# Role of dietary inclusion of *Gracillaria arcuata* extract on growth performance and biochemical responses in grey mullet, *Mugil cephalus* (Linnaeus, 1758)

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#### Abstract

The present paper aims to examine the effects of dietary incorporation of Gracillaria arcuata extract (GE) on growth conduct and some blood biochemical parameters (Cholesterol (CHO), triglyceride (TG), total protein (TP) and glucose (GLU)) of the grey mullet, Mugil cephalus. Four experimental regimens were considered by including GE at the concentration of 0, 5, 10 and 15 mg kg<sup>-1</sup>diet (GE0, GE5, GE10 and GE15 respectively). Each regimen was allocated randomly to three identical groups of fish (n=10 in each tank) with an introductory average weight of roughly 14.95g. Upon a 60 day feeding trial, fish fed with GE10 and GE15 diet had a considerable (p < 0.05) final weight increase (FW), food intake (VFI), weight gain (WG), protein efficiency ratio (PER), lipid efficiency ratio (LER) and specific growth rate (SGR) compared with control group. While, there were no significant difference in FW, WG and LER among fish fed with GE5 diet and fish fed GE0 diet (p>0.05). No considerable difference, in terms of survival rate, was not shown between all of experimental treatments (p>0.05). The highest level of serum total protein was seen in fish on the GE15 diet (p < 0.05). Fish fed GE10 and GE15 diets had lower Glucose, triglyceride and cholesterol concentrations than did fish fed GE0 and GE5 diet (p < 0.05). The outcomes implied that dietary inclusion of G. arcuata extract caused positive influences on growth performance, feed utilization and biochemical responses in M. cephalus.

# Keywords: Gracillaria arcuata, Grey mullet, Biochemical responses, Growth performance

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# Introduction

a commercially influential Being species, grey mullet, Mugil cephalus been bred for centuries has in comprehensive and semiintensive ponds in many Mediterranean cephalus countries. М. has an auspicious market in Europe, East and South Asia. Also, mullets are a vital aquaculture species in Iran. Consumer demand has expedited the aquaculture progression of these species in Asian countries (Yelghi et al. 2012; Akbary, 2019). Even though natural food may contribute required micronutrients to the bred mullet, the utilization of additional supplements caused higher yields and resulted in rich quality fish product (Wassef et al., 2001).

Seaweeds as a rich source of essential nutrient are considered for human and animal nutrition (Xuan et al., 2013). Even though nutritional aspects of macroalgae are not as prevalent compared to those of land plant raw sources, they contain low content in lipids in their chemical compostion (0.3-7.2 g100 g dry weight <sup>1</sup>). They contain moderate protein (10- $30 \text{ g} 100 \text{ g} \text{ dry weight}^{-1}$ ), but have plenty of non-starch polysaccharides (consisting mostly of pectins, alginic acid, agar and carrageenan), minerals and vitamins (Wassef et al., 2001).. Contradictory growth results have been observed using of dietary seaweed supplementation in aqua feeds for fish. Mustafa et al. (1995)observed enhanced performance in red seabream, Pagrus major fed diets up to 5% level of Ulva sp, Ascophyllum sp and Porphyra sp supplementation. Soler-Vila et al. (2009) showed increased growth performance in rainbow trout, Oncorhynchus mykiss fed diets up to 10% level of Porphyra sp supplementation. In contrast, Valente et al. (2006) demonstrated that the use of the macro-algae G. bursa-pastoris at different inclusion levels (5-10 %) as an ingredient for European sea bass, Dicentrarchus labrax diets does not affect growth levels and feed utilization effectiveness. Also, Al-Asgah et al. (2016) reported that the utilization of the macroalgae G. aucuata at high incorporation levels (20-30%)for African catfish (Clarias gariepinus) regimens negatively affects growth performance and feed utilization efficiency. Xuan et al. (2013)demonstrated that the final body weights of black sea bream. Acanthopagrus schlegelii fed on red algae- G.lemuneiformis 5 and 10% diets indicated non-significant fluctuations while 20% determined a considerable depression in final weight from the control. Choi et al. (2014, 2015) showed that red algae extract of Hizika fusiformis and Pyropia vezoensis could have been positively influenced on growth and immunity in juvenile olive flounder. Preceding researches indicated that macroalgae may be utilized as a supplement in striped mullet, M. cephalus diets (Wassef et al., 2001). Moreover, these studies only focused on the examination of growth performance, feed utilization or fish body composition, and did not pay attention to fluctuations in blood biochemistry incited by the utilization of macroalgae Until now, even with the aquaculture influence of the grey mullet, Mugil cephalus, no reports are

