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ON

Effect of Different Stocking Density on Growth Performance, Feed Utilization and Immune Response of Finfish in Biofloc System

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SUBMITTED TO:

Course Instructors

Dr. A. K. M. Aminul Islam
Professor
Dept. of Genetics and Plant Breeding,
BSMRAU.

Prof. **Dr. Md. Mizanur Rahman**
Professor
Dept. of Soil Science,
BSMRAU.

Dr. Dinesh Chandra Shaha
Associate Professor
Dept. of Fisheries Management
BSMRAU.

Dr. Md. Sanaullah Biswas
Associate Professor
Dept. of Horticulture,
BSMRAU.

Major Professor

Dr. Md. Amzad Hossain
Professor & Head
Dept. of Aquaculture,
BSMRAU.

SUBMITTED BY:

Nusrat Zahan
Reg. No: 15-05-3618
MS Student
Department of Aquaculture

BANGABANDHU SHEIKH MUJIBUR RAHMAN AGRICULTURAL UNIVERSITY

SALNA, GAZIPUR-1706

Effect of Different Stocking Density on Growth Performance, Feed Utilization and Immune Response of Finfish in Biofloc System[1]

By

Nusrat Zahan[2]

ABSTRACT

The increasing global population and the limiting global capture fisheries undeniably increase the demand for aquaculture products. An increase in aquaculture production which correlates with the application of intensive aquaculture systems and the increasing of feeding. Intensive aquaculture correlates with increasing stocking density. In aquaculture, stocking density describes the number of fish that are stocked initially per unit area. Increasing stocking density is an important parameter to increase productivity. High stocking density and excessive feeding practices negatively affecting the aquaculture industry by accumulating residual feed, metabolic and toxic compound which has an impact on the emergence of disease or fish death which ultimately reduce productivity. Biofloc is a system in which bacteria convert fish waste into microbial biomass (biofloc), improving the water quality in the culture environment and subsequently resulting in less water use. It is a system that promotes continuous recycling and reuse of nutrients. Various finfish species can be cultured in a biofloc system among them tilapia, catfish are the most suitable species because of their capability to survive in adverse environmental conditions. Different stocking density has different effects on growth performance, feed utilization and immune response of finfish. In biofloc system study related to effects of stocking density are limited. A few study has been conducted using various stocking density to know the effects on tilapia, catfish, common carp. The study on rohu, tilapia, catfish, and common carp reported better growth performance, feed utilization, immune response and proximate composition also at high stocking density in biofloc system. As studies related to the effects of different stocking density on growth performance, feed utilization and immune response of finfish is limited in some fish species so more research should be conducted on other commercially important finfish species.

Keywords: Stocking density, finfish, biofloc, growth performance, feed utilization, immune response.

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[1]A seminar paper presented at seminar course on July 9, 2020

[2]MS student, Department of Aquaculture, BSMRAU, Gazipur-1706

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Chapter I

INTRODUCTION

Aquaculture is the fastest-growing food-producing sectors (FAO, 2018). Aquaculture continues to be the world's fastest growing and most diverse food Production sector, with over 95.6 percent of total aquaculture production being realized within developing countries and the sector growing at an average APR of 6.64 percent per year, compared with1.15percent for economically developed countries (FAO, 2018). According to FAO (2018), Bangladesh ranked 5th in world aquaculture production. Fisheries and aquacultures are the efficient protein production sectors offering ample opportunities to alleviate poverty, hunger and malnutrition (FAO, 2018). About 50% of the world's fish production comes from Aquaculture (FAO, 2018). Since the 1970s this sector growing fast at the rate of ~9% per year (FAO, 2018). This rapid growth of aquaculture depends upon increasing productivity within limited use of resources (water, land and feed) and applying more environmentally friendly culture practice for aquaculture sustainability.

The rapid expansion of aquaculture, however, has raised questions about its sustainability. The rapid growth of aquaculture depends upon environmentally friendly culture practice for aquaculture sustainability. Due to this increasing pressure, Aquaculture is now diverted from the extensive culture system to a more intensive culture system.

Intensive farming depends on high fish densities with the use of large quantities of high-protein levels diets (25–55%) aiming for increasing productivity in closed or semi-closed systems to overcome the limitation of water and lands resources (Avnimelech *et al*., 2009). Increasing the production intensity of fish in culture system is the most possible way for increasing the quantity of fish juveniles due to the limited availability of space and good quality of water. The intensity of an aquaculture production system depends largely on stocking density.

In aquaculture, stocking density (SD) describes the number of fish that are stocked initially per unit area; however, it is generally used to refer to the density of fish at any point of time. Stocking density is an important parameter in fish culture operations, since it has direct effects on the growth and survival and hence on production (Chakraborty *et al*., 2010). Stocking density is a key factor in determining the productivity and profitability of commercial fish farms. The increase in fish stocking density and accordingly the feed input to the culture system consequently leads to the high accumulation of wastes from the uneaten feed, egested fecal materials and excreted metabolic by-products that may ultimately cause water quality deterioration. The low environmental quality in aquaculture systems promotes the emerging of disease by facilitating the proliferation of pathogens and compromising the susceptibility of the animal against the disease (Mydlarz, Jones, & Harvel, 2006). Increasing stocking density has also negative effects on growth, feed utilization capacity of fish.

To overcome this problem, intensive aquaculture system development should be directed to finding a system that can maintain maximum fish growth, but at the same time can also improve the fish resilience against diseases and environmental stress.

Currently, the environmentally friendly technology called biofloc is being developed. The BFT or Bio-flocs technology is hoped to solve the problem of feed inefficiency, production cost, water quality, productivity, and profit optimization in order to reduce aquaculture waste (Avnimelech, 2009). Biofloc technology is an aquaculture technology which is based on the principle of inorganic nitrogen assimilation (ammonia, nitrite, and nitrate) by the microbial community (heterotrophic bacteria) in the culture media as the source of nutrition for bacteria (De Schryver *et al.*, 2008).

A basic factor in designing a biofloc system is the species to be cultured and their ability to ingest and digest suspended particles. Biofloc Systems work best with species that are able to derive some nutritional benefit from the direct consumption of floc. Biofloc systems are also most suitable for species that can tolerate high solids concentration in water and are generally tolerant of poor water quality.

Finfish are those fishes which contains fins. Some finfish species are suitable for biofloc systems. Due to advantage of biofloc as a food resource, biofloc systems are used to grow tilapia, carps, catfish, Climbing Perch (Koi), trout, salmon.

But in case of density related study there is a limited research on finfish in biofloc system. Most of the researcher who studied the effects of stocking density on finfish in biofloc system used rohu,tilapia, common carp and some catfish species.

In this review paper the main focus is on the effects of different stocking density on growth performance, feed utilization and immune response and proximate composition of finfish in biofloc system.

Objectives

After reading this article, readers will be able:

- To find out the effects of different stocking density on -
    - Growth performance and feed utilization of finfish in biofloc system.
    - Immune response and proximate composition of finfish in biofloc system.

