

## **Effect of dietary supplementation of *Padina astraulis* (Hauk, 1887) extract on biochemical response and digestive enzyme activities of grey mullet, *Mugil cephalus* (Linnaeus, 1758)**

**Akbary P.<sup>1\*</sup> ; Shahraki N.<sup>1</sup>**

Received: August 2016

Accepted: April 2017

### **Abstract**

This research aimed to assess the effect methanol extract from *Padina astraulis* (Hauk, 1887) on biochemical response and digestive enzyme activities in grey mullet, *Mugil cephalus* (Linnaeus, 1758). A control diet without and three other empirical regimens were prepared with the addition of of *P.astraulis* extract (PE diet) at the incorporation levels of 5, 10 and 15 g kg<sup>-1</sup> diet, respectively. Three hundred sixty grey mullet with an introductory mean weight of 0.82 g were randomly dispersed into twelve fish tanks and fed twice a day (09:00 and 17:00) for 60 days. After 60 days of the feeding trial, no significantly different was noticed in terms of survival rate between control and the groups supplemented with PE ( $p>0.05$ ). Dietary PE significantly increased serum total protein, globulin levels and amylase, lipase and protease enzyme activities in treated fish ( $p<0.05$ ). Moreover, there was considerable decline ( $p<0.05$ ) in cholesterol, glucose and triglyceride levels in those fish which received PE diet over the control. The current research illustrates the appropriateness of *P. astraulis* extract as a novel dietary additive in grey mullet diet for improving metabolism of lipid and carbohydrate and enhancing digestive enzyme activities in grey mullet.

**Keywords:** *Padina astraulis*, *Mugil cephalus*, Biochemical response, Digestive enzymes, Dietary supplement

---

1-Fisheries Group, Faculty of Marine Sciences, Chabahar Maritime University, Chabahar, Iran

\*Corresponding author's Email: paria.akbary@gmail.com

## Introduction

*Mugil cephalus* (Linnaeus, 1758) known as grey mullet is an important candidate for aquaculture owing to its high demand and fast growth, and has been successfully cultivated in brackish water, marine environments and fresh water throughout the Mediterranean region (Turan *et al.*, 2011 ; Akbary *et al.*, 2016; Akbary and Kakoolaki, 2019). Mullet are established as extremely fascinating species for pond culture in Iran, China ,Egypt, Hawaii, Italy, Japan, Philippine, Taiwan and other countries, where they are mostly bred in polyculture with other species such as shrimp, carps, tilapia and milkfish (El-Dahhar *et al.*, 2000). In order to improve the fish quality, appropriate feeding habits and rich nutrients to the fish are vital factors. Antibiotics and synthetic drugs such as hormones and vitamins are experimented for different activities, such as immunostimulant, growth stimulation and appetizing in aquaculture (Nwabueze, 2012). Although various synthetic products enhance fishes and shrimps production (Sahu *et al.*, 2007), they are not the common choice in commercial aquaculture processes due to their high costs, inclination to produce residues and inappropriate side effects. There is a search for alternative appetizers of natural origin because of concerns expressed by consumers. Natural plant products such as microalgae (Ju *et al.*, 2009), seaweeds (Yeh *et al.*, 2006.) and herbal extract (Sankar *et al.*, 2011) have a role of growth advocates, immunostimulants and appetizers. They

are prosperous in the aquaculture industry in enhancing feed consumption and bettering digestion (Supamattaya *et al.*, 2005).

