Production performance of whiteleg shrimp *Litopenaeus vannamei* at different stocking densities reared in sand ponds using plastic mulch

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Abstract. This study aimed to determine shrimp performance such as growth, survival rate and biomass production of the whiteleg shrimp (*Litopenaeus vannamei*) cultured in sandy ponds using plastic mulch and different densities (100, 200 and 300 shrimp m^{-2}). The experiment was conducted using 9 ponds of 3x4x1 m (12 m^3). Experiments were designed in 3 different stocking densities as treatments and repeated 3 times. Shrimps were cultured in a period of 75 days by measuring daily growth, survival rate (SR), food conversion ratio (FCR), and biomass production. The water quality on daily temperature, pH, salinity, DO and transparency, nitrite, ammonia, and TOM were observed every two weeks. Post larvae (PL9) *L. vannamei* was fed with 30% protein powder and crumbs fed 4 times per day. Water refreshment was done periodically at the time of filling. Observations were conducted on final weight, daily growth, survival rate, FCR, and biomass production using ANOVA uni-variate analysis. The final weight of 9.58-12.93 g, survival rate between 61.75-97.99%, daily growth between 0.1138-0.1655 g, FCR between 0.92 and 2.06 and biomass production 14.99-22.37 kg m^{-2} were recorded. Density affects growth, SR, FCR, and biomass production of shrimp ($P<0.05$). Growth decreased with increasing density ($P<0.05$), survival decreased with increasing density ($P<0.05$), while biomass production was significantly different between all treatments ($P<0.05$). Low density can be applied to aquaculture *L. vannamei* in sandy ponds using mulch.

Key Words: marginal land, plastic-mulch, coastal area, survival rate, food conversion ratio.

Introduction. The southern coastal area of Central Java has potential fishery resources to be utilized as a shrub area. Triyatmo (2012) reported that the development of aquaculture and land suitability of ponds in this area can be applied to shrimp farming, especially in inter tidal and supra tidal areas. The ponds are built on less productive marginal land namely sand dunes and swale lands that are still affected by the tides of the sea (Djumanto et al 2016). Kamiso et al (2001) indicated that sand dunes have sandy soil texture, neutral acid pH (5.7-7.0), low conductivity (24-532 μmho cm^{-1}), the permeability of water of 5 cm $hour^{-1}$ (easy to pass water).

Whiteleg shrimp (*Litopenaeus vannamei*) culture in plastic sand layers began in 2013 in the Special Region of Yogyakarta, and then was developed along the southern coastal area of Central Java. The construction of the pond consists of sand and plastic coated clay, water sources are pumped from groundwater wells made around pond area with salinity of 10-25 ppm (Triyatmo 2012). At present, it is estimated that there are about 1,100 units of ponds measuring size ranging from 1,000 to 4,500 m^2 (Rustadi 2015; Djumanto et al 2016). Rustadi (2015) reported that the success of harvest at the beginning was up to 3 times of culture, however in the next period failed due to the emergence of white feces diseases. Currently, due to its waste disposal contaminated the environment and is compounded by the emergence of disease outbreaks that result in crop failures so that sustainability is not guaranteed. The problem is a result of the erroneous assumption that in order to increase production and implicit profit an increased density of individuals is needed. Meanwhile the capacity of the ponds is limited to the existing technology, limited water quality and resources.

Aquaculture can be performed within semi-intensive and intensive systems. One of the characteristics of both cultivation systems is the stocking density (Briggs et al 2004; Budiardi 2008; Neal et al 2010). In aquaculture the application of different cultivation systems affects the density (Mena-Herrera et al 2006). Density is a parameter that can affect the growth and survival rate of cultured species (Krummenauer et al 2010; Neal et al (2010), Sookying et al (2011). Wasielesky et al (2001) and Gaber et al (2012) states that the density and performance of penaeid shrimp have positive correlation. The optimum density of the penaeid shrimp has been reported even though it varies greatly, such as 9-12 ind m^{-2} for *Penaeus semisulcatus* (Zakai et al 2004), 10 ind m^{-2} for *P. indicus* (Sivanandavel & Soundarapandian 2010), 10 ind m^{-2} for *Fenneropenaeus merguiensis* (Anand et al 2014), and 10-15 ind m^{-2} for *P. monodon* (Duy et al 2012; Hossain et al 2013). While *L. vannamei* has been widely reported although the density and culture systems used are very diverse. So far, it has not been known the optimum density of *L. vannamei* cultured in sandy soils. Therefore, this study is conducted to determine the effect of stocking densities on *L. vannamei* production performance in intensive system using sandy ponds.

