmental impacts, such as the BFS.

In the traditional system (TRS), which relied on water changes throughout the production period, it has been observed that a high density of tilapia can have adverse effects on production parameters. These effects include decreased survival and growth rates, increased feed conversion ratio (FCR), decreased amount of crude protein in the fish carcass, and a lower cost-benefit ratio **(Ridha, 2006; Ferdous *et al*., 2014; Moniruzzaman *et al*., 2015)**. Additionally, it has negative physiological responses such as decreased levels of hemoglobin (Hb), red blood cells (RBCs), and white blood cells (WBCs) and decreased plasma cortisol concentrations **(Barcellos *et al*., 1999; Kpundeh *et al*., 2013)**. Previous studies have examined the impact of several SDs on the Nile tilapia farmed in the BFS **(Haridas *et al*., 2017; Lima *et al*., 2018; Liu *et al*., 2018; Shourbela *et al*., 2021)**. The majority of these studies have used fingerlings, while few studies have addressed the impact of SDs on on-grown or broodstock fish. This study aimed to evaluate how fish culture systems (traditional and biofloc) affect the growth, hematological parameters, serum biochemical markers, and flesh quality of *O. niloticus* reared in different stocking densities for 60 days.

MATERIALS AND METHODS

1. The procedures and management of experimental fish

This study used about 900 Nile tilapias that were in the growing stage. They were acquired from a private farm in Dakahlia Governorate, Egypt. The average initial body weight was 53.17± 3.32g. The fish were kept in a 20m^3 cement tank for 15 days before the experiment as an acclimatization period. Throughout this period, fish were fed with a floating feed comprising 30.02% crude protein and 5.49% crude fat manufactured by the New Hope Aquafeed Company, Dakahlia, Egypt. The water in the tanks was changed twice a week, replacing approximately half of the total amount each time. This research was conducted in the Fish Research Unit, Faculty of Agriculture, Mansoura University, Egypt. Then, fish were dispersed into 12 continuously aerated tanks with four treatments (2 SDs and 2 culture systems (CSs)). Each treatment consisted of three tanks (as replicates; with a total volume of 1m^3). The fish were reared using two different culture systems (BFS and TRS) and stocked at two SD levels (50 and 100 fish/m^3).

After fish were distributed, and 15 days before the trial began, fish in all treatments were fed at a rate of 4% of tank biomass. This phase was intended to promote the establishment of the biofloc system. Molasses (a carbon source) was fed to tanks cultivated with a biofloc system to maintain the nitrogen-to-carbon ratio of 1:10. The amount of molasses added was calculated according to the method followed by **Avnimelech (2009)**. Molasses was added once daily at midday, while feed was added by hand twice daily at 9:00 a.m. and 2:00 p.m. Floc formation was monitored by measuring it daily using an Imnhof funnel (capacity of 1000mL). After the formation of the biofloc system, the experiment was intiated and lasted for 60 days. Every two weeks, the fish were weighed, and the amount of feed and molasses added was modified accordingly. Fish were fed 4% of the biomass in the tank for 30 days during the trial, and then 3% of the biomass until the completion of the experiment. For tanks cultured using the traditional system, the water was changed at a rate of 30% twice a week. As for ponds cultured using the biofloc system, the ponds were only supplied to compensate for the percentage of loss by evaporation every week according to each tank.

Throughout the 60-day experimental period, water quality indicators were inspected weekly. A thermometer was used to check the water's temperature. Dissolved oxygen (DO, mg/L) was measured with a Milwaukee MW600 PRO portable DO-meter, USA, and pH was measured with a HI98129 Waterproof pH & Temperature Tester from Hungary. According to **APHA (1992)**, total ammonia nitrogen (TAN, mg/L) in water was examined using Chemists® test kits from CHEMETRICS, INC., USA, by direct Nesslerization methods.

2. Fish samples

To calculate growth performance criteria, fish in each tank were weighed. Then, ten fish in each tank (replicate) were randomly selected (total n = 30/treatment). Fish were exposed to 50mg/ L clove oil extract to anesthetize. Following that, a 5mL blood

sample was extracted from the fish's caudal vein with a 3mL syringe and placed in tiny plastic vials containing heparin to determine the hematologic parameters. Other blood samples were collected in dry tubes without anticoagulants and centrifuged at 300rpm for 15 minutes to extract serum. The clear serum was stored in plastic Eppendorf tubes at -20°C until biochemical parameters were evaluated.

3. The experimental measurements

3.1. Growth performance and feed utilization parameters

Growth performance parameters were calculated using the following equations:

- Weight gain (WG, g) = FW (g)/ IW (g)
- Average daily gain (ADG, g/fish/day) = WG (g) / the experiment period (days) Body weight index (BWI, %) == [WG (g) / IW (g)] × 100
- Specific growth rate (SGR, %/day) = [(Ln FW – Ln IW)/ the experiment period (days)] × 100
- Survival rate (SR, %) = [Number of survived fish/ Total number of cultured fish] × 100
- FCR = FI (g) / WG (g)
- Protein efficiency ratio (PER) = WG (g) / PI (g)

Where:

IW: initial weight; FW: final weight; FI: feed intake; PI: protein intake

3.2. Hematological parameters

Hematological criteria such as Hb were measured using commercial colorimetric kits (Diamond Diagnostic, Egypt) on an Ao Bright-Line Hämocytometer model. RBCs, platelets (PLT), WBCs, and lymphocytes (%) were counted (Neubauer improved, Precicolor HBG, Germany) following **Decie and Lewis (2006)**. According to the method described by **Stoskopf (2015)**; using the microhematocrit tube, packed cell volume (PCV) was measured.

3.3. Serum biochemical parameters

Total cholesterol (TCH) and triglycerides (TG) were tested using the technique published by **Ellefson and Caraway (1976)**. According to the method outlined by **Tietz (1995)**, total protein (TP) was determined. While, albumin (ALB) was assessed according to the outlines of **Doumas *et al.* (1971)**. **Doumas and Biggs (1972)** described the method for calculating serum globulin (GLB) by subtracting albumin from total protein. Using the Roche (Basel, Switzerland) commercial RIA kits and the Cobas 6000 immunoassay analyzer, cortisol (CORT) levels were measured. Glucose (GLU) was assessed according to the procedure developed by **Henry (1964)**.

3.4. Flesh quality parameters

To evaluate the drip loss (DL), six dorsal muscle samples in each treatment were stored at 4°C for three days using the procedure outlined by **Bosworth *et al.* (2004)**. In order to calculate the frozen leakage rate (FLR), other muscle samples (n = 6/treatment)

were kept at –20°C for a full day, following the protocol described by **Ling Qiao *et al.* (2014)**.

4. Statistical analysis

All data were statistically analyzed using فاتtwo-way analysis of variance (ANOVA) with the SAS® software version 9.1.3 for Windows **(SAS, 2006)**. The purpose was to determine the effects of culture systems (TRS and BFS), different SDs (50 and 100 fish/m^3), and their interaction. Before statistical analyses, all ratios and percentages were transformed using the arcsine method. Tukey's *post hoc* test was used to compare the means of treatments, and statistical significance was determined at a probability level of $P \leq 0.05$.

RESULTS

1. Water quality

Data in Table (1) demonstrated the effect of CSs, different SDs, and their interaction on water quality criteria. Water temperature is unaffected by the CSs or SDs. The TRS shows a substantial rise in DO, TAN, and pH when compared to the BFS ($P \leq 0.05$). Furthermore, the interaction between CSs and SDs revealed that fish reared in a BFS had a significantly lower TAN than fish in the TRS, while no statistical variances were detected in DO and pH.

