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Research Article

The effect of dietary fatty acids on the incidence of the redhead syndrome in white leg shrimp (*Litopenaeus vannamei*) under environmental stress

Mobaraki S.[1]; Javadzadeh N.[1*]; Mabudi H.[1]; Hafezieh M.[1,2]; Khodadadi M.[1]

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Abstract

This study aimed to examine the effect of fatty acid in the diet on changes the incidence of the Red-head Syndrome under temperature stress and hypoxia in white leg shrimp (*Litopenaeus vannamei*) during 8 weeks. For this purpose, shrimps were fed by 4 diets (diet 1: containing 7.02% of total fat; diet 2: containing 6.41% of total fat; diet 3: containing 8.05% of total fat; diet 4: containing 6.41% of total fat with 1750 mg/kg of vitamin E), under 3 stressors (S1: no challenge to environmental stresses; S2: with hypoxic stress (2.0±1.0 ppm); S3: with increasing temperature stress (36±2°C). The results of the hypoxic stress on the incidence of redness in *L. vannamei* fed with different levels of fatty acids showed that there were differences significantly ($p<0.05$). Temperature increase although showed no differences between healthy shrimps($p>0.05$) but, there were significant difference among red-headed shrimp in treatment 1, 2 and 3 ($p<0.05$). The examination in treatments 2 and 3 showed hepatopancreatic were loss the columnar structure so thatelongated cells and dilated tubules were observed. Some B cells had no vacuoles and were not fully developed. The number of R cells was low and basophilic F cells were convex. In treatment 4, the cells have few reserves and were normal. Some B cells were degenerated. The height of the tubules was lower than normal, but no inflammatory reaction was observed in the tissue. In treatment 4, cell showed better conditions than treatments 2 and 3. Accordingly, B cells were present well and R cells showed moderate storage. In general, it can be concluded that the use of vitamin E can reduce the complication of redness and minimal cell damage to hepatopancreatic tissue in *L. vannamei*.

Keywords: Fatty acid, Vitamin E, Redhead, Hepatopancreatic tissue, *Litopenaeus vannamei*, Environmental stresses

1-Department of Fisheries, Ahwaz Branch, Islamic Azad University, Ahwaz, Iran
2-Iranian Fisheries Sciences Research Institute, Agricultural Research Education and Extension Organization (AREEO), P.O. Box: 15745/133. Tehran, Iran
[*]Corresponding author's Email: nargeajavadzadeh@yahoo.com

Introduction

Shrimp farming is developing as one of the most important aquaculture activities in the world and Iran. The use of non-native species to increase food production is increasing worldwide, such as the breeding of *Litopenaeus vannamei* (Peña-Navarro *et al*., 2020). The redhead complication is due to the appearance of orange or red color in the cephalothorax. Penaeid shrimp are very sensitive to various environmental and oxidative stresses such as stresses caused by changes in temperature, ammonia, and oxygen variables (Fazlerohani *et al*., 2019).

The presence of antioxidants in the daily diet can improve the aquatic immune system and reduce the effect of environmental stress (Babin *et al*., 2010). The nutritional needs of shrimp include protein, amino acids, lipids, minerals, vitamins, and carbohydrates prerequisites for pellet production in shrimp farms and should be used in the diet. Different species of Penaeid shrimps have different needs for fatty acids in their diets. The Onzález-Félix *et al*. (2002) reported that the amount of unsaturated required fatty acids in the diet of *L. vannamei* shrimp will be raised by the increase in salinity.

Vitamins are essential for the survival, growth, and natural reproduction of animals as one of the main components of food in the diet. Vitamin E is one of the fat-soluble vitamins and as antioxidant, it also plays an important role in increasing the body's resistance to infection against stressful conditions. It has also been shown to have a positive regulatory effect on stress-related reactions in fish (Harsij *et al*., 2020). Lucien-Brun (2006) found that if the food quality is poor and the breeding ponds are not in good condition in terms of adequate environment and water change, redness or rupture of the hepatopancreas occurs. Studies by Tume *et al*. (2009) and Parisenti *et al*. (2011) showed that non-ester (free) astaxanthin pigment is probably the predominant pigment in red-headed shrimp. Ambati *et al*. (2014) showed that redhead complication in shrimp is due to increased levels of astaxanthin pigment in their tissues. Han *et al*. (2013) studied shrimp body pigments and found that any factor that releases astaxanthin from the surrounding protein structure could intensify the red color in shrimp hepatopancreas.

