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

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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^{-1} 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

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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, Hawai, 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. astraulis* 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. astraulis* 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. astraulis* extract on the

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

Materials and methods

Preparation of Padina astraulis methanol extract

Two kilograms of *P. astraulis* algae was collected from the Chabahar coast, Iran and oven dried at 60℃, 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℃) 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^{-1}. The derived extract was dispread 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^{-1} 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^{-1}, 0.11±0.04 mg L^{-1} 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^{-1}) and chemical composition (%) of the experimental diets.

	Diets			
Ingredients (g kg^{-1})	**PE0**	**PE5**	**PE10**	**PE15**
P.astraulis	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[a]	9.4	9.4	9.4	9.4
Proximate composition(%)				
Crude protein	51.6	51	50.6	51.6

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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

[a]Premix (mg kg^{-1}) KI, 250; $MnSO_4 \cdot H_2O$, 2800; $ZnSO_4 \cdot H_2O$, 2350; vitamin K, 225; biotin , 3500; (2%)niacin, 4850; calcium pantothenate, 11,000; folic acid, 2000; vitamin B1, 1500; vitamin B_2, 2000; vitamin B_6, 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^{-1}) 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 cirumstances. 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

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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±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^{-1}. 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^{-1} 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^{-1} 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^{-1} feed than the control. Albumin: globulin ratio was higher only in fish on the PE diet at 15 g kg^{-1} 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.

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

PE diet, *P. astraulis* extract diet. Values (mean±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^{-1} 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^{-1} feed over the control group apart from fish received PE diet at 15 g kg^{-1} feed (Table 3).

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

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

PE diet, *P. astraulis* 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. astraulis* 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^{-1} 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^{-1} 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^{-1} feed is safe and advantageous to the health of *M. cephalus*. This is in agreement with the findings of Kaleeswaran *et al*. (2012)

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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 macro algae 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^{-1} 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^{-1} 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^{-1} 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. astraulis* is a novel feeding supplement that may be fortuitously utilized in grey mullet feed.

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