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Light Limitation Alters Water Quality, Biofloc Composition, Zooplankton Community, and Performance of the Whiteleg Shrimp (*Litopenaeus vannamei*) Reared with Intensive Biofloc

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ABSTRACT

An 18-week experiment was performed to explore light limitation effects on water profile, biofloc analysis, zooplankton population, growth, and proximate body analysis of whiteleg shrimp (*Litopenaeus vannamei)* grown in biofloc system. The experiment was conducted in six 36m^3 cement tanks with a water volume of 30m^3. Post larvae of *L.vannamei* shrimp (0.02± 0.0001g) were stocked in tanks at a stocking density of 200animal/m^3. The experiment was consisted of two treatments: T1: without light limitation and T2: with light limitation. Significantly higher dissolved oxygen (5.34± 0.080mg/l) was found in T2 compared to (5.01± 0.056mg/l) the units of T1. Moreover, significantly higher pH was observed in T2. Turbidity (NTU) and floc volume (ML/L) were significantly higher in T1 (60.60± 2.51 and 19.73± 0.726, respectively) compared to (48.05± 1.90 and 17.13± 0.41, respectively) the light- limited group. Furthermore, significantly higher survival rate was observed in T2 when compared with T1 (97.20± 0.153 *vs* 94.97± 0.696%, respectively). Additionally, final shrimp biomass (Kg) and biomass increase percentage were significantly greater in T2 (74.02± 0.43 and 411.25± 3.10, respectively) compared to (71.53± 0.55 and 397.38± 3.10, respectively) T1. FCR and PER were significantly improved in the light- limited group (1.32± 0.007 and 1.98± 0.010), as compared with (1.37± 0.008 and 1.92± 0.011, respectively) T1. Furthermore, significantly higher flocs protein content (17.97± 0.40%) was found in T1 compared to (15.62± 0.32 %) T2. Additionally, significantly higher total zooplankton count (141560orgs/m^3 ± 2163.82) was observed in T1 compared to (65350 orgs/m^3 ± 240.90) T2. Under biofloc system, light limitation improved biomass, survival rate, feed utilization, and water quality, while biofloc composition and zooplankton abundance were negatively affected.

INTRODUCTION

Aquaculture technology has been developed worldwide with new systems and higher intensification **(Joffre *et al.*, 2018; Dauda, 2019)**. The higher growth of aquaculture industry may participate in improving food production, which enhances food security for the highly increased populations around the world **(Beveridge *et al.*, 2013;**

Joffre *et al.*, 2018). While, the increased stocking densities and higher feed quantities have been used for the intensive aquaculture units accompanied with greater effluents, resulting in environmental pollution and degradation **(Verdegem, 2013; Bossier & Ekasari, 2017; Dauda, 2019)**.

Biofloc technology (BFT) is a recent technology based on heterotrophic bacteria, algae and nitrifying bacteria in controlling the quality of rearing water and supporting *in situ food* nutrients to the cultured organisms **(De Schryver *et al.*, 2008; Avnimelech, 2015, Dauda, 2019)**. The biofloc industry was regarded as an environmentally friendly aquatic animals farming technology **(Li *et al.*, 2018)**. The addition of carbohydrate in rearing water to keep C/N ratio more than 10 provides suitable environment to enhance heterotrophic bacterial proliferation, which assimilates nitrogen waste converting it into a single-cell protein **(Kuhn *et al*., 2010; Avnimelech, 2015; Bossier &Ekasari, 2017)**.

Light intensity influences significantly the growth, proliferation and types of microorganisms in the culture units **(Llario *et al*., 2019)**. Moreover, some studies reported that light intensity can affect growth, metamorphosis and survival rate of fish and shrimp **(Didrikas & Hansson, 2009; Guo *et al*., 2013; Chen *et al*., 2021)**. Light intensity and photoperiod affect the growth performance of shrimp and their survival in either clear-water or biofloc units in varying ways according to shrimp species, age, and whether the culture system was indoor or outdoor **(El-Sayed, 2021)**.

Regarding biofloc system, more active heterotrophic bacteria in low light intensity were reported, and also higher performance in nitrogenous wastes conversion compared to algae, whose assimilation fluctuates depending on light exposure **(Dauda, 2019)**. While, other experiments reported that outdoor BFT units exposed to sun light had a higher activity of photosynthetic microorganism proliferation than indoor BFT units **(Coyle *et al*., 2011; Fleckenstein *et al*., 2019)**.