Environmental factors including temperature fluctuations and oxygen concentrations have a specific effect on the occurrence of redhead complications (Deniss and Diaz, 2011). So far, several studies have examined the different effects of physicochemical stresses and suggested ways to control and reduce their effects (Lee *et al*., 2004; Parisenti *et al*., 2011; De Sousa *et al*., 2016; Tume *et al*., 2019; Harsij *et al*., 2020; Ebadi *et al*., 2021). Temperature stresses are more effective on shrimp than osmotic stresses (Chien *et al*., 2003). Duan *et al*. (2014) reported that Total Plasma Protein and hepatopancreas glycogen were highly depleted in shrimp by swimming fatigue at various DO concentrations, whereas the plasma lactate accumulated at high levels after swimming fatigue at different velocities. Chen *et al*. (2019) stated both the innate immune response and the environmental stress response have a complex relationship with shrimp diseases. De Souza *et al*. (2016) reported that with the temperature fluctuation from the optimum, enzymatic activities and the amount of oxidative stress increases. This study aimed to investigate the effects of dietary fatty acid composition on the incidence of the redhead and hepatopancreatic tissue of *L. vannamei* under environmental stress and the effect of vitamin E on the improvement of the redhead complication.

Material and methods

Animals

L. vannamei shrimp with an average weight of 8±1 (g), were collected from a farm and stored in 1000 liter tanks containing vitamin C. After 3 days, the shrimps were biometric transferred to 300 liter fiberglass tanks containing 250 liters of seawater with a salinity of 43±1 ppt under laboratory conditions. The density of each tank was 15 pieces of shrimp. After adaptation time (24 hours), each group was kept in a controlled condition for 8 weeks and fed on specially prepared food ration.

Nutrients

The nutrients used in this study as raw materials in the preparation of diets are presented in Table 1. Hammond's method (1986) was used to methylate fatty acids. The analysis of the composition of the experimental diets used based on the percentage of moisture and the percentage of dry matter is shown in Table 2. Table 3 shows the constituents of the diets used in the present study in terms of dry matter. The combination of experimental diets (grams of food per 100 grams) is presented in Table 4.

Table 1: Food supply sources for making diets in the experiment.

Source	Food ingredients
Kilka powder	Pars Kilka
Meto powder	Pars Kilka
Sardin powder	Pars Kilka
Shrimp meal	Havorrush Company
Soy Lecithin	Mazandaran Livestock Feed
Fish oil	Pars Kilka
Gluten	Havorash Company
Vitamin E	Kimia Roshd Company
Cholesterol	Merk Company
Cellulose	Merk Company
Antioxidants	Company VDS
Antifungal	Company VDS
Vitamin premix	Company VDS
Mineral premix	Company VDS

Table 2: Lipid composition level for each experimental feed.

Diets	%Total lipid	Total saturated fatty acids Per 100 grams of the food	Total unsaturated fatty acids Per 100 grams of the food	considerations
Diet 1	7.02	5.0	13.0	-
Diet 2	6.41	1.0	2.0	-
Diet 3	8.05	3.0	7.5	-
Diet 4	6.41	3.0	2.0	With 1750 mg/kg of vitamin E.

Table 3: diet composition for each experiment.

Diets	Moisture %	Dry Matter %			
		Protein	Lipid	Fiber	Ash
Diet 1	10.1±0/.	36.81±0.28	7.02±0.28	3.5±0.06	8.25±0.12
Diet 2	9.8±0.11	35.96±0.28	6.41±0.28	1.0±0.06	8.5±0.12
Diet 3	9.9±0.11	35.14±0.28	8.05±0.28	0.8±0.06	7.75±0.12
Diet 4	9.7±0.11	36.48±0.28	6.41±0.28	0.5±0.06	9.5±0.12

Table 4: percent ingredient composition of each experimental diet (grams of food per 100 grams).

Ingredients %	Diets			
	Diet **1**	Diet **2**	Diet **3**	Diet **4**
Fish meal	40.00	40.61	38.07	40.61
Shrimp meal	15	15	15	15
Binder	1.5	1.5	1.5	1.5
Lisetin	1	1	1	1
Cholesterol	0.5	0.5	0.5	0.5
wheat flour	25.71	25.71	25.71	25.71
Lipid	7.02	6.41	8.05	6.41
Mineral supplement	2	2	2	2
Vitamin supplement	2	2	2	2
Antioxidants	0.02	0.02	0.02	0.02
Antifungal	0.25	0.25	0.25	0.25
Dicalcium phosphate	1.5	1.5	1.5	1.5
cellulose	3	3	3	3
Chromium oxide	0.5	0.5	0.5	0.5
Total	100	100	100	100

Study design

At this stage, four groups (Treatments) were examined (three diets with basic food groups and one basic food containing vitamin). All groups had 3 treatments and each treatment had 3 replications. (diet 1: containing 7.02% of total fat; diet 2: containing 6.41% of total fat; diet 3: containing 8.05% of total fat; diet 4: containing 6.41% of total fat with 1750 mg/kg of vitamin E) and under 3 stressors (S1: no challenge to environmental stresses; S2: with hypoxic stress (2.0±1.0 ppm); S3: with increasing temperature stress (36±2°C))

In group 1 shrimp fed with Diet 1 and treated without the challenge of environmental stresses (S0, water temperature 28±1°C and DO<5 ppm), Diet (1) with hypoxia stress (S1, 2 ppm±1) and Diet (1) with increasing water temperature stress (S2, 36±2°C).

