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The Impact of Carbon to Nitrogen Ratios on the Growth, Water Quality, and Color Quality of Koi Fish (*Cyprinus rubrofuscus*) in a Biofloc System Supplemented with *Spirulina platensis*

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ABSTRACT

Koi fish are freshwater ornamental fish that have high economic value. High koi fish production and good fish color quality are significant factors in the success of koi fish culture. The aim of this study was to evaluate the effect of different carbon to nitrogen ratios on the growth performance, water quality, and color quality of koi fish with *Spirulina platensis* enrichment. Four treatments and three replications were employed in this study's completely randomized design (CRD). The treatments used included control (K) without biofloc + *Spirulina platensis* 5% treatment, (P1) biofloc C/N ratio 12 + *Spirulina platensis* 5% treatment, (P2) biofloc ratio C/N 15 + *Spirulina platensis* 5% treatment, and (P3) biofloc C/N ratio 18 + *Spirulina platensis* 5% treatment. As many as 125 fish were reared in a tarpaulin pond and fed with artificial feed mixed with *Spirulina plantesis* powder three times a day with a feeding rate of 5%. Parameters observed included specific growth rate, feed conversion ratio, feed efficiency, survival rate, fish biomass, the color quality of koi fish, and water quality. The outcomes revealed that the P3 treatment had the highest specific growth rate, survival rate, feed efficiency, fish biomass, and color quality but the lowest feed conversion ratio value among control and other treatments. In addition, application of biofloc technology can decrease ammonia, nitrate, and nitrite parameter in the culture ponds so that it can provide clean water for koi fish.

INTRODUCTION

Cyprinus rubrofuscus, also known as the koi fish, is a fish with significant commercial significance. It is a species of ornamental fish renowned for its exquisite body pattern and color. The shape, physical fitness, behavior, dazzling color, and health status of koi fish and other ornamental fish can all be used as indicators of their beauty. Fish farmers have been prompted to upgrade their systems for intensive cultivation as a result of the rising market demand for koi fish **(Pradana *et al.*, 2022; Amin *et al.*, 2023)**. One way to increase koi fish production is through intensification. However, high productivity is influenced by the high use of feed which can have an impact on production waste which affects the environment. The total nitrogen used in culture ponds is only around 20-30 percent which becomes fish biomass, while the rest will become water pollutants which have high levels of toxicity such as nitrites and ammonia **(Liu *et al.*, 2019)**.

The biofloc technology is one of the technologies being used to increase fish farming production **(Bossier & Ekasari 2017)**. According to **Ogello *et al.* (2021)**, the term bioflocs refers to a heterogeneous macro-aggregate of planktonic materials in the water column. These materials include diatoms, filamentous microalgae, micro-and macro-invertebrates, bacteria that form flocs, protozoa, fecal waste, and uneaten feed.

Biofloc technology is a method of improving the quality of the water by supplementing the aquaculture system with more carbon **(Deng *et al.*, 2018)**. Biofloc technology was able to minimize the buildup of hazardous nitrogen metabolites, including ammonia, nitrite, and others, by adjusting the carbon/nitrogen (C/N) ratio and converting them into microbial clumps without using water recirculation. The main principle of biofloc is to recycle nutrients by keeping the carbon/nitrogen ratio balance in water at a level that encourages the growth of heterotrophic bacteria that turn ammonia into floc **(Najdegerami *et al.*, 2016)**. Providing a carbon source, such as molasses added to ponds with constant aeration, helps maintain a higher C/N ratio **(Ogello *et al.*, 2021)**. The benefits of biofloc technology have been demonstrated in many studies, which cite favorable results in growth and survival through the exclusive consumption of microbial proteins **(Castro *et al.*, 2018)**. By maintaining water quality, this method can sustainably affect fish production positively **(Minabi *et al.*, 2020)**. Apart from that, the biofloc application in aquaculture system supports SDGs 6, namely clean water **(FAO, 2017)**.

The price of ornamental fish like koi fish is determined by color, and fish with good color patterns have a higher price in the market. Fish in their natural environment obtain coloration from wild sources, but the cultivation of ornamental fish at high densities without dietary carotenoid supplementation that causes coloration to fade, which decreases the commercial value of fish. Fish use carotenoids as one of the main groups of natural pigments for their pigmentation. Koi fish are characterized by a wide variety of colors and color patterns, and also more than hundreds of colors have been developed. The economic value of this fish increases with the intensity of skin color caused by the absorption and deposition of carotenoids **(Maiti *et al.*, 2017; Prabath *et al.*, 2019)**. The main components of color pigment formation are carotenoids which are natural pigment components that contribute quite well to red and orange colors **(Nafishi *et al.*, 2016)**. Carotenoids cannot be synthesized by ornamental fish, thereore it must be added to the feed in natural or synthetic form to obtain color pigmentation **(Sathyaruban *et al.*, 2021)**. Thus, the formation of color in ornamental fish depends on the amount of carotenoids in the feed **(Andriyani *et al.*, 2018)**. The addition of *Spirulina* powder can be used to increase the color intensity of koi fish. The protein and carotenoid content of *Spirulina* has the ability to enhance the color and growth of koi fish **(Sudirman *et al.*, 2020)**. *Spirulina* contains 51 pigments which include carotene, chlorophyll and xanthophyll pigments **(Somella *et al.*, 2018)**. Compared to other microalgae, *Spirulina platensis* is more effective at coloring ornamental fish **(Sathyaruban *et al.*, 2021)**.

