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Investigation of Probiotic's Effect on Efficacy of Biofloc in Shrimp Farming

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Abstract

Due to the limited natural resources, applied in the aquaculture industry, less dependent methods have been used. The comprehensive system of bacteria plays an important role as a water purifier from organic matter and toxic nitrogen compounds and waste into food. The method is fieldwork and taking notes of the results. In the form of four treatments (350 liters) with three repetitions in each treatment in the form of 1. Native probiotics 2. Commercial probiotics 3. Native and commercial probiotics 4. Control with specified concentration. Preparation of western white shrimp larvae and preparation of Bacillus subtilis and Bacillus licheniformis in two forms of traders and natives (from the digestion of juvenile huso) and study of shrimp hemolymph (glucose, cholesterol, uric acid, protein) and weight, length, length of carapace and SGR. During the test, the nitrogen-to-carbon ratio is kept constant at around 15.5. Significant changes occurred following the use of both native and commercial probiotics in the studied indices, including the most significant increase in total protein from 5.39 g to 9.32 g and shrimp weight from 10 g to 13.26 and a decrease in coefficient Food conversion up to 40%, while increasing the fertility of shrimp, followed by an increase in uric acid to 1.52 grams. Native probiotics improved indices such as immunoglobulin up to 120 mg with a significant difference compared to other treatments, improved feed length, and conversion ratio, and total weight and total protein.

Keywords: Biofloc, Litopenaeus vannamei, Probiotic, Shrimp farming.

Introduction

Recently, shrimp farming has become increasingly important in developing countries. Following the problems caused by the over-exploitation of its natural resources, effective steps have been taken to improve farming methods and increase shrimp production. The expansion of aquaculture products, due to environmental pressures caused by the polluting effect of the industry's effluent on water resources and also the industry's strong dependence on fish oil and flour in aquatic nutrition, highlights the need to use methods with minimal consumption of natural resources (Huerta-Rábago et al., 2019, Tacon et al., 2002). Biofloc technology offers a new solution to these problems, which is based on using the system without changing the water. This technology is based on adjusting the carbon-to-nitrogen ratio to develop microbial communities (Huerta-Rábago et al., 2019).

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Biofloc method as a safe and efficient method that has been used industrially for aquaculture in recent years has reduced the dependence of the aquaculture industry on the use of environmental resources, minimizes waste and wastewater, and economically has also been cost-effective (Llario et al., 2020, Meskini and Esmaeili, 2018). By adding carbohydrates to water and adjusting the carbon-to-nitrogen ratio (C/N), heterotrophic bacteria absorb nutrients and optimize biomass formation, thereby eliminating nitrogen and nitrite. If the carbon-to-nitrogen ratio is well balanced in the breeding tank, the nitrogenous waste will be converted to microbial biomass, which is consumed by aquatic feed (Tacon et al., 2002, Arias-Moscoso et al., 2018). In this system, the relationship between carbohydrate addition, ammonia reduction, and microbial protein production depends on the microbial conversion efficiency, the carbon-to-nitrogen ratio in the microbial biomass, and the carbon still content of the added material.

Although the use of biofloc systems in aquaculture has helped the economy of this industry and environmental protection, in any case, has created an unnatural environment for aquatic life and growth that is different from the ecosystems of these organisms. Therefore, in the absence of proper biological management of unnatural environments, a lot of damage will be done to the quality and quantity of the final products of this industry (Cabello, 2006, Meskini et al., 2020). The use of probiotics in aquaculture is very wide. It includes a wide range of microorganisms that function not only through food and nutrition but may also be effective through water. The purpose of using live microbial products is its effect on the activity of microbes in the gastrointestinal tract by stabilizing the desired microbes preventing the accumulation of harmful bacteria, and subsequently helping to maintain the health of living organisms. In fact, by applying microbial management in the form of using beneficial bacteria, the ecological compatibility of the host with the breeding environment is increased, and the optimal utilization of water resources is increased (Bentzon-Tilia et al., 2016, Cabello, 2006). A marketing system has two distinct dimensions. One of those dimensions is the institutions, organizations, and firms that participate in a market, and the second is the functions that the above-mentioned participants perform (*L. B. Pinheiro et al., 2022).* In each company, different areas of the company's activities are affected and can lead to issues such as inefficiency in product marketing, inefficiency and inability to properly employ manpower and the like (*Nasrollah Maghsoudi, 2022).* Generally, most beneficiary units and other productive sectors especially the trade unions and agricultural cooperatives have no place for technical- engineering services in their organization (*Mojtaba Ziaeemehr et al., 2018).* Therefore, the possibility of proper use of water resources is well provided, and production efficiency is increased. Types of microorganisms used in the aquaculture industry as probiotics are yeasts, bacilli, lactic acid bacteria, and Pseudomonas. In fact, by applying microbial management in the form of beneficial bacteria, the ecological compatibility of the host with the farming environment, the ability to establish

inhibitory effects against pathogens will increase.