Table 1. Effect of CSs, different SDs, and their interaction on water quality parameters

CS	SD (fish/m^3)	Temperature (°C)	DO (mg/L)	TAN (mg/L)	pH
Traditional		26.65±0.13	6.80±0.30^{a}	0.73±0.21^{a}	8.28±0.08^{a}
Biofloc		26.57±0.17	6.23±0.25^{b}	0.30±0.07^{b}	7.88±0.19^{b}
	50	26.45±0.12	7.03±0.15^{a}	0.26±0.04^{b}	8.21±0.17^{a}
	100	26.77±0.15	6.01±0.24^{b}	0.77±0.20^{a}	7.95±0.15^{b}
Interactions					
Traditional	50	26.50±0.23	7.35±0.03	0.33±0.01	8.45±0.03
	100	26.80±0.12	6.25±0.38	1.14±0.23	8.10±0.06
Biofloc	50	26.40±0.12	6.70±0.06	0.19±0.04	7.97±0.28
	100	26.73±0.32	5.77±0.30	0.41±0.09	7.80±0.30
***P-value* (Two ways analysis)**					
CS		0.705	0.046	0.0076	0.098
SD		0.175	0.002	0.0030	0.025
CS*SD		0.939	0.738	0.0428	0.673

Mean in the same column having different small letters are significantly different ($P \leq 0.05$). CS: Culture systems; SD: Stocking density; DO: Dissolved oxygen; TAN: Total ammonia nitrogen.

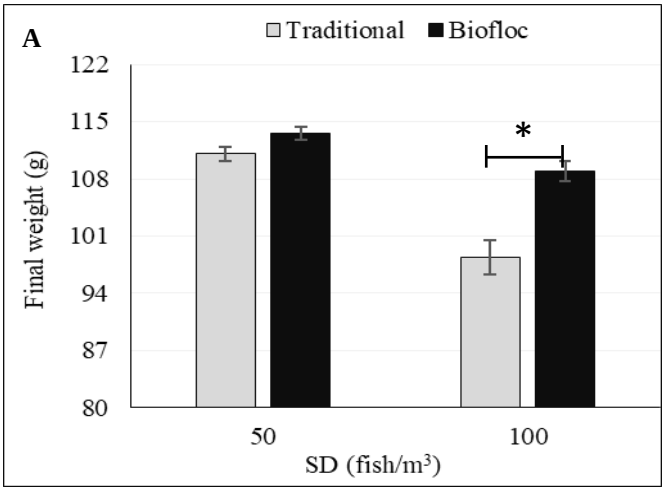
2. Growth performance and feed utilization parameters

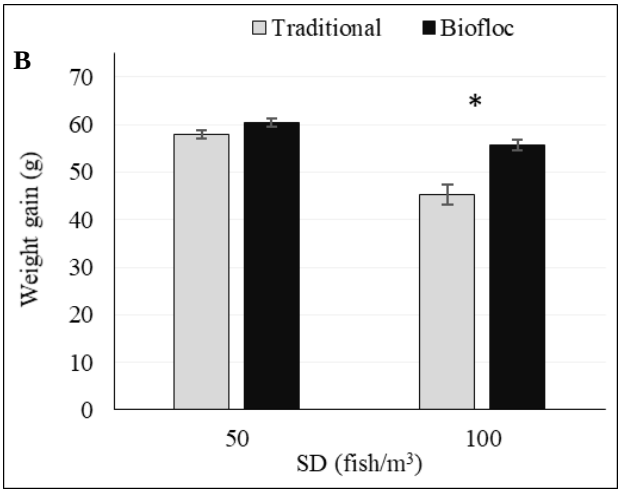
Table (2) displays the effects of CSs, different SDs, and their interaction on the growth performance and feed utilization parameters of the Nile tilapia. Fish reared with the BFS obtained higher values for growth performance and feed utilization (FCR and PER) compared to TRS. When SD reached 100 fish/m^3, growth performance, feed utilization parameters, and S% decreased significantly compared to those stocked at 50 fish/m^3 ($P \leq 0.05$). With regards to fish cultured at a density of 50 fish/m^3, the BFS and TRS exhibited no significant ($P< 0.05$) variances in growth performance or feed efficiency metrics. (Fig. 1). Fish cultivated in a BFS at an SD of 100 fish/m^3 significantly ($P< 0.05$) surpassed those reared in a TRS, in terms of growth and feed utilization induces (Fig. 1).

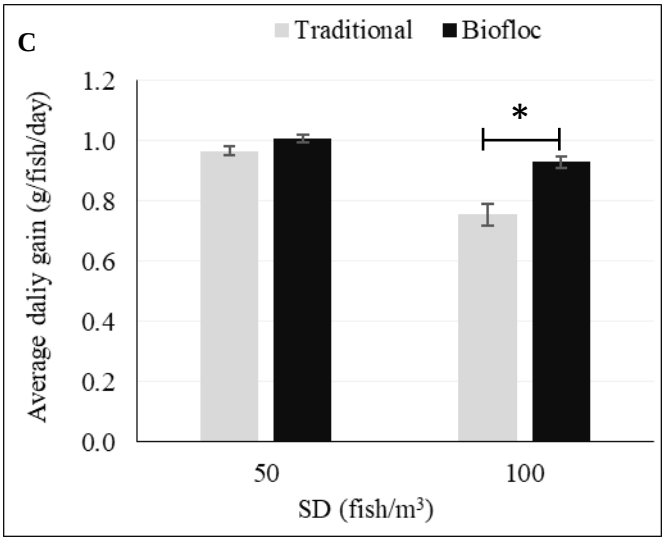
Table 2. The effect of CSs and different SDs on growth performance and feed utilization parameters of the Nile tilapia

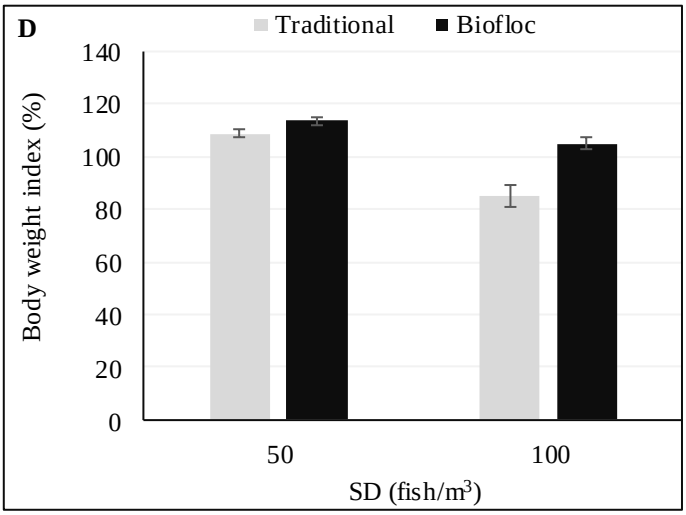
CS	SD (fish/m^3)	FW (g)	WG (g)	ADG (g/fish/day)	BWI (%)	SGR (%/day)	FCR	PER (%)	SR (%)
Traditional		104.70^{b}	51.57^{b}	0.86^{b}	97.00^{b}	1.13^{b}	2.04^{a}	1.66^{b}	97.00
		±3.10	±3.00	±0.05	±5.64	±0.05	±0.09	±0.07	±0.86
Biofloc		111.2^{a}	58.06^{a}	0.97^{a}	109.2^{a}	1.23^{a}	1.78^{b}	1.89^{a}	96.00
		±1.24	±1.24	±0.02	±2.32	±0.02	±0.02	±0.02	±1.15
	50	112.3^{a}	59.15^{a}	0.99^{a}	111.3^{a}	1.25^{a}	1.80^{b}	1.86^{a}	98.00^{a}
		±0.77	±0.77	±0.01	±1.46	±0.01	±0.02	±0.02	±0.73
	100	103.6^{b}	50.48^{b}	0.84^{b}	94.95^{b}	1.11^{b}	2.01^{a}	1.69^{b}	95.00^{b}
		±2.58	±2.57	±0.04	±4.84	±0.04	±0.10	±0.08	±0.86
P-value **(Two ways analysis)**									
CS		0.0012	0.0013	0.0013	0.0012	0.0014	0.0005	0.0002	0.4117
SD		0.0002	0.0002	0.0002	0.0002	0.0002	0.0016	0.0008	0.0317
CS*SD		0.0172	0.0175	0.0175	0.0176	0.0157	0.0061	0.0058	0.4117