Group 2 includes shrimp fed on Diet 2 and treated without the challenge of environmental stresses (S0), with water temperature (28 ± 1 °C)and DO(<5 ppm), Diet (2) with hypoxia stress (S1, 2 ppm±1) and Diet (2) with increasing water temperature stress (S2, 36±2°C).

Group 3 of this study is shrimp fed with Diet 3 and treated without the challenge of environmental stresses (S0), water temperature (28±1°C)and DO(<5 ppm), Diet 3 with hypoxia stress (S1, 2 ppm±1), Diet 3 with increasing water temperature stress (S2, 36±2°C).

Group 4 of this study are shrimp fed with diet 4 and treated without the challenge of environmental stresses, water temperature (S0, 28±1°C and DO<5 ppm), Diet 4 with hypoxia stress (S1, 2 ppm±1), Diet 4 with increasing water temperature stress (S2, 36±2°C).

The challenge of rising temperatures

To increase temperature, the dedicated treatment in each groupwas challenged to Temperature 36±2°C to increase the water temperature for 48 hours by electric aquarium heaters to apply temperature stress) (Qiu *et al*., 2011).

Oxygen deficiency challenge

For hypoxia stress, the shrimps were then keptwhere aeration of the treatment tanks was stopped after 8 weeks until the dissolved oxygen reached 1.5-2 (mg/lit) for 48 hours . No water changes were made during the challenge. Feeding at the time of the challenge was done according to the pre-challenge schedule (Pakravan *et al*., 2018). At the end of the environmental stress period, shrimps of all treatments were harvested separately and bioassayed (Wang et al., 2016).

Prevention of complications with antioxidants

After obtaining the results of each treatment in group4, vitamin E was added to diet at a rate of 1750 mg/kg of food in order to investigate the possibility of preventing redhead syndrome (Díaz *et al.,* 2004). Therefore, group 4 of the study included shrimps that were fed with diet 4 (Liu *et al*., 2007).

Tissue histopathology

Hepatopancreatic tissue was removed in vitro and immediately fixed in Davidson solution to prevent the destruction of cell and tissue structure by the activity of intracellular enzymes. For this purpose, Davidson's solution was injected into the hepatopancreatic tissue of the sampled shrimp. Then, the hepatopancreas tissues remained in the solution for 24-72 hours and then stained using H & E staining method (Lightner, 1996; Yeganeh *et al*., 2020). Accordingly, the tissues were placed in ethyl alcohol and xylene solution to prepare the paraffin to penetrate the tissue. The samples were then placed in molten paraffin and then paraffin-impregnated samples were blocked. Then, incisions 5 to 10 μm thicknesses were prepared using a microtome(Leica jung RM2045,Germany). The tissue was then stained using Hematoxylin and Eosin (H & E), observed using a light microscope (Nikon Eclipse-E200, Japan), and photographed with a digital camera connected to the microscope.

Statistical analysis

The experiment was conducted as a completely randomized plan. First, the data normality condition was examined by Shapiro-Wilk statistical test. Then the means were compared using one-way ANOVA. Tukey test was used to evaluate the significant differences between treatments. All analyzes were performed using SPSS software version 23 and graphs were drawn using Microsoft Office Excel 2013 (Zar, 2013).

Results

In order to select and determine 4 diets (Table 4), six commercial foods collected from shrimp farms of shif Shrimp Site, Bushehr province, where the Red head disease observed. The results obtained from the fatty acid profile analysis are presented in Table 5 and helped us to determine diets (Table 4) applied in this experiment. After reviewing the analysis, 4 diets were made to evaluate the complication of redhead disease and the effect of vitamin E on improving the complication was added. The analysis of diets and the physical and chemical parameters of water on different days used in the experiment are shown in Table 5 and Figure1, respectively.

Table 5: Analysis of commercial foods based on fatty acid profiles in each experiment.