Material and Method

Description of the study sites. This research was conducted from August to October 2017 on the coast of the Keburuhan village, Ngombol Purworejo District, Central Java. This area is consisted of farmlands and aquaculture exploitations with sand dune and clay soil. 60% of land has been used as a cultivated area but is not exploited in the present. A total of 9 ponds of 3x4x1 m (12 m^3) were constructed on sand land and covered with plastic-mulch on its ground. Each pond was separated with a barrier made of sand-filled sacks. The ponds were filled with water up to a height of 80 cm. The source of water came from ground surface water obtained by making a well which was dug around the ponds in a depth of cca. 20 m.

Tools and materials. Each pond is equipped with an electric mill (1 HP) to support the supply of the oxygen. Water quality observation was conducted in situ consisting of temperature (mercury thermometer), salinity (Otago's optical refractometer), dissolved oxygen (DO meter), pH (digital pH meter) and brightness (Petri disk) observed daily. The calorie meter reaction was used for nitrite analysis (Spectrophotometer/sulfanilamide), ammonia (Spectrophotometer/phenate), and total organic matter (titrimetry) was performed in laboratories observed every two weeks. All parameters are sampled on the perimeter of each pond plot. The *L. vannamei* seed (PL9) comes from Central Protein Prima hatchery. The shrimp was fed with commercial shrimp feed contains 30% protein powder and crumble content. Vitamin C and Omega Protein was also used for the shrimp feed mixes.

Experiment design. This research was designed to find out the influence of stocking density on performance of *L. vannamei*, as growth, survival rate, FCR and biomass production. Experiments consisted of three groups of different density treatments (100, 200 and 300 shrimp m^{-2}) with 3 replicates. A total of 9 compartments of 12 cubic meters of ponds were provided with an electric mill to assist in the supply of the oxygen. Parameters tested were: average weight, daily growth, survival rate, feed conversion ratio (FCR) and biomass production. The length and weight of the shrimp was measured by taking a sample of 30 shrimps every 2 weeks of the culture period. Daily growth measurement were based on Ricker (1979) in Effendi et al (2016) and FCR according to Zonneveld et al (1991). At the end of the study, the biomass was calculated by the total weight of the shrimps per ponds. Water quality such as temperature, dissolved oxygen (DO), salinity, nitrite, ammonia and pH were measured daily.

Shrimp culture stages. Shrimp culture includes the preparation, water sterilization, seed spreading, feeding process, water quality management and harvesting stages. During the culture period, shrimps are fed (30% protein content) in the form of powder and crumble for prawns sized <1 g and pellet for 1 g shrimp. The amount of feed at the initial phase was given by blind feeding until the age of 25 days. The amount of feed was given in accordance with the density of each treatment. Then, the feeding was followed by the development of sampled shrimp biomass. Feeding was conducted 4 times a day at 07:00, 11:00, 15:00 and

20:00. Feeds given were mixed with vitamin C, omega protein, and water. Feed were administered by manual spreading in the experimental ponds throughout the edges.

The monitoring of water quality and shrimps conditions was conducted during the culture period. The water quality management, which consists of measurement of several parameters, which includes oxygen, temperature, pH, salinity, and brightness was measured daily. In addition to the measurement of water quality, water change is also undertaken to maintain the water quality of the ponds. Water change was done periodically 1-5% until the second month of cultured period and 5-7% until harvest time. To reduce the content of organic matter at the bottom of the ponds, the siphon was administered after 25 days of prawns, then every week. The sampling for shrimp length was done at the age of 30 because shrimp size was relatively small. Further measurements are performed every 14 days of culture. A total of 30 shrimps were sampled randomly at each pond using a net catcher and then measured the length and weight. After measurements, the shrimps were returned to their ponds. Harvesting was done after shrimps reached the market size of about 12-13 g, or when shrimp was at the 75^{th} day of the maintenance. Harvest was performed in the afternoon by draining the water from the pond. Then, the harvested shrimps were placed in plastic basket and sorted. The shrimp were sampled randomly and measured in length and weighed further measured the total weight of the harvest to determine biomass.

