Research Article

Stressing stocking density and rearing time effect on whiteleg shrimp (*Penaeus vannamei*) reared intensively in floating cages

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ABSTRACT. The stressing effect of stocking density and rearing time was assessed on shrimp *Penaeus vannamei* reared intensively in floating cages. Juvenile shrimp were stocked in 9 m^2 cages at 200, 250, and 300 ind m^{-2} for a 25-day grow-out period. Total soluble protein concentration, superoxide dismutase (SOD), and catalase (CAT) activities were used as stress indicators in shrimp muscle, hepatopancreas, and hemocytes. Two-way ANOVA showed that protein content in hepatopancreas significantly decreased as stocking rate increased (from 3.81 to 2.26 mg mL^{-1}). Density and rearing time interacted significantly to determine SOD activity in muscle and hemocytes where the maximum activity occurred at the densest rate by the end of the study (10.21 and 122.41 U mg^{-1}); CAT activity in hepatopancreas was significantly lower when the highest density (4.1 U mg^{-1}) was used. Final shrimp weight (5.28-5.49 g), survival (90.2-91.9%), feed conversion ratio (0.75-0.78), specific (0.058-0.063), and absolute (1.17-1.30 g $week^{-1}$) growth rates were not significantly affected by density, although yields varied significantly (0.99-1.49 kg m^{-2}). To conclude, despite the stocking rate causing a stressing effect on shrimp, its antioxidant enzymatic activity prevented such development from negatively affecting shrimp growth, survival, feed conversion ratio, and production.

Keywords: *Penaeus vannamei*; oxidative stress; antioxidants; aquaculture; hemocytes; superoxide dismutase

INTRODUCTION

Sea floating cages constitute an alternative to conventional shrimp production systems with advantages over pond farming, such as good water circulation and high oxygen solubility (Effendi et al. 2016). Moreover, aquaculture directly at sea is becoming increasingly attractive, particularly for landless and financially-poor fishing families (Radulovich & Fuentes-Quesada 2019). Several studies have evaluated different stocking densities and cage designs for shrimp nursery and grow-out stages (Paquotte et al. 1998, Lombardi et al. 2006, Zarain-Herzberg et al. 2010, Radulovich & Fuentes-Quesada 2019). Postlarvae have been stocked at 80-700 m^{-2} for the nursery stage, while stocking rates for grow-out have varied from 10 to 550 m^{-2} using a wide variety of cages regarding shape, size, and materials (Paquotte et al. 1998, Cuvin-Aralar et al. 2009, Effendi et al. 2016, Radulovich & Fuentes-Quesada 2019). Intensifying shrimp (*Penaeus vannamei*) farming in floating cages requires high stocking rates, which has led to yields comparable to those reported for shrimp produced in intensive ponds or raceways (Zarain-Herzberg et al. 2010, Bardera et al. 2021, Suwoyo & Hendrajat 2021).

Managing high stocking densities can significantly impact shrimp basal oxidative stress, health, and risk of production losses (Arnold et al. 2006, Tu et al. 2008, Arambul-Muñoz et al. 2019). Many diseases have been linked to environmental deterioration and stress associated with farm intensification, resulting in a major constraint to sustainable growth and economy for shrimp

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aquaculture (Shang et al. 1998, Kumar & Engle 2016). High stocking densities are believed to disrupt homeostasis and cause significant stress in farmed organisms (de la Vega et al. 2007); nonetheless, physiological responses, such as oxidative stress in shrimp intensively reared in marine floating cages, have not been investigated.

Knowledge of antioxidant response, such as super-oxide dismutase (SOD), catalase (CAT), and protein content in tissues, e.g. hemolymph, hepatopancreas, and muscle of farmed shrimp, is helpful for stress indication (Tu et al. 2010). Oxidative stress in aquatic animals occurs when steady-state reactive oxygen species (ROS) concentration has transiently or chronically increased, disturbing cellular metabolism and damaging cellular constituents (Lushchak 2011).

