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RESEARCH ARTICLE

Effects of different carbon sources on growth and some innate immune responses of Russian sturgeon (*Acipenser gueldenstaedtii*) in biofloc systems

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ABSTRACT

The Russian sturgeon (*Acipenser gueldenstaedtii*) species is in high demand owing to its valuable caviar. Therefore, it is in danger of extinction. Since the Russian sturgeon reaches sexual maturity late in its life cycle, this species has a high economic cost for farmers. However, this high cost can be reduced with an environmentally friendly system called biofloc technology. This study compared the growth performance and health indicators of biofloc groups using different carbon sources such as starch (BS), molasses (BM) and dextrose (BD). In the 60-day study, fish with an average initial weight of 106.44±5.79 g were stocked in tanks at a density of 21 fish/tank (0.4 m^3/tank). The water temperature was set at 19 °C degrees throughout the study. On the 30th and 60th days of the experiment, fish were weighted to measure the growth parameters and sampled for immune indices. No mortality was observed in any group throughout the study. A between group comparison of weight gain revealed that BS and BM (105.51±2.26; 100.50±2.18) performed better than the control (BC, without external carbon sources) and BD groups (95.90±2.09; 87.36±2.18) ($P<0.05$). Furthermore, FCR and SGR were calculated from the data obtained at the end of the experiment, and the data shows that the BS and BM groups were statistically more effective than the other groups. Moreover, a comparison of NBT, lysozyme and myeloperoxidase enzyme activities indicated that all BFT groups had a stronger immune system than the control group ($P<0.05$). According to the results, the immune-enhancing effect of BFT for sturgeon was determined, and it was reported that BS and BM are more suitable for use in this species in terms of FCR and SGR, as they result an economic and environmentally friendly production.

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Introduction

Aquaculture is understood to be an important nutrient source, providing essential macro and micronutrients, as well as having a better protein conversion efficiency, lower carbon emissions and carbon footprint than other animal production systems. However, the ever-increasing demand for aquaculture in recent years has led to more intensive production per unit area. Since this situation may cause some environmental and economic problems, it has become crucial to develop new production techniques. The number of studies on BFT, a new production technology, have increased using these criteria.

Biofloc technology (BFT) is an aquaculture technique in which limited water exchange results in an increase in the number of microscopic organisms, such as bacteria, zooplankton, nematodes, fungi and, algae (Dauda et al., 2018; Khanjani et al., 2022). In this technology, the nutrients in the water are consumed by the aquatic organisms, and as a result, the number of microorganisms in the water is increased and they can be consumed as food by the fish. Increasing the number of microorganisms in the water increases the water quality because they consume the fish feces and feed waste that is in the environment (Zhao et al., 2022).

BFT has low investment and maintenance costs for intensive production (Avnimelech, 2015). Intensive aquaculture techniques produce high levels of inorganic nitrogen, ammonia (NH_3) and converted nitrite (NO_2) (Ogello et al., 2021). Bacteria in BFT systems remove nitrogen from the water for protein production (Avnimelech, 2015). This system has several benefits, such as maximizing feed conversion, increasing biosecurity, reducing water use through zero or minimal water changes, and reducing the environmental impact of wastewater (Avnimelech, 2015).

Today, the importance of protecting the environment and eliminating potential waste at its source has increased even more. Therefore, environmentally friendly approaches are supported in the aquaculture sector. Since BFT was understood to be a sustainable method in aquaculture, studies have generally followed water quality. In intensive aquaculture with zero water exchange, high nutrient input encourages the densification of microorganism communities. While some of the nitrogen (N), carbon (C) and phosphorus from the feed is used by aquatic organisms, some of it is released into the aquatic environment (Mugwanya et al., 2021). Heterotrophic bacteria in water incorporate organic pollutants into their biomass by adjusting the appropriate C and N ratio (Emerenciano et al., 2017). Bacteria-consuming communities developed from dissolved organic matter from fish and crustaceans, the water continues to maintain its quality without water exchange in the system (Pérez-Rostro et al., 2014).

Increasing aquaculture production will increase the dependence on fish feed (Liu et al., 2019). This situation returns as feed cost for farmers rises and pollution in the environment increases. In this context, BFT systems, which have increased their popularity recently, keep the wastes in the culture and transform it into biofloc as a food (Khanjani et al., 2022). The presence of heterotrophic bacteria, which is the driving force of BFT, improves the weight gain and feed conversion ratio (FCR) of fish (Bossier & Ekasari, 2017).