Data analysis. Parameters data such as final weight, length and weight of shrimp, daily growth rate, survival rate, FCR and production of shrimp biomass were analyzed during the culture period. The sample size was estimated using a method according to Zar (1999). The growth rate of each density treatments was estimated using the formula GR = Wt-W0/t, where GR is growth rate, W_t and W_0 is the final weight and initial weight; and t is the time of maintenance. Survival rate, FCR and biomass production follows the formula of Zonneveld et al (1991), SR = Nt / N0 x 100%

Statistical analysis. All data were presented as mean±standard deviation (SD) of replicated measurements (n = 30). Bartlett and Kolmogorov-Smirnov tests were applied to test for data normality, independence, and homogeneity. Growth rate, final weight, survival rate, feed conversion ratio and biomass production were analyzed by analysis of variance (ANOVA) using the statistical software SPSS version 19 (uni-variate). Significance of differences was defined at $p<0.05$.

Results. Final weight analysis, survival rate, daily growth, feed conversion ratio (FCR) and biomass production is shown in Table 1.

Table 1

Final weight, survival rate, daily growth, FCR and biomass production of *Litopenaeus vannamei* in sandy ponds

Parameter	100 (shrimp m^{-2})	200 (shrimp m^{-2})	300 (shrimp m^{-2})
Final weight (g)	12.93 ± 0.7^a	10.18 ± 0.66^b	9.58 ± 0.54^c
Survival rate (%)	96.54 ± 1.47^a	83.46 ± 5.72^b	64.98 ± 4.58^c
Absolute growth (g day^{-1})	0.1526 ± 0.011^a	0.1225 ± 0.08^b	0.1118 ± 0.006^c
Feed conversion ratio (FCR)	0.99 ± 0.07^a	1.47 ± 0.005^b	2.00 ± 0.05^c
Biomass production (kg ha^{-1})	14.99 ± 1.09^a	20.33 ± 0.14^b	22.37 ± 0.57^c

Different letters indicate significant differences ($p<0.05$).

The mean scores of final weight varies between 9.58±0.54 and 12.93±0.7 g. Density of 300 shrimps m^{-2} was significantly different with 100 and 200 shrimps m^{-2} ($P<0.05$). The recorded growth rate was between 0.1118±0.006 and 0.1526±0.011 g day^{-1}, with significant differences between low and high density maintenance ($P<0.05$) and showed an inverse relationship ($P<0.05$, R2 = 0.77) between high density and weights (2 g $week^{-1}$) (Figure 1). The effect of density on growth performance was different between all treatments ($P<0.05$) (Figure 2). Survival rate was significantly different among all treatments ($P<0.05$), which was highest in 100 shrimps m^{-2} density (96.54%) followed by 200 and 300 shrimps m^{-2} (83.46% and 64.98%) (Table 1). Feed conversion ratio (FCR) varied between 0.99±0.07 and 2.00±0.05, FCR of the highest density was significantly different comparing to the lowest density ($P<0.05$). Production of *L. vannamei* biomass ranged from 14.99±1.09 to 22.37±0.57 kg m^{-2}. There was a significant difference between all treatments ($P<0.05$), the highest biomass production was found in 300 shrimps m^{-2} (22.37±0.57 kg m^{-2}) and the lowest at 100 (14.99±1.09 kg m^{-2}). The absolute growth and average weight growth of *L. vannamei* during 75 days of all treatments are presented in Figure 1 and Figure 2.