In outdoor biofloc systems with higher algal growth, the algae may participate in transforming nitrogenous wastes in the unit into organic and bioactive compounds that can be utilized by the reared species, which helps improving shrimp performance and immunity **(Ge *et al*., 2017; Fleckenstein *et al*., 2019)**. Higher nutritional composition of algal-abundant flocs (41.9% protein, 2.3% lipid) were reported compared to the flocs in bioflocs bacteria - dominated BFT systems (38.4% protein, 1.2% lipid) **(Ju *et al*., 2008b, Dauda, 2019)**. Moreover, **Xu *et al*. (2016)** and **Dauda (2019)** reported that BFT units based on both bacteria and phytoplankton result in higher performance for the cultured shrimp.

On the other hand, systems in total darkness need more oxygen input in daylight hours, but this is accompanied by reduced harmful algae growth **(Baloi *et al*., 2013)**. **Ray *et al*. (2009)** recorded that, by shading sunlight in biofloc units, culture species can be reared in insulated buildings with controlled environment, reducing energy costs in winter.

Along stocking density effects, C:N ratio, carbohydrate material, & impacts of varies sun light exposure levels on the BFT units need more investigation in order to optimize shrimp production in BFT units. It is necessary to estimate the suitable light intensity for both biofloc and the reared organisms in the culture units. Therefore, this experiment aimed to assess the impact of limited light conditions on water quality, proximate analysis of flocs and shrimp body, zooplankton community, and growth performance parameters, and survival rate of whiteleg shrimp *l. vannamie* in an intensive biofloc system.

MATERIALS AND METHODS

1. Shrimp culture system and experimental design

This study was conducted from May to September 2021 at private *L.vannamei* hatchery located in Damietta, Egypt. The experiment was performed in six $36m^3$- tanks made from cement (3 W *10 L*1.2 D). Experimental tanks with a water volume of $30m^3$were filled with filtered seawater (salinity 32ppt). Post larvae of whiteleg shrimp samples, with an initial body weight of 0.02± 0.00g, were transported from commercial marine shrimp hatchery located on the coast of the Mediterranean (Al-Ekhlas shrimp hatchery). Stocking density of post larvae in tanks was $200orgs/m^3$. All experimental tanks were supported with continuous aeration regime. During the study, no water renewal was experienced except for the compensation of evaporation.

The experiment was designed in a completely randomized design with two treatments: T1 = tanks were shaded with white plastic sheet (without light limitation) and T2 = culture with light limitation (tanks were shaded with black plastic sheet). Light source was the natural light. Light intensity in the treatment without light limitation was 5320 lux ± 254, while it was 1230± 112 in the light- limited treatment. Each treatment had three replicates. Moreover, shrimps were cultured for 18 weeks.

Four meals were introduced to the cultured shrimp daily at 8 AM, 11 AM, 2 PM, and 5 PM with 38% protein shrimp ration (Skretting, Egypt). Shrimp were fed daily at 15% of body weight at the start of the experiment, which was lowered gradually to 2.5% at the end of the study. Feeding quantity was modified every 14 days after weighing a representative shrimp sample from each tank and accounting for any recorded mortality. Throughout the experimental period, shrimp samples were fed with crumbled feed (0.4-0.6mm) and pelleted feed (0.8- 1.5mm).The addition of the carbon source (wheat flour) was done after the last meal to all experimental tanks to promote biofloc formation **(Said *et al.*, 2022a, b)**. Theoretically, carbon source addition was done one time per day depending on the calculations, as reported by **Avnimelech (2009)**. The pre-weighed wheat flour was dissolved in culture water and dispensed on the water surface of each tank. An input C: N ratio of 15: 1 was kept during the experiment; we followed the method of **Said *et al.* (2022a, b)**. The composition of experimental feed and carbon source are shown in Table (1).

Table 1. Proximate analysis of experimental feed and carbon sources used in the study

Constituent	Feed	Wheat flour
Crude protein	38.35	10.44
Ether extract	9.98	3.17
Crude fiber	4.78	5.29
Total Ash	8.98	1.61
Moisture	8.02	10.88
Nitrogen free extract	29.89	68.61

Values are percent of diet on a dry weight basis.

