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Effect of different levels of dietary protein and water salinity on antioxidant enzymes of white leg shrimp (*Penaeus vannamei*) juveniles

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Abstract

In this study, effect of different levels of dietary protein (25, 35 and 45 per cent) and water salinity (0-3, 12-15 and 32-35 ppt) on antioxidant enzymes of white leg shrimp (*Penaeus vannamei*) was studied. 324 P.vannamei (with mean weight±SE: 5.55 ± 0.18 gr) were randomly distributed in twenty seven tanks. Experimental shrimps were fed by formulated diets for 56 days and at the end of the experiment, total antioxidant capacity (T-AOC) and activity of super oxidase dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) of hepatopancreas and muscle tissue were measured. The results showed significant difference in antioxidant enzymes activity of experimental shrimps among different treatments. This study showed that increasing trend on levels of dietary protein (especially 45%) at different water salinity, cause oxidative stress and increase the activity of antioxidant enzymes. Also, changes in water salinity from 32-35 to 12-15 and 0-3 ppt significantly increased (P>0.05) the activity of antioxidant enzymes in the hepatopancreas and muscle tissue of white leg shrimp. In general, level of 35% dietary protein and salinity of 32-35 ppt are appropriate conditions for culturing of juveniles of white leg shrimp.

Keywords: *Protein, salinity, Antioxidant enzymes, White leg shrimp, Penaeus vannamei*

1. Introduction

Protein is the main component of shrimp diet and the main section of the food production expenses (Kureshy and Davis, 2002). Excessive increase in dietary protein, not only increases food expenses, but also increases environmental ammonia and cause stressor condition for the aquatic organism and on the other side, low dietary protein levels led to decrease in growth rate (Thoman et al., 1999). In this respect, using optimal levels of dietary protein and ability of shrimp for protein digestion, ingestion and metabolism in various environmental conditions is effective in production expenses.

Temperature, salinity and dissolved oxygen are the main important environmental factors which have affected fish physiology (Hernandez et al., 2006). Therefore, water salinity can affect growth,

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survival, metabolism, osmolarity and immune system of shrimp (Alvarez et al., 2004). Although, *P. vannamei* belongs to euryhaline aquatics and tolerates wide range of environmental salinity (0.5-45 ppt) and tolerates some physiological stress arising from low salinity, however, low salinity can be a serious challenge for immune system and disease resistance in this species (Briggs et al., 2004; Chong-Robles et al., 2014; Li et al., 2015).

Environmental alterations induce stress in aquatic crustaceans and change their immune system. Various stresses increase free radicals production which has destroyed cell wall, enzymes and genetic material (Liu et al., 2007b). Antioxidant enzymes activity remove free radicals and this process can be affected by diet composition, environmental stressor and health status of aquatic organisms (Xu and Pan, 2013). In stress condition, health of aquatic organisms can be evaluated by assessment of antioxidant enzymes activity, such as SOD, CAT and GPX (Li et al., 2008; Liu et al., 2007a). Furthermore, Total antioxidant capacity (T-AOC), is one of the most important indicators of body antioxidant system. This indicator is represented the enzymatic antioxidants (included of SOD, CAT and GPX) and non-enzymatic antioxidant (included of Ascorbate, Urate, Pyruvate, Vitamin E, Taurine and Hypotaurine) (Yang et al., 2015). Shrimp has enzymatic and non-enzymatic antioxidant systems, like other aerobic organisms, to resist stressors (Castex et al., 2009). Askari Sari et al. (2006) recorded the best growth and survival rate of *P. vannamei* (mean weight: 2.7 gr) on 15-17 ppt and by the diet which has included of 40% protein. Li et al. (2008) and Lin et al. (2012) presented decreased antioxidant activity of hepatopancrease and muscle of *P. vannamei* in low salinity.

Alteration in nutritional and environmental conditions can cause oxidative stress in aquatic organisms and it predisposes various diseases in aquatics. Considering that white leg shrimp has cultured in different areas of the world and Iran in different salinity, administration of optimal levels of dietary protein can enhance shrimp immune system in different water salinity. Based on this statement, alteration of antioxidant enzymes of hepatopancreas and muscle of juveniles of *P. vannamei* was studied.