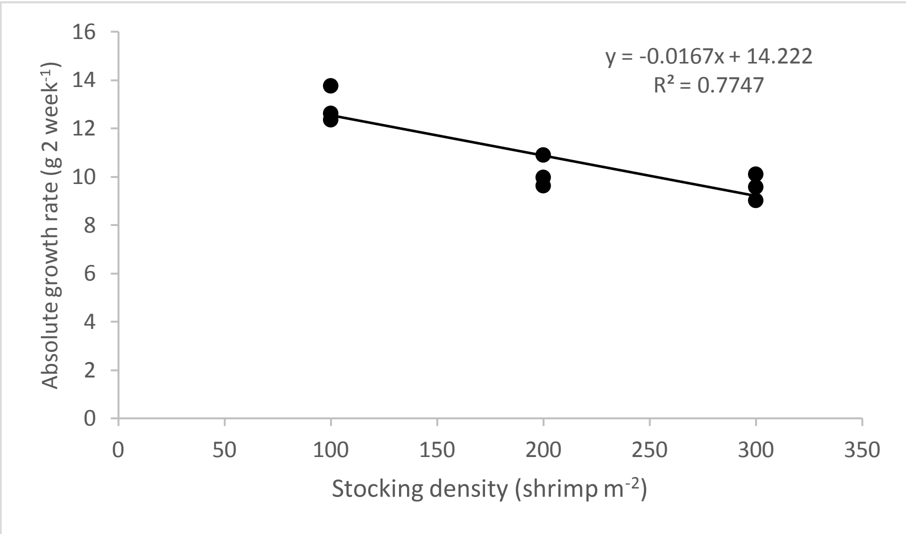

Figure 1. Absolute growth (±SE) of *Litopenaeus vannamei* with different stocking densities grown in sandy ponds.

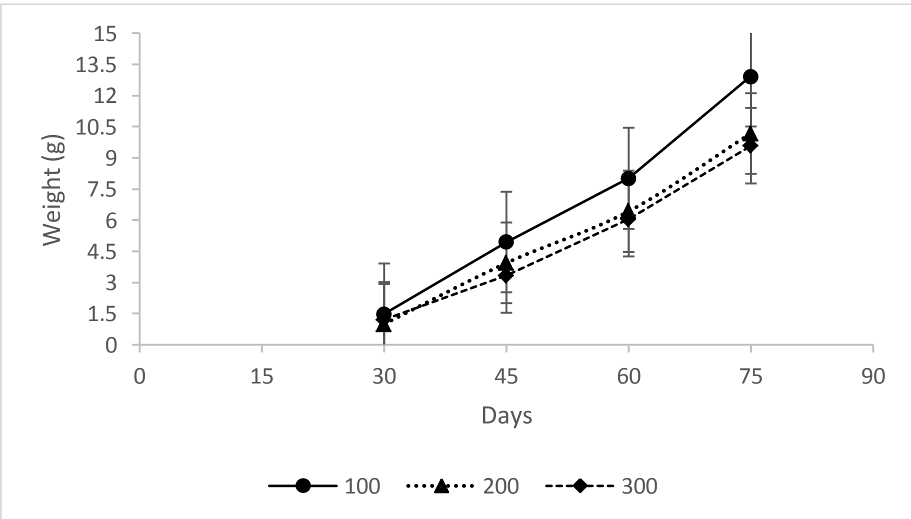

Figure 2. The average weight growth (±SE) of *Litopenaeus vannamei* reared at different stocking densities for a 75 days period.

The results of the water quality parameters analysis during the study showed that the values are still tolerated by *L. vannamei*, but total organic matter (TOM) tend to be higher than the national standard value for penaeid culture in Indonesia (≤90 mg L^{-1}) (Table 2) (Marine and Fisheries Ministry 2016).