	Fatty acid %	Food **1**	Food **2**	Food **3**	Food **4**	Food5	Food **6**
1	C14:0	1.85	1.61	5.42	2.02	2.24	2.18
2	C16:0	20.49	22.56	24.92	15.71	20.2	19.03
3	C16:1	2.67	5.24	5.67	1.54	3.33	2.24
4	C18:0	5.49	5.93	6.68	5.02	5.47	5.2
5	C18:1 n-9	27.85	33.3	25.99	22.99	26.39	26.04
6	C18:2 n-6	26.61	18.99	11.12	39	25.97	34.69
7	C18:3 n-3	4.11	2.34	2.27	5.31	4.48	3.27
8	EPA	2.56	2.1	4.05	1.84	2.44	1.75
9	DHA	6.53	4.34	5.63	4.02	4.39	2.43
10	Total Omega3	13.2	8.78	11.95	11.17	11.31	7.45
11	Total Omega6	26.61	18.99	11.12	39	25.97	34.69
12	Total Omega9	27.85	33.3	25.99	22.99	26.39	26.04
13	Total saturated fatty acid	27.83	30.1	37.02	22.75	27.91	26.41
14	Total unsaturated fatty acid	70.33	66.34	54.73	74.7	67	70.42
15	Total saturated fatty acids in 100 g	5.2	1.35	2.72	1.47	2.95	3.22
16	Total unsaturated fatty acids in 100 g	13.15	2.98	4.02	4.84	7.08	8.6

Physicochemical parameters of water including oxygen, temperature, salinity, and pH are depicted in Figure 1.

Oxygen depletion stress

The results of the effect of hypoxic stress on the incidence of red-head complication in *L. vannamei* fed on different levels of fatty acids during 8 weeks are shown in Table 6. Based on the obtained results, there was a significant difference between treatment 1 and treatments 2, 3, and 4 (p<0.05). While there was no statistically significant difference between treatments 2 and 3 (p>0.05). There was also a significant difference between treatments 2 and 4 (p<0.05). Therefore, it can be concluded that oxygen depletion as an environmental stimulus is effective for oxidative stress and can affect the occurrence of redness (Fig. 2).

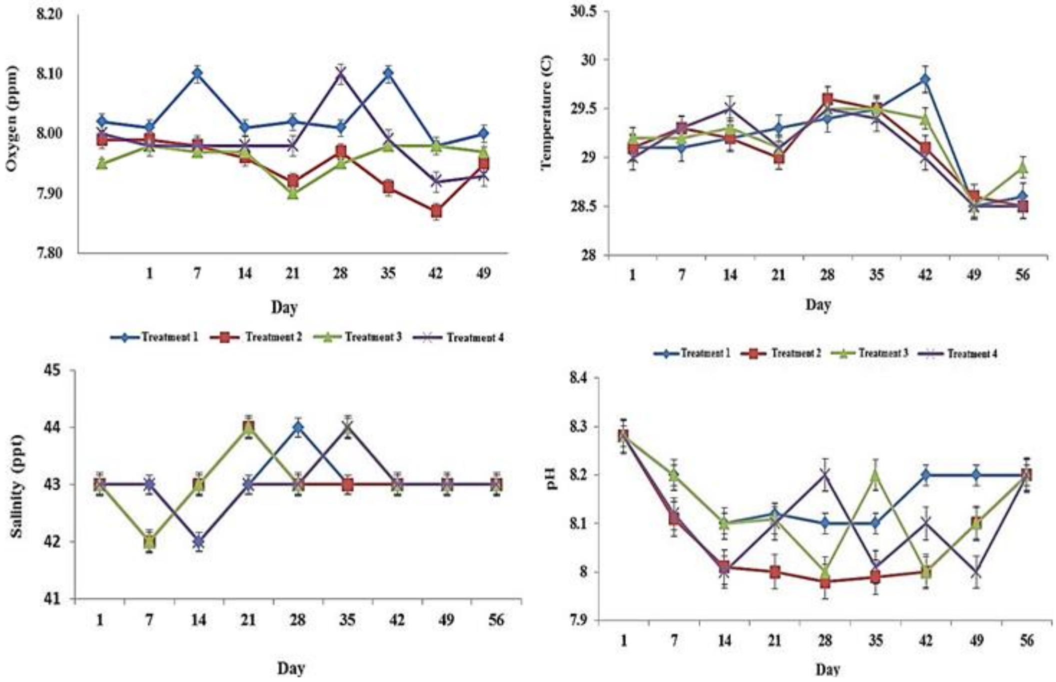

Figure 1: Water quality parameters during the experimental period (mean ± standard deviation). The groups of the experiment are presented as treatments in these graphs.

Table 6: Percentage of a redhead (Mean ± SE) due to oxygen depletion stress in the tested shrimp.

