

1 **Effect of Different Stocking Density to the Growth Performance of Pacific**
2 **White Shrimp *Litopenaeus vannamei* Cultured in Concrete Tank Using**
3 **Air Fine Disc Diffuser**

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23 **Abstract**

24

25 **Key words:** *Density, Growth performance, Concrete tank, Litopenaeus vannamei*

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46 **1. Introduction**

47 Recently, most of the shrimp farms, especially in Indonesia, has shifted from extensive or
48 semi-intensive ponds to the intensive and even supra-intensive technology system with high
49 stocking density ranging from 110 – 500 shrimp/m² or intensive and >500 shrimp/m² for supra-
50 intensive farming system (Zulkarnain et al., 2020). There are advantages and disadvantages of
51 using (supra) intensive technology. According to Samocha (2019), high stocking density of
52 shrimp in intensive system will lead to greater yields and more efficient in the use of culture
53 environment. However, high inputs of nutrients and limitation on water exchange will create
54 water quality problems that do not always arise in traditional or semi-intensive farming system
55 (Samocha, 2019). If not quickly and correctly addressed, it will lead to the increase of ammonia-
56 nitrogen and nitrite-nitrogen levels (Jescovitch et al. 2018), development of dense microbial
57 community (Ray et al., 2011), outbreaks of shrimp diseases due to the number of contacts
58 (Kautsky et al., 2000; Ruiz-Velazco et al., 2010) and loading of oxygen-consuming organic
59 matter (Direkbusarakom et al., 1998).

60 The negative correlation between stocking density and growth performance of shrimp has
61 been well established (Sandifer et al., 1996; Moss and Moss. 2004; Arnold et al. 2009). In the
62 biofloc Technology (BFT) trial, density of 300 shrimp/m² exhibited superior zootechnical
63 performance compared to 450 shrimp/m² (Krummenauer et al., 2011). Meanwhile, the growth
64 and survival of Vannamei cultured in three different densities (100, 200 and 300 shrimp/m²)
65 using sand ponds with plastic mulch decreased with increasing density (Samadan et al. 2018). In
66 addition, using zero water discharge (ZWD)-recirculating aquaculture system, the optimum
67 performance based on survival, feed conversion ratio (FCR), and productivity was reached at the
68 lowest density of 500 shrimp/m³ compared to the 750 and 1000 shrimp/m³ (Suantika et al. 2018).

69 Interestingly, with proper supplementation of amino acid to fulfill the specific nutrient
70 requirement of *Vannamei*, the increase in stocking density significantly increased FCR and yield,
71 while reducing shrimp weekly growth and final body weight (Façanha et al., 2016). Therefore,
72 proper combination of stocking density and water quality management system to increase the
73 environmental carrying capacity could a promising strategy to enhance the productivity.

74 The degradation of environmental carrying capacity conditions, especially dissolved
75 oxygen (D.O) levels, seems to increase with the increases of shrimp density in the ponds. The
76 occurrence of low DO in Southern Brazil was closely associated with shrimp density, where the
77 mean of DO recorded at the density of 450 shrimp/m², 1,32 mg/L, was lower than 300 shrimp/m²
78 at the level of 2,02 mg/L (Krummenauer et al., 2011). In addition, during spring-summer time of
79 culture period, the lowest DO level was also found in the highest stocking density (Mena-Herrera
80 et al., 2006). Brock and Main (1994) suggested that the D.O level of 0 – 1,5 mg/L are lethal for
81 shrimp and level of 1,7 – 3,0 mg/L resulting in low feed conversion and slow growth. As shrimp
82 technology advances, greater productivity and efficiency with respect to density and water
83 quality conditions are required. The aim of this study was to investigate the effects of density and
84 water quality conditions supplied with air fine disc diffusers on the growth performance of pacific
85 white shrimp *Litopenaeus vannamei* cultured in the concrete tank.

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87 **2. Material and methods**

88 This study was performed at the Batam Nara Indonesia (Batam, Riau Island Province,
89 Indonesia). Pacific white shrimp (*L. vannamei*) were obtained from Suri Tani Pemuka (Anyer,
90 West Java, Indonesia), acclimated and nursed in an indoor nursery system for 20 days until
91 reaching a suitable size. At the start of the production trial, juvenile shrimp (mean initial weight

92 of 0,3 g) were then stocked into 32 semi-indoor concrete tanks (8 x 8 x 1 m) at 4 (four) different
93 density: 300, 400, 500 and 600 shrimp/m² over 65 days production period. Tanks were filled with
94 saline water (30 – 33 ‰) and supplied with air disc fine bubble diffuser as the primary source of
95 mechanical aeration and one 0,5-hp paddlewheel (MinipaddTM) for additional aeration system.
96 Water exchange was performed 5 – 10% throughout the trial for 65 days of culture.