2. Material and Methods

The shrimps have been transferred from Shahid Kiani Educational Shrimp Center, (Choebdeh Abadan, Iran) to Imam Khomeini Port Marine Fish Propagation Center. The shrimps were transferred to 10 tons tanks with water salinity: 20 ppt (similar to water salinity of shrimp farms). During the adaptation period, shrimps were fed by the diet no.: 4004- Havorash Company (36% Crude protein, 8% Lipid, 3% Fibers, 14% ash and 10% moisture) as satiation four times a day, for 10 days (Samadi, 2012). Then 324 shrimps (with mean weight±SE: 5.55±0.18 gr and mean length±SE: 8.81±0.15 cm) were randomly introduced to 27 tanks (with capacity 3000 liters and 12 shrimps per tank). Ascending and descending trends of water salinity as 2 ppt/day performed to providing different salinity treatments (Adding marine salt for higher salinity level (32-35 ppt) and adding fresh water for lower salinity levels (12-15 ppt and 0-3 ppt) (Wang et al., 2014), so each group was included of 9 tanks with different salinity levels. Then each group was fed by three different levels of dietary protein (25%, 35% and 45%) in triplicate (Table 1).

Table 1: treatments designs based on different levels of dietary protein and water salinity (3 replicates)

Salinity (ppt)	Dietary protein (% dry weight)		
	45%	35%	25%
3-0	Treatment 3	Treatment 2	Treatment 1
15-12	Treatment 6	Treatment 5	Treatment 4
35-32	Treatment 9	Treatment 8	Treatment 7

Experimental Diets were formulated for three different levels of dietary protein based on AOAC (2000). All nutrients defined by standard analysis (AOAC, 2000). WUFFFDA software used for diet formulation (Table 2). In this experiment, the shrimps were fed four times a day (at 06:00, 12:00, 18:00 and 24:00 O'clock) as satiation (Yang et al., 2010; Zhang et al., 2013). The tanks have cleaned every day and water exchanged 10-15 per cent daily. Temperature, pH, salinity and dissolved oxygen rates were monitored and registered twice a day (in the morning and in the evening) by multiparameter tester (Model WTW, Germany). During the experiment, salinity rate for fresh, brackish and marine water has been registered and presented as mean salinity±standard error: 2.87±0.088, 14.68±0.094 and 34.45±0.086 ppt, respectively. Also, water temperature, dissolved oxygen and pH (Mean±SE)

Table 2: composition and percentage of experimental diet components and approximate biochemical analysis of diets

Raw material (%)	Diet 1 (25% protein)	Diet 2 (35% protein)	Diet 3 (45% protein)
Fish meal[1]	4.00	21.50	39.00
Soybean meal powder[1]	10.00	10.00	10.00
Shrimp head and tail meal[1]	10.00	10.00	10.00
Squid meal[1]	5.00	5.00	5.00
Fish oil	7.00	6.00	5.00
Wheat flour[1]	40.00	26.00	12.00
Vitamin supplement [2]	2.00	2.00	2.00
Mineral supplement [3]	2.00	2.00	2.00
Cholesterol	0.50	0.50	0.50
Lecithin	0.50	0.50	0.50
Gelatin	4.00	4.00	4.00
Filler	15.00	12.50	10.00
Approximate biochemical analysis of diets			
Protein	27.3±1.11	35.17±1.10	46.02±1.11
Lipid	8.9±0.40	9.1±0.72	9.4±0.63
Ash	9.10±0.81	11.45±0.85	14.34±0.91
Moisture	9.70±0.11	9.18±0.10	9.01±0.09
Carbohydrate[4]	45.00	35.10	21.23
Energy [5]	1.77	1.79	1.82

