DEPIK
Jurnal Ilmu-Ilmu Perairan, Pesisir dan Perikanan
Journal homepage: www.jurnal.usk.ac.id/depik

Effect of microalgae (*Tetraselmis chui* Butcher 1959) on growth and survival of nile tilapia (*Oreochromis niloticus* Linnaeus 1758) reared in low salinity biofloc system

Wa Iba[1,2]*, Neneng Wahyuni[1], Muhaimin Hamzah[1], Michael A. Rice[2]

[1] *Department of Aquaculture, Faculty of Fisheries and Marine Sciences, University of Halu Oleo, Kendari, Indonesia.*
[2] *Department of Fisheries, Animal and Veterinary Sciences, College of Environmental and Life Sciences, University of Rhode Island, Kingston, USA*

ARTICLE INFO

Keywords:
Saline
Biofloc
Microalgae

DOI: 10.13170/depik.13.1.36981

ABSTRACT

Addition of microalgae in the biofloc system may improve growth and survival of Nile tilapia. Also, culturing Nile tilapia in saline water biofloc system may be an adaptation for freshwater shortage. In this study, the growth and survival of Nile tilapia cultured in a low salinity biofloc system with added microalgae was investigated. Increasing salinity in the culture system was done by gradually adding sea water into acclimatization container (618 L, v) on weekly basis for 4 weeks until 9 psu salinity was reached. Tilapia were allowed to adapt to salinity change for one week and fed with commercial feed twice a day until satiation. The biofloc experiment was conducted with 3 microalgal density treatments (5, 15 and 25 × 10^4 cells. mL^{-1}) in triplicates. Average initial weight of experimental fish was 4.82 ± 0.76 g. Tilapia were reared for 40 days in aquaria (35 × 35 × 40 cm, l × w × h, 15 L, v) containing 8 individuals fish in each aquarium. The commercial pellets were delivered twice per day at 5% of tilapia biomass until satiation. *T. chui* was added to the tilapia culture every week. This study showed that the addition of microalgae did not affect the fish weight gain, specific growth rate and survival ($p>0.05$). However, feed conversion ratio and culture productivity were better in tilapia supplied with weekly addition of *T. chui* at 15 × 10^4 cells mL^{-1} compared to other densities tested.

Introduction

Nile tilapia is one of the leading aquaculture commodities with an increasing market demand. This species has been widely cultured partially because of their ability to adapt to environmental conditions with a wide range of salinity (El-Sayed, 2006). Culturing tilapia in brackish or saline water has been successful (El-Sayed, 2006; Rimmer *et al.*, 2012; Rahman *et al.*, 2021; Dawood *et al.*, 2022) and maybe a practice that can be applied to adapt with freshwater shortages and changing in environmental conditions due to climate change (Rahman *et al.*, 2021; Dawood *et al.*, 2022). Moreover, culturing Nile tilapia at high density to increase production has been made possible using biofloc technology (Avnimelech, 1999; Assaduzaman *et al.*, 2009; Fimbres-Acedo *et al.*, 2020; Manduca *et al.*, 2020; Khanjani and Sharifinia, 2020)

Biofloc is a general term for the activated sludge which is adopted from the biological waste water culture system by maintaining adequate levels of oxygen, microorganisms, and C/N ratios (Avnimelech, 1999; Putra *et al.*, 2019; Khanjani and Sharifinia, 2020) and also conserve water, thus minimizing environmental impacts (Manduca *et al.*, 2020; Silva *et al.*, 2022). The system is considered very cheap, simple, environmentally friendly and has very high productivity (Crab *et al.*, 2012; Taw, 2014; Mohammadi *et al.*, 2021). The formation of biofloc by microorganisms, especially heterotrophic bacteria, generally aims to increase nutrient utilization, avoid environmental stress, and predation (De Schryver *et al.*, 2008; Mohammadi *et al.*, 2021). The bacterial floc is composed of a mixture of various types of micro-organisms (floc-forming bacteria, filamentous bacteria, fungi and microalgae), suspended particles,

