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The Survey effect of salinity stress on blood parameters of young Litopenaeus vannamei

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Abstract
L. Vannamei is one of the most important species of farmed shrimp that spread quickly across farming areas. Since any changes in environmental factors will always threaten their health, so by measuring of blood parameters, the effects of salinity fluctuations can be examined on growth and health indicators. To evaluate each of variable salinity in this study, four separate experimental groups each with three treatments with 3 replications were used. According to the results, any stress arising from increase or decrease in salinity rate may be associated with abnormalities in the immune system, so that its activity in salinity of 15 and 45 g/L compared to 30 grams of salt per liter was associated with a significant decrease in the values of heamocyte cells and total protein in blood plasma ($P < 0.05$). It can be concluded that the most appropriate salinity degree in L. Vannamei growth is 30 grams per liter.

Keywords: stress, salinity, blood parameters, L. Vannamei.

1. Introduction
Preparation of food requirements, simultaneous to human societies and population growths, will be the most problem of the societies. In order to solve this problem, turning to marine resources is increasing day by day [1]. Along with development of this industry in Iran in recent years, shrimp production rate has been developed due to increasing demand of domestic markets and providing of shrimp export conditions [2]. So, one of the most important issues related to the development of an aquatic species is understanding the relationship between biological and non-biological parameters and their impact on shrimps' growth and survival and is determination of relationships between them. It should be noted that growth of shrimp in addition to gender, depends on the stage of life and environmental factors such as the quantity and quality of food, temperature and salinity of water [3]. It should be noted that shrimps of Penaeidae family are the most important species that is produced commercially today, so that in 2011, the shrimp production rate in the world amounted to 4 million tons, that in the meantime, Litopenaeus Vannamei was assigned 90% of the total share of world production of shrimp to itself [4]. Its order, category, and classification are decapoda (ten-footed), crustacean and arthropoda, respectively. Crustaceans have been able to seize a dominant aquatic ecosystem, due to high compatibility with environment during their developments [5]. Invertebrates live almost in all habitats available on earth and this means that they are able to afford the fight and deal with a wide range of pathogens. Effectiveness of invertebrates' immune system is proved by their survival and evolution over millions of years [6]. Immune system of shrimp is consisted of a non-specific immune system. In the meantime, several factors are involved to improve aquaculture conditions, including optimal growing conditions, proper nutrition, good water quality, improving storage density and use of vaccines, probiotics and immune system stimulants [7]. Nowadays, by investigation of immune system activity and shrimp health factors can ensure the health of the creatures. On the other hand, shrimps are saline friendly creatures that live in water, so any changes in environment impacts on their health. The most important factors that affect the health of the shrimp can be noted in the physicochemical factors such as water temperature, salinity rate and pH. It is worth noting that any changes to these factors may cause changes in health factors of shrimps. In the study of Taheri *et al* (2014), a sharp decline in salinity of 40 grams per liter to 5 grams per liter was observed over a period of 7 days. They observed that total haemocyte count of shrimp has fallen sharply at the first, so that this amount was reduced to 35% by reduction of salinity rate to 35 grams per liter, and with the continuous fall in salinity rate this amount was increased in the salinity of 25 grams per liter [8]. But with regard to non-native of Litopenaeus

Vannamei in Iran, today, we see that in some growing regions of the country this species is growing at more than 57 parts per thousand of salinity, over 36 °C temperature and pH over 8 [2]. On the other hand, due to changes in water quality in the pools of growing shrimp, changes in quality especially salinity may be more than 5 parts per thousand. The changes are usually associated with risks such as stress, increased susceptibility to pathogens, growth decrease, survival, and increase in production costs [9]. Given that the shrimps were Yuri Hallin (halophytes) species and they set their body in environment with using energy of osmotic pressure [10], but created severe fluctuations in salinity can cause severe changes in health factors of shrimps such as the number of blood haemocyte, total protein plasma and density percent of haemocyte cells. Following this case, in addition to stress tolerance and increased consumption of food and Feed Conversion Ratio (FCR), it is usually associated with an increase in cost. Therefore, it is tried in this study, to assess the health factors in different levels of salinity compared with created fluctuations.