There has been prior research on the usage of *Spirulina* as a feed supplement. Previous investigations have demonstrated that *Spirulina* exhibits relatively stable proximate composition, with protein content typically ranging from 55-70%, carbohydrates comprising 15-25%, lipids accounting for 6-13%, and ash content representing 7-13% of dry weight **(Lafarga *et al.*, 2020)**. These values align with findings from **Soni *et al.* (2017)**, who documented protein levels between 60-70%, lipid content of 8-14%, carbohydrate proportions of 10-19%, and ash percentages of 7-11%.

Research by **Sudirman *et al*. (2020)** used *Spirulina* flour at various doses in koi fish and a dose of 5% resulted in the highest absolute growth in length and weight among other treatments. **Kim and Lee (2012)** also on their research about effects of *Spirulina*, astaxanthin, and canthaxanthin consumption on *Cyprinus carpio* var. koi showed that *Spirulina* powder 5% had a high value of specific growth rate and skin total carotenoid when compared with *Spirulina powder* 10% for eight weeks. Other studies regarding *Spirulina* in koi fish include that of **Prabath *et al.* (2019)** on the impact of dietary-supplemented *Spirulina platensis* extracted pigments on the coloring of koi carp, using several treatments, including phycocyanin and carotenoids pigments extracted from *Spirulina*, raw *Spirulina*, and pigments extracted residue. **Abdulrahman *et al.* (2019)** also analyzed the effect of *Spirulina* as a feed additive on blood and biological parameters of common carp *Cyprinus carpio*.

Currently, there is a lack of information regarding research on biofloc in koi fish. Research on koi fish biofloc that has been carried out includes research by **Castro *et al.* (2018)** regarding the application of biofloc on koi fish using different carbon sources. The C/N ratio is one of the crucial factors for successful biofloc technology use **(Minabi *et al.* (2020)**. C/N Ratio values between 10:1 to 20:1 is suggested for biofloc development and effective biofloc system performance **(Wang *et al.*, 2015; Luo *et al.*, 2020)**. Therefore, the aim of this research was to evaluate the effect of different carbon to nitrogen ratios on the growth performance, water quality, and color quality of koi fish with *Spirulina platensis* enrichment.

MATERIALS AND METHODS

Tools and materials

This research was conducted in the Experimental Pond of Omah Koi Farm Indonesia, which is located in Genteng Banyuwangi, East Java, Indonesia. The tools used include 12 round tarpaulin ponds with a diameter of 2 meters, Resun LP100 blower, hose and aeration stone, digital scale, ruler, modified toca color, thermometer, pH paper, test kit dissolved oxygen, and spectrophotometry. The component was koi fish seeds which ranged in size from 3 to 5cm, feed, *Spirulina platensis* powder, and molasses containing bacteria *Bacillus subtilis*, *Bacillus lincheniformis*, and *Bacillus fumillus*. The commercial feed used contains 35% crude protein, 3% crude fiber, 5% crude fat, 12% moisture content, 12% ash content, 2% calcium. In addition, the feed used contains vitamins: A, D3, E, B1, B2, B6, B12, niacin, pantothenic acid, choline, biotin, vitamin C, and menadione (Vit K).

Research design

Four treatments and three replications were used in this study with a completely randomized design. The stocking density of the pond was 100 fish/m^3. The treatments given in this study include control (K): *Spirulina platensis* 5% and without biofloc, P1: *Spirulina platensis* 5% and biofloc C/N ratio 12, P2: *Spirulina platensis* 5% and biofloc C/N ratio 15, P3: *Spirulina platensis* 5% and biofloc C/N ratio 18. For the carbon source, this research used molasses with C organic content was 65% and contained bacteria: *Bacillus subtilis* 2x10 cfu/ml, *Bacillus lincheniformis* 2x10 cfu/ml and *Bacillus fumilus 2x10* cfu/ml and other compositions, namely maltodextrin 0.5g/ ml and organic acid 10%.