For aquaculture to be sustainable, the aquaculture environment must be healthy and free from diseases. Aquaculture has an economic loss of approximately 50% due to fish diseases (Assefa & Abunna, 2018). This loss negatively affects the producer directly and the consumer indirectly. Based on sustainable production, it is necessary to focus on "prevention" instead of "treatment" (Jeney, 2017). For this purpose, BFT systems emerge as an innovative strategy especially for disease resistance.

Shrimp, tilapia, and carp are the most commonly used species in BFT (Mugwanya et al., 2021). The most crucial point here is that it is possible to produce the world's two most cultivated species with sustainable aquaculture. The fact that this system has not been tested for other species and successful results have not been obtained has left this method undeveloped. However, new species have been continuously tried with BFT.

Sturgeon is an anadromous fish species that usually lives in the Sea of Azov, the Black Sea, and the Caspian Sea, with a 200–250-million-year history. Natural sturgeon populations have declined because of environmental pollution and excessive demand for their meat and caviar. As a result, the International Union for Conservation of Nature (IUCN) listed all commercially used sturgeon species worldwide in Appendix II of the CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora) regulations and prohibited their fishing in 1997. After this regulation, sturgeon aquaculture studies started to meet the worldwide demand, and successful results were obtained quickly. Although production has not reached the desired level, meat production has increased threefold, and caviar production has increased approximately fourfold between 1998 and 2017 (Sytova, 2017).

The fact that approximately 20% of the globally produced caviar is obtained from Russian sturgeon has brought this species to a very crucial position in terms of the aquaculture sector (Bronzi et al., 2019). Due to the production difficulties of being an anadromous species, its cultivation is carried out in completely controlled conditions. Temperature and feeding protocol are vital in breeding of this species, and sperm and egg are usually taken with hormone application in spring (Vasilyeva et al., 2019).

Although wide range of production techniques, including recirculating aquaculture system (RAS) and cage are used in the production, the most widely used method is the production in flow-through system with 36% (Bronzi et al., 2019). However, the inability to provide optimum water conditions throughout the year leads the species to grow with RAS, but in this case high electricity and operating cost arise.

RAS has the additional advantage of the helping the species reach sexual maturity 8 years earlier. On the other hand, studies have reported that the taste and quality of the caviar obtained from the fish produced with RAS is suboptimal as a disadvantage (Korchunov, 2012). Therefore, studies on new and combined production techniques are needed. With biofloc technology, an innovative new production model, feed cost can be reduced, feed efficiency can be increased, fish health can be improved, and environmental impact can be minimized because water use will be reduced. Therefore, in this study, the effects of different C sources on growth and some innate immune responses of Russian sturgeon (*Acipenser gueldenstaedtii*) in biofloc systems BFT were investigated.

Material and Methods

Experimental Design and Set-Up

The studies were carried out in the Trabzon Central Fisheries Research Institute (SUMAE). Russian sturgeon produced in SUMAE were used as material. A total of 252 fish grown in a flow-through system (fresh water) in concrete ponds were taken and placed in a total of 12 tanks (0.4 m^3/tank), consisting of 3 replications, with 21 fish in each, after an adaptation period of approximately 10 days. Fish were obtained with an average weight of 106.44±0.63 g. Throughout the studies, the temperature was fixed at 19 ± 1°C. Temperature, oxygen, and pH were recorded daily with the Hach HQ40d probe. During the experiment, all groups were fed twice day (08:00-16:00) with diet containing 45% crude protein as 3% of their total biomass. The feed was obtained from a commercial fish factory (manufactured by Sürsan Aquaculture Company), and the nutritional values of the feed are shown in Table 1. The experiment was conducted for 60 days.

Table 1. Proximate composition of the experiment diet

Diet Content	Amount
Crude protein (%)	45
Crude lipid (%)	20
Ash (%)	6
Vit A (IU)	8800
Vit D3 (IU)	1600
Vit E (mg/kg)	200
Calcium (%)	1.75
Sodium (%)	0.25
Total phosphorus (%)	1

Note: Diet was manufactured by Sürsan Aquaculture Company

Four treatments were considered for the present study, including a control group (BC, without external C sources) and three biofloc treatments with different external C sources (BM: molasses; BS: starch; BD: dextrose). The experiment was carried out according to the principles of the animal experimentation ethics committee of SUMAE.