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98 2.1 Feed management

99 All ponds were offered with the same diet (33 – 35% crude protein, 5% crude lipids)
100 produced by Evergreen (Indonesia Evergreen Agriculture, Lampung Selatan, Indonesia)
101 throughout the growth trial. The amount of feed used in this experiment was calculated based on
102 expected weight gain of 1 g/wk, a feed conversion ratio (FCR) of 1,4 and a weekly mortality of 3
103 % during grow-out period. During the trial, shrimp were fed six times per day and the daily
104 ration was adjusted based on percentage of body weight after sampling the shrimp on weekly
105 basis

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107 2.2 Growth sampling and water quality

108 Shrimp were sampled weekly through the remaining production cycle using hand net (0,5
109 m in diameter and 1 cm mesh) to collect approximately 20 – 30 individuals per tank. Water
110 quality (Dissolved oxygen, pH, temperature, salinity, total dissolved solids, conductivity and
111 oxidative redox potentials) were monitored four times a day (06.00 – 07.00 h; 14.00 – 15.00 h;
112 17.00 – 18.00 h and 23.00 – 24.00 h) using real-time water quality sensors (Aqua Troll 500, In-
113 Situ Inc., Fort Collins, CO, USA) and managed by AquaEasy Smart Aquaculture apps (BOSCH,
114 Singapore). Secchi disk readings were recorded once a week and ammonia nitrogen (NH₃-N)

115 analyzed with Ultraviolet/Visible spectrophotometer (PerkinElmer, Lambda XLS, USA) once a
116 week. Meanwhile, nitrite nitrogen (NO₂-N) and nitrate nitrogen (NO₃-N) were analyzed using
117 HACH DR890 colorimeter (Hach Company, Loveland, CO, USA) twice a week. At the end of
118 growth trial, shrimp were harvested totally, then counted and batched weighed to calculate the
119 final biomass, final weight, percentage weight gain (PWG), feed conversion ratio (FCR),
120 percentage survival (SR), and voluntary feed intake (VFI),

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$$123 \quad \text{PWG} = \frac{(\text{average individual final weight} - \text{average individual initial weight})}{(\text{average individual initial weight})} \times 100$$

$$124 \quad \text{FCR} = \frac{\text{feed given (g)}}{\text{alive weigh gain (g)}}$$

$$125 \quad \text{SR} = \frac{\text{final number of shrimp}}{\text{initial number of shrimp}} \times 100$$

$$126 \quad \text{VFI} = \frac{\text{feed intake (g)}}{\text{shrimp}}$$

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129 2.3 Statistical analysis

130 All growth parameters were analyzed using one-way analysis of variance (ANOVA) to
131 determine the significant differences among treatments followed by Tukey's multishrimpe
132 comparison tests to determine the difference between treatment means in each trial. All statistical
133 analyses were conducted using SAS system (V9.4. SAS Institute, Cary, NC, USA).

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137 **Results**

138 *3.1 Water quality*

139 During the growth trial, the levels of dissolved oxygen, pH, total dissolved solids,
140 oxidative redox potentials, salinity and temperature presented in **Table 1** are within the
141 acceptable range to support the optimal growth of Pacific white shrimp *L. vannamei* (Boyd and
142 Tucker, 1992). The ammonia level ranged from $0,22 \pm 0,09$; $0,21 \pm 0,12$; $0,30 \pm 0,27$; and $0,29 \pm$
143 $0,44$ for 300 shrimp/m²; 400 shrimp/m²; 500 shrimp/m² and 600 shrimp/m², respectively.
144 Meanwhile for NO₂-N and NO₃-N parameter, there is an increasing trend for the concentration
145 level as the shrimp density increases.

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147 *3.1 Growth Trial*

148 The density treatments affected the growth performance of the shrimp (Table 3). Final
149 weight (g), weight gain (g) and the ADG of the shrimp cultured in the density of 300
150 Shrimp/m² were significantly higher compared to shrimp cultured in higher density ($P < 0.05$).
151 However, in terms of biomass, the density of 300 shrimp/m² yielded the lowest quantity
152 compared to higher density treatments. The feed input increases as the density increases
153 ($P < 0.05$). The FCR and survival rate (%) were not significantly different among the dietary
154 treatments ($P < 0.05$).

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156 **Discussion**

157 The results of this study indicated that the *L. vannamei* juvenile grow faster if they are
158 rearing at the lower density. Our study indicated that the growth of 0.17 ± 0.02 g/d was obtained
159 at the stocking density of 300 shrimp/m², which is higher than other density treatments. This

160 result similar to the previous reports by Krummenauer et al. (2011) and Suantika et al. (2018).
161 According to Samocha (2019), stocking density has a significant effect to determine the carrying
162 capacity of the tank and crucial for optimizing feed and water quality during the culture period.
163 In addition, McGraw et al. (2001) suggest that the production yields and profitability of
164 *Vannamei* increases as the carrying capacity and water quality condition increased.

