

The Potential of Ornamental Fish Culture in Biofloc Technology with Different C/N Ratio and Multi-Criteria Decision Making Model: An Example of Goldfish (*Carassius auratus*)

Biyoflok Teknolojisinde Farklı C/N Oranı ve Çok Kriterli Karar Verme Modeli ile Akvaryum Balığı Yetiştiriciliğinin Potansiyeli: Japon Balığı (*Carassius auratus*) Örneği

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Abstract: Biofloc technology (BFT) has become an agenda to meet the need for protein food and ornamental aquaculture with the increasing interest in sustainable aquaculture. In this context, the current study focused on BFT for ornamental goldfish (*Carassius auratus*), which has commercial value. The study was conducted with control and two different C/N ratios (15:1 and 20:1). On the other hand, increasing the C/N ratio had a positive effect on total suspended solids and total bacteria count in the culture water. The specific growth rate and weight gain were observed to be significantly higher at a high C/N ratio. The feed conversion ratio was lower in the C/N 20 group, indicating a more efficient feed utilization. According to the liver histological results, the vacuolization symptom is more severe in BFT groups. Considering all the results, the suitability of *C. auratus* cultivation in the BFT system with a C/N ratio of 20 was proven according to nine different evaluation criteria. In conclusion, rearing *C. auratus* in BFT systems is advised due to its economic and ecological benefits.

Keywords

- Ornamental aquaculture
- Sustainable aquaculture
- Nitrogenous compounds
- Fish
- Histology

Özet: Biyoflok teknolojisi (BFT), yalnızca protein ihtiyacını karşılamak için değil, aynı zamanda akvaryum balığı yetiştiriciliği için de gündeme gelmiştir. Bu bağlamda, mevcut çalışma ticari değere sahip bir tür olan Japon balığı (*Carassius auratus*) üzerine odaklanmıştır. Kontrol grubu ve iki farklı C/N oranı (15:1 ve 20:1) ile gerçekleştirilen çalışmada, BFT gruplarında azot döngüsü hızlı bir şekilde gerçekleşmiştir. Diğer yandan, C/N oranının artırılması, kültür suyunda toplam askıda katı madde ve toplam bakteri sayısı üzerinde olumlu bir etki göstermiştir. Büyüme performansı açısından, yüksek C/N oranında spesifik büyüme hızı ve ağırlık artışı önemli ölçüde daha yüksek gözlemlenmiştir. Yem değerlendirme oranı ise C/N 20 grubu için daha düşük oranla daha verimli bir sonuç göstermiştir. Karaciğer histolojik sonuçlarına göre, vakuolizasyon semptomunun BFT gruplarında daha şiddetli olduğu tespit edilmiştir. Tüm sonuçlar göz önünde bulundurulduğunda, 20 C/N oranına sahip BFT sisteminde *C. auratus* yetiştiriciliğinin uygunluğu dokuz farklı değerlendirme kriterine göre kanıtlanmıştır. Sonuç olarak, *C. auratus*'un BFT sistemlerinde yetiştirilmesi hem ekonomik hem de ekolojik açıdan önerilmektedir.

Anahtar kelimeler

- Süs balıkları yetiştiriciliği
- Sürdürülebilir su ürünleri yetiştiriciliği
- Azotlu Bileşikler
- Balık
- Histoloji

1. INTRODUCTION

The main purpose of the growth in aquaculture is to produce more without

increasing the use of basic natural resources such as water and soil and harming the environment (Mizuta et al., 2023). Another goal is to design



systems that provide an appropriate cost/benefit ratio to support economic and social sustainability (Avnimelech, 2009). With reduced access to water resources, many alternative strategies for sustainable systems in aquaculture are being studied (Turcios and Papenbrock, 2014) and the flow-through system is a production model that has been discussed in recent years in terms of sustainable water use (Cullis et al., 2018). Recirculating aquaculture systems (RASs) use significantly less water than traditional flow-through farming systems (Ahmed and Turchini, 2021), but they involve high initial investment and operating costs, as well as management challenges, which can pose economic and logistical difficulties (Zhang et al., 2024).

Feeding costs in aquaculture facilities constitute approximately 60-70% of total production costs (Verdegem et al., 2023). Fish meal and fish oil, which are gold ingredients for protein and lipid sources, have become extremely finite items in aquafeeds. With biofloc technology (BFT), it is possible to reduce feed costs in aquaculture, the use of high water volumes, and mitigate potential environmental pollution with effluent water (Avnimelech, 1999). The BFT concept is a partially innovative method in which a suitable living environment is provided for heterotrophic bacteria (Crab et al., 2012). Thus, bacteria remove ammonia from the water and form a floc that aquatic organisms can consume. The appropriate C/N ratio in water for heterotrophic bacteria is directly or indirectly (via feed content) provided by an external carbon source added (Zhao et al., 2024). Different kind of C sources such as molasses, sucrose, and starch were used in previous studies (Minaz and Kubilay, 2021). It has been reported that successful results were obtained with the C/N ratio between 10:1 and 20:1 (Panigrahi et al., 2018; Xu et al., 2016). Additionally, temperature is vital for the proliferation of bacteria in BFT systems (Avnimelech, 1999; Minaz et al., 2023). In order to adapt to the living standards of heterotrophic bacteria, water must be at adequate temperatures. Therefore, breeding warm water fish in BFT systems can provide more economic advantages. BFT systems in aquaculture are utilized not only for food production but also for ornamental fish cultivation (Besen et al., 2021; da Cunha et al., 2020; Yu et al., 2020). Biofloc

