



Effect of restricted feeding on the growth and body composition of European seabass *Dicentrarchus labrax* (Linnaeus, 1758)

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ABSTRACT

The aim of this study was to determine the effects of restricted feeding on the growth and body composition in juvenile European seabass *Dicentrarchus labrax*, (Linnaeus, 1758). Four different feeding regimes were tested in fishes having average weight of 6.57 ± 0.09 g. The three treatment groups were fed three times a day adopting three different regimes: 6 days feeding/1 day starvation (6F/1S), 5 days feeding/2 days starvation (5F/2S) and 4 days feeding/3 days starvation (4F/3S) for a period of 60 days. Control group (C) was fed daily three times a day. At the end of the study the average weights were 31.21 ± 1.39 g (C), 36.47 ± 1.33 g (6F/1S), 29.01 ± 1.01 g (5F/2S) and 24.21 ± 0.82 g (4F/3S) respectively ($p < 0.05$). SGR were 2.54 ± 0.17 (C), 2.64 ± 0.20 (6F/1S), 2.37 ± 0.06 (5F/2S) and 2.10 ± 0.09 (4F/3S) ($p < 0.05$) and FCR values recorded were 1.29 ± 0.05 (C), 1.12 ± 0.01 (6F/1S), 1.21 ± 0.01 (5F/2S) and 1.24 ± 0.01 (4F/3S). Crude protein and lipid values in restricted feeding groups were higher than those of the control group ($p < 0.05$) while SFA and MUFA values increased with starvation period while DHA, EPA, PUFA and Omega-3 values decreased with starvation period ($p < 0.05$). Results of the study clearly showed that restricted feeding had effect on the growth parameters, biochemical composition and fatty acid compositions in European seabass.

Keywords: Biochemical composition, *Dicentrarchus labrax*, European seabass, Fatty acid composition, Growth performance, Restricted feeding

Introduction

Growth and metabolic response to starvation and refeeding in various species were studied by several workers (Perez-Jimenez *et al.*, 2007; Eroldogan *et al.*, 2008). In their natural environment, fish are exposed to feed deprivation during certain periods (McCue, 2010). Fish farms adopt modifications in aquaculture practices as well as in feeding strategies and expose farmed fish to short or long term starvation periods during certain times of the year for obtaining better growth and feed conversion ratios. Fish can exhibit different adaptation strategies during starvation periods (Takagi, 2001; Guderley *et al.*, 2003). These adaptations vary with environmental conditions, fish species, starvation period and refeeding protocols (De Silva *et al.*, 1997; Jobling and Johansen, 1999; Simkins, 2002; Perez-Jimenez *et al.*, 2007; Peres *et al.*, 2011). Studies have shown that, following starvation period, feed intake and growth increased very rapidly in European seabass with the renormalisation of the living environment or start of refeeding (Pastoureaud, 1991; Perez-Jimenez *et al.*, 2007; Comoglio *et al.*, 2008). Seabass can withstand long-term starvation conditions, however water temperature plays an important role. Starvation affects metabolic activities and stored energy

reserves are used during this period which may result in weight loss.

The aim of the present study was to determine the effect of restricted feeding on the growth performance as well as on the biochemical and fatty acid compositions in juvenile seabass.

Materials and methods

The study was conducted at Sinop University Faculty of Fisheries, Aquatic Research Building (Sinop, Turkey). Fish having initial average weight of 6.57 ± 0.09 g were used and four different feeding regimes were adopted for a period 60 days. The three treatment groups were fed three times a day: 6 days feeding/1 day starvation (6F/1S), 5 days feeding/2 days starvation (5F/2S) and 4 days feeding/3 days starvation (4F/3S) respectively. Control group (C) was fed three times daily. The fish were fed with commercial feed having 48:16 protein/fat ratio. Twelve 100 l fiberglass tanks were used with 3 replications for each treatment.

Temperature, oxygen, pH and salinity of the rearing water were measured twice a day before feeding throughout the study using WTW multi-parameter device. The

average water temperature, oxygen level, pH and salinity values recorded were: 25.61±0.01°C, 6.41±0.13 mg l⁻¹, 7.21±0.06 and 18.30±0.01 ppt respectively.

