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Research Article

The effect of Biofloc system on water quality, growth and hematological indices of Juvenile great sturgeon (*Huso huso*)

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Abstract

The basis of biofloc technology is biomaterials, dead organic particles, microbes, algae, protozoa and other organisms. The biofloc acts as a biological filter and is an environment for bacteria that can remove the ammonia. In this study, for evaluate the effect of biofloc system on physical and chemical parameters of water, growth performance and blood indices, 120 juvenile *Huso huso* with an initial mean weight of 168.2±2.9 g were introduced in 6 fiberglass tanks with volume 2000 liters and reared for 8 weeks. Treatments included control and a biofloc system with 3 replications in each treatment. During the experiment, physical and chemical parameters of water, growth, and blood indices in fish were measured. Based on the results, a significant decrease was observed in feed conversion ratio (FCR). Specific growth rate (SGR), weight gain and weight gain percentage increased significantly in biofloc system compared to the control ($p<0.05$). The lowest number of white blood cells, neutrophils and the highest number of red blood cells, hematocrit and mean corposcular volume (MCV) were observed in the biofloc treatment and there was a significant difference compared to control treatment ($p<0.05$). Physicochemical parmeters results such as pH, NO_3, NH_3, NH_4 and TAN values showed a significant difference in biofloc system compared to the control ($p<0.05$). In addition, biofloc treatment had more bacteria than the control ($p<0.05$). Salinity, nitrogen to phosphorus ratio, alkalinity and orthophosphate levels showed no significant difference between control and biofloc system ($p>0.05$). However, the total amount of suspended solids (TDS) in biofloc treatment was significantly higher in bifloc system than the control ($P<0.05$). Therefore, biofloc system can be selected as the better treatment than control according to its good performance in farmed beluga.

Keywords: Biofloc, Water quality, Growth, Hematological indices, *Huso huso*

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Introduction

Sustainable development of aquaculture should be such that it provides maximum production without further use of primary resources such as water and soil, no harm to the environment and the optimal cost-benefit ratio in the economic and social dimension simultaneously. Biofloc system, which is currently one of the most important technologies in the industry, especially fish farming, can help the development of the aquaculture industry with these conditions. By maintaining water quality, biofloc reduce the need for water exchange and reduce the feed conversion ratio of farmed shrimp by producing consumable microbial protein (Abdirad and Ghaednia, 2016).

A biofloc system is a microscopic particle made of bacteria, diatoms and algae, food particles and dead organisms. Biofloc technology is known as the new "water revolution" in the field of aquaculture, because nutrients can be recycled continuously. Reuse in culture medium was obtained using minimal or zero water exchange. Such an environmentally friendly technique is based on the production of microorganisms that play three main roles (1): maintaining water quality by absorbing nitrogen compounds produced (2) feeding, increasing cultivation capabilities by reducing feed conversion ratio (FCR) and feed costs; (3) competition with pathogens as well as correction of carbon to nitrogen ratio in culture medium. In addition, the sustainable approach of such a system is based on high production of small fish or shrimp. In this method, fish or shrimp in an intensive method (at least 300 g per square meter) with minimal water exchange or at zero (Emerenciano *et al.*, 2012a). In addition, biofloc is a rich source of protein and natural fats from locally available foods that are available 24 hours a day due to the complex interaction between organic matter, physical substrate and a wide range of microorganisms. This natural productivity plays an important role in nutrient circulation and maintaining water quality. Using biofloc with fish or shrimp has many benefits such as improved growth rate, reduced FCR, and feed costs (Avnimelech, 2009).

The cost of food accounts for more than 50% of total production costs, most of which are related to the supply of dietary protein sources (Bender *et al.*, 2004). In addition, climate change in recent decades, the occurrence of numerous droughts and lack of fresh water resources are other problems facing this industry (Bakhshi *et al.*, 2016). Due to the valuable freshwater resources, today 41% of the world's people live near rivers facing drought. About 70% of the world's population is projected to face water scarcity by 2050 (Avnimelech, 2009).

Currently, due to higher biosafety and greater environmental benefits of RAS aquaculture systems, the use of these systems has increased. These systems reduce the entry of parasites and species and the burden of bio-pollution (Ray, 2012). The rotational system is based on the principle of reuse of water that is excreted after the breeding

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process (Putra and Dan Setiyanto, 2011). The use of biofloc technology to breed species that are more adapted to environmental conditions is more successful (Souza *et al.,* 2014). The aim is to reduce the contamination of the breeding system by the bacteria in them, and the biofloc, in addition to reducing the contamination of the pool, is eaten by fish. Studies on the effect of biofloc on physical and chemical parameters of water, blood indices and growth of various aquatic animals have been conducted, by Bakhshi *et al*. (2014) on the efficiency of using biomass production system in common carp (*Cyprinus carpio*), Khanjani *et al.* (2016), on the production and evaluation of bioflocs for use in systems without water exchange, Haghparast Radmard *et al.* (2018), on the effect of different carbon-nitrogen ratios in intensive breeding system Biofloc on growth and health indices of common carp, Khanjani (2019), on the application of biomass technology in aquaculture with emphasis on ornamental fish, Crab *et al*. (2010), on the application of biofloc technology to protect shrimp saline water (*Artemia franciscana*) against *Vibrio harveyi,* Mahanand and Srinivasa (2012), on optimal feed formulation for *Labeo rohita* with biofloc, Suantika *et al*. (2015), on the function of the drainage system with nitrifying bacteria and microalgae *Chaetoceros calcitrans* in super-intensive breeding of white shrimp (*Litopenaeus vannamei*). Biofloc effect on sturgeon has not been yet studied.