systems can play a crucial role in farming ornamental species by optimizing water quality and maintaining fish health (Wang et al., 2015). The microorganisms' ability to biologically process waste into nutrients improves feed efficiency and promotes sustainable production (Faizullah et al., 2015). Additionally, it can reduce disease pressure, minimizing the need for antibiotics. However, proper biofloc management is necessary as water turbidity can be undesirable for aesthetic species. In this context, goldfish (*Carassius auratus*) has high value with its interesting color and is one of the most popular ornamental species in the world (Sinha and Asimi, 2007). The improvement of skin pigmentation in goldfish cultured in biofloc technology enhances the overall appeal of the fish (da Cunha et al., 2020).

In biofloc terms, the 'waste'—nitrogen formed by uneaten feed and feces from the cultured organisms—is converted into protein feed available for the same aquatic organisms. This feature enables the minimization of water exchange without compromising water quality, thereby reducing the total amount of nutrients discharged into the environment (Lezama-Cervantes and Paniagua-Michel, 2010). Despite its well-documented benefits in commercial aquaculture, the application of BFT in the ornamental fish industry remains underexplored. Ornamental fish species, including goldfish, are among the most widely traded species globally, yet their production still relies heavily on traditional systems that require frequent water exchanges, leading to increased operational costs and environmental concerns. Given the rising demand for sustainable and cost-effective production systems in the ornamental fish industry, investigating the applicability of BFT for goldfish production is essential. This study hypothesizes that different C/N ratios in BFT systems can significantly influence the growth performance, liver histology, and water quality parameters of goldfish (*Carassius auratus*). To address this hypothesis, the study compares two C/N ratios (15 and 20) against a control group to determine the most suitable protocol for goldfish culture in BFT systems. Additionally, this study employs the PROMETHEE method, a multi-criteria decision-making model, to evaluate the effectiveness of different BFT treatments

systematically. This model integrates key parameters, including water quality variables, histological indicators, and growth performance metrics, to establish a comprehensive ranking of BFT conditions. By utilizing this structured approach, the study aims to provide a scientifically sound recommendation for optimizing BFT systems in ornamental fish aquaculture.

2. MATERIAL and METHODS

This study was carried out over a period of four weeks at the Aquaculture Application and Research Center in Recep Tayyip Erdoğan University, Türkiye. Current study was checked and approved by the Ethical Local Committee of the Recep Tayyip Erdoğan University (Decision No: 2023/05). The methods were carried out in accordance with the relevant guidelines and regulations.

2.1. Experimental design

The fish were transferred to the facility 14 days before the trial to adapt to environmental conditions. During this adaptation period, the biofloc system was initiated by inoculating the tanks with aquarium water to promote the gradual development of a heterotrophic bacterial community. Three treatments were tested in triplicates, consisting of two BFT groups with C/N ratios of 15 (C/N 15) and 20 (C/N 20) and a control group. A total of 240 healthy goldfish (*Carassius auratus*), with an average weight of 3.27 ± 0.19 g, were used in this study. The fish were obtained from a local aquarium shop, and before the experiment, all individuals were examined for bacterial and parasitic diseases to ensure their health status. Each aquarium had a volume of 100 L, with dimensions of approximately 40 cm in width, 100 cm in length. The fish were fed twice daily (10:00 A.M. and 04:00 P.M.) at a rate of 4% of their biomass. The commercial diet used in the study was specifically formulated for goldfish, and its composition is provided in Table 1. The feed pellets had a diameter of approximately 1.5 mm, and no additional vitamins or supplements were added to the formulation since the study primarily focused on the effects of the C/N ratio. The feed was stored in a closed jar in a dry environment to maintain its quality. The composition of the feed included various cereals,

vegetable protein extracts, derivatives of vegetable origin, fish and fish derivatives, oils and fats, minerals, algae, and yeasts. The trial was conducted in a controlled indoor environment where the air temperature was regulated. A 12-hour light and 12-hour dark photoperiod was applied throughout the study. Starch was used as the carbon source to maintain the appropriate C/N ratio in the BFT groups, with a carbon content of 45%. The C/N ratio was calculated based on a previously determined model (Crab et al., 2012). For the control group, water was treated using an aquarium filter system (sand bed), and 80% of the water was renewed weekly. All aquaria were continuously aerated for 24 hours using an air stone system connected to a central air pump. Any water loss due to evaporation was replenished accordingly.

Table 1. Analytic constituents of commercial feed.