Chapter II
MATERIALS AND METHODS

All the information of this seminar paper has been collected from the secondary sources as it is just a review paper. Various relevant books, journals, proceedings, reports, published paper, internet etc. has been reviewed during the preparation of the review paper to collect key information. Suggestion and valuable information from my major professor and my course instructors helps me to improve this paper. I compiled and prepared this seminar manuscript systematically and chronologically to enrich this paper.

Chapter III

REVIEW OF FINDINGS

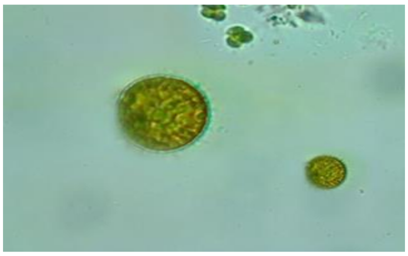
The detailed on the selected topic so far extracted and reviewed is discussed below under different sub-headings.

3.1 Biofloc

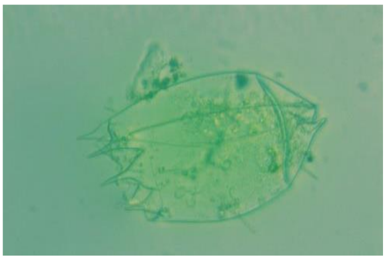
Biofloc is a compound which is made of 60 to70% of organic substance and 30 to 40% of in organic substances (Wilen *et al.*, 2003). In biofloc, organic matter includes a heterogeneous mixture of microorganisms (algae, protozoans, rotifers, diatom, cyanobacteria and fungus) and inorganic matter which includes dead cell, colloids etc. They can reach up to 1000µm in size and irregular shape in shape (Chu &Lee, 2004).

3.1.1. Microbial Community in Biofloc

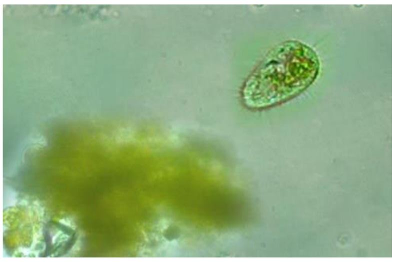
Biofloc contains about 24.4% phytoplankton, 3-5% bacterial community, a Small amount of protozoan community and 33.2% detritus and the remaining quantity is ash (39.3%) (Ju *et al.*, 2008). The number of bacteria is between 10^6 and 10^9/ml of floc plug (Avnimelech, 2007). Monroy *et al.*, (2013), identified heterotrophic bacterial communities of genus *Sphingomonas, Pseudomonas, Bacillus, Nitrospira, Nitrobacter* and yeast *Rhodotorula* sp in biofloc that favor water quality and physiological well-being of cultured organisms.



Coscinodiscus sp.



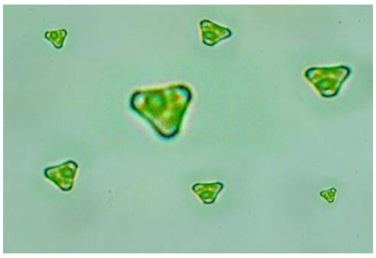
Brachionus sp.



Cyclidium sp.

Paramecium sp.

Triceratium sp.



Desmodesmus sp.

Figure 1. A pictorial view of microbial community in biofloc.

(Source: Google and Wikipedia)

3.1.2. Nutritional Value of Biofloc

Biofloc meet nutritional standards to serve as aquaculture feed in general, research has shown that the capacity of the technique to control the water quality in the culture systems and the nutritional properties of the flocs are influenced by the sources of carbon used to produce the floc (Crab, 2010).

Anand *et al*., (2013) reported that proximate composition of biofloc contained 24.30% protein, 3.53% crude lipid, 29.24% nitrogen free extract, 31.98% ash content and 10.75% acid insoluble ash content. Rostika, (2014) also recorded the proximate composition of floc with 53.5% protein, 2.6% crude protein, 4.0% crude fiber and 7.5% ash content.

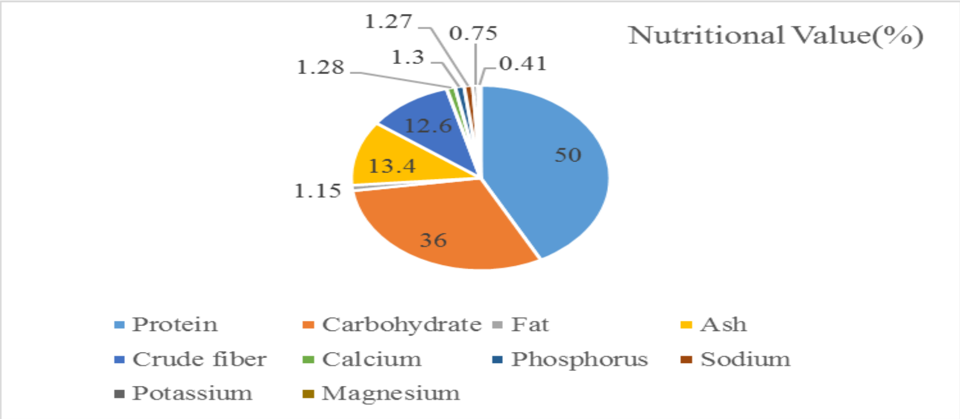


Figure 2. Nutritional content of biofloc on dry matter basis.

(Source: Kuhn *et al*., 2009)

3.2. Biofloc Technology and Its Principle

Biofloc system is an innovative and cost-effective technology in which toxic and harmful materials to fish and shellfish such as nitrate, nitrite, and ammonia are transformed into a useful product, i.e., proteinaceous feed. The basic principle of biofloc technology (BFT) is the microbial conversion of nutrient waste, in particular nitrogenous wastes, in aquaculture systems into microbial biomass that can be utilized back by the cultured organisms (Avnimelech,2009).The principle of this technology is to generate nitrogen cycle by maintaining higher C: N ratio through stimulating heterotrophic microbial growth.

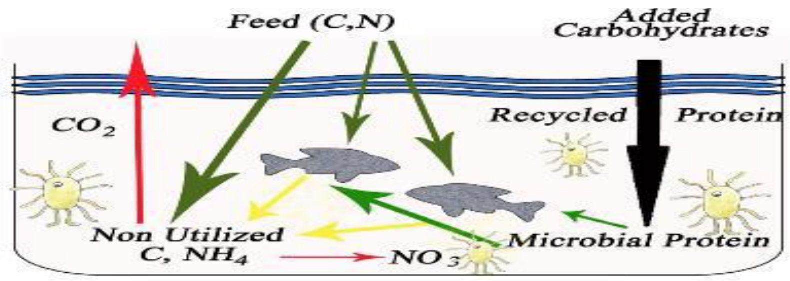


Figure 3. A Scheme of biofloc technology.