2. Target traits measurement

2.1. Water quality

The temperature and dissolved oxygen were daily estimated by an electronic probe (HANNA, HI9146-04), measuring pH and turbidity every 48h by a portable pH meter (Milwaukee, MW102), and turbidity meter (Lovibond, TB211 IR), respectively. Ammonia and nitrite were both daily monitored by a photometer (HANNA, HI97715, and HI97708, respectively), while light intensity was recorded using lux meter (HANNA, HI 97500). On the other hand, floc volume was monitored every 48h by Imhoff cone.

2.2. Growth, feed utilization, and survival

For the determination of growth performance and feed utilization. shrimps from all tanks were collected and weighed as final weight (FW), weight gain (WG), average daily weight gain (ADWG), weekly weight gain (G/W), specific growth rate % (SGR%), total biomass, and biomass increase as a percentage.

The determination of feed utilization was determined through measuring feed conversion ratio (FCR), feed efficiency (FE), and protein efficiency ratio (PER) **(Tacon *et al.*, 2002)**. To determine the overall survival rate, the number of shrimps was counted at the beginning and the end of the trial.

2.3. Bioflocs and shrimp body composition

The proximate composition analysis ofbiofloc and shrimp was conducted using the method described by **AOAC (2005)**. Samples of flocs were gathered during the last week of the experiment from the experimental tanks by a 100µm mesh for biochemical analysis. Shrimp samples from each experimental tank were obtained at the harvesting time. Floc samples were dried in a drying oven at 60°C and after that they were grounded, and the moisture content was determined by a previously defined weight of samples that were dried in a drying oven at 105°C for six hours. For ash content, a previously defined weight of dried samples was burnt in a muffle furnace at 550°C for

four hours. The determination of crude protein was conducted using the Kjeldahl method (FOSS, KjelTecTM 8400). Additionally, the determination of crude lipid was conducted using the automatic fat extraction method (FOSS, SoxtecTM 8000), and estimation of crude fiber by automatic fiber analysis method (FOSS, FibertecTM 8000). While, nitrogen-free extract was counted from the difference **(Tacon, 1990)**.

2.4. Zooplankton

A plankton net of 55µm mesh size was used in zooplankton collection from the units during the last week of the experiment; 5 liters were filtered through the mesh from each tank. The samples collected were transferred to clean bottles, labeled and directly fixed with 4% formalin. Three subsamples (one ml) were taken from each homogenized plankton sample and assessed for zooplankton species counting and identification. The subsamples were identified using a binocular microscope with magnification varying from 100to 400X. Zooplankton population density was counted as the number of individuals per m^3 using the model reported by **APHA (1995)**: No. X m-3 = (c X v′) / (v″ x v‴) x 1000

Where, - c= number of animals counted.
v′= volume of concentrated sample, ml.
v″ = volume counted, ml.
v‴= volume of the grab sample, liters.

The identification of zooplankton species was conducted using the method described by **Edmondson (1963)**, **Ruttner (1971)**, **Pennek (1978)**, **Pontin (1978)**, **Wallace and Snell (1991)** and **Foissner and Berger (1996)**.

3. Statistical analysis

Data were statistically analyzed by IBM SPSS Statistics 25 software (IBM Corporation, NY, USA). Independent sample *t-test* was used in analyzing the influence of treatments on growth performance, feed utilization, survival rate, and proximate composition of flocs and shrimp. Water quality parameters were analyzed using two-way repeated-measures analysis of variance, with treatment as the main factor and sampling date as the repeated measures factor. Zooplankton numbers were analyzed using an independent sample *t-test* (IBM SPSS Statistics version 25). The results were presented as mean ± *SE*. Mean differences between treatments were found by Duncan's multiple range test. A probability value (*p*) of less than 0.05 was used to show the statistically significant differences.

RESULTS AND DISCUSSION

1. Water quality

Significantly higher dissolved oxygen concentration average (5.34± 0.080mg/l) was found in T2 units (with light limitation) compared with (5.01± 0.056 mg/l) the units of T1 (without light limitation). Moreover, slightly higher ammonia and nitrite concentrations were reported in T1 without significant differences from T2 units. Additionally, significantly higher pH value was observed in the units reared with light limitation (T2). Furthermore, higher turbidity and floc volume were found in T1 (60.60± 2.51 and 19.73± 0.72, respectively) compared to (48.05± 1.90 and 17.13± 0.41, respectively) the light- limited group (Table 2).