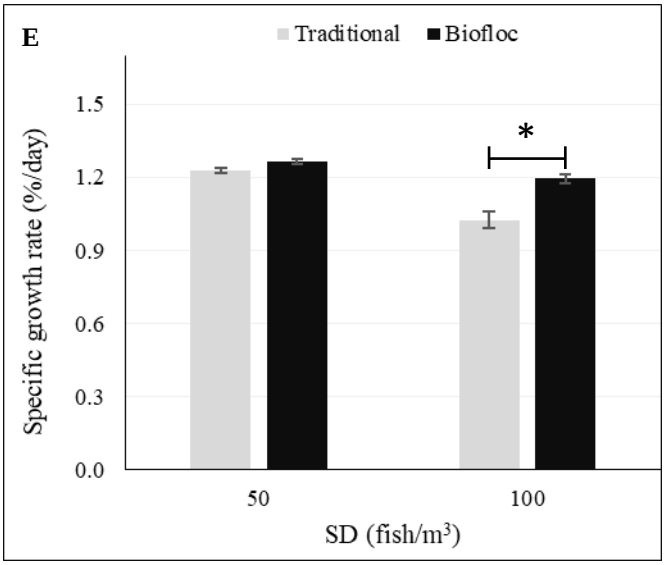
-Mean in the same column having different small letters are significantly different ($P \leq 0.05$). CS: culture systems; SD: stocking density; FW: final weight; WG: weight gain; ADG: average daily gain; BWI: body weight index; SGR: specific growth rate; FCR: feed conversion ratio; PER: protein efficiency ratio; S%: survival.

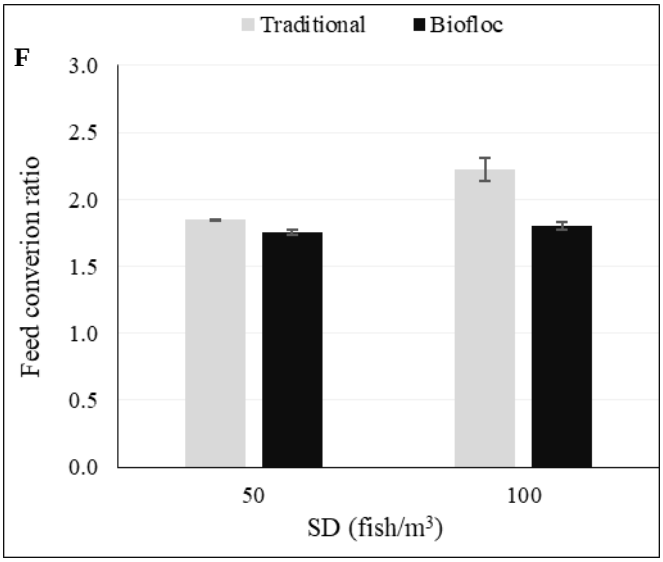

A
Traditional
Biofloc
Final weight (g)
122
115
108
101
94
87
80
*
50
100
SD (fish/m³)


B
Traditional
Biofloc
Weight gain (g)
70
60
50
40
30
20
10
0
*
50
100
SD (fish/m³)


C
Traditional
Biofloc
Average daliy gain (g/fish/day)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
50
100
SD (fish/m³)


D
Traditional
Biofloc
Body weight index (%)
140
120
100
80
60
40
20
0
50
100
SD (fish/m³)


E
Traditional
Biofloc
Specific growth rate (%/day)
1.5
1.2
0.9
0.6
0.3
0.0
*
50
100
SD (fish/m³)


F
Traditional
Biofloc
Feed converion ratio
3.0
2.5
2.0
1.5
1.0
0.5
0.0
50
100
SD (fish/m³)

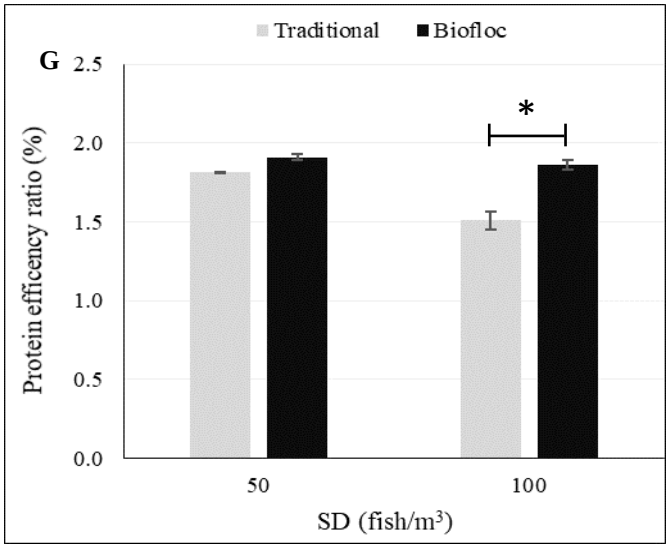


Fig. 1. Effect of interaction between culture systems (Traditional and biofloc) and different stocking densities (SDs) on growth performance and feed utilization parameters of Nile tilapia. Vertical bars indicate standard error; means with an asterisk indicate significant differences between culture systems for each stocking density ($P \leq 0.05$)

3. Hematological parameters

Fish grown in the BFS had significantly ($P \leq 0.05$) greater Hb, RBCs, and PCV levels, as well as lower levels of lymphocytes compared to fish reared in the TRS (Table 3). No effect of CSs was observed on WBCs and PLT ($P \geq 0.05$). In terms of SD, fish reared at 100 fish/m^3 showed significant increases in Hb, RBCs, PCV, and WBCs, but PLT and lymphocytes were significantly lower than those reared at 50 fish/m^3($P \leq 0.05$). The interaction between CSs and SDs did not show any statistically significant variations in any of the hematological parameters ($P \geq 0.05$).