Oxidative stress broadly includes biological pheno-mena associated with ROS generation and molecular mechanisms protecting or repairing cells from oxidative damage (Brol et al. 2021). Reactive oxygen species are usually produced in living organisms during normal aerobic metabolism and immune response (Guo & Dixon 2021). Several stressing conditions increase their production, resulting in oxidative stress inside the cell (Zenteno-Savín et al. 2006, Guo & Dixon 2021), and the antioxidant defense mechanisms counteract ROS toxicity (Li et al. 2016).

Protein content in shrimp muscle is governed by many factors, including species, growth stage, molting process, feed, and season (De Oliveira-Cesar et al. 2006, Sriket et al. 2007). In this regard, related stress proteins (i.e. antioxidant enzymes) are involved during environ-mental stress that sometimes results in a shrimp disease outbreak (Li & Xiang 2013).

According to the reviewed literature, no previous evidence of stressing effect in reared shrimp at different densities in floating cages has been published. Thus, this study aims to analyze the effect of high stocking density and rearing time on shrimp *P. vannamei* protein content and antioxidant enzymatic activity in muscle, hepatopancreas, and hemocytes when raised intensi-vely in seawater floating cages.

MATERIALS AND METHODS

Study area and experimental conditions

This study was part of a 58 day experimental *Penaeus vannamei* intensive grow-out in floating cages conducted in Bahia de Navachiste (25°29'8.5"N, 108°52'38.64"W), Sinaloa, Mexico.

For farming, 9 m² (3×3×1 m) cages were cons-tructed with a floating support frame made of polyvinyl chloride (PVC)-tubes and PVC-covered polyester nets

(Sansuy®, São Paulo, BR) with a mesh size of 1.5×2.5 mm. Sanitary certified postlarvae (SyAqua-USA nine-year-old broodstock) from a local hatchery (SyAqua México S. de R.L. C.V.) were used for the experimental trial. Postlarvae weighed from 0.012 g (PL18) to 0.04 g (PL26) and were transported to the farming site at 20°C water temperature. For acclimatization, the water temperature was increased at a 2°C h⁻¹ rate until the farming site temperature of 23°C was reached.

After 38 days of the nursery stage, juveniles (0.5-1 g) were transferred for grow-out using stocking densities of 200, 250, and 300 ind m⁻² by triplicate in 9 m² cages. Commercial 2 mm-pellets (Malta Cleyton® 35% protein, CDMX, MX) were used for feeding. Feed was offered twice a day (at sunrise and sunset) in amounts recommended by the manufacturer. Further adjustments were made when feed accumulated at the bottom of the cages (particularly during the molting stages) to minimize feed waste. The feed composition was: protein (35.03%), fat (9.04%), fiber (2.84%), humidity (8.84%), ash (6.09%) and nitrogen-free extract (38.16%). The PVC tubes and cage nets were cleaned by hand every two weeks to facilitate water renewal. Further details of the experimental trial are explained in Zarain-Herzberg et al. (2010).

Sample collection of muscle, hepatopancreas, hemo-cyte, and sample preparation

Random samples of nine shrimp were collected from each treatment after 1, 8, 15, and 25 days of grow-out (i.e. a total of 36 shrimp for each treatment). Each shrimp muscle (2 g) and hepatopancreas samples were collected and stored at -80°C until use. After eight days of rearing, hemolymph (~100-600 µL) was collected from the pleopod base of the first abdominal segment, near the genital pore. Hemolymph was obtained using insulin or a 27-gauge hypodermic needle on a 1.0 or 3.0 mL syringe, containing 400-600 µL of cooled (4°C) anticoagulant solution with ionic and osmotic shrimp hemolymph strength values (450 mM NaCl, 10 mM KCl, 10 mM EDTA-Na₂, 10 mM HEPES, pH 7.3, 850 mOsm kg⁻¹) to obtain 600-1000 µL of total hemolymph (Campa-Córdova et al. 2002). The hemolymph was placed in sterile glass tubes, kept in an ice bath during sampling, and transferred to Eppendorf tubes (Eppendorf AG, Hamburg, DE) for centrifugation (5724 g at 4°C for 5 min). The supernatant was discarded, and 100 µL anticoagulant was added to each tube for cell suspension and stored at -80°C until use.