Feature	1	2	3	4
Non-redhead (%)	100.0±0.0[a]	66.67±2.72[b]	71.11±3.14[b]	91.11±3.14[c]

Different letters in the row indicate a significant difference between experimental treatments (p<0.05).

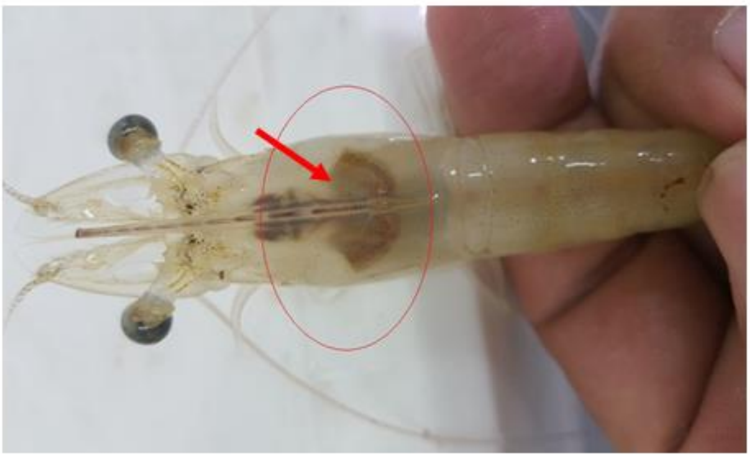

Figure 2: Ruptured hepatopancreas due to environmental stress.

Stress by rising temperature

The results of *caused* temperature rise as an environmental factor in the incidence of redhead syndrome in the tested shrimp are shown in Table 7. These results show that healthy shrimp do not differ significantly in treatments 1 and 4 (p<0.05). Shrimps in treatment 1 showed a significant difference with treatments 2 and 3 (p <0.05). There is no statistically significant difference in red-headed shrimps in treatments 1 and 4 (p>0.05). Therefore, if the composition of the diet is not suitable in terms of total fat content and the temperature rises, the prevalence and progression of redhead disease will increase. Treatment 2 with the lowest total fat content of 6.41% showed the highest prevalence of redhead complication. The results also showed that the use of vitamin E antioxidant in treatment 4 significantly reduced the complication.

Table 7: Percentage of redhead incidence (Mean ± SE) due to temperature stress in tested shrimp.

Treatments	1	2	3	4
Non-redhead (%)	98.89±1.57[a]	64.29±6.64[b]	70.00±4.71[b]	90.48±3. 37 [a]

Different letters in the row indicate a significant difference between experimental treatments (p<0.05).

The effect of vitamin E on the complication

The results of the experiment showed that the use of vitamin E supplemented diet is effective on reducing the occurrence and progression of the complication. In shrimp of treatment 4 enriched with vitamin E in the conditions of environmental stress, the prevalence of the complication did not expand, which was similar to those of treatment 1 as control diet (p<0.05).

Histopathology observations

Based on pathological studies of shrimp fed with experimental diets containing different profiles of fatty acids, cellular changes were observed in the hepatopancreatic organs of *L. vannamei*. Cellular examination of hepatopancreatic tissue of shrimp fed on diet 1 containing 7.02% of total fat revealed that most of B cells (blasenzellen) were significantly depleted. Some of the foci identified in the figure have been destroyed and some cells have receded in this area. But in general, the cells are in a normal state (Fig. 3A, B). Figure 3B shows the cross-section of the tubule in which a large number of vacuolated R cells have accumulated (H & E/ Pheoxin. X: 400). Other epithelial cells are very rare. Some myoepithelial cells (MECs) are visible. Also, some cell structures are seen that are phagolysosomes. Cellular examination of hepatopancreas related to treatment 2 (a diet containing 6.41% of total fat) showed that cell height was reduced. The normal and optimal height of these cells in the tissue should be about 50 micrometers. Vacuoles and their reserves are reduced (Fig. 3C, D). Figure 3C shows the cross-section of the initial tubular region in western white leg shrimp (*Litopenaeus vannamei*) hepatopancreatic tissue (H & E/ Pheoxin. X: 10), the "restzellen" (R) cells are present and marked by yellow arrows. Several B cells ("blasenzellen") have seen that look like stars in this image. Areas of the cell that are lower in height (Ihcz) are visible on the tubules. In this image, a cluster of cells can be seen. The vacuoles have shrunk and their reserves have been reduced. B cells are shown in Figure 3D (H & E / Pheoxin. X: 400). But it is lower than normal conditions and their cellular storage levels are reduced and their amount is much less than normal.