Sturgeon has high economic and fishery value (Webster and Lim, 2002). *Huso huso* is a valuable sturgeon species whose population has been decreased in recent years due to damage to spawning grounds (Peterson *et al.*, 2006). The aim of this study was to evaluate the efficiency of biofloc system on water quality, growth and blood indices of *H. huso* with the ultimate goal of improving the quality of physical and chemical parameters of rearing environment, increasing growth performance, and improving blood indices.

Materials and methods

This experiment was performed in the International Sturgeon Research Institute of the Caspian Sea in 2019. For this study, 120 beluga fish with mean inintial weight 168.2±2.9 g were distributed in six tanks under the same environmental conditions after adaptation and reared in 6 fiberglass tanks with 4000 liter in an indoor environmental condition using well water during 8 weeks. Each of the tanks equipped with an aeration system with a flow water rate of 3 liters per minute. Biofloc treatment and control were considered. Chemosynthesizing and nitrifying bacteria such as *Nitrosmonas, Nitrozococcus*, *Nitrozira, Nitrozbolus*, and Vibrio were used in the BFT system. Prior to the start of the experiment, the fish were fed for adaptation with a basic diet for one week. The required amount of food was weighed using a digital scale and fed to the fish according to the desired treatments. Fish were fed about 3% of body weight in 4 meals in 8, 12,

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16 and 20 hours manually (Sener *et al*., 2006). Total length recorded to the nearest 1 mm and total weight recorded to the nearest 0.1 g , all measurements were done in all treatments and replications at the beginning and end of the study. Fish feeding was stopped 24 hours before and after the bioassay to reduce stress. According to the length and weight of fish obtained from bioassay, growth parameters were estimated to find the growth trend of fish in the treatments.

- Daily growth (g/day) GR (Merrifield *et al*., 2011)
  Bwi= average initial weight per tank, Bwf= average final weight per tank, n= number of breeding days
- Weight gain percentage (% BWI) (Merrifield *et al.*, 2011)
  Bwi = average initial weight per tank, Bwf= average final weight per tank
- Specific growth rate (percentage per day) SGR (Merrifield *et al*., 2011)
  Wo=mean of primary biomass (g), Wt=mean of final biomass (g), T= number of days of rearing
- Obesity coefficient (K or CF) (Merrifield *et al.*, 2011)
  BW=mean final body weight (g), TL= mean total final length (cm)

Feed conversion ratio (FCR): (Merrifield *et al.*, 2011) FCR= F/(Wt - Wo)
  F=amount of food consumed by fish, Wo=mean of primary biomass (g), Wt=mean of final biomass (g)

- Using Rees solution with melange and neobar slide (Klontz, 1994). Hemoglobin was measured using the cyan-meth hemoglobin method and by spectrophotometer (Model 2100-VIS made by Unico USA) with a wavelength of 540 nm (Klontz, 1994). Microhematocrit tubes and a Hettich microcentrifuge at 7000 rpm for 5 minutes were used to measure Survival rate (SR) (Grisdale-Helland *et al.*, 2009) SR=100% (N / T)
  N=fish number at the end of the period, T: fish number at the beginning of the period

Blood samples were taken from the fish after bioassay and 24 hours after feeding. Blood was drawn from the tail vein using a 2 cc syringe. 0.5 cc of blood inside heparin-containing epinephric tubes for blood counts (Torrecillas *et al.*, 2011). Isolation of serum from blood cells was performed using a centrifuge (Labofuge 200 model, manufactured by Heraeus Sepatech, Germany), at a speed of 3000 rpm in 10 minutes. Serum was isolated using a microsampler and transferred to a vial (Pottinger and Carrik, 2001). Red and white blood cells were counted hematocrit.

96% methanol and 10% Giemsa solution (made by Merck Germany) were used to stain white blood cells and white blood cell counts such as neutrophils, lymphocytes, monocytes and eosinophils were counted by the method. Zigzag took place (Klontz, 1994; Ameri Mahabadi, 1999).

Glucose levels were determined using the Glucose C_2-test Wako kit and by enzymatic method by motarotase and glucose oxidase (Kubokawa *et al.*, 1999).

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Water temperature was measured daily using a conventional thermometer (Bakhshi *et al.*, 2014). pH levels were measured daily for pH dynamics in each biofilter substrate using Mettler Toledo pH meter (Suantika *et al.*, 2016).

Ammonium, nitrite and nitrate were measured daily for chemical parameters using Nessler, diazotized and Nitrate HCl methods, respectively (Ahn *et al.*, 2005). Ammonia water content was measured by spectroscopy using a spectrophotometer (Model 9200CE, Cecil) (Moopam, 1999). Total ammonia nitrogen (TAN) concentration was measured using Palin photometer 7500 (made in England) (Khanjani *et al.*, 2016). Bacterial counting was performed using the whole plate counting method (Suantika *et al.*, 2012). Each sample was added to a 0.85% NaCl solution at a ratio of 1:17 v / v and exposed to ultrasonic vibration for 10 minutes using a sonicator (Bransonic 3510-DTH) to separate the cells. Be fragmented. Then 100 μL of the solution dissolved in Vinovogradsky agar medium for the growth of oxidizing bacteria ammonium and nitrite and in the nutrient agar medium for the growth of heterotrophic bacteria, each was distributed in three pieces (Cappuccino and Sherman, 2012).