Various researches relevant to dietary seaweeds such as *P. australis* in *Litopenaeus vannamei* (Akbary and Aminikhoei, 2018), *Porphyra purpurea* (Roth) in thick-lipped grey mullet, *Chelon labrosus* (*A. risso*), (Davies *et al.*, 1997), *Hizikia fusiformis* (Harvey) in olive flounder, *Paralichthys olivaceus* (Choi *et al.*, 2014), *Pyropia yezoensis* in olive flounder (Choi *et al.*, 2015) and *Sargassum ilicifolium* (Turner) in rainbow trout, *Oncorhynchus mykiss* (Walbaum), (Zamannejad *et al.*, 2016) diets were advantageous for the growth and immunity of fish. Interestingly, the effect of supplementation of dietary seaweed also variably depends on dietary seaweed is also dependent on dietary seaweed's species and concentrations (Yeh *et al.*, 2006.; Zamannejad *et al.*, 2016). Also, Choi *et al.* (2015) reported that total protein and glucose levels increased and decreased respectively when the inclusion level of dietary *P. yezoensis* extract was high. Although reports are available about beneficial effect of seaweed on growth, immunity and biochemical responses, as a dietary supplementation in aquaculture species, no reports are available in supporting the dietary duty of *P. australis* extract on biochemical responses and digestive enzyme activities in grey mullet. The main objective the present research was to evaluate the influence of dietary incorporation *P. australis* extract on the

biochemical response and digestive enzyme activities in grey mullet, *M.cephalus*.

## Materials and methods

### *Preparation of Padina australis methanol extract*

Two kilograms of *P. australis* algae was collected from the Chabahar coast, Iran and oven dried at 60°C, the powdered by mortar and pestle then strained. After, 50 g its powder was left during 48 h in 99 % methanol 10 L (10%w/v) at room temperature (24±1.2°C) and the derived extract was concentrated to 300 ml via a rotary evaporator (IKA, Germany) which provided the extract 6.1 g of powder ml<sup>-1</sup>. The derived extract was dispersed on the diet after dilution in 300 ml of distilled water(Choi et al., 2015).

### *Experimental diets and Feeding conditions*

Four regimens were designed with PE as a supplement at incorporation levels of 0, 5, 10 and 15 g kg<sup>-1</sup> diet evaluated proximate compositions are shown in Table 1. For the preparation of four experimental regimens of size 1.6 mm

commercial extruded pellet (Beyza Feed Mill, Iran) was ground into a powder (0.5 mm particle size). After grinding, 30% distilled water was included and mixed further. The mixture was pelletized at a 1mm particle size via a chopper machine. The empirical regimens were freeze-dried overnight at 40° C and then kept at -20 °C until use(Choi et al., 2015). The feeding test was carried out at Offshore Fisheries Research Center, Chabahar, Iran. Three hundred- sixty grey mullet with an introductory mean weight of 0.82 g were randomly divided into twelve tanks (60 L) at a stocking density of 20fish/tank (three tests per treatment) and hand fed amply twice (09:00 and 17:00) every day for a period of 60 days and feed intake was recorded on daily basis . Dissolved oxygen levels, ammonia nitrogen levels and pH were measured about 7.01±0.87 mg L<sup>-1</sup>, 0.11±0.04 mg L<sup>-1</sup> and 7.8±0.4 correspondingly. The photoperiod was regulated as a 12:12 h (dark/light) cycle. Biometry was done during the first and last days of the experiment.

**Table 1: Ingredients (g/kg<sup>-1</sup>) and chemical composition (%) of the experimental diets.**

Ingredients (g kg <sup>-1</sup> )	Diets			
	PE0	PE5	PE10	PE15
<i>P.australis</i>	0	5	10	15
Fish meal	427	427	427	427
Soybean meal	192.5	192.5	192.5	192.5
Wheat flour	93	93	93	93
Dried yeast	37.5	37.5	37.5	37.5
Fish oil	55	55	55	55
Soy oil	27.5	27.5	27.5	27.5
Choline chloride	2	2	2	2
Bi calcium phosphate	3.7	3.7	3.7	3.7
Lecithin	28.15	28.15	28.15	28.15
Premix <sup>a</sup>	9.4	9.4	9.4	9.4
Proximate composition(%)				
Crude protein	51.6	51	50.6	51.6

**Table 1 continued:**

Crude lipid	11.9	11	11.4	11.2
Crude ash	12.1	12	11.8	12.6
Dry matter	92.2	92.1	92	92

<sup>a</sup>Premix (mg kg<sup>-1</sup>) KI, 250; MnSO<sub>4</sub>·H<sub>2</sub>O, 2800; ZnSO<sub>4</sub>·H<sub>2</sub>O, 2350; vitamin K, 225; biotin, 3500; (2%) niacin, 4850; calcium pantothenate, 11,000; folic acid, 2000; vitamin B<sub>1</sub>, 1500; vitamin B<sub>2</sub>, 2000; vitamin B<sub>6</sub>, 2000; and vitamin C, 50,000.