Physico-chemical indicators of the environment and microbial-bacterial indicators are very effective parameters in creating optimal conditions that should be used with bio-additives to create optimal conditions for the growth and optimal life of aquatic animals, especially shrimp, which is the subject of this study (Kathia et al., 2017). So far, many studies have been conducted on the effect of using probiotics on water quality indicators, growth, nutrition, and safety in shrimp and other farmed species. Based on the results of these studies, the positive effects of these supplements in the shrimp diet on the increasing growth rate, survival, nutrition, water quality, and other biological characteristics have been proven (Nayak, 2010). However, not many studies have been performed on probiotics in the water of breeding ponds and earthen ponds. On the other hand, the biofloc system is a suitable and useful system that can return nutrients to the system continuously and use them repeatedly. Such a system is based on the growth of microorganisms in the breeding environment and is a good system due to low water exchange or lack of water exchange. These microorganisms have two important roles, which include maintaining water quality (absorption of nitrogen compounds in the production of microbial protein) and nutrition (reducing food costs)(Yuniasari and Ekasari, 2010, Cordero et al., 2015).

The great variety of probiotics in the aquaculture industry and the ambiguity in the effects of these additives in different conditions have attracted many researchers to increase productivity. In addition, there are limited reports on the effect of commercial and native Bacillus bacteria as probiotics on the safety indices of western white-footed shrimp in our country. Therefore, this study aimed to use the biofloc system, the effect of *Bacillus subtilis* and *Bacillus licheniformis* probiotics in commercial, commercial-native, and native forms on some safety indicators and microbial flora of western white shrimp. Also, the beneficial effects of the biomass system and the basic formulas for regulating the carbon-to-nitrogen ratio were examined.

Method and Material

Preparation of Tank, Biofloc, and probiotics

The method of this research is experimental and taking notes of the results. First, seawater passed through a sand filter (pH = 8), salinity 36 g/L, oxygen level 6 mg/L, ammonia level 0.006 mg/L at the rate of 3.5 cubic meters (3500 L) in a fiber tank Glass with a volume of 4 cubic meters, which was pre-washed and disinfected. This tank is prepared in an indoor hall with 12 hours of light and 12 hours of darkness. Then to prepare the initial inoculum and create a biofloc production system from organic materials including commercial feed shrimp 42% protein (40 g), bran and wheat flour (10 g), molasses (48 g), urea 46% nitrogen (1 g) and soil Clay (1 g) was used (Avnimelech, 2009). At the end of the four weeks, 1 liter of water was taken from the tank to evaluate the flakes produced and transferred to a 1-liter graduated cylinder, then 1 hour of stillness, 950 ml of liquid by siphoning, and 50 ml. The remaining volume, which

contained flakes and precipitated microorganisms, was transferred to a 1000 ml container. The samples were transferred to a planktonology laboratory. Three ml of the homogenized sample was poured into a 3% slide in a Sedgwick-rafter chamber with 2% Lugol's Iodine solution during three cutting steps and examined under a microscope. This method was used to measure the density of bioflocs and the size of bioflocs produced in water.

Preparation of Litopenaeus vannamei

Litopenaeus vannamei larvae with an average weight of 5.5 g and an average body length (from the edge of the back of the eye to the end of Telson) of 5 mm were obtained from the Persian Gulf Aquatic Reproduction and Reconstruction Center located in Kolahy Port. For this experiment, 600 pieces of *vannamei* shrimp larvae were prepared, and after the initial adaptation and bioassay, they were transferred to 4 hypothetical treatments, each consisting of 350-liter tanks. In other words, twelve 350-liter tanks, each containing 285 liters of water containing suitable and mature bioflacs with high bacterial density, host 50 posts of *vannamei* shrimp larvae with a density of 175 pieces per cubic meter.

Preparation and reproduction of commercial probiotics

Spores of commercial probiotic bacilli used in this experiment were obtained from Protexin Aquatec Company. This commercial product is in the form of bacterial suspension (mixture of *B. subtilis* and *B. licheniformis*) from the suspension of the above product using the instructions of Protexin Company. The concentration of 1.5×10^6 spores per ml was activated and prepared for eight hr. Spores were cultured on Trypticase soy agar and incubated for 30 h at 30°C.