1. Approximate biochemical analysis of diets is based on dry matter percentage: fish meal (crude protein: 65.5% and crude lipid: 6.7%), soybean meal powder (crude protein: 44% and crude lipid: 1.3%), shrimp head and tail meal (crude protein: 42.8% and crude lipid: 2%), squid meal (crude protein: 71.5% and crude lipid: 2.3%), Wheat flour (crude protein: 12.6% and crude lipid: 1.1%)
2. Each kilogram of vitamin supplement included (mg): A= 1600000, K_3= 2000, E=40000, D_3= 400000, B_1=6000, B_2=8000, B_3= 12000, B_5= 40000, B_6=4000, B_9= 2000, B_{12}= 8, H_2= 40, C= 60000, Inositol= 20000
3. Each kilogram of mineral supplement included (mg): Fe= 6000, Zn= 10000, Se=20, Co=100, Cu= 6000, Mn= 5000, I= 600, choline chloride= 6000
4. Carbohydrate was calculated by mines of total protein, lipid, ash and moisture from 100.
5. Total energy of the diet is calculated by multiplying 0.017, 0.0398 and 0.0237 (Mj/g) for carbohydrate, lipid and protein.

were 26.10±0.10 ^{o}C, 6.64±0.09 mg/L and 7.95±0.01, respectively

The experiment lasted for 56 days (8 weeks). At the end of the experiment, three shrimps were randomly selected from each replicate (9 samples from each treatment). The shrimps were euthanized by using 20 ppm Eugenol (Mousavi et al., 2012). After necropsy, hepatopancreas and muscle tissues were dissected and immediately stored in liquid nitrogen up to analysis (Parrilla-Taylor and Zenteno-Savin, 2011). Hepatopancreas and muscle tissues were weighted and homogenized and diluted in ratio of 1 to 9 (W/V), in Phosphate-buffered saline (KCl 0.0027 M, NaCl 0.14 M, pH 7.4). The diluted samples were centrifuged (12000 rpm for 10 min in 4^{o}C) and the supernatant was separated and used for measurement of antioxidant activity (Li et al., 2008; Parrilla-Taylor et al., 2013; Chen et al., 2014). T-AOC was measured by T-AOC kit manufactured by Biorex® by enzymatic-colorimetric method and by using spectrophotometer (Specord 250, Germany) on wavelength 660 nm (Erel, 2004).

SOD was measured by SOD kit manufactured by Biorex® by enzymatic-colorimetric method and by using spectrophotometer (MR-96A, China) on wavelength 505 nm (Imai et al., 2000). CAT activity was measured by CAT kit manufactured by ZellBio® by enzymatic-colorimetric method and by using spectrophotometer (Specord 250, Germany) on wavelength 405 nm (Aebi, 1984). GPX was measured by GPX kit manufactured by Biorex® by enzymatic-colorimetric method and by using spectrophotometer (UV-Visible, model 331, Japan) on wavelength 660 nm (Flohe and Gunzler, 1984). All antioxidant activity assays were conducted in Fisheries Laboratory of Khorramshahr University of Marine Science and Technology.

This experiment was done as a completely randomized design and Shapiro-wilk test used for randomized data. IBM SPSS Statistics software (version 19) applied for analysis of the results. Levene's test used for homogeneity of variance and two-way analysis of variance was used for statistical analysis of the results. Duncan multiple range test was used for comparing means with a confidence level of 95%. All data presented as mean±standard error.

3. Results

The results of antioxidant capacity (T-AOC, SOD, CAT and GPX) in hepatopancreas and muscle tissue of white leg shrimp under different levels of salinity and dietary protein are presented in Tables 3 and 4 and Figures 1 to 4. Based on the results, T-AOC and SOD activity of hepatopancreas and muscle tissue of *Penaeus vannamei*, were affected by different levels of dietary protein and water salinity levels, so the maximum rate of T-AOC and SOD activity was seen in treatment 3 (45% protein and 0-3 ppt salinity) and the minimum T-AOC and SOD activity of hepatopancreas and muscle tissue observed in treatment 7 (25% protein and 32-35 ppt salinity) and 8 (35% protein and 32-35 ppt salinity), respectively.