Work procedures

The rearing pond is filled with water as much as 50% of the water level of 80cm, then given molasses with bacteria according to the treatment dose, strongly aerated, and left for four days in order to grow floc-forming bacteria. The addition of molasses to the culture media was carried out by adapting the calculations based on **De Schryver *et al.* (2008)**. Several things are considered in calculating molasses requirements, namely feed protein content 35%, nitrogen content in feed 16%, feed nitrogen content wasted into the culture media 75%, feeding rate of fish feed, and C/N ratio for each treatment.

After four days, the water was filled to 100%. Henceforth, the molasses was added once a week. The fish were reared for 49 days. Fish were given commercial feed that had added *Spirulina platensis* 5%. The process of mixing *Spirulina* in commercial feed is the preparation of commercial feed which is weighed as needed. Furthermore, *Spirulina* powder is dissolved in 500ml water to form a solution. Furthermore, the *Spirulina* solution was poured evenly on the feed and stirred slowly until the feed was completely coated. The feed that has been mixed with *Spirulina* is then dried by aerating in a shady place for 2 to 4 hours. After drying, the feed is stored in an airtight container in a cool and dry place. The feed is used within a week maximum to maintain nutrient quality. Feeding was given 3 times a day with a feeding rate of 5%.

Water quality measurements are carried out every day in the morning and evening (temperature and pH) every week for dissolved oxygen, and nitrate, nitrite, ammonia on week 1, week 4, and week 7. Sampling of fish length and weight was carried out once a week with as many as 30 fish in each pond. Observation of fish color is carried out at the end of the maintenance period visually by at least three different people using modified toca color.

Test parameters

Specific growth rate (SGR)

The formula for specific growth rate (SGR) is as follows **(Najdegerami, 2016)**.

$$\mathbf{SGR = (ln\ Wt - Ln\ Wo)/t \times 100}$$

SGR = specific growth rate (%)
Wt = average weight at the end (grams)
Wo = average weight at the beginning (grams)
t = length of maintenance (days)

Survival rate

According to **Zaman *et al.* (2020)**, survival can be calculated by the formula:

$$\mathbf{SR\ (\%) = [Nt/No] \times 100}$$

SR = survival rate (%)
Nt = number of fish at the end of rearing
No = number of fish at the beginning of maintenance

Feed efficiency

Feed efficiency was calculated using the following formula **(Shofura *et al.*, 2017):**

$$\mathbf{FE = weight\ gain/\ feed\ intake \times 100\%}$$

Feed conversion ratio

The feed conversion ratio was calculated using the following formula **(Küçük *et al.*, 2014);**

$$\mathbf{FCR = Feed\ intake/weight\ gain}$$

Fish biomass

Fish biomass can be calculated using the formula **(Zonneveld *et al.*, 1991):**

$$\mathbf{Fish\ Biomass\ (g) = w \times Nt}$$

Fish Biomass = weight of fish biomass (g)

w = average weight of fish (g)
Nt = total number of fish

Fish color observation

Observation of the color of koi fish is done visually by at least 3 different people and is matched with modified toca color. The score starts from 1 to 30 with yellow to red color **(Yunisari, 2017)**.

Data analysis

The data obtained were processed using analysis of variance (ANOVA). The calculation was continued with Duncan's test if there were significant results **(Kusriningrum, 2008)**. Data analysis was conducted using SPSS software version 22.

RESULTS

1. Survival rate

The application of koi fish culture using biofloc systems with different C/N ratios and *Spirulina plantesis* enrichment showed that survival value was not significantly different compared to the control (P>0.05), which ranged from 82.93-91.20%. However, the lowest SR value was found in the control treatment with a value of 82.93±11.87%, while the highest SR was in the P3 treatment with a value of 91.20±6.40% (Fig. 1). It showed that the combination of the biofloc system and *Spirulina plantesis* could increase the survival rate of koi fish.