Preparation and reproduction of native probiotics

The bacteria *B. subtilis* and *B. licheniformis* used in this project were isolated from the digestive tract of *Huso huso* juveniles. To prepare these probiotics, the suspensions of both bacteria were first cultured on TSA and incubated for 30 h at $30^{\circ C}$. Then, from the cultured colonies, using 0.5 McFarland solution and spectrophotometer, the desired concentrations were determined based on CFU/mL by determining the light absorption at a wavelength of 600 nm. The same procedure was followed to prepare commercial-native probiotic doses (in equal proportions of each). Finally, 1.5×10^6 CFU/g of the bacterium was added to the shrimp feed surface by spraying, and no probiotic supplement was added to the control treatment diet. The diet used in feeding samples of isoenergetic products made by Hoorash Company and containing 38% protein, 9% fat, and an energy level of 4523 calories per gram based on 7% biomass per day with an equal amount at 6, 14, and 22 which were fed during 60, 90 and 120 days.

Feed the shrimp

Shrimps were fed with four identical diets in the dose of probiotics during the experimental period: a control diet (without probiotics), a diet supplemented with commercial probiotics with a concentration

of 1.5×10^6 CFU/g, a diet supplemented with commercial-native probiotics with a concentration of 1.5×10^6 CFU/g, and a diet supplemented with native probiotics with a concentration of 1.5×10^6 CFU/g.

Measuring changes in biochemical factors

At the end of each experiment, 2.5 ml of hemolymph from the pericardial cavity in the cephalothorax was sampled. To measure plasma biochemical parameters, hemolymph samples were placed in a centrifuge at 12 Krpm at $4^{\circ C}$ for 15 minutes to separate plasma. Then the supernatant was separated and measured by autoanalyzer using quantitative detection kits of Pars Azmoun Company. Total plasma protein content according to Biuret method; glucose, cholesterol, and triglycerides by photometric method; enzyme level of uric acid; Plasma high-density lipoprotein cholesterol (HDL) value was evaluated by HDL cholesterol direct enzymatic; low-density lipoprotein cholesterol (LDL) was evaluated by LDL cholesterol enzyme colorimetric.

Investigation of growth factors in each period

At the end of each experiment period, shrimp growth factors including total weight, feed conversion ratio (FCR), survival rate (SR), specific growth rate (SGR), average daily growth (ADG), total length, and carapace length were evaluated in each treatment.

Statistical analysis

Using One-way ANOVA and Duncan's test based on the studied indicators, using SPSS and Excel software, the number of changes in each period of the experiment was calculated.

Result

Measuring changes in glucose index

The analysis results on the glucose index during the shrimp growth period in Table 1 showed that repetition on this index was not significant. The effect of different levels of probiotic treatments on this index did not have a significant effect. Also, this indicates that changes in native, commercial, and native-commercial probiotics have not affected glucose levels in shrimp. According to the diagram (Figure 1A), the highest amount of glucose in the control treatment and native-commercial probiotics is 861 mg, which is not significantly different from other treatments. The lowest value of this index was obtained in the control treatment at the rate of 848.5 mg.

Measuring changes in triglyceride index

The analysis results on the triglyceride index during the shrimp growth period in Table 1 showed that repetition on this index is not significant. Also, the effect of different treatment levels on this index is not significant. These results indicate that changes in native and commercial probiotics have not affected the triglyceride index of shrimp. According to Figure 1B, the lowest triglyceride index in the control treatment was 32.54, which is not significantly different from other

treatments. The highest value of this index was obtained in the native probiotic treatment at 36.35.

Measuring changes in total protein index

The analysis results of the total protein index during the shrimp growth period (Table 1) showed that repetition on this index is not significant. Furthermore, the effect of different probiotic treatments on this index is also significant at the level of 1% probability. This indicates that changes in native and commercial probiotics have affected the total protein content of shrimp. According to Figure 1C, the highest total protein in the native probiotic treatment is 9.32 g, which is significantly different from other treatments. The lowest value of this index in the control treatment was 8.26.