Catalase activity was decreased in treatment 7 (25% protein and 32-35 ppt salinity) in hepatopancreas and treatment 8 (35% protein and 32-35 ppt salinity) in muscle tissue and the maximum rate of catalase activity was in treatment 3 (45% protein and 0-3 ppt salinity).

Glutathione peroxidase activity in hepatopancreas and muscle tissues was in maximum level in treatment 3 (45% protein and 0-3 ppt salinity) and minimum of GPX activity was seen in treatment 7 (25% protein and 32-35 ppt salinity).

Maximum capacities of T-AOC, SOD, CAT and GPX activity were observed in protein level: 45% and salinity: 0-3 ppt. There was no interaction effect of different levels of dietary protein and water salinity on T-AOC, CAT, SOD and GPX activity ($p>0.05$).

Table 3: Antioxidant enzymes activity rate in hepatopancreas of white leg shrimp under different levels of dietary protein and water salinity (Mean±SE, n=3).

Treatment	T-AOC* (mmol Trolox equivalent/mg protein)	SOD* (U/ml)	*CAT (U/ml)	GPX * (U/l)
Mean of antioxidant enzyme activity in hepatopancreas (One-way Anova variance analysis)				
1 (p25/s3)	157.88±13.64 ^{bc}	3.13±0.24 ^{bc}	15.87±2.96 ^{bc}	148.61±9.81 ^{cd}
2 (p35/s3)	187.21±8.68 ^{ab}	3.54±0.17 ^{b}	19.26±2.35 ^{ab}	190.67±6.95 ^{b}
3 (p45/s3)	211.92±12.65 ^{a}	4.24±0.18 ^{a}	23.47±1.33 ^{a}	236.94±7.86 ^{a}
4 (p25/s15)	134.21±9.56 ^{cd}	1.70±0.19 ^{f}	12.72±1.21 ^{bcd}	113.56±11.13 ^{ef}
5 (p35/s15)	147.84±8.56 ^{c}	2.48±0.17 ^{de}	14.86±2.77 ^{bcd}	141.60±13.81 ^{cde}
6 (p45/s15)	156.84±10.35 ^{bc}	2.77±0.13 ^{cd}	16.20±2.67 ^{bc}	164.03±13.52 ^{bc}
7 (p25/s35)	90.83±8.25 ^{e}	0.87±0.09 ^{g}	8.72±0.59 ^{d}	91.13±5.65 ^{f}
8 (p35/s35)	112.54±9.34 ^{de}	0.97±0.07 ^{g}	10.24±1.59 ^{cd}	117.77±8.76 ^{def}
9 (p45/s35)	126.75±13.57 ^{cd}	2.05±0.17 ^{ef}	12.21±0.90 ^{cd}	126.18±5.46 ^{de}
Mean of the main effects				
25% protein	127.64±11.19 ^{b}	1.90±0.34 ^{c}	12.44±1.40 ^{b}	117.77±9.54 ^{c}
35% protein	149.20±11.66 ^{a}	2.33±0.38 ^{b}	14.87±1.73 ^{ab}	150.01±11.89 ^{b}
45% protein	165.17±13.90 ^{a}	3.02±0.33 ^{a}	17.30±1.88 ^{a}	175.72±16.94 ^{a}
Salinity 0-3 ppt	185.67±9.80 ^{a}	3.63±0.19 ^{a}	19.53±1.60 ^{a}	192.07±13.41 ^{a}
Salinity 12-15 ppt	146.30±5.78 ^{b}	2.32±0.18 ^{b}	14.59±1.27 ^{b}	139.73±9.73 ^{b}
Salinity 32-35 ppt	110.04±7.45 ^{c}	1.29±0.20 ^{c}	10.39±0.75 ^{c}	111.69±6.28 ^{c}
(Two-ways Anova variance analysis)				
Protein	0.00	0.00	0.03	0.00
Salinity	0.00	0.00	0.00	0.00
Salinity×Protein	0.71	0.19	0.83	0.12

Different letters on any column show significant difference between experimental groups ($p<0.05$)

* (TAOC= total antioxidant capacity, SOD= Superoxide dismutase, CAT= Catalase, GPX= Glutathione peroxidase)

Table 4: Antioxidant enzymes activity rate in muscle tissue of white leg shrimp under different levels of dietary protein and water salinity (Mean±SE, n=3).