(Source: Avnimelech, 1999)

In biofloc technology, regulation of the predominantly heterotrophic bacterial community over autotrophic microorganisms is attained by the utilization of high carbon to nitrogen ratios (Avnimelech, 1999). The uptake of ammonia by bacteria improves water quality as well as increases microbial biomass production (Avnimelech, 1999). These processes serve as fuel for operating the ‘‘floc system’’ (Burford *et al*., 2004). Furthermore, a nutrient-rich feed source is available 24 h per day and could reduce artificial feed inputs and costs (Avnimelech, 2007).

The survival rate of cultivable finfish in BFT culture system is higher than that of in recirculating aquaculture system and control culture system (Suresh and Lin, 1992). Craig and Helfrich, (2002) observed that biofloc do not allow for a complete replacement of traditional fish feed but still can bring about a substantial decrease in the total production costs.

3.3. Effect of Different Stocking Density on Finfish in Biofloc System

Recently, BF technique have widely been used to maximize fish production for its ability to support high densities cultivation, to improve water quality and simultaneously recycling feed and protein production in the same culture unit (Avnimelech, 2007).

Among finfish, most of the study related to effects of different stocking density has been conducted on rohu, tilapia, common carp and some catfish species.

3.3.1. Growth Performance

Increasing the stocking density is an important strategy to increase the productivity as well as profitability of fish production (Fauji *et al*., 2018). Various studies stated that fish treated with biofloc provides satisfactory growth performances than conventional method at high density.

3.3.1.1. Growth Performance of Indian Major Carps (IMC)

Labeo rohita is the most commercial fish in our country. In biofloc system, *Labeo rohita* showed a greater growth performance in view of weight gain, FCR, SGR in various studies.

Table 1. Growth performance of *Labeo rohita* in different stocking densities

Stocking Density (fish/m^2)	**Culture Period (Days)**	**Weight Gain (%)**	**SGR(Day^{-1})**	**FCR**	**Reference**
1.3	90	162.51±8.0	1.07±0.014	1.93±0.33	Mahanand *et al*., 2013
2.6		98±2.00	0.76±0.08	3.55±0.49	
3.9		100±1.3	0.77±0.028	3.66±0.69	
50 (wheat based biofloc)	60	70.71±2.1	2.57 ± 0.01	1.67±0.09	Ahmad *et al*., 2017
30 fry (40% protein supplied)	80	4363.70±141.94	1.83 ± 0.02	1.5 ± 0.0	Sawant *et al*.,2020

In table 1, *Labeo rohita* showed better growth performance at comparatively lower stocking density in biofloc system because of their less adaptability on low water quality.

3.3.1.2. Growth Performance of Tilapia

Tilapia is the most suitable species for biofloc systems because it has great capability to survive in adverse environmental condition. Several Studies has been conducted on Nile tilapia to know the growth performance of different stocking density in biofloc system.

Table 2.Growth performance of Nile tilapia in different stocking densities

Stocking Density (Fish/m^3)	Culture Period	Weight Gain (g)	SGR (%day^{-1})	FCR	Reference
20		64.48±1.17	0.77±0.66	1.95±0.04	
40	84	85.97±1.25	1.02±0.07	1.79±0.02	Zaki *et al*., 2020
60		47.50±1.78	0.57±0.06	2.47±0.09	
166		16.11±0.59	2.90±0.15	1.21±0.01	
333	120	15.8±0.75	2.90±0.18	1.25±0.04	Liu *et al*., 2018
600		13.38±0.88	2.75±0.22	1.44±0.05	
333 (control)		13.04±0.46	2.73±0.18	1.50±0.10	
20		189.67	1.09	1.52	
40	112	187.79	5.90	1.50	Manduca *et al*., 2020
60		146.34	5.52	1.68	
80		107.07	5.57	1.70	
25		8.30 ± 0.81	151.19±9.12	1.44	Ekasari & Maryam ,2012
50	98	10.82 ±1.60	104.98±9.01	1.76	
100		12.70	65.84	2.09	
125	104	318.8 ± 19.7	1.01 ±0.06	1.40 ±0.06	Ridha, 2006
200		263.2 ± 9.6	0.87 ±0.19	1.55±0.04	

In table 2, Growth performance of Nile Tilapia vary among different stocking density.

In most of the studies, growth parameters was preferable in case of medium and high stocking density in biofloc system than comparatively traditional culture practice.

3.3.1.3. Growth Performance of Catfish

In biofloc system, stocking density has great effects on growth performance of Catfish. Several study has been conducted on African Catfish (*Clarias gariepinus*) to observe growth performance at different stocking density.

Table 3. Growth performance of African Catfish in different studies related to stocking density

Stocking Density (Fish/m^3)	Culture Period (Days)	Weight Gain (g)	SGR (%day^{-1})	FCR	Reference
10		4.81±0.04	0,72±0.13	0.99±0.01	
20	45	5.69±0.02	6.17±0.10	0.97±0.02	Battisti *et al.*, 2020
30		9.92±0.01	4.36±0.10	0.46±0.01	
4		2.94±0.02	6.77±0.07	0.92±0.01	
6	20	3.78±0.01	7.07±0.12	0.73±0.02	Fauji *et al.*, 2018
8		3.34±0.01	6.39±0.07	0.72±0.01	
4(control)		3.13±0.01	6.63±0.07	0.97±0.01	
1000	60	94.19±22.8	7.34±0.40	1.10±0.07	Putra *et al.*, 2017

In table 3, growth performance of African Catfish (*Clarias Gariepinus*) was more or less highest in case of high stocking density which is a good indicator for culture catfish in biofloc.

Another study on African catfish (*Clarias gariepinus)* was conducted by Soyedibya *et al.*, (2017) in biofloc system. On this study, African catfish (*Clarias gariepinus)* was reared for 40 days at an initial weight of 1.85±0.09g. The different stocking density which was used for the experiment was 1,000 fish/m^3; 1,500 fish/m^3; 2,000 fish/m^3 and 2,500 fish/m^3.