2. Method

This study was performed from Jun 2015 until Sep 2015. A number of young shrimps weighing 8 to 10 g were used, in this study. It is worth noting that the shrimp was selected from centers that no history of the disease had been reported, till that time. Thus, by referring to the disease-free shrimp of Persian Gulf-Harbour Research Station, 90 young shrimp was selected and transferred to the Iran's Institute of Research Shrimp Laboratory in Bushehr, they were then sorted in three 300 liter tanks containing sterilized sea water with salinity of 35-37 grams per liter. To adapt shrimp to new circumstances, their adaptation lasted in vitro for a week [2, 9]. During the study period, shrimp feeding treated was carried out using Havorrash growth stage food (code 4003) to 2.5-3 percent of body weight in 9, 15 and 22 hours [12].

To provide saltwater, seawater was filtered first by sand filters and disinfected with sodium hypochlorite at a rate of 20 parts per million, the process of water disinfection was carried out for 12 hours. In the following, sodium thiosulfate at a rate of 8-10 parts per million with vigorous aeration was used to neutralize the chlorine in the water [2, 13].

In this study, an experimental group was composed of three treatments each with 3 repetitions and arranged randomly in facing hall. On the other hand, 30 young shrimps were storage in each repetition.

In experimental treatments of salinity stress, salinity degrees were set on 15, 30 and 45 grams per liter. For this purpose, disinfected fresh urban water and salt was used to set different levels of salinity. Therefore, by calculation the amount of fresh and salty water or salt, through water salinity-equation (N1: raw salt, V1: initial volume, N2: secondary salinity, V2: secondary volume), setting of different levels of salinity was done after mixing them with each other by ocular salinity meter (ATAGO).

It is worth noting that in this study, salt treatment degree of 30 were considered as control treatment and salt treatment degree of 15 and 45 were considered as experimental treatments (14).

$N_1V_1=N_2V_2$

It is worth noting that treatment lasted 30 days during the study period related to salinity stress. During this time, measurement of shrimp health factors such as the number of total heamocyte count, total protein plasma and differentiate heamocyte count were analyzed on 1, 8, 21 and 30 days of study. After random selection of 6 pieces of shrimp from each of the repetitions of experimental treatments, bloodletting from their abdominal sinus were done separately. The results of the studies were analyzed by SPSS 18 statistical software and ANOVA statistical test.

3. Results

The results of this study indicate that in the first week of study no significant difference was found between average of blood haemocyte cells of shrimps in different treatments ($P> 0.05$) (Table 1). According to the results, it was found that average of blood haemocyte cells in shrimp salinity treatment 15 grams per liter was significantly higher than shrimp salinity treatment 30 and 45 grams per liter ($P <0.05$). On the other hand, the average of blood haemocyte cells in shrimp salinity treatment 45 grams per liter was significantly lower than shrimp salinity treatment 30 grams per liter ($P <0.05$) (Table 1). The results showed that during the third week, the average of blood haemocyte cells in shrimp salinity treatment 30 grams per liter was significantly higher than shrimp salinity treatment 45 and 15 grams per liter ($P <0.05$). Meanwhile, despite the higher rate of total blood haemocyte in shrimp salinity treatment 45 grams per liter than shrimp salinity treatment 15 grams per liter, no statistically significant difference was found ($P <0.05$) (Table 1). Based on the results of the study it was observed that the average of blood haemocyte cells in shrimp salinity treatment 15 grams per liter was significantly higher than shrimp salinity treatment 45 grams per liter ($P <0.05$). But despite the higher average of blood haemocyte cells in shrimp salinity treatment 15 grams per liter than shrimp salinity treatment 30 grams per liter and also shrimp treatment 30 grams per liter compared to shrimp treatment 45 grams per liter, no significant difference was found ($P <0.05$) (Table 1). The results showed that the average of blood haemocyte cells in shrimp salinity treatment 15 grams per liter in the second week was significantly higher than those measured in the first, third and fourth weeks ($P <0.05$). So the average of blood haemocyte cells in the fourth and third weeks was significantly lower than the first week ($P <0.05$). Meanwhile, despite the lower rate of blood haemocyte cells in third week compared to the fourth week, no statistically significant difference was found ($P <0.05$) (Table 1). Regarding to shrimp salinity treatment of 30 ppt, results showed that the average of blood haemocyte cells in third week was significantly higher than the second, first and fourth weeks ($P <0.05$). However, despite the higher average of blood haemocyte cells in the third week than the first week, there was found no statistically significant difference ($P <0.05$) (Table 1). Results showed that the average of blood haemocyte cells in first week was significantly higher than third, second and fourth weeks ($P <0.05$). But despite higher obtained values in the second week compared to the fourth week, no statistically significant difference was found ($P <0.05$) (Table 1).