Treatment	T-AOC* (mmol Trolox equivalent/mg protein)	SOD* (U/ml)	*CAT (U/ml)	GPX * (U/l)
Mean of antioxidant enzyme activity in muscle tissue (One-way Anova variance analysis)				
1 (p25/s3)	118.54±8.59 bc	2.95±0.24 ab	26.41±1.67 bc	140.20±9.81 ab
2 (p35/s3)	140.67±11.15 ab	2.62±0.40 abc	30.10±2.68 ab	152.82±6.11 a
3 (p45/s3)	165.83±12.47 a	3.18±0.24 a	33.33±2.06 a	130.39±7.87 abc
4 (p25/s15)	101.63±7.93 cd	2.37±0.26 abcd	19.24±2.07 de	107.95±9.23 c
5 (p35/s15)	89.71±8.90 de	2.37±0.42 abcd	17.28±1.58 def	128.98±9.44 abc
6 (p45/s15)	120.71±7.75 bc	2.51±0.31 abc	21.91±1.44 cd	116.36±9.81 bc
7 (p25/s35)	74.13±6.57 de	1.64±0.24 cd	14.83±2.30 ef	44.86±8.53 e
8 (p35/s35)	63.92±4.88 e	1.49±0.34 d	12.63±1.48 f	74.31±6.11 d
9 (p45/s35)	88.05±8.65 de	2.05±0.21 bcd	15.64±2.02 def	81.32±9.81 d
Mean of the main effects				
25% protein	98.10±7.54 b	2.32±0.22 a	20.16±1.97 b	97.67±14.74 b
35% protein	97.77±12.20 b	2.16±0.26 a	20.00±2.79 b	118.70±12.19 a
45% protein	124.86±12.30 a	2.58±0.21 a	23.63±2.75 a	109.36±8.63 ab
Salinity 0-3 ppt	141.68±8.72 a	2.91±0.17 a	29.95±1.48 a	141.13±5.18 a
Salinity 12-15 ppt	104.02±6.10 b	2.42±0.17 a	19.48±1.09 b	117.77±5.65 b
Salinity 32-35 ppt	75.03±5/00 c	1.72±0.16 b	14.37±1.08 c	66.83±6.96 c
(Two-ways Anova variance analysis)				
Protein	0.00	0.26	0.06	0.03
Salinity	0.00	0.00	0.00	0.00
Salinity×Protein	0.24	0.94	0.46	0.16

Different letters on any column show significant difference between experimental groups ($p<0.05$)

* (TAOC= total antioxidant capacity, SOD= Superoxide dismutase, CAT= Catalase, GPX= Glutathione peroxidase)

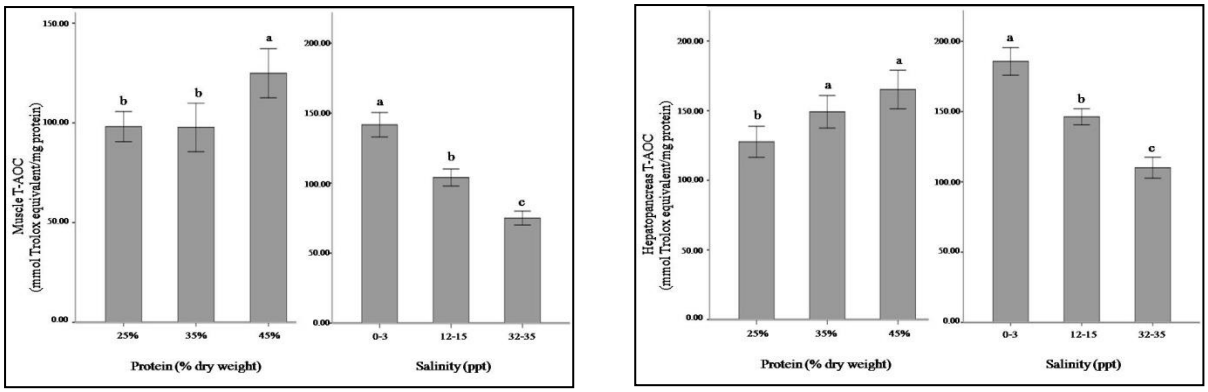

Figure 1: effects of different levels of dietary protein and water salinity on T-AOC in hepatopancreas and muscle tissue of *Penaeus vannamei*. Different letters represent significant differences between experimental groups ($p<0.05$).