For cell disruption, 100 mg of frozen shrimp muscle, hepatopancreas, or 100 µL of hemocyte sus-pension were added to a mechanical homogenizer containing 0.5 mL of phosphate buffer (50 mM, pH 7.8). The homogenate was centrifuged (5724 g at 4°C

for 5 min), and the supernatant was recovered and heated at 65°C for 5 min. After the second centrifugation (crude extract), a new supernatant was obtained and stored at -80°C. Samples were permanently maintained on ice throughout the process.

Protein content analysis and superoxide dismutase and catalase activity assays

Total soluble protein concentration in each crude extract was measured according to Bradford (1976), with serum albumin as a standard. Each sample was analyzed by triplicate and expressed as mg mL^{-1} of protein content. These data were used for comparing stocking density and elapsed rearing time effects on protein content.

Following Beauchamp & Fridovich (1971), manganese superoxide dismutase activity was determined using nitro-blue tetrazolium (NBT) in the presence of riboflavin. For this purpose, 2 mL of the reaction mixture (0.1 mM EDTA, 13 µM methionine, 0.75 mM NBT, and 20 µM riboflavin in 50 mM phosphate buffer at pH 7.8) and 0-100 mL of crude extract were placed under fluorescent light for 2 min or until A$_{560}$ reached an optical density value of 0.2-0.25 in control tubes. The specific activity (units per mg of protein) was calculated using the computer program described by Vázquez-Juárez et al. (1993). The specific SOD activities from shrimp groups (200, 250, and 300 ind m^{-2}) were used for comparing stocking density and elapsed rearing time effects on SOD activity. The samples were analyzed by triplicate, and SOD activity was expressed as U mg^{-1} of protein.

Catalase activity was measured using the method by Aebi (1984), following the decrease in absorbance at 240 nm caused by hydrogen peroxide (H$_2$O$_2$) disappearance in 1 min and expressed in U mg^{-1} of protein. Each sample was analyzed in triplicate. Stocking densities and elapsed rearing time effects on CAT activity were compared using the specific CAT activity from each shrimp group.

Zootechnical parameters

The specific growth rate (SGR) was estimated as G in the exponential equation (Hopkins 1992): shrimp growth was determined by weighing a 100-shrimp random sample from each cage with a digital balance (Ohaus Scout Pro C200, 0.01 g precision, Parsippany, NJ) at 1, 8, 15 and 25 days. Survival was estimated for the same periods from the initial and surviving populations at the end of the grow-out trial (58 days), assuming a constant daily mortality rate. Biomass was calculated as a percent survival function of stocking density and mean individual shrimp weight. Feed conversion ratio (FCR) was estimated as feed offered/ estimated biomass ratio:

$$w_t = w_i e^{Gt}$$

where w_t is mean shrimp weight as a function of time t, and w_i is the initial shrimp weight. The absolute growth rate (AGR) was also estimated as:

$$AGR = (w_f - w_i)/t$$

where w_f is the final shrimp weight and time is in weeks.

Statistical analyses

Two-way ANOVA of protein concentration and enzymatic activity over rearing time was used to detect differences in stress indicator values among the stocking density groups. Following Zar (2010), the lack of independence in the repeated-measure variables was corrected with the Huynh-Feldt factor. Multiple comparisons among density or time levels were conducted when any of the factors were significant, and no significant interaction between them was observed. The factors' levels were not compared when the interaction was significant. Instead, multiple compa-rison testing was performed among cell (density-time combinations) mean values. Tukey's *post-hoc* test was used to separate mean values.

