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Growth yield, carcass traits, biochemical and non- specific immune parameters in grey mullet, *Mugil cephalus* Linnaeus, 1758 under cyclic starvation and L- carnitine supplementation

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Abstract

The main objective for the present research was to assess the effect of cyclic starvation and L- carnitine supplementation on growth (body final weight, hepatosomatic index (HSI), daily growth rate (DGI), feed conversion ratio (FCR) and voluntary feed intake (VFI)), body composition (crude protein, crude lipid, ash and moisture), biochemical (plasma total protein, glucose, cholesterol, triglyceride and liver glycogen) and immunological (lysozyme activity) parameters in grey mullet. To satisfy the foregoing end, a total of 240 fish with an average weight of 1.33±0.26 g were randomly divided into four groups (three replicates, 20 fish per tank). Two groups were fed on a daily basis and the other two ones were kept starved for 7 days once in 2 weeks. Two groups were fed a carnitine free basal diet and the remaining were fed a diet with 800 mg carnitine kg^{-1}. Cyclic starvation led to significantly decreased body final weight, DGI, FCR, total protein, triglyceride and cholesterol. On the contrary, no significant differences were found across all groups for FCR, HSI, glucose, liver glycogen and lysozyme concentrations. At the same time, the combined effects of cyclic starvation and carnitine supplementation were manifested in DGI and VFI parameters. In light of the above results, it can be noted that in both feeding regimes, growth, feed utilization and carcass quality in grey mullet increased upon adding 800 mg carnitine kg^{-1} diet

Keywords: *Mugil cephalus*, Cyclic fasting, L-carnitine, Growth performance, Cholesterol, Lysozyme

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Introduction

In recent times, the aquaculture industry has been becoming a popular and fast growing food production source in many countries. To keep good food quality and to improve productivity, some commercially important marine species including grey mullet, grouper, sea bream and sea bass are cultured intensively (Liao *et al.*, 2001). To optimize feeding strategy is of great importance while managing commercial fish aquaculture during overproduction. Prior to applying fasting or food deprivation to induce growth compensation in aquaculture practices, it is necessary to determine which feed deprivation duration is more suitable to induce compensatory growth among others (Ali *et al.*, 2003; Eroldogan *et al.*, 2006). However metabolic responses to starvation may differ in gender, species, size, feed deprivation duration and environmental conditions (Park *et al.*, 2012).

Tanks to its wide adaptability to captivity conditions and high commercial value, the grey mullet, *Mugil cephalus*, belonging to Mugilidae, is cultured in the Mediterranean region frequently.

Literature is fraught with enormous studies dealing with growth and metabolic responses of fishes to feed deprivation and re-feeding (Vosyliene and Kazlauskiene, 1999; Falahatkar *et al.*, 2012). Only recently, some researches addressed biochemical (Chatzifotis *et al.*, 2011; Turan *et al.*, 2011; Park *et al.*, 2012) and immunological (Caruso *et al.*, 2010; Feng *et al.*, 2011; Caruso *et al.*, 2012) responses of various fish to starvation treatment. To the best of our knowledge, this is the first study on grey mullet shedding much more light on the effect of a short-time fasting period in fish and their responses to re-feeding on metabolic and physiological responses. However, the foregoing subject deserves many investigations.

L- carnitine is found to be a new and important nutrient essential to strike a balance between growth rate of fish and its optimal uptake of food (Ma *et al.*, 2008; Mohseni *et al.*, 2008). L-carnitine is a quaternary amine characterized as being water-soluble having great contributions in lipid β-oxidation to facilitate incorporating activated long-chain fatty acids into mitochondria and side intermediate compounds out of the mitochondrial matrix (Harpaz, 2005). Given its involvement in fish lipid metabolism, carnitine dietary supplementation enhances protein synthesis as well as promotes growth performance (Ozorio *et al.*, 2001; Nogueira *et al.*, 2011). During the past two decades, different cultured fish species were studied for effects of L-carnitine supplementation on fish culture and nutrition. Some examples are *Huso huso* (Mohseni *et al.*, 2008), hybrid striped bass (*Morone chrysops*×*M. Saxatilis*) (Twibell and Brown, 2000), Black Sea bream and rainbow trout among others (Jalali Haji-abadi *et al.*, 2010). Most studies have focused on growth rates and food conversion efficiencies, however recently, several studies have dealt with biochemical (Ozorio, 2001; Mohseni *et*

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al., 2008) and immunological responses.

