Aquaculture Reports 16 (2020) 100257

Contents lists available at ScienceDirect

Aquaculture Reports

journal homepage: www.elsevier.com/locate/aqrep

Effect of supplementing heterotrophic and photoautotrophic biofloc, on the production response, physiological condition and post-harvest quality of the whiteleg shrimp, *Litopenaeus vannamei*

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ARTICLE INFO

keywords:
Heterotrophic biofloc
Autotrophic biofloc
Pacific white shrimp
BFT system
Postharvest quality
Shrimp physiology
Microbial-based systems

ABSTRACT

The effect of supplementing biofloc produced under heterotrophic and photoautotrophic conditions, on the production response, physiological condition and postharvest quality of juvenile *L. vannamei*, intensively farmed under greenhouse conditions was assessed. Heterotrophic bioflocs were produced under a restricted light condition, using an unspecific marine consortium as inoculum and maintaining the C:N ratio around 10-12. The photoautotrophic biofloc were produced under direct light exposition; using *Navicula incerta* as inoculum and maintaining the C:N ratio around 2-3. No significant differences on the water quality variables were observed among treatments except for TAN which was higher in the control. The production response was affected and a lower survival and higher FCR were recorded with heterotrophic bioflocs. The hemolymph parameters were similar in shrimp between groups, except for cholesterol which increased by more than 4-fold in the control. The postharvest quality of shrimp was qualified as good in general terms, without significant differences between groups, but the mean of the total qualifiers was slightly better in the treatment with photoautotrophic biofloc. The results of the study suggest that supplementation of both types of biofloc has not negative effect on the water quality, on the physiological condition of shrimp and on their postharvest quality.

1. Introduction

Diverse investigations on the benefits of biofloc in the culture of fish and shrimp have placed this technology as a promising strategy towards sustainability. The presence of these microbial communities do not only have a positive effect on the production response of shrimp, but an antagonist effect on potential pathogens, and also play a role as immunomodulatory agent for shrimp (Ekasari et al., 2014). Most of these works are related to the productive, zoo-technical and reproductive responses of the organisms farmed in many types of biofloc technology systems (BFT) (Martınez-Cordova et al., 2015). However, the effects of aquafeeds on the physiology and post-harvest quality of farmed organisms has not been addressed enough, being an important issue because their implications on the production and marketing (Rivas-Vega et al., 2001; Porchas-Cornejo et al., 2011). In this regard, whether the contribution of biofloc on shrimp nutrition has been investigated enough, their effect on the physiological and post-harvest quality of shrimp are still poorly addressed topics. There are reasons to support the hypothesis that the consumption of these kinds of alternative feeds may affect some biological conditions such as changes in the immune and antioxidant responses of shrimp after using microbial biomass as direct food source (bioflocs, biofilms, peryphyton) (Becerra-Dorame et al., 2014; Kim et al., 2014). The physiological condition is an important aspect to consider for the culture of any species because it may be ultimately associated to overall production and economic profitability (Cuzon et al., 2004); while the post-harvest quality of shrimp is associated to the protein denaturation (Rivas-Vega et al., 2001), and consequently, the storage shelf life, aspect, price, and consumer preference of the product. It has been addressed that the post-harvest quality of aquaculture products is significantly influenced by the consumption of natural feed such as insects and other (Martinez-Cordova et al., 2013).

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https://doi.org/10.1016/j.aqrep.2019.100257
Received 1 July 2019; Received in revised form 23 October 2019; Accepted 25 November 2019

In the conventional BFT systems, the microbial consortiums are produced into the same units growing the farmed organisms; however, it is possible to produce the bioflocs separately, and then incorporate to the culture. In this way, it is possible to avoid or diminish some problems such as the excess of suspended solids, eutrophication and hypernutrification, as reported in some investigations (Gaona et al., 2017).