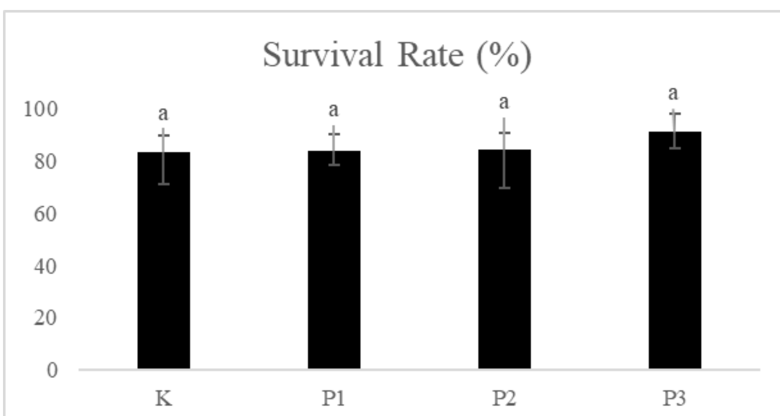

Fig. 1. The survival rate of koi fish

2. Specific growth rate

The specific growth rate in the control treatment was not significantly different from P1, P2, and P3 (P>0.05). However, the control was significantly different from P3 (P<0.05). The P3 treatment showed the highest value compared to the control treatment, P1, and P2, which was 1.72±0.10%. At the same time, the control treatment had the lowest specific growth rate, namely 1.16±0.50% (Fig. 2). It indicates that the biofloc system with a C/N ratio of 18 and *Spirulina plantesis* enrichment gave the best specific growth rate for koi fish.

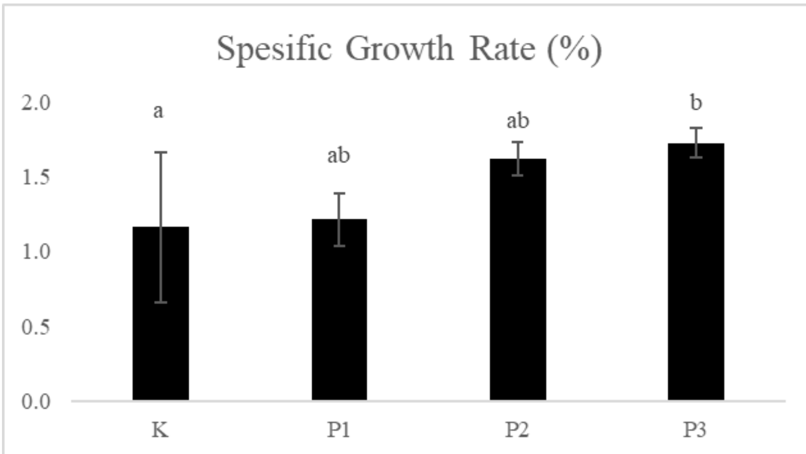

Fig. 2. The specific growth rate of koi fish

3. Fish biomass

Fish biomass in the P3 treatment produced the highest biomass compared to the control treatments, P1, and P2, which was 709.51±79.58 grams. At the same time, the control treatment had the lowest biomass, namely 503.11±150.50 grams (Fig. 3). There was no significant difference between the control treatment and other treatments (P>0.05). However, treatment with biofloc technology and *Spirulina plantesis* enrichment gave a higher biomass value than the control.

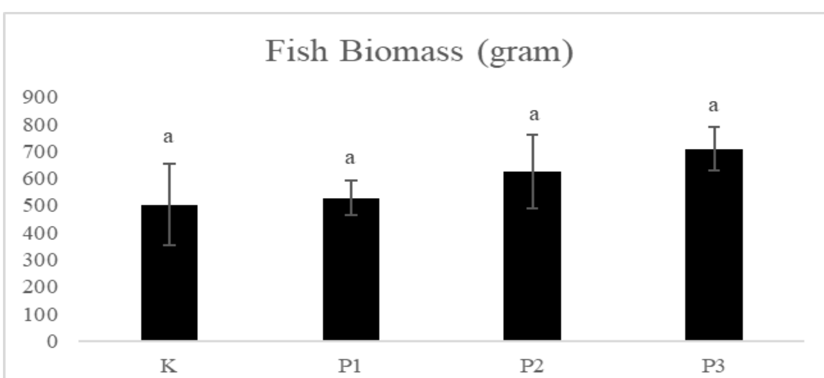

Fig. 3. Biomass of koi fish

4. Feed conversion ratio

The feed conversion ratio in the P3 treatment with biofloc C/N ratio 18 and *Spirulina plantesis* enrichment resulted in the lowest value compared to the control treatments, P1, and P2, which was 1.45±0.33. At the same time, the control treatment had the highest feed conversion ratio, which was 2.64±1.63 (Fig. 4). There was no significant difference between all treatments (P>0.05), but the combination of biofloc and *Spirulina platensis* was able to reduce the FCR value.