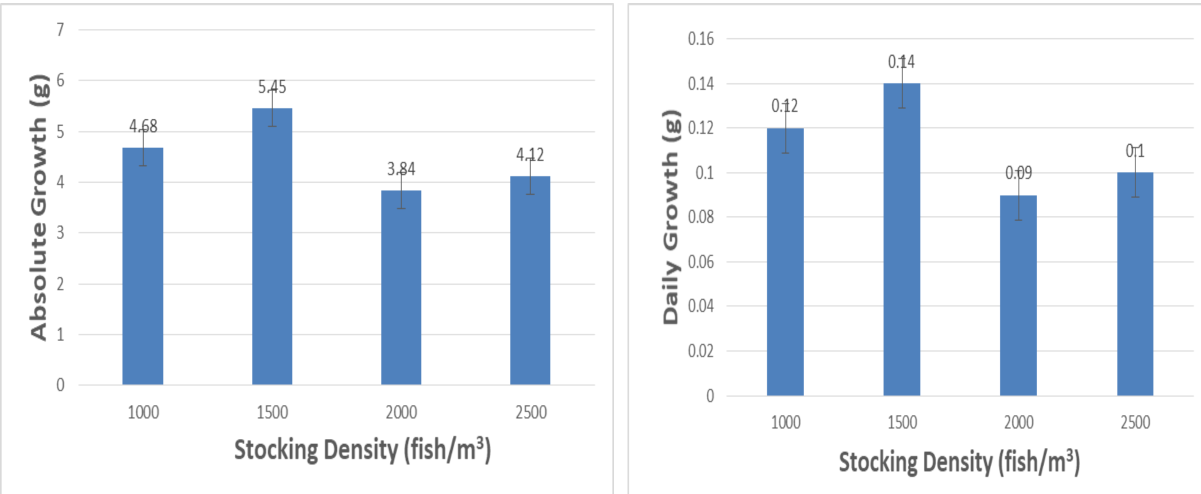


Figure 4. Absolute growth and daily growth of African catfish (*Clarias gariepinus)* in biofloc pond at the stocking density of 1,000fish/m^3; 1,500 fish/m^3; 2,000 fish/m^3; and 2,500 fish/m^3.

(Source: Soedibya *et al.*, 2017).

In figure 4, results showed that growth rate increased when stocking density decreases.

It is expected that it caused by the reason that fish will get more space to move, more feed, more dissolved oxygen and low competition.

Growth rate was observed on African catfish (*Clarias gariepinus)* fingerling among three comparative stocking density which was designated as treatment P_1:1100, P_2:1200, P_3:1300 fingerlings /m^3 in an experiment of Basuki *et al*, (2018). Duration of this experiment was 16 weeks or 4 months. Initial weight of Catfish fingerlings was 8±0.2 g each.

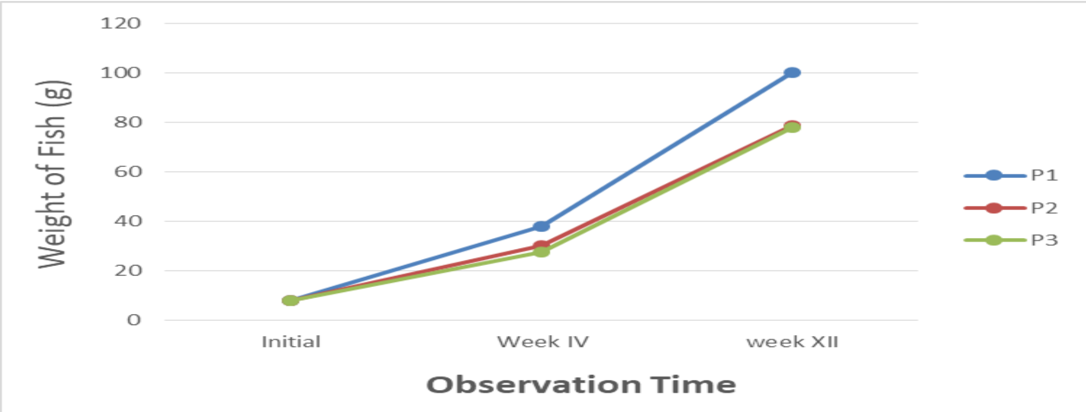


Figure 5. The dynamics of African Catfish growth rate based on the weight in various densities which are maintained in biofloc.

(Source: Basuki *et al*., 2018)

In figure 5, Basuki *et al*. (2018), found that a low stocking density of 11000 *Clarias gariepinus*/m^3 showed better growth performance compared to other higher stocking densities due to improved water quality at low density.

3.3.1.4. Growth Performance of Common carp

Different stocking density has different effects on common carp's growth performance.

Table 4. Growth performance of common carp in different stocking density

Stocking Density	Culture Period (days)	Weight Gain (%)	SGR (day^{-1})	FCR	Reference
6 kg m^{-3} 12kg m^{-3}	49	133.95±16.47 114.34±11.61	1.72±0.14 1.55±0.11	1.32±0.11 1.55±0.08	Adineh *et al*., 2019
15 fish/cistern(Biofloc) 15fish/cistern (control)	91	18.77 28.6	1.49 8.66	1.13 2.31	Dinda *et al*.,2019

In table 4, growth performance was better also in highest stocking density in which is a good indicator for culturing common carp in biofloc system.

3.3.2. Feed Utilization

Stocking density has a great effect on feed utilization of fish in biofloc. As biofloc act as proteinaceous feed so better performance also observed at high stocking density also.

3.3.2.1. Feed Utilization of Indian Major Carps (IMC)

Feed utilization capacity of *Labeo rohita* at three different stocking density was observed by Mahanand *et al*., (2013) at three different stocking density 1.3 fish/m^2, 2.6 fish/m^2 and 3.9fish/m^2.

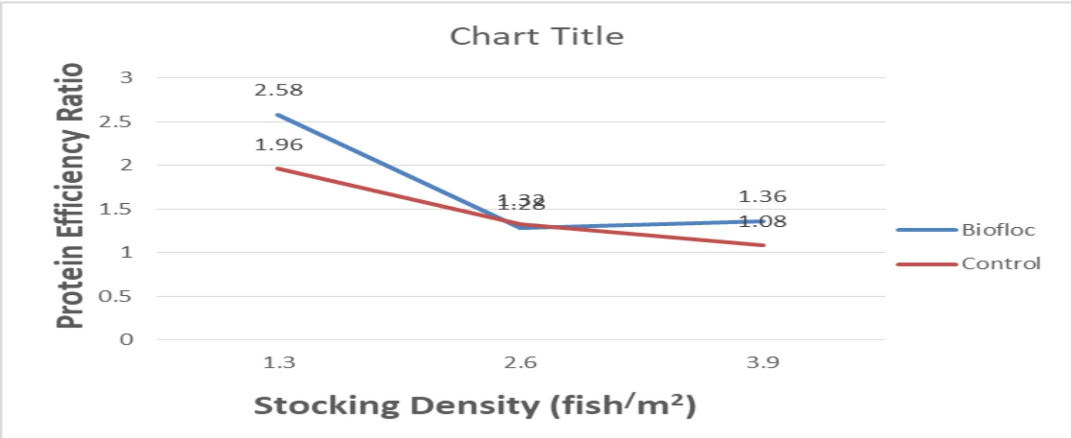


Figure 6. Protein Efficiency Ratio of *Labeo rohita* at different stocking density.

(Source: Mahanand *et al*., 2013)

In figure 6, Protein Efficiency Ratio was highest at lowest stocking density of biofloc system due to better protein retention capacity of fish.

Another study at a stocking density of 50 fingerlings of *Labeo rohita* /m^3 in biofloc system was conducted by Ahmad *et al*., (2017) for 60 days with different wheat as a carbon source for biofloc and without carbon source for control.

