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Contribution of Biofloc System on Growth Performance and Feed Utilization of Tilapia Reared in High Salinity Media

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ABSTRACT

Tilapia strain Srikandi is known to grow quickly in brackish water. However, the best feeding management for efficient production in a marine biofloc system has not been extensively studied. This study aims to evaluate the impact of the biofloc system on the growth performance and feed utilization of tilapia raised in high salinity environments. The experiment involved a completely randomized design with four treatments: control (standard feeding rate in a clear-water system), N (standard feeding rate with a biofloc system), NA (25% reduction in standard feeding rate with a biofloc system), and NB (50% reduction in standard feeding rate with a biofloc system). The standard feeding rate used was 4% of fish biomass, and biofloc was prepared using molasses as a carbon source with a C/N ratio of 10. The experimental fish used were tilapia strain Srikandi (5-5.5 cm), stocked into 16 glass containers with a working volume of 96 L and a stocking density of 20 individuals per container. The fish were fed a commercial floating feed (40% protein content) three times a day for 30 days. The study found that a lower feeding rate resulted in higher weight gain and specific growth rate for tilapia strain Srikandi (8.01 ± 0.44 g; 5.00 ± 0.14 %/day) compared to higher feeding rates in the marine biofloc system ($P<0.05$). A 50% reduction in the standard feeding rate led to the lowest feed conversion ratio (0.38 ± 0.04) and higher protein efficiency ratio, protein retention, and lipid retention (6.62 ± 0.61; 115.56 ± 8.44%; 24.72 ± 13.36%), compared to higher feeding rates in both clear-water and biofloc systems. This suggests that implementing a biofloc system in marine tilapia farming enhances the growth performance of tilapia strain Srikandi. The water quality parameters remained within acceptable ranges for cultivating tilapia strain Srikandi, with dissolved oxygen concentration: 3.2-6.9 mg/L, temperature: 27.4-32.6 °C, pH: 6.75-8.08, salinity: 21-38 ppt, and total ammonia nitrogen: 0.10-1.50 mg/L. A 50% reduction in feeding rate in the biofloc system resulted in lower total ammonia nitrogen levels compared to higher feeding rates in both the biofloc and clear-water systems.

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Key words
Biofloc, Feed conversion ratio, Feeding rate, Growth, Salinity, Tilapia strain Srikandi

INTRODUCTION

Aquaculture practices in Indonesia are mainly oriented toward freshwater culture. On the other hand, Indonesia has a potential brackish water pond area of around 1,270,982 ha with a utilization level of 21,75% (MMAF, 2018). This condition creates an opportunity to develop underutilized space by cultivating a high range of adaptable commodities to increase fish production and ensure global food security (Mirera and Okemwa, 2023).

Tilapia is one of the primary commodities in the global aquaculture business. This commodity has a rapid growth rate, a high tolerance to environmental stressors, high reproduction performance, feeding on low trophic levels, and euryhaline characteristics (Gunadi *et al*., 2021). Based on these characteristics, tilapia can be developed to cultivate in brackish water or marine waters.

Tilapia farming in Indonesia is involved in the priority sector of national aquaculture production, so many efforts have been employed to reach a significant increase in national tilapia production that resulted in a production value of 6.88 million tons in 2018 and made Indonesia the second most extensive tilapia producer worldwide (FAO, 2020). Ministry of Marine Affairs and Fisheries of the Republic of Indonesia has performed several efforts to apply tilapia farming in high-salinity environments by assigning the Research Institute for Fish Breeding Sukamandi to carry out some research in order to create a tilapia strain that can survive in high-salinity media. In 2012, the Research Institute for Fish Breeding

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Sukamandi has been released tilapia strain Srikandi (*Oreochromis aureus* × *Oreochromis niloticus*) based on Minister of Marine Affairs and Fisheries Decree No. KEP.09/MEN/2012. This strain has been reported to grow quickly in brackish water media (Gunadi *et al*., 2021). Some hybrids of tilapia have also been observed to exhibit salinity tolerance. Tilapia shows good growth performance and survival in salinity of up to 20 ppt (Malik *et al*., 2018). Tilapia farming in brackish water ponds has attained some success reports, while the success of marine farming of tilapia is still limited and has been recently experimented by some researchers (Ridha, 2014).