The biological and biochemical composition of bioflocs which are mainly constituted by organic matter and aerobic microbes, may vary depending on diverse factors such as source of water, microbial inoculum (if so), carbon/nitrogen ratio, substrate, temperature, salinity, light intensity, DO concentration, turbulence of the water column among some other (Martınez-Cordova et al., 2015). Previous studies on our research group, has demonstrated the possibility of forming photoautotrophic, heterotrophic or mixotrophic bioflocs depending on the initial inoculum (Gomez-Ramirez et al., 2019). Biofloc and biofilm mass based on photoautotrophic microorganisms (also known as peryphyton when attached to submerged surfaces), have usually low protein contents but they have a high content of lipids and carbohydrates (Gangadhara and Keshavanath, 2008). Contrarily, high protein and low lipid concentrations are commonly constituting bioflocs or biofilms based on heterotrophic microorganisms (Emerenciano et al., 2014).

The aim of this study was to evaluate the effect of adding two types of bioflocs (photoautotrophic and heterotrophic) produced exogenously to the culture system, on the physiological condition (as indicated by some hemolymph parameters) and postharvest quality of the white leg shrimp, *Litopenaeus vannamei* intensively farmed under greenhouse conditions.

2. Materials and Methods

The study was conducted over 10 weeks in the facilities of DICTUS, the University of Sonora at Hermosillo, Sonora, Northwestern Mexico. A greenhouse (6 m x 3 m) was used to install nine experimental units consisting of plastic tanks with an operative volume of 50 L, as well as two bioreactors to produce the bioflocs.

The bioreactors consisted of plastic tanks (500 L of capacity and operated at 450 L). These were supplied with filtered marine water (35 PSU) and constant aeration provided through a porous tube to achieve dissolved oxygen (DO) levels over 4.5 mg/L, while maintaining the water column with an adequate turbulence to avoid the biofloc sinking. For the production of heterotrophic biofloc, molasses was supplied each week to reach a C:N ratio of 10-12. The tank was covered with black plastic to avoid light penetration. For the production of photoautotrophic bioflocs, the water was fertilized with Triple17^R (an agricultural fertilizer with 17 % N; 17 % P and 17 % K) to have a C:N ratio of 2-3. The tank was covered with transparent plastic allowing the penetration of light during the day. The bioreactor for producing the heterotrophic biofloc was inoculated with 5 mg/L of an unspecific bacterial marine consortium (lyophilized), whereas the bioreactor to produce photoautotrophic bioflocs was inoculated with 500 mL/m^3 of the benthic microalgae *Navicula incerta* at a concentration of 1×10^6 cel/ml. Both bioreactors were provided with 1.5 g/L amaranth seeds as a floating substrate in order to have a nuclei to accelerate the biofloc formation. After 15 days, the biofloc from each reactor was ready to be collected (with a plastic net mesh 300 μm in order to have a size capable to be efficiently captured by the shrimp), and used for juvenile shrimp culture. The remaining volume was maintained as inoculum to continue the production of bioflocs.

To evaluate the effect of biofloc types on the production response, the physiological condition, and the post-harvest quality of white shrimp, a single-factor completely randomized experimental design with three replicates per treatment was performed. The treatment evaluating the heterotrophic biofloc was named TH; the treatment evaluating the photoautotrophic biofloc was named TP, and the control without biofloc was identified as TC. Experimental units consisting of plastic containers (50 L) were provided with constant aeration and covered with plastic mesh to avoid shrimp escape; these were stocked with 12 (300 org/m^3) juvenile *L. vannamei* (1.5 g) obtained from a shrimp farm located near to our facilities. Shrimp were fed twice a day (0800 and 1400) with a formulated commercial feed (Ziegler, 35 % CP), adjusting the daily ration based on apparent consumption. Additionally to the formulated feed, every three days, the harvested bioflocs were added to the respective units at a rate of 3 % of the shrimp biomass (this percentage was based on previous trials experiences). Once a day the unconsumed feed, feces, and molts were removed by siphoning. Contrarily to the conventional BFT systems, every week 50 % of water was replaced and freshwater was used to replace loss by evaporation and maintain salinity levels.