* Corresponding author.
Email address: wa.iba@uho.ac.id

p-ISSN 2089-7790; e-ISSN 2502-6194
Received 13 February 2024; Received in revised form 15 March 2024; Accepted 27 April 2024
Available online 30 April 2024

various colloids and organic polymers, various cations and dead cells (De Schryver *et al.,* 2008) with sizes varying in the range of 100 - 1000 µm (Azim *et al.*, 2007; De Schryver *et al.*, 2008; Mohammadi *et al.*, 2021). Also, the composition of microbes in the floc affects the biofloc structure and nutrient content (Pacheco-Vega *et al.*, 2018; Angela *et al.*, 2021). Moreover, Ju *et al.* (2008) reported that biofloc dominated by bacteria and green microalgae contained higher protein (38 and 42%) than diatom-dominated biofloc (26%) that in turn affected the growth and survival of culture animals.

The function of microalgae in the floc is to assimilate inorganic carbon, nitrate and phosphate (Angela *et al.*, 2021). Addition of microalgal biomass to the bio-floc system aids in maintaining water quality and may therefore increase growth and survival of Nile tilapia. Several microalgae species that belong to Chlorophytes or green microalgae have been identified as components of some biofloc systems, including *Chlorella* sp. and *Schizochytrium* sp. (Schrader *et al.*, 2011; Yunos *et al.*, 2017; Llario *et al.*, 2019). Moreover, the density of microalgae in the floc has been known to also affect the quality of the biofloc (Yunos *et al.*, 2017; Araujo *et al.*, 2019). Therefore it is probable that adding microalgae cells regularly to the system may increase the biofloc quality (Angela *et al.*, 2021; Silva *et al.*, 2022). Several microalgae species has been added to the bioflocs system include *Chlorella* sp., *Ankistrodesmus* sp., *Chlorella vulgaris, Spirulina platensis* and *Scenedesmus obliquus* (Lima *et al.,* 2019; Rijal *et al.*, 2020; Angela *et al.*, 2021; Silva *et al.*, 2022). These species is considerably smaller cells size compared except for filamentous cyanobacteria, *S. platensis*, to *T. chui* (Iba, 2016). *T. chui* is a green microalgae that can tolerate a wide range of salinity up to 35 psu (Iba *et al.*, 2018) and has been used in aquaculture as live feed for various species include fish larvae, shrimp and bivalves (Iba *et al.*, 2014). Despite being widely used in hatcheries as live feed, the effect of addition of a green microalgae *T. chui* in low salinity biofloc culture system on growth and survival of Nile tilapia has not been investigated, so this is the aim of the present study.

Materials and Methods

Experimental Animals and Bio-floc Preparation

Tetraselmis chui initial stock was obtained from Brackish Aquaculture Research Center at Takalar, South Sulawesi and were cultured at the Productivity and Aquatic Environment Laboratory Faculty of Fisheries and Marine Sciences (FFMS) Halu Oleo University, Kendari, South East Sulawesi and Nile tilapia juveniles were reared in brackish bio-floc system at the Hatchery and Production Unit Laboratory that are associated with the teaching laboratory at the FFMS.

Salinity acclimatization for tilapia juveniles (4 ± 1 cm average length) was carried out in a 618 L fiberglass aquaculture tank. Water salinity was increased weekly by gradually and slowly adding of seawater using an infusion hose into the container. Salinity was measured after the addition of seawater and the tilapia were allowed to adapt for one week. During the acclimatization process, tilapia was fed on commercial feed (Prima Feed with 39-41 % crude protein) twice a day until satiation. Water salinity reached 9 psu after one month of acclimatization and tilapias were moved into aquaria (35 × 35 × 40 cm, 15 L), (w × l × h, v) containing brackish (9 psu) biofloc system with aeration. The aquaria were sterilized prior experimental culture using chlorine and detergent and then rinsed with fresh water and air-dried for two days.

The low salinity biofloc system was set up according to method by Putra *et al.*, (2019) by adding 280 µL of EM4 (Effective Microorganism, http://emindonesia.com) containing *Lactobacillus* sp., *Rhodopseudomonas* sp., *Actinomycetes* sp, *Streptomyces* sp., and yeast, 10 mL of molasses and different initial densities of *T. chui* to each aquaria at 5, 15 and 25 × 10^4 cells.mL^{-1} or BFT_5, BFT_{15}, BFT_{25}, respectively, in triplicates. The aquarium containing bioflocs were covered using aluminum foils, aerated and incubated for 14 days before stocked with acclimatized tilapias (5 ± 1 cm average length, 4 ± 1 g average weight, 8 individual fish per aquaria). The culture experiment was conducted for 40 days. The fish was fed with the same commercial feed as in the conditioning diet twice a day (morning, 8 AM and afternoon, 5 PM) at ad libitum/satiation (Putra *et. al.*, 2020).