Table 3. Effect of CSs and different SDs, and their interaction on hematological parameters of the Nile tilapia

CS	SD (fish/m^3)	Hb (g/dL)	RBCs ($\times 10^6$/µL)	PCV (%)	WBCs ($\times 10^3$/µL)	PLT ($\times 10^3$/µL)	Lymphocytes (%)
Traditional		7.61±0.54^{b}	2.91±0.35^{b}	22.20±1.31^{b}	14.18±0.93	1.79±0.21	78.67±0.21^{a}
Biofloc		9.56±0.51^{a}	3.93±0.38^{a}	29.76±1.88^{a}	13.81±1.06	1.51±0.14	75.00±0.14^{b}
	50	7.41±0.46^{b}	2.83±0.31^{b}	22.09±1.25^{b}	12.84±0.54^{b}	1.88±0.20^{a}	78.50±0.20^{a}
	100	9.76±0.48^{a}	4.01±0.38^{a}	29.87±1.85^{a}	15.15±1.15^{a}	1.42±0.12^{b}	75.17±0.12^{b}
Interactions							
Traditional	50	6.40±0.27	2.30±0.09	19.20±0.80	12.90±0.41	2.26±0.21	82.00±0.21

	100	8.83±0.58	3.51±0.56	25.20±1.17	15.45±1.68	1.32±0.15	75.33±0.15
Biofloc	50	8.43±0.46	3.36±0.49	24.98±1.07	12.78±1.10	1.51±0.22	75.00±0.22
	100	10.70±0.38	4.51±0.46	34.54±0.32	14.85±1.83	1.52±0.20	75.00±0.20
P-value **(Two ways analysis)**							
CS		0.0008	0.0377	<.0001	0.7963	0.1881	0.0124
SD		0.0002	0.0197	<.0001	0.0180	0.0364	0.0203
CS*SD		0.8664	0.9489	0.0715	0.8656	0.0317	0.0203

Mean in the same column having different small letters are significantly different ($P \leq 0.05$). CS: Culture system; SD: Stocking density; Hb: Hemoglobin; RBCs: Red blood cells; PCV: Packed cell volume; WBCs: White blood cells; PLT: Blood platelets.

4. Serum biochemical

Table (4) demonstrates the serum biochemical characteristics values of *O. niloticus* reared in different CSs with varying SD rates, as well as their interaction between CSs and SDs. In terms of CSs, fish cultivated in the TRS had significantly higher levels of CORT, GLU, TCHO, TG, and ALB, although GLB levels were significantly decreased compared to fish reared in the BFS ($P \leq 0.05$). Regarding SD, the Nile tilapia raised at 100 fish/m^3 exhibited the highest concentrations of CORT, TCHO, TG, and GLB, as well as the lowest levels of GLU and ALB as compared to fish cultivated at 50 fish/m^3 ($P \leq 0.05$). However, there are no significant variances observed in TP levels under the effect of CSs and SDS ($P \geq 0.05$). Furthermore, the interaction between CSs and SDs does not result in significant differences in TCHO, TG, TP, ALB, and GLB levels ($P \geq 0.05$).

Table 4. Effect of CSs and different SDs, and their interaction on serum biochemical parameters of the Nile tilapia

CS	SD (fish/m^3)	Stress indicators		Lipid profile		Serum proteins		
		CORT (ng/mL)	GLU (mg/dL)	TCHO (mg/dL)	TG (mg/dL)	TP (g/dL)	ALB (g/dL)	GLB (g/dL)
Traditional		9.00±1.88^{a}	72.79±9.07^{a}	150.0±9.63^{a}	183.8±11.00^{a}	4.65±0.05	2.26±0.25^{a}	2.39±0.25^{b}
Biofloc		7.55±0.79^{b}	69.96±2.35^{b}	129.1±4.97^{b}	147.4±4.97^{b}	4.54±0.09	1.53±0.15^{b}	3.01±0.10^{a}
	50	5.11±0.60^{b}	84.79±4.94^{a}	132.1±5.54^{b}	158.6±8.52^{b}	4.58±0.10	2.18±0.27^{a}	2.40±0.22^{b}
	100	11.44±1.05^{a}	57.96±3.54^{b}	147.0±10.10^{a}	172.6±12.42^{a}	4.61±0.04	1.61±0.16^{b}	3.00±0.16^{a}
Interactions								
Traditional	50	4.30±0.87	96.33±3.12	135.7±9.47	174.0±10.82	4.65±0.10	2.78±0.11	1.88±0.10
	100	13.70±1.03	49.25±2.21	164.3±14.38	193.7±19.58	4.65±0.03	1.75±0.32	2.90±0.33
Biofloc	50	5.93±0.71	73.25±3.94	128.5±6.70	143.3±8.02	4.50±0.19	1.58±0.31	2.93±0.20
	100	9.18±0.79	66.67±1.70	129.7±8.36	151.5±6.30	4.58±0.06	1.48±0.06	3.10±0.04
P-value **(Two ways analysis)**								
CS		0.0170	0.3440	0.0615	0.0119	0.3293	0.0082	0.0095
SD		<.0001	<.0001	0.1671	0.2796	0.7405	0.0328	0.0120
CS*SD		0.0038	<.0001	0.2027	0.6486	0.7405	0.0708	0.0580

Mean in the same column having different small letters are significantly different ($P \leq 0.05$). CSs: Culture system; SD: Stocking density; CORT: Cortisol; GLU: Glucose; TCHO: Total cholesterol; TG: Triglycerides; TP: Total protein; ALB: Albumin; GLB: Globulin.

The interaction between CSs and SDs led to a noticeable increase in the concentration of CORT in fish that were raised in the BFS and SD of 50 fish/m^3, compared to those raised in the TRS (Fig. 2; $P \leq 0.05$). Conversely, fish stocked at a density of 100 fish/m^3 and reared in the TRS exhibited the highest CORT levels when compared to fish cultivated in the BFS ($P \leq 0.05$). Regarding GLU, fish SD of 50 fish/m^3 showed a significant increase in the TRS. In contrast, when the fish were cultured in BFS at an SD of 100 fish/m^3, the concentration of GLU in the fish showed a significant increase compared to those cultured in TRS (Fig. 2; $P \leq 0.05$).

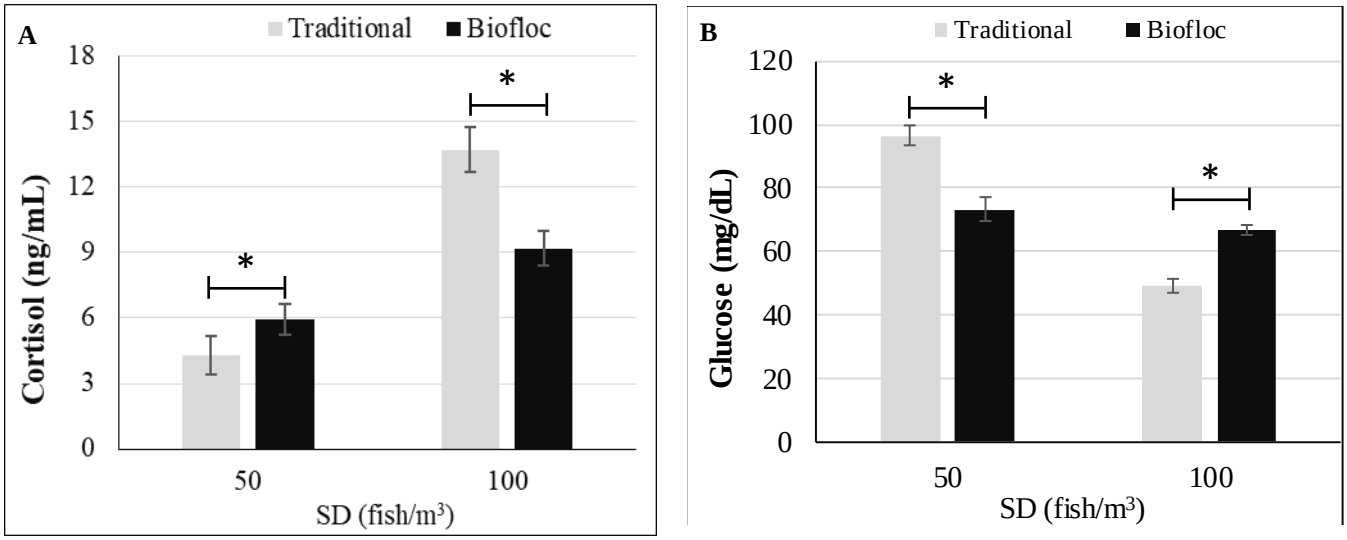


Fig. 2. Serum cortisol (A) and (B) glucose of the Nile tilapia reared with different stocking densities (SDs) and culture systems (Traditional and biofloc). Vertical bars indicate standard error; means with an asterisk indicate significant differences between culture systems for each stocking density ($P \leq 0.05$)