One-way ANOVA was used for detecting possible differences in zootechnical parameter mean values. Previously to analysis, percentage survival values were transformed to arcsine values using Zar (2010) tables. Tukey's *post-hoc* test separated mean values when ANOVA was significant. Possible differences in SGR were analyzed using nonlinear regression and an invariance test (Ratkowsky 1983), determining whether the stocking rate effect is reflected in changes in *G* values of the exponential equation. The invariance test is based on the principle referred to as the "extra sum of squares" (Draper & Smith 1981).

The normality and variance homogeneity assumptions were tested with Shapiro-Wilk's and Bartlett's tests. Stata 17.0 (StataCorp, College Station, TX, USA), Statistica 6.0 (StatSoft, Inc., Tulsa, OK, USA), and GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA) were used for analysis, setting significance at α = 0.05.

RESULTS

Water quality parameter values observed during the 25-day grow-out trial are shown in Table 1. The two-way ANOVAs showed significant density and rearing time effects on stress indicators (Tables 2-3).

Table 1. Minimum, maximum, and mean ± standard error (SE < values of water quality parameters observed during *Penaeus vannamei* study) (Zarain-Herzberg 2010).

Parameter	Minimum	Maximum	Mean
Dissolved oxygen (mg L^{-1})	3.6	4.9	4.2 ± 0.34
Temperature (°C)	22.7	31.6	28.6 ± 3.33
pH	7.9	8.2	8.02
Salinity	35	39	37.0 ± 1.03

Table 2. Effect of stocking density (D) and rearing time (T) on *Penaeus vannamei* stress indicators and tissues. Means ± standard errors with different letters indicate significant differences (*P* < 0.05). SOD: superoxide dismutase, CAT: catalase, HP: hepatopancreas, HC: hemocytes, ND: Not determined.

Factors	Protein (mg mL^{-1})			SOD (U mg^{-1})	CAT (U mg^{-1} in thousands)		
	Muscle	HP	HC	HP	Muscle	HP	HC
Density (ind m^{-2})							
200	6.01 ± 0.64	3.81 ± 0.25a	5.89 ± 1.69	18.90 ± 1.99	0.88 ± 0.16	4.25 ± 0.06a	3.08 ± 0.70
250	7.23 ± 0.64	2.57 ± 0.32b	4.36 ± 1.37	23.11 ± 3.52	1.48 ± 0.89	4.17 ± 0.06ab	11.75 ± 4.94
300	7.67 ± 1.29	2.26 ± 0.31b	7.02 ± 2.01	27.14 ± 6.17	1.02 ± 0.35	4.10 ± 0.05b	7.53 ± 4.45
Time (d)							
1	7.59 ± 1.05ab	2.92 ± 0.57	ND	19.90 ± 4.85	0.69 ± 0.07	4.26 ± 0.04^a	ND
8	9.04 ± 1.20a	3.18 ± 0.25	5.73 ± 1.54a	19.58 ± 2.95	0.41 ± 0.09	3.90 ± 0.04b	8.66 ± 3.22
15	6.50 ± 0.69ab	3.13 ± 0.38	10.52 ± 0.95b	21.73 ± 6.55	0.59 ± 0.02	4.24 ± 0.02a	9.06 ± 5.32
25	4.74 ± 0.62b	2.29 ± 0.36	1.02 ± 0.53c	30.98 ± 4.62	2.82 ± 1.17	4.31 ± 0.04a	4.64 ± 3.03
Two-way ANOVA (*P*-values)							
D	0.341	0.033	0.310	0.696	0.734	0.030	0.378
T	0.028	0.151	1.15×10^{-05}	0.104	0.099	1.54×10^{-08}	0.726
Interaction							
(D × T)	0.190	0.084	0.940	0.086	0.644	0.450	0.927