As mentioned above, to the best of our knowledge, this is the first on the individual and combined effects of cyclic fasting and carnitine supplementation on growth performance, carcass quality, biochemical and immunological responses in grey mullet.

The ongoing study aims to answer to this research question as to whether cyclic fasting, and L- carnitine supplementation and their interaction affects the health state of grey mullet, and in turn optimizes its production in aquaculture. To this end, we evaluated growth yield (body final weight, hepatosomatic index (HSI), daily growth rate (DGI), feed conversion ratio (FCR) and voluntary feed intake (VFI), body compositions (crude protein, crude lipid, ash and moisture), biochemical (plasma total protein, glucose, cholesterol, triglyceride and liver glycogen) and immunological (lysozyme activity) parameters.

Materials and Methods

Fish and Rearing condition

In the present study, a total number of 240 *M. cephalus* weighing 1.33±0.26 g (mean±SD), were captured from the coastal water of Chabahar in mid-February 2014 for quarantine and health check purposes. Fish were accustomed for a week in two 400-L tanks separately and were fed twice a day with a commercial diet for rainbow trout (Beyza Feed Mill Co, Shiraz, Iran). Then the fish were randomly divided into four groups in triplicate so that 20 fish 60 L^{-1} were selected and distributed in flow-through tanks. Each tank was maintained at 28.2±0.5˚C and was continuously supplied with seawater (37 g L^{-1}) at a flow rate of 25 L min^{-1}. The dissolved oxygen concentration, ammonia nitrogen concentration and pH measured and recorded daily were about 7.01±0.87 mg L^{-1}, 0.11±0.04 mg L^{-1} and 7.8±0.4, respectively. The photoperiod was regulated as a 12:12 dark/light cycle.

Experimental design and diets

Having been subjected to seven days acclimation, four groups of the eight groups were fed on a daily basis (hereafter referred as C0 and C800) while the remaining groups were kept starved for seven days once in two weeks (referred as SRf 0 and SRf 800). Two groups were fed with an L-carnitine-free basal diet and the other two were fed an L- carnitine supplemented diet (800 mg kg^{-1}) (Table 1). To prepare both experimental diets, dry ingredients were ground into a powder (0.5 mm particle size). After grinding, 30% distilled water was added to it and mixed further. The mixture was pelletized to a particle size of 1 mm using a chopper machine. The experimental diets were freeze-dried at 40˚ C overnight and then stored at −20 °C until used. During the feeding trials, all fish were fed by hand twice (09:00 and 17:00) a day for 9 weeks.

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Table 1: Formulation (% unless otherwise stated) and proximate composition (%) of the experimental diets.

Nutrient material (%)	Dietary L- carnitine(mg kg $^{-1}$)	
	0	800
Fish meal	45.5	45.5
Soya meal concentrate	20.5	20.5
Wheat	4	4
Wheat gluten	10	10
Sunflower meal	0.04	0.04
Dried yeast	7	7
Fish oil	0.05	0.05
Soybean oil	3	3
Colza oil	2	2
lecithin	4	4
Carniking	-	0.2
Vitamins and minerals[a]	2	2
Choline chloride	0.2	0.2
Dicalcium phosphate	0.4	0.4
Carnintine (mg kg $^{-1}$)[b]	0	800
Proximate composition (%)		
Crude protein	45	45.05
Crude lipid	15.34	15.37
Crude Ash	13.45	13.42
Phosphorus	1	1
Dry matter	92.2	92.1

[a]Vitamins and minerals supplied per kilogram of feed. Vitamins: Vitamin A, 2250000 IE; vitamin D, 250001 IE; vitamin E, 20001 IE. Minerals: 501 mg $CuSO_4$;15000 mg $ZnSO_4$ ،.، ;0.01$MnSO_4$ 500 $CoSO_4$;500 KI; 35 Na_2SeO_3
[b]50 % L-arnitine, 35% silica and 15% water (Lonza, Basel, Switzerland)

