Received: 27 January 2021 | Revised: 11 July 2021 | Accepted: 13 July 2021

DOI: 10.1111/are.15493

Aquaculture Research WILEY

ORIGINAL ARTICLE

Growth, body composition and antioxidant status of *Litopenaeus vannamei* juveniles reared at different stocking densities in the biofloc system using inland saline groundwater

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Abstract

Growth, body composition and antioxidant status of *Litopenaeus vannamei* under zero water exchange biofloc-based culture system using inland saline groundwater (ISGW) were evaluated during the 60-day culture period in four varying stocking densities of 60 (T1), 100 (T2), 140 (T3) and 180 (T4) juvenile shrimps m^{-3}. The carbon : nitrogen ratio was maintained at 15:1 using sugarcane molasses. Shrimp in the BFT treatments performed significantly better ($P < 0.05$) than control in terms of survival (98%-100%), weight gain (10.83 to 7.68g), feed conversion ratio (1.19 to 1.30) and feed efficiency ratio (0.88–0.84) at the end of the experimental period. Higher level of crude protein (29.1% to 33.0%), lipid (5.0% to 5.6%) and ash (1.4% to 1.6%) content was observed in the tissues of the shrimps from the BFT treatments when compared to control. Antioxidant enzyme activities such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in gills, hepatopancreas and muscle were higher in the BFT treatments than in the control. These results indicate that microbial protein from recycled waste functions as nutrient, growth promoter and antioxidant capacity stimulator in shrimp reared in BFT using ISGW.

KEYWORDS
antioxidant enzymes, biofloc system, body composition, inland saline ground water, *Litopenaeus vannamei*

1 | INTRODUCTION

Inland salinization has become a major threat to environmental resources, affecting an area of almost one billion hectares worldwide or about 7% of the earth's continental extent (Shrivastava & Kumar, 2015). Two decades ago, around 20% of the global cultivated area and 33% of irrigated agricultural lands got seriously affected by secondary salinization (Pitman and Lauchli, 2002). The trend of salinization in inland regions is increasing at a rate of 10%, and by around 2050, more than 50% of the arable land would be affected (Jamil et al., 2011). In India itself, around 30.8 million hectares' area are salt-affected, out of which 6.88 million hectares are cultivated land (Bhumbla, 1978). These regions are in the arid tracts and semi-arid tracts of various states of India including Punjab and Haryana (Patel & Dave, 2011; Shrivastava & Kumar, 2015).

Possible utilization of saline resources for agricultural purposes has been experimented in multiple ways, from the flushing of the affected region with fresh water to engineering environments, animals and plants to increase their tolerance (Vijayvargiya & Kumar, 2011). However, effective means to reclaim it through agriculture cropping have been rare because most of the terrestrial crops are dependent on freshwater or at least require large quantities of freshwater as a conjunctive effort. Culturing of halotolerant and hardy fish species using inland saline groundwater (ISGW) has been

recommended as having one of the greatest potentials to reclaim/revitalize the saline-affected resources. For the culture of shrimp, fortification of essential nutrients such as magnesium and potassium ions using commercially available muriate of potash (MOP) or potassium chloride (KCl) is required as these minerals are critical for growth and survival (Antony et al., 2015). Inland saline aquaculture (ISA) needs to ensure that saline water used for farming is not posing threats to the neighbouring areas. Low or zero water exchange systems using *in situ* water management technologies such as biofloc technology (BFT) would be a remunerative and sustainable means to reclaim these salt-affected resources for food production.

In BFT, the waste feed and faeces are recycled and converted into microbial protein within the system (De Schryver et al., 2008). The microbial biomasses in the system keep the water clean by reducing obnoxious nitrogenous compounds (Avnimelech, 1999; De Schryver et al., 2008). As it is zero water exchange system, the target culture species selected should tolerate water quality with high turbidity, suspended solids and intermediate dissolved oxygen (Burford et al., 2004; McIntosh, 2000; Tacon et al., 2002). The white leg shrimp, *L. vannamei*, owing to its conformity to the above criteria and due to its high commercial value has emerged as the most popular candidate species cultured in the BFT system (Emerenciano et al., 2012). This shrimp is also able to graze the microbial flocs and convert them into useful animal protein, which is demonstrated by increased feed utilization and growth performance, and enhanced digestive enzyme activities and immune system (Xu & Pan, 2012). However, stocking density may also affect shrimp growth performance and antioxidant status due to various factors such as competition for food, space, territory and stress owing to crowding as demonstrated by other authors (Appelbaum et al., 2002; Araneda et al., 2008; Babu et al., 2014; Samocha et al., 2004; Williams et al., 1996).

Crab (2010) reported that bioflocs contain adequate protein, lipid, carbohydrate and ash required for an aquafeed. Previous findings suggested that bioflocs are rich in various amino acids, fatty acids and vitamins (Ekasari et al., 2014; Emerenciano et al., 2013; Kuhn et al., 2010; Logan & A., 2010). The consumption of biofloc by shrimp or fish has demonstrated numerous benefits such as supplying of various nutrients by the microbial community, increasing feed utilization and growth performance, stimulating digestive enzyme activities, lowering FCR and enhancing immune system (Burford et al., 2004; Ballester et al., 2010; Emerenciano et al., 2012; Moss et al., 2006; Wasielesky et al., 2006; Xu & Pan, 2012). Aquatic animals are peculiarly susceptible to oxidative stress as a result of pathogen pressure and environmental perturbations (Castex et al., 2010; Liu & Chen, 2004). To protect against toxicity and eliminate reactive oxygen species (ROS) such as superoxide anion (O_2), hydrogen peroxide (H_2O_2) and hydroxyl radical, organisms have evolved protective enzymatic systems such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). The present study is designed to evaluate the growth, body composition and antioxidant status of *L. vannamei* reared in inland saline water using zero water exchange biofloc-based system under different stocking densities.

2 | MATERIALS AND METHODS

2.1 | Experimental set-up

The study was conducted at the Wet Laboratory of the ICAR-Central Institute of Fisheries Education (CIFE), Rohtak Centre, Haryana, India. Uniform-sized fibreglass-reinforced plastic (FRP) circular tanks of 500 L capacity with 0.98 m diameter filled with 350 L of water were used for the experiment. ISGW of 15 g L^{-1} salinity used for the experiment was pumped out from a depth of 20 m from underground and stored in two large storage cemented reservoir tanks (9000 L capacity) for 10 days for settlement of solids. The ionic composition of the water used for the experiment was 4560 mg L^{-1} Na^+, 163 mg L^{-1} K^+, 568 mg L^{-1} Mg^{2+} and 161 mg L^{-1} Ca^{2+}. Mineral fortification of the ISGW was done using commercial-grade fertilizer muriate of potash (MOP) containing 50% K^+ and magnesium chloride ($MgCl_2$) having 27% Mg^{2+} content, following the methods of Davis et al., (2005).