### Biochemical analysis

At the end of experiment, nine fish from each treatment were anesthetized (with clove oil at 5 mg L<sup>-1</sup>) and blood samples were extracted after excising caudal peduncle and were poured into un-heparinized sterile tubes 1–1.5 ml for the serum biochemical experimental objectives (Shaluei *et al.*, 2012).

Plasma glucose concentration was measured based on the approach by Trinder (1969). Plasma total protein and albumin content were measured using the approach by Wootton (1964). By subtracting albumin level from total protein, globulin was determined. Triglyceride and cholesterol level measurement took place based on the method by Sankar *et al.* (2011). Biochemical estimation of blood glucose, protein, cholesterol and triglyceride were determined by means of standard analyses kits (Pars Azmon, Iran) using automatic analyzer (Furuno, CA-270, Japan).

### Digestive enzyme activity

For preparation of enzyme extracts, three fish from each tank were chosen randomly and sacrificed. The digestive parts were cautiously taken out, completely washed with sterile refined water, weighed and separately homogenized using a cooled buffer phosphate (0.65 %, pH 7, 1: 10 w/v). The supernatant, extracted by

centrifugation (3000 g for 20 min at 4°C) (Centrifuge EBA21, Hettich, Germany), was used for enzyme assays. Amylase movement was assessed by 3, 5- dinitrosalicylic acid (DNS) method (King, 1965). 0.1 ml tissue homogenated, 0.05 M tris phosphate buffer (pH 7.8), 0.01 N NaOH and 2.5 ml of 1% (w/v) consisted the reaction concoction. The mixture was incubated at 30°C for 10 min and stopped by 2.5 ml, 10% trichloroacetic acid (TCA) and filtered. The reagent blank consisted of just tissue homogenate before preventing the resulting reaction and with no incubation. The absorbance was recorded at 320 nm. Unit amylase activity was calculated as the weight (mg) of maltose liberated for duration of 10 min at 30°C. The activity of unit protease was shown as the amount of tyrosine liberated under the duration 15 min under the assay circumstances. Lipase movement was assessed by King (1965) method. Olive oil emulsion, phosphate buffer (pH 7.8, 0.1 M), tissue homogenate and distilled water consisted the reaction mixture. The reaction concoction was then incubated at a temperature of 30°C for the duration of 24 h and added two drops phenolphthalein indicator and 95% alcohol for titration against 0.05 N NaOH until a permanent pink color was achieved. Activity levels of unit lipase shown as the amount of 0.025 N NaOH needed to neutralize the fatty acids freed during an 18 h incubation period

at pH 6.9 and at a temperature of 30°C. Digestive enzymes were determined as enzyme unit per gram tissue.

#### Statistical analysis

Every measurement was carried out twice. The one-way analysis of variance (ANOVA) method was utilized for data analysis. Each group was assumed to be considerably different if  $p < 0.05$ . When a considerable  $F$  value was gained for ANOVA the fluctuations among every group was examined by utilizing the Duncan multiple comparisons test. SPSS for windows versions 16 was utilized for the statistical purposes. Data are determined as means  $\pm$  standard Error.

### Results

#### Biochemical indices of serum

The total protein content of the serum was considerably ( $p < 0.05$ ) enhanced for

fish on the diet in question over the control. The highest total protein content was determined in those fish groups that were fed PE at 15 g kg<sup>-1</sup>. There was no compelling difference ( $p > 0.05$ ) in serum glucose observed in those fish group which were on the PE diet over the control except received PE diet at 15 g kg<sup>-1</sup> feed. There was significant ( $p < 0.05$ ) decline in cholesterol and triglycerides levels in those fish on the PE at 10 and 15 g kg<sup>-1</sup> over the control group (Table 1). Serum albumin and globulin content was only considerably ( $p < 0.05$ ) higher in fish on the PE diet at 15 g kg<sup>-1</sup> feed than the control. Albumin: globulin ratio was higher only in fish on the PE diet at 15 g kg<sup>-1</sup> feed and the fish fed remaining levels of PE diet had shown the same level to control (Table 2).