SPSS 22 software was used to analyze all the data and Excel 2020 program was used to draw the graphs. This study was conducted in a completely randomized design. At first, the normality of the data was tested by Shapiro-Wilk test. When the data distribution was normal, to compare the mean of the data between different treatments of the analysis of variance, one One-way ANOVA and Tukey test were used to isolate homogeneous groups at 5% probability level and non-parametric Kruskal-Wallis test was used when the data were not normal. Data presented as standard±SD.

Results

Physical and chemical parameters

The results of water physical and chemical parameters showed no significant difference in water temperature and NO_2 between treatments (p>0.05). NO_3, NH_3, NH_4, TAN, pH and total bacteria count showed significant difference between the treatments (p<0.05) (Table 1).

Growth performance

Based on the obtained results, no significant difference was observed in final weight, final length, average daily growth, survival percentage and obesity coefficient between treatments (p>0.05). There was a significant difference in feed conversion ratio, specific growth rate, weight gain and weight gain percentage between treatments (p<0.05) (Table 2).

Table 1: Results of water physical and chemical parameters during 8 weeks.

Indices	Control	Biofloc
Temprature (ºC)	18.50 ± 0.20[a]	18.47 ± 0.15[a]
Dissolved oxygene (mg L^{-1})	7.90 ± 0.01[a]	8.20 ± 0.02[a]
pH	7.8 ± 0.09 [a]	7.60 ± 0.12[a]
NO_3 (ppm)	0.018 ±0.008[a]	0.076 ± 0.001[b]
NO_2 (ppm)	0.007 ± 0.002 [a]	0.004 ± 0.002[b]
NH_3 (ppm)	0.0154 ± 0.002[a]	0.006 ± 0.036[b]
NH_4 (ppm)	0.245 ± 0.016[a]	0.2133 ± 0.025[b]
TAN (ppm)	0.263 ± 0.015[a]	0.217 ± 0.021[b]
Total bacteria (n cm^{-3})	351.6 ± 350[a]	31467.5 ± 18631[b]
Salinity (ppt)	0.20 ± 0.01[a]	0.22 ± 0.01[a]
TSS (mg L^{-1})	550 ± 30[a]	600 ± 35[b]
N/P	1:0.003[a]	1:0.001[a]
Alkality (Calcium carbonate) (mg L^{-1})	176.50 ± 13.70[a]	192.30 ± 15.10[a]
Ortophosphate	0.771 ± 0.064[a]	0.576 ± 0.003[b]

Various letters in each row indicate the significant difference between the treatments (p<0.05).

Table 2: Growth performance of *H. huso* in different treatments during 8 weeks.

Parameters	Control	Biofloc
Initial weight (g)	168.20 ± 2.90[a]	168.20 ± 2.90[a]
Final weight (g)	653.70 ± 28.94[a]	664 ± 31.06[a]
Initial length (cm)	35.50 ± 0.41[a]	35.8 ± 0.58[a]
Final length (cm)	48.60 ± 0.90[a]	51.20 ± 1.10[a]
FCR	1.50 ± 0.16[a]	1.20 ± 0.33[b]
SGR (%)	1.80 ± 0.06[a]	2.00 ± 0.01[b]
Body weight gain (g)	488.70 ± 29[a]	496.50 ± 32[a]
Body weight increase (%)	281.80 ± 12.10[a]	288 ± 7.60[b]
Mean daily growth (g day^{-1})	6.80 ± 0.90[a]	7.10 ±0.30[a]
CF (%)	0.50 ± 0.00[a]	0.52 ± 0.01[a]
Survival rate (%)	100 ± 0.00[a]	100 ± 0.00[a]

Various letters in each row indicate the significant difference between the treatments (p<0.05).

Blood indices

Based on the results of blood indices, there was no significant difference in hemoglobin, MCH and MCHC between the treatments (p>0.05). Differential count results of white blood cells showed a significant difference in neutrophil and lymphocyte count (p<0.05), but there was no significant difference in monocyte and eosinophil percent between the treatments (p>0.05). White blood cell count, red blood cell count, hematocrit and MCV showed significant difference between the treatments (p<0.05) (Table 3).

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Table 3: Blood indices changes of *H. huso* in different treatments during 8 weeks.

Indices	Control	Biofloc
White blood cells (n m^{-3})	7800.67 ± 450.92 [a]	6500 ± 458.26[b]
Red blood cells (n mm$^{-3)}$	6.30 ± 0.50[a]	6.90 ± 0.58[b]
Hb (g dl^{-1})	5.83 ± 0.12[a]	6.10 ± 0.15[a]
Hct (%)	22 ± 1[a]	24.60 ± 1.53[b]
MCV (fl)	331.20 ± 6.11[a]	340.67 ± 10.01[b]
MCH (pg)	90.10 ± 1[a]	85.33 ± 1.53[a]
MCHC (g dl^{-1})	28.80 ± 0.35[a]	25.47 ± 0.25[a]
Neutrophile (%)	15.33 ± 0.58[a]	11.67 ± 1.53[b]
Lymphocyte (%)	78 ± 1.73[a]	85 ± 1.73[b]
Mnocyte (%)	5 ± 1[a]	4.33 ± 1.16[a]
Eosinophile (%)	0.87 ± 0.58[a]	1 ± 0.72[a]

Various letters in each row indicate the significant difference between the treatments (p<0.05).