Components		Content
Crude protein (%)		28
Crude oils and fats (%)		3.5
Crude fibre (%)		2.0
Moisture (%)		7.0
Additives (IU/kg)	Vitamin A	16000
	Vitamin D	1890
Trace elements (mg/kg)	Manganese ($MnSO_4 \cdot H_2O$)	81
	Zinc (H_2O_5SZn)	48
	Iron ($FeSO_4 \cdot H_2O$)	31

2.2. Water quality parameters and growth performance

Water temperature, dissolved oxygen (DO), pH, and electrical conductivity (EC) were daily measured as routine water quality parameters by a portable multi-parameter (Hach, HQ40D 58258-00). The weight of total suspended solids (TSS) was measured by filtering 50 mL of aquarium water through glass fiber Whatman GF/F filter paper with 45 µm pore size. The filter papers were then dried at 60 °C for 24 hours and TSS was calculated based on the final weight of filter glass. TSS measurements were conducted every other day throughout the experimental period. The volume of settling flocs was measured once every two days by Imhoff cone (Avnimelech and Kochba, 2009). A sedimentation period of 15 minutes was allowed to measure settleable solids. Ammonia, nitrite, and nitrate were measured weekly using a

spectrophotometric method (Rice et al., 2017). Commercial test kits were used for these analyses: Hach LCK 304 for ammonia, Hach NitriVer[®] 2 for nitrite, and Hach NitraVer[®] 6 for nitrate. The total bacterial count was performed on a plate count agar (PCA) at the end of the study with three replicates.

At the end of the four-week trial period, the fish were collected to calculate final weights (FW), specific growth rate (SGR), weight growth rate (WGR), and feed conversion ratio (FCR). Fish weights were measured with a precision scale at the beginning and end of the study for growth performance calculations. Growth parameters were calculated as per the following equations:

$$SGR\%/d = (\ln \ln FW - \ln \ln IW) / t \times 100$$

$$WGR = (FW - IW) / FW \times 100$$

$$FCR = FI / WG$$

Where, FW (g) and IW (g) are the weights of fish in the final and initial stages of the trial, respectively. In addition, FI (g), WG (g), and t (day) represents feed intake, weight gain of fish, and trial period (4 weeks), respectively.

2.3. Histological examination

Anesthetized fish (60 mg/L clove oil) were cut from the abdomen and liver tissues were fixed in 10% neutral buffered formalin. Fixed tissues were transferred into 50% ethyl alcohol after 48 hours. Liver tissues were passed throughout the alcohol series and placed one night in the liquid paraffin at 65 °C. The next day, livers were embedded into the paraffin. Samples were cut with a thickness of 5 µm using a microtome. Samples taken from microscope slides were subjected to alcohol and xylene series again. Before this process, paraffin was removed at 65 °C. Then, tissue sections were stained with hematoxylin and eosin. Tissues covered with cover-slip were examined under a light microscope (Minaz et al., 2022).

2.4. Multi-criteria decision model- PROMETHEE

A multi-criteria decision model (MCDM) was used to determine which of the two C/N ratio scenarios was more beneficial (Demirel et al., 2021). This model includes a total of 4 basic phases: (1) examination of biofloc on *C. auratus*, (2) formulation of evaluation criteria and weights for each C/N ratio, (3) scoring two C/N ratios depending on evaluation criteria, and (4) final

decision the best C/N ratio with PROMETHEE method. This model considers a set of evaluation criteria for each alternative. Each evaluation criterion contains several weight values, the sum of which is "1" (It means percentage importance of criteria).

2.4.1. Determination of evaluation criteria and its weight percentage

According to the MCDM principle, alternatives must be evaluated based on predetermined criteria. The decision maker evaluates and reaches a conclusion by considering these criteria. The evaluation criteria for determining the optimal C/N ratio are based on water quality, bacterial community, fish growth performance, and the histological state of the fish. In this study, we identified 9 different evaluation criteria, which were categorized into three main headings: (1) water quality parameters, (2) growth performance, and (3) histological alterations. The evaluation criteria and their weightings, determined by expert opinion based on their importance factor, are presented in Table 2. In the model, both quantitative parameters and qualitative parameters that could be quantified were used to determine the evaluation criteria. The weight values for each criterion were established based on expert opinion, reflecting the importance of each factor. To ensure accuracy in the MCDM analysis, a clear and structured process was followed for determining the weights. A panel of experts, all highly knowledgeable in aquaculture and fish welfare, was consulted to assess the relative significance of each criterion. These experts were selected due to their extensive experience and specialized expertise in histological and blood parameter analysis. The Analytic Hierarchy Process (AHP) was employed to compile expert opinions and assign weights, as this method effectively captures expert judgment while minimizing potential biases. A total of 15 experts independently reviewed and evaluated the criteria to finalize the weights. In the multi-criteria decision analysis of groups with two different C/N ratios using biofloc technology, a Consistency Index (CI) of 0.013 and a Consistency Ratio (CR) of 0.010 were obtained, indicating an acceptable level of consistency in the prioritization of the ratios based on various criteria. In PROMETHEE software, the weight

scores of the criteria must always add up to 1 (Ozturk, 2018). Therefore, weight scores are distributed according to the importance of the criteria. The most effective criteria in choosing the best C/N ratio are SGR, FCR, ammonia, and nitrite. Because the growth performance of the fish is the primary parameter showing the effectiveness of the biofloc, while ammonia and nitrite are indicators of toxicity for fish.

Table 2. Evaluation criteria and weighting scale.