**Table 2: Serum biochemical parameters of *Mugil cephalus* fed different levels of PE diet for 60 days.**

Parameter	PE diet (g kg <sup>-1</sup> feed)			
	0	5	10	15
Total protein (mg ml <sup>-1</sup> )	3.42 $\pm$ 0.13 <sup>a</sup>	3.83 $\pm$ 0.18 <sup>b</sup>	3.92 $\pm$ 0.24 <sup>b</sup>	4.16 $\pm$ 0.38 <sup>c</sup>
Albumin (mg ml <sup>-1</sup> )	0.5 $\pm$ 0.2 <sup>a</sup>	0.5 $\pm$ 0.2 <sup>a</sup>	0.6 $\pm$ 0.2 <sup>a</sup>	0.9 $\pm$ 0.2 <sup>b</sup>
Globulin (mg ml <sup>-1</sup> )	1.2 $\pm$ 0.5 <sup>a</sup>	1.2 $\pm$ 0.2 <sup>a</sup>	1.3 $\pm$ 0.1 <sup>a</sup>	1.6 $\pm$ 0 <sup>b</sup>
Albumin: globulin ratio (mg ml <sup>-1</sup> )	0.42 $\pm$ 0.1 <sup>a</sup>	0.42 $\pm$ 0.2 <sup>a</sup>	0.46 $\pm$ 0.3 <sup>a</sup>	0.56 $\pm$ 0 <sup>b</sup>
Glucose (mg dl <sup>-1</sup> )	5.5 $\pm$ 0.13 <sup>c</sup>	4.86 $\pm$ 0.2 <sup>b</sup>	4.83 $\pm$ 0.18 <sup>b</sup>	3.83 $\pm$ 0.34 <sup>a</sup>
Triglycerides (mg dl <sup>-1</sup> )	208.8 $\pm$ 15.23 <sup>c</sup>	205.1 $\pm$ 15.12 <sup>c</sup>	191.3 $\pm$ 14.78 <sup>b</sup>	172.5 $\pm$ 12.8 <sup>a</sup>
Cholesterol (mg dl <sup>-1</sup> )	282.5 $\pm$ 23.4 <sup>c</sup>	251.02 $\pm$ 21.5 <sup>c</sup>	237 $\pm$ 22.34 <sup>b</sup>	180 $\pm$ 24 <sup>a</sup>

PE diet, *P. astraulis* extract diet. Values (mean $\pm$ SE of three replications. In each row without a common superscript are considerably different ( $p < 0.05$ ))

#### Digestive enzyme activities

Amylase activity level was significantly higher for fish on the PE diet compared with control. The maximum specific amylase activity was determined in those fish fed PE diet at 15 g/kg<sup>-1</sup> of feed. There was no considerable

changes in the activity levels of protease and lipase observed in those fish fed PE diet at 5 and 10 g kg<sup>-1</sup> feed over the control group apart from fish received PE diet at 15 g kg<sup>-1</sup> feed (Table 3).

**Table 3: Digestive enzyme activities of *Mugil cephalus* fed different levels of PE diets for 60 days.**

Specific activity of enzyme (unit mg <sup>-1</sup> protein)	PE diet (g kg <sup>-1</sup> feed)			
	0	5	10	15
Amylase	4±0.23 <sup>a</sup>	5.2±0.71 <sup>b</sup>	5.32±0.87 <sup>b</sup>	6.8±0.65 <sup>c</sup>
Protease	0.12±0.07 <sup>a</sup>	0.17±0.05 <sup>a</sup>	0.22±0.09 <sup>ab</sup>	0.35±0.06 <sup>b</sup>
Lipase	0.19±0.03 <sup>a</sup>	0.22±0.02 <sup>a</sup>	0.28±0.07 <sup>ab</sup>	0.35±0.06 <sup>b</sup>

PE diet, *P. australis* extract diet. Values (mean ±SE of three replication). In each row without a common superscript are considerably different ( $p < 0.05$ ).