Discussion

An important factor in order to increase profitability in the aquaculture industry is to achieve the desired growth (Zakeri, 2009). Growth is one of the factors that affect the commercial production capacity of fish (Sharif Rouhani and Iran, 2010). Water temperature, feeding rate, and fish size are the most important growth factors in fish (Sener *et al.*, 2006). The results of the present study showed that there was a significant difference between growth performances, feed conversion ratio factors, specific growth rate, weight gain, and weight gain percentage. There was no significant difference in other growth indicators. However, the most appropriate values were observed in most cases in the biofloc treatment compared to the control. Perhaps the reason for the high growth indices in the treatments containing biomass was explained by the fact that the presence of bacteria in them acted as a factor to improve environmental conditions and the existence of these conditions led to better life of fish.

Numerous studies have been shown that biofloc increases growth in Mozambican tilapia (*Oreochromis mossambicus*) (Avnimelech, 2007), freshwater shrimp (*Macrobrachium rosenbergii*) (Asaduzzaman *et al.,* 2008) and western white shrimp (*Litopenaeus vannamei*) (Xu *et al.,* 2012). The use of biofloc system in ruho fish significantly increased the growth of this fish compared to the control treatment (Kamilya *et al.,* 2017). Avnimelech (1999) stated that biofloc could meet the need for protein and other nutrients for Mozambican tilapia. Khademi Hamidi *et al.* (2019), by studying carp, stated that growth and nutrition performance was significantly higher in the molasses-containing treatment than the control treatment. Mahanand *et al*. (2013), in a study of *Labeo rohita* juveniles, found that fish raised in the biofloc system had a higher mean final weight and higher specific growth rate than the control treatment. However, they had lower feed conversion ratio and protein yield rate than the control treatment and concluded that the biofloc system is suitable for breeding *Ruho*. This result indicates that the type of food and diet and species of fish affect the efficiency of the biofloc system. The results of the above studies are somewhat consistent with the results of the present study. Andriani *et al*. (2019), by studying tilapia, stated that the results of weight gain did not differ significantly between different treatments. However, the highest value was observed in the biofloc treatment and the lowest in the control treatment. Using a biofloc system for fish farming has advantages such as improving growth rate (Wasielesky *et al.,* 2006) and reducing feed conversion ratio (Burford *et al.,* 2004).

The results of the present study showed that among the different treatments, the biofloc treatment had better conditions in terms of blood indices. The lowest number of white blood cells, monocytes, and neutrophils and the highest number of red blood cells, hemoglobin, hematocrit, and MCV were observed in this treatment. Blood composition changes under the influence of physiological and pathological factors (Jamalzadeh *et al.,* 2008). The study of blood composition leads to the recognition of health status (Bani and Haghi-Vayghan, 2011) and aquatic physiology (Hued and Bistoni, 2002). Indicators such as white blood cells, red blood cells, hematocrit, hemoglobin, MCHC, MCV, and MCH indicate the health status of fish (Michael *et al.,* 2019). Studies have shown that the number of total homocytes in shrimp in biofloc treatments was significantly higher than the control group (Xu and Pan, 2013). The results of the above studies are consistent with the results of the present study. The use of biofloc system for breeding African catfish (*Clarias gariepinus*) increased glucose at high density (1000 per square meter) (Hastuti and Subandiyono, 2018).

Water pollution is a physical, chemical, and biological change that eventually leads to disease in ecosystem organisms (Svobodova and Vykusova, 1991). Water quality directly affects aquatic health (Yildiz *et al.,* 2017). Biofloc system improves water quality and aquatic environmental conditions (Azimi *et al.,* 2016).

The results of the present study showed that biofloc treatment has lower (better) values than the control in many physical and chemical factors of water. The best and most appropriate values of pH, NO_3, NO_2, NH_3, NH_4 and TAN were observed in this treatment, which is also due to the presence of bacteria in the biomass. The effect of temperature on the biofloc technology used for tilapia did not show a significant difference compared to the control group (Long *et al.,* 2015) which in this study was not observed in this regard. Andriani *et al.* (2019), by examining the Nile tilapia in 4 different environments, showed that there was a more suitable pH in the treatment containing biomass compared to the control treatment. In an intensive breeding system, contamination by ammonia nitrogen and nitrite is an important stress factor that also affects the aquaculture system (Tovar *et al.,* 2000). The number of nitrifying bacteria in the water determines the concentration of ammonia, nitrate, and nitrite (Andriani *et al.*, 2018). Khademi Hamidi *et al*. (2019), by studying carp, stated that although compared to other experimental treatments, the water change of the control treatment was 10% more daily, but there was no significant change in its quality, which indicates good performance of the biofloc system. The study of the effect of biofloc technology on tilapia showed that the concentration of nitrate and nitrite in the biofloc treatment was significantly lower than the control treatment (Long *et al.*, 2015). The results of a study on *Carassius auratus* showed that the biofloc system significantly reduced ammonia nitrogen after 14 days, nitrite, and nitrate after 7 days (Wang *et al.*, 2015). In addition, Azimi *et al*. (2016), by studying common carp, stated that the amount of total ammonia nitrogen decreased in different treatments due to the formation of biofloc in the system and the presence of nitrogen-digesting bacteria in water. Adineh and Hersij (2018) studied the effect of different levels of biofloc on water quality, growth performance, and survival of *Litopenaeus vannamei* shrimp larvae and stated that water quality and some growth indices improved. Ammonia and nitrite levels decreased and total suspended solids increased.