Protein content

No significant interaction was observed between density and rearing time in the protein content of the tissues analyzed (Table 2). Density affected content significantly in hepatopancreas (*P* = 0.033) where the maximum (3.81 mg mL^{-1}) and minimum (2.26 mg mL^{-1}) concentrations occurred when stocking 200 and 300 ind m^{-2}, respectively. A significant effect of rearing time on protein content was detected in muscle (*P* = 0.028) and hemocytes (*P* = 1.15×10^{-5}), where maximum concentrations occurred after eight (9.04 mg mL^{-1}) and 15 (10.52 mg mL^{-1}) days, respectively. After that, protein content diminished, reaching minima after 25 days, at the end of the study period (4.74 and 1.02 mg mL^{-1}, respectively).

No significant influence of stocking density was recorded on protein content in muscle (*P* = 0.341, 6.01-7.67 mg mL^{-1}) and hemocytes (*P* = 0.310, 4.36-7.02 mg mL^{-1}). No evidence was obtained of protein content in hepatopancreas significantly changing during the rearing period (*P* = 0.151, 2.29-3.18 mg mL^{-1}).

Superoxide dismutase activity (SOD)

SOD activity in muscle was significantly (*P* = 0.027) influenced by density and rearing time interaction (Table 3) and varied significantly during the rearing period (*P* = 0.025), although the stocking rate did not have a significant influence (*P* = 0.511). Tukey's *post-hoc* test showed that the factor interaction caused more conspicuous changes in enzymatic activity occurring within the highest stocking rates with a clear tendency to increase from the lowest activity (1.56 U mg^{-1}) at the start of the trial to the maximum activity by the end of the study period (10.21 U mg^{-1}). The rest of the activity values were similar throughout the rearing period.

A significant interaction effect between stocking density and rearing time was obtained on SOD activity in hemocytes (*P* = 0.002, Table 3). Both factors significantly affected SOD activity in this tissue when they were analyzed separately (*P* = 0.010, *P* = 0.0009, Table 3). Evident changes in enzymatic activity occurred until day 25 when the effect of both factors influenced it, and their interaction resulted in the highest stocking rates showing the maximum activity values (83.86 and 122.41 U mg^{-1}).

No evidence was found of a significant interaction between stocking rate and rearing time affecting SOD activity in the hepatopancreas (*P* = 0.086). Moreover, no significant effects were detected when both factors were analyzed separately (*P* = 0.696, *P* = 0.086), and the overall activity ranged from 18.9 to 30.98 U mg^{-1} (Table 2).

Table 3. Effect of stocking density (D) (ind m^{-2}) and rearing time (T) (days) on *Penaeus vannamei* superoxide dismutase activity (U mg^{-1}) in muscle and hemocytes. Means ± standard errors with different letters indicate significant differences ($P < 0.05$).

Cells (D:T)	Muscle	Cells (D:T)	Hemocyte
300:1	1.56 ± 0.25[a]	250:8	0.68 ± 0.25[a]
300:8	1.95 ± 0.23[a]	250:15	0.69 ± 0.11[a]
250:8	3.22 ± 0.69[a]	200:15	0.70 ± 0.20[a]
250:1	3.75 ± 1.44[a]	300:15	0.73 ± 0.18[a]
200:15	3.90 ± 0.23[ab]	300:8	1.63 ± 0.17[a]
200:1	3.91 ± 1.97[ab]	200:8	6.06 ± 2.15[a]
250:25	4.11 ± 0.48[ab]	200:25	6.30 ± 2.71[a]
250:15	4.32 ± 0.88[ab]	250:25	83.86 ± 24.41[b]
200:25	4.61 ± 0.90[ab]	300:25	122.41 ± 17.02[b]
200:8	5.06 ± 2.19[ab]		
300:15	5.78 ± 0.24[ab]		
300:25	10.21 ± 2.39[b]		
Two-way ANOVA (*P*-values)			
D	0.511		0.010
T	0.025		8.0×10^{-4}
Interaction (D × T)	0.027		0.002

Catalase (CAT) activity

There was no significant interaction between stocking density and rearing time influencing CAT activity in the tissues analyzed (Table 2). Furthermore, no significant differences in mean CAT activity were observed in muscle when both factors were analyzed separately ($P = 0.734$, $P = 0.099$); the overall activity varied within 412.4-2 820.8 U mg^{-1} (Table 2).