Four BFT treatments and a clear water control were set up in triplicates using a completely randomized design (CRD). The BFT treatments T1, T2, T3 and T4 were stocked with juvenile shrimp having an average body weight of 1.54 ± 0.05 g at 60, 100, 140 and 180 Nos. m^{-3} and control at 60 Nos. m^{-3}. All the tanks were filled with mineral fortified ISGW up to 350 L each. The four biofloc treatments were filled with 5 L of 2-day-old biofloc inoculums. The biofloc inoculum was prepared using 20 g L^{-1} pond soil, 10 mg L^{-1} ammonium sulphate and 200 mg L^{-1} fermented sugarcane molasses following Avnimelech (1999) in an FRP tank of 500 L filled with fortified 15 g L^{-1} ISGW. Continuous aeration (7 mg L^{-1} of dissolved oxygen) was provided in all the experimental tanks from a centralized aeration unit connected to 2 air pumps of 210W and 90W with an output capacity of 300 L min^{-1} and 120 L min^{-1}. The aeration pipe in each tank was provided with air stones and a regulator to control the air pressure in all the tanks. Twenty grams of carbohydrate was added per gram of TAN released, which was estimated on considering that the added carbohydrate contains 50% carbon and that 50% of the dietary protein input was converted to ammonia. In consequence, 0.53 kg carbon was applied for each kg of the 34.5% dietary protein feed administrated (Rajkumar et al., 2015).

2.2 | Stocking and rearing

Specific pathogen-free (SPF) *L. vannamei* juveniles were procured from Geekay Hatcheries, Nellore, Andhra Pradesh, India, and were transported to the experimental site. The juvenile shrimp were acclimatized and nursed in 1000-L FRP tanks at a stocking density of 60 m^{-3} for a period of 10 days before stocking into the experimental units. Acclimation was done by lowering the salinity by 0.5 g L^{-1} every 2 hour to avoid excess stress to the shrimp (Nunes & Lopez, 2001). Juvenile shrimp of average weight (1.54 ± 0.05 g) were randomly stocked in the experimental tanks when floc volume and total suspended solids (TSS) reached above 5 mL L^{-1} and 100 mg L^{-1},

respectively, as recommended by Rajkumar et al., (2015). All the treatments and control were fed with a commercially available feed having 35% crude protein, 5% crude fat, 4% crude fibre and 12% moisture. The shrimp were initially fed at 6% body weight using starter feed for 10 days and for the rest of 50 days with pre-grower feed at 3% body weight twice a day at 09:00 and 19:00 hours. Feeding rates were adjusted based on growth sampling. The rearing trials did not require ethical approval.

2.3 | Water Quality Parameters

The biofloc tanks were maintained at zero water discharge except for the compensation of evaporation loss. In the control units, 10% water exchange was done daily using fortified ISGW following Boyd and Fast (1992). Water quality parameters such as temperature, salinity, floc volume, pH and dissolved oxygen (DO) were measured on a daily basis from the experimental tanks during morning hours between 8:00 and 9:00 hours. Water temperature was checked using a digital thermometer (TC-902, Agarwal Electronics), salinity using a hand-held refractometer (Atago S/Mill-E), floc volume (FV) using a Imhoff cone (Merck Specialties) and pH using a portable pH meter (pHep Pocket-sized pH Meter; Hanna Instrument). Though there was a gradual reduction in pH from initial to final culture period, there was no need to correct the pH throughout the trial. DO content of the water was estimated using Winkler's titrimetric method, and total alkalinity and hardness were estimated by titrimetrically following the standard methods (APHA, 2005) using phenolphthalein and methyl orange and Eriochrome Black T as indicators respectively. Total ammonia nitrogen (TAN), nitrite-N and nitrate-N of the water samples were estimated using Spectroquant TAN, nitrite-N and nitrate-N test kits (Merck) following the prescribed guidelines under Merck Spectroquant (NOVA 60). Settling tanks were not used throughout the trial to remove excess TSS. TSS was determined through gravimetry by filtering aliquots of 20 m L of water through GF 50-A glass fibre filters, according to AOAC (2000); Strickland and Parsons (1972).

2.4 | Growth parameters of shrimp

Growth was assessed by measuring the body weight of random test animals (n=10) from each tank at a regular interval of 10 days during the 60-day experimental period and the biomass yield at the end of the experiment from all the tanks. Shrimp were harvested at the end of the experiment after draining the tanks and were weighed. Survival rate, average body weight (ABW), specific growth rate (SGR) and total weight gain were calculated according to the formulae given below:

ABW (g) = Total weight (g)/ Total number of animals

SGR (%) = [(ln final weight - ln initial weight) × 100] / No. of trial days

FCR = Feed given (DW)/weight gain (WW).

Total weight gain (wet weight, g) = Final weight (g) - Initial weight (g)

DW: dry weight, WW: wet weight

2.5 | Body composition of shrimp

Body composition of the shrimp was estimated at the end of the 60-day experimental period by following AOAC (2000) standard protocols using shrimp samples (n = 10) from each treatment for each parameter. Whole shrimp samples were weighed and dried in a hot air oven at 105°C till a constant weight was achieved. Total nitrogen was estimated using an automated nitrogen analyser (KEL Plus Classic DX VA; Pelican Equipment), and crude protein was calculated by multiplying the total nitrogen with a factor 6.25. Ether extract was estimated using an automatic fat extraction system (SOCS PLUS-SCS 08 AS; Pelican Equipment, India) using petroleum ether (boiling point 60-80°C) as the solvent. Total ash content was estimated in a muffle furnace (AI-7981, Expo Hi-Tech) at 550°C for 6 hours.