During the experimental period, fishes were sampled from each group and growth parameters of the fish and biochemical compositions of fish meat were determined. Biometric data of the fish were collected and growth performance, viserosomatic index (VSI), hepatosomatic index (HSI), carcass yield (CY) and condition factor (CF) values were calculated (Skalli and Rabin, 2004; Hossu *et al.*, 2005; Cui *et al.*, 2006) using the following formulae:

$$\text{Specific growth rate (SGR) (\%)} = \frac{\text{Ln Final weight (g)} - \text{Ln Initial weight (g)}}{\text{Day}} \times 100$$

$$\text{Specific feed rate} = \text{SGR} \times \text{FCR}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Total amount of feed consumed (g)}}{\text{Total weight gain (g)}}$$

$$\text{DFC (Average daily feed consumption)} = \text{Feed consumption} / \text{days}$$

$$\text{Protein efficiency rate (PER) (\%)} = \frac{\text{Live weight gains (g)}}{\text{Protein intake (g)}} \times 100$$

$$\text{HSI (\%)} = \frac{\text{Liver weight (g)}}{\text{Total body weight (g)}} \times 100$$

$$\text{VSI (\%)} = \frac{\text{Vicera weight (g)}}{\text{Total body weight (g)}} \times 100$$

$$\text{Carcass yield (CY) (\%)} = \frac{\text{Edible fillet weight (g)}}{\text{Total body weight (g)}} \times 100$$

$$\text{Condition factor (CF)} = \frac{W}{L^3} \times 100$$

The fillet crude protein (%), crude lipid (%) and moisture (%) analysis were carried out according to Weende method, acid hydrolysis in Soxtec System and by drying method respectively following Association of Official Analytical Chemists (AOAC, 2000). The fillets were stored at -20°C until used for biochemical analyses. Fatty acid analysis was done by Gas chromatography (IUPAC, Firestone and Horwitz, 1979) at TUBITAK Marmara Research Center Food Institute. The fish were stored at -80°C until analysis.

The data obtained were tested with one way ANOVA using SPSS 21 software. The differences between the average values were compared using Tukey's multiple comparison tests at p<0.05 significance level.

Results and discussion

On termination of feeding, weight gain (36.47±1.33 g), specific growth rate (2.64±0.20) and FCR values of 6F/1S group were found better than those of other groups (Table 1, Fig. 1, 2). Starvation period was found to have an effect on weight gain and growth. The lowest weight gain and SGR values were recorded in 4F/3S group while the lowest FCR value was detected in control group (p<0.05). The differences between the groups in terms of PER values were not significant (p>0.05), while the lowest CY and CF values were determined in 4F/3S group (p<0.05). Wu *et al.* (2004) reported that growth performance and feed consumption values in 1-day starvation group and continuous feeding groups were similar while Turkmen *et al.* (2011) reported slowest growth in groups subjected to restricted feeding. Hu *et al.* (2001) observed a more rapid growth during feeding following the starvation

Table 1. Growth performance, whole body and fillet proximate composition of European seabass on termination of experimental feeding

Growth parameters	Treatment group			
	Control	6F/1S	5F/2S	4F/3S
Initial weight (g)	6.57±0.08	6.57±0.08	6.58±0.01	6.57±0.02
Final weight (g)	31.21±1.39 ^b	36.47±1.33 ^c	29.01±1.01 ^b	24.21±0.82 ^a
SGR (%)	2.54±0.17 ^c	2.64±0.20 ^c	2.37±0.06 ^b	2.10±0.09 ^a
FCR	1.29±0.05 ^b	1.12±0.01 ^a	1.21±0.01 ^b	1.24±0.01 ^b
DFC	6.78±0.16	7.13±0.23	8.02±0.35	8.03±0.59
PER	2.86±0.08 ^a	2.86±0.01 ^a	2.96±0.01 ^a	2.89±0.03 ^a
HSI	2.31±0.20 ^b	2.58±0.12 ^b	2.07±0.18 ^{ab}	1.70±0.12 ^a
VSI	7.56±0.40 ^a	8.36±0.57 ^a	8.65±0.45 ^a	8.79±0.33 ^a
CY	41.49±0.41 ^b	40.82±0.51 ^{ab}	38.97±0.30 ^{ab}	38.35±0.56 ^a
CF	1.23±0.02 ^b	1.28±0.02 ^b	1.17±0.02 ^{ab}	1.13±0.02 ^a
Proximate composition (Fillet)*				
Protein	19.29±0.38 ^a	20.32±0.08 ^{ab}	20.17±0.16 ^{ab}	20.71±0.07 ^b
Lipid	2.73±0.30 ^a	2.80±0.15 ^a	4.15±0.17 ^b	4.03±0.09 ^b
Dry mater	24.29±0.05 ^a	24.51±0.14 ^a	24.64±0.02 ^a	24.24±0.02 ^a
Ash	1.44±0.02 ^a	1.24±0.04 ^a	1.36±0.03 ^a	1.35±0.02 ^a

Values in the same row with different superscripts are significantly different (p<0.05).