The proximate composition of the commercial feed and both biofloc types was determined by following the AOAC (2019) methods. Biofloc samples were taken at the beginning, at the middle, and at the end of the trial.

Environmental variables such as temperature, salinity, dissolved oxygen, pH and ORP were monitored twice a day (0800 and 1400 h) by means a multi-parameter sonde YSI 660. The concentration of total ammonia (TAN) was determined each week, based on indophenol formation with sodium salicylate, using a Hanna programmable spectrophotometer.

Growth and survival of shrimp were monitored weekly, by weighing all the survivors in a digital balance. After 10 weeks, the organisms were harvested, counted and weighed to record the final survival, final biomass and calculate the feed conversion ratio (FCR).

At the end of the trial, a half (8–9) of the survivor shrimp from each unit were considered for analyzing hemolymph parameters and the other half for assessing the post-harvest quality.

Samples of shrimp hemolymph were obtained from the ventral sinus at the base of the first abdominal segment with a 3-mL insulin syringe previously rinsed with EDTA as an anticoagulant. Levels of glucose, lactate, proteins, triglycerides, and cholesterol were measured as indicators of shrimp physiological condition, using commercial kits by Randox (Randox Laboratories, Oceanside, CA, USA).

To assess the post-harvest quality of the shrimp, a sensory test was performed using head-off and peeled shrimp cooked at 100 °C for 10 min. Each one of the five panelists analyzed the shrimp for the different tests. Panelists were five experienced judges who received special training to evaluate sensory characteristics such as flesh odor, color, consistency, fracturability, juiciness, fibrousness, cohesiveness, and gumminess. The different descriptors were evaluated accordingly to the European Council Regulation (1996). Four rank categories were considered: 5 to 4.6 (excellent), 4.5 to 3.6 (good), 3.5 to 2.6 (fair), and 2.5 to 1.0 (rejectable).

Finally, after normality and homoscedasticity tests, a one-way analysis of variance was performed followed by a post-hoc Tukey test (NCSS). A confidence interval of 95 % was considered.

3. Results

Proximate composition of bioflocs revealed that the heterotrophic biofloc (TH) contained a higher proportion of protein (46.7 %) than the photoautotrophic (TP: 19.9 %) and the control diet (35.0 %), whereas lipids registered values of 7.8, 4.9 and 0.8 % for control, TH and TP, respectively. Carbohydrates registered 39.6, 38.6 and 17.6 %, respectively.

The water environmental variables ranged most of the time into the levels acceptable for shrimp culture, and no significant differences were observed among groups, except for TAN. Salinity ranged from 35 to 37‰, dissolved oxygen 5.9–6.3 mg/L, pH 7.7–8.2, ORP 167-199. Total ammonia nitrogen registered a mean value of 1.90 (± 0.4) mg/L in TP, followed by TH (2.80 ± 0.5 mg/L) and the control (3.40 ± 0.6 mg/L), respectively (Table 1).

Significant differences were observed among treatments regarding

Table 1
Environmental variables (± *SD*) during the trial in the treatments using biofloc and feed for shrimp (TH and TP) and the control, and production parameters of juvenile L. *vannamei* on the treatments and control.

	Temperature (°C)	Salinity (PSU)	Dissolved Oxygen (mg/L)	pH	ORP	TAN (mg/L)
Control	26.8 ± 2.1a	36.5 ± 2.7a	6.1 ± 0.2a	7.7-8.0	178 ± 11a	3.40 ± 0.6b
TH	26.4 ± 1.9a	36.6 ± 2.5a	6.1 ± 0.2a	7.8-8.1	181 ± 12a	2.80 ± 0.5ab
TP	26.6 ± 2.0a	36.5 ± 2.6a	6.2 ± 0.2a	7.9-8.2	188 ± 11a	1.90 ± 0.4a

Different letter in the same column means significant differences at P < 0.05.

Table 2
Production parameters of juvenile L. *vannamei* on the treatments and control.