Sampling and analysis

Clove powder was used anaesthetize fish for 2 min (20 mg L^{-1}), subsequently fish were individually measured and weighed using an electronic balance (0.001g) on days 21, 42 and 63 after the experiment began. Totally in three sampling periods, growth yield was expressed as final weight (g), DGI, FCR and VFI. At the end of the experiment, the HSI was determined. Fifty fish from each group were slaughtered by spinal section. The liver was removed and weighed immediately. Livers were stored at -20˚ C for liver glycogen analysis. The following parameters were calculated as follows: HSI=100× (liver weight/total body weight), DGI=(total weight gain×100)/ [{(initial total weight + final total weight +dead fish wt.)/2}/days], FCR= feed consumed/ weight gain and VFI (%BW day^{-1}) = (100× crude feed intake/ (final weight+initial weight/2)/day). Eventually, five fish per tank were randomly collected and pooled for carcass composition analysis. To determine liver glycogen level, the livers were homogenized in 200µL distilled water, centrifuged for 10 min at 3000 rpm followed by supernatant separation. Simultaneously, the blood specimens (4 fish per tank) were collected through cutting of the caudal peduncle, transferred to Eppendrof tubes (1.5 mL) and then centrifuged (3000g, 15 min), and the plasma was stored at −25 °C until

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biochemical and immunological analyses.

Carcass chemical analysis

Analysis of proximate composition was carried out as per AOAC standard methods (1995). Upon drying samples at 105 °C to a constant weight and subtracting the initial weight of sample the moisture content was calculated. The moisture content was used to determine nitrogen content by Kjeldahl method. By multiplying nitrogen percentage by 6.25, the crude protein content was determined. To determine crude lipid the solvent extraction method by Soxtec system was considered in which diethyl ether (boiling point, 40–60 °C) was used as a solvent. Ash content was determined by incinerating the samples in a muffle furnace at 550 °C for 24 h.

Biochemical analysis

The method described By (Trinder, 1969) was used to measure plasma glucose concentration. Plasma total protein content, triglycerides and cholesterol level, liver glycogen concentration were determined via methods put forward by Wootton (1964), Bidinotto *et al*. (1997) respectively. Standard analyses kits (Pars Azmon, Iran) using automatic analyzer was used to evaluate biochemical estimation of blood glucose, protein, cholesterol, triglyceride (Furuno, CA-270, Japan).

Determination of immunological parameter

Turbid metric assay according to the method described by Ellis (1990) was used to determine serum lysozyme activity. Briefly, test serum (100 µL) was added to 100 µL *Micrococcus lysodeikticus* suspension (Sigma-Aldrich, St. Louis, MO, USA) (0.2 mg mL^{-1}) in a 0.05 M PBS (pH 6.2). The reaction was carried out at 25˚C and absorbance was measured at 0.5 and 4.5 min in a spectrophotometer at 550 nm.

Statistical analysis

To calculate mean±S.E.M. for each group, SPSS software ver. 16.0 was used (Statistical Package for the Social Sciences, Inc, IBM, USA). For mean separation, two-way ANOVA according to General Linear Method procedure with feed frequency (apparent satiation and cyclic starvation) and dietary L- carnitine (0 and 800 mg kg^{-1} diet) supplementation as fixed variables and its interactions were considered. Tukey's test with probability level of 5% (p<0.05) was used to compare means. Normality was tested using the Kolmogorov–Smirnov test. To confirm variance homogeneity Leven's test was used. Non homogenous data were arcsine transformed before further statistical analysis.

Results

The results of growth performance of grey mullet subjected to the different diet regimes and experimental diets were presented in Table 2. Experimental fish were grown from an initial weight 1.33g to a mean body final weight ranging from 6 to 12g for 9

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week experimental diets. The HSI did not differ among all the groups in a significant way (p>0.05). At the end of the experiment L- carnitine supplementation and their interaction affected final body weight; VFI and DGI were significantly affected by the feeding regimes. L-carnitine decreased significantly VFI, while FCR showed higher values in the fasted groups. The unrestricted fed group accounted for the highest DGI and final body weight.

Table 2: Growth performance of grey mullet subjected to the experimental diets (0 or 800 mg L – carnitine kg $^{-1}$) and different feeding regimes (fed every day: starved 7 days every 2 weeks) during three experimental periods.