Microalgae culture

T. chui was cultured semi continuously in 3 L plastic bottles with culture volume of 2 L containing filtered and sterilized seawater using Walne's concentrated culture media. The culture bottles filled with *T. chui* stock with an initial density of 10 × 10^4 cells.mL^{-1} were then placed into the culture rack equipped with white fluorescence light (1000-1500 lux) and 12:12 photoperiod. Microalgae was harvested at late log or at the onset of stationary phase culture at day 7 based on our previous study by calculating the cells density using an improved Neubauer hemocytometer under a light microscope. Microalgal density was calculated every harvest time to determine the volume to be added to the biofloc system according to the treatment using the following equation:

$$V2 = \frac{C1C2}{V1}$$

when, C refers to cell concentrations in tanks 1 or 2 and V is the volume (Anggraini *et al.*, 2022)

Growth and Survival of Nile Tilapia

Growth of Nile tilapia (W) or weight gain was calculated based on the difference between initial and final weight (g) whereas the specific growth rate (SGR) was calculated as the percentage of weight gain differences over the culture period (Silva *et al.*, 2022) as follows:

$$W = Wt - Wo$$

$$\text{SGR} = \frac{lnWt - lnWo}{t}\ x\ 100\ \%$$

when, W_t is the weight at time (t) and W_o is weight at time zero.

The survival of the cultured Nile tilapia was calculated as percentage of survived fish at the end of experiment (Silva *et al.*, 2022) using the following equation:

$$\text{SR} = \frac{Nt}{No}\ x\ 100\ \%$$

when N_t = Fish alive at time t and N_o is the initial number of fish.

Culture productivity and feed conversion ratio

Culture productivity was calculated as the biomass gain of the cultured fish in each *T. chui* addition for every cubic liter of biofloc system ($kg.m^{-3}$) (Araujo *et al.*, 2019). Feed Conversion Ratio (FCR) was calculated using the following equation (Silva *et al.*, 2022):

$$\text{FCR} = \frac{Feed\ Consumed\ (g)}{AverageWeightGain(g)}$$

Water quality

Temperature and salinity were measured at the beginning of the experiment and maintained to be constant throughout the study period. Whereas pH, dissolved oxygen, ammonia and nitrate level in the culture system were measured at the beginning and at the end of the study (Table 1).

Table 1. Water quality parameters observed during this study.

No	Parameters	Unit	Methods
1.	Temperature	°C	Thermometer
2.	Salinity	psu/ppt	Hand refractometer
3.	pH	-	pH meter
4.	DO	$mg.L^{-1}$	Winkler Titration
5.	Ammonia	$mg.L^{-1}$	Spectrophotometer
6.	Nitrate	$mg.L^{-1}$	Spectrophotometer

Data analysis

All data except water quality were analyzed using one way ANOVA in SPSS for Windows 16.0 version. The data showed a significant level ($p<0.05$) were subjected to Duncan post-hoc test.

Results

Growth, survival and culture productivity

There was no mortality of Nile tilapia juveniles observed during the study period. Average weight gain of Nile tilapia was 6.04-6.57 g with no significant effect of *T. chui* addition on weight gain ($p>0.05$) and specific growth rate ($p>0.05$). FCR of Nile tilapia was tend to get better when cultured in a biofloc system with weekly addition of 15×10^4 cells· mL^{-1} of *T. chui* at 1.09 (Table 2, p> 0.05).

Table 2. Growth parameters of *O. niloticus* cultured in low salinity biofloc system with *T.chui* addition (mean ± SD).

