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Growth, antioxidant system, and immunological status of shrimp in bioflocs and clear water culture systems

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Abstract – The objective of this work was to evaluate the effect of the traditional culture system in clear water and of the one in microbial flocs on the antioxidant and immunological status of *Litopenaeus vannamei* shrimp. Samples (gill, hemolymph, and hepatopancreas) were collected on days 15, 30, and 60 of the experimental period. The following immunological parameters were analyzed at each evaluation time: granular and hyaline hemocytes, total protein, and apoptosis. Assays on glutamate-cysteine ligase (GCL) activity and on the concentrations of reduced glutathione (GSH) and thiobarbituric acid reactive substances (TBARS) were also performed. The evaluated immunological parameters did not differ significantly between treatments. Shrimp reared in clear water showed higher levels of lipid peroxidation in the gills and of GCL activity in the hemolymph on days 15 and 30. Shrimp in microbial floc had a higher survival rate, and the water did not have to be renewed during the experimental period. The microbial floc system improves production levels and provides a healthier culture environment.

Index terms: *Litopenaeus vannamei*, BFT, oxidative stress, TBARS.

Crescimento, sistema antioxidante e estado imunológico do camarão nos sistemas de cultivo em bioflocos e água clara

Resumo – O objetivo deste trabalho foi avaliar o efeito do sistema de cultivo tradicional em água clara e do em bioflocos sobre os estados antioxidante e imunológico do camarão *Litopenaeus vannamei*. Amostras (brânquia, hemolinfa e hepatopâncreas) foram coletadas nos dias 15, 30 e 60 do período experimental. Os seguintes parâmetros imunológicos foram analisados a cada tempo amostral: hemócitos granulares e hialinos, proteína total e apoptose. Também foram realizados ensaios da atividade da glutamato-cisteína ligase (GCL) e das concentrações de glutatona reduzida (GSH) e de substâncias reativas ao ácido tiobarbitúrico (TBARS). Os parâmetros imunológicos avaliados não diferiram significativamente entre os tratamentos. Os camarões criados em água clara apresentaram maiores níveis de peroxidação lipídica nas brânquias e de GCL na hemolinfa nos dias 15 e 30. Camarões em bioflocos apresentaram maior taxa de sobrevivência, e a água não precisou ser renovada durante o período experimental. O sistema de bioflocos melhora os níveis de produtividade e promove um ambiente de cultivo mais saudável.

Termos para indexação: *Litopenaeus vannamei*, BFT, estresse oxidativo, TBARS.



Introduction

Aquaculture has increased substantially over the years. Shrimp now ranks second in terms of value, being responsible for 15% of the total value of internationally traded fishery products in 2012 (FAO, 2016). *Litopenaeus vannamei* is the penaeid shrimp species most cultured worldwide. In the 1980s, shrimp production, in the traditional system in clear water, was performed with low stocking density in ponds with a high surface area and a great amount of water use and discharge, which not only caused negative environmental impacts, but also economic losses. Subsequently, alternative strategies were developed to increase shrimp production and turn shrimp culture into an environmentally friendly activity. These strategies include: fertilization, use of feeding trays, increased stocking densities, and the use of the biofloc technology (BFT) system (Wasielesky Jr. et al., 2006).

BFT is a shrimp production system based on the manipulation of a microbial community through the addition of a carbon source that promotes the development of heterotrophic bacteria (Souza et al., 2014). One of the benefits of this system is the bacterial uptake of nitrogen, including ammonia, and its ulterior conversion into cellular protein, providing a supplemental source of nutrition (Burford et al., 2004; Wasielesky Jr. et al., 2006) and possibly reducing the demand for protein in feed. Another important advantage is there is no water exchange, which drastically reduces water use and also avoids the damage caused by the release of effluents to the environment (Souza et al., 2014). The assimilation of nitrogen compounds by heterotrophic bacteria in BFT allows the same water to be used for several production cycles with no negative environmental impact (Krummenauer, 2014). In addition, the BFT system can promote an enhancement in the immune cellular response and antioxidant status of shrimp (Xu & Pan, 2013; Martins et al., 2015). The immune and antioxidant systems play an important role in animal physiology and are crucial to control their health and growth performance.

The antioxidant system is also fundamental in the protection against damage due to reactive oxygen species, which includes lipid peroxidation (LPO), a form of oxidative damage usually quantified by determining the content of thiobarbituric acid reactive substances (TBARS) in tissues (Oakes & Van der Kraak, 2003).