Measuring changes in immunoglobulin index

The analysis results of variance (Table 1) on immunoglobulin values showed that the effect of repetition on this index is not significant. The effect of different treatment levels on this index is significant at the level of 1% probability. This indicates that changes in probiotics have been shown to affect the shrimp immunoglobulin. According to the diagram in Figure 1D, the highest amount of immunoglobulin in the native probiotic treatment is 120 mg, which significantly differs from other treatments. The lowest value of this index was obtained in the control treatment of 99.27 mg.

Measuring changes in the cortisol index

The results of analyses on the cortisol index during the shrimp growth period showed that repetition on this index is not significant. The effect of different treatment levels on this index is significant at the level of 1% probability. This suggests that changes in probiotics may affect cortisol levels in shrimp. According to Figure 1E, the highest amount of cortisol in the native probiotic treatment is 1.38 mg per ml of blood, which significantly differs from other treatments combining carpentry and native. The lowest value of this index was obtained in the treatment of the commercial and local probiotic combinations of 1.08.

Measuring changes in LDL index

The analysis of variance on the LDL index during the shrimp growth period (Table 1) showed that the effect of repetition on this index is not significant. The effect of different treatment levels on this index is significant at the level of 1% probability. This shows that changes in probiotics have been able to affect the LDL index of shrimp. According to the diagram of Figure 1F, the highest LDL index in the control treatment was 31.25, which is a significant difference from the native probiotic treatment. The lowest value of this index was obtained in treating native probiotics at the rate of 29.55.

Measuring changes in HDL index

The analysis of variance (Table 1) on the HDL index during the growth period showed that the effect of repetition on this index is not significant. The effect of

different treatment levels on this index is significant at the level of 1% probability. This indicates that changes in commercial and local probiotics have affected the HDL index of shrimp. According to the diagram in Figure 1G, the highest HDL index in the control treatment was 90.75, which is a significant difference from other treatments. The lowest value of this index was obtained in treating native probiotics at the rate of 79.5.

Table 1. Results of analysis of variance on glucose, triglyceride, total protein, immunoglobulin, cortisol, LDL, and HDL indexes during the shrimp growth period

Sources of changes	Degrees of freedom	Mean of squares						
		Glucose	Triglyceride	Total protein	Immunoglobulin	Cortisol	LDL	HDL
Repetition	2	0.85ns	1.11 ns	0.04 ns	4.1 ns	0.0003 ns	0.76 ns	5.73 ns
Treatment	3	64.3 ns	0 .99 ns	0.86 **	295 **	0.12 **	10.67 ns	306.8 **
Error	6	11.86	0.43	0.06	6.8	0.003	0.64	2.56
Coefficient of variation	0.43	1.66	2.9	2.43 ns	4.8	2.57	1.8	