	Initial Weight (g)	Weight gain (g)	Survival (%)	Biomass (g)	SGR %·day^{-1}	FCR
Control	1.5 ± 0.2a	5.5 ± 0.5a	86 ± 2b	72 ± 6b	21.6 ± 1.2a	2.2 ± 0.2a
TH	1.4 ± 0.1a	5.0 ± 0.3a	78 ± 2a	61 ± 4a	21.0 ± 0.9a	2.1 ± 0.3a
TP	1.4 ± 0.2a	5.2 ± 0.4a	86 ± 2b	70 ± 5ab	21.5 ± 1.1a	1.7 ± 0.2b

Different letters in the same column means significant differences at P < 0.05.

some parameters of shrimp productive response (Table 2). The weight gain registered no-significant differences between shrimp groups (a mean of 0.5 g/week), but mean survival varied from 77 to 86 %, with the lowest record in TH compared to TP and the control, respectively. The FCR was significantly lower in TP (1.7) compared to TH and the control (≥2.1).

Regarding the hemolymph parameters, the levels of glucose, lactate, protein, and acylglycerides were similar among treatments, and only cholesterol resulted to be significantly higher in the control, while the lowest levels were found in TP (Table 3).

With respect to the post-harvest quality, no significant differences were observed among treatments and control for any of the descriptors considered (Table 4). Most of these recorded values ranging into the category “good”, and only a few were qualified as “fair”. Any of the descriptors recorded values into the category “rejectable”.

Table 4
Post-harvest variables of cooked shrimp from the treatments and the control done by expert panelists.

Descriptors	Treatments		
	Control	TH	TP
Odor	3.8 ± 0.4a	3.7 ± 1.1a	3.6 ± 0.5a
Color	3.6 ± 0.8a	3.0 ± 1.1a	3.4 ± 0.5a
Consistency	4.2 ± 0.4a	3.6 ± 0.8a	4.0 ± 0.7a
Fracturability	3.8 ± 1.1a	3.0 ± 0.8a	3.8 ± 0.8a
Juicines	4.0 ± 1.0a	3.6 ± 0.8a	3.8 ± 0.4a
Fibrousness	3.4 ± 1.3a	3.4 ± 1.4a	4.0 ± 0.7a
Cohesiveness	3.4 ± 0.8a	3.5 ± 0.5a	3.6 ± 0.5a
Firmness	4.2 ± 0.8a	3.6 ± 0.5a	4.4 ± 0.5a
Gumminess	3.4 ± 1.3a	3.0 ± 0.5a	3.6 ± 0.7a
MEAN OF DESCRIPTORS	3.77 ± 0.7	3.37 ± 0.6	3.80 ± 0.5

Categories: 5-4.6 (excellent), 4.5-3.6 (good), 3.5-2.6 (fair) and 2.5-1.0 (rejectable).
Different letter in the same row means significant differences at P < 0.05.

4. Discussion

With the exception of TAN, all the water quality parameters ranged into the values considered as suitable for shrimp culture (Boyd and Tucker, 2012). Total ammonia nitrogen recorded in some punctual measurements (particularly in the control units), concentrations considered dangerous for the development and survival of shrimp (Schuler et al., 2010). However, no massive mortalities were recorded for any of the treatments. The lower values of TAN recorded in TP, when compared to the control, could be attributed to the effect of the photo-autotrophic microorganisms associated to the bioflocs, which can remove diverse species of nitrogen, but mostly ammonia from the water column to use it as a nutrient in their metabolism. Also in TH, the TAN concentration were lower than in the control, although without significant differences; in that case, the decrease was probably due to the nitrifying microorganisms originally present in the inoculum or associated to the bioflocs during the trial, which transform ammonium nitrogen into nitrites and nitrates; but also to the direct degradation of organic matter by the heterotrophic bacteria (Lønborg et al., 2009). Furthermore, after a using a targeted metagenomic approach, Vargas-Albores et al. (2019) reported the occurrence of nitrogen metabolism (including denitrification) in heterotrophic bioflocs.