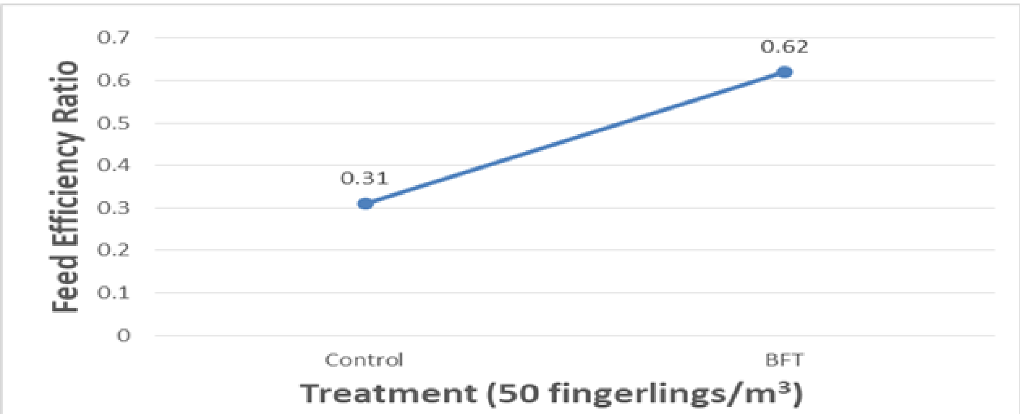


Figure 7. Feed Efficiency Ratio of *Labeo rohita* at 50 fingerlings/m^3 stocking density.

(Source: Ahmad *et al*., 2017)

In figure 7, *Labeo rohita* showed better feed efficiency in biofloc than control treatment at 50 fingerlings /m^3 due to efficient utilization of biofloc protein.

3.3.2.2. Feed Utilization of Tilapia

The effects of different stocking density on feed utilization of Nile tilapia (*Oreochromis niloticus*) was reported on several study.

Table 5 .Feed utilization of tilapia at different stocking density in biofloc

Stocking Density (Fish/m^3)	**Culture Periods (Days)**	**FER**	**PER**	**Reference**
200		0.85 ±0.0058	2.43 ±0.017	Haridas *et al.*, 2017
250		0.93 ±0.007	2.65 ±0.020	
300	90	0.85 ±0.033	2.44 ±0.096	
350		0.81 ±0.033	2.32 ±0.095	
150 (control)		0.20 ±0.0132	0.58 ±0.038	
20		0.51±0.05	1.95±0.04	Zaki *et al.*, 2020
40	84	0.56±0.04	1.79±0.02	
60		0.40±0.05	2.47±0.09	
30 (Biofloc)		1.20±0.03	2.59±0.09	Long *et al.*, 2015
30(Control)	56	1.03±0.01	2.20 ± 0.03	

Here, PER-Protein Efficiency Ratio, FER-Feed Efficiency Ratio

In table 5, feed utilization capacity of Nile tilapia was highest in biofloc comparatively higher stocking density than traditional culture practice.

A study on Red tilapia (*Oreochromis* sp.) was conducted by Ekasari & Maryam, (2012) using three different stocking density such as 25, 50 and 100 fish/m^3.It was conducted for 14weeks.

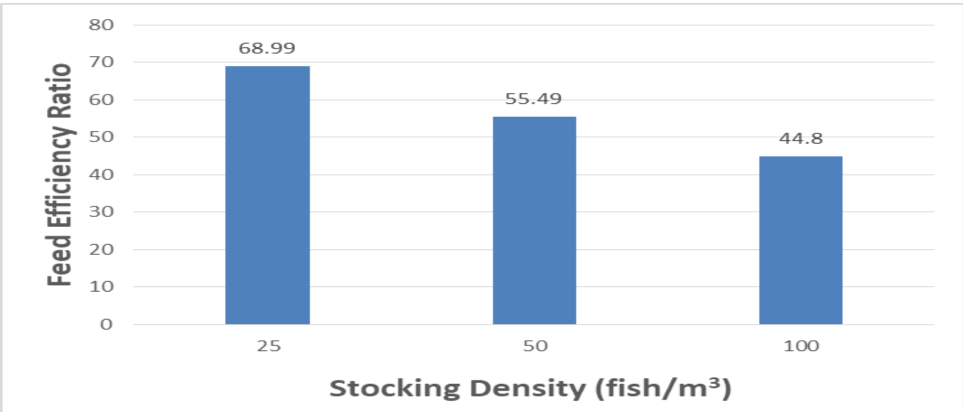


Figure 8 .Feed Efficiency Ratio of Red tilapia (*Oreochromis* sp.) at different stocking density.

(Source: Ekasari &Maryam, 2012)

In figure 8, Ekasari &Maryam, (2012) showed that better Feed Efficiency Ratio was highest in case of lower stocking density due to low competition for feed and biofloc protein.

3.3.2.3. Feed Utilization of Catfish

Fauji *et al*., (2018) evaluated feed utilization on African catfish (*Clarias gariepinus*) to know the effects of different stocking density on. The stocking density was used to conduct this experiment was 4, 6 & 8 fish/L which was designed as BFT4, BFT6 and BFT8 with a control group containing 4 fish/L.

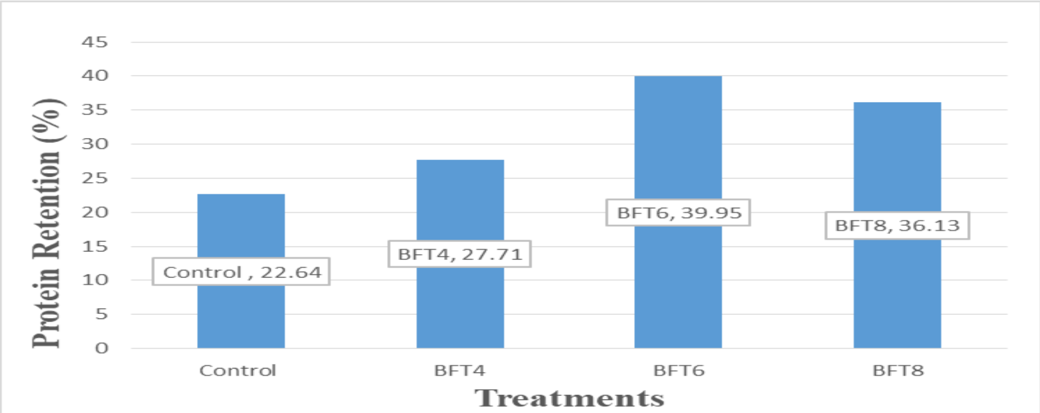


Figure 9. Protein Retention of African catfish at different stocking density in biofloc.

(Source: Fauji *et al*., 2018)

In figure 9, Among biofloc treatments higher protein retention was observed at medium stocking density in BFT6 because fish were able to utilize both dietary and biofloc protein.

Feed Utilization was observed on African catfish (*Clarias gariepinus*) by Soedibya *et al*., (2017).Utilized stocking density were 1,000 fish/m^3; 1,500 fish/m^3; 2,000 fish/m^3 and 2,500 fish/m^3. Feed were given at 3% body weight at two times a day.