Because glutamate-cysteine ligase (GCL) is a major determinant of the levels of the cellular antioxidant reduced glutathione (GSH), studies have investigated its activity in several aquatic organisms (Longaray-Garcia et al., 2013; Souza et al., 2016).

The objective of this work was to evaluate the effect of the traditional culture system in clear water and of the one in microbial flocs on the antioxidant and immunological status of *Litopenaeus vannamei* shrimp.

Materials and Methods

A 60-day trial was conducted at the marine aquaculture station at Universidade Federal do Rio Grande, located in the state of Rio Grande do Sul, in Southern Brazil (32°12'13.5"S, 52°10'40.3"W). The experimental system consisted of six 200-L tanks assigned to the following treatments: BFT and clear water (CW) at 27°C and salinity of 30 g L⁻¹. *Litopenaeus vannamei*, weighing 1.91±0.22 and 2.26±0.24 g for BFT and CW, respectively, were acclimatized for ten days and stocked at a density of 111 shrimp per square meter. To promote the development of the microbial flocs, BFT tanks received an inoculum of 75 L from a heterotrophic shrimp culture, as adapted from Souza et al. (2012). The animals were fed twice daily, via a feeding tray, with feed containing 38% crude protein (Wasielesky Jr. et al., 2006).

The water from the CW treatment was renewed approximately 70% every three days to avoid the increase of ammonia. When ammonia reached 1 mg L⁻¹ in the BFT tanks, a molasses dose calculated according to Ebeling et al. (2006) and Avnimelech (1999) was applied. At the end of the experimental period, the following were evaluated: survival, as the final number of shrimp/initial number of shrimp x 100; final weight; specific growth rate, as $(\ln(\text{final weight}) - \ln(\text{initial weight}))/\text{trial duration} \times 100$; and productivity, as total biomass/water volume.

Throughout the experimental period, the following water quality parameters were recorded daily: water temperature, salinity, pH, and dissolved oxygen. Water samples were collected three times a week to determine the concentrations of total ammonia (Chemical methods..., 1983) and nitrite (Bendschneider & Robinson, 1952). Alkalinity and nitrate were monitored once a week as proposed by Chemical methods... (1983) and Baumgarten et al. (1996), respectively. Total

suspended solids (TSS) were evaluated following the methodology adapted from Strickland & Parsons (1972).

For the immunological analysis, hemolymph was collected at 30 and 60 days directly from the heart of six shrimp per treatment using sterile syringes (Söderhäll & Smith, 1983). Granular and hyaline hemocytes were counted with a Neubauer chamber (Maggioni et al., 2004). Total protein concentration in the serum of six shrimp per treatment was determined according to the method of Bradford (1976), using bovine serum albumin as a standard (Maggioni et al., 2004). For the detection of apoptosis, 5 μ L shrimp hemolymph were smeared onto a clean glass slide and air-dried; five clean slides were used per treatment. Apoptotic hemocytes were counted by Tunel staining with the ApopTag® Plus Peroxidase In Situ Apoptosis Detection Kit (EMD Millipore Corporation, Temecula, CA, USA) according to Charriaut-Marlangue & Ben-Ari (1995) and Wang & Zhang (2008).

For the antioxidant system analysis, the hemolymph, gills, and hepatopancreas of nine shrimp per treatment were sampled randomly on days 15, 30, and 60. For protein quantification and the antioxidant enzyme analysis, hemolymph was centrifuged twice to obtain a pellet or cell lysate. After centrifugation, the cell lysate was resuspended in a buffer solution and stored in an ultra-low freezer (Souza et al., 2016). The samples from the gills and hepatopancreas were weighed and then added at a ratio of 1:5 to a buffer solution. Tissue samples were then centrifuged, and the supernatant was stored in an ultra-low freezer at -80°C . Total protein content was determined by the Biuret method using the “Proteínas Totais” commercial kit (Dolles Reagentes e Equipamentos para Laboratórios Ltda., Goiânia, GO, Brazil) and read at 550 nm with the Victor2 microplate reader (PerkinElmer do Brasil Ltda., São Paulo, SP, Brazil).