Salinity is one of the determining parameters in tilapia farming that influences its growth, which correlates with alterations in other parameters such as temperature, dissolved oxygen level, ammonia level, and feed regimes (FAO, 2016; Durigon *et al*., 2020; Dawood *et al*., 2021). Salinity can affect growth, osmoregulatory systems, metabolic modifications, biochemical and physiological parameters and heat shock protein gene expressions (Vargas-Chacoff *et al*., 2015; Mashaii *et al*., 2016). In a restricted limit, tilapia can adjust to water salinity fluctuation through osmoregulation mechanisms that cost high energy and lead to growth disturbance and mortality (Gunadi *et al*., 2021).

Biofloc technology is a microbial-based aquaculture production system in which microorganisms present in the system with particular roles, including water quality maintenance, natural food source provision that will decrease feed conversion ratio, and pathogen competition acting as a biocontrol (Khanjani *et al*., 2023). Proteinaceous microbial aggregates, produced from interaction among carbon and nitrogen balance from traditional aquafeeds incorporated with affordable external carbon sources, such as molasses and grains, contain vitamins, lipids, and carbohydrates, which are produced in situ and reduce aquafeed consumption up to 20% (Crab *et al*., 2012; Ogello *et al*., 2014; Pérez *et al*., 2014; Martínez-Córdova *et al*., 2017). The positive impacts of the biofloc system on feed utilization expressed by a lower feed conversation ratio are also associated with the improvement of growth and survival of cultivated species. A low feed conversion ratio indicates effective feed management and is vital to increasing production efficiency (Khanjani *et al*., 2024). In a biofloc system, microbial aggregates present in the rearing media are consumed by shrimp and fish, positively impacting feed utilization (Emerenciano *et al*., 2013). Daily feed requirement in Pacific white shrimp can be replaced by flocs up to 29%, resulting in an improved feed conversion ratio (Burford *et al*., 2004).

Moreover, several studies have reported that dietary flocs can improve non-specific immune responses and the antioxidant status of shrimp due to their rich content in natural beneficial microorganisms and bioactive compounds (Xu and Pan, 2013). Applying a biofloc system also can significantly reduce water consumption in aquaculture, making it more convenient for the site with poor water resources or urban centres (Khanjani *et al*., 2024). Tilapia farming in high salinity media using a biofloc system creates a new approach to utilizing some open space in brackish water and marine environment. However, some basic procedures, such as determining the best feeding management and water quality management to achieve an efficient production system, have yet to be widely studied. Different strains of tilapia require different feeding management when reared in the biofloc system. The red hybrid tilapia shows the best growth performance when fed at a rate of 4% of body weight per day, while the GIFT strain demonstrates excellent growth at a feeding rate range of 4.3-6.1% of body weight per day (Jong *et al*., 2024; Oliveira *et al*., 2024). Additionally, different strains exhibit different growth performances when cultivated using different feeding frequencies. The red hybrid tilapia achieves optimum growth performance when fed twice daily, while black tilapia shows the best growth when fed four times daily (Hisano *et al*., 2020; Putra *et al*., 2022). Several factors, including water quality and fish size, affect the feeding rate. Enhancing our understanding of ideal feeding management will facilitate growth promotion, chemical composition monitoring, and mitigation of water quality issues due to overfeeding (Ertan *et al*., 2015). Therefore, it is necessary to conduct an experiment to determine the optimum feeding rate of the hybrid tilapia strain Srikandi when reared in high salinity water using the biofloc system. This study aimed to evaluate the contribution of the biofloc system on growth performance and feed utilization of hybrid tilapia strain Srikandi reared in a high salinity media.