5. Flesh quality

Data in Table (5) display that fish cultured in the BFS system had a significantly lower DL and FLR compared to those in the TRS ($P \leq 0.05$). Fish at an SD of 100 fish/m^3 had significantly higher DL and FLR than those stocked at an SD of 50 fish/m^3 ($P \leq 0.05$). When the interaction between CSs and various SDs was analyzed, there were no discernible changes in DL and FLR ($P > 0.05$).

Table 5. Effect of CSs & different SDs, and their interaction on flesh quality parameters of the Nile tilapia reared

CS	SD (fish/m^3)	Drip loss (%)	Frozen leakage rate (%)
Traditional		2.96±0.34	2.18±0.15
Biofloc		2.37±0.15	1.92±0.12
	50	2.31±0.19^{b}	1.98±0.09^{b}
	100	3.02±0.31^{a}	2.13±0.18^{a}
Interactions			
Traditional	50	2.64±0.32	2.17±0.14
	100	3.29±0.59	2.20±0.28
Biofloc	50	1.98±0.13	1.79±0.09
	100	2.76±0.20	2.05±0.23
P-value **(Two ways analysis)**			
CS		0.0108	0.0196
SD		0.0547	0.0467
CS*SD		0.8555	0.5601

Mean in the same column having different small letters are significantly different ($P \leq 0.05$). CS: Culture system; SD: Stocking density.

DISCUSSION

Increased fish productivity is always associated with a higher SD of farmed fish. However, increased SD leads to adverse changes in water quality indices. These changes include a decrease in DO and pH, as well as an increase in the accumulation of nitrogenous residues **(M'balaka *et al.*, 2012; Mehrim *et al.*, 2017; Shourbela *et al.*, 2021)**. This aligns with the current findings, which demonstrate that increasing the SD led to a rise in TAN and a reduction in pH and DO levels. At the same time, it is noted that the water quality parameters of the BFS are improved compared to the TRS, especially in terms of decreasing TAN levels in the water. This indicates the BFS's positive impact in mitigating the detrimental effects of increasing SD. Under the BFS, ammonia nitrogen converts from its toxic form into biofloc (a microbial protein) **(Azim & Little, 2008; Avnimelech, 2009)**, thereby reducing TAN levels. It is also noted that the level of DO in the BFS is lower compared to the TRS, which is due to the increase in living mass inside the system as a result of the increase in the microbial load that makes up the floc. Despite constant aeration in biofloc tanks, a reduction in DO levels was associated with increased fish at SD (100 fish/m^3), which was attributed to increasing biomass (fish and microorganisms) that increased DO demand. This outcome aligns with the results stated by **Das *et al.* (2022)** in pengba (*Osteobrama belangeri*) reared in a BFS. Additionally, the reduction of pH (still at the acceptable level for rearing the Nile tilapia) in both the BFS and the high SD may be associated with the decomposition of the accumulated fish waste in tanks and its conversion to flocs by microorganisms **(Das *et al.*, 2005)**. In addition to waste decomposition, the increased carbon dioxide output **(Paterson *et al.*, 2003)** is involved.

Numerous studies have found that increasing SD harms the Nile tilapia growth performance **(Mehrim, 2009; Mehrim *et al*., 2017; Liu *et al*., 2018; Ahmed *et al*., 2021; Mansour *et al*., 2022)**. As the current study found, increasing SD reduced growth performance criteria. This decrease might be connected to the stress that fish experience as a result of rising SD. Conversely, the BFS-reared fish showed better growth performance than the TRS-reared fish. Raising fish in the BFS has a positive effect on minimizing the negative effects of developing SD, as seen by the interaction of CSs and SDs. Compared to fish raised at the same density and cultured in the TRS, fish grown in the BFS at an SD of 100 fish/m^3 showed superior growth and FCR. This enhancement may be attributed to the ability of the BFS to enhance water quality by converting the ammonia from its toxic form into biofloc **(Avnimelech, 2009; Bossier & Ekasari, 2017)**. Additionally, it can be ascribed to the enhancement of the health situation of fish raised in the BFS by the production of floc, which functions as a natural probiotic. Floc comprises beneficial microbes and their cell components that encourage growth and boost immunity **(Crab, 2010; Khanjani *et al*., 2020)**.

Moreover, higher floc intake as a natural diet enhanced with microbial protein, lipid, carbohydrate, and ash contents can be responsible for the enhanced growth and FCR of fish raised in the BFS, especially at high SD (100 fish/m^3). These components aid in the fingerlings' survival and growth **(Crab *et al*., 2010; Das *et al*., 2022; Pellegrin *et al*., 2022)**. The improved FCR in the BFS may be linked to an increment in digestive enzyme activities. Fish obtain exogenous enzymes from the microbes present in the floc, which ultimately enhances feed efficiency **(Xu & Pan, 2012; Das *et al*., 2022)**. Nonetheless, some research indicates that SD has no impact on the FCR and SGR of the Nile tilapia raised in the BFS **(Lima *et al*., 2018)**. The small size of the Nile tilapia fingerlings employed in these trials may be the cause of this lack of influence.

Hematological markers are important indicators for assessing the physiological condition and stress of fish **(Zafar *et al*., 2022)** and can reflect the nutritional and environmental circumstances **(Yu *et al*., 2023)**. Numerous studies have shown that high SD has a deleterious impact on the Nile tilapia's hematological parameters **(Mehrim, 2009; Telli *et al*., 2014; Mehrim *et al*., 2017)**. This study shows a significant rise in hematological parameters in fish reared in the BFS, as well as those cultured at high SD. This increase is due to the stress caused by the increase in SD, as indicated by elevated CORT levels (as an indicator of stress). As a response to this stress, fish increase their RBCs, Hb, and PCV to fulfill the increased demand for oxygen **(Das *et al*., 2022)**. This increase in these characteristics is most noticeable in fish cultivated in the BFS, especially at high SD, because fish raised in the BFS require more oxygen to deal with density stress as well as fish growth than fish raised in the TRS. RBC, WBC, Hb, and PCV increases have been noted in the juvenile great sturgeon, *Huso huso* **(Aghabarari *et al*., 2021)** and the peacock cichlids, *Aulonocara* sp. reared in the BFS **(Mahalakshmi *et al*., 2024)**. Fish cultured in the BFS have been found to possess improved

immunostimulatory qualities due to the unique composition of the flocs. Floc contains some materials like carotenoids and phytosterols, which may contribute to enhancing the general health of the cultured fish **(Crab *et al*., 2010)**. **Babin *et al*. (2010)** postulated that carotenoids have probiotic benefits that may improve the health of fish, boost animal immunity, raise stress lenience, and carry out an antioxidant function. The variety of bacteria in the BFS may function as probiotics, enhancing fish physiological and metabolic reactions, antioxidant status, and illness resistance according to **Ekasari *et al*. (2014)**.