	T. chui addition				
Parameter	**BFT**	**BFT_5**	**BFT_{15}**	**BFT_{25}**	**p-value**
Initial weight (g)	5.12 ± 0.63	4.80 ± 0.61	4.87 ± 0.77	4.51 ± 0.86	0.045
Final weight (g)	11.30 ± 0.76	10.83 ± 0.83	11.34 ± 0.98	11.08 ± 0.91	0.190
Weight gain (g)	6.18 ± 0.99	6.04 ± 1.12	6.47 ± 0.90	6.57 ± 1.44	0.355
SGR ($\%.day^{-1}$)	2.00 ± 0.38	2.05 ± 0.42	2.14 ± 0.36	2.24 ± 0.58	0.144
SR (%)	100	100	100	100	
FCR	1.48 ± 0.11	1.50 ± 0.11	1.09 ± 0.11	1.50 ± 0.11	0.154
Biomass gain (g)	49.65 ± 4.54	48.31 ± 3.14	51.74 ± 1.71	49.65 ± 2.05	0.526
Culture Productivity ($kg.m^{-3}$)	3.31 ± 0.30	3.22 ± 0.21	3.45 ± 0.11	3.50 ± 0.14	0.528

Water quality

All water quality parameters measured in this study were still in the range that can be tolerated by tilapias. Temperature remained stable throughout the study at 29 °C close to the upper limit of optimal temperature for tilapia culture similar to pH (Table 3).

Depik Jurnal Ilmu-Ilmu Perairan, Pesisir dan Perikanan
Volume 13, Number 1, Page 161-167

Table 3. Water quality parameters during study period.

Parameters	Observed	Optimal	Ref.
pH	7	6.8-8.0	Emerenciano *et al.* (2017)
Temperature (°C)	29	25-31	Emerenciano *et al.* (2017)
DO ($mg.L^{-1}$)	7.8 and 10.1	≥4	Emerenciano *et al.* (2017)
Nitrate ($mg.L^{-1}$)	0.043 and 0.052	< 20	Avnimelech *et al.* (2015)
Ammonia ($mg.L^{-1}$)	0.05 and 0.06	0.02–0.05	Avnimelech *et al.* (2015)
Salinity (ppt)	9	1.84–8.04	Dawood *et al.* (2022)

Discussion

The survival of tilapia juveniles in this study was better than the study by Putra *et al.* (2019), and Menduca *et al.* (2020) who obtained 61-84 % and 33.75 %, respectively. This difference may be attributed to the presence of *T. chui* and probiotic bacteria derived from EM4. Different microalgae species that was added into biofloc systems was known to produce different survival rate. Araujo and co-workers (2019) found that a weekly addition of different densities of *C. vulgaris* into Nile tilapia cultured in biofloc system produced 80-85 % survival. Also, addition of *Chlorella* sp. and *Ankistrodesmus* sp. in biofloc system of cultured giant prawn resulted in >84 % survival which was lower compared to biofloc system without microalgae addition. Moreover, shorter culture period compared to other studies may also play roles in excellent survival in this study. Majority of studies with biofloc system was conducted for 60 days or more which in turn affect water qualities of the zero water exchange system (Menduca *et al.*, 2020; Angela et al., 2021; Araujo *et al.*, 2019). However, study by Putra *et al.* (2019) found that survival of Nile tilapia cultured in biofloc system without microalgae addition for 40 days was 61-92 %, depended on carbon sources used to develop biofloc. This present study suggested that addition of *T. chui* enriched the biofloc microbiome in the biofloc as a good additional feed source for Nile Tilapia juveniles and at the same time improved water quality thus increased survival. Furthermore, the presence of *T. chui* in biofloc might help in reducing the stress of the cultured tilapia thus increasing health status and survival. Microalgae presence in biofloc aid in nitrate reduction in culture water as a result of nitrification of ammonia into nitrite (NO_2) by nitrifying bacteria such as *Nitrobacter* sp. and *Nitrosomonas* sp which further oxidize into nitrate (NO_3) by Nitrobacter and Nitrospira bacteria (Yunos *et al.*, 2017; El Sayed, 2020). The amount of nitrate that can be removed by *Chlorella vulgaris* and *Tetradesmus obliquus*) in *Oreochromis niloticus* culture using RAS at a density of 17 kg/m3in average was 78% and 80%, respectively and phosphate was 97% and 99%, respectively (Tejido-Nuñez *et al.*,2019). Moreover, microalgae are excellent carbon sequester both in form of Co_2 and simple organic carbon derived from dissolved organic matter from aquaculture waste (Yu *et al.*, 2019). Therefore, good water quality and sufficient food provided by biofloc containing microalgae are valuable to Nile tilapia culture.