GCL activity and GSH synthesis were determined according to White et al. (2003). The adopted method makes use of the reaction of naphthalene dicarboxaldehyde with GSH or γ -glutamylcysteine, forming fluorescent cyclic products that can be detected on the Victor2 fluorescence microplate reader (PerkinElmer do Brasil Ltda., São Paulo, SP, Brazil) at the wavelengths of 485 and 530 nm for excitation and emission, respectively. LPO was measured by determining TBARS, according to the methodology of Oakes & Van Der Kraak (2003), measured by

fluorescence at the wavelengths of 520 and 580 nm for excitation and emission, respectively. The concentration of lipid peroxides was expressed as nmol TBARS per milligram of protein, and tetramethoxypropane (Thermo Fisher Scientific, Acros Organics, Geel, Belgium) was used as a standard.

Data are given as the mean \pm standard error of the mean and were analyzed by the one-way analysis of variance, followed by the Newman-Keuls post-hoc mean comparison. Assumptions of normality and of homogeneity of variances were previously checked by the Kolmogorov-Smirnov and Levene tests, respectively. In all cases, results were considered to be significant at 5% probability.

Results and Discussion

Although significant differences were observed in pH, salinity, alkalinity, total ammonia, nitrite, and nitrate between treatments, water quality parameters remained at concentrations suitable for shrimp culture in both studied systems (Table 1).

The values obtained for pH and alkalinity were lower in the BFT system than in the CW one. This was probably due to the respiration of heterotrophic organisms, which increased the carbon dioxide concentration in the water of the biofloc treatment, and to nitrification (Wasielesky Jr. et al., 2006; Furtado et al., 2011), as shown by the

Table 1. Water physicochemical parameters in the experimental tanks⁽¹⁾.

Parameter	Treatment	
	BFT at 27°C	CW at 27°C
Temperature ($^{\circ}\text{C}$)	26.89 \pm 0.15a	26.73 \pm 0.09a
pH	7.68 \pm 0.02a	8.23 \pm 0.01b
Salinity (g L^{-1})	31.88 \pm 0.24a	29.28 \pm 0.18b
DO (mg L^{-1})	5.91 \pm 0.03a	6.13 \pm 0.02a
Alkalinity (mg L^{-1})	93.14 \pm 5.91a	148.70 \pm 1.15b
TAN (mg L^{-1})	0.15 \pm 0.01a	0.33 \pm 0.03b
Nitrite (mg L^{-1})	0.07 \pm 0.006a	1.40 \pm 0.23b
Nitrate (mg L^{-1})	42.02 \pm 4.77a	7.77 \pm 1.78b
TSS (mg L^{-1})	30 \pm 15.23a	450 \pm 40.56b

⁽¹⁾Means followed by equal letters in the rows do not differ among treatments by the one-way analysis of variance, at 5% probability. DO, dissolved oxygen; TAN; total ammonia nitrogen; TSS, total suspended solids; BFT, biofloc technology; and CW, clear water. Data are mean \pm standard error of the mean.

higher level of nitrate in biofloc tanks. Total ammonia nitrogen (TAN) was also lower in BFT tanks due to the nitrification process performed by the microorganisms present in this system. This result is in agreement with those found by Ray et al. (2017), who tested shrimp culture in CW and BFT in a recirculating aquaculture system. The authors also reported higher levels of TAN in the CW treatment, showing that the microorganisms present in the BFT play an important role in controlling these levels.

Shrimp reared in the BFT system presented a survival rate of $73.33 \pm 1.4\%$, higher than that of $59.16 \pm 3.0\%$ of those reared in CW (Table 2). However, shrimp in CW showed a greater final weight, although specific growth rate did not differ between treatments. The improved performance (survival and productivity) of *L. vannamei* reared in BFT systems can be related to the consumption of bioflocs by the shrimp. According to Burford et al. (2004), up to 29% of the daily feed intake of this species can come from particles in heterotrophic culture systems. Therefore,

the consumption of natural products and of other constituents of bioflocs by shrimp can increase the efficiency of feed use (Wasielesky Jr. et al., 2006; Emerenciano et al., 2012). Viau et al. (2013) confirmed that the consumption of microorganisms by shrimp represents a complementary food source.

No statistical differences were detected between treatments regarding immunological parameters (Table 2). Souza et al. (2014) also found no differences between treatments when assessing the immunological parameters hyaline and granular hemocyte count and total protein. It is assumed that the large concentration of bacteria associated with bioflocs may contribute to enhance the immunity and growth performance of shrimp when bioflocs are consumed (Kim et al., 2014); however, this was not observed in the present study.