Table 2

The average value of water quality parameters of *Litopenaeus vannamei* on different stocking densities on sandy ponds

Parameter	*Treatment shrimp m^{-2}*		
	100	*200*	*300*
Temperature (^{0}C)	29.23±0.42	29.6±0.35	28.77±0.45
pH	7.97±0.04	7.99±0.03	7.96±0.02
Salinity (ppt)	22.07±2.72	25.07±1.1	24.80±1.39
Dissolved oxygen (mg L^{-1})	4.51±0.08	4.24±0.06	4.27±0.05
NO_2 (mg L^{-1})	1.67±0.29	1.96±0.06	1.35±0.48
NH_3 (mg L^{-1})	1.63±0.05	2.25±0.32	2.49±0.17
Total organic matter (mg L^{-1})	185.15±24.34	188.83±9.62	248.20±18.84
Transparency (cm)	39.63±0.06	38.07±0.81	38.60±0.53

During cultured, water quality parameters are often changed, although not significantly different between treatments. Temperature values vary between 29.23-9.60°C, pH 7.96-7.99, salinity 22.07-25.07 ppt, dissolved oxygen between 4.24-4.51 mg L^{-1}, nitrite 1.35-1.96 mg L^{-1}, ammonia 1.63-2.49 mg L^{-1}, TOM 185.15-248.20 mg L^{-1} and transparency 38.07-39.636 cm.

Discussion. The results of the present research show the feasibility of *L. vannamei* culture in sand soil ponds using mulch plastic. Performance of *L. vannamei* showed significant differences between low and high density populations (P<0.05, Table 1). It was showed at parameters such as average final weight, survival rate, daily growth and FCR, which has higher value. However, the density of 300 shrimp m^{-1} produces higher biomass production than the density of 100 and 200 shrimp m^{-1}. It appears that stocking density affects average weight, survival rate, daily growth and FCR of *L. vannamei*. Some researchers have used stocking density as their treatment, although the density used was relatively smaller as Suriya et al (2016) reported maintenance of 65 and 85 shrimp m^{-2} density to obtain survival rate of *L. vannamei* of 80-90%. Sookying et al (2011) obtained survival rate between 45% and 47% with density of 10 and 20 shrimp m^{-2}. While Gaber et al (2012) reported survival rate of 51.60-89.0% with density of 5, 15 and 25 shrimp m^{-2}. Araneda et al (2008), reported a density of 90 (76.1%), 130 (68.9%) and 180 (65.9%) shrimp m^{-2} with 76.1%, 68.9% and 65.9% survival rate respectively, where the survival rate decreased with increasing of the density.

As with survival rate, density also plays an important role in the growth of aquatic organisms (Araneda et al 2008). The growth rate in low spread density is higher than in high-density (Figure 1) (Araneda et al 2008). It is further explained that under certain conditions individual growth rates may be slowed down and also rapidly, depending on the area of culture such as aquaculture, brackish water and freshwater (low salinity). In addition, other factors such as water temperature, initial and final weight of individuals, and feed types also play an important role in the growth of organism. Fluctuations in temperature and salinity often occur during culture period. Williams et al (1996) and Davis & Arnold (1998) reported the growth rate of *L. vannamei* between 0.50 and 0.95 g week^{-1} at high salinity at density of 107 and 100 m^{-2}. Samocha et al (2004) and Sowers & Tomasso (2006) get high growth rate of 1.17 and 1.23 g week^{-1} in low salinity. Duy et al (2012), at a salinity of 20-23 ppt with a density of 10 shrimp m^{-2} found in *Penaeus monodon* a lower growth rate than in 30-33 ppt salinity.

At relatively low temperatures, feed consumption is slower than in high temperatures (Babu & Mude 2014). Araneda et al (2008) reported in *L. vannamei* a better feed consumption at 35°C temperature with density of 90 shrimp m^{-2}, in contrast with 180 shrimp m^{-2} where the feed consumption was lower. However, there have been variations in growth, reported in previous studies, although, temperature conditions have shown optimal values (Krummenauer et al 2010; Ruiz-Velazco et al 2010; Ray et al 2011; Gaber et al 2012; Napaumpaiporn et al 2013; Saraswathy et al 2013; Tharavathy 2014; Kumar & Krishna 2015).

Stocking density applied in this research is quite high, namely 100, 200 and 300 shrimps m^{-2}, survival rate values are 96.65, 83.46 and 64.98%, respectively. The average weight of *L. vannamei* was 12.93, 10.18 and 9.58 g respectively. Despite of the land area of the pond that is marginal and less productive (Djumanto et al 2016), the results obtained are optimal and can be used as a density standard for aquaculture of *L. vannamei* in sandy mulch.