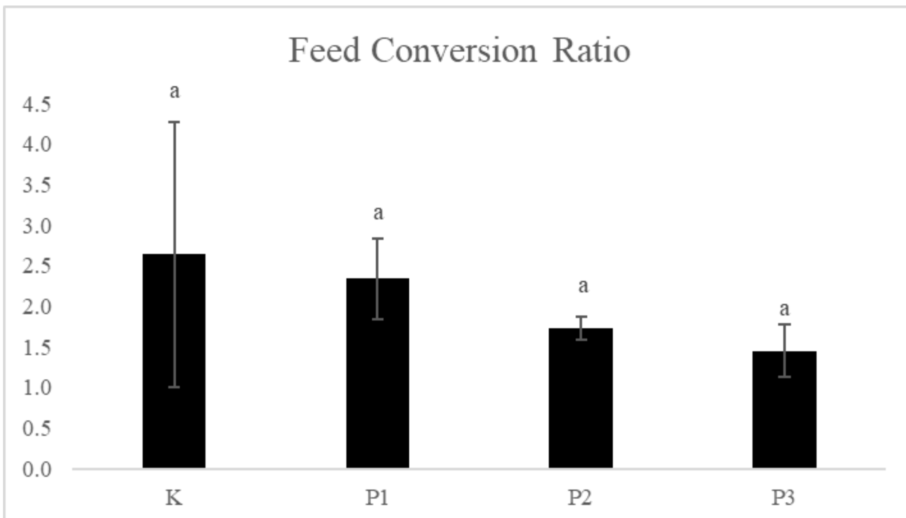

Fig. 4. FCR of koi fish

5. Feed efficiency

Feed efficiency in the P3 treatment had the highest value compared to the control treatments, P1, and P2, which was 70.43±13.47% (Fig. 5). There was no significant difference between all treatments (P>0.05), but the treatment with biofloc system and *Spirulina platensis* enrichment was able to increase the feed efficiency value.

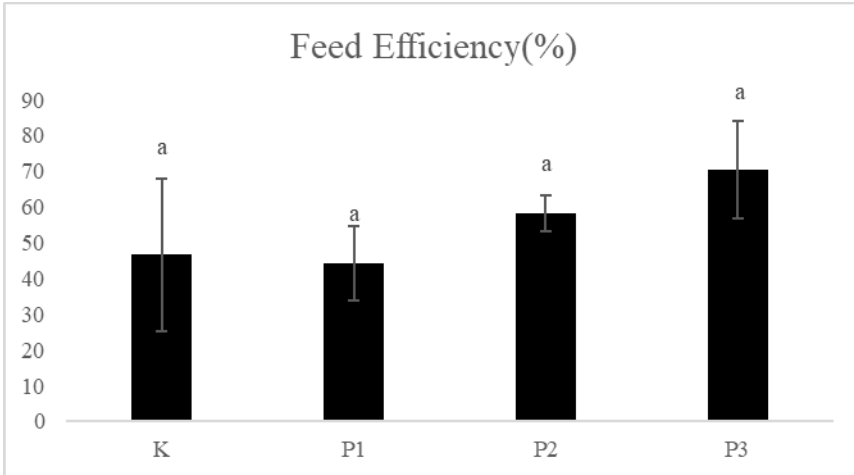

Fig. 5. Feed efficiency of koi fish

6. Color quality

At the end of the study, the color quality was found that the control treatment was significantly different from all treatments. But treatment P1 was not significantly different from P2 (P>0.005) but significantly different from P3 (P<0.005); P2 was not significantly different from P3 (P>0.05). The highest color quality value was in the P3 with an average score of 26.27±0.64, and the lowest was in the control with a value of 18.47±1.10 (Fig. 6). The treatment of biofloc system and *Spirulina platensis* enrichment was able to improve the color quality of koi fish.

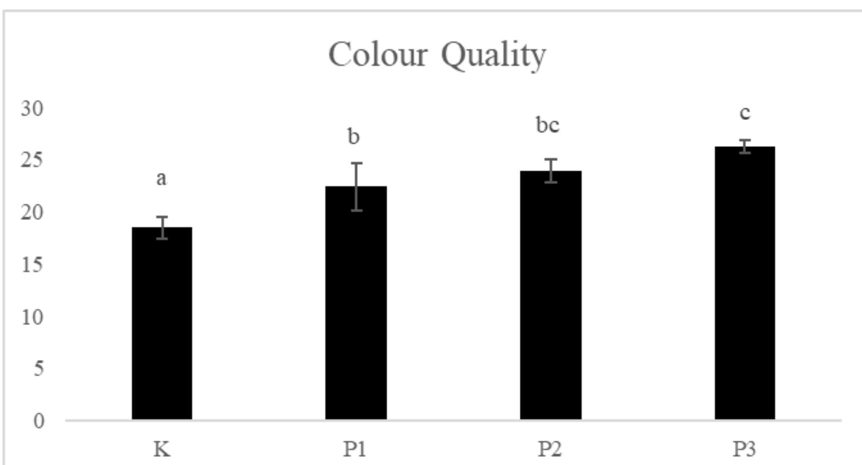

Fig. 6. The color quality of koi fish

7. Water quality

Based on the results, the temperature of the water is still in the optimal range, although there is a temperature of 27^{0}C in the P1 treatment (Table 1). According to **Indoesian National Stadard (SNI) (2011)**, the optimal temperature for koi living media ranges from 20– 26^{0}C. For a good pH parameter, according to **SNI (2011)**, it is in the range of 6.5–8, the minimum dissolved oxygen parameter is 5mg/ l, the maximum nitrate is 50mg/ l, and the maximum nitrite is 0.2mg/ l. Meanwhile, according to **Boyd (1982)**, the optimal concentration of ammonia is < 0.2mg/ l.