Table 1: The average of total blood haemocyte cells in shrimp salinity treatment 15, 30 and 45 grams per liter at different times

Week / Salinity	Total blood haemocyte cells (cells per ml)			
	First	Second	Third	Fourth
15 grams per liter	$7.22 \times 10^{-6} \pm 0.65 \times 10^{-6b}$	$1.46 \times 10^{-6a} \pm 10.36 \times 10^{-6}$	$0.87 \times 10^{-6c} \pm 5.41 \times 10^{-6}$	$0.90 \times 10^{-6c} \pm 5.71 \times 10^{-6}$
30 grams per liter	$0.80 \times 10^{-6b} \pm 6.55 \times 10^{-6}$	$1.71 \times 10^{-6b} \pm 7.37 \times 10^{-6}$	$1.61 \times 10^{-6a} \pm 8.27 \times 10^{-6}$	$0.91 \times 10^{-6c} \pm 4.66 \times 10^{-6}$
45 grams per liter	$0.88 \times 10^{-6b} \pm 6.57 \times 10^{-6}$	$0.86 \times 10^{-6c} \pm 3.84 \times 10^{-6}$	$0.74 \times 10^{-6b} \pm 5.56 \times 10^{-6}$	$0.37 \times 10^{-6c} \pm 3.21 \times 10^{-6}$

Values are given based on mean ± SD 95% (dissimilar letters in each row indicate significance and similar letters indicate no significance).

Results showed that average of total protein plasma in shrimp salinity treatment 15 grams per liter was significantly higher than shrimp treatment 30 grams per liter (P <0.05). Also, despite the higher average of total protein plasma in shrimp salinity treatment 45 grams per liter compared to shrimp treatment 30 grams per liter, no significant difference was found (P <0.05) (Table 2). According to the results, the rate of total protein plasma in shrimp salinity treatment 15 grams per liter compared to the ones that obtained treatment 30 and 45 grams per liter no statistically significant difference was found (P <0.05) (Table 2). The results of the study showed that the average of total protein plasma in shrimp salinity treatment 15 grams per liter was significantly higher than shrimp salinity treatment 45 grams per liter (P <0.05). Despite the higher rate of total protein plasma in shrimp salinity treatment 15 grams per liter compared to shrimp treatment 30 grams per liter and also lower rate of total protein plasma in shrimp salinity treatment 45 grams per liter compared to shrimp treatment 30 grams per liter, there was found no statistically significant difference (P <0.05) (Table 2). The results showed that the average of protein plasma in shrimp treatment 15 grams per liter than measured values in shrimp treatment 45 and 30 grams per liter no statistically significant difference was found (P <0.05). According to the results, total protein plasma in first week was significantly higher than those obtained in second, third and fourth weeks (P <0.05). But despite the higher rate of total protein plasma in third week than fourth and second weeks, no statistically significant difference was found (P <0.05) (Table 2). Results showed that the average of total protein plasma in first week than second, third and fourth weeks, no statistically significant difference was found (P <0.05) (Table 2). The results showed that average of total protein plasma in first week were significantly higher than those obtained in third and second weeks (P <0.05). But despite the higher values obtained in first week than fourth week, no statistically significant difference was found (P <0.05). On the other hand, despite the lower rate of total protein plasma in second week than third week, no statistically significant difference was found (P <0.05) (Table 2).

Table 2: The average of total blood protein plasma in salinity treatment 15, 30 and 45 grams per liter at different times

Week / Salinity	Total protein plasma (milligram per ml)			
	First	Second	Third	Fourth
15 grams per liter	9.61 ± 0.75^{a}	5.89 ± 0.38^{b}	7.29 ± 1.18^{b}	6.41 ± 0.68^{b}
30 grams per liter	7.72 ± 0.92^{a}	5.19 ± 1.38^{a}	6.39 ± 0.72^{a}	5.52 ± 0.68^{a}
45 grams per liter	8.73 ± 1.48^{a}	4.46 ± 0.63^{b}	5.07 ± 0.49^{b}	6.23 ± 0.75^{ab}

Values are given based on mean ± SD 95% (dissimilar letters in each row indicate significance and similar letters indicate no significance).