Widanari *et al*. (2012). Evaluated the effect of biofloc technology (BFT) application on water quality and production performance of red tilapia (*Oreochromis* sp.) at different stocking densities. Three different fish densities were applied, i.e. 25, 50, and 100 fish.m^{-3}, and for each density there were Control (without external carbon input) and BFT treatments. Mixed sex red tilapia with an initial average body weight 77.89±3.71 g was cultured in 3 m^3 concrete tanks for 14 weeks. Molasses was added on BFT treatments as the organic carbon source at a C/N ratio of 15. Control treatments of each density tested showed more fluctuated water quality parameters throughout the experimental period. The highest TAN and nitrite-nitrogen were observed in control treatment at a stocking density of 100 fish.m^{-3} (3.97 mg TAN/L and 9.29 mg NO2-N/L, respectively). The highest total yield was observed in control treatment at the highest density treatment (43.50 kg), whereas the highest survival was obtained by BFT treatment at a density of 25 fish.m^{-3} (97.78±0.77%). Total feed used in BFT was lower than that of control treatments in particular at 50 fish.m^{-3} density (p<0.05).

By adding the right amount of carbohydrates to water and adjusting the carbon to nitrogen ratio, heterotrophic bacteria absorb the resulting food and optimally form a biofloc, in which case ammonia and nitrite in the water are reduced (Asaduzzaman *et al.*, 2008). It should be noted that excessive consumption of biofloc disturbs the balance of heterotrophic bacteria in the farming system (Adineh and Hersij, 2018). The total amount of suspended solids is a true indicator of biomass or biofloc (Avnimelech *et al.*, 1994). In a biofloc system, the management of the floc control is very important. Because excessive production of total dissolved solid (TDS) in the aquatic environment can create a negative response in the process of growth and survival (Adineh and Hersij, 2018).

Heterotrophic bacteria use carbon dioxide and excess nitrogen from water to produce microbial proteins and reduce the concentration of inorganic nitrogen in water (Burford *et al.*, 2004). Khanjani *et al*. (2016), using the biofloc system in western white shrimp farming systems, stated that the system increases the density of heterotrophic bacteria and expands the flock by the system without changing the water and adding organic matter. Suantika *et al.* (2016), examining different substrates, stated that although ammonium removal was observed in all experimental groups, nitrification bacteria, the rate of ammonium oxidation and nitrification bacteria increased the amount of ammonium significantly. They decreased more than the control treatment. Microbial flakes caused the residual food and excretory material to be re-spun and reused by fish, improving food absorption, especially in conditions without water exchange (Xu and Pan, 2012). Andriani *et al*. (2018), by studying tilapia, stated that the type of filter in the aquaponic system affects the amount of nitrosomonas bacteria in the water. In a general conclusion, the treatment containing biomass can be selected as the best treatment according to their performance, because they improved the conditions and increased the efficiency of the breeding system. Even if they are not used directly by aquatic animals, their presence improves living conditions.

References

Abdirad, Z. and Qaednia, B., 2016. Shrimp Farming with Biofloc System, Shrimp and Crustaceans Quarterly, 1(**2**), 12-12.

Adineh, H. and Hersij, M., 2018. The effect of different levels of biofloc on water quality, growth performance, and survival of *Litopenaeus vannamei* shrimp larvae. *Journal of Veterinary Research*, 73(**4**), 401-393. DOI: 10.22059/jvr.2019.227254.2584

Ahn, Y., Park, E.J., Oh, Y.K., Park, S., Webster, G. and Andrew, J.W., 2005. Biofilm microbial community of a thermophilic trickling biofilter used for continuous biohydrogen production. *FEMS Microbiology Letter*, 249, 31-38. DOI: 10.1016/j.femsle.2005.05.050.

Ameri Mahabadi, M., 1999. Laboratory methods of veterinary hematology. University of Tehran Press. 126 P.

Andriani, Y., Dhahiyat, Y., Hasan, Z., Subhan, U., Iskandar, I., Zidni, I. and Mawardiani, T., 2018. Effect of water irrigation volume on *Capsicum frutescens* growth and plankton abundance in aquaponics system. IOP Conference Series Earth and Environmental Science. The 2nd International Symposium on Marine and Fisheries Research.

Andriani, Y., Dhahiyat, Y. and Hasan, Z., 2019. The productivity of Nile tilapia (*Oreochromis niloticus*) and water quality condition in different filters in aquaponics system. *Global Scientific Journals*, 7(**6**), 591-597.

Asaduzzaman, M., Wahab, M.A., Verdegem, M.C.J., Huque, S., Salam, M.A. and Azim, M.E., 2008. C/N ratio control and substrate addition for periphyton development jointly enhance freshwater prawn (*Macrobrachium rosenbergii*) production in ponds. *Aquaculture*, 280, 117-123 DOI:10.1016/j.aquaculture.2008.04.019.

Avnimelech, Y., Kochva, M. and Diab, S., 1994. Development of controlled intensive aquaculture systems with a limited water exchange and adjusted carbon to nitrogen ratio. Isr J Aquac Bamidgeh 46,119–131. DOI: 10.1016/S0044-8486(99)00085-X.