Criteria number	Evaluation criteria	Weight value	Preference function
C1	Nitrate	0.05	Linear
C2	Nitrite	0.15	V-shape
C3	Ammonia	0.15	V-shape
C4	Temperature	0.05	Linear
C5	Total bacteria	0.05	Linear
C6	Settleable solids	0.05	Linear
C7	SGR	0.15	V-shape
C8	WGR	0.10	Linear
C9	FCR	0.15	V-shape
C10	Histology	0.10	Level

2.4.2. Selection of best C/N ratio based on PROMETHEE analysis

The best C/N ratio for *C. auratus* culture in the BFT system was determined based on the PROMETHEE decision model (Visual PROMETHEE 1.1.0.0). Firstly, the evaluation criteria and the weight values of these criteria were determined. Afterwards, a decision matrix was created. The PROMETHEE decision model allows decision-makers to either select a particular option based on an evaluation factor or restrict the evaluation factor to predetermined values. The approach involves a seven-step process to arrive at a decision.

(1) The data matrix is prepared using equations 1, 2, and 3. Criterion weights are determined for *k* number criteria (*k*=10 in the current study).

$$w = w_1, w_2, \dots, w_k \tag{1}$$

$$w: \text{criteria weight} \\ c = f_1, f_2, \dots, f_k \tag{2}$$

$$c: \text{criteria weight and } f: \text{function} \\ S = (A, B, C, \dots) \tag{3}$$

S: decision alternatives

(2) Preference functions for the criteria are determined according to Equation 4 (linear preference function) and Equation 5 (usual preference function).

$$p(d) = \begin{cases} 0 & d \leq q \\ (d - p)/(p - q) & q < d \leq p \\ 1 & d > p \end{cases} \tag{4}$$

q: indifference value

p: sufficient biggest difference

q: difference between two decision alternatives

$$p(d) = \begin{cases} 0 & d \leq 0 \\ 1 & d > 0 \end{cases} \tag{5}$$

(3) The common preference function for decision alternatives “x” and “y” is calculated with Equation 6.

$$p(x, y) = \begin{cases} 0 & f(x) \leq f(y) \\ p[f(x) - f(y)] & f(x) > f(y) \end{cases} \tag{6}$$

According to Equation 6, it is determined whether the evaluation factor is maximization or minimization.

(4) The preference index of “x” and “y” decision options evaluated according to the *k*-number criterion was calculated using Equation 7.

$$\pi(x, y) = \sum_{i=1}^K w_i P_i(x, y) \tag{7}$$

(5) Determining positive ϕ^+ and negative ϕ^- advantages for alternatives with Equations 8 and 9.

$$\phi^+(x) = \frac{1}{n-1} \sum \pi(x, y) \tag{8}$$

$$\phi^-(x) = \frac{1}{n-1} \sum \pi(y, x) \tag{9}$$

(6) Partial priorities are determined with PROMETHEE I. Equations 10 and 11 show the difference between “x” and “y” decision alternatives. If any of the following conditions occur, decision option “x” is indistinguishable from decision option “y”.

$$\phi^+(x) = \phi^+(y) \tag{10}$$

$$\phi^-(x) = \phi^-(y) \tag{11}$$

Similar to the example below, if any of the conditions in Equation 12, 13, 14 occur, the “x” decision option is superior to the “y” decision option.

$$\phi^+(x) > \phi^+(y) \text{ and } \phi^-(x) < \phi^-(y) \tag{12}$$

$$\phi^+(x) > \phi^+(y) \text{ and } \phi^-(x) = \phi^-(y) \tag{13}$$

$$\phi^-(x) < \phi^-(y) \text{ and } \phi^+(x) = \phi^+(y) \tag{14}$$

In the condition where decision alternative “x” cannot be compared with decision alternative “y”, equations 15 and 16 are used.

$$\phi^+(x) > \phi^+(y) \text{ and } \phi^-(x) > \phi^-(y) \tag{15}$$

$$\phi^+(x) < \phi^+(y) \text{ and } \phi^-(x) < \phi^-(y) \tag{16}$$

(7) The ranking of decision options is performed with PROMETHEE II. The exact priorities of the decision options are determined by Equation 17. All calculated priority values are sorted from high priority to low priority. Thus, all decision options are evaluated in a similar way and a complete ranking is obtained.

$$\varphi(x) = \varphi^+(x) - \varphi^-(x) \quad (17)$$

The decisions given in equations 18 and 19 can be reached according to the full priority value calculated from the “x” and “y” decision alternatives.

$$\varphi(x) > \varphi(y) \quad (18)$$

Decision alternative “x” is superior.

$$\varphi(x) = \varphi(y) \quad (18)$$

Decision alternatives “x” and “y” are not superior.

2.5. Statistical analyzes

All data are presented as the means \pm standard deviation (SD). As a preliminary test, it was proven that the data sets exhibited normal distribution based on the Kolmogorov-Smirnov test. Student T-test was used for the comparison of total bacteria counts between groups. In addition, significant differences between groups for growth performance were determined with one-way ANOVA test. In case of significant differences between C/N groups, the Tukey post-

hoc test was used to assess differences. Differences were considered statistically significant when the calculated p value was <0.05 . All analyses were performed in SPSS software (Version 23, IBM Corp., Armonk, New York, USA).