available on the effects of macroalgae on the species' physiology.

Thus, the purpose of the current research was to investigate the effects of dietary incorporation of Gracillaria arcuata extract (GE) on growth performance and some blood biochemical parameters (Cholesterol (CHO), triglyceride (TG), total protein (TP) and glucose (GLU)) of the grey mullet, Mugil cephalus.

#### Material and methods

Fish

A total of 120 healthy fish, M. cephalus (mean weight 14.94 2.01 g mean length 21.10±1.06 cm) were naturally compiled from the Chabahar Coast in the southeast of Iran. The fish were then to Offshore **Fisheries** transferred Research Center in south of Iran and kept in a general tank prefilled with filtered seawater for 10 days. Fish were randomly divided (with equal densities) into twelve 60 L fiberglass tanks prefilled with 400 L of filtered wellaerated seawater (150 L h<sup>-1</sup> of flow rate) with 10 fish per tank. The water was kept at 28-30 °C, dissolved oxygen  $7.5\pm0.7$  mg L<sup>-1</sup>, pH  $7.8\pm0.1$ , and salinity  $33.3\pm0.30$  g L<sup>-1</sup>. Fish were administrated diets supplemented with 0, 5, 10 and 15 mg kg<sup>-1</sup> GE as experimental groups in triplicates. All of them were hand-fed (3-5% of body weight) four times daily to apparent satiation for 60 days.

#### Experimental diet and herbal extract

The G. arcuata algae were gathered around the from Chabahar coast, Iran. Two kg of G. arcuata were dried at 60°C in oven, powdered by mortar, pestle and strained. The powder (50 g) were added to 10 L (10%w/v) of methanol 99% in room temperature  $(24\pm1.2$  °C) within 48 h. The derived extract was concentrated to 300 ml via a rotary evaporator (IKA, Germany). The GE, which was diluted in 300 ml of distilled water, was then added to the staple diet to achieve the suitable concentrations of analysis and control at incorporation level of 0, 5, 10 and 15 mg kg<sup>-1</sup> diet. Evaluated proximate compositions are shown in Table 1. The wet dough was pelletized at 1mm particle size via a chopper machine The empirical (National, Japan). regimens were dried, and strained into the required particle size (1.2 mm) and then kept at 4-8 °C until use (Choi et al., 2015).

	Diets						
Ingredients (g kg <sup>-1</sup> )	GE0	GE5	GE10	GE15			
Gracillaria arcuata	0	5	10	15			
$(g kg^{-1})$							
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			

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

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Table 1 continued:							
Ingredients (g kg <sup>-1</sup> )		Diets					
	GE0	GE5	GE10	GE15			
Premix <sup>a</sup>	9.4	9.4	9.4	9.4			
Proximate							
composition(%)							
Crude protein	51.6	51.3	50.2	51.1			
Crude lipid	11.9	11.2	11.8	11.4			
Crude ash	12.1	12.2	12	12.3			
Dry matter	92.2	92.3	91.8	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 B1, 1500; vitamin B<sub>2</sub>, 2000; vitamin B<sub>6</sub>, 2000; and vitamin C, 50,000.