## Discussion

This research took place to examine the influences of dietary supplementation of *P. australis* extract on biochemical response and digestive enzyme activities of grey mullet (*M. cephalus*). The outcomes of the current research demonstrate that serum glucose level reduced in fish fed dietary PE. Serum insulin level is typically enhanced by reducing the blood glucose when immunostimulant regimen is used (Kaleeswaran *et al.*, 2012). Immunostimulant diet is thought to potentate the action of insulin through the increase of insulin binding, insulin receptor number and function through lowering glucose and lipids, thereby regulating the uptake of glucose in to cells (Ahmed and Sharma, 1997). A possible explanation of the beneficial effects of PE diets attributed that it has stimulated the insulin activity, an accordingly reduced the glucose level. Moreover, the present physiological findings are in agreement with those reported by Choi *et al.* (2015) who revealed that serum glucose decreased by increasing the level of *P. yezoensis* extract in olive flounder (*Paralichthys olivaceus*), as well as in Asian seabass (*Lates calcarifer*) fed *Mentha piperita* which also significantly decreased serum glucose (Talpur, 2014). The present study revealed that feeding PE

diets at 10 and 15 g kg<sup>-1</sup> feed to *M. cephalus* led to a decrease in serum triglycerides and cholesterol content in fish, which is in concurrence with the findings of Choi *et al.* (2015) who reported that feeding *P. yezoensis* extract in *P. olivaceus* has shown the potential to reduce these contents in fish. Kaleeswaran *et al.* (2012) concluded that using ethanolic extract of *Cynodon dactylon* showed effects on blood cholesterol levels in *Catla catla*. The current research demonstrates the decrease in triglycerides and cholesterol have potentially improved fish cardiovascular activity (Kaleeswaran *et al.*, 2012). Total protein, globulin and albumin contents are recognized prime factors for retaining a healthy immune system and immune activity in the blood and are well known to show strong elemental immunity (Wu *et al.*, 2007; Talpur and Ikhwanuddin, 2012; Kaleeswaran *et al.*, 2012). In the present study, significant positive effects of dietary PE diet at 15 g kg<sup>-1</sup> feed on serum total protein, albumin, globulin and albumin: globulin ratio was detected compared with the other levels of PE or the control group. Generally it could be stated that the PE diet at 15 g kg<sup>-1</sup> feed is safe and advantageous to the health of *M. cephalus*. This is in agreement with the findings of Kaleeswaran *et al.* (2012)

who found that using 5% ethanolic extract of *Cynodon dactylon* significantly increased serum total protein, albumin and globulin concentrations and albumin globulin ratio of *C. catla*. The data are also comparable with the significantly higher serum total protein determined in *P. olivaceus* fed with *P. yezoensis* extract (Choi *et al.*, 2015).

Macronutrients such as carbohydrates and protein effect fish digestive enzyme activities (Lara-Flores *et al.*, 2003). The presence of macroalgae and microalgae even at low levels in the gut can cause the formation of digestive enzymes (Reitan *et al.*, 1993; Mian *et al.*, 2014). Higher digestive enzyme specific activities such as amylase, protease and lipase determined in dietary PE diet at 15 g kg<sup>-1</sup> feed may promote protein, starch and fat digestion. Identically, escalated digestive enzymes levels were noticed in fishes (Lara-Flores *et al.*, 2003) on probiotic and *Zingiber officinalis* as herbal appetizer (Venkatramalingam *et al.*, 2007), regimens with supplements compared to control. This implies that dietary PE diet at 15 g kg<sup>-1</sup> feed like all spices may reduce feed transit time. The minimization of transit time may have had an advantageous effect on digestive enzymes and could may expedite the total digestive action (Venkatramalingam *et al.*, 2007). Also, Ansarifard *et al.* (2018) showed that the activities of protease, amylase and lipase in dietary supplementation of 10% *A. platensis* were significantly higher than those of the control group.