	Experimental diets				*p* value[a]		
	C0	**C800**	**S/Rf0**	**S/Rf 800**	**C**	**S**	**C×S**
Period 1 (1-21st day)							
Initial weight (g)	1.32±0.03	1.28±0..05	1.30±0.04	1.40±0.07	NS	NS	NS
Final weight(g)	3.30±0.06	3.35±0.01	2.56±0.02	2.96±0.05	NS	*	NS
VFI(%BWday^{-1})	1±0.04	0.97±0.03	0.72±0.02	0.94±0.0	NS	*	NS
DGI	3.12±0.17	3.25±0.12	2.16±0.08	2.55±0.09	NS	*	NS
FCR	1.07±0.10	0.97±0.08	1.38±0.04	1.58±0.04	NS	*	NS
Period 2 (22-42nd day)							
Final weigh (g)	8.26±0.05	8.63±0.07	6.09±0.15	4.34±0.16	*	*	*
VFI (%BWday^{-1})	0.79±0.01	0.97±0.01	1.53±0.03	1.63±0.04	*	*	*
DGI	2.50±0.02	2.82±0.02	2.06±0.05	1.36±0.07	*	*	*
FCR	0.99±0.03	0.94±0.02	1.48±0.03	1.81±0.01	NS	*	NS
Period3(43-64th day)							
Final weight (g)	11.26±0.07	11.70±0.05	8.07±0.28	6.19±0.13	*	*	*
VFI (%BWday^{-1})	2.36±0.04	2.64± 0..07	3.37±0.02	3.87±0.04	*	*	*
DGI	0.99± 0.03	1.02± 0.05	0.67±0.07	0.78±0.01	NS	*	NS
FCR	0.95±0.02	0.93±0.04	1.23±0.05	1.09±0.08	NS	*	NS
1-64th day							
VFI (%BWday^{-1})	1.36±0.03	1.48±0.03	1.97±0.01	1.97±0.06	*	*	*
DGI	5.16±0.04	5.41±0.04	3.51±0.14	2.51±0.06	*	*	*
FCR	0.94±0.02	0.94±0.01	1.25±0.01	1.32±0.11	NS	*	NS
HSI	1.62±0.11	1.52±0.13	1.58±0.15	1.42±0.10	NS	NS	NS

[a] Data means were compared using two- way ANOVA (p<0.05). NS: Not significant. Asterisks indicate significant (p<0.05) differences between groups. Values (mean±SE of three replicates) (n=60) VFI: Voluntary feed intake, DGI: Daily growth index, FCR: Feed conversion rate and HSI: Hepatosomatic index. C: effect of carnitine. S: effect of starvation and C×S: interaction effect.

Table 3 presents effects of the feeding regimes and L- carnitine level on chemical composition of carcass According to the results, L- carnitine imposed marked impacts on crude lipid of carcass as the lowest lipid content was observed in the fish fed continuously and feeding with 800 mg L- carnitine kg^{-1} diet (C800). Conversely cyclic starvation did not affect crude lipid content alone. Crude protein, crude ash and moisture among all groups did not differ significantly.

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Table 3: Carcass chemical composition (% tissue, dry matter) of grey mullet subjected to the experimental diets (0 or 800 mg L – carnitine kg^{-1}) and different feeding regimes (fed every day: starved 7 days every 2 weeks) for 64 days.

	Experimental diets				*p* value[a]		
	C0	**C800**	**S/Rf0**	**S/Rf 800**	**C**	**S**	**C×S**
Crude protein	18.40±0.65	18.82±0.60	17.84±1.01	18.05±1.01	NS	NS	NS
Crude lipid	5.91±0.91	2.11±0.84	3.62±1.46	2.14±0.84	*	NS	NS
Crude ash	6.37±0.54	7.14±0.91	5.84±0.13	6.27±0.29	NS	NS	NS
Moisture	72.58±0.21	73.76±1.25	71.98±1.94	74.76±1.35	NS	NS	NS

[a] Data means were compared using two- way ANOVA (p<0.05). NS: Not significant. Asterisks indicate significant (p<0.05) differences between groups. Values (means± SE of three replicates) (n=60). C: effect of carnitine. S: effect of starvation and C×S: interaction effect

Table 4 and Table 5 present biochemical responses of plasma and liver of grey mullet on feeding regimes and L- carnitine level respectively. Both the L- carnitine content and the cyclic starvation alone significantly affected plasma total protein, cholesterol, triglyceride, whereas cyclic starvation alone in turn had a considerable impacts on plasma and liver glycogen (p<0.05). The highest total protein, cholesterol and triglyceride were observed in the fish fed continuously on 800 mg carnitine kg^{-1} diet (C800) and the lowest their levels were detected in the starved fish fed basal diet (S/RF0).