#### Growth performance

10 fish in each group and duplications) and the data were utilized to determine growth conduct according to Harikrishnan *et al.* (2012) and Akbary *et al.* (2011) as follows:

WG (%) = Wf–Wi/Wi  $\times 100$ 

:Where, WG was weight gain and mean final body weight (Wf) of every treatment was determined by dividing total fish weight in each tank by number of fish.

VEL (g) = $\frac{100 \times \text{ crude feed intake} / (Wf + Wi/2)}{100 \times \text{ crude feed intake} / (Wf + Wi/2)}$
t t
Where, VFI was voluntary feed intake
$DGR (g) = \frac{[(WG \times 100)/(Wi + Wf)/2)]}{[(WG \times 100)/(Wi + Wf)/2)]}$
t t
Where, DGR, Wi and Wf were daily
growth rate, initial weight and final
weight, respectively.
SGR (specific growth rate)(%) = $\left[\frac{\text{Ln}(\text{Wi}) - \text{Ln}(\text{Wf})}{\Delta t}\right] \times 100$
Feed given (g.dry weight)
body weight gain (g)
WG (g body weight gain)
protein fed (g)
WG (g. body weight gain)
lipid fed (g)
Surviva (%) = $\frac{\text{final number of fish}}{\text{initial number of fish}} \times 100$

#### 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 in to un-heparinized sterile tubes 1–1.5 mL for the serum biochemical experimental objectives (Shaluei *et al.*, 2012; Akbary *et al.*, 2016).

Serum glucose concentration was determined based on the approach provided By Trinder (1969). Serum total protein was measured using the approach explained by Wootton (1964). Triglyceride and cholesterol level measurements were taken place based on the approach explained by Sankar (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).

#### Statistical analysis

All experiments were repeated twice. Data were assessed via one- way analysis of variance (ANOVA). Each group was assumed to be considerably different if p<0.05. When a considerable F value resulted from ANOVA the fluctuations among all groups were tested via the Duncan

multiple comparisons test. SPSS was utilized for statistical purposes for windows versions 16. Data are determined as means±standard Error.

#### Results

#### Growth performance

The achieved results from varying mean growth and nutritional criterion have has been presented in Table 2. The addition of 10 and 15 mg of GE to the regimen resulted in a significant enhancement in final weight (FW), food intake (VFI), specific growth rate

(SGR), protein efficiency ratio (PER) and lipid efficiency ratio (LER) in comparison with the control group (p<0.05). Peak levels of criterion mentioned previously were seen in fish fed on the GE15 regimen, while the FW, weight gain (WG) and LER in fish fed with GE5 regimen did not show considerable variances rather than those in control (p>0.05). No considerable difference was observed when it came to the survival rate of grey mullet on the experimental diets (p>0.05).

Table 2: Growth	performance of gro	ey mullet fed t	he experimental	diets for 60 days.