Fish utilize several chemical and physiological adaptations to manage stress when they are subjected to environmental stressors like crowding conditions. These changes are indicators of the degree of stress **(Iwama *et al*., 1998)**. **Dawood *et al*. (2020)** have identified GLU and CORT as bio-indicators of stress, particularly crowding stress in the Nile tilapia. The study's findings showed that CORT levels increased with increasing SD in the TRS compared to the BFS. CORT levels have been used as an indicator of crowding stress in many fish species **(Refaey *et al*., 2018; Dawood *et al*., 2020)**. However, CORT levels significantly decreased in fish raised in the BFS compared to the TRS, particularly in the case of fish SD of 100 fish/m^3. This suggested that the BFS could mitigate the deleterious effects of increased SD. Chronic stress results from increasing SD leads to the depletion of glycogen reserves, resulting in a drop in GLU levels **(Olsen *et al*., 2002)**. As demonstrated in the current investigation, a significant reduction in GLU levels in both the BFS and with increasing SD. Additionally, the like pattern was noted by **Liu *et al*. (2018)** in *O. niloticus* cultured in a BFS. Changes in GLU levels are a normal response to stress and serve as a measure of the metabolism's mobilization to generate additional energy **(Barton & Iwama, 1991)**. **Liu *et al*. (2018)** observed significantly decreased CORT and GLU levels in the Nile tilapia reared at high SD in the BFS, which demonstrated that the BFS has a protective effect against stress.

It wasobserved that fish bred at high SD had significantly higher TCHO and TG levels, but the BFS witnessed a significant decrease compared to the TRS. This increase is due to the fact that fish cultivated at high SD are exposed to crowding stress, which requires more energy to reach equilibrium. **Menezes *et al*. (2015)** showed that the stress resulting from high SD stimulates lipid metabolism, leading to increasing levels of TCHO and TG. Other fish species, including the channel catfish, *Ictalurus punctatus* **(Refaey *et al*., 2018)**, the juvenile blunt-snout bream, *Megalobrama ambylcephala* **(Yadata *et al*., 2020)**, adn peacock cichlid, *Aulonocara* sp. **(Mahalakshmi *et al*., 2024)**, have also been found to have a parallel response. The decline in TCHO and TG in fish stored at high SD under the BFS may be related to the biofloc technology itself, which reduces stress on the fish as observed through reduced cortisol and glucose (as bioindicators of stress). **Das *et al*. (2022)** have reported that fish raised in the BFS and subjected to crowding stress have elevated activities of carbohydrate metabolic enzymes, including lactate dehydrogenase and malate dehydrogenase. To meet the increased energy demand needed to handle

stress, this may initiate the Krebs cycle and change the anaerobic route **(Chatterjee *et al*., 2006; Kumar *et al.*, 2011)**.

Increased SD led to negative changes in flesh quality **(Refaey *et al.*, 2018; Wu *et al.*, 2018)**. In addition, many researches have revealed the harmful effect on the flesh quality of fish exposed to stress **(Refaey *et al.*, 2017)**. This is reliable with the findings obtained in this study, which show a significant rise in both DL and FLR with increasing SD, as well as in fish cultivated in the TRS compared to the BFS. In this context, **Bjørnevik and Solbakken (2010)** stated that the augmentation in DL and FLR is disapproved by industries and consumers. Fish exposed to stress causes a decrease in muscle pH **(Jørpeland *et al*., 2015; Refaey *et al.*, 2017)**, which reduces its water-holding capacity and rises water loss. Furthermore, **Refaey *et al.* (2018)** indicated that *I. punctatus* stocked at high SD causes a reduction in muscular bundle area and an increase in intramuscular connective tissue, reducing flesh quality. Furthermore, results showed that fish rearing in the BFS enhanced flesh quality (decreased DL and FLR), especially in fish stocked at 100 fish/m^3. This indicated the positive effect of the BFS that diminishes the undesirable effect of high SD. This enhancement may be related to improving the health of fish reared in the BFS.

CONCLUSION

Generally, increasing SD has a negative effect on the Nile tilapia. However, it has been observed that fish raised in a biofloc system (BFS) tend to show an improved growth performance, hematological parameters, flesh quality, and a notable reduction in serum cortisol levels, especially when stocked at high densities (100 fish/m^3). These findings suggest that the BFS can mitigate the adverse effects of high density, thereby promoting the overall health of fish. This implies that using biofloc to increase fish production and produce a nutrient-rich product is a feasible approach.

REFERENCES

Aghabarari, M.; Abdali, S. and Yousefi Jourdehi, A. (2021). The effect of Biofloc system on water quality, growth and hematological indices of Juvenile great sturgeon (*Huso huso*). Iran. J. Fish. Sci. 20, 1467–1482.

Ahmad, I.; Babitha Rani, A.M.; Verma, A.K. and Maqsood, M. (2017). Biofloc technology: an emerging avenue in aquatic animal healthcare and nutrition. Aquac. Int. 25, 1215–1226.

Ahmed, S.A.A.; Ibrahim, R.E.; Farroh, K.Y.; Moustafa, A.A.; Al-Gabri, N.A.; Alkafafy, M. and Amer, S.A. (2021). Chitosan vitamin E nanocomposite ameliorates the growth, redox, and immune status of Nile tilapia (*Oreochromis niloticus*) reared under different stocking densities. Aquaculture 541, 736804.

APHA (1992). APHA (American Public Health Association). Standard Methods for the Examination of Water and Wastewater. 18th Edition, (APHA), American Water Works Association (AWWA) and Water Pollution Control Federation (WPCF), Washington DC.

Avnimelech, Y. (2009). Biofloc technology: a practical guide book. World Aquaculture Society. ISBN (Hardback): 978-1-888807-16-5

Azim, M. E. and Little, D.C. (2008). The biofloc technology (BFT) in indoor tanks: Water quality, biofloc composition, and growth and welfare of Nile tilapia (*Oreochromis niloticus*). Aquaculture 283, 29–35. https://doi.org/10.1016/j.aquaculture.2008.06.036

Babin, A.; Biard, C. and Moret, Y. (2010). Dietary supplementation with carotenoids improves immunity without increasing its cost in a crustacean. Am. Nat. 176, 234–241.

Barcellos, L.J.G.; Nicolaiewsky, S.; De Souza, S.M.G. and Lulhier, F. (1999). The effects of stocking density and social interaction on acute stress response in Nile tilapia *Oreochromis niloticus* (L.) fingerlings. Aquac. Res. 30, 887–892.

Barton, B.A. and Iwama, G.K. (1991). Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annu. Rev. fish Dis. 1, 3–26.

Bjørnevik, M. and Solbakken, V. (2010). Preslaughter stress and subsequent effect on flesh quality in farmed cod. Aquac. Res. 41, e467--e474.

Bossier, P. and Ekasari, J. (2017). Biofloc technology application in aquaculture to support sustainable development goals. Microb. Biotechnol. 10, 1012–1016.