2.6 | Antioxidant enzyme activities

At the end of the experiment, 10 shrimp samples were randomly taken from each tank for tissue extraction (muscles, hepatopancreas and gill). About 10 mg of different tissues was collected from the samples, pooled up and stored in freshly prepared 0.25 molar sucrose solution (chilled) at the ratio of 1:19 (tissues : sucrose). The tissues were then homogenized in a mechanical homogenizer and centrifuged at 5700 × g for 4 min at 4°C. The supernatant was recovered and collected into new sterile microcentrifuge tubes and stored at −80°C to avoid protein denaturation. Total protein in the shrimp tissues was quantified following Lowry's method (Lowry, 1951) reading in a UV-Vis spectrophotometer (Genetix) at 660 nm using bovine serum albumin as standard. SOD activity was estimated using an SOD Assay Kit (Cayman Chemical Company) following the correspondent protocols (Misra & Fridovich, 1972); the absorbance was measured at 440 nm using an ELISA plate reader, and specific activity was expressed as SOD units m L^{-1} protein. CAT was assayed according to the method described by Takahara et al., (1960), and the specific activity was expressed as CAT units m L^{-1} protein. GPx activity was measured using a Cayman GPx Assay Kit (Cayman Chemical Company) following the specified protocols. The absorbance was measured at 340 nm using a ELISA plate reader, and specific activity was expressed as GPx μmol $min^{-1}g$ $protein^{-1}$.

2.7 | Statistical analysis

Statistical analysis was carried out using PROC UNIVARIATE, PROC GLM and PROC MEANS of SAS version 9.3 (SAS Institute, Cary, NC, 2002) in which data were subjected to one-way analysis of variance

(ANOVA). The difference between treatment means was tested using Duncan's multiple range test. The analyses were run at 5% significance level.

3 | RESULTS

3.1 | Water quality parameters

During the culture period, water quality parameters such as temperature (25-26°C), pH (7.5-8.0), DO (5.76-6.5 mg L^{-1}), TAN (0.2-0.5 mg L^{-1}), nitrite-N (0.1-0.53 mg L^{-1}), nitrate-N (1.84-5.55 mg L^{-1}) and TSS (230-255 mg L^{-1}) were recorded as indicated in Table 1. Both floc volume and TSS gradually increased by the culture period in the BFT treatments, while in the control, it remained almost nil. To maintain optimum water quality in the control units, daily 10% water exchange was done using fortified ISGW.

3.2 | Growth parameters

All growth parameters of the shrimp in the treatment groups with different stocking densities were significantly different ($P < 0.05$) from each other and from the control as represented in Table 2. The best growth performance in terms of mean final body weight and total biomass was recorded in the BFT treatments T1 and T4 with 12.37 ± 0.21 g and 1868.98 g, respectively, while the least was in control with 7.01 ± 0.14 g and 462.48 g respectively. FCR was lower in the BFT groups than the control of which T1 recorded the lowest value.

3.3 | Body composition

The crude protein, lipid and ash contents of the shrimp from the BFT treatments were significantly higher ($P < 0.05$) when compared to control, while there was no significant difference ($P > 0.05$) among the BFT treatments as shown in Table 3. There was no significant difference ($P > 0.05$) in moisture content of whole body of the shrimp between the BFT treatments and the control.

3.4 | Antioxidant enzyme activities

In all type of shrimp tissues, antioxidant activities were significantly higher ($P < 0.05$) in the BFT treatments than in the control as represented in Table 4. SOD, CAT and GPx activities in muscle, hepatopancreas and gill were recorded to be the highest in T4 and lowest in control. Among the three tissues, the SOD activity was the highest in gills followed by hepatopancreas and the least in muscle. CAT activity was the highest in gills, while hepatopancreas and muscle exhibited similar values. GPx activity in T4 was significantly higher ($P < 0.05$) than all other experimental groups, while the rest of the biofloc treatments showed no significant difference ($P > 0.05$) among them. GPx activity in all the tissues exhibited almost similar values, though gills indicated a slightly higher value compared with others.

4 | DISCUSSION

With continuous aeration, DO in all the experimental tanks was well above the safe levels of 4.6 mg L^{-1} recommended by McGraw et al., (2001) for shrimp farming. The concentrations of nitrogenous compounds were maintained within the safe limits for shrimp culture recommended for TAN (2.44 mg L^{-1}), nitrite-N (6.1 mg L^{-1}) and nitrate-N (177 mg L^{-1}) (Furtado et al., 2014; Lin & Chen, 2001, 2003). The ability to maintain these nitrogenous compounds within the limits in zero water discharge systems may be attributed to the periodical addition of carbon sources (De Souza et al., 2014; Rajkumar et al., 2015). Higher nitrate-N with low TAN and nitrite-N indicates the active role of the nitrifying bacteria and the oxidation of ammonia into nitrate, the compound that is least harmful to penaeid

TABLE 1 Mean water quality parameters of different experimental groups during the experimental period of 60 days in which shrimp *Litopenaeus vannamei* was reared in inland saline groundwater-based experimental units

Treatments→ Parameters↓	C	T1	T2	T3	T4
Temperature (°C)	$25.0^{a} \pm 0.60$	$26.0^{a} \pm 0.70$	$25.0^{a} \pm 0.30$	$26.0^{a} \pm 0.40$	$25.0^{a} \pm 0.10$
Dissolved oxygen (mg L^{-1})	$6.50^{a} \pm 0.01$	$6.25^{a} \pm 0.04$	$6.00^{a} \pm 0.01$	$6.00^{a} \pm 0.01$	$5.76^{a} \pm 0.05$
pH	$8.00^{a} \pm 0.14$	$7.50^{a} \pm 0.24$	$7.50^{a} \pm 0.31$	$7.50^{a} \pm 0.03$	$7.50^{a} \pm 0.12$
TAN (mg L^{-1})	$0.20^{a} \pm 0.07$	$0.30^{b} \pm 0.09$	$0.35^{b} \pm 0.11$	$0.40^{c} \pm 0.05$	$0.50^{d} \pm 0.02$
Nitrite-N (mg L^{-1})	$0.10^{a} \pm 0.05$	$0.32^{b} \pm 0.12$	$0.38^{b} \pm 0.04$	$0.45^{c} \pm 0.07$	$0.53^{d} \pm 0.05$
Nitrate-N (mg L^{-1})	$1.84^{a} \pm 0.27$	$5.21^{b} \pm 0.33$	$5.52^{c} \pm 0.12$	$5.57^{c} \pm 0.56$	$5.55^{c} \pm 0.45$
Floc volume (mL L^{-1})	$0.53^{a} \pm 0.05$	$21.49^{b} \pm 1.86$	$22.43^{b} \pm 1.98$	$23.28^{b} \pm 1.95$	$24.75^{b} \pm 2.05$
Total suspended solids (mg L^{-1})	NA*	$230.0^{a} \pm 15.00$	$236.0^{a} \pm 20.00$	$240.0^{b} \pm 12.01$	255.0 ± 19.03

Values (mean ± SE) in the same column with different superscripts differ significantly (Duncan's multiple range test ($P < 0.05$)). Clear water—C: 60 shrimp m^{-3}; and biofloc treatments—T1: 60 shrimp m^{-3}, T2: 100 shrimp m^{-3}, T3: 140 shrimp m^{-3} and T4: 180 shrimp m^{-3}. NA*: TSS in control was not measured.