The FCR is one of the important parameters in the cultivation of *L. vannamei*. The value of FCR increased along with the treatment of stocking density with significantly differences between treatments ($P<0.05$). The FCR for the density of 300 shrimps m^{-2} (2.00±0.05) was higher than for densities of 100 and 200 shrimps m^{-2} (0.99±0.07 and 1.47±0.005 respectively). Same results were reported by Sookying et al (2011), Silva et al (2015) and Shakir et al (2014), despite the different density applied. Increased organic matter derived from ineffective feed stuffs, metabolism and excretion remaining high, especially organic content (TOM) (Perez-Velazquez et al 2012). Budiardi (1999, 2008) reported that this condition influenced shrimp appetite so that the growth was slower. Avnimelech et al (2004) stated that the accumulation of excessive organic matter trigger anaerobic environmental conditions, the high oxygen demand in the sediment, and the decline in environmental quality ultimately impacts on low growth on the cultivation period.

L. vannamei culture on the coast of Central Java generally applies semi-intensive and intensive cultivation systems. However, the farmers do not pay attention to the aspect of spreading density as one of the conditions in the success of cultivation. This study applied high density stocks using commercial feeds. During the 75 day culture period, biomass production on high-density 300 shrimps m^{-2} (22.37±0.57 kg m^{-2}) is higher than in the case of density 100 shrimps m^{-2} (14.99±1.09 kg m^{-2}) and 200 shrimp m^{-2} (20.33±0.14 kg m^{-2}). However, in terms of the individual weight, in lower density higher body weight was recorded. Sookying et al (2011) obtained biomass yields with 17 shrimps m^{-2} of 2,660.8 kg ha^{-1}, with 26 shrimps m^{-2} of 3,052.8 kg ha^{-1}, with 35 shrimps m^{-2} of 4,612.5 kg ha^{-1}, with 45 shrimps m^{-2} of 6149.6 kg ha^{-1}. Saraswathy et al (2013), reported density of 80 shrimps m^{-2} with 2,000 kg ha^{-1}, and density of 160 shrimps m^{-2} with 3,560 kg ha^{-1}. Kumar & Krishna (2015), at density of 20, 30, 40, and 50 shrimps m^{-2} obtained 2,401.490 kg ha^{-1}, 3,434.496 kg ha^{-1}, 4,307.625 kg ha^{-1} and 4,942.913 kg ha^{-1}.

The decreased of growth parameters and survival rates in shrimp farming and increased biomass production has also been reported (Reid & Arnold 1992; Williams et al 1996; Davis & Arnold 1998; Appelbaum et al 2002; Samocha et al 2004). Larger organisms dominate against small organisms at the time of feeding will trigger competition for greater use of space at higher densities. This shows that interpersonal interactions have an effect on inhibiting growth and survival especially in increased density (Harán et al 2004; Arnold et al 2006). Although in the present study the cannibalism aspect has not been observed, often *L. vannamei* looks to prey on other shrimps that have died. When compared to the initial spread, the population density of shrimps decreased, greatly influencing the growth rate of the three density treatments by increasing the utilization of space (Abdussamad & Thampy 1994; Arnold et al 2006).

The water quality during the study showed that the values were suitable for *L. vannamei*, with an exception in the case of the relatively high total organic matter (TOM). The salinity, pH and temperature parameters often change, although they are still in normal limits (Boyd 1998). The concentrations of nitrate and ammonia has increased during the rearing period, particularly in high-density treatments, due to the increasing number of feedings and the resulted droplets (Arnold et al 2006; Krummenauer et al 2010).

Conclusions. The *L. vannamei* culture in sand ponds is effective using mulch with the best density of 100 shrimps m^{-2}. This density influence showed best result on GR, SR, and FCR. The GR value obtained was 0.1526 g day^{-1}, the SR was 96.54%, and the FCR was 0.99. However, the density of 300 shrimps m^{-2} tend to produce the highest biomass production, namely 22.37 kg m^{-3}, but in a low final weight and SR compared to low stocking density variant. For *L. vannamei* culture in sandy ponds using plastic-mulch it is advisable to pay attention on the season as well on the water management during the culture period.

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