3. RESULTS

3.1. Assessment of water quality

The water quality parameters measured over the study period are shown in Table 3. Accordingly, no significant differences were observed between the groups in terms of temperature, pH, DO, EC and settleable solids ($P>0.05$). On the other hand, the total bacteria count and TSS were significantly higher in the C/N 20 group than C/N 15 group ($P<0.01$). Even though ammonium concentrations were relatively higher in the biofloc groups in the first two weeks, there was a rapid decrease in the third week (Figure 1). On the contrary, a rapid increase was noted as of the third week in the control. Nitrite and nitrate concentrations increased rapidly in the biofloc groups, especially after the third week. The control group showed lower results than the BFT groups initially and then remained stable throughout the trial.

Table 3. Routine water quality parameters monitored throughout the study. C/N 15: biofloc group with 15 C/N ratio, C/N 20: biofloc group with 20 C/N ratio. EC: electrical conductivity, DO: dissolved oxygen, TSS: total suspended solids.

	Control	C/N 15	C/N 20
pH	7.19 \pm 0.56	6.93 \pm 0.72	6.95 \pm 0.70
Temperature ($^{\circ}$ C)	29.07 \pm 0.25	28.50 \pm 0.73	28.45 \pm 0.55
DO (mg/L)	6.75 \pm 0.27	7.28 \pm 0.26	7.12 \pm 0.21
EC (μ S/cm)	113.68 \pm 15.4	140.1 \pm 15.3	150.93 \pm 45.2
Settleable solids (mL/L)		10.25 \pm 2.12	13.91 \pm 3.30
TSS (mg/L)		346.45 \pm 38.8 ^b	456.24 \pm 48.4 ^a
Total bacteria (log CFU/mL)		10.56 \pm 0.41 ^b	12.46 \pm 0.27 ^a

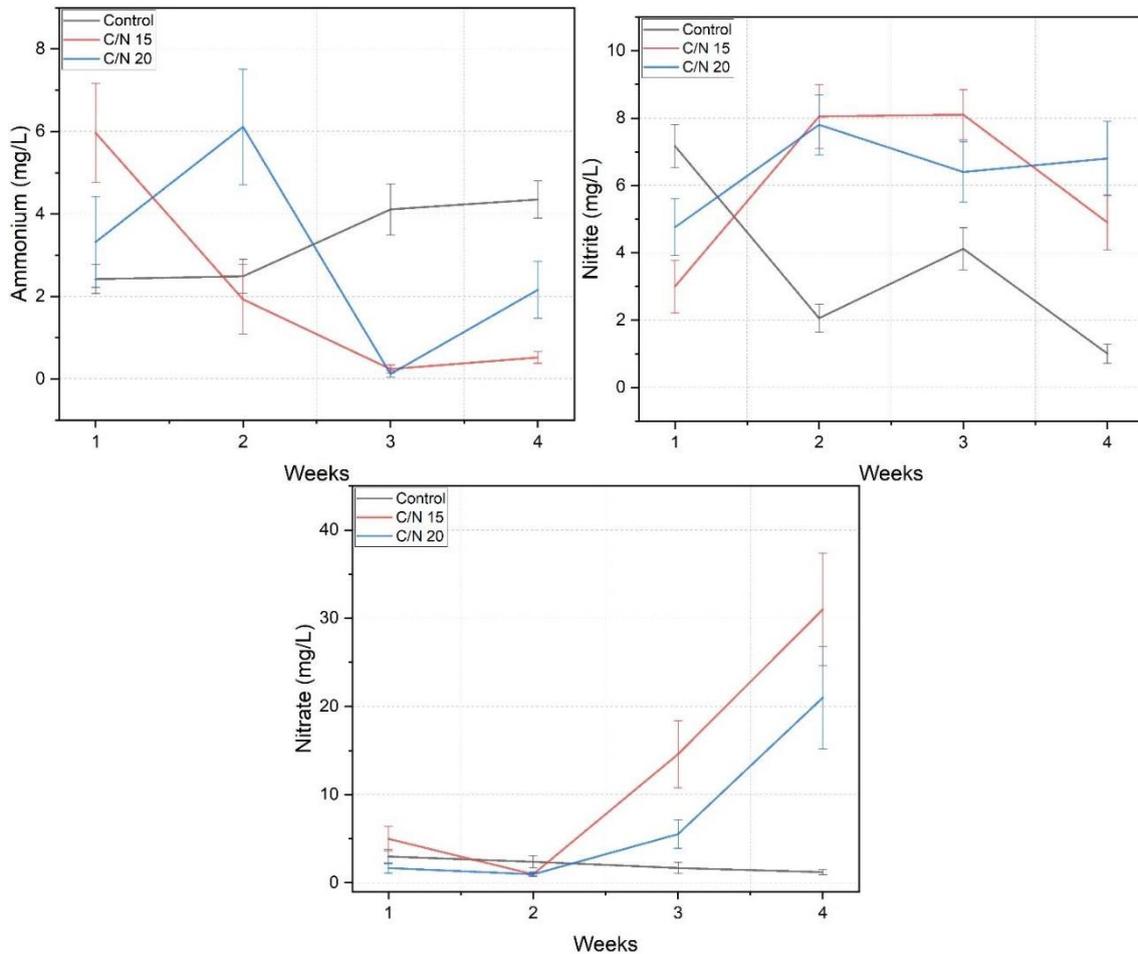


Figure 1. Changes in ammonium, nitrite and nitrate depending on time (4 weeks). C/N 15: biofloc group with 15 C/N ratio, C/N 20: biofloc group with 20 C/N ratio.