	Growth parameters		Diet (mg kg <sup>-</sup> )				
	_	GE0	GE5	GE10	GE15		
	Initial weight	$14.98 \pm 1.12^{ns}$	$14.81 \pm 2.08^{ns}$	$14.85 \pm 2.10^{ns}$	14.96±1.09 <sup>ns</sup>		
	(g fish <sup>-1</sup> )						
	Final weight (g fish <sup>-1</sup> )	$37.86 \pm 2.90^{b}$	$41.87 \pm 1.08^{b}$	$47.55 \pm 1.89^{a}$	48.68±1.03 <sup>a</sup>		
	Voluntary feed intake	$1.64 \pm 0.13^{a}$	$1.27 \pm 0.04^{b}$	$1.18\pm0.06^{b}$	$1.26 \pm 0.06^{b}$		
	Weight gain (%)	$152.39 \pm 18.97^{b}$	$182.79 \pm 7.70^{b}$	$215.46 \pm 6.18^{a}$	$8^{a}$ 225.40±6.70 <sup>a</sup>		
	Daily growth rate	$6.26 \pm 0.82^{ns}$	6.22±0.35 <sup>ns</sup>	$6.84\pm0.42^{ns}$	$7.47\pm0.42^{ns}$		
	(%)						
	Feed conversion rate	$1.04\pm0^{ns}$	$1.03\pm0^{ns}$	$1.03\pm0^{ns}$	$1.04\pm0^{ns}$		
	Specific growth rate	1.46±0.13 <sup>c</sup>	$1.72 \pm 0.04^{b}$	$1.91\pm0.03^{ab}$	1.96±0.03 <sup>a</sup>		
	(%)						
	Protein efficiency	$12.08 \pm 1.59^{\circ}$	$16.61 \pm 1.07^{b}$	$19.01 \pm 1.52^{ab}$	$20.12 \pm 1.59^{a}$		
	ratio						
	Lipid efficiency ratio	$3.07 \pm 0.38^{b}$	$3.68 \pm 0.15^{b}$	$4.37 \pm 0.12^{a}$	$4.54 \pm 0.13^{a}$		
_	Survival (%)	100±0 <sup>ns</sup>	100±0 <sup>ns</sup>	99.79±0.03 <sup>ns</sup>	99.85±0.06 <sup>ns</sup>		

Values (mean±SE of three replications), n=10. In each row not sharing a common superscript are significantly different (p < 0.05).<sup>ns</sup> =Not significant.

#### Biochemical responses

Blood criterion of fish on the experimental regimens are presented in Table 2. Data analysis in Table 2 implied that the dietary chlorella mostly influenced the criterion relevant to protein/ lipid metabolism of grey mullet. The serum total protein content was considerably (p<0.05) increased for fish fed GE10 and GE15 diets over the control group. The maximum total

protein content was recorded in the fish on the GE15 regimen. There was considerable (p<0.05) decline in cholesterol, glucose and triglycerides levels in those fish on the GE diet over the control (Table 3). The lowest cholesterol and triglycerides levels of serum were observed in fish fed GE15. The lowest glucose level was observed among fish fed GE10 and GE15 diets.

GE diet (mg kg <sup>-1</sup> feed)								
Parameter	0	5	10	15				
Total protein (g dl <sup>-1</sup> )	$4.42\pm0.43^{\circ}$	$4.80 \pm 0.17^{bc}$	4.96±0.12b	$5.06 \pm 0.12^{a}$				
Glucose (mg $dl^{-1}$ )	$55 \pm 1.57^{a}$	$24.86 \pm 1.40^{b}$	17.16±1.01 <sup>c</sup>	15.13±1.24 <sup>c</sup>				
Triglycerides (mg dl <sup>-1</sup> )	227.33±11.76 <sup>a</sup>	$194.67 \pm 3.88^{b}$	$184 \pm 2.30^{\circ}$	$140.33 \pm 2.60^{d}$				
Cholesterol (mg dl <sup>-1</sup> )	$105.20{\pm}2.37^{a}$	$99.33{\pm}1.88^{a}$	82.66±3.71 <sup>b</sup>	$59.66 \pm 2.60^{\circ}$				

Table 3: Serum	biochemical	parameters	of Mugil	cephalus	fed (	GE d	diet at	different	levels	for	60
days.											

GE diet, G. aucuata diet . Values (mean $\pm$ SE of three replication). In each row not sharing a common superscript are significantly different (p < 0.05)