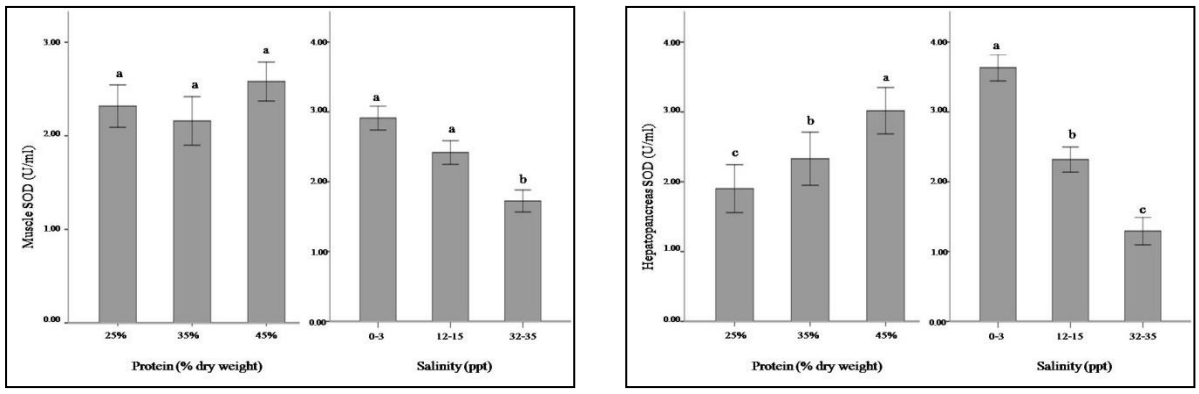

Figure 2: effects of different levels of dietary protein and water salinity on SOD activity in hepatopancreas and muscle tissue of *Penaeus vannamei*. Different letters represent significant differences between experimental groups ($p<0.05$).

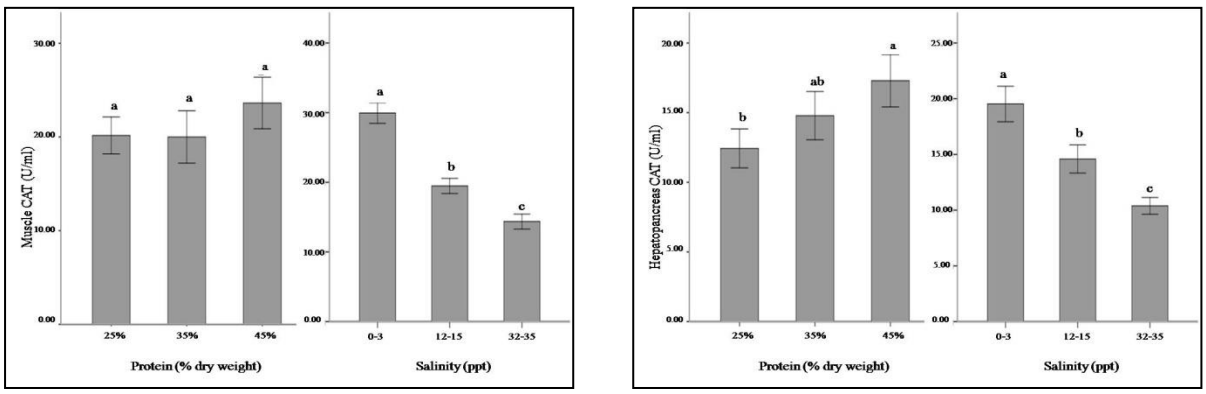

Figure 3: effects of different levels of dietary protein and water salinity on CAT activity in hepatopancreas and muscle tissue of *Penaeus vannamei*. Different letters represent significant differences between experimental groups ($p<0.05$).