165
166 However, evidence showed that the higher biomass will be obtained when *Vannamei* cultured
167 with 600 Shrimp/m² compared to other treatments when rearing intensively in the concrete tank.
168 XX (20XX) reported that carrying capacity is really important to

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255 **Table 1.** Summary of water quality characteristics for the four treatments over 65 d of culture
 256 period. Data obtained by using sensors and recorded using AquaEasy Smart Aquaculture apps
 257 (BOSCH, Singapore). Values are presented as mean \pm standard deviation of eight replicates per
 258 treatment.

	Treatments			
	300 shrimp/m ²	400 shrimp/m ²	500 shrimp/m ²	600 shrimp/m ²
Dissolved Oxygen (mg/L)				
<i>06.00 – 07.00 h</i>	4,76 \pm 0,14	4,82 \pm 0,16	4,72 \pm 0,33	4,89 \pm 0,26
<i>14.00 – 15.00 h</i>	4,99 \pm 0,22	4,92 \pm 0,18	4,75 \pm 0,17	4,70 \pm 0,25
<i>17.00 – 18.00 h</i>	5,09 \pm 0,24	4,99 \pm 0,16	4,76 \pm 0,24	4,72 \pm 0,19
<i>23.00 – 24.00 h</i>	4,76 \pm 0,33	4,81 \pm 0,27	4,75 \pm 0,33	4,89 \pm 0,24
pH				
<i>06.00 – 07.00 h</i>	7,33 \pm 0,14	7,31 \pm 0,28	7,29 \pm 0,17	7,28 \pm 0,09
<i>14.00 – 15.00 h</i>	7,35 \pm 0,19	7,25 \pm 0,23	7,23 \pm 0,23	7,18 \pm 0,11
<i>17.00 – 18.00 h</i>	7,44 \pm 0,20	7,24 \pm 0,28	7,41 \pm 0,26	7,37 \pm 0,19
<i>23.00 – 24.00 h</i>	7,29 \pm 0,17	7,18 \pm 0,38	7,38 \pm 0,28	7,24 \pm 0,11
Total Dissolved Solids (mg/L)				
<i>06.00 – 07.00 h</i>	31,03 \pm 0,11	32,14 \pm 2,31	32,19 \pm 0,35	32,28 \pm 0,49
<i>14.00 – 15.00 h</i>	29,31 \pm 2,14	31,27 \pm 0,19	32,19 \pm 0,43	32,32 \pm 0,51
<i>17.00 – 18.00 h</i>	30,04 \pm 1,19	30,12 \pm 2,52	31,57 \pm 1,63	31,62 \pm 4,47
<i>23.00 – 24.00 h</i>	30,33 \pm 1,28	31,39 \pm 0,29	32,39 \pm 0,88	32,44 \pm 0,31
Oxidative Redox Potential (V)				
<i>06.00 – 07.00 h</i>	251,89 \pm 32,09	263,28 \pm 23,66	261,78 \pm 32,61	262,49 \pm 25,87
<i>14.00 – 15.00 h</i>	259,27 \pm 23,37	264,25 \pm 42,22	260,33 \pm 11,89	267,14 \pm 30,36
<i>17.00 – 18.00 h</i>	260,14 \pm 22,58	262,55 \pm 19,29	262,47 \pm 27,73	261,23 \pm 26,55
<i>23.00 – 24.00 h</i>	258,32 \pm 41,55	263,18 \pm 29,71	262,31 \pm 44,03	261,32 \pm 32,86

Salinity (‰)

<i>06.00 – 07.00 h</i>	32,81 ± 0,30	33,05 ± 0,23	32,83 ± 0,60	32,79 ± 0,59
<i>14.00 – 15.00 h</i>	33,07 ± 0,18	32,99 ± 0,72	32,94 ± 1,08	33,15 ± 0,59
<i>17.00 – 18.00 h</i>	32,67 ± 0,78	32,78 ± 0,29	32,67 ± 0,49	32,34 ± 1,08
<i>23.00 – 24.00 h</i>	32,23 ± 1,54	33,19 ± 0,48	33,35 ± 0,28	33,29 ± 0,37

Suhu (° c)

<i>06.00 – 07.00 h</i>	29,22 ± 0,17	29,14 ± 0,27	29,20 ± 0,18	29,17 ± 0,33
<i>14.00 – 15.00 h</i>	29,58 ± 0,39	29,35 ± 0,11	29,42 ± 0,18	29,40 ± 0,25
<i>17.00 – 18.00 h</i>	29,61 ± 0,22	29,43 ± 0,48	29,42 ± 0,44	29,54 ± 0,52
<i>23.00 – 24.00 h</i>	29,29 ± 0,18	29,19 ± 0,33	29,22 ± 0,19	29,24 ± 0,28

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261 **Table 2.** Summary of nitrogen characteristics dissolved in the water for the four treatments over
 262 65 d of culture period. Values are presented as mean ± standard deviation of eight replicates per
 263 treatment.