TABLE 2 Growth performance and survival of shrimp *Litopenaeus vannamei* reared for 60 days in inland saline groundwater-based experimental units

Treatments→ Parameters↓	C	T1	T2	T3	T4
Stocking density (shrimp m^{-3})	60	60	100	140	180
Initial body weight (g)	$1.55^{a} \pm 0.01$	$1.54^{a} \pm 0.01$	$1.52^{a} \pm 0.02$	$1.54^{a} \pm 0.04$	$1.54^{a} \pm 0.03$
Final body weight (g)	$7.01^{e} \pm 0.14$	$12.37^{a} \pm 0.21$	$11.40^{b} \pm 0.21$	$10.63^{c} \pm 0.27$	$9.22^{d} \pm 0.26$
Total weight gain (g)	$5.47^{e} \pm 0.02$	$10.83^{a} \pm 0.12$	$9.87^{b} \pm 0.07$	$9.09^{c} \pm 0.09$	$7.68^{d} \pm 0.04$
Specific growth rate (%)	$2.52^{e} \pm 0.01$	$3.47^{a} \pm 0.03$	$3.36^{b} \pm 0.01$	$3.24^{c} \pm 0.05$	$2.95^{d} \pm 0.02$
Survival rate (%)	$95.24^{c} \pm 1.12$	100^{a}	100^{a}	100^{a}	$97.88^{b} \pm 0.53$
Final biomass (g)	$462.48^{e} \pm 16.53$	$732.5^{d} \pm 22.41$	$1108.3^{c} \pm 28.07$	$1536.1^{b} \pm 36.18$	$1869.98^{a} \pm 44.72$
Feed conversion ratio	$1.73^{a} \pm 0.01$	$1.19^{c} \pm 0.02$	$1.21^{c} \pm 0.01$	$1.26^{b} \pm 0.01$	$1.30^{b} \pm 0.01$
Feed efficiency ratio	$0.58^{d} \pm 0.00$	$0.84^{a} \pm 0.01$	$0.83^{a} \pm 0.00$	$0.80^{b} \pm 0.01$	$0.77^{c} \pm 0.01$

Values (mean ± SE) in the same row with different superscripts differ significantly (Duncan's multiple range test ($P < 0.05$)). All the values in percentage were arcsine-transformed for ANOVA. Clear water—C: 60 shrimp m^{-3}; and biofloc treatments—T1: 60 shrimp m^{-3}, T2: 100 shrimp m^{-3}, T3: 140 shrimp m^{-3} and T4: 180 shrimp m^{-3}.

TABLE 3 Body compositions of whole shrimp *Litopenaeus vannamei* reared in inland saline groundwater at the end of 60 days of the experimental period

Parameters→ Treatments↓	Moisture (%)	Crude protein (%)	Crude lipid (%)	Ash (%)
C	$78.2^{a} \pm 0.10$	$29.1^{b} \pm 0.14$	$5.0^{b} \pm 0.01$	$1.4^{b} \pm 0.02$
T1	$78.0^{a} \pm 0.08$	$33.0^{a} \pm 0.16$	$5.6^{a} \pm 0.02$	$1.6^{a} \pm 0.02$
T2	$78.1^{a} \pm 0.09$	$33.0^{a} \pm 0.10$	$5.6^{a} \pm 0.02$	$1.5^{a} \pm 0.03$
T3	$78.0^{a} \pm 0.08$	$33.0^{a} \pm 0.12$	$5.6^{a} \pm 0.01$	$1.6^{a} \pm 0.02$
T4	$78.1^{a} \pm 0.06$	$33.0^{a} \pm 0.10$	$5.6^{a} \pm 0.01$	$1.5^{a} \pm 0.03$

Values (mean ± SE) in the same column with different superscripts differ significantly (Duncan's multiple range test ($P < 0.05$)). All the values in percentage were arcsine-transformed for ANOVA. Clear water—C: 60 shrimp m^{-3}; and biofloc treatments—T1: 60 shrimp m^{-3}, T2: 100 shrimp m^{-3}, T3: 140 shrimp m^{-3} and T4: 180 shrimp m^{-3}.

shrimp (Cohen et al., 2005; Ebeling et al., 2006; Wyk et al., 1999;). TSS observed were within the recommended level of 500 mg L^{1} (Samocha et al., 2007). This indicates that *L. vannamei* could effectively harvest sizeable microbial aggregates, which in turn might have helped to maintain low water turbidity and overall water quality of the system.

There were high survival rates in the BFT treatments with almost no mortality, while the control had significantly lower survival. It may be because of the fact that the biofloc developed in the system might have created a favourable environment for the shrimp to adapt without stress. Moreover, the suspended flocs could have acted as *in situ* natural feed for the shrimp to graze on. Enhanced growth performances in terms of body weight gain, SGR and biomass were observed in all BFT treatments compared with control. This may be attributed to the presence of protein-rich microbial flocs naturally available as feed for the shrimp in BFT apart from the commercial feed provided. Xu and Pan (2013) also suggested that the microbial flocs could influence the digestive enzymes, gut microflora and overall nutrient assimilation by the shrimp. The fact that shrimp might have utilized and assimilated the nutrient-rich flocs effectively is further supported by the higher content of crude protein, lipids and ash observed from the shrimp reared in the BFT treatments when compared to control. Rajkumar et al. (2015) reported that the addition of carbohydrate in the culture system led to an increase in protein utilization and supply of essential lipids and vitamins for the growth of shrimp. Sriket et al., (2007) and Xu and Pan (2012) also reported that consumption of microbial flocs can enrich the nutritional value, sensory qualities and shelf life of the shrimp.