The activity in hepatopancreas was significantly affected by stocking density ($P = 0.030$), where the minimum (4103.2 U mg^{-1}) occurred when the highest density was used, and the maximum (4252.3 U mg^{-1}) was obtained by stocking at the lowest one (Table 2). The activity in hepatopancreas varied significantly during the rearing period ($P = 1.54×10^{-08}$) with the minimum activity occurring after eight days (3896.79 U mg^{-1}) and maximum after 25 days (4306.2 U mg^{-1}) (Table 2).

There was no significant CAT activity in hemocytes among the stocking density levels varying within 3075.9-11,747.3 U mg^{-1} among the stocking densities ($P = 0.378$, Table 2). Similarly, CAT activity did not vary significantly during the rearing period ($P = 0.726$), ranging from 4640.5 to 9062.5 U mg^{-1} (Table 2).

Zootechnical parameters

No significant effects of stocking density on final shrimp weight, survival, FCR, SGR, and AGR were observed, although shrimp biomass varied significantly, ranging from 0.99 to 1.49 kg m^{-2} (Table 4). The growth curves observed during the study period are shown in Figure 1, where fitting the exponential equation resulted adequate, and the invariance test showed that SGR did not differ significantly among the stocking rates.

DISCUSSION

This investigation showed that stocking density had a stressing effect on *Penaeus vannamei* when reared intensively in floating cages, and the strength of the effect varied significantly throughout the period analyzed. The results in this study agree with Li & Brouwer (2009) and Trasviña-Arenas et al. (2013), who noted that the dynamics of antioxidant enzymatic indices might demonstrate varied time-courses.

In this report, stress indicators rendered similar sensitivity regarding the number of tissues where the significant influence of stocking rate occurred (one tissue for every indicator) and the variability of the effect during the study (two tissues for all the indicators). However, the SOD activity was particularly sensitive when muscle and hemocytes were analyzed, and significant synergistic interactions occurred between stocking rate and rearing time. The stressing effect was higher in both tissues by the end of the study, but the effect was much more amplified at the densest stocking rate.

In this investigation, SOD activity was the most reliable indicator considering its sensitivity to stress

Table 4. Effect of stocking density on *Penaeus vannamei* zootechnical parameters after 25 days of grow-out. Means ± standard errors with different letters indicate significant differences ($P < 0.05$). FCR: feed conversion ratio, SGR: specific growth rate, AGR: absolute growth rate.

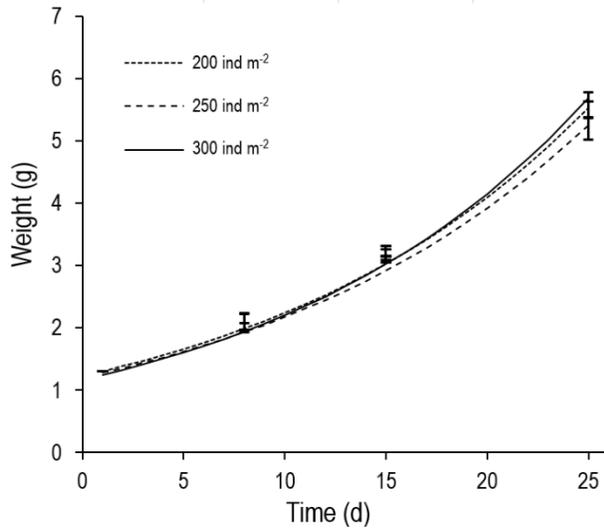| Stocking density (ind m^{-2})	Final weight (g)	Survival (%)	Biomass (kg m^{-2})	FCR	SGR	AGR
200	5.41 ± 0.14	91.90 ± 0.59	0.99 ± 0.026a	0.78 ± 0.023	0.060 ± 0.003	1.25 ± 0.038
250	5.28 ± 0.18	91.54 ± 0.30	1.21 ± 0.036b	0.84 ± 0.034	0.058 ± 0.003	1.17 ± 0.049
300	5.49 ± 0.07	90.26 ± 0.95	1.49 ± 0.036c	0.75 ± 0.012	0.063 ± 0.002	1.30 ± 0.020
P-value	0.57	0.27	8.59E-05	0.13	0.56	0.10