Floc Formation

In order to accelerate flocculation, 3 separate tanks were prepared for each type of C source. After adding 25 mg/l N to each preparation tank, molasses, starch, and dextrose were added separately to the tanks as a C source. After the biofloc level in the tanks reached 20 ml/l, floc was inoculated into the treatment tanks to support the formation of floc, and the experiment was started later. Oxygen for the fish was provided by an air blower; 3 lines were drawn for each tank, and cylindrical air stones were used.

The daily amount of external C sources (once a day) for each group was determined, as reported by Avnimelech (1999). The C:N ratio was considered 15:1. During the experiment, the biofloc density in the tanks was monitored with the Imhoff funnel. No complete water changes were made; we only added water to replace the water lost by evaporation.

Growth Parameters

The fish weights were measured at the beginnings, day 30, and the end of the experiment. Growth parameters; weight gain (WG), specific growth rate (SGR), survival rate (SR), and feed conversation ratio (FCR) were calculated as reported by Khanjani et al. (2017).

Formulas for determining growth parameters;

$$SR(\%) = 100 \times \frac{Final\ number\ of\ fish}{Initial\ number\ of\ fish} \quad (1)$$

$$WG(g) = Final\ weight - Initial\ weight \quad (2)$$

$$SGR(\%) = 100 \times \frac{\ln(Final\ weight\ (g)) - \ln(Initial\ weight\ (g))}{Time\ (Exp\ days)} \quad (3)$$

$$FCR = \frac{Feed\ provided\ (g)}{Weight\ gain\ (g)} \quad (4)$$

Blood Sample Analysis

In days 30 and 60, 7 fish were taken from each tank for blood sample collection and analyses. The fish were stunned with clove oil (dose: 30 mg/l), a widely used natural product. After thoroughly cleaning the posterior part of the anal fin with alcohol to prevent blood from mixing with mucous, blood was drawn from the caudal fin by inserting a 5 ml plastic syringe without harming the fish. Collected blood samples were placed in K3EDTA and gel serum tubes and immunological analyses were performed.

Immunological Analyses

Respiratory burst activity

The respiratory burst activity of neutrophils and monocytes was determined by NBT (nitro blue tetrazolium) activity in blood. For NBT analysis, 100 µl of blood samples were incubated for 30 minutes with NBT solution. 50 µl of this mixture for each sample, was added to tubes containing N, N-dimethyl formamide. Subsequently, the centrifuged tubes were read at 540 nm in a 1 ml spectrophotometer cuvette. NBT activity was calculated as mg NBT formazan/ml (Siwicki et al., 1993).

Lysozyme activity

In order to determine the lysozyme activity, phosphate buffer solution (PBS) was added at the same rate to 100 µl of the serum sample. *Micrococcus lysodeicticus* was added to this mixture and read in a multiscan microplate reader at 530 nm at 0.5 for 4.5 minutes. Analysis results were calculated as µg/mL (Ellis et al., 1990).

Myeloperoxidase activity

For myeloperoxidase activity (MPO), 10 µl of the serum sample was diluted with 90 µl of hanks balanced salt solution (HBSS) solution. Afterwards, a solution containing 3,3′,5,5′-tetramethylbenzidine dihydrochloride and hydrogen peroxide was added to this mixture, and the reaction was stopped with 35 µl of sulfuric acid after 2 minutes, and readings were taken at 450 nm in a multiscan microplate reader (Quade & Roth, 1997).

Data Analysis

In this study, analyses of variance were used to evaluate the relations between the experimental groups, and Tukey multiple comparison tests were used for the differences between the groups using the SPSS v22 statistical program.

Results

At the end of the treatment, the best FCRs were found as 2.18±0.01 and 2.16±00 in the BS and BM groups, respectively (P<0.05). The highest SGRs were also in the BS and BM groups (P<0.05), as were the highest degrees of weight gain (P<0.05). The group with the highest feed consumption was BS, whereas the groups with the lowest were BM and BD (P<0.05), as shown in Table 2.