Table 2. Water quality parameters for whiteleg shrimp reared under biofloc system (zero water exchange) with and without light limitation for 18 weeks

Parameter	**Light limitation**		***P*-value**
	Without	With	
DO (mg/L)	5.01±0.05	5.34±0.08	0.00
NH_3 (mg/L)	0.03±0.00	0.02±0.00	0.63
NO_2(mg/L)	0.35±0.01	0.34±0.02	0.72
pH	6.62±0.09	6.96±0.06	0.00
Turbidity (NTU)	60.60 ± 2.51	48.05 ± 1.90	0.00
Floc volume (ml/L)	19.73±0.72	17.13±0.41	0.00
Heterotrophic Bactria count (CFU/ ml)	**$4.1 \times 10^5 \pm 0.05$**	**$2.2 \times 10^5 \pm 0.15$**	**0.07**

Probability value (*P*) of less than 0.05 was used to indicate statistically significant differences.

Appropriate water quality **(Panigrahi *et al*., 2019; Hoang *et al*., 2020)** was maintained in both treatments within the experiment indicating the positive impact of biofloc on water profile. Oxygen, ammonia, nitrite, and pH were kept within the appropriate levels for *L.vannamei* intensive culture **(Krummenauer *et al*., 2011; Furtado *et al*., 2015; Samocha, 2019; Martins *et al*., 2020)**. **Briones and Raskin, (2003)** and **Deng *et al*. (2018)** reported that the presence of more diverse bacterial species in the BFT system removes nitrogenous wastes more effectively, more stable system, and resistance to problems that threats the system.

Light represents a limiting factor for photosynthesis process and algal blooming and as a result, nitrogen wastes removal rate and floc formation in biofloc systems **(El-Sayed, 2021)**. The results of the current study revealed significantly higher DO concentration and pH levels in the limited light tanks. Insignificant higher ammonia and nitrite concentrations were reported in T1 (unlimited light treatment). The relative superiority of water quality profile in the light- limited treatment is compatible with **Hargreaves (2006)**, who reported that units receive full sunlight- showed unstable levels of pH and dissolved oxygen.

The accumulation of toxic nitrogen wastes is a major problem in intensive culture of shrimp, therefore it is necessary to remove them or transfer these to less detrimental compounds or to single-cell protein **(Avnimelech, 2015)**. In light-limited units that do not contain shrimp, the results agreed that the biofloc formulation is suitable for shrimp, and water conditions can be controlled by BFT **(Azim *et al*., 2008)**. This optimization can be reached by the removal of ammonia through heterotrophic bacteria assimilation or by the nitrification of autotrophic bacteria in natural sunlight **(Ebeling *et al*., 2006; Hargreaves, 2006; Xu *et al*. 2016)**. These mechanisms overlap in biofloc systems and improve water quality parameters **(Dauda, 2019)**. Nitrogen removal in biofloc system relies on various parameters, including microbial community, system volume, culture period **(Lezama- Cervantes &Paniagua-Michel, 2010; Dauda, 2019)**, carbohydrate material, and C:N ratio **(Vilani *et al*., 2016; Xu *et al*., 2016)**.

Avnimelech (2015) mentioned that heterotrophic bacterial assimilation is the most reliable pathway in nitrogen waste removal. On the other hand, **Dauda (2019)** postulated that biofloc consists of a mixture of algae–bacteria may use nitrogenous nutrients efficiently compared to biofloc depended on bacteria only. Nitrifying bacteria can just transfer the unionized ammonia to less toxic nitrate-N **(Crab *et al*., 2007)**.

The current findings of ammonia and nitrite match with those of **Khao *et al*. (2020)**, who mentioned lower level of TAN in the single shaded units, as compared with sun exposed units. Higher nitrogenous compounds in the unlimited light group may be due to the inhibiting mechanism between microalgae and bacteria within the sunlight-exposed systems **(Fuentes *et al*., 2016; Dauda, 2019)**. Theoretically, biofloc systems with outspread of heterotrophic bacteria would improve the conversion of nitrogenous wastes since algal conversion is affected by light intensity and exposure **(Dauda, 2019)**. Moreover, **Reis *et al*. (2019)** observed that, units with light limitation enhances nitrification process compared to natural light exposed units, which may be related to the inhibition of the viability of nitrifying bacteria by light exposure (photosensitivity) and competition by other micro-organisms with the nitrifying bacteria for nutrients **(Guerrero & Jones 1996; Vergara *et al*., 2016)**. This photosensitivity can explain why nitrification occurred better and more readily in light- limited biofloc

systems. Furthermore, light limitation supported better culture environment for nitrifying bacteria proliferation.