In conclusion, the results presented in present study show that diet containing 15 g kg<sup>-1</sup> PE enhanced total protein, albumin and globulin and declined cholesterol, triglyceride and glucose. It may be related to increase protein sparing effect of lipids. Supplementation of dietary PE had marked effect on digestive enzyme activities of grey mullet. It can be one of the effective ways to stimulate appetite in grey mullet. These elements are all vital in the formation of rich quality grey mullet. These results indicate *P. australis* is a novel feeding supplement that may be fortuitously utilized in grey mullet feed.

#### Acknowledgements

We would like to thank the personnel of the Offshore Fisheries Research Center, Chabahar, Iran. Special thanks are also due to Dr. N. Tayari Sadaf laboratory expert, Chabahar, Iran, for her valuable help.

#### References

- Ahmed, R.S. and Sharma, S.B., 1997. Biochemical studies on combined effects of garlic *Allium sativum* (Linn.) and ginger *Zingiber officinale* (Rose) in albino rats. *Indian Journal of Experimental Biology*, 35, 841-843.
- Akbary, P. and Aminikhoi, Z., 2018. Effects of *Padina australis* (Hauck) polysaccharide extract on growth, antioxidant and nonspecific immune parameters of the western white leg shrimp, *Litopenaeus vannamei* (Boone). *Iranian Journal of Aquatic Animal Health*, 4(2), 73-85.

- Akbary, P. and Kakoolaki, S., 2019.** Growth, hematological, innate immune responses of Mugil cephalus fed with Echinacea purpurea-supplemented diet against Photobacterium damsela infections. *International Journal of Environmental Science and Technology*, 16(1), 325-334.
- Akbary, P., Kakoolaki, S., Salehi, H., Sepahdari, A., Mehrabi, M. and Jadgal, S., 2016.** The efficacy of Echinacea (*Echinacea purpurea*) methanol extract on growth performance in grey mullet (*Mugil cephalus*). *Iranian Journal of Aquatic Animal Health*, 2(2), 14-24.
- Choi, Y.H., Kim, K.W., Han, H.-S., Nam, T.J. and Lee, B.-J., 2014.** Dietary Hizikia fusiformis glycoprotein-induced IGF-I and IGFBP-3 associated to somatic growth, polyunsaturated fatty acid metabolism, and immunity in juvenile olive flounder *Paralichthys olivaceus*. *Comparative Biochemistry and Physiology. Part A*, 167, 1-6.
- Choi, Y.H., Lee, B.J. and Nam, T.J., 2015.** Effect of dietary inclusion of *Pyropia yezoensis* extract on biochemical and immune responses of olive flounder *Paralichthys olivaceus*. *Aquaculture*, 435, 347-353.
- Davies, S.J., Brown, M.T. and Camilleri, M., 1997.** Preliminary assessment of the seaweed *Porphyra purpurea* in artificial diets for thick-lipped grey mullet (*Chelon labrosus*). *Aquaculture*, 152, 24-258.
- El-Dahhar, A.A., Salama, M.E., Moustafa, Y.T. and Zahran, S.M., 2000.** Effect of salinity and salinity acclimatization on survival and growth of the wild hatched mullet larvae using different feeds in glass aquaria. *Egyptian Journal of Aquatic Biology and Fisheries*, 4, 137-155.
- Ju, Z.Y., Forster, I.P. and Dominy, W.G., 2009.** Effects of supplementing two species of marine algae or their fractions to a formulated diet on growth, survival and composition of shrimp (*Litopenaeus vannamei*). *Aquaculture*, 292, 237-243.
- Kaleeswaran, B., Ilavenil, S. and Ravikumar, S., 2012** Changes in biochemical, histological and specific immune parameters in *Catla catla* (Ham.) by *Cynodon dactylon* (L.). *Journal of King Saud University - Science*, 24, 139-152.
- King, J., 1965.** Practical clinical enymology. D'van Nostrand Company Ltd, New York. 363 P.
- Lara-Flores, M., Olvera-Novoa, M.A., Guzmán-Méndez, B.E. and López-Madrid, W., 2003.** Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus* and the yeast *Saccharomyces cerevisiae* growth promoters in Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, 216, 193-201.
- Mian, J., Hussain, S.M., Siddiqui, P.J.A. and Immink, A., 2014.** Haematological, biochemical and immunological changes on growth enhancement of grey Mullet fingerlings (*Mugil cephalus* L). On shrimp head protein hydrolysate and macroalgae based diets world.