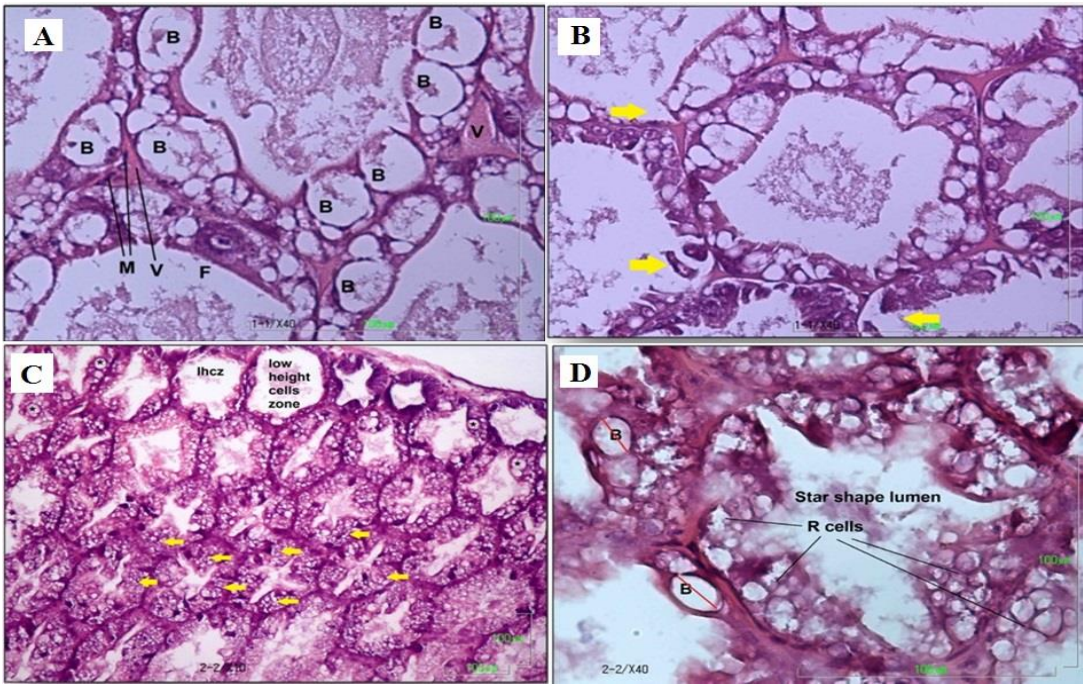

Figure 3: Histopathology of *L. vannamei* hepatopancreas tissue in treatments 1 (a diet containing 7.02% of total fat; Figs. A and B) and 2 (a diet containing 6.41% of total fat; Figs. C and D). Sections of tissue were stained using Hematoxylin and Eosin (H & E).

In treatment 4 (a diet containing 6.41% of total fat with 1750 mg/kg of vitamin E) the cells have few reserves and the available reserves were abnormal. Some B cells are depleted. The height of the tubules is lower than normal, but no inflammatory reaction was observed in the tissue (Fig. 4A and B). Figure 4A shows the atrophied cross-section of the tubules in the hepatopancreatic tissue of western white leg shrimp (*Litopenaeus vannamei*), which have very few reserves. No infiltration of hemocytes was observed and there is no inflamed part (H & E/ Pheoxin. X: 10). Figure 4B shows a convex section of a tubule. The amount of reserves was average. Some of the B cells have been emptied. No inflammatory reaction was observed. Some tubules appear softer and less swollen (H & E/ Pheoxin. X: 10). The condition of the cells is better than treatment 2 (a diet containing 6.41% of total fat) and B cells were present well. R cells have moderate reserves (Fig. 4B). Figure 4C shows a convex image of hepatopancreatic tubules. The reserves were in good condition. There were several R cells with moderate reserves. The interstitial arteries were in good condition and no abnormalities were observed (H & E/ Pheoxin. X: 400). Figure 4D shows a cross-section of a tubule that has become partially atrophied. The height of the tubule was lower than normal. The number of reserves was very low or destroyed. But no inflammatory reaction was observed (H & E/ Pheoxin. X: 400).