At high light intensity, algal growth could highly increase, resulting in slight-shading, which prevents light penetration and may result in phytoplankton collapse and sudden death. This means that systems depends on algae mainly results in daily variability in the levels of DO, CO_2, pH and toxic ammonia **(Hargreaves, 2006)**. Under these conditions, biofloc system conditions stays in continuous fluctuations between day and night. Likewise, **Avnimelech (2015)** reported that biofloc technology provides better culture environment when processed in greenhouses or other indoor units.

Additionally, better water quality in the light- limited units, in terms of higher dissolved oxygen concentration and lower ammonia and nitrite level with suitable turbidity and floc volume, may be due to the lower abundance of cyanobacteria in these units. **Jiang *et al*. (2020)** recorded that, the cyanobacteria and proteobacteria were highly abundant in the light- exposed units. The higher proliferation of cyanobacteria could cause detrimental algal growth and adversely affects water quality in shrimp production units **(Xu *et al*., 2019)**. Thus, limiting light by shading the biofloc units may control cyanobacteria in the cultured units.

Higher biofloc volume together with higher turbidity were reported in T1 units. Similar findings were recorded by **Khoa *et al*. (2020)** when they compared units exposed to natural light and treatments with limited light conditions produced by covering the tank surface with one light constriction shading net of one, two, or three layers. Higher floc volume was found in the more sunlight exposed units. Furthermore, **Esparza-Leal*et al*. (2017)** reported that, the highest TSS concentrations were found in the aquaculture systems exposed to natural sunlight. While, lower turbidity in light limited units was also recorded by **Jiang *et al*. (2020)**. The lower turbidity in the light- restricted units may be due to the growth of phylum proteobacteria in these units which can get rid of the organic matter from the cultured units. These bacteria had high dominance in the units with light restriction **(Jiang *et al.*, 2020)**. Moreover, these bacteria can remove the presented organic matter in the culture systems **(Miura *et al*., 2007; Rud *et al*., 2017)**.

2. Growth performance and survival rate

Final weight, weight gain, average daily gain, weekly weight gain and specific growth rate were all slightly higher in T2 (with light limitation) without significant statistical differences between the two treatments. A significantly higher biomass (kg) and biomass increase percentage were found in T2 (with light limitation) system (74.02± 0.43 and 411.25± 3.10, respectively) compared to (71.53± 0.55 and 397.38± 3.10, respectively) T1. Additionally, a statistically significant better survival rate was obvious

in T2 than the unlimited light treatment (97.20± 0.153 *vs* 94.97± 0.696%, respectively) (Table 3).

Table 3. Growth performance and survival of whiteleg shrimp reared under biofloc system with and without light limitation for 18 weeks

Parameter	Light limitation		*P*-value
	Without	With	
Initial weight (g)	0.02 ± 0.00	0.02 ± 0.00	1
Final weight (g)	12.55 ±0.06	12.69 ± 0.06	0.13
Weight gain (g)	12.53 ± 0.06	12.67 ± 0.06	0.13
ADG (g /day)	0.09±0.00	0.10 ±0.00	0.09
G/Week	0.69 ± 0.00	0.70 ±0.00	0.13
SGR %	5.11 ± 0.00	5.12 ±0.00	0.14
Biomass (Kg)	71.53± 0.55	*74.02 ±0.43	0.02
Biomass increase percentage	397.38 ± 3.10	*411.25±3.10	0.02
Survival rate%	94.97±0.69	*97.20±0.15	0.03

Probability value (*P*) of less than 0.05 was used to indicate statistically significant differences.