MATERIALS AND METHODS

Experimental design

The feeding trial was conducted at Multispecies Teaching Factory, Marine and Fisheries Polytechnic of Jembrana, Bali, Indonesia. One factor experiment was employed through a completely randomized design with four treatments and quadruplicate. The treatments applied were different feeding rates on tilapia marine farming reared under a biofloc system, including control (standard feeding rate reared under a clear-water system), N (standard feeding rate with a biofloc system), NA (25% standard feeding rate reduction with a biofloc system), and NB (50% standard feeding rate reduction with a biofloc system). A standard feeding rate used was 4% of fish biomass. Mohammady *et al*. (2023) found this feeding rate as an optimum feeding rate, resulting in tilapia's optimum growth and feed efficiency under a freshwater biofloc system.

Preparation of rearing media and experimental fish

Prior to the experiment, the containers were cleaned and dried. Then, they were filled with seawater and chlorinated at a dose of 30 mg L^{-1} combined with strong aeration for a day. The chlorine residue was neutralized using sodium thiosulfate at a dose of 10 mg L^{-1} and was strongly aerated. The disinfected containers were then dried again and were filled with seawater at a working volume of 96 L (Jatayu *et al*., 2023).

Before the feeding trial was started, the biofloc system in each container was prepared 7 days beforehand. Biofloc preparation was conducted by adding molasses as a carbon source and using a C/N ratio of 10 (Wankanapol *et al*., 2017). The floc formation was also stimulated by adding a probiotic (Paraqua Bioflok, CV. Pradipta Paramita, Indonesia) at a dose of 5 g/m^3.

The experimental animals used, tilapia strain Srikandi, were sourced from the Research Institute for Fish Breeding Sukamandi, West Java, Indonesia. These fish were acclimated into a concrete container with a dimension of 285 × 285 × 100 cm^3 for a period of 2 weeks prior to the start of the experiment. During this acclimation period, the fish were fed with commercial floating pellets (MS PF-800, PT. Matahari Sakti, Indonesia) with manufacture's proximate compositions (Protein, Min. 39%; Lipid, Min. 5%; Fiber, Max. 6%; Ash, Max. 12% and Moisture, Max. 10%) at a feeding rate of 4% of fish biomass and a feeding frequency of three times a day at 08:00 am, 12:00 am, and 04:00 pm (Mohammady *et al*., 2023).

Fish rearing techniques

The fish with an initial size of 5-5.5 cm were randomly stocked into 16 units of glass containers with a dimension of 60 × 40 × 50 cm^3 and a working volume of 96 L with a stocking density of 20 individuals per container. The experimental fish were fed with commercial floating pellets (MS PF-800, PT. Matahari Sakti) with feeding rates based on treatments applied in this experiment three times a day at 08:00 am, 12:00 am, and 04:00 pm. The feeding trial was performed for 30 days.

To maintain the floc formation in the rearing media during the feeding trial, molasses was added into the rearing media every day, 2 h after the fish feeding in the morning (Ekasari *et al*., 2014b). Water exchange was done every 10 days at 4-6% of total water volume (Khanjani *et al*., 2021).

Sampling and measurement of experimental parameters

The test fish was sampled every 10 days to adjust the amount of respective feed during the feeding trial. All fish in each container were counted and weighed at the beginning and the end of the feeding trial to record experimental parameters, including weight gain (WG), specific growth rate (SGR), survival (SR), feed conversion ratio (FCR), protein efficiency ratio (PER), protein retention (PR), lipid retention (LR), initial protein content (IPC), final protein content (FPC), initial lipid content (ILC), and final lipid content (FLC). Protein and lipid intake was measured by evaluating protein and lipid content of the feed, while protein and lipid retained in the fish body were measured by determining protein and lipid content of the fish body at the beginning and the end of the feeding trial. The evaluation of protein and lipid content of the feed and the fish body was done in Technical Implementation Unit for Quality Testing and Development of Marine and Fisheries Products Banyuwangi according to AOAC (2000). The experimental parameters were calculated according to Mirzakhani *et al*. (2019).