The growth of the shrimp reduced with an increase in stocking density in the BFT treatments, which may be due to the crowding and competition for food and space. At high stocking density, the dominance by larger organisms over the smaller ones in food, space and territory can create inhibitory effects on animal growth (Babu et al., 2014). Similar reports on growth reduction with an increase in stocking density were reported by earlier authors (Appelbaum et al., 2002; Bratvold & Browdy, 2001; Samocha et al., 2004; Williams et al., 1996). It was observed that the BFT treatments with stocking density of 180 shrimp m^{-3} still performed better in terms of survival and weight gain when compared to control at 60 shrimp m^{-3}. The biomass of shrimp increased with an increase in stocking density in the BFT treatments. It may, thus, be construed that BFT has a higher carrying capacity when compared to conventional clear water culture system in ISGW also. Rajkumar et al. (2015) and Krummenauer et al., (2011) also reported good

TABLE 4 Antioxidant enzyme activities of shrimp *Litopenaeus vannamei* reared in inland saline groundwater in different treatment groups at the end of the 60-day experiment

Tissues→ Treatments↓	Muscles	Gills	Hepatopancreas
SOD activity (units mg protein^{-1})			
C	$3.31^{d} \pm 0.12$	$3.78^{c} \pm 0.18$	$3.29^{c} \pm 0.21$
T1	$3.63^{c} \pm 0.09$	$5.84^{b} \pm 0.23$	$5.08^{b} \pm 0.33$
T2	$3.72^{b} \pm 0.08$	$5.82^{b} \pm 0.17$	$5.11^{b} \pm 0.21$
T3	$3.75^{b} \pm 0.11$	$5.81^{b} \pm 0.18$	$5.17^{b} \pm 0.21$
T4	$3.98^{a} \pm 0.12$	$6.22^{a} \pm 0.21$	$5.44^{a} \pm 0.18$
Catalase activity (μmol g protein^{-1})			
C	$0.30^{c} \pm 0.01$	$0.35^{c} \pm 0.03$	$0.32^{c} \pm 0.02$
T1	$0.41^{b} \pm 0.01$	$0.51^{b} \pm 0.01$	$0.44^{b} \pm 0.03$
T2	$0.43^{b} \pm 0.02$	$0.53^{b} \pm 0.01$	$0.45^{b} \pm 0.03$
T3	$0.44^{b} \pm 0.02$	$0.53^{b} \pm 0.02$	$0.46^{b} \pm 0.02$
T4	$0.51^{a} \pm 0.02$	$0.59^{a} \pm 0.03$	$0.52^{a} \pm 0.03$
Glutathione peroxidase (μmol g protein^{-1})			
C	$2.01^{c} \pm 0.03$	$2.09^{c} \pm 0.05$	$2.56^{c} \pm 0.06$
T1	$2.38^{b} \pm 0.04$	$2.99^{b} \pm 0.04$	$2.83^{b} \pm 0.07$
T2	$2.39^{b} \pm 0.04$	$2.96^{b} \pm 0.05$	$2.82^{b} \pm 0.06$
T3	$2.40^{b} \pm 0.03$	$3.11^{ab} \pm 0.04$	$2.81^{b} \pm 0.04$
T4	$2.73^{a} \pm 0.03$	$3.25^{a} \pm 0.04$	$3.07^{a} \pm 0.04$

Values (mean ± SE) in the same column with different superscripts differ significantly (Duncan's multiple range test ($P < 0.05$)). Clear water—C: 60 shrimp m^{-3}; and biofloc treatments—T1: 60 shrimp m^{-3}, T2: 100 shrimp m^{-3}, T3: 140 shrimp m^{-3} and T4: 180 shrimp m^{-3}. SOD: superoxide dismutase.

growth of *L. vannamei* in the BFT system at a stocking density of 130 m^{-3} and 300 m^{-3} respectively.

It is a well-known fact that the lower the FCR, the higher the economical sustainability of the system. In the present study, the lowest FCR was obtained in the BFT treatments having stocking densities of 60 m^{-3} and 100 m^{-3} respectively. All the BFT treatments registered improved FCR as compared to control, which indicates that biofloc can improve the feed and nutrient utilization in culture organisms. It was also reported that biofloc can influence the digestive enzymes, gut microflora and, hence, overall nutrient assimilation (Xu & Pan, 2013). Enhanced growth performances and low FCR of the shrimp may be attributed to the protein-rich microbial biofloc aggregates that served as *in situ* natural feed, which is available round the clock.

Invertebrates such as shrimp do not possess an adaptive immune system, and hence, they rely on efficient immune defences to protect against invading agents (Liu et al., 2007). Hence, improving the innate immunity and/or enhancing antioxidant defence capacity of the animal is necessary to keep it healthy. Earlier authors have reported that antioxidant enzyme activities in animals are largely regulated by dietary nutrients, environment and stress level (Ninawe & Selvin, 2009; Smith et al., 2003). In normal physiological conditions, reactive oxygen species (ROS) such as the superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (^-OH) and singlet oxygen (O_2) are normally produced to counter any foreign invaders (Schwarz, 1996). These free radical generations are enhanced when exposed to stress, and the radical damage can be significant because it can proceed as a chain reaction (Chien et al., 2003). The higher ROS production may induce oxidative stress and can result in cell membrane damage, inactivation of enzymes and damage to genetic material and other vital cell components (Liu et al., 2007). The rapid elimination of these free radicals is essential for the proper functioning and survival of organisms. This is performed by antioxidant defence enzymes including SOD that scavenges the superoxide anion, CAT that acts on H_2O_2 and reduces it into two molecules of water and one molecule of oxygen, and GPX that scavenges H_2O_2 and lipid hydroperoxides (Campa-Córdova et al., 2002; Li et al., 2008). These antioxidant enzymes are generally present in all oxygen-respiring organisms.

The present study recorded higher antioxidant enzyme activity in the BFT treatments than in the control. The result is in agreement with that of Liu et al., (2007) who reported an increase in the enzyme activity in *L. vannamei* when fed with an immunostimulant-supplemented diet. Xu and Pan (2013) also reported an increased antioxidant enzyme activity in BFT with an increased C/N ratio. The reason may be due to the presence of microbial flocs, which are rich in various vitamins such as tocopherol and citric acid (Crab, 2010; Emerenciano et al., 2013) that can serve as antioxidants and can increase the immune status of the animal. The microbial flocs can confer benefits to the shrimp immune system, which may be due to the presence of carotenoids, retinoids, poly-β-hydroxybutyrate and exoenzymes (Aguilera-Rivera et al., 2014) and thus can act as immunostimulants to improve innate immunity and/or enhance antioxidant capacity of shrimp (Becerra-Dorame et al., 2012; Ninawe & Selvin, 2009; Smith et al., 2003). Moreover, the activities of the antioxidant enzymes are regulated by dietary nutrients, health status and environmental stress.