The productive parameters in the treatments and control ranged into the values considered suitable for intensive shrimp culture. The survival of 86.1 % recorded in TP and the control is inclusively higher than most of the reported for intensive farming of white shrimp, while the growth rate of around 0.5 g/week, is on the average range for this type of culture (Schveitzer et al., 2013). The best response of TP, particularly the low FCR is attributed to the nutritional contribution of microalgae and other microorganisms associated to photoautotrophic bioflocs, which complemented the formulated feed supplied, as previously reported by Marinho et al. (2017). Contrarily, despite the heterotrophic biofloc registered a protein content exceeding by more than 2-fold times the photoautotrophic biofloc, the production response was lower in TH, probably because of the quality of protein, but also due to

Table 3
Haematic parameters of L. *vannamei* in the treatments and the control.

	Glucose (mg dL^{-1})	Lactate(mg dL^{-1})	Acylglycerides(mg dL^{-1})	Cholesterol(mg dL^{-1})	Protein(mg mL^{-1})
Control	18.0 ± 5.1a	20.2 ± 3.9a	115.4 ± 16.0a	119.7 ± 37.2b	130.2 ± 14.1a
TH	12.1 ± 2.2a	15.3 ± 4.1a	106.8 ± 20.7a	48.6 ± 12.4a	130.6 ± 25.7a
TP	16.0 ± 5.0a	14.4 ± 3.9a	108.1 ± 25.5a	29.3 ± 6.6a	132.7 ± 26.4a

Different letter in the same column means significant differences at P < 0.05.

the heterotrophic biofloc resulted to be deficient in lipids, compared to the photoautotrophic. Surely in the control and the two treatments, bioflocs were also produced in situ, incentivized by the unconsumed feed, feces, nitrogenous compounds excreted by shrimp, etc.

The physiological condition of shrimp after the trial (as indicated by the hemolymph parameters), showed no differences between experimental groups (except for cholesterol), suggesting that the use of biofloc as complementary food may not alter the physiological status of shrimp, at least judging by these parameters. Regarding cholesterol, the concentration observed in the control was significantly greater than the recorded in TP (almost the quadruple). This is a remarkable finding that needs to be further investigated and confirm if this decrease also reflected in muscle, because of the possibility of farming shrimp with low cholesterol levels considering that several people do not consume shrimp due to its high cholesterol content (Venugopal and Gopakumar, 2017). However, it should be considered that neither shrimp nor bacteria can synthesize cholesterol *de novo*, while some algae can produce some sterols (demosterol), while bacteria synthetize hopanoids as functional analogues of cholesterol (Kannenberg and Poralla, 1999); thus it is possible that the cholesterol contained in the formulated feed would be sufficient for shrimp.

Regarding post-harvest quality (as indicated by the sensory analysis), no significant differences were found among the treatments and the control. Most of the descriptors were qualified into the range considered “good” and only a few of them into the range considered “fair”, while none of them in the range “rejectable”. These results could be positive for the cause of BFT, since the type of food consumed by shrimp, particularly bacteria and algae, can negatively influence its post-harvest quality and organoleptic characteristics. For example, some decades ago, pond-cultured penaeid shrimp imported into the United States from Ecuador were reported to have undesirable organoleptic characteristics including an intense earthy-musty flavor which made them unmarketable. Later Lovell and Broce (1985) concluded that the consumption of geosmin-producing blue-green algae was responsible of this unmarketable characteristics.

Finally, these results demonstrate that neither consuming photoautotrophic nor heterotrophic bioflocs affect the physiological performance and post-harvest quality of shrimp. As expected, the biofloc presence favored the water quality, and promoted a better production response exclusively observed in TP which had a more balanced proximate composition. However, additional experiments considering protein and lipid quality of bioflocs, as well as nutrient utilization and shelf life of shrimp could provide additional information to these findings.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

This article does not contain any studies with animals performed by any of the authors

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Acknowledgements

We want to thank CONACYTfor the financial support to the project 222722, from which the results of this paper were obtained.

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