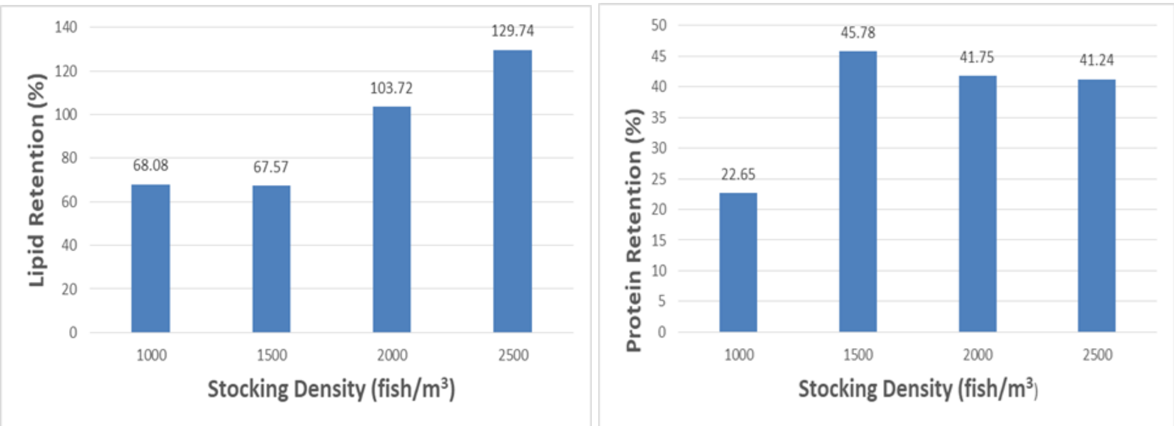


Figure 10. Average lipid and protein retention of African catfish (*Clarias gariepinus*) at different stocking density.

(Source: Soedibya *et al*., 2017)

In figure 10, it is expected that lipid retention was higher due to their less movement on the treatment tank for space limitation at highest stocking density. Protein retention were higher in case of 1500 fish/m^3 stocking density due to better utilization of protein from artificial feed and biofloc.

A 10 week study was conducted by Hastuti *et al*., (2016) on African Catfish (*Clarias gariepinus*) at three different stocking density (500,1000,1500 fish/m^3).

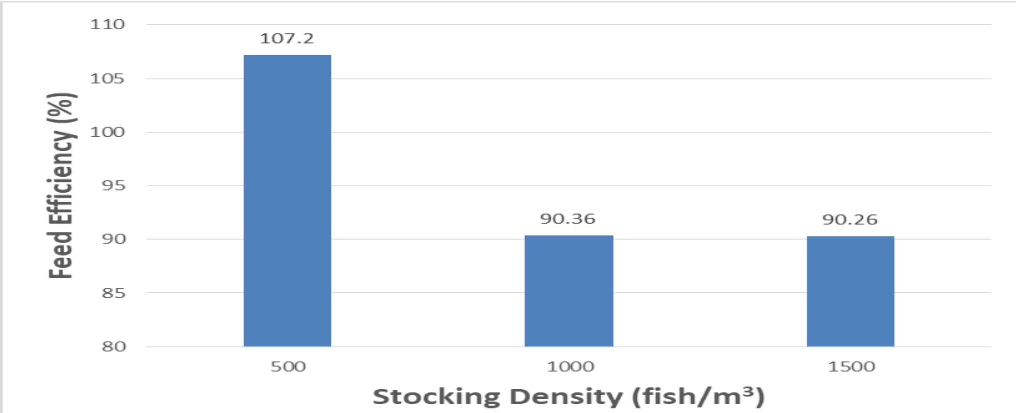


Figure 11. Feed Efficiency of African Catfish at different stocking density.

(Source: Hastuti *et al*., 2016)

In figure 11, Feed Efficiency was highest in low stocking density due to capacity of better utilization of feed.

3.3.2.4. Feed Utilization of Common carp

Feed utilization was lowest in control treatment than biofloc treatment at two different stocking density (CW6 (clear water, 6 kg m^{-3}), CW12 (clear water, 12 kg m^{-3}), BFT6 (biofloc, 6 kg m^{-3}), and BFT12 (biofloc, 12 kg m^{-3})) in Common carp. (Adineh *et al*., 2018)

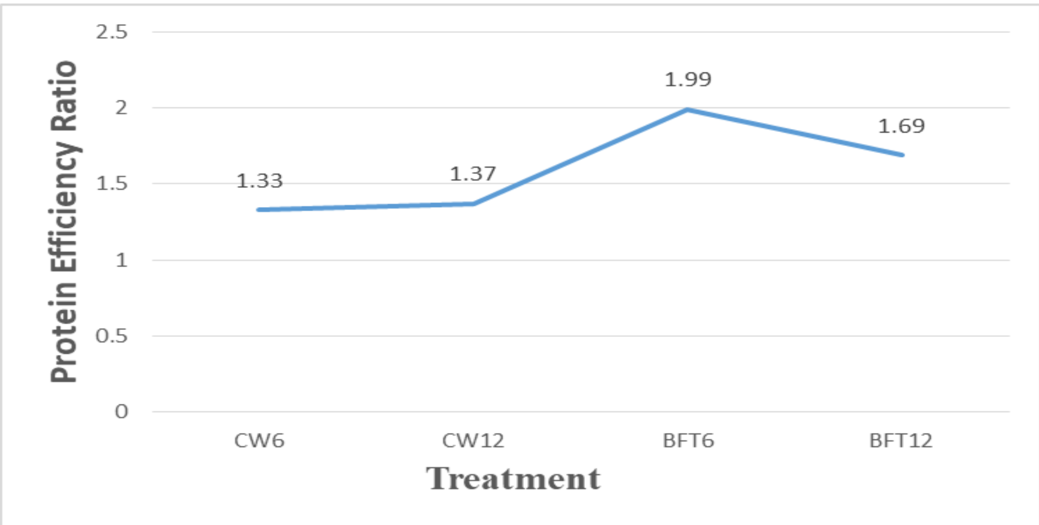


Figure 12. Protein Efficiency Ratio of Common carp in biofloc.

(Source: Adineh *et al*., 2018)

In figure 10, highest PER was observed in BFT6.It is expected that fish in treatment BFT6 was able to utilize both the dietary protein and the biofloc protein without any competition among them due to comparatively low stocking density than BFT12.

3.3.3. Immune Response

High stocking density stress could influence fish innate immune responses negatively, which would make fish more susceptible to acute stress and ultimately impair fish welfare. Biofloc has a great potential to increase immunity of fish as it acts as probiotic. Research related to the effects of stocking density on immune response in biofloc system are limited.

3.3.3.1. Immune Response of Indian Major Carps (IMC)

Among Indian Major carps a few studies has been conducted on *Labeo rohita* re;ated to stocking density.