** Significance at 0.05 and 0.1%, ns: not significant in Duncan

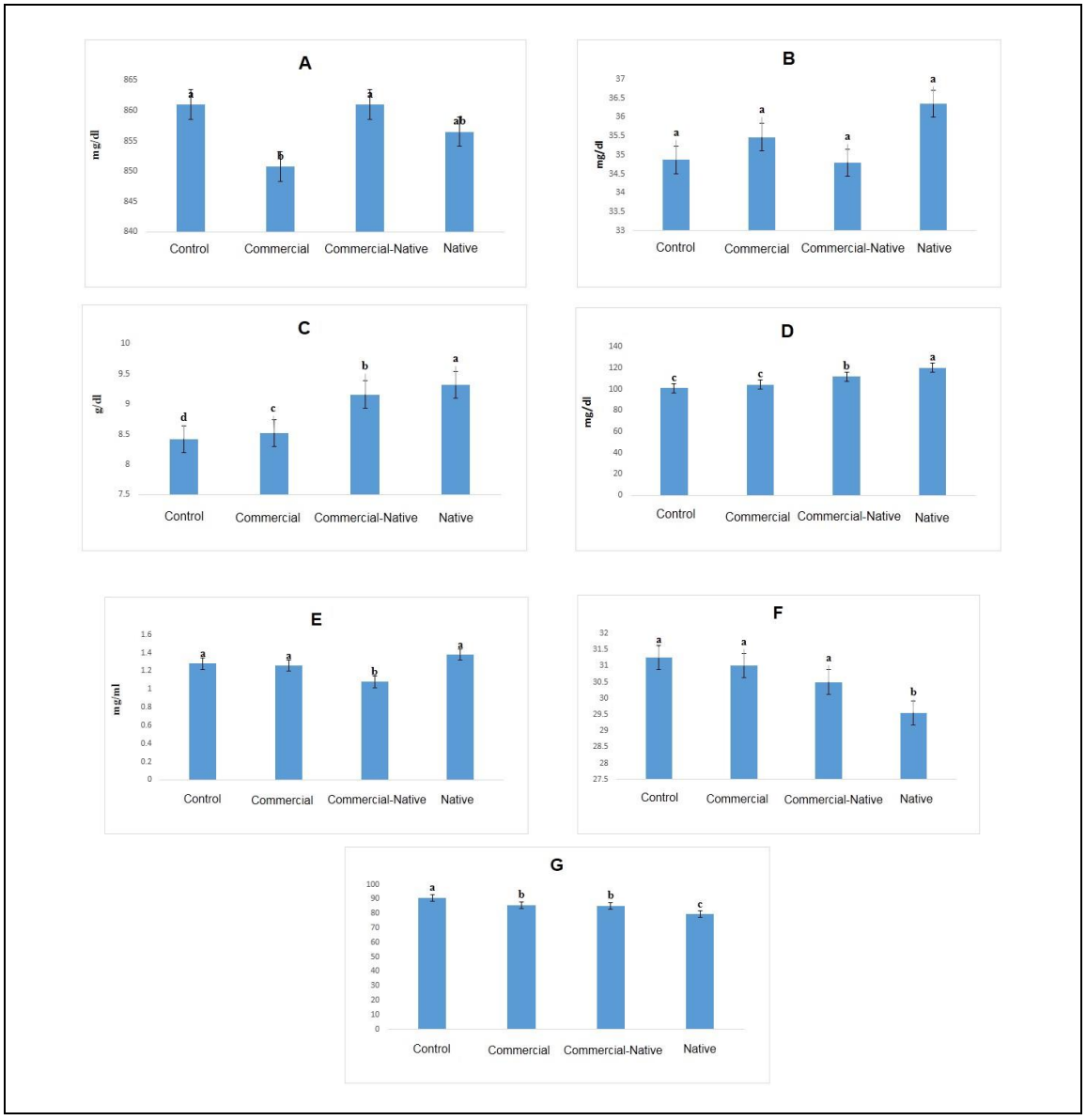

Figure 1. Measuring change in biochemical factors. A: Glucose changes in different treatments, B: Triglyceride changes in different treatments, C: Total protein changes in different treatments, D: Immunoglobulin changes in different treatments, E: Cortisol changes in different treatments, F: LDL changes in different treatments, and G: HDL changes in different treatments

Measuring changes in feed conversion ratio index

The analysis of variance (Table 2) on the amount of feed conversion ratio index during the growth period of shrimp showed that the effect of repetition on this index is not significant. In addition, the effect of different levels of probiotic treatments on this index is significant at the level of 1% probability. This shows that changes in the type of probiotics have affected the feed conversion ratio of shrimp. According to the diagram in Figure 2H, the highest feed conversion ratio in the native probiotic combination treatment is 2.14, which is a

significant difference from other treatments. The lowest value of this index in the control treatment was 1.86.

Measuring changes in the SGR index

The analyzed results (Table 2) on the SGR index during the growth period showed that repetition on this index is not significant. The effect of different treatment levels on this index is significant at the level of 1% probability. This indicates that changes in local and commercial probiotics have been able to affect the SGR of shrimp. According to Figure 2I, the highest amount of SGR in native probiotic treatment is 6.69, which is a significant difference between the control and commercial types. The lowest value of this index was obtained in the commercial-type treatment at the rate of 5.66.

Measuring changes in carapace length index

The analysis of variance (Table 2) on the carapace length index during the shrimp growth period showed that the effect of repetition on this index is not significant. Also, the effect of different treatment levels on this index is significant at the level of 1% probability. This indicates that changes in native and commercial types of antibiotics have affected the length of the carapace in shrimp. According to the diagram in Figure 2J, the maximum length of the carapace in the control treatment is 2.43 cm, which is significantly different from other treatments. The lowest value of this index was obtained in the treatment of native probiotics at the rate of 0.31 cm.

Measuring changes in the percentage of survival index

The analysis of variance (Table 2) on the percentage of survival during the growth period showed that the effect of repetition on this index is not significant. The effect of different treatment levels on this index is significant at the level of 1% probability. This shows that changes in local and commercial probiotics have affected the survival rate of shrimp. According to the diagram of Figure 2K, the highest survival rate in native probiotic treatment is 87.6%, which is a significant difference between the control and commercial types. The lowest value of this index in the control-type treatment was 79.33%.