Avnimelech, Y., 1999. Carbon nitrogen ratio as a control element in aquaculture systems. *Aquaculture*, 176, 227-235. DOI: 10.1016/S0044-8486(99)00085-X.

Avnimelech, Y., 2007. Feeding with microbial flocs by tilapia in minimal discharge bioflocs technology ponds. *Aquaculture*, 264, 140-147. DOI: 10.1016/j.aquaculture.2006.11.025.

Avnimelech, Y., 2009. Biofloc technology: A practical guide book. World Aquaculture Society, Baton Rouge. Louisiana, USA. 182 P.

Azimi, A., Jafarian, H., Hersij, M. Gholipoor, H. and Patimar, R., 2016. The effect of different ratios of carbon to nitrogen on water quality parameters and growth performance of common carp juveniles in the biofloc system. *Aquaculture Development*, 10(**4**), 89-75.

Bakhshi, F., Malekzadeh Viayeh, R. and Hossein Najd Gerami, A., 2014. Investigating the efficiency of using biomass production system in intensive breeding of common carp (*Cyprinus carpio*). *Journal of Animal Environment*, 6(**3**), 53–45.

Bakhshi, F., Hossein Najd Gerami, A ., Imani, A. and Moghanloo Survey, K., 2016. The effect of using biofloc technology on growth parameters, carcass composition and reduction of production costs in intensive breeding of common carp juveniles. *Journal of Veterinary Research*, 71(**2**), 169–162.

Bani, A. and Haghi-Vayghan, A., 2011. Temporal variations in haematological and biochemical indices of the Caspian kutum, *Rutilus frisii kutum*. *Ichthyological Research*, 58, 126–133. DOI: 10.1007/s10228-010-0199-6.

Bender, J., Lee, R., Sheppard, M., Brinkley, K., Philips, P., Yeboah, Y. and Wah, R.C., 2004. A waste effluent treatment system based on microbial mats for black sea bass *Centropristis striata* recycled water mariculture. *Aquacultural Engineering*, 31(**1-2**), 73-82. DOI: 10.1016/j.aquaeng.2004.02.001.

Burford, M., Thompsona, P.J., McIntoshb, R.P, Baumanb, R.H. and Pearson, D.C., 2004. The contribution of flocculated material to shrimp (*Litopenaeus vannamei*) nutrition in a high-intensity, zero-exchange system. *Aquaculture*, 232, 525-537. DOI: 10.1016/S0044-8486(03)00541-6.

Cappuccino, J.G. and Sherman, N., 2012. Microbiology: A Laboratory Manual. 9th Ed. Benjamin Cummings, Redwood City.

Crab, R., Lambert, A., Defoirdt, T., Bossier, P. and Verstraete, W., 2010. The application of bioflocs technology to protect brine shrimp (*Artemia franciscana*) from pathogenic *Vibrio harveyi*. *Journal of Applied Microbiology*, 109(**5**), 1643-1649. DOI: 10.1111/j.1365-2672.2010.04791.x

Emerenciano, M.G.C., Balester, E.L.C., Cavali, R. and Wasioleski, W., 2012a. Biofloc technology application as a food source in a limited water exchange nursery system for Pink shrimp (*Farfantepenaeus brasiliensis*) (Latreille, 1817), *Aquaculture Research*, 43(**3**), 447 - 457, DOI: 10.1111/j.1365-2109.2011.02848.x

Grisdale-Helland, B., Helland, S.J. and Gatlin, D.M., 2009. The effects of dietary supplementation with mannanoligosaccharide, fructooligosaccharide or galactooligosaccharide on the growth and feed utilization of Atlantic salmon (*Salmo salar*). *Aquaculture*, 283, 163-167. DOI: 10.1016/J.AQUACULTURE.2008.07.012.

Haqhparast Radmard, M.M., Mojtaba Alishahi, M., Ghorbanpour, M. and Shahriari, A., 2018. The effect of different carbon-nitrogen ratios in dense biofloc breeding system on growth and health indices of common carp (*Cyprinus carpio*). *Journal of Animal Environment*, 10(**2**), 170–169.

Hastuti, S. and Subandiyono, S., 2018. Haematological parameters of the North African catfish *Clarias gariepinus* farmed using biofloc technology. *AACL Bioflux*, 11(**4**), 1415-1424.

Hued, A. and Bistoni, M.A., 2002. Effects of water quality variations on fish communities in the Central Part of Argentina, South America. *Proceeding of the International Association of Theoretical and Applied Limnology*, 28, 112-116.

Jamalzadeh, H., Keyvan, A., Naked, S.H. and Ghomieh Marzdashti, M.R., 2008. Levels of some blood and biochemical characteristics of Caspian salmon (*Salmo trutta* caspius). *Iranian Journal of Fisheries*, 17(**3**), 54-47.

Kamilya, D., Debbarma, M., Pal, P., Kheti, B., Sarkar, S. and Singh, S.T., 2017. Biofloc technology application in indoor culture of *Labeo rohita* (Hamilton, 1822) fingerlings: The effects on inorganic nitrogen control, growth and immunity. *Chemosphere*, 182, 8-14. DOI: 10.1016/j.chemosphere.2017.05.021.

Khademi Hamidi, M., Adineh, H., Harsij, M. and Gholipour Kanani, H., 2019. Effects of adding molasses in water and diet of common carp on growth, blood biochemical indices, digestive enzymes and water quality in a biofloc system. *Fish and Shellfish Immunology*, 95, 440-448. DOI: 10.1016/j.fsi.2019.10.057.