The values of SOD, CAT and GPx activities in gills, hepatopancreas and muscles of shrimp from the BFT treatments were comparatively higher than control. All the values were within the range as reported by earlier findings (Li et al., 2008; Liu et al., 2007). Liu et al., (2007) reported that the diet supplemented with immunostimulant enhanced the activity of various antioxidant enzymes in crustaceans. In crustaceans, a higher level of antioxidant enzyme activities indicated the presence of higher production of ROS (Chien et al., 2003). Higher activities of SOD, CAT and GPx in the present study at higher stocking density suggest the possible stress with higher free radicals, which caused enhanced antioxidant enzyme activity to scavenge the free radicals. Liu et al., (2007) suggested that these enzymes can eliminate the radicals effectively and rapidly due to the direct stimulatory action of vitamin E in stressed condition. Further, these values can be correlated with growth and yield to provide evidence for the positive impact of the presence of biofloc to ameliorate density-induced stress.

Therefore, the present study leads to a finding that, in spite of water quality and higher stocking density-related stressors in the

shrimp in the BFT treatments, the presence of immunostimulatory properties in the biofloc might accelerate the free radical scavenging activity of the antioxidant enzymes. Xu and Pan (2013) reported increased SOD activity in hepatopancreas in the BFT treatments and suggested that the biofloc is a source of abundant natural microbes and bioactive compounds that could exert a positive effect on the physiological health of cultured shrimp. Both microbial components such as polysaccharides and bioactive compounds such as carotenoids in the biofloc can exert an immune-stimulating effect, and this action was continuous as long as shrimp consumed biofloc (Xu & Pan, 2013). The higher activity found in hepatopancreas in the present study may be due to the reason that this tissue acts as the main immune site of the animal (Jiang et al., 2014). Thus, the increase in antioxidant enzyme activity at lower stocking densities might not necessarily be due to stress but due to an increase in the immune status of the animals. Any possible water quality or density-induced stress might have been counterbalanced by the activity of the antioxidant enzymes. The higher enzyme activity in the gills might reveal the response of the latter to the higher suspended solids, turbidity and other water quality impacts.

5 | CONCLUSION

The study concluded with the fact that BFT can enhance the productivity of inland saline water through zero water discharge mode. The results provide evidence that this technology can enhance the growth, biochemical composition and antioxidant status of shrimp reared in underutilized inland saline water. There was also indication that the technology provides stress mitigation using environmental manipulation through conversion and assimilation of nitrogenous waste from the system. It provides auto-recycling within the system, which can be projected as a sustainable method of shrimp farming in inland areas.

ACKNOWLEDGEMENTS

The authors are thankful to Director, ICAR-Central Institute of Fisheries Education, Mumbai, India, for providing all the facilities to conduct the present research and for the fellowship for the student for carrying out the research.

CONFLICTS OF INTEREST

The authors have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS

Ms. Dorothy, M.S, was a student author and executed the research work. Mr. Harikrishna designed the experiment, performed monitoring and edited the manuscript. Dr. Arun Sudhagar performed research and laboratory analysis supervision and edited the manuscript. Dr. A.K. Reddy performed wet laboratory trial and water quality management monitoring, and laboratory analysis. Dr. Babitha Rani, A.M, was a major advisor, planned and executed the experiment, performed statistical analysis and edited the manuscript.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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REFERENCES

Aguilera-Rivera, D., Prieto-Davó, A., Escalante, K., Chávez, C., Cuzon, G., & Gaxiola, G. (2014). Probiotic effect of FLOC on Vibrios in the pacific white shrimp *Litopenaeus vannamei*. *Aquaculture*, *424*, 215–219

Antony, J., Vungurala, H., Saharan, N., Reddy, A. K., Chadha, N. K., Lakra, W. S., & Roy, L. A. (2015). Effects of salinity and Na+/K+ ratio on osmoregulation and growth performance of black tiger prawn, *Penaeus monodon Fabricius*, 1798, juveniles reared in inland saline water. *Journal of the World Aquaculture Society*, *46*(2), 171–182

(Association of Official Analytical Chemists), 2000. Official Methods of Analysis of AOAC (16th edn). Patricia Cunniff (editora), Washington DC.

APHA (2005). *Standard methods for the examination of the water and wastewater*, 22nd ed. American Public Health Association

Appelbaum, S., Garada, J., & Mishra, J. K. 2002. Growth and survival of the white leg shrimp (*Litopenaeus vannamei*) reared intensively in the brackish water of the Israeli Negev desert

Araneda, M., Pérez, E. P., & Gasca-Leyva, E. (2008). White shrimp *Penaeus vannamei* culture in freshwater at three densities: condition state based on length and weight. *Aquaculture*, *283*(1–4), 13–18

Avnimelech, Y. (1999). Carbon/nitrogen ratio as a control element in aquaculture systems. *Aquaculture*, *176*(3–4), 227–235

Babu, P. S., Razvi, S. S. H., Venugopal, G., Ramireddy, P., Mohan, K. M., Rao, P. S., Patnaik, R. R. S., Narasimhacharyulu, V., & Ananthan, P. S. (2014). Growth and production performance of Pacific white leg shrimp *Litopenaeus vannamei* (Boone, 1931) in low stocking short term farming in earthen pond conditions. *Indian Journal of Fisheries*, *61*(4), 68–72

Ballester, E. L. C., Abreu, P. C., Cavalli, R. O., Emerenciano, M., De Abreu, L., & Wasielesky, W. Jr (2010). Effect of practical diets with different protein levels on the performance of *Farfantepenaeus paulensis* juveniles nursed in a zero exchange suspended microbial flocs intensive system. *Aquaculture Nutrition*, *16*(2), 163–172

Becerra-Dorame, M. J., Martínez-Porchas, M., Martínez-Córdova, L. R., Rivas-Vega, M. E., Lopez-Elias, J. A., & Porchas-Cornejo, M. A. 2012. Production response and digestive enzymatic activity of the Pacific white shrimp *Litopenaeus vannamei*. (Boone, 1931) intensively pregrown in microbial heterotrophic and autotrophic-based systems. The Scientific World Journal, 2012.