*Biochemical analysis were performed on wet weight basis

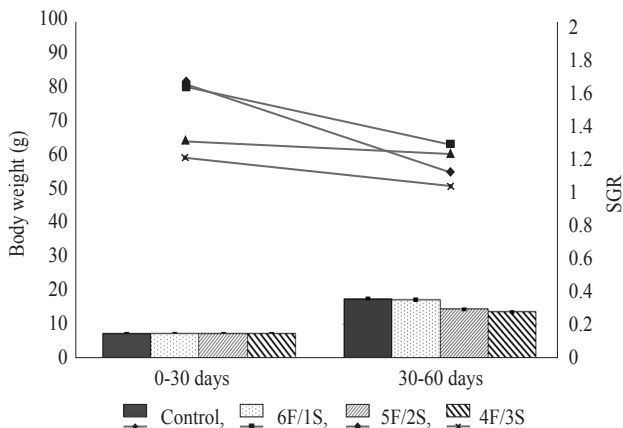


Fig. 1. Weight gain values and specific growth rates in European seabass during the experimental period

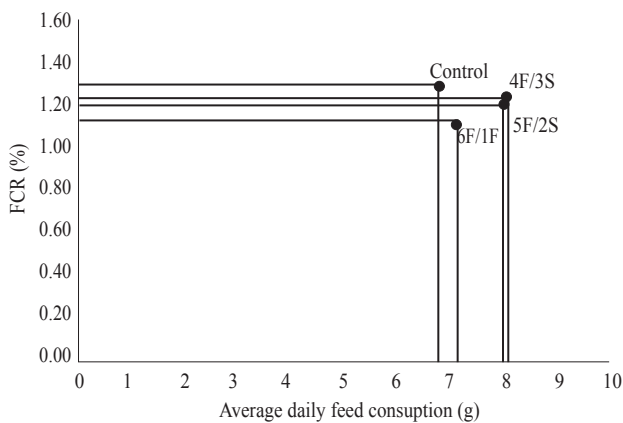


Fig. 2. Daily average feed consumptions and feed conversion ratios in European seabass during the experimental period

period. Studies have also reported reduction in the SGR during starvation period (Eroldogan *et al.*, 2006; Jobling, 2010).

In the present study, the highest feed consumption was recorded in 4F/3S group (8.03 ± 0.59 g) while the lowest value was observed in the control group (6.78 ± 0.16 g). Better FCR value were obtained in limited feeding groups compared to those of the control group and the highest value was found in 6F/1S group (1.12 ± 0.01). It has been reported that limited feeding methods increased the amount of feed given daily and decreased FCR (Wang *et al.*, 2000; Eroldogan *et al.*, 2006; Adaklı and Tasbozan, 2015).

VSI values of limited feeding groups were higher than that of the control group whereas HSI values were lower than that of the control group except in 6F/1S group. A linear relationship was observed between starvation period and VSI value. McCue (2010) observed that with starvation weight loss started initially in the digestive system. Starvation may lead to decrease in HSI values

(Echevarria *et al.*, 1997; Sevgili, 2007) and increase in VSI values (Eroldogan *et al.*, 2006).

Protein, fat, dry matter and ash values recorded at the beginning of the study were: 17.97 ± 0.29 , 1.27 ± 0.05 , 22.94 ± 0.24 and $1.52 \pm 0.06\%$ respectively. On termination of the study, crude protein and crude fat values in limited feeding groups were found higher than those of control group ($p < 0.05$), whereas there were no significant differences between the dry matter values ($p > 0.05$). It is a known fact that starvation leads to changes in biochemical composition of the body (Jobling, 2010). Some limited feeding studies have reported decreased protein and lipid values during restricted feeding (Peres *et al.*, 2011; Adaklı and Tasbozan, 2015; Gao *et al.*, 2015; Halder and Ali, 2015), whereas some have reported decreased protein and increased lipid values (Simkins, 2002; Wu *et al.*, 2004). It was found that, both low growth parameters and high lipid values especially in 5F/2S and 4F/3S groups which exhibited low growth rates indicate that a significant proportion of the protein sources taken *via* feed were used for energy needs during starvation periods. It was found that during limited feeding in juvenile fish which require high levels of protein and energy, significant proportion of the protein sources in feed were used for energy needs.