Bosworth, B.G.; Small, B.C. and Mischke, C. (2004). Effects of transport water temperature, aerator type, and oxygen level on channel catfish *Ictalurus punctatus* fillet quality. J. World Aquac. Soc. 35, 412–419.

Chatterjee, N.; Pal, A.K.; Das, T.; Mohammed, M.S.; Sarma, K.; Venkateshwarlu, G. and Mukherjee, S.C. (2006). Secondary stress responses in Indian major carps *Labeo rohita* (Hamilton), *Catla catla* (Hamilton) and *Cirrhinus mrigala* (Hamilton) fry to increasing packing densities. Aquac. Res. 37, 472–476.

Cohen, J.M.; Samocha, T.M.; Fox, J.M.; Gandy, R.L. and Lawrence, A.L. (2005). Characterization of water quality factors during intensive raceway production of juvenile *Litopenaeus vannamei* using limited discharge and biosecure management tools. Aquac. Eng. 32, 425–442.

Crab, R. (2010). Bioflocs technology: an integrated system for the removal of nutrients and simultaneous production of feed in aquaculture. PhD Thesis, Faculty of Bioscience Engineering, Ghent University.

Crab, R.; Chielens, B.; Wille, M.; Bossier, P. and Verstraete, W. (2010). The effect of different carbon sources on the nutritional value of bioflocs, a feed for *Macrobrachium rosenbergii* postlarvae. Aquac. Res. 41, 559–567.

Das, P.C.; Ayyappan, S. and Jena, J. (2005). Comparative changes in water quality and role of pond soil after application of different levels of organic and inorganic inputs. Aquac. Res. 36, 785–798.

Das, P.C.; Nayak, A.; Sarkar, S.; Choudhary, P.; Kumari, R. and Mohanty, S. (2022). Growth performance and immune responses of pengba (*Osteobrama belangeri*) during high-density fingerling rearing in biofloc system. Aquac. Res. 53, 6378–6388. https://doi.org/https://doi.org/10.1111/are.16111

Dawood, M.A.O.; Metwally, A.E.-S.; El-Sharawy, M.E.; Atta, A.M.; Elbialy, Z.I.; Abdel-Latif, H.M. and Paray, B.A. (2020). The role of β-glucan in the growth, intestinal morphometry, and immune-related gene and heat shock protein expressions of Nile tilapia (*Oreochromis niloticus*) under different stocking densities. Aquaculture 523, 735205.

Decie, S.I.V. and Lewis S.M. (2006). Practical hematology. 10th Edn, Churchill Livingstone, London. ISBN: 13: 978- 443,.

Doumas, B.T. and Biggs, H.G. (1972). In: Cooper, G.R. (Ed.), Determination of Serum Albumin in Standard Method of Clinical Chemistry.Academic Press, New York., in: Standard Methods of Clinical Chemistry. Elsevier, pp. 175–188.

Doumas, B.T.; Watson, W.A. and Biggs, H.G. (1971). Albumin standards and the measurement of serum albumin with bromcresol green. Clin. Chim. acta 31, 87–96.

Ekasari, J.; Azhar, M.H.; Surawidjaja, E.H.; Nuryati, S.; De Schryver, P. and Bossier, P. (2014). Immune response and disease resistance of shrimp fed biofloc grown on different carbon sources. Fish Shellfish Immunol. 41, 332–339.

Ellefson, R.D. and Caraway, W.T. (1976). Fundamentals of clinical chemistry. In: Tietz, N.W., Saunders, W.B. (Eds.), Lipids and Lipoproteins, Ed Tietz NW.

FAO (2024). The State of World Fisheries and Aquaculture 2024. Blue Transformation In Action. Rome, FAO.

Ferdous, Z.; Masum, M.A. and Ali, M.M. (2014). Influence of stocking density on growth performance and survival of monosex tilapia (*Oreochromis niloticus*) fry. Int. J. Res. Fish. Aquac. 4, 99–103.

Haridas, H.; Verma, A.K.; Rathore, G.; Prakash, C.; Sawant, P.B. and Babitha Rani, A.M. (2017). Enhanced growth and immuno-physiological response of Genetically Improved Farmed Tilapia in indoor biofloc units at different stocking densities. Aquac. Res. 48, 4346–4355.

Henry, R.J. (1964). Clinical chemistry: principles and technics. New-York Hoeber Med. Div. Harper Row.

Iwama, G.K.; Afonso, L.O.B. and Vijayan, M.M. (1998). Stress in fish. Ann. York Acad. Sci. 851, 304–310.

Jørpeland, G.; Imsland, A.; Stien, L.H.; Bleie, H. and Roth, B. (2015). Effects of filleting method, stress, storage and season on the quality of farmed Atlantic cod (*Gadus morhua* L.). Aquac. Res. 46, 1597–1607.

Khanjani, M.H.; Alizadeh, M. and Sharifinia, M. (2020). Rearing of the Pacific white shrimp, *Litopenaeus vannamei* in a biofloc system: The effects of different food sources and salinity levels. Aquac. Nutr. 26, 328–337.

Khanjani, M.H.; Mozanzadeh, M.T.; Sharifinia, M. and Emerenciano, M.G.C. (2023). Biofloc: A sustainable dietary supplement, nutritional value and functional properties. Aquaculture 562, 738757.

Khanjani, M.H.; Zahedi, S. and Mohammadi, A. (2022). Integrated multitrophic aquaculture (IMTA) as an environmentally friendly system for sustainable aquaculture: functionality, species, and application of biofloc technology (BFT). Environ. Sci. Pollut. Res. 29, 67513–67531.

Kpundeh, M.D.; Xu Pao X.P.; Yang Hong, Y.H.; Qiang Jun, Q.J. and He Jie, H.J. (2013). Stocking densities and chronic zero culture water exchange stress' effects on biological performances, hematological and serum biochemical indices of gift tilapia juveniles *(Oreochromis niloticus)*. J. Aquac. Res. Development, 4(189), 2. DOI: 10.4172/2155-9546.1000189

Kumar, S.; Sahu, N.P.; Pal, A.K.; Subramanian, S.; Priyadarshi, H. and Kumar, V. (2011). High dietary protein combats the stress of *Labeo rohita* fingerlings exposed to heat shock. Fish Physiol. Biochem. 37, 1005–1019.

Lima, P.C.M.; Abreu, J.L.; Silva, A.E.M.; Severi, W.; Gálvez, A.O. and Silva, L.O.B. (2018). Nile tilapia fingerling cultivated in a low-salinity biofloc system at different stocking densities. Spanish J. Agric. Res. 16, 9.

Liu, G.; Ye, Z.; Liu, D.; Zhao, J.; Sivaramasamy, E.; Deng, Y. and Zhu, S. (2018). Influence of stocking density on growth, digestive enzyme activities, immune responses, antioxidant of *Oreochromis niloticus* fingerlings in biofloc systems. Fish Shellfish Immunol. 81, 416–422. https://doi.org/https://doi.org/10.1016/j.fsi.2018.07.047

M'balaka, M.; Kassam, D. and Rusuwa, B. (2012). The effect of stocking density on the growth and survival of improved and unimproved strains of *Oreochromis shiranus.* Egypt. J. Aquat. Res. 38, 205–211.

LingQiao, M.; Qi, C.; JingJing, C. and DaPeng, L. (2014). Comparative study on muscle texture profile and nutritional value of channel catfish (*Ictalurus punctatus*) reared in ponds and reservoir cages. J. Fish. China 38, 532–537.