Table 4: Plasma total protein, cholesterol, triglyceride and glucose concentrations and Liver glycogen concentration in grey mullet subjected to the experimental diets (0 or 800 mg L – carnitine kg $^{-1}$) and different feeding regimes (fed every day: starved 7 days every 2 weeks) for 64 days.

	Experimental diets				*p* value[a]		
	C0	C800	S/Rf0	S/Rf 800	C	S	C×S
Biochemical parameters							
Total protein (g dL^{-1})	4.62±0.06	6.07±0.28	4.32±0.04	5.37±0.03	*	*	NS
Cholesterol (mg dL^{-1})	194±2.16	152.25±3.05	163±2.38	121.25±2.25	*	*	NS
Triglyceride (mg dL^{-1})	59.50±2.72	46.25±0.85	64.75±2.49	56.25±4.76	*	*	NS
Glucose (mg dL^{-1})	18±0.72	17.92±0.15	15.25±0.5	14.03±0.13	NS	*	NS
Glycogen (mmol glucose g^{-1})	74±1.08	72.50±2..21	88.75±3.42	86.25±3.11	NS	*	NS

[a] Data means were compared using two- way ANOVA (p<0.05). NS: Not significant. Asterisks indicate significant (p<0.05) differences between groups. Values (mean±SE of three replicates) (n=60). C: effect of carnitine. S: effect of starvation and C×S: interaction effect

Table 6 presents lysozyme concentrations of grey mullet upon feeding the experimental diet. Plasma lysozyme concentration was affected by cyclic starvation to a great extent, while starved fish fed the basal diet accounted for the highest concentration (S/RF0). Conversely L- carnitine supplementation alone had no effect on lysozyme concentration (p>0.05). Also interaction effect of L- carnitine and the feeding regime was not observed in the experimental treatment (p>0.05).

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Table 6: Lysozyme concentration measured in the plasma of grey mullet subjected to the experimental diets (0 or 800 mg L – carnitine kg $^{-1}$) and different feeding regimes (fed every day: starved 7 days every 2 weeks) for 64 days.

	Experimental diets				*p* value[a]		
	C0	**C800**	**S/Rf0**	**S/Rf 800**	**C**	**S**	**C×S**
Lysozyme (Unit mL^{-1})	30.50±0.64	31±0..91	37.72±0.73	37±0.40	NS	*	NS

[a] Data means were compared using two- way ANOVA (P<0.05). NS: Not significant. Asterisks indicate significant (p<0.05) differences between groups. Values (means± SE of three replicates) (n=60). C: effect of carnitine. S: effect of starvation and C×S: interaction effect

Discussion

In this research, in grey mullet subjected to the different feeding regimes growth yield, carcass chemical composition, biochemical and non-specific immune responses were studied (starved fish for 7 days and re-fed for 2 weeks in continuous manner) and two L- carnitine levels. The rationale behind the present research was to investigate whether cyclic fasting, L- carnitine supplementation and their interaction can lend to keep grey mullet healthy to optimize its production in aquaculture.

According to the present study upon different feeding regimes, final body weight increased, but in the control fish, the weight gain was lower than in the continuously fed fish. The feeding regimes affected VFI, DGI and FCR significantly. In the starved groups, FCR and VFI were higher and DGI was lower than continuously fed fish. Conversely, using a similar regime of cyclic fasting in previous observations Rueda *et al*. (1998) Our results are in line with Nogueria *et al*. (2011) in red porgy subjected to cyclic fasting for 7 days once in 2 weeks implying that presumably fasting applied protocol was not sufficiently long to induce a mechanism of compensatory growth. Here, no significant differences were observed for the HSI and survival suggesting the importance of the liver reserves of lipids during short time feed deprivation periods in grey mullet, similar to that found by Nogueira *et al*. (2011)