Measuring changes in the uric acid index

The analysis of variance (Table 2) on the uric acid index during the growth period showed that the effect of repetition on this index is not significant. The effect of different treatment levels on this index is significant at the level of 1% probability. This indicates that changes in native and commercial probiotics have affected the uric acid content of shrimp. According to the diagram in Figure 2L, the highest amount of uric acid in commercial probiotic treatment is 1.52, which is a significant difference between the control and the native type. The lowest value of this index in the control-type treatment was 1.19.

Measuring changes in the total weight index

The analysis of variance (Table 2) on the total weight index during the growth period showed that the effect of repetition on this

index is not significant. The effect of different treatment levels on this index is significant at the level of 1% probability. This shows that changes in local and commercial probiotics have affected the total weight of shrimp. According to the diagram in Figure 2M, the maximum total weight in the native probiotic treatment is 13.26 g, which is a significant difference between the control and commercial types. The lowest value of this index in the control-type treatment was 11.8 g.

Measuring changes in the total length index

The analysis of variance (Table 2) on the total length index during the growth period showed that the effect of repetition on this index is not significant. The effect of different treatment levels on this index is significant at the level of 1% probability. This shows that changes in local and commercial probiotics have affected the total length of shrimp. According to the diagram in Figure 2N, the maximum total length in native probiotic treatment is 11.2 cm, which is a significant difference between the control and commercial types. The lowest value of this index in the control-type treatment was 10.83 cm.

Table 2. Results of analysis of variance on feed conversion ratio, SGR, the length of the carapace, survival rate, uric acid, total weight, total length, and indexes during the shrimp growth period

Sources of changes	Degrees of freedom	Mean of squares						
		Feed conversion ratio	SGR	The length of the carapace	Survival percentage	Uric acid	Total weight	Total length
Repetition	2	0.001ns	1.11 ns	0.0007 ns	0.25 ns	0.00001 ns	0.003 ns	0.003 ns
Treatment	3	0.06 **	0.99 ns	3.34 **	38.5 **	0.08 **	1.24 **	0.06 **
Error	6	0.002	0.43	0.001	0.36	0.003	0.002	0.002
Coefficient of variation		2.57	1.66	1.78	2.12 ns	4.54	1.38	1.42

** Significance at 0.05 and 0.1%, ns: not significant in Duncan

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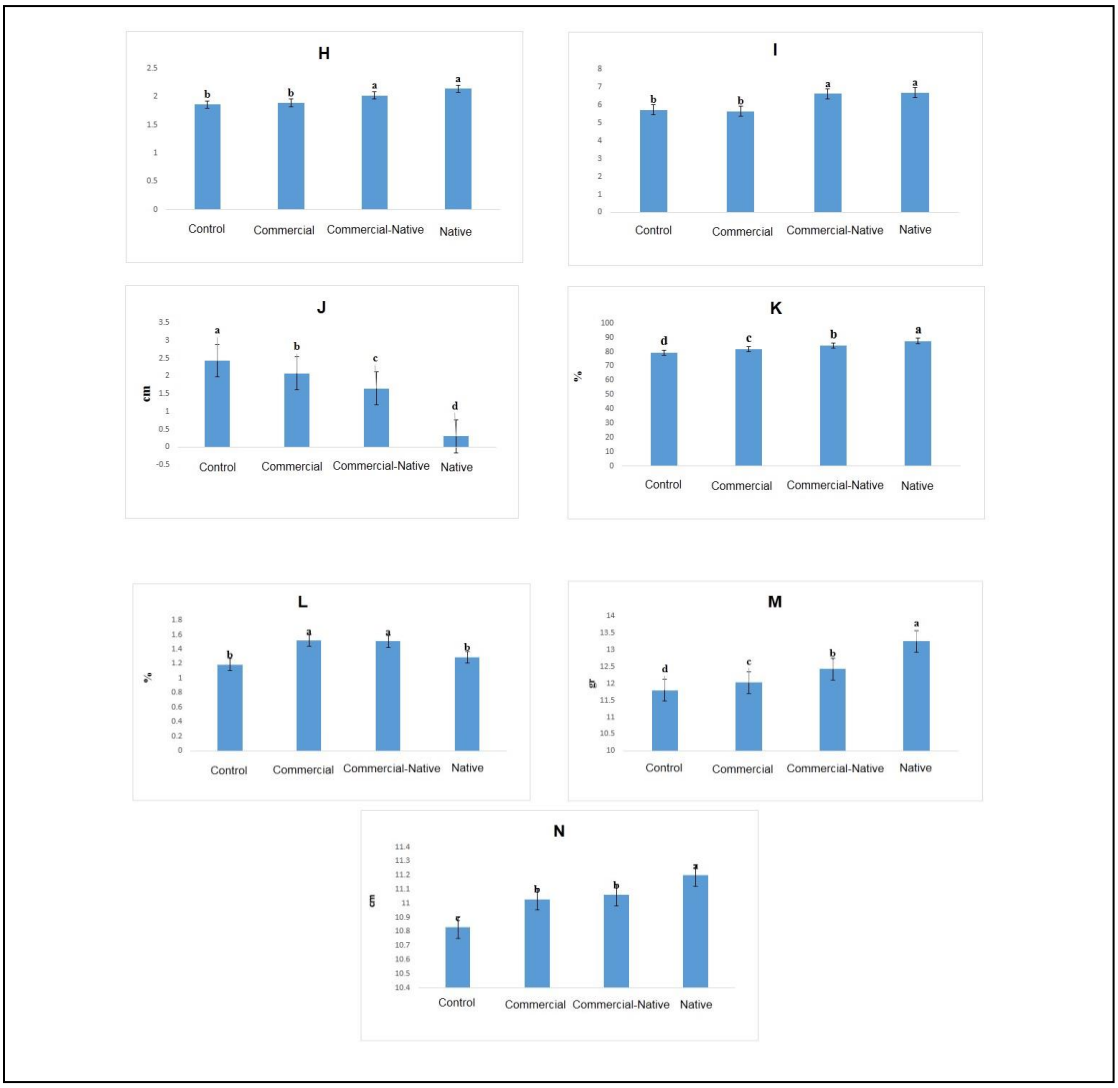