Growth and survival in both experimental treatments idicated the positive impact of biofloc on growth performance parameters and survival rates **(Durigon *et al*., 2020; Hoang *et al*., 2020)**. Better performance of shrimp under BFT system may be due to better water quality, higher nutritional value of biofloc **(Fleckenstein *et al.*, 2020; Khanjani & Sharifinia, 2020)**. Shrimp physiological functions, feeding behaviors, molting, growth, and survival were all previously reported to be affected with light **(Gardner & Maguire, 1998; Baloi *et al.*, 2013; Fleckenstein *et al.*, 2019)** and also the proliferation of light dependent organisms **(Samocha, 2019)**. Significant differences in the shrimp survival rate and total biomass between the two treatments indicated that light has an effect on shrimp performance and survival under biofloc system.

Numerical differences of WG, ADG, SGR, G/Week between the two treatments agree with the findings of **Esparza-Leal*et al*. (2017)**, who reported numerical differences between shrimp growth either in the light- exposed units or in darkness. **Baloi *et al*. (2013)** also reported that the Pacific white shrimp could be reared in complete darkness with good performance. Furthermore, others studied rearing *L. vannamei* at lower stocking densities reported that white leg shrimp can accept different light exposure levels, without any adverse effect on survival **(You *et al*., 2006; Neal *et al*., 2010)**.

The significantly higher final biomass of T2 (with light limitation) in the present study is compatible with **Khao *et al*. (2020)**, who recorded an increased shrimp weight after 90 days of culture in single- shaded units when compared to exposed units. The significantly higher biomass in T2 may be ascribed to the favorable differences in water quality over T1. In addition, the ability of shrimp to consume the increased abundance of

heterotrophic bacteria in T2 may explain the higher biomass. Reduced algae concentration in these units resulted in reduced variability of the measured water quality parameters compared to the more phytoplankton dependent T1.

Under conditions of high light intensity, much higher phytoplankton growth is achieved, resulting in light shading that causes phytoplankton to collapse or die. Under these conditions, the performance of biofloc systems is highly unsteady between daylight and dark conditions, negatively affecting growth performance and survival rates of shrimp.

The increased floc concentration in the T1 may explains their lower survival rate. **Avnimelech (2015)** reported that higher floc volume more than 15ml/L negatively affects shrimp health since the excessive solids contaminate shrimp gills and limit oxygen exchange. Moreover, higher proliferation of some species of bacteria in T2 can inhibit pathogenic bacteria. In this context, more studies should be done to assess the effect of microbial population on inhibiting the growth of pathogenic bacteria in biofloc systems, which may lead to the proliferation of beneficial strains of bacteria that can be produced commercially **(Khao, 2020)**.

Van Quach *et al*. (2017) and **Nguyen *et al*. (2019)** mentioned that climate change problems are rising and affect directly the cultured shrimp performance. Lower survival of shrimp, diseases, and higher costs of production as a result of the high-water temperature and unsteady climate conditions have been reported **(Mackay & Russell, 2011; Van Quach *et al*., 2017; Nguyen *et al*., 2019)**. To decrease the sensibility of the cultured shrimp to climate change effects, shrimp production in more controlled units may be applied. Limiting sunlight exposure and controlling water temperature result in better growth and survival rates of the reared shrimp **(Hai *et al*., 2016; Samocha *et al*., 2019)**.

3. Feed utilization

FCR, FE and PER were all significantly improved in group T2 (1.32± 0.007, 0.75± 0.003, and 1.98± 0.010) compared to(1.37± 0.008, 0.73± 0.005, and 1.92± 0.011, respectively) group T1 (Table 4).

Table 4. Feed utilization of whiteleg shrimp reared under biofloc system, with and without light limitation for 18 weeks

Parameter	Light limitation		*P*-value
	Without	With	
Feed intake	97.96 ± 0.40	98.03 ± 0.57	0.93
FCR	1.37 ±0.00	1.32 ± 0.00	0.01
FE	0.73 ± 0.00	0.75 ± 0.00	0.01
PER	1.92 ± 0.01	1.98 ± 0.01	0.01

Probability value (*P*) of less than 0.05 was used to indicate statistically significant differences.

Acceptable feed utilization and protein utilization efficiencies were noted in the two treatments which may result in the continual presence of biofloc particles as a natural food source **(Burford & Lorenzen, 2004; Ju *et al*., 2008; Hastuti & Subandiyono, 2014; Bakhshi *et al*., 2018)**, and in good water conditions **(Avnimelech, 2007; Emerenciano *et al*., 2011)**. Moreover, biofloc enhances the ingestion and digestion of the supplied feeds with the production of extracellular enzymes helping with food digestion **(Tacon *et al*., 2002)**.