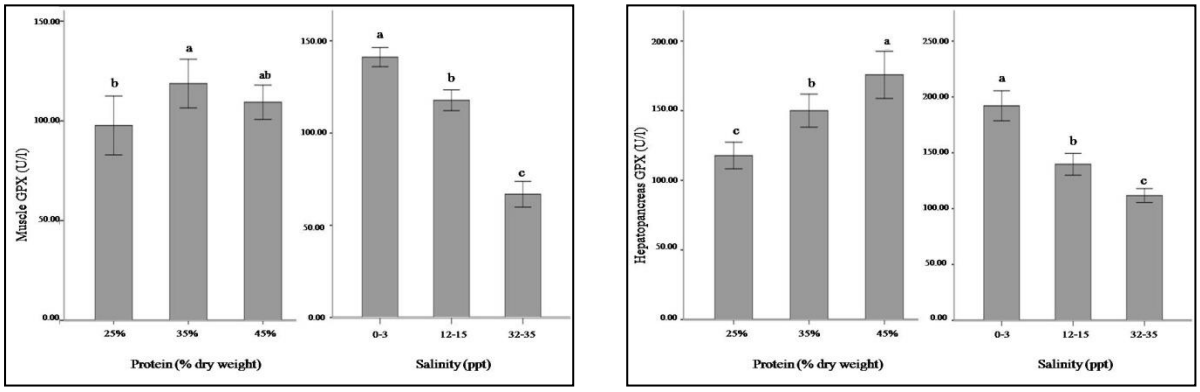

Figure 4: effects of different levels of dietary protein and water salinity on GPX activity in hepatopancreas and muscle tissue of *Penaeus vannamei*. Different letters represent significant differences between experimental groups ($p<0.05$).

4. DISCUSSION AND CONCLUSION

By investigating the prevailing

Previous studies have shown that dietary protein levels can influence the health status of the shrimp in vitro (Pascual et al., 2004; Goimier et al., 2006). Antioxidant parameters are more sensitive than other sections of immune system in response to imbalance of dietary protein levels in shrimp. These parameters can be used as physiological health indicators for assessment of dietary protein levels (Chen et al., 2014). Some of the amino acids (Arginine, Citrulline, Glycine, Taurine and Histidine), small peptides (GSH and Carnosine) and nitrogen metabolites (Keratin and Uric acid) directly remove oxygen free radicals; so, dietary protein deficiency, not only causes disrupting in antioxidant enzymes synthesis, but also decreases antioxidants compounds concentration in tissues and leads to alteration in antioxidant capacity. On the other hand, increased dietary protein level causes oxidative stress. Actually, existence and presence of some protein compounds such as homocysteine leads to increase in superoxide ion and causes oxidative stress. Also, increasing protein consumption, leads to induction of ROS production and lipid peroxidation in leukocytes and mononuclear cells (Fang et al., 2002). In the current study, increased T-AOC activity of both hepatopancreas and muscle tissues has been observed in increased dietary protein levels from 25 to 45 per cent. SOD and CAT activity has been increased only in hepatopancreas but there was not significant difference in SOD and CAT activity in muscle tissue. Furthermore, GPX activity has been increased in hepatopancreas by increased dietary protein levels from 25% to 45% whit it decreased by increased water salinity. But, GPX activity has been increased in muscle tissue by increased dietary protein levels from 25% to 35%, then, decreased on 45% level of dietary proteinas well as by increased water salinity. These alterations in antioxidant enzymes activity, can be resulted from response of immune system to oxidative stress which resulted from excessive increase of dietary protein and ROS production in tissues (Fang et al., 2002), so, decrease in dietary protein to optimum levels (25 and 35%) and decrease in ROS production, levels of antioxidant enzymes activity have partially decreased.

SOD, CAT and GPX are the main enzymes of antioxidant immune system. SOD converts O2.- to H2O2 and O2 (Krishnamurthy and Wadhwani, 2012).