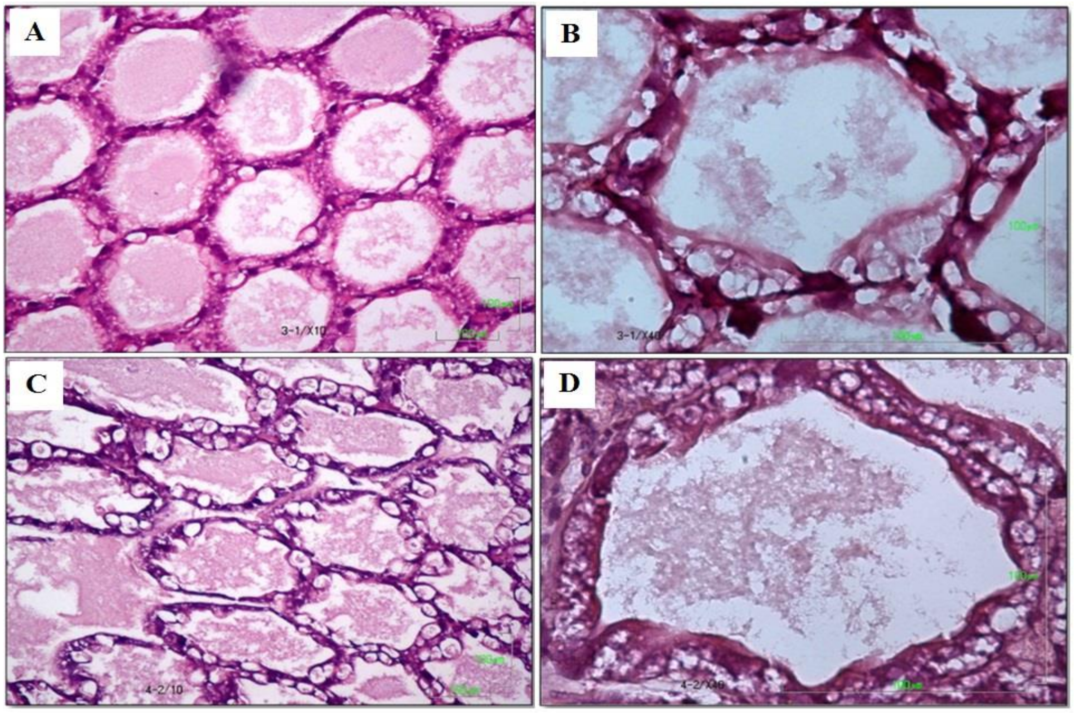

Figure 4: Histopathology of L. vannamei hepatopancreas tissue in treatments 3 (a diet containing 8.05% of total fat; Figs. A and B) and 4 (a diet containing 41 6.6% of total fat with 1750 mg/kg of vitamin E; Figs. C and D). Sections of tissue were stained using Hematoxylin and Eosin (H & E).

Discussion

Various factors cause redness in shrimp. Poor food quality and poor nutrition management are the main reasons for this complication. The use of substandard compounds in the diet, including the use of substandard fish meal, undesirable fish oil, or their replacement with other plant proteins, has a direct effect on hepatopancreatic tissue and causes oxidation in fats. The occurrence of redheads has been one of the quality problems of western white leg shrimp (*L. vannamei*) in recent years and loose the shrimp cost in trade. Although this complication does not have a significant effect on the health, smell, and taste of shrimp and is only an apparent defect, it causes the appearance of shrimp to deteriorate and reduce marketability, and ultimately reduces its price in shrimp markets (Peña-Navarro *et al*., 2020; Ebadi *et al*., 2021). The best way to prevent this complication is to use food diets that contain fatty acids and antioxidants to reduce the effects of stress. Vitamin E has a special role as an intracellular antioxidant and is essential for maintaining metabolism in cells. This vitamin is fat-soluble and causes the best response in shrimp in the face of stressful conditions (An *et al*., 2020). Lipid performance by enriched Artemia with determined fatty acids or increasing the immunity level by adding extracts of Persian walnut to improve diets fed to shrimp brood-stock or post-larvae in order to develop ovaries or resist against stresses (Adloo *et al.,* 2020; Forouzani *et al*., 2021). On the other hands, adding 4-6% of *Bohadschia ocellata* meal to commercial diet of *P. vannamei juveniles* clearly improved some biochemical activities such as blood cholesterol content to enhance growth and immunity performance (Javanmardi *et al.*, 2020). In another study, *Zingiber officinale* extract, which has lipid contents was appropriate for increasing growth performance, antioxidant activity and disease resistance of *L. vannamei* (Shahraki *et al*., 2021).

In the present study, temperature stress increased the incidence of redhead complications in farmed shrimps. The shrimp fed with diet 2 had lower quality than the other tested diets. Therefore, these shrimps had lower reserved fat and had difficulty providing metabolic energy. Shrimp fed with diet 1 (with higher quality and dietary compositions) showed the least complication effects. Liñán-Cabello *et al*. (2002) have shown that tolerance to environmental stresses increases in the presence of carotenoids. As explained by Lucien-Brun (2006), poor food quality, along with other adverse environmental conditions, causes redness or rupture of the hepatopancreas, which eventually spreads due to stress on shrimp. These results were consistent with the results of the studies by Chien *et al*. (2003) and De Souza *et al*. (2016). They showed that during the beginning of stress due to temperature changes, the resistance of shrimp decreases, and the function of the hepatopancreas decreases. Also, enzymatic activities increase and the amount of oxidative stress increases with temperature fluctuations from the optimum limit. Qiu *et al*. (2011) found that exposing *L. vannamei* to non-optimal temperature conditions for 3 hours significantly increases the effect of oxidative stress and lipid oxidation. Based on these results, temperature changes are considered as a very effective environmental factor on the occurrence of damage caused by environmental stress in shrimp, which is very important to maintain in natural conditions and following the needs of the breeding period to improve production conditions.