The antioxidant system and TBARS levels differed significantly. Shrimp reared in the CW system on days 15 and 60 exhibited an increase of 431.04 and 4,193.80%, respectively, in GCL activity in hemolymph, in comparison with those reared in BFT (Figure 1). On day 15, shrimp reared in the BFT system showed a higher GSH concentration of 252.70% than those reared in CW. A higher GCL activity of 364.15 and 234.60%, respectively, was observed in the hepatopancreas of shrimp reared in the BFT system on days 15 and 30 (Figure 2). GSH synthesis is GCL-activity dependent and plays an important role against oxidative damages (Irvine, 1996). Zhang et al. (2007) reported that the addition of a suitable dose of GSH to the diet of tilapia improves the antioxidant capacity of muscle tissues and promotes their growth.

On day 30, shrimp reared in the CW system presented a higher level of 351.51% of TBARS in hemolymph. In gills, shrimp reared in the CW system showed a higher lipid peroxidation of 656.25% on day 15. It should be noted that LPO is one of the major outcomes associated with the failure of the antioxidant system (Castex et al., 2010). In the present work, the higher LPO in gills of shrimp reared in CW indicated a failure of the antioxidant system to counteract free radicals (Figure 3).

Based on its composition characteristics, bioflocs should present an antioxidant activity (Ju et al., 2008; Martins et al., 2015) and also stimulate digestive enzyme activities, improving feed utilization (Xu et al., 2013) and increasing dietary antioxidant assimilation.

Table 2. Immunological and zootechnical parameters of *Litopenaeus vannamei* shrimp reared during a 60-day experimental period (at 30 and 60 days) in biofloc technology (BFT) and clear water (CW) systems at 27°C⁽¹⁾.

Parameter	Period (days)	Treatment	
		BFT at 27°C	CW at 27°C
GH, granular hemocytes (%)	30	66.2±2.2	64.8±1.6
	60	67.8±1.9	69.2±2.1
HH, hyaline hemocytes (%)	30	33.8±2.2	35.2±1.5
	60	32.2±1.9	30.8±2.1
TP (mg mL ⁻¹)	30	119.6±2.1	119.2±1.2
	60	119.4±1.0	121.0±1.7
Apoptosis (%)	30	2.0±0.3	2.4±0.05
	60	3.2±0.5	2.6±0.6
SV (%)		73.33±1.4a	59.16±3.0b
FW (g)		7.03±0.3a	9.84±0.6b
SGR (%)		2.30±0.07a	2.46±0.08a
Productivity (kg m ⁻³)		0.95±0.2a	0.19±0.3b

⁽¹⁾Means followed by equal letters in the row do not differ among treatments by the one-way analysis of variance, at 5% probability. GH, granular hemocytes; HH, hyaline hemocytes; TP, total protein; SV survival; FW, final weight; and SGR, specific growth rate. Data are mean±standard error of the mean.