In this respect, increase in SOD activity presented the high production of O2.-. On the other hand, increase in SOD activity leads to more H2O2 production and this is one of the main factors for increase in CAT and GPX activity in this research (Najafi, 2013). Of course, it is noted that decreasing trend of dietary protein levels to under 20%, led to excessive reduction of antioxidant capacity and redox process in farmed shrimp and can decrease immune system capacity of the shrimp (Pascual et al., 2004). Optimal protein level, not only led to animal growth, but also prepared amino acids as osmolytes that in turn, can increase defense and survival against salinity stress. Free amino acids (such as Glycine, Alanine, Proline and Tourine) can accumulate in cell and have noticeable role as organic osmolytes in osmoregulation of marine invertebrates (Li et al., 2015). SOD and CAT are the primary enzymes for inhibition of free radicals that these processes have joint to oxidative and phagocytosis processes in injured tissues. These defense mechanisms and their activity depend on animal condition, diet quality and environmental factors. Usually, increased activity of SOD and CAT is an indicator of increased production of free radicals. Therefore, significant increase in SOD and CAT activity in experimental shrimps on low salinity condition may indicate accumulation of free radicals in tissues. If these free radicals produced on low salinity condition, the animal would experience a severe oxidative damage (Li et al., 2008). In this research, the maximum activity of SOD and CAT in muscle and hepatopancreas tissues of white leg shrimp was observed in salinity of 3 ppt. in this respect, increased activity of SOD and CAT for removing produced free radicals can be one of the main protective mechanisms against decreased salinity levels (Li et al., 2008). Previous studies showed that white leg shrimp is vulnerable and very sensitive to environmental stress on salinity of 3 ppt in comparison with 15, 25 and 35 ppt (Lin and Chen, 2001; Lin and Chen, 2003; Li et al., 2007). By transferring white leg shrimp from salinity 35 ppt to 25, 20 and 15 ppt, considerable decrease on immune system was observed after 1-6 hours due to decrease in hyaline cells, granular cells, total haemocyte count, phenoloxidase activity, respiratory burst and SOD activity. These parameters are more affected when the shrimps were infected by Vibrio alginolyticus (Li et al., 2010). It is reported that, 48 hours after transferring farmed white leg shrimp in marine water (salinity 32 ppt, nitrite 0.007 mg/l) to marine water with salinity 15 ppt and nitrite 20 mg/l, T-AOC activity, GPX, CAT activity of hepatopancreas decreases but SOD increases (Wang et al., 2015). Any positive alteration in shrimp diets in different salinity is a novel and effective method for decreasing effects of environmental salinity fluctuation from optimum condition in aquatic farms (Romano and Zeng, 2012).

Current study showed that decreased water salinity from 32-35 ppt, leads to increased antioxidant enzymes activity of hepatopancreas and muscle tissues of white leg shrimp which may be resulted from oxidative stress from high salinity levels and whereby increases in ROS production and their accumulation in tissues. Usually, activity of Na+/K+-ATPase mechanism is increased when the marine crustaceans are exposed to low salinity. This mechanism is included the maximum energy intake in ion exchange process and subsequently, increasing in energy intake for osmoregulation leads to increased oxygen consumption (Romano and Zeng, 2012). Increasing in oxygen consumption by gills in low salinity can be a reason for increased production of superoxide radicals (Pallavi et al., 2012), and subsequently increased antioxidant enzymes activity of shrimp in low salinity. So, in this research, it seems that excessive increase in dietary protein for increasing growth rate (especially in low salinity), causes increase in oxidative stress and decrease in defense and immune responses in white leg shrimp.

Overall, dietary protein levels and different levels of salinity are affect activity of T-AOC, SOD, CAT and GPX in white leg shrimp and change the immune system. Therefore, any increase in dietary protein over the optimal level (35%) and decrease in water salinity levels from the range of 32-35 ppt, cause oxidative stress and as a result, increase the levels of antioxidant enzymes activity in juveniles of white leg shrimp.

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