Bhumbla, D. R. (1978). Saline and sodic soils. *Soils and Rice*, 719–734

Boyd, C., & Fast, A. W. (1992). Pond monitoring and management. In A. W. Fast, & L. J. Lester (Eds.), *Marine shrimp culture: principles and practices. Developments in aquaculture and fisheries science*, Vol. *23*. Elsevier Science Publisher B.V

Bratvold, D., & Browdy, C. L. (2001). Effects of sand sediment and vertical surfaces (AquaMatsTM) on production, water quality, and microbial ecology in an intensive *Litopenaeus vannamei* culture system. *Aquaculture*, *195*(1–2), 81–94

Burford, M. A., Thompson, P. J., McIntosh, R. P., Bauman, R. H., & Pearson, D. C. (2004). The contribution of flocculated material to shrimp (*Litopenaeus vannamei*) nutrition in a high-intensity, zero-exchange system. *Aquaculture*, *232*(1–4), 525–537

Campa-Córdova, A. I., Hernández-Saavedra, N. Y., De Philippis, R., & Ascencio, F. (2002). Generation of superoxide anion and SOD activity in haemocytes and muscle of American white shrimp (*Litopenaeus*

vannamei) as a response to β-glucan and sulphated polysaccharide. *Fish & Shellfish Immunology*, *12*(4), 353–366

Castex, M., Lemaire, P., Wabete, N., & Chim, L. (2010). Effect of probiotic Pediococcus acidilactici on antioxidant defences and oxidative stress of *Litopenaeus stylirostris* under Vibrio nigripulchritudo challenge. *Fish & Shellfish Immunology*, *28*(4), 622–631

Chien, Y. H., Pan, C. H., & Hunter, B. (2003). The resistance to physical stresses by *Penaeus monodon* juveniles fed diets supplemented with astaxanthin. *Aquaculture*, *216*(1–4), 177–191

Cohen, J. M., Samocha, T. M., Fox, J. M., Gandy, R. L., & Lawrence, A. L. (2005). Characterization of water quality factors during intensive raceway production of juvenile *Litopenaeus vannamei* using limited discharge and biosecure management tools. *Aquacultural Engineering*, *32*(3–4), 425–442

Crab, R. (2010). *Bioflocs technology: an integrated system for the removal of nutrients and simultaneous production of feed in aquaculture* (Doctoral dissertation, Ghent University)

Davis, D. A., Boyd, C. E., Rouse, D. B., & Saoud, I. P. (2005). Effects of potassium, magnesium and age on growth and survival of *Litopenaeus vannamei* post-larvae reared in inland low salinity well waters in west Alabama. *Journal of the World Aquaculture Society*, *36*(3), 416–419

De Schryver, P., Crab, R., Defoirdt, T., Boon, N., & Verstraete, W. (2008). The basics of bio-flocs technology: The added value for aquaculture. *Aquaculture*, *277*(3-4), 125–137. https://doi.org/10.1016/j.aquaculture.2008.02.019

de Souza, D. M., Suita, S. M., Romano, L. A., Wasielesky, W. J., & Ballester, E. L. C. (2014). Use of molasses as a carbon source during the nursery rearing of *Farfantepenaeus brasiliensis* (Latreille, 1817) in a Biofloc technology system. *Aquaculture Research*, *45*(2), 270–277

Ebeling, J. M., Timmons, M. B., & Bisogni, J. J. (2006). Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia–nitrogen in aquaculture systems. *Aquaculture*, *257*(1–4), 346–358

Emerenciano, M., Ballester, E. L. C., Cavalli, R. O., & Wasielesky, W. (2012). Biofloc technology application as a food source in a limited water exchange nursery system for pink shrimp Farfantepenaeus brasiliensis (Latreille, 1817). *Aquaculture Research*, *43*(3), 447–457. https://doi.org/10.1111/j.1365-2109.2011.02848.x

Emerenciano, M., Gaxiola, G., & Cuzon, G. (2013). Biofloc technology (BFT): a review for aquaculture application and animal food industry. *Biomass now-cultivation and Utilization*, 301–328

Furtado, P. S., Gaona, C. A., Poersch, L. H., & Wasielesky, W. (2014). Application of different doses of calcium hydroxide in the farming shrimp *Litopenaeus vannamei* with the biofloc technology (BFT). *Aquaculture International*, *22*(3), 1009–1023

Jamil, A., Riaz, S., Ashraf, M., & Foolad, M. R. (2011). Gene expression profiling of plants under salt stress. *Critical Reviews in Plant Sciences*, *30*(5), 435–458

Jiang, H., Li, F., Zhang, J., Zhang, J., Huang, B., Yu, Y., & Xiang, J. (2014). Comparison of protein expression profiles of the hepatopancreas in *Fenneropenaeus chinensis* challenged with heat-inactivated Vibrio anguillarum and white spot syndrome virus. *Marine Biotechnology*, *16*(1), 111–123

Krummenauer, D., Peixoto, S., Cavalli, R. O., Poersch, L. H., & Wasielesky, W.. (2011). Superintensive culture of white shrimp, *Litopenaeus vannamei*, in a biofloc technology system in southern Brazil at different stocking densities. *Journal of the World Aquaculture Society*, *42*(5), 726–733

Kuhn, D. D., Lawrence, A. L., Boardman, G. D., Patnaik, S., Marsh, L., & Flick, G. J. Jr (2010). Evaluation of two types of bioflocs derived from biological treatment of fish effluent as feed ingredients for Pacific white shrimp, *Litopenaeus vannamei*. *Aquaculture*, *303*(1–4), 28–33

Li, Y., Jiang, B., Zhang, T., Mu, W., & Liu, J. (2008). Antioxidant and free radical-scavenging activities of chickpea protein hydrolysate (CPH). *Food Chemistry*, *106*(2), 444–450

Lin, Y. C., & Chen, J. C. (2001). Acute toxicity of ammonia on Litopenaeus vannamei Boone juveniles at different salinity levels. *Journal of Experimental Marine Biology and Ecology*, *259*(1), 109–119

Lin, Y. C., & Chen, J. C. (2003). Acute toxicity of nitrite on Litopenaeus vannamei (Boone) juveniles at different salinity levels. *Aquaculture*, *224*(1–4), 193–201

Liu, C. H., & Chen, J. C. (2004). Effect of ammonia on the immune response of white shrimpLitopenaeus vannamei and its susceptibility to Vibrio alginolyticus. *Fish & Shellfish Immunology*, *16*, 321–334