It was found that similar values were obtained for certain fatty acids (lauric acid, tridecanoic acid, pentadecanoic acid, arachidic acid, behenic acid, tricosanoic acid, lingoceric acid, myristoleic acid, eicosenoic acid, erucic acid, nervonic acid, linoleic acid, γ -linolenic acid, eicosadienoic acid, eicosatrienoic acid, cis-8.11.14-eicosatrienoic acid, arachidonic acid, docosadienoic acid) in limited feeding groups and control group. Palmitic acid, stearic acid, palmitoleic acid and oleic acid values were higher in limited feeding group, whereas heptadecanoic acid, α -linolenic acid, eicosadienoic acid and docosapentaenoic acid values were higher in control group ($p < 0.05$) (Table 2).

Myristic acid (C14:0) ($5.26\% \pm 0.03$), palmitic acid (C16:0) ($23.25\% \pm 0.05$), heptadecanoic acid (C17:0) ($0.59\% \pm 0$), stearic acid (C18:0) ($5.78\% \pm 0$) and docosapentaenoic acid (C22:5N3) ($0.89\% \pm 0.01$) values decreased in all groups at the end of the study. Differences between the control group and the limited feeding groups were significant ($p < 0.05$). Palmitoleic acid (C16:1) ($4.97\% \pm 0.02$), oleic acid (C18:1n9c) ($17.56\% \pm 0.01$) and α -linolenic acid (C18:3n3) ($0.94\% \pm 0$) values increased in all groups at the end of the study. The differences between the control group and the limited feeding groups were significant ($p < 0.05$). \sum SFA and \sum MUFA values were higher in limited feeding groups compared to the

Table 2. Fatty acid composition of fish meat (%) in different experimental groups of European seabass

Fatty acids	Day 0	Day 60			
		Control	6F/1S	5F/2S	4F/3S
C12:0 Lauric acid	0.07±0 ^a	0.05±0 ^a	0.05±0 ^a	0.05±0 ^a	0.05±0 ^a
C13:0 Tridecanoic acid	0	0.04±0 ^a	0.04±0.01 ^a	0.03±0 ^a	0.04±0.01 ^a
C14:0 Myristic Acid	5.26±0.03 ^c	4.46±0.01 ^b	4.31±0.01 ^a	4.31±0.01 ^a	4.26±0 ^a
C15:0 Pentadecanoic Acid	0.69±0.01 ^b	0.72±0.01 ^a	0.68±0 ^a	0.70±0 ^a	0.70±0.01 ^a
C16:0 Palmitik Acid	23.25±0.05 ^c	20.32±0.01 ^a	20.77±0.01 ^b	20.66±0.01 ^b	20.73±0 ^b
C17:0 Heptadecanoic Acid	0.59±0 ^b	0.57±0 ^c	0.54±0 ^a	0.55±0 ^a	0.55±0.01 ^a
C18:0 Stearic Acid	5.78±0 ^c	3.95±0 ^a	4.16±0 ^b	4.27±0.01 ^d	4.19±0.01 ^c
C20:0 Arachidic Acid	0.48±0.01 ^c	0.39±0 ^a	0.38±0 ^a	0.41±0.01 ^{ab}	0.42±0 ^b
C22:0 Behenic Acid	0.15±0.01 ^c	0.12±0 ^a	0.12±0 ^a	0.13±0 ^{ab}	0.14±0 ^{bc}
C23:0 Tricosanoic Acid	0	0.04±0.01 ^a	0.03±0 ^a	0.04±0 ^a	0.04±0 ^a
C24:0 Lingoceric Acid	0.14±0.01 ^c	0.09±0 ^a	0.09±0 ^a	0.10±0 ^{ab}	0.11±0 ^b
∑SFA	36.39±0.1 ^c	30.70±0.01 ^a	31.16±0.01 ^b	31.24±0.02 ^b	31.21±0.01 ^b
C14:1 Myristoleic Acid	0.04±0 ^a	0.08±0.01 ^b	0.08±0 ^b	0.07±0 ^b	0.07±0 ^b
C16:1 Palmitoleic acid	4.97±0.02 ^a	5.16±0 ^b	5.27±0 ^c	5.27±0.05 ^c	5.30±0 ^c
C18:1n9c Oleic Acid	17.56±0.01 ^a	21.46±0.01 ^b	22.41±0.01 ^c	21.63±0.01 ^c	21.93±0 ^d
C20:1n9c Eicosenoikasil	1.23±0.01 ^d	0.90±0 ^{ab}	0.88±0.01 ^a	0.91±0 ^{bc}	0.93±0.08 ^c
C22:1n9 Erucic Acid	0.21±0.01 ^b	0.14±0 ^a	0.14±0 ^a	0.15±0 ^a	0.16±0.01 ^a
C24:1 Nervonic acid	0.49±0.01 ^b	0.46±0 ^a	0.45±0 ^a	0.50±0.01 ^b	0.54±0.01 ^c
∑MUFA	24.48±0 ^a	28.20±0.01 ^b	29.22±0.01 ^c	28.52±0.01 ^c	28.92±0.01 ^d
C18:2n6c Linoleic Acid	8.18±0.01 ^c	7.07±0.01 ^b	6.89±0.01 ^a	7.06±0 ^b	7.06±0.01 ^b
C18:3n6 γ-Linolenic Acid	0.15±0.01 ^a	0.17±0 ^b	0.16±0 ^{ab}	0.17±0.01 ^b	0.17±0.01 ^b
C18:3n3α-Linolenic Acid	0.94±0 ^a	1.22±0 ^d	1.17±0.01 ^b	1.20±0 ^c	1.16±0.01 ^b
C20:2 Eicosadienoik acid	1.25±0.01 ^a	1.36±0 ^c	1.33±0 ^b	1.35±0 ^c	1.32±0 ^b
C20:3n3 EikosatrienoikAsit	0.08±0 ^a	0.11±0 ^a	0.10±0 ^a	0.11±0 ^a	0.11±0 ^a
C20:3n6 cis-8.11.14-Eikosatrienoik asit	0.09±0.01 ^a	0.09±0.01 ^a	0.08±0 ^a	0.09±0 ^a	0.09±0 ^a
C20:5n3 EPA	5.19±0.01 ^a	6.01±0.01 ^c	5.98±0.01 ^c	6.01±0.01 ^c	5.88±0.01 ^b
C 20:4n6 Araşidomik acid	0.83±0.02 ^c	0.57±0.01 ^b	0.54±0.01 ^b	0.57±0.01 ^b	0.48±0 ^a
C22: 6n3 DHA	12.31±0.09 ^a	14.49±0.01 ^c	13.77±0.01 ^b	13.98±0.01 ^b	13.84±0.03 ^b
C22:5n3 Dokosapentanoik acid	0.89±0.01 ^d	0.78±0 ^c	0.75±0 ^{ab}	0.77±0.01 ^{bc}	0.74±0.01 ^a
C22:2 Dokosadienoik acid	0.27±0 ^a	0.35±0 ^b	0.34±0 ^b	0.35±0.01 ^b	0.34±0 ^b
∑PUFA	30.16±0 ^a	32.20±0.01 ^d	31.09±0.01 ^b	31.63±0.03 ^c	31.16±0.01 ^b
Omega 3	19.41±0.09 ^a	22.60±0.01 ^d	21.76±0.01 ^b	22.06±0.02 ^c	21.71±0.01 ^b
Omega 6	9.23±0.02 ^d	7.89±0 ^c	7.66±0 ^a	7.88±0.01 ^c	7.79±0.01 ^b
Omega3/omega6	2.10±0.01 ^a	2.86±0.01 ^c	2.84±0.01 ^c	2.80±0.02 ^b	2.80±0.03 ^b
Omega 9	18.99±0.01 ^a	22.50±0.01 ^b	23.42±0.01 ^c	22.69±0.01 ^c	23.02±0.03 ^d