Water quality parameters measured included dissolved oxygen, temperature, pH, salinity, and total ammonia nitrogen. Dissolved oxygen, temperature, pH, and salinity were measured everyday using DO meter, thermometer, pH meter, and hand refractometer, respectively. Total ammonia nitrogen was measured every 10 days using ammonia marine test kit (Salifert®, Salifert, Duive, the Netherlands).

Statistical analysis

Statistical analysis was performed using SPSS version 26. Data normality and homogeneity of variance were assessed using Shapiro-Wilk's test and Levene's test, respectively. The growth performance and feed utilization data were then analysed using one-way ANOVA at a confidence level of 95%, followed by Duncan's post-hoc test, while water quality data were analysed using descriptive statistics.

RESULTS

Growth performance

Different feeding rates affected the tilapia strain Srikandi's growth performance reared under the marine biofloc system (Table I). A lower feeding rate resulted in higher WG and SGR of tilapia strain Srikandi (8.01 ± 0.44 g; 5.00 ± 0.14 %/ day) compared to higher feeding rates applied in the marine biofloc system ($P<0.05$). A reduction of standard feeding rate at 25-50% also did not influence the survival of tilapia strain Srikandi reared under the marine biofloc system ($P>0.05$).

Table I. Growth performance of tilapia strain Srikandi fed with different feeding rates reared under marine biofloc system.

Parameters	Treatments			
	K	N	NA	NB
Weight gain (g)	7.89 ± 0.56[b]	4.05 ± 0.83[a]	4.87 ± 0.50[a]	8.01 ± 0.44[b]
Specific growth rate (%/day)	4.96 ± 0.19[c]	3.37 ± 0.43[a]	3.78 ± 0.23[b]	5.00 ± 0.14[c]
Survival rate (%)	92.50 ± 9.57[a]	92.50 ± 6.45[a]	93.75 ± 4.79[a]	92.50 ± 6.45[a]

K, control with standard feeding in a clear water system; N, is with standard feeding rate with a biofloc system; NA, is with 25% reduction in standard feeding rate with a biofloc system; NB, is with 50% reduction in standard feeding rate with a biofloc system. Data are expressed as mean ± standard deviation. Data in the same row with different superscript letters are significantly different (P<0.05).

Table II. Feed utilization of tilapia strain Srikandi fed with different feeding rates reared under marine biofloc system.

Parameters	Treatments			
	K	N	NA	NB
Feed conversion ratio	0.77 ± 0.08[b]	1.37 ± 0.24[c]	0.86 ± 0.10[b]	0.38 ± 0.04[a]
Protein efficiency ratio	3.26 ± 0.34[b]	1.86 ± 0.35[a]	2.93 ± 0.32[b]	6.62 ± 0.61[c]
Protein retention (%)	56.47 ± 6.60[b]	33.66 ± 5.50[a]	52.14 ± 5.94[b]	115.56 ± 8.44[c]
Lipid retention (%)	11.17 ± 4.26[a]	3.09 ± 2.48[a]	8.07 ± 6.72[a]	24.72 ± 13.36[b]
Initial protein content (%)	10.15 ± 0.00[a]	10.15 ± 0.00[a]	10.15 ± 0.00[a]	10.15 ± 0.00[a]
Final protein content (%)	16.46 ± 0.67[a]	16.52 ± 0.32[a]	16.47 ± 0.37[a]	16.60 ± 0.41[a]
Initial lipid content (%)	0.90 ± 0.00[a]	0.90 ± 0.00[a]	0.90 ± 0.00[a]	0.90 ± 0.00[a]
Final lipid content (%)	0.59 ± 0.19[a]	0.39 ± 0.18[a]	0.42 ± 0.19[a]	0.37 ± 0.13[a]

Data are expressed as mean ± standard deviation. Data in the same row with different superscript letters are significantly different (P<0.05). For details of treatment groups see Table I.

Table III. Water quality of rearing media of tilapia strain Srikandi fed with different feeding rates reared under marine biofloc system.