Khanjani, M.H., Sajjadi, M.M., Alizadeh, M. and Surinejad, A., 2016. Production and evaluation of bioflocks for use in systems without water exchange. *Development of Aquaculture* (*Biological Sciences*), 10(**1**), 42–33. DOI: 10.22092/ijfs.2021.123873.

Khanjani, M.H., 2019. Application of biomass technology in aquaculture with emphasis on biological fish. *Ornamental Aquatic Animals*, 6(**2**), 48–35. DOI. 10.1111/raq.12412

Klontz, G.W., 1994. Fish Hematology. In Techniques in Fish Immunology. Edited by JS Stolen.; TC Fletcher., A.F Rowley., T.C Kelikoff., S.L. Kaattari and S.A. Smith. SOS Publications, pp. 121–132.

Kubokawa, K., Watanabe, T., Yoshioka, M. and Iwata, M., 1999. Effects of acute stress on plasma cortisol, sex steroid hormone and glucose levels in male and female sockeye salmon during the breeding season. *Aquaculture*, 172, 335-349. DOI: 10.1016/S0044-8486(98)00504-3.

Long, L., Yang, J., Li, Y., Guan, C. and Wu, F., 2015. Effect of biofloc technology on growth, digestive enzyme activity, hematology, and immune response of genetically improved farmed tilapia (*Oreochromis niloticus*). *Aquaculture*, 448, 135-141. DOI:10.1016/J.AQUACULTURE.2015.05.017.

Mahanand, S.S. and SrinivasaRao, P., 2012. Optimum formulation of feed for Rohu, *Labeo rohita* (Hamilton), with biofloc as a component. *Aquaculture International*, 21, 347-360. DOI: 10.1007/s10499-012-9557-x.

Mahanand, S.S., Moulick, S. and Srinivasa Rao, P., 2013. Water Quality and Growth of Rohu, *Labeo rohita*, in a Biofloc System. *Journal of Applied Aquaculture*, 25, 121–131. DOI: 10.1080/10454438.2013.788898.

Masłon, A. and Tomaszek, J.A., 2015. A study on the use of the Bioball as a biofilm carrier in a sequencing batch reactor. *Bioresource Technology*, 196, 577-585. DOI: 10.1016/j.biortech.2015.08.020.

Merrifield, D.L., Bradley, G., Harper, G.M., Baker, R.T.M., Munn, C.B. and Davies, S.J., 2011. Assessment of the effects of vegetative and lyophilized *Pediococcus acidilactici* on growth, feed utilization, intestinal colonization and health parameters of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Nutrition*, 17(**3**), 73-79. DOI: 10.1111/j.1365-2095.2009.00712.x.

Michael, S.E., Abarike, E.D. and Cai, J., 2019. A Review on the Probiotic Effects on Haematological Parameters in Fish. *American-Eurasian Journal of Scientific Research*, 6(**1**), 32-38.

Moopam, 1999. Manual of Oceanographic Observation and

Pollutant Analyses Methods. Third Edition. Regional Organization for the Protection of the Marine Environment. ROPME Publishing, Kuwait. 574 P.

Peterson, D., Vecsei, P. and Hochleithner, M., 2006. Threatened fishes of the world: *Acipenser ruthenus* Linnaeus, 1758 (Acipenseridae). *Environmental Biology of Fishes*, 78, 211-212. DOI: 0.1007/s10641-006-6659-1.

Pottinger, T.G. and Carrick, T.R., 2001. A comparison of plasma glucose and plasma cortisol as selection markers for high and low stress-responsiveness in female *rainbow trout*. *Aquaculture Research*, 175, 351-363.

Putra, I. and dan Setiyanto, D., 2011. Pertumbuhan Dan Kelangsungan Hidup Ikan Nila (*Oreochromis niloticus*) Dalam Sistem Resirkulasi. *Jurnal Perikanan dan Kelautan*, 16(**1**), 56-63

Ray, A.J., 2012. Biofloc technology for superintensive shrimp culture. In Biofloc Technology- a practical guide book Edited by Y. Avnimelech. 2nd Ed. The World Aquaculture Society, Baton Rouge. Louisiana, USA. pp. 167-188. DOI: 10.13140/2.1.4575.0402.

Rotllant, J., Balm, P.H.M., Perez-Sanchez, J., Wendelaar-Bonga, S.E. and Tort, L., 2001. Pituitary and interrenal function in gilthead sea bream (*Sparus aurata* L., Teleostei) after handling and confinement stress. *General and Comparative Endocrinology*, 121, 333-342. DOI: 10.1006/gcen.2001.7604.

Sener, E., Yıldız, M. and Savaş, S., 2006. Effect of vegetable protein and oil supplementation on growth performance and body composition of Russian sturgeon juveniles (*Acipenser gueldenstaedtii*) at low temperatures. *Turkish Journal of Fisheries and Aquatic Sciences*, 6, 23-27.

Sharif Rouhani, M. and Iran, A.M., 2010. Thematic-product technical instructions. Volume: Fourth, Sturgeon. Ministry of Jihad Agriculture, Agricultural Research, Education and Extension Organization, Iran Fisheries Research Institute. 84 P.