3.2. Growth performance of fish

Table 4 shows the growth performance parameters of goldfish. Final weights did not differ significantly between groups ($p>0.05$). However, the specific growth rate and weight

gain ratios were significantly higher in the BFT group with C/N 20 compared to the other two groups ($p<0.01$). In addition, a significant difference was observed in terms of FCR between the control and C/N 20 group ($p<0.05$).

Table 4. Growth performance parameters of goldfish. C/N 15: biofloc group with 15 C/N ratio, C/N 20: biofloc group with 20 C/N ratio. IW: initial weight, FW: final weight, SGR: specific growth rate, WGR: weight growth, FCR: feed conversion ratio.

Growth performance	Control	C/N15	C/N20
IW	3.35±0.35	3.21±0.17	3.24±0.08
FW	5.47±1.11	4.98±1.18	6.84±1.01
SGR	1.43±0.12 ^b	1.31±0.21 ^b	2.08±0.14 ^a
WGR	38.75±5.27 ^b	35.54±4.78 ^b	52.63±6.41 ^a
FCR	1.96±0.32 ^a	1.72±0.41 ^{ab}	1.37±0.25 ^b

*Values in the same row with different superscripts are significantly different.

3.3. Histological assessment of liver tissue

Liver histological examinations of goldfish in BFT and control treatments are shown in Figure 2. No histological differences were noted

between the groups. More severe vacuolization symptoms were observed only in the liver tissues of the C/N 20 group compared to the control and C/N 15 groups.

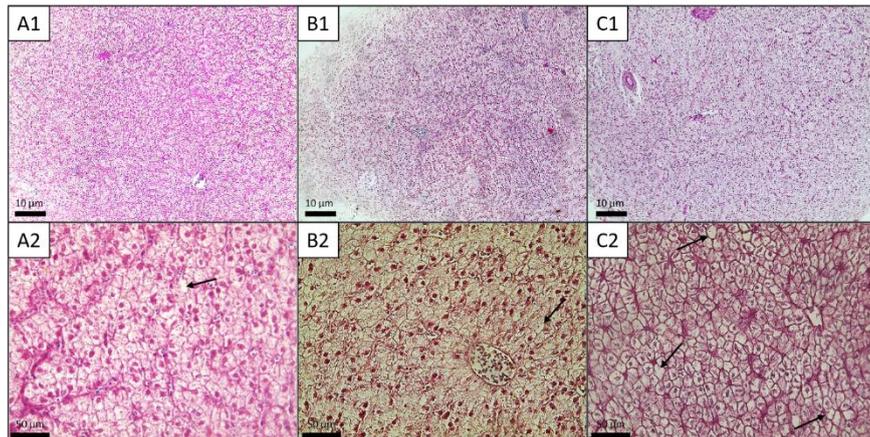


Figure 2. Histological comparison between BFT and control groups. A: Control, B: biofloc group with 15 C/N ratio (C/N 15), C: biofloc group with 20 C/N ratio (C/N20) groups. 1: 4x and 2: 10x magnification. Arrow: vacuolization.

3.4. Determination of the best C/N ratio (PROMETHEE-MCDM)

In accordance with the PROMETHEE decision model, the alternatives were ranked based on their respective scores, as presented in Table 5. Depending on the positive and negative superiority values, the control group is the least preferable alternative. Additionally, the C/N 20 ratio is the most preferable alternative depending on the evaluation criteria. Figure 3 presents the distribution of criteria affecting the ranking of treatments. The main criteria that make C/N 20

treatment the preferable alternative are the variables related to growth performance.

Table 5. Ranking of culture alternatives for *C. auratus*.

Treatments	$\phi^+(i)$	$\phi^-(i)$	$\phi(i)$	Ranks
Control	0.1464	0.4189	-0.2726	3
C/N 15	0.3077	0.3433	-0.0356	2
C/N 20	0.4873	0.1791	0.3082	1