Figure 2. Measuring change in biochemical factors. H: Feed conversion ratio changes in different treatments, I: SGR changes in different treatments, J: The length of the carapace changes in different treatments, K: Survival rate changes in different treatments, L: Uric acid changes in different treatments, M: Total weight changes in different treatments, and N: Total length changes in different treatments**.**

Statistical analysis (correlation)

Based on the correlation between the studied indexes, it was observed that glucose and triglycerides have no significant relationship with any of the indexes. The protein content is not significantly related to cortisol, glucose, and triglycerides and is correlated with other indexes at a probability level of 1%. The highest significant correlation is between carapace length and survival percentage, which is 0.984, which is a negative or inverse relationship.

Discussion

In this study, 14 indexes of shrimp were measured and analyzed in a randomized

design with three replications under Duncan's test, analysis of variance, and comparison of mean values. In this study, we tried to maintain a balanced amount of nitrogen and carbon. Observations showed that the amount of total protein increased significantly compared to the control in both commercial and native probiotics. According to the study's main hypotheses, the results showed that HDL levels, LDL, uric acid, and cortisol indexes did not change significantly in different treatments. Still, glucose levels showed significant changes in the treatments. Also, there was no significant change in immunoglobulin and commercial-type probiotics, but significant changes were observed in commercial-native probiotics and native probiotics compared to control and commercial treatments. In each of the studied treatments, compared to the control treatment, the total weight of shrimp significantly increased. The feed conversion ratio did not change in control and commercial probiotic treatments, but it changed and increased significantly in both native probiotic and native-commercial probiotic treatments. The indices of carapace length, SGR, and shrimp length were significantly different in different treatments. In general, it can be said that the use of native probiotics can have a significant impact on many growth indicators of shrimp. Also, the number of chemical indices of this product had significant changes in both native probiotics and commercial probiotics treatments, but native types of these probiotics are more recommended.

Based on the correlation between the studied indexes, it was observed that glucose and triglycerides have no significant relationship with any of the indexes. The protein content is not significantly related to cortisol, glucose, and triglycerides and is correlated with other indexes at a probability level of 1%. The highest significant correlation is between carapace length and survival percentage, which is 0.984, which is a negative or inverse relationship. In other words, the longer the carapace, the lower the survival rate of shrimp, according to the study by Chun, SJ et al. (Chun et al., 2018) and Zhang, QH et al. (Zhang et al., 2011) and the length of the carapace in the shrimp will increase accordingly.