Although initial weight of tilapia juveniles used in this study was not uniform (Table 2, p>0.05), the weight and biomass gain at the end of experiment were similar (Table 2, p>0.05,). The feed availability due to floc formation supported the growth of tilapia and this could be attributed to almost similar growth of Nile tilapia without mortality. The proteinaceous bacterial and green algae biomass could be utilized by tilapia as an additional food source with good nutrition. Moreover, the composition of organisms in the floc affected the structure and the nutritional content of biofloc. Ju *et al.* (2008) reported that bioflocs dominated by bacteria and green microalgae contained higher protein (38 and 42 % protein) than bioflocs dominated by diatoms (26 %). This present study found that the biofloc formation using molasses, EM4 and microalgae was able to be digested and utilized by cultured fish. Although application of biofloc technology has been shown to make a significant contribution to increasing growth and production of closed system tilapia cultivation (De Schryver et al., 2008; Azim and Little 2008), using *T. chui* as enrichment agent might increase the benefit even further. Ekasari (2014) stated that biofloc that was dominated by green microalgae contained higher protein up to 42 % that might contributed to better growth and survival compared to those with less green microalgae biomass.

The availability of heterotrophic bacteria and yeast from EM4 in biofloc system might also contributed to good growth and survival of Nile tilapia in this study. According to Ferreira *et al.* (2015) the presence of heterotrophic bacteria such as *Bacillus* or *Lactobacillus* that also act as probiotic was able to decompose organic matter in the waters thus provided good water quality. Several studies found that the existence of *Bacillus* spp. in the biofloc system reduced the number of pathogenic bacteria. Combining effects of photosynthesis microalgae, such as *T. chui* in this study, and heterotrophic bacteria from EM4 might improve the immune system of the Nile tilapia and increased their resistant to pathogenic infection, therefore, no mortality was observed during the 40 days culture period. Moreover, the enzymes secreted from these microbes association improved feed ingestion and digestion that in turn increased protein retention and growth (Pérez-Fuentes *et al*., 2018; Huang *et al*., 2019; Kumar *et al*., 2021).

This study confirmed the finding from other studies that Nile tilapia, as omnivorous and filter feeders, was able to utilize the microbial community enrichment from EM4 and *T. chui* associated with biofloc both as nutritious natural feed and may be as enzymes sources for better ingestion and digestion of feed. The low FCR value at 15 × 10^4 cells.mL^{-1} of weekly *T.chui* addition in this study suggested that microalgae might increase the biomass of microbial associations as additional food sources and aid in better nutrient digestion of tilapia. This is in accordance with the study of Mohammadi *et al.* (2021) who found that increased biofloc microbial biomass and diversity directly affected the feed efficiency, immunity thus survival of cultured fish.

However, culture productivity in this current study that ranged from 3.22 to 3.50 kg.m^3 was lower than the study of Araújo et al. (2019) with *C. vulgaris* addition in biofloc system of tilapia and cultured for 63 days that was at 4.33-4.74 kg.m^3. Different size of microalgae species that was used might be the reason for this differences. *C. vulgaris* is a small cell size green microalgae that has an average diameter around 3 µm (Wiel *et al*., 2017) and thus contributed to small size bioflocs that might contain better nutrition particularly those of amino acids and nitrogen retention from biofloc by cultured animal (Ekasari *et al*., 2014) compared to *T. chui* cells that have a larger cells size with an average diameter around 13 µm and an estimated volume of about 260 μm^3 (Iba, 2016; Lima *et al*., 2021). However, small-sized bioflocs contained more abundant candidate pathogens particularly for shrimp (Huang *et al*., 2020) and it may be preferable to maintain the large sized biofloc in culturing Nile tilapia by adding a large size microalgae such a *T. chui* for enrichment of the system.