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	Unit	Treatments			
		300 shrimp/m ²	400 shrimp/m ²	500 shrimp/m ²	600 shrimp/m ²
Ammonia	mg/L	0,22 ± 0,09	0,21 ± 0,12	0,30 ± 0,27	0,29 ± 0,44
Nitrit (NO ₂ -N)	mg/L	0,09 ± 0,12	0,11 ± 0,08	0,14 ± 0,30	0,14 ± 0,31
Nitrat (NO ₃ -N)	mg/L	42,88 ± 12,47	64,87 ± 20,56	78,55 ± 28,94	80,94 ± 30,22

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268 **Table 3.** Growth performance of shrimp (mean initial weight of 0,3 g) offered experimental diets for 65 d. Values represent the mean
 269 of eight reshrimpicates. Results in the same columns with different superscript letter are significantly different ($P<0.05$) based on
 270 analysis of variance followed by the Tukey's multiple comparison test.

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Density (shrimp/m ²)	Biomass (Kg)	Final weight (g)	Weight gain (g)	ADG (g)	Feed input (Kg/tank)	FCR ¹	Survival rate (%)
300	133.2±5.1 ^b	13.0±0.2 ^a	12.5±0.2 ^a	0.17±0.02 ^a	210.00±0.00 ^a	1.58±0.06	53.36±2.17
400	156.9±15.7 ^{ab}	11.6±0.5 ^b	11.2±0.5 ^b	0.16±0.00 ^b	220.25±36.30 ^a	1.40±0.18	52.98±4.72
500	172.8±10.0 ^{ab}	10.3±0.4 ^c	10.0±0.4 ^c	0.15±0.01 ^c	245.75±27.29 ^a	1.43±0.20	52.36±2.70
600	198.7±32.0 ^{ab}	9.3±1.0 ^d	8.8±0.9 ^d	0.14±0.02 ^d	280.88±34.48 ^{ab}	1.43±0.10	55.94±7.73
<i>P</i> -value	<0.0001	<0.0001	<0.0001	0.0002	<0.0001	0.0850	0.4822
PSE ²	19.8827	0.5728	0.5515	0.0139	28.5431	0.1468	4.8442
Linear Regression							
r ²	0.6173	0.8762	0.8849	0.7778	0.4858	0.0988	0.0074
p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0798	0.6388

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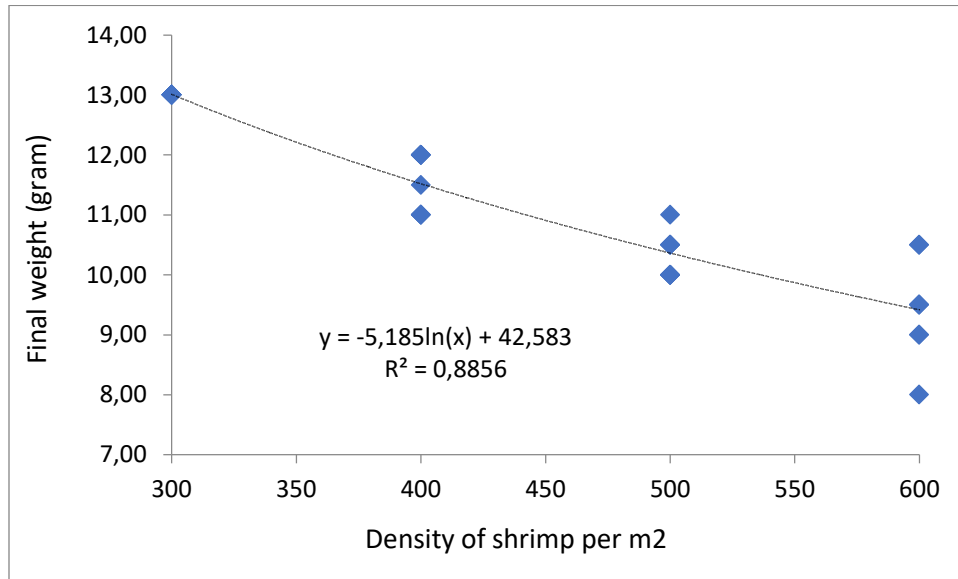
273 ¹ FCR = Feed conversion ratio

274 ² PSE = Pooled standard error

275 ³ ADG = Average Daily Growth

1 **Figure 1.** Logarithmic regression line

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5 **Figure 2.** Shrimp growth at four densities measured weekly in 32 concrete tanks during 11-week

6 experimental grow-out. Points are mean values \pm SD for eight replicates