Siwicki, A.K. and Anderson, D.P., 1993. Nonspecific defence mechanisms assay in fish. II. Potential killing activity of neutrophils and macrophages, lysozyme activity in serum and organs and total immunoglobulin (T-Ig) levels in serum. Fish Diseases Diagnosis and Prevention’s Methods. FAO-Project GCP/INT/526/JPN, IFI Olsztyn, pp. 105-112.

Souza, D.M.D., Suita, S.M., Romano, L.A., Jr, W.W. and Ballester, E.L.C., 2014. Use of molasses as a carbon source during the nursery rearing of *Farfantepenaeus brasiliensis* (Latreille, 1817) in a Biofloc technology system. *Aquaculture Engineering*, 45, 270-277. DOI: 10.1111/j.1365-2109.2012.03223.x.

Suantika, G., Astuti, D.I., Arief, R.R., Rusni, M. and Turendro, O.R., 2012. Use of zero water discharge technology through the application of nitrifying bacteria and textile vertical substrate in grow-out phase of *Macrobrachium rosenbergii*. *Journal of Aquaculture Research and Development*, 3, 139 P.

Suantika, G., Lumbantoruan, G., Muhammad, H. and Aditiawati, P., 2015. Performance of zero water discharge (ZWD) system with nitrifying bacteria and microalgae *Chaetoceros calcitrans* components in super intensive white shrimp (*Litopenaeus vannamei*) culture. *Journal of Aquaculture Research and Development*, 6, 1-6. DOI: 10.4172/2155-9546.1000359.

Suantika, G., Pratiwi, M.I., Situmorang, M.L., Djohan, Y.A., Muhammad, H. and Astuti, D.I., 2016. Ammonium Removal by Nitrifying Bacteria Biofilm on Limestone and Bioball Substrate Established in Freshwater Trickling Biofilter. *Poultry, Fisheries and Wildlife Sciences*, 4(**2**), 157. DOI: 10.4172/2375-446X.1000157.

Svobodova, Z. and Vykusova, B., 1991. Diagnostic preventionand therapy of fish disease and intoxications. Manual for international training collrse on Freshwater fish disease and intoxication, pp. 156-157.

Torrecillas, S., Makol, A., Caballero, M.J., Montero, D., Gines, R., Sweetman, J. and Izquierdo, M.S., 2011. Improved feed utilization, intestinal mucus production and immune parameters in Sea bass (*Dicentrarchus labrax*) fed mannan oligosaccharides (MOS). *Aquaculture Nutrition*, 17(**2**), 223-233. DOI: 10.1111/j.1365-2095.2009.00730.x.

Tovar, A., Moreno, C. and Manuel-Vez, M.P., 2000. Environmental impacts of intensive aquaculture in marine waters. *Water Research*, 34(**1**), 334–342.

Wasielesky, W., Atwood, H., Stokes, A., Browdy, C.L., 2006. Effect of natural production in a zero exchange suspended microbial floc based super-intensive culture system for white shrimp, *Litopenaeus vannamei*. Aquaculture 258, 396–403. DOI: 10.1016/j.aquaculture.2006.04.030.

Wang, G., Yu, E., Xie, J., Yu, D., Li, Z., Luo, W., Qiu, L. and Zheng, Z., 2015. Effect of C/N ratio on water quality in zero-water exchange tanks and the biofloc supplementation in feed on the growth performance of crucian carp, *Carassius auratus*. *Aquaculture*, 443, 98–104. DOI: 10.1007/s10695-015-0151-9.

Webster, C.C. and Lim, C.E., 2002. Nutrient requirement and feeding of finfish for aquaculture. CAB International, CABI Publishing. 418 P. DOI: 10.1079/9780851995199.0000

Widanari, W., Ekasari, J. and Siti, M., 2012. Evaluation of biofloc ttechnology Application on water quality and pproduction pperformance of Red Tilapia (*Oreochromis* sp.) Cultured at

Different Stocking Densities. *HAYATI Journal of Biosciences*, 19(**2**), 73-80. DOI.10.4308/hjb.19.2.73**.**

Xu, W.J. and Pan, L.Q., 2012. Effects of bioflocs on growth performance, digestive enzyme activity and body composition of juvenile *Litopenaeus vannamei* in zero-water exchange tanks manipulating C/N ratio in feed. *Aquaculture*, 356-357, 147-152. DOI:10.1016/j.aquaculture.2012.05.022.

Xu, W.J., Pan, L.Q. and Zhao, D.H., 2012. Preliminary investigation into the contribution of bioflocs on protein nutrition of *Litopenaeus vannamei* fed with different dietary protein levels in zero-water exchange culture tanks. *Aquaculture*, 350–353,147–153.
DOI:10.1016/j.aquaculture.2012.04.003.

Xu, W.J. and Pan, L.Q., 2013. Enhancement of immune response and antioxidant status of *Litopenaeus vannamei* juvenile in biofloc-based culture tanks manipulating high C/N ratio of feed input. *Aquaculture*, 412–413(**1**), 117-124.

Yildiz, H.Y., Robaina, L., Pirhonen, J., Mente, E., Dominguez, D. and Parisi, G., 2017. Fish Welfare in Aquaponic Systems: Its Relation to Water Quality with an Emphasis on Feed and Faeces: A Review. *Water*, 9(**1**), 13 P. DOI: 10.3390/w9010013.

Zakeri, M., 2009. Effects of different levels of dietary protein and fat on biological function in *Acanthopagrus latus* breeders. PhD. thesis. Khorramshahr University of Marine Sciences and Technology. 205 P.

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