$\phi^+(i)$ positive superiority values

$\phi^-(i)$ negative superiority values

$\phi(i)$ final superiority values

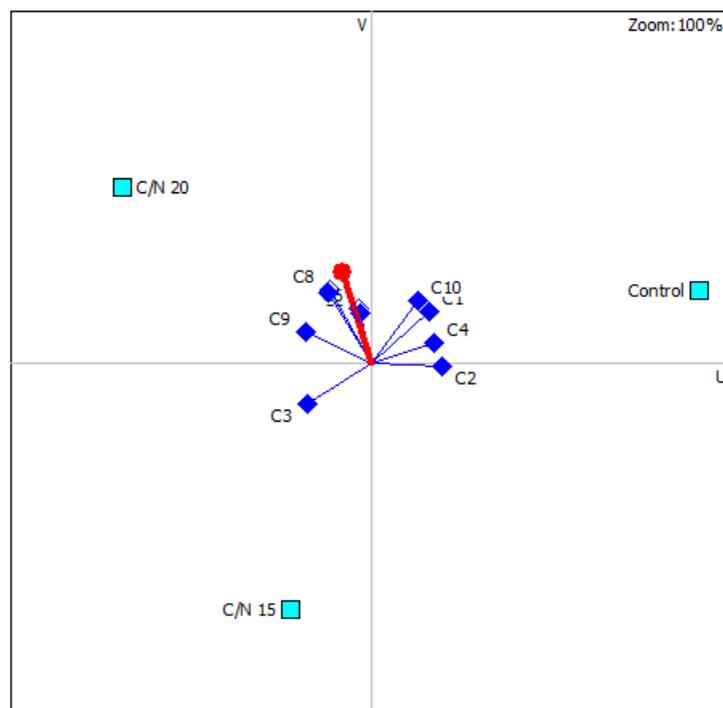


Figure 3. Distribution of criteria affecting the ranking of treatments.

4. DISCUSSION

Physicochemical water parameters are an important factor for the health of cultured organisms in aquaculture (Khanjani et al., 2021). In the current study, the pH level was within the acceptable range (around 7) for goldfish. The pH level in BFT systems is generally more stable compared to conventional systems (Boyd et al., 2011). However, significant fluctuations can occur, especially during the initial stages of the nitrogen cycle, which may impact other physical and chemical water quality parameters. Water temperature was also appropriate and within the optimum range for goldfish (between 22-30 °C) throughout the study (Avnimelech, 2009; Fraser et al., 2002). In BFT systems, the concentration of DO must be at adequate levels to meet the needs of the cultured organism and bacteria (Khanjani et al., 2020). High DO concentrations in the control group in the present study can be attributed to a low microbial community (Mirzakhani et al., 2019). The EC values increased with increasing C/N ratios due to the high organic matter concentrations resulting from C/N ratio adjustment (Crab et al., 2012). In BFT systems, TSS affects growth and survival rate of cultured organisms (Qiao et al., 2018). Low TSS concentrations (<100 mg/L) prevent sustainable high water quality and negatively affect the nitrification process (Gaona et al., 2017). However, if TSS is above 800 mg/L, it may cause blockages in the gills of fish (Schweitzer et al., 2013). In the current study, TSS concentrations (300-500 mg/L) were within appropriate levels for BFT systems. TSS, as an indicator of biofloc volume, increases with more incubation time and higher C/N ratio (Harun et al., 2019; Panigrahi et al., 2018). The fundamental principle of the BFT system relies on the development of a heterotrophic bacterial community stimulated by the added carbon source. An increased C/N ratio serves as an indicator of microbial growth and elevated electrical conductivity (EC) (Khanjani et al., 2021).

Ammonia and nitrite nitrogen are stressors that affect organisms in the intensive aquaculture environment (Tovar et al., 2000). Therefore, the maximum acceptable values for ammonia and nitrite nitrogen concentration are 0.1 and 5 mg/L, respectively (Boyd, 2017). Contrary to these low concentrations, long-term exposure is only harmful when the nitrate concentration exceeds

60 mg/L (Miranda-Filho et al., 2009). While ammonia decreased rapidly in the BFT groups after the second week, it increased in the control group in the current study. Heterotrophic bacteria in the biofloc systems remove ammonia concentration faster than nitrifying bacteria (Hargreaves, 2006; Minaz and Kubilay, 2021). The growth rate and biomass efficiency per unit substrate of heterotrophic bacteria are higher than nitrosomonas and nitrobacter. It has also been claimed that heterotrophic bacteria in BFT systems can consume ammonia nitrogen for growth and reproduction (Yun et al., 2012). As a result of the ammonia conversion, nitrite and nitrate concentrations showed an increasing trend in the BFT groups over time. Since the denitrification process of heterotrophic bacteria is longer, the removal rate of ammonia nitrogen cannot reach 100% and their nitrogen removal efficiency is in the range of 77-98% (De Schryver and Verstraete, 2009). The C/N ratio is very important in BFT systems for the stability of operation. The C/N ratio greater than 10:1 in aquaculture has shown that it can assimilate 0.2 g of nitrogen per square meter per day depending on several factors such as temperature, biomass, and illumination level (Azim and Little, 2008). Similarly, it was determined that the BFT system with sufficient C/N ratio completely converted 10 mg/L ammonia nitrogen within 5 hours (Asaduzzaman et al., 2008). Similar to our study, C/N ratio above 15:1 had a decreasing effect on ammonia concentration (Wang et al., 2015).