#### Discussion

The possible utilization of macroalgae in fish feeds is dependent on relevant costs in their production, harvesting and processing before their incorporation in fish regimens. Valente et al. (2006) showed that the utilization of the macroalgae G.bursa-pastoris at different incorporation levels (5-10 %) as an ingredient for European sea bass, Dicentrarchus labrax diets does not affect growth conduct and feed utilization efficiency. Also, Al-Asgah et al. (2016) recorded that the utilization of the macroalgae G. aucuata at high incorporation levels (20-30%) for African catfish (Clarias gariepinus) diets suppresses growth performance and feed utilization efficiency. These complied with data achieved by Xuan et al. (2013) who showed that the final body weights of black sea bream, Acanthopagrus schlegelii fed on red algae- G. lemuneiformis 5 and 10% regimens showed non-significant discrepancies while 20% reported a considerable decrease in final weigh from the control. In contrast, in the our study, the use of the macroalgae G. aucuata extract (GE) at high inclusion levels (10-15 mg kg<sup>-1</sup>) significantly growth promoted the and feed utilization in grey mullet, indicating that the omnivorous grey mullet well utilization plant protein sources. The effective composition of microalgae resulting in growth enhancement has obviously defined, not but the advantage has been connected to their content of vitamins and minerals, lipid mobilization and enhanced absorption and consumption efficiency ratios (Xuan et al., 2013). Increased VFI and PER were observed in grey mullet fed increasing GE diets, which may explain the reason for the increased growth in the present study. LER of GE10 and GE15 diet was considerably higher in comparison to GE5 and GE0. This may be due to *G*. arcuata extract which influentially supplements stimulated the lipid metabolism (Choi et al., 2015). Similarly Choi et al. (2015) discovered identical growth performances and protein usage efficiencies in olive flounder. **Paralichthys** olivaceus when on regimens containing 15 g of red algae, *P.* yezoensis extract  $kg^{-1}$ diet. These results revealed that the most suitable algae incorporation levels in fish diets may be dependent on the feeding manners of the fish and algae species.

5-15 mg GE in diets could significantly improve the blood parameters of grey mullet. In this research, considerably lower serum total cholesterol levels were noticed at all GE levels compared to the ones on the control diet. In congruence with our results, Casas- Valdez et al. (2006) recorded the significant minimization of serum cholesterol level in brown shrimp, Farfantepenaeus californiensis fed diets containing 4 % Sargassum. Also Akbary and Shahraki et al. (2017) observed the considerable reduction of serum cholesterol level in grey mullet fed regimens with 15g Padina astralis *extract* supplementation. kg<sup>-1</sup> diet. Identical inclination of reducing blood cholesterol level also been obtained in olive flounder fed diets containing 15 g P.yezoensis extract kg<sup>-1</sup> (Choi et al., suggesting 2015), that algae supplementation stimulate could hormonal control of lipid metabolism (Choi et al., 2015). In this study, significantly higher serum total protein concentrations was noticed at all GE levels compared to the ones on the Similar tendency control diet. of increasing the serum total protein also been achieved in olive flounder fed diets containing 5-20 g P.yezoensis extract kg<sup>-1</sup> (Choi et al., 2015) suggesting that lipids are conversely utilized as an energy sources prior to protein. In the present study. significantly lower triglyceride and glucose levels were noticed at all GE levels compared to the ones on the control regimen. Similarly, Choi et al. (2015) indicated a decreeing tendency as dietary P.yezoensis extract level escalates. The same case also found by Akbary and Shahraki et al. (2017), They reported glucose and triglyceride levels in grev mullet fed wit 15g P. kg<sup>-1</sup> extract diet astralis were significantly lower than the control. An glucose relationship of inverse detected concentration was when macroalgae level is increased. This fact might be an indirect consequence of the reduction in digestible carbohydrates as the incorporation level of algae was increased.

The outcomes of this research showed that marine macroalgae *G*. *arcuata* 10-15mg kg<sup>-1</sup> incorporation levels have huge potential as possible ingredients in regimens for grey mullet with enhanced effects on growth conduct, feed utilization efficiency and blood biochemical criterion. Hence, this research implies that optimum dietary supplementation level of *G. arcuata* extract could be roughly 15 mg kg<sup>-1</sup> of regimen for the positive influences on growth conduct and feed utilization efficiency with no blood biochemical criterion in grey mullet.

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