For the pH parameter, all treatments had a pH value of 6. Dissolved oxygen ranged from 4.39– 5.28mg/ l (Table 1). Nitrite and TAN parameters decreased except for the control at the beginning until the end of the experimental period, but the values were still within optimal limits (Tables 2, 4). Nitrate also decreased in all treatments; while the initial maintenance was recorded with a high value above the optimal limit for koi fish, nitrate then decreased (Table 3).

Table 1. Data of temperature, pH, and dissolved oxygen in treatment

Parameter	Time	K	P1	P2	P3
Temperature (^{0}C)	Morning	25	25-26	25-26	25-26
	Evening	25-26	25-27	25-26	25-26
pH	Morning	6	6	6	6
	Evening	6	6	6	6
Dissolved oxygen (mg/l)	Morning	5.22	5.22	5.11	5.00
	Evening	4.39	5.11	5.06	5.28

Table 2. Data of nitrite (NO_2) in treatment (mg/l)

Treatment	Week 1	Week 4	Week 7
K	0.024	0.038	0.089
P1	0.110	0.041	0.008
P2	0.066	0.043	0.040
P3	0.169	0.042	0.051

Table 3. Data of nitrate (NO_3) in the treatment (mg/l)

Treatment	Week 1	Week 4	Week 7
K	87.667	45.000	30.000
P1	67.667	35.000	33.333
P2	79.667	36.667	33.333
P3	97.667	33.333	31.667

Table 4. Data of total ammonia nitrogen TAN in the treatment (mg/l)

Treatment	Week 1	Week 4	Week 7
K	0.060	0.048	0.153
P1	0.092	0.045	0.009
P2	0.072	0.002	0.012
P3	0.053	0.026	0.000

DISCUSSION

The specific growth rate of treatment using biofloc system resulted in higher growth than the control. The highest specific growth rate value is P3 (C/N ratio 18 with *Spirulina platensis* enrichment), with a value of 1.72±0.10%. The results of this study are also higher than those of **Gouveia *et al.* (2003)**, who illustrated an SGR value of 0.2 ±0.1 % because of using only *Spirulina* in the feed. The P3 treatment also produced a higher SGR value than that recorded in the study of **Minabi *et al.* (2020)** regarding the effects of different C/N ratios in the biofloc system on water quality, growth, and body composition of common carp (*Cyprinus carpio*). In that study, the C/N ratio 19 had the highest SGR value, namely 1.60 ± 0.03%.

The use of biofloc media proved to have an effect on the SGR. It is because koi fish eat the floc formed in the media, so it has a higher SGR value when compared to the control treatment. Biofloc is able to accelerate growth in weight and length because the organic materials in biofloc are natural food for fish that contain good nutrition and are able to be paired with natural feed, thus causing fish growth to be good, even though the amount of feed given can be reduced **(Septiani & Wijaya, 2014)**. **Hermawan *et al.* (2014)** also added that biofloc technology could improve growth and feed utilization efficiency. This technology can provide additional protein feed in the form of bacterial floc. In addition, in the treatment using biofloc, carbon sources which added to the culture media can be converted by heterotrophic bacteria as an energy source, resulting in bacterial biomass with protein and can be used by koi fish as additional food with good nutrition. This finding is comparable to the research of **Zhao *et al.* (2012)**, which explained that the use of biofloc system technology was able to provide a higher growth rate than the control. Besides biofloc media, the presence of *Spirulina* in the feed also causes the SGR value to increase.

The highest survival rate obtained in this study was the P3 treatment, with a value of 91.20%. The addition of biofloc can increase immunity and affect the survival rate. The high survival rate in biofloc systems is due to the role of bacteria. The increased abundance of bacteria in aquaculture waters occurs due to the addition of carbon. According to **Mohammadi *et al.* (2021)**, potential probiotics and bioactive substances found in biofloc may strengthen fish immune systems. In this respect, **Zhao *et al.* (2016)** examined the bioactive content in the biofloc system with a carbon source of 100 percent molasses containing poly-β hydroxybutyrate, polysaccharides and carotenoids which functions to improve the immune system of fish. Poly-β hydroxybutyrate could protect from pathogenic infection and polysaccharide useful as antioxidant, anti-inflammatory and immunomodulatory activiy. Furthermore, it has been documented that carotenoids supply vital nutrients and perform a variety of bioactive physiological tasks in animal tissues, such as boosting immune systems, promoting stress tolerance, and promoting growth. **Haghparast *et al.* (2020)** added that the biofloc system based on sugar cane molasses could boost not only health but also antioxidant capacity and resistance to bacterial infection.