In the current study, the BFT system affected growth performance. Particularly in the C/N 20 group, specific growth rate and weight gain ratios were significantly improved. The higher floc volume observed in the C/N 20 group suggests that these flocs could serve as a potential feed source. Consequently, the significantly lower FCR in the C/N 20 group compared to the control may be attributed to this effect (Panigrahi et al., 2018). Biofloc mass has previously been used as an additive to commercial feeds, with results of improved FCR (Khanjani et al., 2021; Mirzakhani et al., 2019). A study on another species from the same family reported that weight gain rate (WGR) and specific growth rate (SGR) increased as total suspended solids (TSS) concentration rose, reaching an optimal threshold at 800 mg/L. However, when TSS levels exceeded 1000 mg/L, growth performance began

to decline, indicating that 800 mg/L serves as a critical limit for optimal fish growth (Qiao et al., 2018). The increase in C/N ratio positively affected SGR, WGR and protein efficiency ratio for *C. auratus* (Wang et al., 2015). However, in another study conducted on fish of similar size to our study, a positive result of SGR was observed in favor of the BFT group (Faizullah et al., 2015). Biofloc can respond to the protein and other nutrients that fish need (Avnimelech, 1999). Bioflocs contain poly-beta-hydroxybutyrate organic compounds as well as chlorophylls, phytosteroids and carotenoids (De Schryver et al., 2010). In addition, organic particles contained in biofloc support the growth of protozoa and algae in the floc (Emerenciano et al., 2012). As a result, this creates a variety of foods available for consumption by fish (Cavalcante et al., 2017). Considering its effect on BFT systems, using biofloc as a feed additive instead of fishmeal may increase weight in fish (Kuhn et al., 2009).

The liver is a reliable indicator tissue in the evaluation of nutritional metabolism in fish (Ostaszewska et al., 2005). To the best of our knowledge, the current study is the first on the histological evaluation of BFT in *C. auratus*. In the current study, significant differences in liver tissue among the groups were due only to vacuolization. More severe vacuolization was observed in the liver tissues in the C/N 20 group. The literature findings report that the over vacuolization symptoms represent high nutritional yield and energy storage rather than a pathological finding (Fontagné et al., 1998; Mosconi-bac, 1987; Segner and Witt, 1990). Therefore, it is expected that the size and volume of hepatocytes may be a physiological response to an excess of dietary energy (Najdegerami et al., 2016). B-cells in aquatic crustaceans are primarily involved in the storage of nutrients and metabolic processes (Vogt, 2021). Previous BFT studies on shrimp have reported an increase in B cells in biofloc groups (Kaya et al., 2019; Suita et al., 2015). The vacuolization observed in fish hepatocytes resembles the endocytosis and vacuole formation processes in crustacean B cells. In both groups of organisms, intracellular vacuoles are associated with metabolic waste processing, detoxification, and nutrient storage. This similarity suggests that biofloc consumption may influence cellular processes related to

metabolic regulation across different aquatic species. This finding also indicates that biofloc may play a role in modulating the immune system, similar to its effects observed in shrimp.

As a new approach in the field of aquaculture, the PROMETHEE-MCDM is a model designed for decision-makers to rank a limited number of alternatives according to conflicting criteria (De Smet et al., 2009). It has the potential in BFT studies to rank all alternative scenarios based on important criteria for the system and organism. In the current study, PROMETHEE-MCDM suggested that the C/N 20 BFT system is the most feasible alternative for the *C. auratus* culture. The positive superiority of C/N 20 was achieved, especially thanks to the growth performance criteria (C7-C9). According to the criteria, the growth performance variables were determined according to the opinion of experts from the academy and private farms. Expectedly, the growth performance of the cultured organism has been considered as one of the criteria of high weight value in this study. On the other hand, water quality parameters (ammonia and nitrite), which are of great importance for the sustainability of the BFT system, were weighted according to their toxicity potential (Kim et al., 2019; Tomasso, 1994). Although growth performance parameters were observed to be lower in the C/N 15 group, the ammonia concentration in the system made this alternative superior to the control. The PROMETHEE method provides a structured and transparent ranking process, allowing for a more objective evaluation of multiple criteria. Its ability to integrate both qualitative and quantitative data makes it a valuable tool in complex decision-making scenarios, such as aquaculture system optimization. Additionally, PROMETHEE has been increasingly applied in the aquaculture sector to assess different production strategies, sustainability measures, and management decisions (Minaz et al., 2024; Minaz, 2024). Based on the findings obtained through this model, the use of a 20 C/N ratio in BFT-based goldfish culture is supported as the most favorable option.

5. CONCLUSION

The current study aimed to explain the suitability of biofloc technology in ornamental

aquaculture. In both BFT groups, the nitrogen cycle occurred much faster than in the control group. Increasing the C/N ratio increased TSS and total bacterial loads. The growth and nutrient utilization performance of *C. auratus* was higher in the group with a C/N ratio of 20:1. Hepatic vacuolizations were observed more severely in fish on BFT treatments, which is an indicator for higher accumulation of energy. According to the multi-criteria decision-making method, C/N ratio of 20 for *C. auratus* is the best applicable method in *C. auratus* aquaculture. In conclusion, the use of biofloc systems in Japanese fish farming offers economic benefits by lowering feed costs, conserving water, reducing disease risks, and enhancing productivity. However, its effectiveness depends on proper management and the maintenance of optimal rearing conditions.

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CONFLICT OF INTEREST

The authors have no relevant financial or non-financial interests to disclose.

AUTHOR CONTRIBUTIONS

Mert Minaz: Conceptualization, Visualization, Writing - Original Draft, Data Curation.

ETHICAL STATEMENTS

Current study was checked and approved by the Ethical Local Committee of the Recep Tayyip Erdogan University (Decision No: 2023/05)

DATA AVAILABILITY STATEMENT

All datasets used during the current study are available from the corresponding author on reasonable request.

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