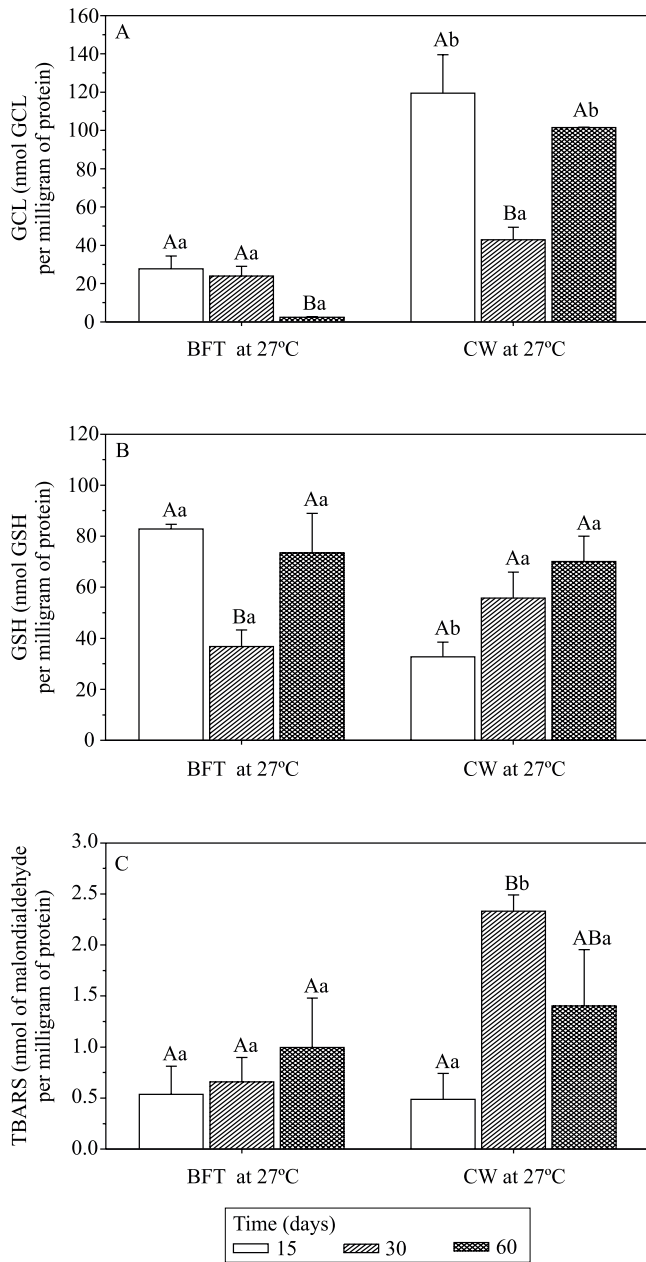


Figure 1. Glutamate-cysteine ligase (GCL) activity (A), reduced glutathione (GSH) concentration (B), and thiobarbituric acid reactive substances (TBARS) (C) in the hemolymph of *Litopenaeus vannamei* shrimp reared in biofloc technology (BFT) and clear water (CW) systems. Data are expressed as means±standard error of the mean. Different lowercase letters indicate significant differences between treatments over the same time period by the analysis of variance, followed by Newman-Keuls post-hoc test, at 5% probability. Different uppercase letters indicate significant differences of each treatment over the experimental period.

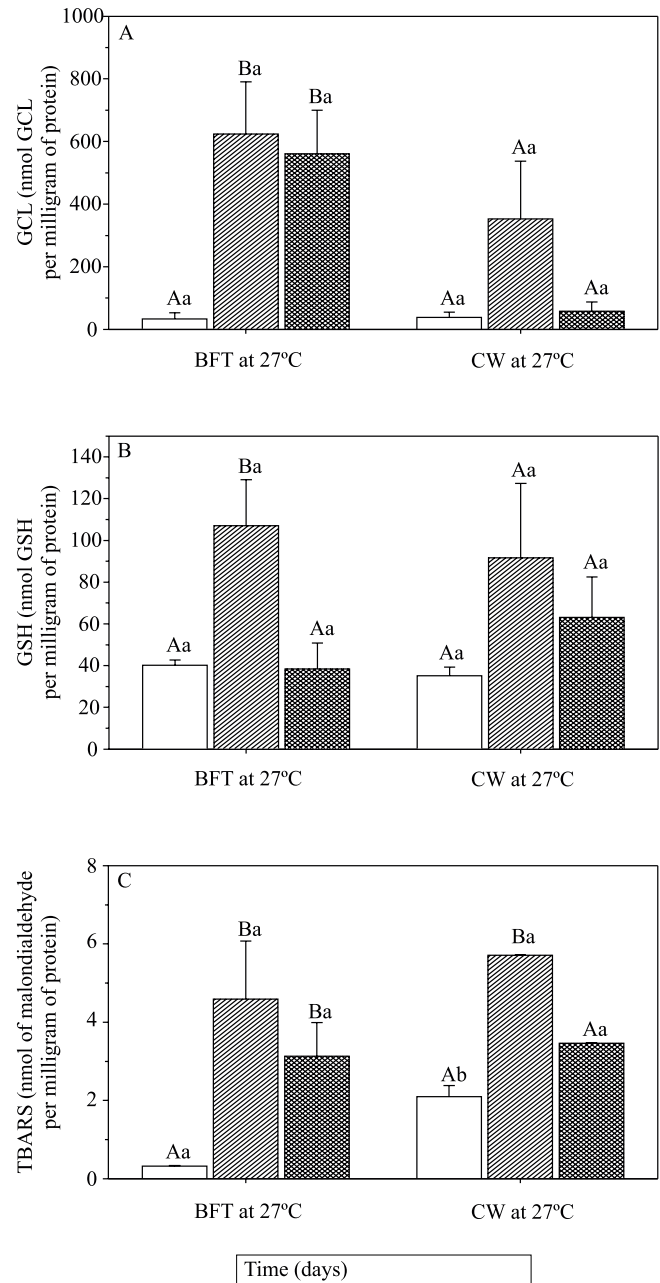


Figure 2. Glutamate-cysteine ligase (GCL) activity (A), reduced glutathione (GSH) concentration (B), and thiobarbituric acid reactive substances (TBARS) (C) in the hepatopancreas of *Litopenaeus vannamei* shrimp reared in biofloc technology (BFT) and clear water (CW) systems. Data are expressed as means±standard error of the mean. Different lowercase letters indicate significant differences between treatments over the same time period by the analysis of variance, followed by Newman-Keuls post-hoc test, at 5% probability. Different uppercase letters indicate significant differences between each treatment over the experimental period.