As shown in Table 3, Figure 1, Figure 2 and Figure 3, the results of the NBT analyses on 30th and 60th days indicate that the biofloc groups had a higher NBT than the BC (P<0.05), with the BS group having the highest NBT of all biofloc groups (1.39±2.13;2.53±0.05). In another analysis examining the effects of different C sources on lysozyme and MPO, as immunological parameters, the BS group also had the highest lysozyme and highest MPO (P<0.05) (Figure 1, Figure 2). Despite showing no difference between them, the biofloc groups were significantly different from the BC (P<0.0.5) in terms of MPO and lysozyme.

Table 2. Growth performance of fish reared in biofloc system with different carbon sources

Parameters	BS	BD	BM	BC
Initial weight (g)	106.73±1.26	105.20±1.25	108.11±1.27	105.72±1.26
Final weight (g)	212.24±1.70^{a}	192.57±1.69^{c}	208.61±1.62^{a}	201.62±1.62^{b}
Weight gain (g)	105.51±2.26^{a}	87.36±2.18^{c}	100.50±2.18^{a}	95.9±2.09^{ab}
FCR (g)	2.18±0.01^{a}	2.46±0.01^{c}	2.16±0.00^{a}	2.39±0.00^{b}
SGR (%)	1.14±0.02^{a}	1.00±0.02^{b}	1.09±0.02^{a}	1.07±0.11a^{b}
Feed Intake (g)	77.44±0.33^{a}	72.23±0.28^{b}	72.71±0.29^{b}	76.44±0.22^{c}

Note: Values are provided as mean ± standard error (n=21). Each treatment has three replications. Values with no superscripts in horizontal row are not significantly different (P>0.05). BS: starch, BD: dextrose, BM: molasses, BC: control (without external C)

Table 3. Immunological indices of Russian sturgeon reared in biofloc system

		BS	BD	BM	BC
	Initial	0.87±1.97^{a}	0.82±0.03^{a}	0.85±0.00^{a}	0.86±0.06^{a}
NBT	30th day	1.39±2.13^{a}	1.36±0.05^{a}	1.36±0.07^{a}	0.88±0.06^{b}
(OD at 540 nm)	60th day	2.53±0.05^{a}	2.40±0.09^{a}	2.46±0.13^{a}	0.89±0.06^{b}
	Initial	20.20±0.85^{a}	20.09±0.84^{a}	20.08±0.84^{a}	20.3±0.85^{a}
Lysozyme	30th day	30.30±1.27^{a}	30.14±1.27^{a}	30.13±1.27^{a}	20.7±0.95^{b}
(µg/ml)	60th day	36.06±1.52^{a}	35.87±1.51^{a}	35.85±1.51^{a}	20.9±0.73^{b}
	Initial	39.18±1.27^{a}	38.97±1.64^{a}	38.97±1.64^{a}	39.38±1.66^{a}
Myeloperoxidase	30th day	46.66±1.51^{a}	46.41±1.96^{a}	46.40±1.96^{a}	39.58±1.67^{b}
(U/l)	60th day	50.48±1.65^{a}	50.21±2.12^{a}	50.19±2.12^{a}	40.17±1.69^{b}

Note: Data are presented as means ± SEM. values with different superscripts in horizontal are statistically different (P<0.05, *n* = 7). Each treatment has three replications. BS: starch, BD: dextrose, BM: molasses, BC: control