- Journal of Fish and Marine Sciences*, 6, 295-304.
- Nwabueze, A.A., 2012** The effect of Garlic (*Allium sativum*) on growth and haematological parameters of *Clarias gariepinus* (Burchell, 1822). *Sustainable Agriculture Research*, 1, 222-228.
- Reitan, K.I., Rainuzzo, J.R., Oie, G. and Olsen, Y., 1993.** Nutritional effects of algal addition in first-feeding of turbot (*Scophthalmus maximus* L.) larvae. *Aquaculture*, 118, 257-275.
- Sahu, S., Das, B.K., Mishra, B.K., Pradhan, J. and Sarangi, N., 2007.** Effect of *Allium sativum* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*. *Journal of Applied Ichthyology*, 23, 80-86.
- Sankar, G., Elavarasi, A., Sakkaravarthi, K. and Ramamoorthy, K., 2011.** Biochemical changes and growth performance of black tiger shrimp larvae after using *Ricinus communis* extract as feed additive. *International Journal of PharmTech Research*, 3, 201-208.
- Shaluei, F., Hedayati, A., Jahanbakhshi, A. and Baghfalaki, M., 2012.** Physiological responses of great sturgeon (*Huso huso*) to different concentrations of 2-phenoxyethanol as an anesthetic. *Fish Physiology and Biochemistry*, 38, 1627-1634.
- Supamattaya, K., Kiriratnikom, S., Boonyaratpalin, M. and Borowitzka, L., 2005.** Effect of a *Dunaliella* extract on growth performance, health condition, immune response and disease resistance in black tiger shrimp (*Penaeus monodon*). *Aquaculture*, 248, 207-216.
- Talpur, A.D., 2014.** *Mentha piperita* (Peppermint) as feed additive enhanced growth performance, survival, immune response and disease resistance of Asian seabass, *Lates calcarifer* (Bloch) against *Vibrio harveyi* infection. *Aquaculture*, 420-421, 71-78.
- Talpur, A.D. and Ikhwanuddin, M., 2012.** Dietary effects of garlic (*Allium sativum*) on haematoimmunological parameters, survival, growth, and disease resistance against *Vibrio harveyi* infection in Asian sea bass, *Lates calcarifer* (Bloch). *Aquaculture*, 364-365, 6-12.
- Trinder, P., 1969.** Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Annals of Clinical Biochemistry*, 6, 24-27.
- Turan, C., Gürlek, M., Ergüden, D., Yağlıoğlu, D. and Öztürka, B., 2011** Systematic status of nine Mullet species (Mugillidae) in the Mediterranean Sea. *Turkish Journal of Fish Aquatic Science*, 11, 315-321.
- Venkatramalingam, K., Godwin Christopher, J. and Citarasu, T., 2007.** Zingiber officinalis an herbal appetizer in the tiger shrimp *Penaeus monodon* (Fabricius) larviculture. *Aquaculture Nutrition*, 13, 439-443.
- Wootton, L.I., 1964.** Micro-analysis in medical biochemistry in micrometer,

4th.ed. J &A Churchill, London. 264 P.

**Wu, G., Yuan, C., Shen, M., Tang, J., Gong, Y., Li, D., Sun, F., Huang, C. and Han, X., 2007.**

Immunological and biochemical parameters in carp (*Cyprinus carpio*) after Qompsell feed ingredients for long-term administration. *Aquaculture Research*, 38, 246-255.

**Yeh, S.T., Lee, C.S. and Chen, J.C., 2006.**

Administration of hot-water extract of brown seaweed *Sargassum duplicatum* via immersion and injection enhances the immune resistance of white shrimp *Litopenaeus vannamei*. *Fish and Shellfish Immunology*, 20, 332-345.

**Zamannejad, N., Emadi, H. and Hafezieh, M., 2016.**

Effects of supplementation of algae (*Sargassum ilicifolium*) on growth, survival and body composition of rainbow trout *Oncorhynchus mykiss*. *Iranian Journal Fisheries Sciences*, 15, 194-205.