Mahalakshmi, S.; Antony, C.; Ravaneswaran, K.; Uma, A. and Somu Sunder L.R. (2024). Influence of biofloc and stocking density on physiological responses and disease resistance in peacock cichlid (*Aulonocara* sp). Aquaculture 592, 741168. https://doi.org/https://doi.org/10.1016/j.aquaculture.2024.741168

Mansour, A.T.; Fayed, W.M.; Alsaqufi, A.S.; Aly, H.A.; Alkhamis, Y.A. and Sallam, G.R. (2022). Ameliorative effects of zeolite and yucca extract on water quality, growth performance, feed utilization, and hematobiochemical parameters of European seabass reared at high stocking densities. Aquac. Reports 26, 101321.

Mehrim, A.I. (2009). Effect of dietary supplementation of Biogen®(Commercial probiotic) on mono-sex Nile tilapia *Oreochromis niloticus* under different stocking densities. J Fish Aquat Sci 4, 261–273.

Mehrim, A.I.; Khalil, F.F.; Refaey, M.M. and Abdalqadir, A.M. (2017). Impacts of Pro-Grow®on growth performance, physiological, immune responses and economic efficiency of adult *Oreochromis niloticus* (Linnaeus, 1758) under stocking density stress. Sci. Int 5, 113–126.

Menezes, C.; Ruiz-Jarabo, I.; Martos-Sitcha, J.A.; Toni, C.; Salbego, J.; Becker, A.; Loro, V.L.; Martínez-Rodríguez, G.; Mancera, J.M. and Baldisserotto, B. (2015). The influence of stocking density and food deprivation in silver catfish (*Rhamdia quelen*): a metabolic and endocrine approach. Aquaculture 435, 257–264.

Moniruzzaman, M.; Uddin, K.B.; Basak, S.; Mahmud, Y.; Zaher, M. and Bai, S.C. (2015). Effects of stocking density on growth, body composition, yield and economic returns of monosex tilapia (*Oreochromis niloticus* L.) under cage culture system in Kaptai Lake of Bangladesh. J. Aquac. Res. Dev 6, 357–363.

Oliveira, L.K.; Wasielesky Jr, W. and Tesser, M.B. (2022). Fish culture in biofloc technology (BFT): Insights on stocking density carbon sources, C/N ratio, fish nutrition and health. Aquac. Fish. 9, 522–533.

Olsen, R.E.; Sundell, K.; Hansen, T.; Hemre, G.-I.; Myklebust, R.; Mayhew, T.M. and Ringø, E. (2002). Acute stress alters the intestinal lining of Atlantic salmon, *Salmo salar* L.: An electron microscopical study. Fish Physiol. Biochem. 26, 211–221.

Paterson, B.D.; Rimmer, M.A.; Meikle, G.M. and Semmens, G.L. (2003). Physiological responses of the Asian sea bass, *Lates calcarifer* to water quality deterioration during simulated live transport: acidosis, red-cell swelling, and levels of ions and ammonia in the plasma. Aquaculture 218, 717–728.

Pellegrin, L.; Nitz, L.F.; Pinto, D. de S.B.; Copatti, C.E.; Wasielesky, W. and Garcia, L. (2022). Effects of suspended solids in the survival and haematological parameters of pacu juveniles (*Piaractus mesopotamicus*) in a biofloc technology culture system. Aquac. Res. 53, 276–284.

Piedrahita, R.H. (2003). Reducing the potential environmental impact of tank aquaculture effluents through intensification and recirculation. Aquaculture 226, 35–44.

Refaey, M.M.; Li, D.; Tian, X.; Zhang, Z.; Zhang, X.; Li, L. and Tang, R. (2018). High stocking density alters growth performance, blood biochemistry, intestinal histology, and muscle quality of channel catfish *Ictalurus punctatus*. Aquaculture 9, 522–533. https://doi.org/10.1016/j.aquaculture.2018.04.003

Refaey, M.M.; Tian, X.; Tang, R. and Li, D. (2017). Changes in physiological responses, muscular composition and flesh quality of channel catfish *Ictalurus punctatus* suffering from transport stress. Aquaculture 478, 9–15.

Ridha, M.T. (2006). Comparative study of growth performance of three strains of Nile tilapia, *Oreochromis niloticus*, L. at two stocking densities. Aquac. Res. 37, 172–

179.

Schneider, O.; Blancheton, J.P.; Varadi, L.; Eding, E.H. and Verreth, J.A.J. (2006). Cost price and production strategies in European recirculation systems, in: Conference: Aqua 2006: Linking Tradition & Technology Highest Quality for the Consumer, Firenze (Florence), Italy, 9-13 May 2006. p. 855.

Shourbela, R.M.; Khatab, S.A.; Hassan, M.M.; Van Doan, H. and Dawood, M.A.O. (2021). The effect of stocking density and carbon sources on the oxidative status, and nonspecific immunity of Nile tilapia (*Oreochromis niloticus*) reared under biofloc conditions. Animals 11, 184.

Stoskopf, M.K. (2015). Biology and management of laboratory fishes, in: Laboratory Animal Medicine. Elsevier, pp. 1063–1086.

Telli, G.S.; Ranzani-Paiva, M.J.T.; de Carla Dias, D.; Sussel, F.R.; Ishikawa, C.M. and Tachibana, L. (2014). Dietary administration of Bacillus subtilis on hematology and non-specific immunity of Nile tilapia *Oreochromis niloticus* raised at different stocking densities. Fish Shellfish Immunol. 39, 305–311.

Tietz, N.W. (1995). Clinical guide to laboratory tests, in: Clinical Guide to Laboratory Tests. p. 1096.

Wu, F.; Wen, H.; Tian, J.; Jiang, M.; Liu, W.; Yang, C.; Yu, L. and Lu, X. (2018). Effect of stocking density on growth performance, serum biochemical parameters, and muscle texture properties of genetically improved farm tilapia, *Oreochromis niloticus*. Aquac. Int. 26, 1247–1259.

Xu, W.-J. and Pan, L.-Q. (2012). Effects of bioflocs on growth performance, digestive enzyme activity and body composition of juvenile *Litopenaeus vannamei* in zero-water exchange tanks manipulating C/N ratio in feed. Aquaculture 356, 147–152.

Yadata, G.W.; Ji, K.; Liang, H.; Ren, M.; Ge, X. and Yang, Q. (2020). Effects of dietary protein levels with various stocking density on growth performance, whole body composition, plasma parameters, nitrogen emission and gene expression related to TOR signaling of juvenile blunt snout bream (*Megalobrama ambylcephala*). Aquaculture 519, 734730.

Yu, Y.-B.; Choi, J.-H.; Lee, J.-H.; Jo, A.-H.; Lee, K.M. and Kim, J.-H. (2023). Biofloc technology in fish aquaculture: A review. Antioxidants 12, 398.

Zafar, M.A.; Talha, M.A. and Rana, M.M. (2022). Effect of biofloc technology on growth performance, digestive enzyme activity, proximate composition, and hematological parameters of Asian stinging catfish (*Heteropneustes fossilis*). J. Appl. Aquac. 34, 755–773.

Zenhom, O.A.; Refaey, M.M.; Ayyat, A.N. and El-Sayed, H.G. (2020). Impact of natural zeolite on growth performance, feed efficiency, and carcass composition of Nile tilapia (*Oreochromis niloticus* L.) cultured at different stocking densities. Abbassa Int. J. Aqua 13, 191–210.