Liu, Y. C., Li, F. H., Dong, B., Wang, B., Luan, W., Zhang, X. J., Zhang, L. S., & Xiang, J. H. (2007). Molecular cloning, characterization and expression analysis of a putative C-type lectin (Fclectin) gene in Chinese shrimp Fenneropenaeus chinensis. *Molecular Immunology*, *44*(4), 598–607

Logan, A., 2010. Biofloc by the truckload: A new aquafeed ingredient from Oberon FMR, Inc., World Aquaculture (2010). *Meeting Abstract*. San Diego

Lowry, O. H. (1951). Rosebrough NJ, Farr Al, and Randall RJ. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, *193*, 265–275

McGraw, W., Teichert-Coddington, D. R., Rouse, D. B., & Boyd, C. E. (2001). Higher minimum dissolved oxygen concentrations increase penaeid shrimp yields in earthen ponds. *Aquaculture*, *199*(3–4), 311–321

McIntosh, R. P. (2000). Changing paradigms in shrimp farming: IV. Low protein feeds and feeding strategies. Global Aquaculture. *Advocate*, *3*(2), 44–50

Misra, H. P., & Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological Chemistry*, *247*(10), 3170–3175

Moss, S. M., Forster, I. P., & Tacon, A. G. (2006). Sparing effect of pond water on vitamins in shrimp diets. *Aquaculture*, *258*(1–4), 388–395

Ninawe, A. S., & Selvin, J. (2009). Probiotics in shrimp aquaculture: avenues and challenges. *Critical Reviews in Microbiology*, *35*(1), 43–66

Nunes, A. J. P., & Lopez, C. V. (2001). Low-salinity, inland shrimp culture in Brazil and Ecuador–economics, disease issues move farms away from coasts. *Global Aquaculture Advocate*, *4*(3), 62–64

Patel, B. B., & Dave, R. S.. 2011. Studies on infiltration of saline-alkali soils of several parts of Mehsana and Patan districts of North Gujarat. Journal of Applied Technology in Environmental Sanitation, 1(1), pp.87-92.Pitman, M.G. and Läuchli, A., 2002. Global impact of salinity and agricultural ecosystems. In Salinity: environment-plants-molecules (pp. 3-20). Springer, Dordrecht. Rajkumar, M., Pandey, P.K., Aravind, R., Vennila, A., Bharti, V. and Purushothaman, C.S., 2016. Effect of different biofloc system on water quality, biofloc composition and growth performance in Litopenaeus vannamei (Boone, 1931). Aquaculture Research, 47(11), pp.3432-3444

Samocha, T. M., Lawrence, A. L., Collins, C. A., Castille, F. L., Bray, W. A., Davies, C. J., Lee, P. G., & Wood, G. F. (2004). Production of the Pacific white shrimp, *Litopenaeus vannamei*, in high-density greenhouse-enclosed raceways using low salinity groundwater. *Journal of Applied Aquaculture*, *15*(3–4), 1–19

Samocha, T. M., Patnaik, S., Speed, M., Ali, A. M., Burger, J. M., Almeida, R. V., Ayub, Z., Harisanto, M., Horowitz, A., & Brock, D. L. (2007). Use of molasses as carbon source in limited discharge nursery and grow-out systems for *Litopenaeus vannamei*. *Aquacultural Engineering*, *36*(2), 184–191

Schwarz, K. B. (1996). Oxidative stress during viral infection: a review. *Free Radical Biology and Medicine*, *21*(5), 641–649

Shrivastava, P., & Kumar, R. (2015). Soil salinity: a serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. *Saudi Journal of Biological Sciences*, *22*(2), 123–131

Smith, V. J., Brown, J. H., & Hauton, C. (2003). Immunostimulation in crustaceans: does it really protect against infection? *Fish & Shellfish Immunology*, *15*(1), 71–90

Sriket, P., Benjakul, S., Visessanguan, W., & Kijroongrojana, K. (2007). Comparative studies on chemical composition and thermal properties of black tiger shrimp (Penaeus monodon) and white shrimp (*Penaeus vannamei*) meats. *Food Chemistry*, *103*(4), 1199–1207

Strickland, J. D. H., & Parsons, T. R. (1972). *A Practical Handbook of Seawater Analysis* (2nd edn), Bulletin 167. Fisheries Research Board of Canada

Tacon, A. G. J., Cody, J. J., Conquest, L. D., Divakaran, S., Forster, I. P., & Decamp, O. E. (2002). Effect of culture system on the nutrition and growth performance of Pacific white shrimp *Litopenaeus vannamei* (Boone) fed different diets. *Aquaculture Nutrition*, *8*(2), 121–137

Takahara, S., Hamilton, H. B., Neel, J. V., Kobara, T. Y., Ogura, Y., & Nishimura, E. T. (1960). Hypocatalasemia: a new genetic carrier state. *The Journal of Clinical Investigation*, *39*(4), 610–619

Van Wyk, P., Davis-Hodgkins, M., Laramore, R., Main, K. L., Mountain, J., & Scarpa, J. 1999. *Farming marine shrimp in recirculating freshwater systems* (pp. 128-138). Harbor Branch Oceanographic Institution

Vijayvargiya, S., & Kumar, A. (2011). *Inluence of salinity stress on plant growth and productivity: Salinity stress influences on plant growth* (p. 170). Lap Lambert Academic Publishers.

Wasielesky, W. Jr, Atwood, H., Stokes, A., & Browdy, C. L. (2006). Effect of natural production in a zero exchange suspended microbial floc based super-intensive culture system for white shrimp *Litopenaeus vannamei*. *Aquaculture*, *258*(1–4), 396–403

Williams, A. S., Davis, D. A., & Arnold, C. R. (1996). Density-dependent growth and survival of *Penaeus setiferus* and *Penaeus vannamei in* a semi-closed recirculating system. *Journal of the World Aquaculture Society*, *27*(1), 107–112

Xu, W. J., & Pan, L. Q. (2012). Effects of bioflocs on growth performance, digestive enzyme activity and body composition of juvenile *Litopenaeus vannamei* in zero-water exchange tanks manipulating C/N ratio in feed. *Aquaculture*, *356*, 147–152

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

How to cite this article: M. S, D., Vungarala, H., Sudhagar, A., Reddy, A. K., & Rani Asanaru Majeedkutty, B. (2021). Growth, body composition and antioxidant status of *Litopenaeus vannamei* juveniles reared at different stocking densities in the biofloc system using inland saline groundwater. *Aquaculture Research*, 52, 6299–6307. https://doi.org/10.1111/are.15493