According to Badiola M et al. (Badiola et al., 2018), the levels of toxic metabolites were constantly monitored during the experiment. Ammonia nitrogen concentrations remained below the level reported by Lin & Chen (Liu et al., 2017), with the addition of molasses as a carbohydrate source in the experiment and its consumption by bacteria. Heterotrophs saw a decrease in ammonia nitrogen levels, followed by a reduction in water toxicity, which, according to Samocha et al. (Samocha et al., 2007), molasses effectively kept ammonia nitrogen low levels. On the other hand, during each growth period, the amount of re-nitrogen increased, which was controllable, and according to Cohen et al. (Levitt-Barmats et al., 2019) and Correia et al. (Correia et al., 2014), this is common. In this study, a pH=8 and a salinity of 36 mg/L were controlled, which according to FríasEspericueta & Páez-Osuna (Frías-Espericueta and Páez-Osuna, 2001), 2001; Lin & Chen (Liu et al., 2017), Vinatea et al. (VINATEA, 2010), Sudaryono et al.

(Sudaryono et al., 2015) a decrease follows this in the toxicity of ammonia nitrogen.

Arpanahi et al. (Abdollahi-Arpanahi et al., 2018) and Guardiola et al. (Zhang et al., 2011) showed that the amount of glucose in the control treatment (without probiotics) was statistically similar to its native type in *Litopenaeus vannamei*. This research is one of the most similar researches with the present research, which has similar results. Niroumand et al. (NIROUMAND, 2016) reported the highest and lowest values of this index: 41.5 and 18.58 mg, which follows the obtained results in this research. Using biofloc technology and adjusting the carbon-to-nitrogen ratio by forming biofloc biomass, this mass is consumed by microorganisms and these microorganisms are used as protein mass by shrimp and other aquatic animals. The proper ratio of carbon to nitrogen for the expansion and growth of biofloc masses is about 10 (Banerjee and Ray, 2017). This ratio is about 15.5 in the shrimp diet with 35-30% protein, which will be very suitable for biofloc mass growth. The suitable carbon-to-nitrogen ratio for the expansion and growth of biofloc masses is about 15 (Guo et al., 2016). This ratio in the shrimp diet with 35-30% protein is about 15, which will be very suitable for the growth of biofloc masses. In *Vannamei* shrimp, up to 29% of the daily water and food consumption of shrimp in the pre-feeding stage can be provided from the biofloc mass. Research has shown that the presence of biofloc in shrimp farming systems increases the growth of *Vannamei* shrimp by up to 15% and reduces the feed conversion ratio by up to 40%.

Arpanahi et al. (Abdollahi-Arpanahi et al., 2018) reported that the highest amount of protein in shrimp was 5.79 g. This number is lower than the current study, which was probably related to the type of shrimp. Also, they reported the highest and lowest levels of immunoglobulin, around 160 and 120 mg. The numbers obtained in the present study are almost consistent with other researchers in this respect. Furthermore, Arpanahi et al. (Abdollahi-Arpanahi et al., 2018) reported the highest amount of cortisol at 1.08 mg. This number is somewhat similar to the results of this study. Akbarzadeh et al. (Akbarzadeh Gholam Ali, 2006) obtained the highest amount of cholesterol in the control treatment without probiotics at 24.25 and the lowest in commercial-local probiotic treatment at 8.81; these values are similar to the calculated values in this study. Native probiotics, according to various research, have a higher efficiency than their commercial type (Akbarzadeh Gholam Ali, 2006). Also, Reeisi et al. (Reiesi) obtained the highest value of carapace length 2.64 cm.

Najmi et al. (Najmi Nasim, 2011) showed that the probiotic bacteria used had a positive effect on the resistance of shrimp and reported that Lactobacillus could improve the resistance and survival indices of *L. vannamei* larvae. Adineh and Hersij (Harsige, 2018) reported that replacing 25% of biofloc (25B) with concentrate feed can improve growth performance and water quality.

Biofloc technology to reduce the water change of breeding farms to zero is a goal followed by researchers. The added value of biofloc to reduce food consumption is quite

significant. Nutrition is one of the most important pillars of aquaculture, which is well supplied with the supply of fish meal and powder as the two main nutrients, essential amino acids, and fatty acids. In 2013, Emerenciano et al. (Emerenciano et al., 2013) examined the effect of natural products on reproductive performance, biochemical compositions, and fatty acid profiles of western white shrimp breeders under biofloc conditions. The results showed that new diets (squid and oysters) in biofloc conditions cause better efficiency of western white shrimp breeders. Types of microorganisms used in the aquaculture industry as probiotics are yeasts, bacilli, lactic acid bacteria, and Pseudomonas. In fact, by applying microbial management in the form of beneficial bacteria, the ecological compatibility of the host with the breeding environment, and the ability to establish inhibitory effects against pathogens will increase.

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