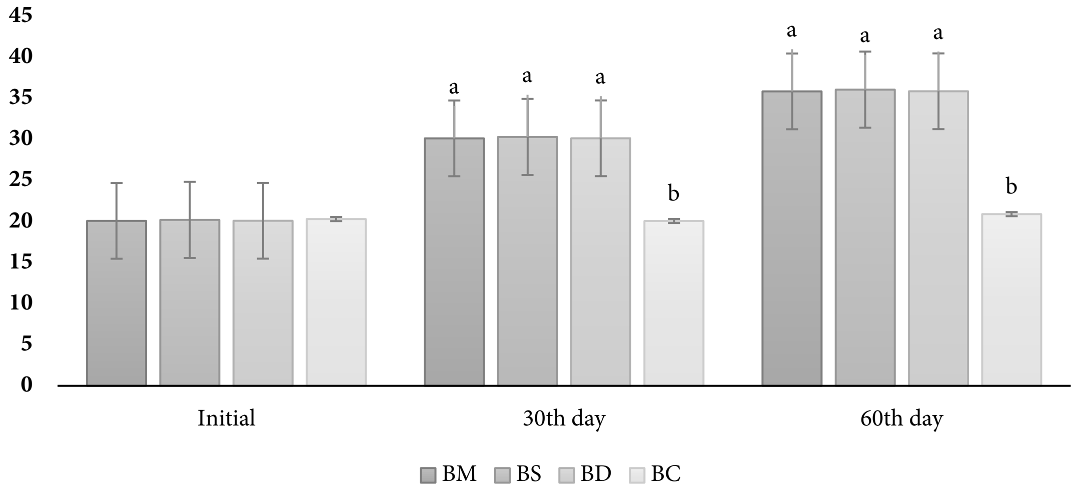

Figure 1. Lysozyme activity of Russian sturgeon reared in different biofloc systems (Error bar showing standard deviation of three replicates (*n*=7). Significance between different groups (P<0.05) marked with asterisk. Error bar with no superscripts is not significantly different (P>0.05). BS: starch, BD: dextrose, BM: molasses, BC: control (without external C))

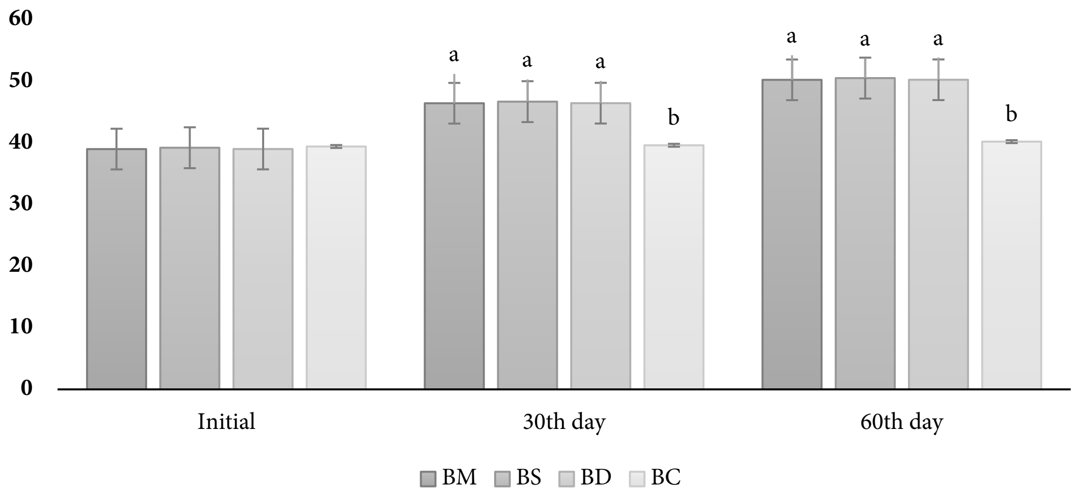

Figure 2. Myeloperoxsidase activity of Russian sturgeon reared in different biofloc systems (Error bar showing standard deviation of three replicates (*n*=7). Significance between different groups (P<0.05) marked with asterisk. Error bar with no superscripts is not significantly different (P>0.05). BS: starch, BD: dextrose, BM: molasses, BC: control (without external C))