Table 6.Innate-Immune response of *Labeo rohita* in different stocking density

Stocking Density(fish/ m^3)	**Culture Periods (Days)**	**NBT (OD at 540 nm)**	**MPO (OD at 450 nm)**	**Serum Cortisol((ng dL-1)**	**Serum Glucose (mg dL-1)**	**Reference**
20 (Biofloc)	112	0.65± 0.17	1.40± 0.25	15.59 ± 0.69	196.78± 4.85	Kamilya *et al.*, 2017
20 (Control)		0.47± 0.19	1.22± 0.16	16.37 ± 0.29	201.99± 9.72	
50 (Biofloc added with tapioca flour)	60	0.33± 0.09	1.99± 0.08	17.34 ± 0.40	47.49 ± 3.42	Ahmad *et al.*, 2017
50 (Control)		1.99 ± 0.08	0.60 ± 0.01	58.86 ± 0.71	99.33±0.34	

Here, NBT- Nitroblue Tetrazolium Activity, MPO-Serum Myeloperoxidase content

In table 06, Immune response and stress tolerance was greater in biofloc treatment than control treatment at different stocking density.

3.3.3.2. Immune Response of Tilapia

Tilapia is the most cultivable species among finfish because of their high tolerance to adverse environmental quality and disease resistance.

Immune response of fish was evaluated on Nile tilapia (*Oreochromis niloticus*) through several study.

Table 7. Blood Parameters of Nile tilapia at different stocking density

Stocking density (fish/m^3)	Culture Period (Days)	RBC (10^9L^{-1})	WBC ($10^{12}L^{-1}$)	Hb(Uml^{-1})	Ht (Uml^{-1})	Reference
20		4.14±0.15	241.00±6.57	98.00±0.08	41.18±0.84	Zaki *et al.*, 2020
40	84	3.59±0.14	322.90±6.62	89.66±0.58	39.00±0.44	
60		2.91±0.08	256.00±3.57	68.33±0.84	27.33±0.75	
30	56	1.33±0.09	7.54±0.94	5.02 ±0.21	23.86±2.57	Long *et al.*, 2015

Here, RBC-Red blood cells, WBC -White Blood Cells, Hb-Hemoglobin and Ht-Hematocrit. In table 07, overstocking density adversely affects RBCs, hemoglobin and hematocrit values of Nile tilapia due to poor water quality.

Immunophysiological response of GIFT strain of Tilapia was evaluated by Haridas *et al.*, 2017. Treatments were designated as stocking densities as 200 (SD1), 250 (SD2), 300 (SD3) and 350 (SD4) fish/m^3 in biofloc-based treatments and 150 (C) fish/ m^3 in control (clear water).

Table 8. Respiratory burst activity, myeloperoxidase activity and serum lysozyme activity, serum cortisol level at 200 (SD1), 250(SD2), 300 (SD3) and 350 (SD4) fish/m^3 stocking density

Parameters	Control	SD1	SD2	SD3	SD4
NBT (OD at 540 nm)	0.17±0.009	0.31±.012	0.29±.008	0.26±0.21	0.25±.021
MPO (OD at 450 nm)	0.24±0.030	0.70±.047	0.65±0.93	0.53±.025	0.39±0.60
Lysozyme (Units mL^{-1})	77.67±2.85	155.33±13.28	143.33±4.81	121.00±8.50	117.67±8.25
Cortisol (ng mL^{-1})	56.09±6.59	30.52±1.47	31.96±0.37	37.92±4.11	39.17±2.09

(Source: Haridas *et al.*, 2017)

Here, NBT- Nitroblue Tetrazolium Activity; MPO-Myeloperoxidase Activity.

In Table 8, Higher NBT, MPO and lysozyme values in biofloc support the hypothesis of natural probiotic effect, whereas the decrease in values with increasing stocking density may be due to the crowding stress.

3.3.3.3. Immune Response of Catfish

Several studies has been conducted on Catfish species to observe the immune response at different stocking density. But most of the study was conducted on Catfish.

Table 9. Blood performances of catfish in response to different stocking density

Catfish Species	Stocking Densities (fish/m^3)	Culture Period (Days)	RBC (10^6 cells/mm^3)	Haematocrit (%)	Haemoglobin (g /dL)	Reference
Silver Catfish (*Rhamdia quelen*)	10	45	1.63±0.19	25.33±4.36	6.82±0.98	Battisti *et al.*, 2020
	20		2.89±0.76	25.66±1.86	7.76±1.99	
	30		2.15±0.57	25.50±3.56	6.69±0.52	
African Catfish (*Clarias gariepinus*)	500	70	1.83± 0.06	23.53± 0.50	6.60 ± 0.26	Hastuti *et al.*, 2016
	1000		1.75± 0.20	26.56± 0.20	7.43 ± 0.08	
	1500		1.57± 0.15	23.23± 0.23	6.62 ± 0.12	

In table 9, in studies African catfish showed better blood parameter performance in biofloc system at high density also which express capability to prevent various pathogenic disease and as well as increase survivability in stress condition.

Immune response of African catfish against *Aeromonas hydrophila* was studied by Fauji *et al*., (2018) at different stocking density (4, 6 & 8 fish/L with a control group without carbon source addition at a stocking density of 4 fish/L) which was designated as BFT4, BFT6, BFT8 and Control. Observation of blood parameters through challenge test with *Aeromonas hydrophila* were performed on the final day in 20 days of the experiment.

Table 10. Blood parameters of African catfish cultured in control (4 fish/L) and biofloc systems with different stocking density (4, 6, 8 fish/L) after challenge test with *Aeromonas hydrophila*

Blood Parameters	Control	BFT4	BFT6	BFT8
Haemoglobin (g %)	3.33±0.15	3.53±0.05	3.60±0.17	3.40±0.17
Haematocrit (%)	36.88±0.24	27.94±1.09	28.23±0.58	33.68±0.53
Red blood cells (10^6 cells/mm^3)	3.51±0.70	3.49±1.27	4.15±0.15	4.07±0.25
White blood cells (10^4 cells/mm^3)	3.20±0.10	3.77±0.15	4.17±0.25	4.30±0.17
Phagocytic activity (%)	25.33±1.15	28.33±0.58	32.67±0.58	30.67±1.15

(Source: Fauji *et al*., 2018)

In table 10, after challenge test with *A. hydrophila*, the phagocytic activity and WBCs in African catfish from biofloc treatments at higher stocking density were consistently higher than that of the control and other bioflc treatment due to better fish innate immunity against pathogenic bacterial infection.

3.3.3.4. Immune Response of Common carp

The results of innate immune response parameters of common carp reared in CW and BFT systems at different stocking densities (6 and 12 kg m^{-3}) for 49 days which was reported by Adineh *et al*., (2019).