The results of the study showed that granular cells compared to semi-granular and hyaline cells had the highest rates. On the other hand, results showed that the average percentage of granular cells in shrimp salinity treatment 15 grams per liter was significantly higher than shrimp salinity treatment 30 grams per liter (P <0.05). However, despite the lower average percentage of granular cells in shrimp salinity treatment 45 grams per liter compared to shrimp salinity treatment 15 grams per liter, no statistically significant difference was found (P <0.05) (Table 2). On the other hand, the results showed that the average percentage of hyaline cells in shrimp treatment 30 grams per liter, was significantly higher than shrimp treatment 45 and 15 grams per liter (P <0.05). Despite the higher average percentage of hyaline cells, no statistically significant difference was found in shrimp treatment 45 grams per liter than shrimps 15 grams per liter (P <0.05). The results of the semi-granular cell count showed that despite the higher average percentage of these cells in shrimp treatment 15 grams per liter no statistically significant difference was found (P <0.05) (Table 3). According to the results, the percentage of granular cells in the second week was significantly higher than hyaline and semi-granular cells (P <0.05). On the other hand, the results showed that the average percentage of granular cells was significantly higher in shrimp salinity treatment 15 grams per liter compared to shrimp salinity treatment 45 and 30 grams per liter (P <0.05). The results showed that the average percentage of these cells of shrimp salinity treatment 30 grams per liter was significantly lower than granular cells of treatment 30 grams per liter (P <0.05). While the percentage of hyaline cells in shrimp salinity treatment 30 and 45 grams per liter was significantly higher than in shrimp salinity treatment 15 grams per liter (P <0.05). On the other hand, despite the higher percentage of hyaline cells in shrimp salinity treatment 30 than shrimp treatment 45 grams per liter, no significant difference was found (P <0.05). The results of the study showed that the average percentage of semi-granular cells in shrimp salinity treatment 30 grams per liter was significantly higher than counted cells at shrimp treatment 45 and 15 grams per liter (P <0.05) (Table 3). According to the results in the third week-study, the percentage of granular cells was significantly higher than counted semi-granular and hyaline cells in different salinity treatments (P <0.05). The results showed that despite the higher average percentage of granular

cells in shrimp salinity treatment 15 grams per liter than any other salinity treatment, no statistically significant difference was found (P <0.05). While the average percentage of hyaline cells in shrimp salinity treatment 45 grams per liter was significantly higher than those counted in shrimp treatment 30 and 15 grams per liter (P <0.05). However, there was no statistically significant difference in the average percentage of counted semi-granular cells in salinity treatment (P <0.05) (Table 3). According to the results, it was found that the average percentage of granular cells in the second week significantly higher than the third week (P <0.05), therefore, despite the higher average percentage of granular cells in the second week compared to the first and third weeks there was no statistically significant difference (P <0.05). Also in conjunction with the semi-granular and hyaline cells, despite higher average percentage of cells in the third week than the first weeks, there was no statistically significant difference (P <0.05) (Table 3). Results showed that the average percentage of granular cells in the third week was significantly higher than the second week (P <0.05), however, despite lower average percentage of granular cells in the first week than the third week, there was no statistically significant difference (P <0.05). While the average percentage of semi-granular cells in the second week was significantly higher than the third and first weeks (P <0.05), there was no statistically significant difference between average percentage of semi-granular cells in the third week with the first week (P <0.05). It was also observed that the percentage of hyaline cell density in the first week, second and third weeks is significantly more (P <0.05). It was also observed that the density percentage of hyaline cells in the first week was significantly higher than the second and third weeks (P <0.05). Despite the higher density percentage of these cells in the second week than the third week, there was no statistically significant difference (P <0.05) (Table 3). According to the results, it was found that despite the higher average percentage of granular cells in the first week compared to the second and third weeks, no statistically significant difference was found between granular cells in different weeks (P <0.05). On the other hand, results showed that despite higher average density of semi-granular cells in the second week than the first and third week, there was no statistically significant difference (P <0.05). In conjunction with hyaline cells, despite higher average cells in the third week than the second and first weeks, there was no statistically significant difference (P <0.05) (Table 3).