Parameters	Treatments				
	K	N	NA	NB	Optimum
Dissolved oxygen (mg/L)	3.7-6.9	3.5-6.8	3.4-6.9	3.2-6.9	> 3.0*
Temperature (°C)	28.9-32.6	29.1-32.4	27.4-31.8	29.1-31.5	28-31*
pH	7.23-8.05	7.23-8.05	7.23-8.07	6.75-8.08	6.5-8.5*
Salinity (ppt)	25-35	25-35	21-35	23-38	10-30*
Total ammonia nitrogen (mg/L)	0.15-1.5	0.15-1.5	0.15-1.5	0.10-1.5	< 1,0**

*Setyawan *et al*. (2022), ** Abakari *et al*. (2021). For details of treatment groups, see Table I.

Feed utilization

Applying a biofloc system in marine tilapia farming produced a better feed utilization of tilapia strain Srikandi (Table II). Tilapia strain Srikandi showed the best feed utilization in the lowest feeding rate when reared under the marine biofloc system. It indicated that applying a biofloc system in tilapia marine farming contributed to up to 50% feed utilization efficiency. A reduction of standard feeding rate at 50% resulted in the lowest FCR (0.38 ± 0.04) and the highest PER, PR, and LR as well (6.62 ± 0.61; 115.56 ± 8.44%; 24.72 ± 13.36%), compared to higher feeding rates both under clear-water system and biofloc system. Applying biofloc in marine tilapia farming improved the utilization of the protein taken from the feed. It also reduced the lipid content of the harvested fish in this experiment. It was shown by the improvement of protein content and the decrease of lipid content of the fish body at the end of this feeding trial than at the beginning.

Water quality

The water quality parameters were within the acceptable range for cultivating tilapia strain Srikandi. The specific ranges during the experiment were as follows: dissolved oxygen concentration ranged from 3.2 to 6.9 mg/L, temperature from 27.4 to 32.6 °C, pH from 6.75 to 8.08, salinity from 21 to 38 ppt, and total ammonia nitrogen from 0.10 to 1.50 mg/L (see Table III). Dissolved oxygen and pH levels were optimal, while temperature and total ammonia nitrogen were within acceptable tolerances. Reducing the feeding rate by 50% in the biofloc system resulted in lower total ammonia nitrogen levels compared to higher feeding rates in both the biofloc and clear-water systems. This suggests that reducing the feeding rate can help in maintaining better water quality, especially in high salinity media when using the biofloc system for culturing tilapia strain Srikandi.

DISCUSSION

Numerous studies indicate that salinity plays a crucial role in regulating fish growth and survival. When exposed to high-salinity environments, freshwater fish use energy from their food to maintain internal balance, rather than using it for growth. Tilapia, for example, performs well in salinity levels ranging from 0-18 ppt without impacting its survival (Figueiredo *et al*., 2022). A specific strain called Srikandi has shown remarkable adaptability to high salinity, with an optimum range of approximately 30 ppt and a tolerance level of up to 40 ppt (Robisalmi *et al*., 2020; Setyawan *et al*., 2022). This strain is a hybrid resulting from the crossbreeding of female tilapia strain Nirwana and male blue tilapia. The salinity range used in the present study falls within the tolerance range of the tilapia strain Srikandi.

Reducing the feeding rate to 50% of the standard rate led to better growth performance in tilapia rearing using a biofloc system compared to higher feeding rates. Lower feeding rates also resulted in higher growth performance of shrimp (Kaya *et al*., 2020). Microbial aggregates formed in the rearing water, known as flocs, could serve as an additional food source for farmed fish, potentially reducing the need for external feeding using aquafeed (Meritha *et al*., 2018). Another experiment suggested that a 25% reduction in the feeding regime did not negatively affect the development of common carp and reduced feed costs (Najdegerami *et al*., 2016). The protein content of flocs is relatively high, ranging from 42-48%. The nutritional composition of flocs may vary and is influenced by factors such as carbon sources and microbial composition (Ju *et al*., 2008; Ekasari *et al*., 2014b). The high protein content of flocs is supported by their amino acid composition and can be considered a sufficient protein source for tilapia (Ekasari *et al*., 2014a). In a separate study, the survival of tilapia GIFT reared in a biofloc system at a salinity range of 0-20 ppt was found to be similar to this present study, indicating that high-salinity rearing media does not adversely affect the survival of tilapia, provided the fish have significant tolerance to saline water (Luo *et al*., 2017).