Beside that, *Spirulina platensis* can increase the immune system of koi fish and improve survival rate. This is in accordance with research of **Jana *et al.* (2014)** postulating that adding *Spirulina* 5% to the feed can improve survival rate of *Pangasius sutchi* than feed without *Spirulina*. **Mahmoud *et al.* (2018)** added that *Spirulina* contains phytopigments, such as phycobilins, phycocyanin, allophycocyanin, and xanthophylls, and carotenoid, with antioxidant activity which shields the cell from oxidative stress. **Shofura *et al.* (2017)** added that internal factors, including gender, age, reproduction, resistance to disease, and external factors like water quality, stocking density, and feed have an impact on survival rate.

In this research, the feed conversion ratio value from the treatment of biofloc had a lower value than the control. The best FCR value is the P3 treatment, with a value of 1.45±0.33. The lower FCR value is better because the lower FCR value means the less amount of feed used. In the biofloc treatment, other than the feed provided, there is biofloc which can be used as a source of in situ feed. According to **Zablon *et al.* (2022)**, biofloc contains probiotic bacteria which are able to digest and absorb commercial feed. Therefore, it produces the best feed conversion ratio value. **Bakhsi *et al.* (2018)** stated that biofloc technology is an economical alternative for use in reducing commercial fish feeds in ponds while reducing potential environmental problems. **Khanjani and Sharifinia (2020)** deduced that this method lowers the cost of production by reducing the feed conversion ratio.

Increasing feed efficiency is an attempt to reduce production costs in aquaculture and to achieve sustainability for the aquaculture business **(De Verdal *et al.*, 2017)**. The efficiency of feed utilization with biofloc media was higher than the control, and P3 had the highest feed efficiency value of 70.43±13.47 %. According to **Shofura *et al.* (2017)**, *Bacillus* sp. can reduce the number of pathogenic bacteria in the digestive tract and increase feed absorption by increasing the concentration of protease enzymes in the digestive tract, where protease enzymes are biocatalysts for protein-breaking reactions. **Ekasari *et al.* (2015)** found that biofloc can enhance the feed utilization efficiency of fish and meanwhile can offer extra essential nutrients and exogenous digestive enzymes.

The color quality of koi fish in the treatment of biofloc and *Spirulina platensis* enrichment had a higher value than the control, with the P3 treatment having the highest color quality value. The presence of carotenoids affects the color quality of fish. Both *Spirulina* and biofloc contain carotenoids **(Zhao *et al.*, 2016; Sudirman *et al.*, 2022)**. The process of increasing color intensity begins with carotene (color pigment) present in the feed being absorbed and flowing through the bloodstream and stored in adipose tissue. The pigment is then deposited on the color cells (chromatophores), which are present in the dermis. The number of pigment cells in the fish's body can change so that it can affect the color of the fish. If the pigment cells are spread evenly, the color of the fish's body will appear darker, but if the pigment cells collect at one point in the nucleus of the cell, the body color will become pale **(Noviyanti, 2015)**. The increase in color brightness occurs due to morphological and physiological changes in the chromatophore cells. Morphological changes are the increase and decrease in the number of organisms' chromatophore pigment cells, influenced by the amount and composition of feed containing carotenoid sources in the feed. At the same time, physiological changes are changes caused by the activity of the movement of chromatophore pigment cells. Apart from feed containing carotenoid sources, another factor that affects the brightness of fish color is water quality **(Nafsihi *et al.*, 2016)**. The existence of biofloc is one of the efforts to maintain water quality. Furthermore, biofloc technology affects the absorption of nutrients, including the carotenoids present in *Spirulina platensis* and biofloc, in improving the color quality of fish. It is supported by the statement of **Arief (2013)** that by converting carbohydrates into lactic acid, which can lower pH and stimulate the production of endogenous enzymes that increase nutrient absorption, feed consumption, and growth and inhibit pathogenic organisms, bacteria play a role in balancing the digestive tract microbes and improving the digestibility of fish.

Water quality parameters are measured as supporting parameters. By manipulating the C/N ratio, this study proves that biofloc media affect water quality. For temperature parameters during the study, all treatments were still in the optimal range of koi fish. According to **Kelabora (2010)**, temperature plays an essential role in the growth and survival of fish because temperature can affect fish appetite. Temperature also affects respiratory activity and even fish reproduction. Drastic changes in temperature up to 5^0C can cause stress or death of fish.