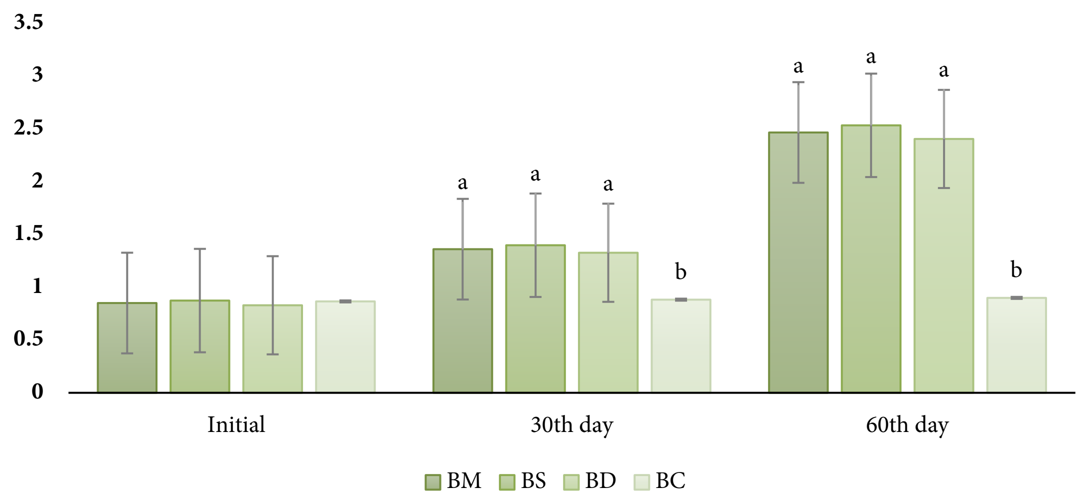

Figure 3. NBT analysis of Russian sturgeon reared in different biofloc systems (Error bar showing standard deviation of three replicates (*n*=7). Significance between different groups (P<0.05) marked with asterisk. Error bar with no superscripts is not significantly different (P>0.05). BS: starch, BD: dextrose, BM: molasses, BC: control (without external C))

Discussion

In this study, they identified other C sources that affect the sturgeon. No studies have reported comparable growth parameters of Russian sturgeon BFT using various C sources. Therefore, it is necessary to compare the growth parameters of the current study with those of Aghabarari et al. (2021). Using only a single C source in the beluga sturgeon (*Huso huso*) and Khanjani et al. (2021) studied the effect of different C sources on the growth performance of Nile tilapia (*Oreochromis niloticus*). Aghabarari et al. (2021) cultured beluga sturgeon (120 fish, 3% feeding rate) with an average initial weight of 168.2±2.9 g in 4 m^3 liter fiberglass tanks for 8 weeks. In another study, Khanjani et al. (2021) cultured Nile tilapia fingerlings (average weight: 1.7 ± 0.1 g, 160 fish/tank, 6% feeding rate) in 160-liter tanks for 30 days. The current study is a small-volume aquaculture model and was carried out in 0.4 m^3 tanks in a controlled environment (21 fish/tank, 3% feeding rate). The protein ratio of the feed used in the present study is 45%. Khanjani et al. (2021) gave the protein content of the feeds as 35% in his research, while the protein ratios of the feeds were not reported in the study of Aghabarari et al. (2021).

Biofloc contains nutrients such as fatty acids, amino acids, and minerals that improve the growth performance of aquatic organisms (Ju et al., 2008; Najdegerami et al.,2016; Ahmad et al., 2017; Panigrahi et al., 2019; Adineh et al., 2019). Khanjani et al. (2021) reported the group using corn, starch, molasses and barley as C sources in Nile tilapia achieved better weight gain than the control group. According to the same study, the group that used molasses and starch had significantly higher survival rates than the other group that used corn and barley. Likewise, Durigon et al. (2020), Hoang et al. (2020), Khanjani et al. (2020) and Mirzakhani et al. (2019) reported that the growth performance was better in BFT in tilapia. In contrast to previous studies, Aghabarari et al. (2021) found no significant difference between biofloc and control groups in terms of final weight and survival rate in beluga sturgeon. Although, present study using molasses, starch and dextrose as a C source, the C/N ratio was optimized to be 1/20, Aghabarari et al. (2021) did not report the C source and the C/N ratio. In terms of weight gain, unlike Khanjani et al. (2021), it was found that only the starch performed better in the current study. Upon closer examination of the details, it should be noted that the current study used tanks with a water volume of 0.4 m^3 and surface area of 1 m^2. The study conducted by Aghabarari et al. (2021) used larger tanks (4 m^3) in a greenhouse, which could induce the development of algae and zooplankton resulting in, additional nutrient sources in the water beyond the biofloc. Furthermore, Khanjani et al. (2021), conducted a study for 30 days, providing twice the daily nutrient input compared to the current study, indicating a higher nutritional input for Nil tilapia compared to the current study. Although the molasses and control groups showed similar differences in the current study compared to Aghabarari et al. (2021), a full comparison could not be made due to the lack of reported C source used by the researcher. Despite the same duration of the study (8 weeks) difference may be explained by the lower water volume (160-400 liters) and larger fish size used in the study by Aghabarari et al. (2021) as well as the higher initial bodyweight of the great sturgeon compared to the Russian sturgeon used in the current study.