Culture productivity of Nile tilapia is also affected by culture density and period. High stocking density was negatively correlated with growth performance and yield thus culture productivity (Abaho *et al*., 2020; Kunda *et al*, 2021). Stocking density at 8 fingerlings per aquaria that applied in this study was considered optimal which could converted at about 180-200 fingerlings per m^3 as suggested by Abaho *et al.* (2020) and Fimbres-Acedo *et al.* (2020). Culture period of Nile tilapia is usually 160 to 180 days to reach market size at about 200-500 g per individual fish depending on culture system and feed (Abaho *et al*., 2020; Fimbres-Acedo *et al.,* 2020), although bioeconomic model showed that 225 days of culture with average yield of 450 g per individual fish increased the profit of Nile tilapia producers (Domínguez-May *et al*., 2024). Dividing culture period of Nile tilapia in microalgae based biofloc system into nursery and grow out phase also increased the culture productivity. High density could be applied during nursery phase with up to 200 fish.m^3 for 20 weeks and then low density at 55 fish.m^3 to 40 weeks that reached the final weight of 500 g per individual fish (Fimbres-Acedo *et al*., 2020).

Water quality

The slightly higher salinity than optimal range tested in this study seemed to have no effect on growth parameters and survival of Nile tilapias. Figueiredo *et al.* (2022) found that 2 and 8 psu was the optimal salinity for tilapia cultured in recirculating system without biofloc. Other study by Dawood *et al.* (2022) observed that higher survival of Nile tilapia was at 15 ppt compared to low salinity levels tested in their study although blood glucose level was increased indicating stress in cultured tilapia. A high salinity up to 17 and 20 psu was also used to culture red tilapia with different carbon sources and the survival rate was around 61-92 % (Putra *et al*., 2019 and Bu *et al*., 2021). Salinity was not the limiting factor in this study but carbon sources suggested that salinity up to 20 psu may still be optimal for tilapia culture.

Dissolved oxygen during culture period was above the optimal range suggested for tilapia culture that is ≥4 mg.L^{-1} (Emerenciano *et al*., 2017). The addition of microalgae *T. chui* may contribute to high dissolved oxygen throughout this study. Study by Fimbres-Acedo *et al.* (2020) confirmed that addition of photoautotrophic microalgae (*Chlorella* sp and *C. sorokiniana* 2805) resulted in higher dissolved oxygen during the first 10 days of culture period at 9.2 - 9.3 mg.L^{-1} and then decreased to 5.9 - 6.0 mg.L^{-1} at the end of the experiment. Conversely, in this study dissolved oxygen was higher at the end of experiment indicating that microalgae may aid in the production of oxygen in culture water as by product of their photosynthesis.

Nitrate content during the study was in the range of 0.043 mg.L^{-1} at the beginning of the study and only increased slightly at 0.052 mg.L^{-1} at the end of experiment. Ammonia content at the beginning of the study was 0.047 while at the end was 0.061 mg.L^{-1}. There was no accumulation of both these compounds at the end of the study suggesting that nitrifying bacteria could convert ammonia into nitrite and then nitrate efficiently in this no water exchange biofloc culture system. Based on the water quality parameters, biofloc formation using EM4 and *T. chui* addition in low salinity intensive system with no water exchange was recommended to be used for Nile tilapia production.

Conclusion

The weekly addition of *T. chui* at different densities in intensive-biofloc systems did not affect the survival, weight gain and specific growth rate of tilapia although better feed conversion ratio was found in addition of *T. chui* at 15×10^4 cells·mL^{-1}. Water quality in low salinity and zero water exchange biofloc system with addition of *T. chui* for 40 days was able to be maintained in optimal conditions without compromise the growth and survival of Nile tilapia. Future research needs to be focused on the composition and nutritional value of biofloc with microalgae addition in low to up to 20 psu salinity and zero water exchange biofloc systems using low protein formulated feed.

Acknowledgments

This research was partly funded by Directorate of Higher Education and Research, The Ministry of Education and Culture Republic of Indonesia with contract No. 39/UN29.20/PG/2022. This research is difficult to carry out without the help of volunteer students in field work, so we are grateful to person/people.

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How to cite this paper:
Iba, W., N. Wahyuni, M. Hamzah, M.A. Rice. 2024. Effect of microalgae (Tetraselmis chui Butcher 1959) on growth and survival of nile tilapia (Oreochromis niloticus Linnaeus 1758) reared in low salinity biofloc system. Depik Jurnal Ilmu-Ilmu Perairan, Pesisir dan Perikanan, 13(1): 161-167.