The degree of acidity (pH) is one of the important water quality factors for aquatic biota. The pH has a significant impact on biochemical activities occurring in the waters; for instance, the nitrification process will stop at low pH. If there is a decrease in the pH value, there will be a decrease in dissolved oxygen concentration, an increase in respiratory activity, and a decrease in the organism's appetite. The range of pH values that can still be tolerated is around 6.0–9.0, and the optimal pH value for organisms is between 7.0–8.5 **(Ombong & Salindeo, 2016)**. A good pH parameter, according to **SNI (2011)**, is in the range of 6.5–8, and according to **Widinata *et al.* (2016)**, the optimum pH for koi fish growth ranges from 5.5 to 9.0. The pH value during the study was 6 in all treatments. This value is still in the optimal range for koi fish, the fish that are kept still have tolerance, so their growth and survival are still relatively good. It is slightly different from the research of **Minabi *et al.* (2020)**, which has a pH range of 8 in common carp biofloc in each treatment.

Oxygen is needed by aquatic organisms. A good DO value in waters for koi fish is between 5– 6mg/ L **(Homoki *et al.*, 2021)**. DO values obtained during the study ranged from 5– 6mg/ L. This amount of dissolved oxygen is quite good and optimal for aquatic organisms. The nitrite parameter was still at the optimal limit for all treatments. The nitrate parameter in the initial measurement still exceeds the quality standard, but in the next measurement, it is at the optimal limit. As for the total ammonia nitrogen parameter, all values are still at the optimal limit, and P3 C/N ratio 18 has the smallest ammonia value. It can be an indication of the high growth rate and survival rate of koi fish in the P3 treatment. At the beginning of the study, in all treatments, the nitrate parameter was higher but decreased at the end of the study. Initially, the nitrite and ammonia parameters had high values, but at the end of the study decreased in the C/N ratio treatment, except for the control treatment, the ammonia and nitrite values increased at the end of maintenance. The C/N ratio has a significant effect on nitrogen concentration reduction by using biofloc technology. In this study, ammonia and nitrate decreased as the C/N ratio increased.

This research is similar to **Minabi *et al.* (2020)**, TAN and nitrite showed an increasing trend at first, followed by a decreasing one until the end of the experimental day in the C/N ratio treatment. In the control treatment, the increasing trend continued until the 35^{th} day of the experiment and overall C/N ratio 19 in that research improved the water quality and growth performance. According to **Furtado *et al.* (2015)**, nitrogenous substances can damage gill tissue, which can impair an organism’s ability to use oxygen during rearing or even result in mortality. **Rajkumar *et al.* (2015)** explained that addition of carbon in biofloc can lower the TAN content of water and significantly increased heterotrophic bacteria. Metabolic residues in fish that are discharged into the waters and unconsumed feed will decompose through a bacterial process into ammonia. Ammonia in the biofloc system can undergo nitrification to become nitrite and then become nitrate or be assimilated by heterotrophic bacteria into new biomass. **Hafsha *et al.* (2016)** argued that the application of biofloc is one of the management methods used to utilize ammonia and provide additional feed by utilizing bacteria in the waters. According to **Aalimahmoudi and Mohammadiazar (2019)**, manipulating the C/N ratio in the biofloc system has a significant effect on the nitrogen content of both ammonia, nitrite and nitrate.

Ammonia and nitrite parameters have become the main stress factors affecting the aquaculture environment for intensive aquaculture. Based on **Wang *et al.* (2015)**, ammonia and nitrites can be potentially harmful in aquaculture when greater than 0.1 and 5mg/ L, respectively. Nitrates can also be harmful if the concentration is greater than 60mg/ L and the animal is exposed for a longer period. Therefore, ammonia and nitrogen nitrites are considered to be more harmful to aquatic animals than nitrates.

Biofloc aims to improve water quality by adding the C element to the culture media, which can trigger the growth of heterotroph bacteria **(Crab *et al*., 2012, Ahmad *et al*., 2017)**. The intensive microbial community in biofloc technology can function as a pond water quality treatment system **(Deng *et al*., 2018)**. Therefore, the use of different C/N ratios as carbon sources in the biofloc system affects the number of toxic nitrogen compounds (TAN and nitrite) and keeps these parameters within a non-hazardous range for *Cyprinus carpio*. Besides, increasing the amount of organic carbon can increase the C/N ratio, leading to the biofloculation process and the expansion of the heterotrophic bacterial mass **(Minabi *et al*., 2020)**.

CONCLUSION

The results showed that P3 treatment with the biofloc C/N ratio 18 and *Spirulina plantesis* enrichment had the highest specific growth rate, survival rate, feed utilization efficiency, and color quality and the lowest feed conversion ratio value among control and other treatments. From our result, treatment with biofloc technology also effectively reduce water quality parameter, including ammonia, nitrogen, nitrite, and nitrate.

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