The observed results of diet 4 containing antioxidant vitamin E showed that shrimp fed with this diet under the conditions of stress decreased oxygen (8.89%) and increased temperature (9.52%) compared to shrimp fed with foods 2 and 3 have the lowest incidence of redness. This indicates the positive effect of the presence of vitamin E antioxidants in improving the complication. According to Gerald Combs *et al*. (2016), this vitamin acts as a fat-soluble biological antioxidant, protects fats from peroxidation by depleting free radicals, and minimizes the saturation of unsaturated lipids by stabilizing unsaturated lipids. As an effective and effective antioxidant, this vitamin also increases the resistance of shrimp in the face of acute environmental fluctuations. This finding is consistent with the findings of other researchers such as Lee *et al*. (2004) researched the vitamin E requirements of juvenile grass shrimp, *Penaeus monodon*, and effects on non-specific immune responses, Díaz *et al*. (2004) researched antioxidant activity in the hepatopancreas of the shrimp (*Pleoticus muelleri*) by electron paramagnetic spin resonance spectrometry, and Liu *et al*. (2007) researched the effects of dietary vitamin E supplementation on antioxidant enzyme activities in *Litopenaeus vannamei* (Boone, 1931) exposed to acute salinity changes. According to these researchers, the presence of vitamin E in the shrimp diet is very important to achieve maximum growth and nonspecific immune responses, and slows down the oxidation of fats, and is effective in reducing the formation of hydroperoxides. They also showed that this vitamin accumulates in muscle tissue and hepatopancreas. Li *et al*. (2014) showed that the concentration of vitamin E in hepatopancreas and muscle tissue increases with increasing vitamin E supplementation in the diet of juvenile shrimps.

In the present study, hepatopancreatic tissue was examined in fed shrimp with four experimental diets. These studies showed that the cells in the hepatopancreatic tissue of fed shrimp with diet 1 were in a normal state. In fed shrimp with diet 2, cell height and vacuoles were reduced, and their reserves were depleted. The cells were in an abnormal state. In fed shrimp with diet 3, the height of the cells was reduced, and, the vacuoles were not fully formed in some cells, and generally, the normal structure of the cells is not seen. Finally, hepatopancreatic tissue cells in fed shrimp with diet 4 were in poor condition compared to normal condition, and their reserves were severely depleted, but they were in a better situation than the tissue cells in treatment 2. Díaz *et al*. (2004) reported that diet-fed shrimp containing different levels of vitamin E showed some tissue changes. Changes in hepatopancreatic tissue cells, hypertrophy, tissue irregularities, shrinkage, and hydration of the cells were some of the cases observed in these cells. However, the same research showed that the use of 1750 mg of vitamin E per kg of food did not show any pathological changes in the organ and the tissue was in a normal state. Wang et al. (2016) reported that with decreasing temperature, the number and volume of secretory cells in *Litopenaeus vannamei* hepatopancreas increase significantly, the tubular lumen appears dilated and the epithelial cell layer becomes thinner. Nutrients such as vitamin E in the shrimp diet play a very important role in their growth and survival rates. Shrimp cannot make these substances in their bodies, so they must get them through their diet. Therefore, the use of supplements containing a variety of vitamins in the diet is essential for them (Arshadi *et al*., 2020). In the present study, it was found that the use of vitamin E in the diet reduced stress in shrimp and therefore the complication of redness in shrimp fed a diet containing vitamin E was reduced. Therefore, it can be concluded that the use of vitamin E supplement antioxidants in the shrimp diet, especially in the harvest diet (at the end of the rearing period) can greatly reduce the stress of harvest time and reduce the risk of redhead complications.

The results of the present study showed that the use of vitamin E supplements is effective in preventing the occurrence and progression of the complication and reduces its severity. In the tested treatment 4 which was enriched with vitamin E supplementation, the prevalence of the complication did not increase in the conditions of environmental stress, but in the conditions of treatment 1 which was the control diet, it did not show a significant difference ($p<0.05$). Therefore, it can be concluded that the use of antioxidant supplements of vitamin E in the diet of shrimp, especially in the diet used by breeders at the end of the rearing period, can greatly reduce the existing stress and greatly reduce the risk of redness.

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