Better feed utilization efficiency that was recorded in T2 group can be explained with improved and less variable water quality in the T2 group. Algal presence in bifloc system may cause unsteadiness of dissolved oxygen, pH, and alkalinity values, and as a result, affecting the growth and feed utilization efficiency of the reared animals **(Martins *et al*., 2003; Furtado *et al*.; 2011; Martins *et al*., 2017)**. Cultured units exposed to sunlight with abundant algae present many challenges including the proliferation of detrimental algal taxa **(Alonso-Rodriguez & Paez-Osuna, 2003; Hargreaves, 2006)**, unreliable nitrogen cycling, and shifts in algal composition related to sunlight availability **(Ray *et al*., 2009; Sookying *et al*., 2011)**. Generally, the exclusion of light and consequential elimination of algae may lead to greater environmental consistency.

Light restriction minimizes the risks of growth of harmful algal species that may rapidly grow in shrimp culture water **(Ray e*t al*., 2009)**. These detrimental algae exert countless bad effects since they form a bad base for the aquatic food chain in the biofloc system; and their blooms can result in dissolved oxygen drop as a result of the increased proliferation and the collapse of the cyanobacteria, where some species of cyanobacteria can exert toxins, which have bad impact on shrimp performance and FCR **(Ju *et al*., 2008a, Schrader *et al*., 2011)**.

4. Biofloc composition

The proximate analysis of composition of bioflocs is shown in Table (5). Significantly higher protein content (17.97± 0.40) was found in the un-limited light group compared to (15.62± 0.32) T2. Moreover, significantly higher fiber content was observed in the un-limited light units (15.86 ± 0.38) compared to (14.19 ± 0.26) the light- limited group. Additionally, there were no statistically significant differences in the lipid percentage between the two treatments. Similarly, numerical differences were observed in the contents of biofloc for ash and carbohydrates according to light limitation.

Avmnielech (2015) and **Samocha *et al*. (2019)** reported that the differences in biofloc proximate analysis depends on the ecosystem conditions, carbon sources added, light intensity, TSS concentration, stocking density, salinity, microalgae and bacteria species. Additionally, **Samocha *et al*. (2019)** concluded that, light intensity affects phytoplankton dominance in the units which most likely explain the difference in biofloc nutrient compositions.

Table 5. Proximate composition of bioflocs for whiteleg shrimp reared with and without light limitation for 18 weeks under biofloc system

	Light limitation		*P*-value
	Without	**With**	
Protein %	17.97 ± 0.40	15.62 ± 0.32	0.00
Lipids %	1.36 ± 0.03	1.39 ± 0.05	0.84
Ash %	15.34 ± 0.12	15.20 ± 0.18	0.12
Fiber %	15.86 ± 0.38	14.19 ± 0.26	0.04
Carbohydrate %	49.47 ± 0.53	53.59± 0.50	0.05

Probability value (*P*) of less than 0.05 was used to indicate statistically significant differences.

In the present study, with C:N ratio of 15:1, crude protein (15.62–17.97%) and crude lipid (1.36–1.39%) contents in bioflocs were slightly lower than those noticed by **Xu and Pan (2012)** and **Khao *et al*. (2020)**, and similar to those recorded by **Martins *et al*. (2017)**. The results of this experiment revealed significantly lower protein and fiber contents of bioflocs from T2 as compared to group T1, which may be explained with differences in light availability, biofloc volume, and composition of microorganisms community including zooplankton, phytoplankton, and /or bacteria.

The higher protein content of bioflocs obtained from the unlimited light group agrees with **Resis *et al*. (2019)** who recorded that, the proximate analysis of flocs revealed higher levels of proteins in the unlimited light group compared to the light-limited group ($P < 0.05$). Previous studies noted a desirable impact of microalgae on biofloc contents **(Browdy *et al*., 2006; Anand *et al*., 2014; Fleckenstein *et al*., 2019)**. BFT systems characterized by the presence of both bacteria and phytoplankton are more effective compared to the heterotrophic bacterial abundance for producing shrimp **(Xu *et al*., 2016)**.

5. Proximate shrimp analysis

The proximate analysis of shrimp body is shown in Table (6). Numerical differences between the two treatments were observed in protein, lipid, ash and fiber composition of shrimp body, while statistically significant higher carbohydrate content was noticed in the light- limited units.