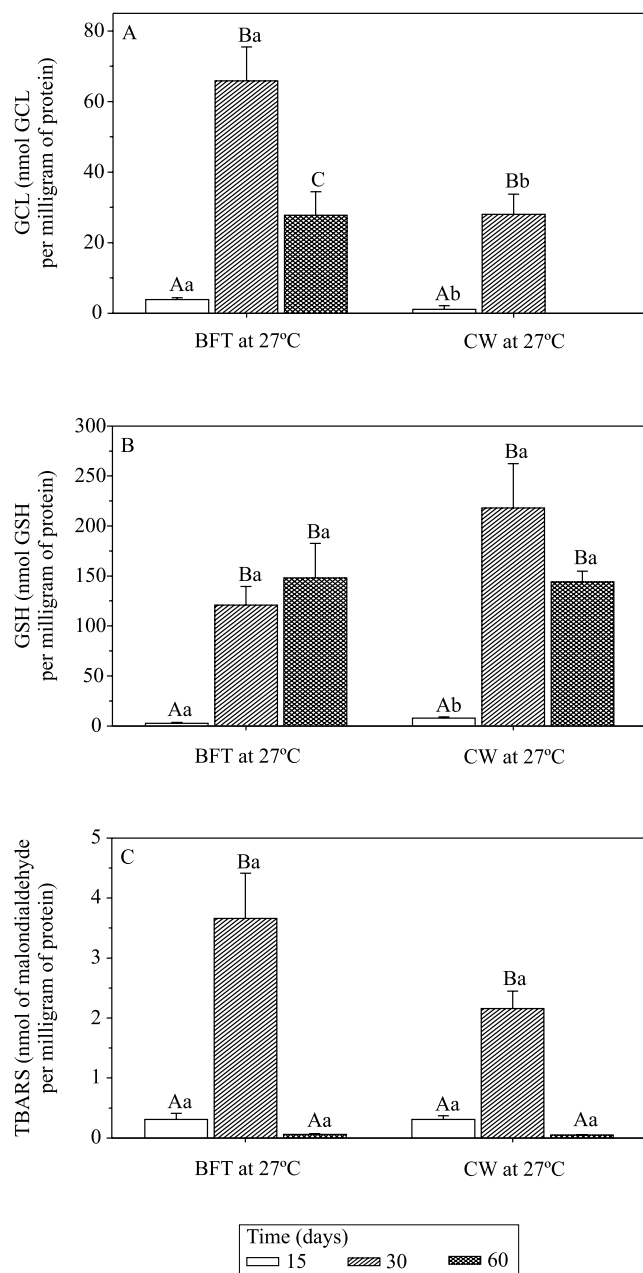


Figure 3. Glutamate-cysteine ligase (GCL) activity (A), reduced glutathione (GSH) concentration (B), and thiobarbituric acid reactive substances (TBARS) (C) in the gills of *Litopenaeus vannamei* shrimp reared in biofloc technology (BFT) and clear water (CW) systems. Data are expressed as means±standard error of the mean. Different lowercase letters indicate significant differences between treatments over the same time period by the analysis of variance, followed by Newman-Keuls post-hoc test, at 5% probability. Different uppercase letters indicate significant differences between each treatment over the experimental period.

The BFT system presents many advantages that are applicable to shrimp farming, including zero water exchange. Currently, the concern worldwide is developing strategies to increase biosecurity and more environmentally friendly handling to reduce the negative impacts of aquaculture practices.

Conclusions

1. *Litopenaeus vannamei* shrimp reared in the biofloc technology (BFT) system present higher productivity and a higher survival rate than those reared in clear water.

2. Shrimp reared in clear water show oxidative damage, with reduced survival.

3. The immunological system of shrimp does not differ between rearing in clear water and in BFT.

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