Table 3: Percentage of blood cells different shrimp treatments in the first, second and third weeks

Treatment	Blood cells	Week		
		First	Second	Third
15 grams per liter	Granular (percent)	73.44 ± 5.50 ^{ab}	78.33 ± 4.81 ^{a}	63.44 ± 4.12 ^{b}
	Semi-granular (percent)	10.11 ± 2.00 ^{a}	8.00 ± 1.86 ^{a}	11.67 ± 1.67 ^{a}
	Hyaline (percent)	16.44 ± 4.96 ^{a}	14.78 ± 4.07 ^{a}	24.89 ± 4.52 ^{a}
30 grams per liter	Granular (percent)	54.56 ± 6.45 ^{ab}	50.33 ± 8.02 ^{b}	72.11 ± 3.63 ^{a}
	Semi-granular (percent)	8.56 ± 2.90 ^{b}	23.44 ± 5.73 ^{a}	13.56 ± 2.01 ^{b}
	Hyaline (percent)	36.89 ± 7.84 ^{a}	26.22 ± 3.95 ^{b}	14.33 ± 2.52 ^{b}
45 grams per liter	Granular (percent)	70.78 ± 6.44 ^{a}	65.11 ± 4.40 ^{a}	61.89 ± 4.33 ^{a}
	Semi-granular (percent)	8.78 ± 2.87 ^{a}	9.56 ± 0.99 ^{a}	7.33 ± 1.98 ^{a}
	Hyaline (percent)	20.78 ± 4.36 ^{a}	25.33 ± 4.13 ^{a}	30.78 ± 4.44 ^{a}

Values are given based on mean ± SD 95% (dissimilar letters in each row indicate significance and similar letters indicate no significance).

The results obtained in the first week of the study showed that the rate of granular cells in all shrimp treatment 15, 30 and 45 grams per liter was significantly higher than the semi-granular and hyaline cells (P <0.05), it was also observed that the percentage of semi-granular cells was significantly higher than the hyaline cells (P <0.05). Such results were also observed in the second and third week, with the difference that in spite of the higher rate of hyaline cells no statistically significant difference was found than the semi-granular cells in shrimp salinity treatment 30 grams per liter, (P <0.05) (Figure 4).

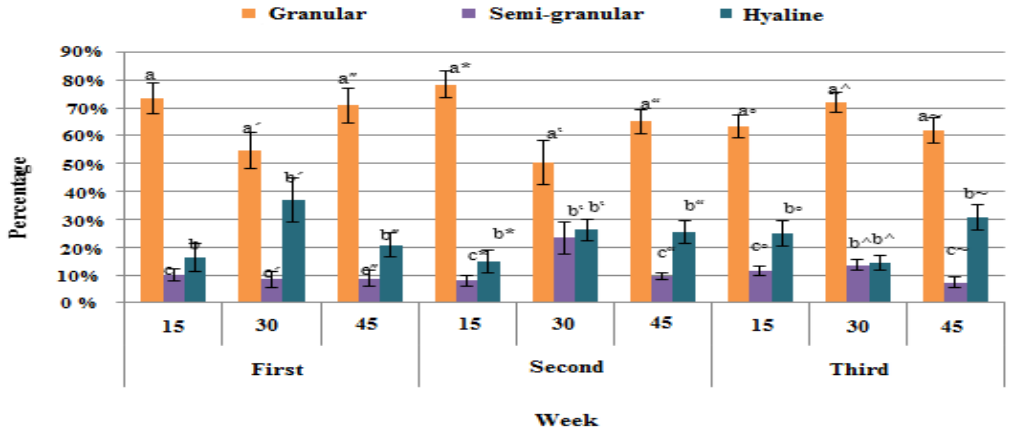

Fig 4: Percentage of granular, semi-granular and hyaline blood cells in different salinity treatment 15, 30 and 45 grams per liter in different weeks

Values are given based on mean ± SD 95% (dissimilar letters in each row indicate significance and similar letters indicate no significance).

4. Discussion

Given the importance of salinity and its effect on growth rate, survival, and physiological function of shrimps, it is said that sudden changes in salinity of water is associated with a sharp decline in activities related to hematological parameters in shellfish; this kind of adaptability occurs in new conditions. Following the adaptability, hematological parameters returns gradually to their initial position [15].

According to the results, the density of haemocyte blood cells in all salinity treatment during the first week, significantly caused reduction in density of semi-granular cells compared with normal levels of total haemocyte blood cells. This occurred due to stress caused by fluctuations in salinity rate of water. From the second week, followed by adaptation of shrimp salinity treatment 15 and 30 grams per liter to new circumstances, recovery of blood parameters return to normal, due to the relative increase in granular cells and density of total haemocyte blood cells. While in shrimp salinity treatment 45 grams per liter, the reduction in total heamocyte blood cells continued until the second week. However, Lu-Qing (2005) stated that hematological factors of shrimp are able to recover after 6 days of salinity changes. It may be noted that shrimps need longer time to adapt to new conditions, due to the stress caused by high levels of salinity [16].