Biofloc provides a supplemental natural food that improves the production of shrimp **(Wasielesky *et al*., 2006; Kuhn *et al*., 2008; Xu & Pan, 2012)**. Photoautotrophic organisms could contribute positively to shrimp quality **(de Carvalho & Caramujo, 2017; Wade *et al*., 2017; Beal *et al*., 2018; Camacho *et al*., 2019; Han *et al*., 2019)**. **Khao *et al*. (2020)** recorded that, low change in protein percentage of the diets in the biofloc systems did not influence the proximate composition of shrimp body. These findings support the non-significant differences in the shrimp body composition between limited and unlimited light treatments in the current study (Table 6).

Table 6. Proximate composition of whiteleg shrimp body reared under biofloc system, with and without light limitation for 18 weeks

	Light limitation		*P*-value
	Without	**With**	
Protein %	73.11 ± 0.23	72.83 ± 0.16	0.33
Lipids %	4.02 ± 0.05	4.1 ± 0.05	0.35
Ash %	15.83 ± 0.11	15.50 ± 0.12	0.05
Fiber %	5.76 ± 0.19	5.38 ± 0.07	0.08
Carbohydrate %	1.26 ± 0.34	2.18 ± 0.25	0.04

Probability value (*P*) of less than 0.05 was used to indicate statistically significant differences.

Zooplankton assessment

Zooplankton numbers and species in both study treatments are shown in Table (7). Non-limited light group T1 showed significantly higher total zooplankton counts (141560orgs/m^3 ± 2163.82) compared to the limited light group T2 (65350orgs/m^3 ± 240.90). We identified five groups of organisms in both treatments: Protozoa, Cladocera, Rotifera, Copepoda, and Meroplankton. Different genera under each zooplankton group are listed in Table (7).

Table 7. Zooplankton abundance and species from biofloc units cultured with and without light limitation for 18 weeks

Zooplankton group	Types of zooplankton	Count orgs/ m^3		*P*-value
		Without	With	
Protozoa	*Arcella* sp.	0	0	
	Leprotintinnus sp.	*23000	12000	0.0001
	Tintinnopsis sp.	*18000	10700	0.0001
	Globigerina sp	*1500	1200	0.001
Cladocera	*Bosmina* sp.	*3050	850	0.022
	Daphnia sp.	*3960	2550	0.0001
Rotifera	*Asplanchna* sp.	*25000	4000	0.0001
	Argonotholca sp	0	0	
	Brachionus sp.	6000	6100	0.073
	Filinia sp.	0	0	
	Keratella sp.	1500	*2000	0.012
Copepoda	*Clausocalanus* sp.	*1600	400	0.0001
	Oithona sp.	*900	700	0.0001
	Euterpina sp.	*1500	1400	0.014
	Microsetella sp.	*700	400	0.0001
	Copepodite stages	*50000	20000	0.0001
Meroplankton		*4850	3050	0.0001
Total zooplankton count		*141560	65350	0.0001

Probability value (*P*) of less than 0.05 was used to indicate statistically significant differences.

The zooplankton groups obviously decreased with light limitation under biofloc system. Zooplankton like Copepoda and Cladocera increased in abundance according to light, and it was reported that light may affect animal performance, and some zooplankton species depends on light **(Pagano *et al*., 1993; Atkinson *et al*., 1996)**. The zooplankton density increased in group T1 due to the proliferation of phytoplankton in the biofloc units. The current results coincide with those of **Thurman (1997)** who reported that, the primary and secondary sources of zooplankton variability are phytoplankton availability followed by light availability.

CONCLUSION

In this experiment, exploring the effects of light limitation on *L.vannamie* production in an intensive biofloc system, the following effects were observed: 1) Water quality was improved in the form of numerically higher dissolved oxygen and reduced ammonia and nitrite concentrations; 2) No statistically significant differences were found in the measured growth performance parameters due to treatment; 3) Survival rate, final group biomass, and feed utilization increased significantly due to light limitation; 4) Biofloc composition of protein and fiber contents were both significantly decreased, and 5) Total zooplankton count decreased significantly. In summary, we can support the hypothesis that shrimp production could be improved in biofloc systems through the practice of light limitation to the culture units. Further work should be performed to more clearly understand the optimal light levels for efficient shrimp production in biofloc systems.

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