These differences between studies suggest that weight difference could be considered.

FCR is an important parameter used to determine the effectiveness, suitability, and acceptability of specially prepared feeds for fish. Aghabarari et al. (2021) reported an FCR as 1.50±0.16 for control and 1.20±0.33 for BFT, with a survival rate of 100% in each group. Since there were no mortalities in the current study, it can be said that the studies are similar. FCR in the current study were as follows: BS: 2.18±0.01, BD: 2.46±0.02, BM: 2.16±0.00, F: 2.39±0.00, depending on the groups. both studies had a feeding rate of 3%, but objective comparison is not possible, considering that Aghabarari et al. (2021) did not report the protein content of the feed used in the study. The FCR of beluga sturgeon in both the biofloc and control groups was lower than in the current study. Differences may be due to initial fish weight, protein ratio of the feed, and system differences, as discussed above. Therefore, FCR must be supported by other critical parameters such as stock density and survival rate, as it is insufficient to measure efficiency without comparing it with other parameters.

Water temperature, feeding rate and fish size are crucial growth factors for fish (Şener et al., 2006). The current study found that the best final weight results and SGR were in the BFT groups using starch and molasses, with no significant difference observed between the dextrose and control groups. Similar findings were reported in other studies conducted on tilapia (*Oreochromis mossambicus*) (Avnimelech, 2007), freshwater shrimp (*Macrobrachium rosenbergii*) (Asaduzzaman et al., 2008), and western white shrimp (*Litopenaeus vannamei*) (Xu et al., 2012), which showed that the biofloc groups had better results than the control group. Although Aghabarari et al. (2021) reported no significant difference in final weight between the groups, SGR was found to be better in BFT. Therefore, the high growth indexes of BFT can be attributed to the presence of bacteria in the water, which acts as a factor that improves environmental conditions.

Many studies have been published on improving the immune system. Researchers have found that various supplements can increase immunity in fish, including tulsi (Das et al., 2015), levan (Gupta et al., 2008), levamisole (Maqsood et al., 2009) and β-glucan (Misra et al., 2006). Additionally, increasing the number of beneficial bacteria in the water has been shown to elevate the presence of components such as carotenoids, Poly b-hydroxybutyrate (PHB), chlorophylls, and polysaccharides in the water, this strengthens the immune system of fish, and also helps to reduce viral, bacterial, and pathogenic effects In the current study, the effects of different C sources on the immune system were examined, and when the results were evaluated, no difference was found between C sources. The results indicate that it is possible to grow sturgeon in the BFT, and this system positively affected non-specific immune indicators such as NBT. This accords with the findings from Ekasari et al. (2014), Komara et al. (2022), and Ahmad et al. (2017).

MPO and lysozyme are known as enzymes that defend against bacterial infections in aquatic organisms. The detection of the highest MPO and lysozyme activity in the BFT explains that BFT improves the immune system in sturgeon fish and creates a stronger defense system against disease factors. The increase in MPO and lysozyme activity is similar to previous studies by Mansour & Esteban (2017) in *O. niloticus*, Ahmad et al. (2017) in Indian major carp (*L. rohita)*, and Zhang et al. (2018) in gibel carp (*Carassius auratus gibelio)*.

Conclusion

The growth performance and health characteristics of sturgeon, which are in danger of extinction, were compared in the present study using different C sources in BFT, a new aquaculture technology. It has been determined that BFT saves water by minimizing the water content change, and thus, ecosystem damages that occur in aquaculture activities carried out in traditional methods have been significantly reduced. In this study, where different C sources were used, growth parameters such as weight gain, SGR and FCR were positively affected in the groups using molasses and starch, compared to the control and dextrose groups. On the other hand, results showed that each different C source used strengthened the immune system.

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Compliance With Ethical Standards

Authors' Contributions

SB: Conceptualization, Supervising
TÖ: Conceptualization
İSY: Writing, Review, Editing
All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

The protocol was approved by the Animal Use and Care Committee of Central Fisheries Research Institute (SUMAE), Republic of Türkiye Ministry of Agriculture and Forestry via document No. Etik-2023/14.

Data Availability Statements

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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