Table 11. Innate immune responses of common carp reared in the CW and BFT systems at different stocking densities (6 and 12 kg m-^{3})

Parameters	Experimental groups			
	CW6	CW12	BFT6	BFT12
Cortisol (ng mL^{-1})	36.78±2.39	43.41±7.83	31.00±1.03	33.07±1.09
Glucose (mg dL^{-1})	135.18±3.68	140.01±2.00	124.35±1.18	128.94±0.92
Lysozyme (U mL^{-1})	34.70±3.85	28.10±1.68	33.13±0.93	32.04±1.03
ACH50 (U mL^{-1})	139.39±3.04	126.59±2.85	136.19±1.05	135.20±0.84
ALP (U L^{-1})	151.94±3.43	131.83±2.07	148.44±1.19	150.98±2.25

(Source: Adineh *et al*., 2019)

Here, ALP-alkaline phosphatase, ACH- Alternative complement.

In Table 11, serum cortisol concentrations in the BFT groups were significantly reduced compared with the CW groups. It express that, BFT can prevent the increase in serum cortisol concentration induced by acute crowding and it acts as anti-stressor.

3.3.4. Proximate Composition

Stocking density has great effects on proximate composition of fish in biofloc system.

Several study has resulted better proximate composition of fish in biofloc systems.

3.3.4.1. Proximate Composition of Indian Major Carps (IMC)

Among various Indian Major Carps species several studies has been conducted on *Labeo rohita* in biofloc systems to evaluate proximate composition of fillet.

Table 12. Proximate composition of *Labeo rohita* at different stocking density

Stocking Density (Fingerling/L)	Culture Period (Days)	Crude Protein (%)	Ether Extract (%)	Moisture (%)	Ash (%)	Reference
50	60	52.40±0.06	4.18±0.03	74.01 ± 0.3	12.75± 0.05	Ahmad *et al.*, 2017
3	90	35.40±0.63	1.1 ± 0.26	4.1 ± 0.10	15.38± 0.35	Mahanand *et al.*, 2013

In table 12, studies showed variable proximate composition of *Labeo rohita* at different stocking density. Proximate composition was also good in higher stocking density,

3.3.4.2. Proximate Composition of Tilapia

Several study has been conducted on tilapia in biofloc systems where proximate composition of fillet was observed.

Table 13. Proximate composition of Nile tilapia at different stocking density

Stocking Density (Fish/m^3)	Culture Period (Days)	Dry Matter (%)	Crude Protein (%)	Ash (%)	Reference
20		27.52±0.9	56.69±0.38	20.67±0.27	
40	84	27.5±0.4	56.34±0.11	20.84±0.9	Zaki *et al.*, 2020
60		27.69±0.7	57.16±0.24	20.49±0.18	
20		92.96	51.89	15.04	
40	112	93.08	48.95	14.43	Manduca *et al.*, 2020
60		93.31	47.16	15.40	
80		93.51	49.19	14.69	
30 (biofloc)	56	24.30±2.20	51.687±0.99	18.80±0.91	Long *et al.*, 2015
30 (control)		28.30±0.62	53.65±0.25	21.30±0.04	

In table 13, proximate composition of Nile tilapia showed best result at high stocking density also. Better proximate composition of fillet was obtained due to better utilization of feed and biofloc protein.

.3.3.4.3. Proximate Composition of Catfish

Few studies has been conducted on African Catfish (*Clarias gariepinus*) to observe the proximate composition in biofloc.

Table 14. Proximate Composition of *Clarias gariepinus* in different studies

Stocking Density (fish/m^3)	**Culture Period (Days)**	**Crude Lipid (%)**	**Crude Protein (%)**	**Ash (%)**	**Reference**
10		9.25±0.85a	19.21±0.57	0.04±0.01	Battisti *et al.*,
20	45	5.27±0.34b	18.27±0.36	0.04±0.01	2020
30		4.20±1.51b	18.93±0.12	0.03±0.01	
150 (biofloc)	42	4.43 ± 0.50	13.82 ± 0.26	2.94 ± 0.09	Dauda *et al.*,
150 (control)		4.60 ± 0.30	13.58 ± 0.17	2.98 ± 0.11	2017

In table 14, proximate composition of *Clarias gariepinus* showed better performance in biofloc treatment than control treatment at highest stocking density also.

3.3.4.4. Proximate Composition of Common carp

Bakhshi *et al*., (2018) conducted an experiment on Common carp in biofloc system where proximate composition was observed with carbon source in 10 weeks.

Table 15. Proximate composition of Common carp in biofloc

Contents	Stocking Density (300 fingerlings/m^3)	
	Control	Biofloc+ Corn Starch
Protein%	43.3 ± 5.4	56.4 ± 0.33
Lipid (%)	14.3 ± 2.6	23.6 ± 3.2
Carbohydrate (%)	31.2 ± 0.9	8.2 ± 3.4
Moisture (%)	55.2 ± 8.6	56.2 ± 4.7
Ash (%)	10.6 ± 0.3	10.7 ± 0.11

(Source: Bakhshi *et al*., 2018)

In table 15, at a stocking density of 300 fingerlings/m^3 proximate composition of common carp was higher in case of Biofloc treatment than control due to consumption of biofloc protein.

Chapter IV
CONCLUSIONS

- For culturing farmed fish stocking density plays an important role in aquaculture. Stocking density has a great effect on growth performance of fish. Normally, the higher the stocking density, the lower the growth performance observed because growth rate are increased through the free movement of fish in a culture environment. Growth rate decreased due to poor water quality which actually occurs when stocking density are high. Biofloc is an improved technique to reduce the water quality problems and also to reduce the feed cost in aquaculture. In case of biofloc system, higher stocking density do not hamper the growth rate of fish. Growth performance (FW, FCR, SGR) are significantly higher for rohu, tilapia, catfish, common carp in biofloc system than traditional culture practice at high density also. Normally feed utilization capacity of fish hampered at high stocking density due to high competition among fish for food, space. But in case of biofloc system fish can uptake protein from dietary protein and also from biofloc which reduce the competition among fish for feed. Better feed intake, PER, Lipid retention, protein retention observed in rohu, tilapia, catfish and some other finfish at a higher stocking density also.
- Due to causing stress condition at higher stocking density, breakdown of immunity system of fish occurs in traditional culture practice. But in case of biofloc system, floc's microbes act as probiotics and enhance the immune system of fish against various diseases. So, various finfish showed better immune response in case of higher stocking density than traditional aquaculture systems. There was a better Haemoglobin & Haematocrit levels, WBC & RBC counts, ALP, Serum lysozyme activity, Serum cortisol level of rohu, tilapia, catfish, common carp at higher density also in case of biofloc systems than traditional aquaculture practice. Proximate composition reflects the nutritive status of fish. Proximate composition of fish fillet in case of rohu, tilapia, catfish and Common carp was highest in case of biofloc treatment than non-biofloc treatment.at different stocking density.

Increasing Stocking density in aquaculture practice is necessary to increase the production of fish in a limited space. To remove the problems associated with increasing stocking density, biofloc can be a better solution. Culturing fish in biofloc can improves growth performance, survivability, feed utilization and immunity against disease at high density also.

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