Figure 1. Shrimp growth curves after 25 days of grow-out testing different stocking rates. Data represent mean ± standard error individual weight. The exponential equation fitting to each data set for specific growth rate estimation is indicated.

and positive correlation between higher values and stocking rate (in hemocytes) and rearing duration (in muscle and hemocytes). In contrast, overall protein content and CAT activity showed significantly lower hepatopancreas values when using the highest stocking rate or at the end of the study (protein content in muscle and hemocytes, and CAT activity in hemocytes).

Stress provokes an improvement in antioxidative efficiency in shrimp (Tovar-Ramírez et al. 2010, Lushchak 2011, Medina-Félix et al. 2017). Thus, according to the hypothesis of "preparation to oxidative stress" (Hermes-Lima et al. 1998), lower protein content may indicate improved antioxidant activity efficiency where a low protein concentration is compensated by highly effective antioxidant enzymatic activity. This phenomenon may have occurred in this study, where low protein content was observed under highly stressful rearing conditions.

The regulation of the redox system is mainly integrated by antioxidant enzymes such as SOD, CAT, and glutathione peroxidase which has been deemed multifactorial and nested (Regoli et al. 2011, Trasviña-Arenas et al. 2013). Any of those enzymes' decreasing or increasing activity may be compensated by the subsequent increasing or decreasing activity of the other two (Hermes-Lima et al. 1998).

Stress by rearing conditions is well-known to provoke antioxidant or immune response changes in shrimp (Li et al. 2006, Tovar-Ramírez et al. 2010, Lushchak 2011, Medina-Félix et al. 2017). However, the literature is scarce, specifically addressing shrimp antioxidant and immune response to stocking density. Lin et al. (2015) studied antioxidant, and immune parameters in *P. vannamei* (10.4-14.1 g) reared in 100 L fiberglass circular tanks at 2, 10, 20, 30, and 40 ind L^{-1} after 3, 6, and 12 h. The authors found that exposure of pre-adult shrimp to high stocking densities reduced SOD activity and immune response, showing the importance of maintaining proper stocking densities to prevent decreased immunity and avoid stressing rearing conditions. Similar to those studies, in this investigation, stressing rearing conditions resulted in lower protein content and antioxidant CAT activity in 5 g shrimp hepatopancreas and hemocytes.

The evaluation of oxidative stress markers is critical in investigating oxidative stress in aquatic organisms (Li & Brouwer 2009, Wang et al. 2009, Cottin et al. 2010, Lushchak 2011). The metabolic pathways in cells are influenced by antioxidant enzymes, which may be valuable biomarkers of shrimp's general health status (Tu et al. 2012, Oliveira et al. 2018). The SOD enzyme has been considered a multi-biomarker system that could monitor shrimp *P. vannamei* under stressful rearing conditions (Zhou et al. 2010, Brol et al. 2021). The results from this investigation confirm the usefulness of SOD activity as a sensitive indicator of stressing conditions for *P. vannamei*, mainly when reared at high stocking rates. In this study, SOD activity was the best stress indicator detecting the interactions between stocking rate and rearing time.