Under different regimes carnitine supplemented diets significantly improved DGI, VFI and final body weight in grey mullet. While the present results are in close agreement with those of Twibell and Brown (2000) on hybrid striped bass (*Morone chrysops×M. Saxatilis)*, Ma *et al*. (2008) on Black Sea bream and Jalali Haji-abadi *et al.* (2010) on rainbow trout, obtained some results opposite to those reported by Nogueira *et al*. (2011) on red porgy. Nogueira *et al*. (2011) showed that the 630 mg kg $^{-1}$ carnitine contained in diets did not improve both growth yield or utilization in red porgy reporting so that species, age and concentration of L- carnitine might affect fish growth.

Carcass chemical composition of grey mullet did not vary upon cyclic starvation which can be attributed to the fact that energy reserves in the liver such as liver glycogen were undertaken to satisfy the demands during starvation. Similarly, Heide *et al*. (2006) on Atlantic halibut and

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Teskeredzic *et al*. (1995) on rainbow trout reported that carcass chemical composition was constant under cyclic fasting. Here, crude protein rate in the fasted group was identical to that in the continuously fed fish denoting that lipids are the principle energy source during short period of feed deprivation (Rueda *et al.*, 1998). Conversely, L-carnitine significantly decreased crude lipid in grey mullet. Similar studies in several fish species confirm our results (Mohseni *et al.*, 2008; Akbari *et al.*, 2014) reporting that L- carnitine can increase the catabolism of body lipid, sparing body protein for anabolic processes. In contrast, Nogueira *et al*. (2011) N reported that whole body lipid content was not significantly affected by 630 mg carnitine kg^{-1} diet in red porgy shedding light that effects of carnitine supplementation are inconsistent.

Similarly, in the present research, plasma glucose concentrations were not affected by both the feeding regime and L- carnitine level in grey mullet. In fact, all groups did not differ in this trend suggesting that the increased glycogenolysis and gluconeogenesis kept plasma glucose concentration constant (Laiz-Carrión *et al.*, 2012). As it was shown here, the increased liver glycogen in starved fish led to hepatic production. Serum glucose levels in chinook salmon, *Oncorhynchus tshawytcha* (Barton *et al.*, 1988) and rainbow trout, *O.mykiss* (Farbridge and Leatherland, 1992) significantly decreased at 20, 28 and 42 days of feed deprivation. In contrast, between 47 and 96 day after fasting in European eel (Dave *et al.*, 1975) and compared to the fed group 4 weeks after fasting in olive flounder (Park *et al.*, 2012), a significant increase was found in serum glucose levels of starved group. Whereas serum glucose levels did not differ in red porgy (Costas *et al.*, 2011) and *Senegalese sole* (Caruso *et al.*, 2012) after 14 and 21 days feed deprivation respectively, which is in agreement with our results.

Under both the feeding regimes, L-carnitine supplementation did not affect plasma glucose concentration. While working on African catfish, Ozorio (2001) pointed out that diets offered with high-carnitine showed low plasma glucose level. Such contradictory results indicate that concentration of glucose varies depending on age and species, and the present results provide additional support for the hypothesis. (Ozorio, 2001)

In the present study, in both the feeding regimes, L- carnitine had a marked effect on plasma total protein as its values were higher compared to continuously fed fish. Increased serum total protein in those fish fed with L-carnitine in their diet that may have emerged in response to the increased transport of fatty acids from tissues for oxidation process and at the same time and in turn this denotes the saving effect of beta-oxidation of fatty acids which reserves more protein and results in high level of protein in blood serum (Fellows *et al.*, 1980).

In contrast, Ozorio (2001) reported that plasma protein level decreased (from 36.0 to 32.9 g L^{-1}) upon adding 550 mg kg^{-1} carnitine supplements in

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diet, suggesting that such effects are varied in nutritional factors, such as the natural carnitine content and fat.

Starved grey mullet and continuously fed fish differed significantly in plasma cholesterol and triglyceride concentrations. In both the experimental diets starvation significantly decreased plasma cholesterol. This is in line with Ince and Thorp (1976) on *Esox lucius*. There are conflicting results from previous studies on the effect of starvation on total cholesterol. These varying responses are consistent with the suggestion that the concentration of cholesterol varies depending on the duration of starvation (Chatzifotis *et al.*, 2011).