Taheri *et al*. (2014) stated that shellfish are able to opposition with changes in the number of haemocyte blood cells against rapid changes in salinity. They can adopt themselves with new situation and these changes make the immune system of shrimp to be protected against pathogens and stressful factors [8]. On the other hand it was observed that the total haemocyte blood cells in salinity treatment 15 grams per liter in the third week of the study was reduced significantly compared to the second week. There is the possibility that the shrimps in a hypertonic environment (low salinity), are associated with an increase in the volume of hemolymph and with reduce of hemocytes cells, due to osmotic pressure balance [17, 18]. While, followed by an increase in salinity rate, the shrimp salinity treatment 45 grams per liter increased to some extent in amounts of heamocyte blood cells in the third week compared to the second week, due to hypotonic environment. But disruption and delay in enzyme activity associated with the immune system was observed due to high levels of salinity stress [10]. In addition to reduction in haemocyte blood cells, reduction in related values to total protein plasma was significantly observed in shrimp salinity treatment 45 grams per liter, compared to shrimp salinity treatment 30 and 15 grams per liter, in the fourth week of the study; as the observed decline in the hematological factors, was more caused by reduction in hyaline and granular density. The studies suggest that shrimps of Penaeidae family, because of having a regulator of the immune system, are able to maintain activities related to immune, to stand partly against to environmental fluctuations and to prevent decline of the immune defense levels against pathogens [19]. Li *et al*. (2002) found that the density of haemocyte blood cells of Japanese shrimp was significantly reduced in salinity of water from 25 grams per liter to 9 and 33 grams per liter over a period of 4 and 8 days, respectively, while their phenoxidizing activity increased [20]. It was stated in [21] that following the change in salinity from 30 to 15 grams per liter in Litopenaeus Vannamei and Chinese shrimp during a short period (10 hours), their bactericidal and antibacterial activities were gradually decreased, while their phenoxidizing activity increased. It was observed in another study that the amount of blood haemocytes cells will increase in freshwater shrimps (rosenbergii) due to increase in salinity rate from zero to 5, 10 and 15 grams per liter in more than 7 days, [22]. Perazzolo *et al* (2002) stated if the salinity rate in shrimp Farfantepenaeus Paulensis reduces from 34 grams to 22 and 13 grams per liter in more than 2 or 3 days, the number of haemocyte blood will gradually decrease [23]. However, according to the results of the present study it was observed that the levels of haemocyte blood cells in shrimp salinity treatment 45 grams per liter was reduced significantly, due to the rate of salinity and stress of it. On the other hand it was also observed that the correlation relation of total protein plasma with salinity levels of 15 and 30 grams per liter was a positive relationship, while this correlation was negative in shrimp salinity treatment 45 grams per liter. So, it is likely that the sensitivity of shrimp to pathogens increases by reduction of total protein plasma due to increased salinity [26, 28]. Also, there is the possibility that the sensitivity of shrimp to pathogens increases by the reduction of haemocyte blood cells due to increased salinity [26, 28].

Total plasma protein levels in the fourth week study compared to the first week were significantly decreased. So that the maximum level of plasma protein was 15 and 45 grams per liter in shrimp salinity treatment. It can be said that the shrimps must increase the volume of blood plasma proteins to adapt themselves to their circumstances. According to the results of the present study it was observed that due to the rate of salinity and stress of it, the levels of heamocyte blood cells was reduced significantly in shrimp salinity treatment 45 grams per liter. On the other hand it was also observed that the correlation relation of total protein plasma with salinity levels of 15 and 30 grams per liter was a positive relationship, while this correlation was negative in shrimp salinity treatment 45 grams per liter. So there is the possibility that sensitivity of shrimp to pathogens increases by reduction of total protein plasma due to increased salinity [26, 28]. Also, there is the possibility that sensitivity of shrimp to pathogens increases due to reduction of haemocyte blood cells by increased salinity, [26, 28].

5. Conclusion

According to the results, it was found that any stress of increase or decrease the salinity rate may be associated with impaired hematological factors activity of shrimp and it can be concluded that appropriate salinity rate in growth of Litopenaeus Vannamei is 30 grams per liter.

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