Increased SOD activity has been previously reported in shrimp hemocytes and hepatopancreas, the primary sites where immune reactions occur (Zhou et al. 2010, Tassanakajon et al. 2013). In agreement with those studies, in this investigation, hemocytes were the tissue showing the highest sensitivity to the stressing effects of stocking density and rearing time when SOD activity was used as a stress indicator.

The effects of stocking density on *P. vannamei* zootechnical parameters have been extensively studied under a wide variety of rearing conditions, thus making it difficult to compare results among reports. No significant differences were detected in growth, survival, and feed conversion ratio among the stocking rate groups in this investigation. Moreover, no differences were observed in those parameters after day 58 when the grow-out trial concluded (Zarain-Herzberg et al. 2010); the mean growth rate was 1.2 g week[-1] and feed conversion ratio was 0.88, which were similar to the mean values observed during the period covered in this report (1.24 g week[-1] and 0.79).

Shrimp growth rates as high as 1.75 g week[-1] have been observed when reared in floating cages (Zarain-Herzberg et al. 2006). Other studies using cages for rearing *P. vannamei* have reported (g week[-1]): 0.8 (Paquotte et al. 1998), 0.77 to 1.68 (Cuvin-Aralar et al. 2009), 1.05-1.33 (Effendi et al. 2016), 0.84-1.26 (Setiawan et al. 2017), and 1.03-1.16 (Radulovich & Fuentes-Quesada 2019). On the other hand, *P. vannamei* shrimp feed conversion ratio in cages varied within 2.58-3.15 (Paquotte et al. 1998), 1.35-3.7 (Lombardi et al. 2006), 1.14-1.63 (Cuvin-Aralar et al. 2009), 76-3.43 (Effendi et al. 2016), 1.18-1.65 (Setiawan et al. 2017), and 1.79-1.93 (Radulovich & Fuentes-Quesada 2019).

In nature, shrimp feed on bacteria, microalgae, protozoa, and detritus suspended in the water (Gleason & Wellington 1988, Thompson et al. 2002, Zarain-Herzberg et al. 2006). When reared in cages, shrimp grazing on cage surfaces significantly satisfies their nutritional requirements (Abreu et al. 2007). Setiawan et al. (2017) studied the effects of artificial substrates and stocking density on *P. vannamei* growth when reared in floating cages and concluded that the substrates in the cages have a positive influence on shrimp because microorganisms growing on the substrate surface can be used as a natural feed source for shrimp.

Natural feed available in the cages and careful monitoring of pelleted feed consumed by the shrimp is the main cause for the shrimp growth rate and feed conversion ratio obtained in this investigation, which compare well with those reported by other studies using floating cages. The composition and quality of the pelleted feeds used could also contribute to determining differences in those parameters when comparing grow-out trials in floating cages. Unfortunately, no information is provided on diet composition in those reports. Overall, from reviewing the previously cited investigations, the main factors affecting zootechnical shrimp parameters are stocking density and availability of natural feed in cage surfaces and artificial substrates.

Despite the evident stressful conditions caused by high shrimp population density in the cages, the zootechnical parameters in this study were satisfactory. The yields obtained at the end of the grow-out trial were as high as 2.6 kg m^{-2}, comparable to those obtained in intensive inland systems (Zarain-Herzberg et al. 2010).

CONCLUSIONS

Evidence has confirmed that stocking density and rearing time have a stressing effect on *P. vannamei* juveniles when reared intensively in a floating-cage system. However, the stocking rate did not affect shrimp growth, survival, and FCR, most likely due to the cellular antioxidant system in shrimp preventing the negative effect of oxidative stress. The antioxidant response varied among tissues at higher densities and at the end of the study, and SOD activity in hemocytes showed being the most sensitive stress indicator for *P. vannamei* juveniles.

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