With their high calorific value, triglycerides as lipids can be catabolized to produce glycerol and free fatty acids (FFA) during starvation (Ida Coordt *et al.*, 2012). Here, in the starved fish, plasma triglyceride concentration significantly was increased rather than in the continuously fed fish. Yar Mohmmadi *et al*. (2012), found increased levels of total tissue lipids and triglycerides, in the plasma of the starving juvenile Persian sturgeon, *Acipencer persicus* and concluded that triglycerides may be the preferred fuel over the other nutrients for mobilization, during fasting as it was shown in the present research .

In the present study, in both of the feeding regimes L- carnitine application significantly decreased plasma cholesterol and triglyceride. the above results suggest that L- carnitine supplementation in fish feeds increases the fatty acid oxidation through mitochondria and given its involvement in lipid metabolism in fish, dietary carnitine supplementation enhances protein synthesis and promote growth performance (Ozorio *et al.*, 2001; Nogueira *et al.*, 2011). Sang *et al*. (2012) reported that the diet with L- carnitine significantly reduced serum cholesterol and triglyceride of large yellow croaker similar to our results implying that different levels of carnitine had different effects on serum biochemical index.

In our study, cyclic fasting significantly affected plasma protein and the values were significantly lower than those in continuously fed fish and taken together this adds to the role of total protein as a fuel source in grey mullet (Cho, 2009). Several fish species accounting for significant decreases in plasma total protein concentrations have been observed, similar to Friedrich and Stepaniswska (2001) on *Cyprinus carpio* and Costa *et al*. (2011) on *Senegales sole*.

In this research, cyclic starvation alone affected liver glycogen concentration to a large extent. In the starved fish, its concentration was higher than in continuously fed fish. These results are in confirmation with previous researches which reported elevated liver glycogen levels having been subjected to 7 days fasting, which exceeded the control values by 2- to 5-fold (Mendez and Wieser, 1993; Bandeen and Leatherland, 1997). All of the above discussion show that stored carbohydrate in the liver as glycogen is found to be first substrate to

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be used during fasting and increased hepatic glycogen deposits after re-feeding in the fasted fish seems to be a factor for food energy storage while synthesising body components (Mendez and Wieser, 1993; Bandeen and Leatherland, 1997; Peterson and Small, 2004).

The results of the present research illustrated that under both the diet treatments, L- carnitine did not affect liver glycogen concentration so that the best of our knowledge it has not been studied in fish until now. As plasma glucose concentration of grey mullet in both the feeding regimes did not vary under L- carnitine supplementation, no cortisol stimulation of liver glycogenolysis and gluconeogenesis was expected in fish fed L-carnitine.(Mommsen *et al.*, 1999).

The effects of L- carnitine on immune parameters have received only limited attention in the present study, in both the feeding regimes, L- carnitine supplemented diet did not play any significant effect on plasma lysozyme concentration. Conversely, fasting for 7 days and re-feeding for 2 weeks resulted in a significant increase in lysozyme concentration implying that starvation as a stressor triggers an innate response. Such significant decrease of lysozyme activity in European eel (Caruso *et al.*, 2010) having been subjected to feed deprivation of 31 days and significant increase of serum lysozyme activity in juvenile Chinese sturgeon *A. sinensis* (Feng *et al.*, 2011) is similar to our results. Nonetheless Caruso *et al*. (2012) pointed out that this effect is temporary, as once the re-feeding period lasts for a longer time, all the values returned to those recorded during fasting. These contradictory results demonstrated that lysozyme activity may vary upon species, age, size, gender and environmental factors (Feng *et al.*, 2011; Caruso *et al.*, 2012).

In conclusion, cyclic fasting for 7 days once in 2 weeks in grey mullet is not sufficiently long to attain the same weight as unrestricted fed fish. To trigger compensatory growth mechanism, much more research is needed during short feed deprivation periods. L- carntine supplementation influenced growth, food utilization, crude lipid of carcass, plasma cholesterol, total protein and triglyceride. Cyclic fasting affected liver glycogen and plasma lysozyme levels alone. DGI and VFI was affected by interaction between carnitine supplementation and feeding regime in turn led to the recovery of growth rates of fish and optimal diet utilization.

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