A higher feed utilization in the biofloc system may result in a lower feed intake or feeding rate, leading to a lower FCR. Tilapia is an omnivorous species known to graze on various types of feed, including algae and detritus (Wang and Lu, 2015). Several studies have shown that protein utilization in the biofloc system is twice as efficient as in the clear-water system, resulting in reduced FCR and increased growth (Wasielesky *et al*., 2006). In some cases, the biofloc system allows for a reduction in dietary protein from aquafeed, as reported in shrimp, Nile tilapia, and Pacu (*Piaractus mesopotamicus*) (Azim and Little, 2008; Shao *et al*., 2017; Durigon *et al*., 2019; Olier *et al*., 2020; Sgnaulin *et al*., 2021; Tubin *et al*., 2023). This is due to the high protein and amino acid content in flocs. The protein content of flocs is higher than its lipid content. This finding is supported by the results of this experiment, which showed that the experimental fish stored more protein than lipids in their bodies, reducing the overall lipid content. Ekasari *et al*. (2014a) stated that flocs are rich in valine, lysine, leucine, phenylalanine, and threonine. Additionally, some researchers reported that flocs lack essential fatty acids (Dantas *et al*., 2014; Ruby *et al*., 2017), which may contribute to the reduction of lipid content in the fish body in this study.

It is essential to manage the water physico-chemical parameters within the tolerance range of cultivated species for optimal production performance in the biofloc system (Alkhamis *et al*., 2023; Islam *et al*., 2023). Several studies have demonstrated that the biofloc system has minimal impact on crucial factors such as temperature, pH, dissolved oxygen, alkalinity, and hardness in Pacific white shrimp (*Penaeus vannamei*) and giant freshwater prawn (*Macrobrachium rosenbergii*). Similarly, in our experiment involving tilapia strain Srikandi, the biofloc system did not significantly alter temperature, pH, dissolved oxygen, and salinity. However, we observed a notable impact on the total ammonia nitrogen levels in the rearing water. Notably, a lower feeding rate combined with the biofloc system resulted in a lower range of total ammonia nitrogen compared to higher feeding rates in both the biofloc and clear water systems. Additionally, it was found that a higher feeding rate can lead to elevated levels of total ammonia nitrogen in Pacific white shrimp (Padilla *et al*., 2024). Furthermore, in an experiment involving giant freshwater prawn, it was concluded that the biofloc system effectively reduced the total ammonia nitrogen levels in the rearing water, resulting in lower levels compared to the clear water system (Islam *et al*., 2023). This reduction in total ammonia nitrogen can be attributed to an increase in the total heterotrophic bacterial population, which has the capacity to consume total ammonia nitrogen, as well as the autotrophic nitrification process. These insightful findings emphasize the significance of maintaining a proper balance between carbon and nitrogen sources to effectively reduce the total ammonia nitrogen levels in the biofloc system (Alkhamis *et al*., 2023).

CONCLUSION

Applying a biofloc system in marine tilapia farming promoted the growth performance of tilapia strain Srikandi. The application of biofloc also contributed to 50% of feed utilization efficiency.

DECLARATIONS

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IRB approval

The study adheres to ethical principles and regulations established by Ministry of Marine Affairs and Fisheries of the Republic of Indonesia.

Ethical statement

This study was conducted in compliance with ethical standards and regulatory guidelines for aquaculture research. Efforts were made to minimize stress and ensure fish welfare by following ethical guidelines for aquaculture research and Good Aquaculture Practices (GAqP) as outlined by Ministry of Marine Affairs and Fisheries of the Republic of Indonesia.

Statement of conflict of interest

The authors have declared no conflict of interest.

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