∑SFA: Total saturated fatty acid, ∑MUFA: Total mono unsaturated fatty acid, ∑PUFA: Total poly unsaturated fatty acid
 Values in the same row with different superscripts are significantly different (p<0.05).

control group (p<0.05). ∑PUFA value was higher in the control group compared to those in limited feeding groups (p<0.05).

Studies have shown that starvation period has effect on the fatty acid compositions of the fish (Akpınar and Aksoylar, 1988; De Silva *et al.*, 1997). In various studies, changes have been observed in ∑SFA, ∑MUFA and ∑PUFA values during starvation periods and this was found associated especially with fish species, energy metabolism and ambient conditions and it was additionally reported that the intensity of fish feed deprivation is also an important factor (Jezierska *et al.*, 1982; Ringo *et al.*, 1990; De Silva *et al.*, 1997; Enien *et al.*, 1998; Baki

et al., 2013). ∑SFA and ∑MUFA values increased in limited feeding groups whereas ∑PUFA value decreased (Fig. 3).

It was found that omega-3 fatty acid which is composed of the essential fatty acid alpha-linolenic acid increased in all groups during the study. Omega-3 value of the control group was higher than those of the limited feeding groups (p<0.05). Unlike the omega-3 fatty acids, omega-6 fatty acid which is composed of linoleic acid decreased in all groups at the end of the study. The difference between the control group and 5F/2S groups in terms of omega-6 fatty acid was not significant (p>0.05), however the differences between other groups were significant (p<0.05). Omega-3/omega-6 ratio which

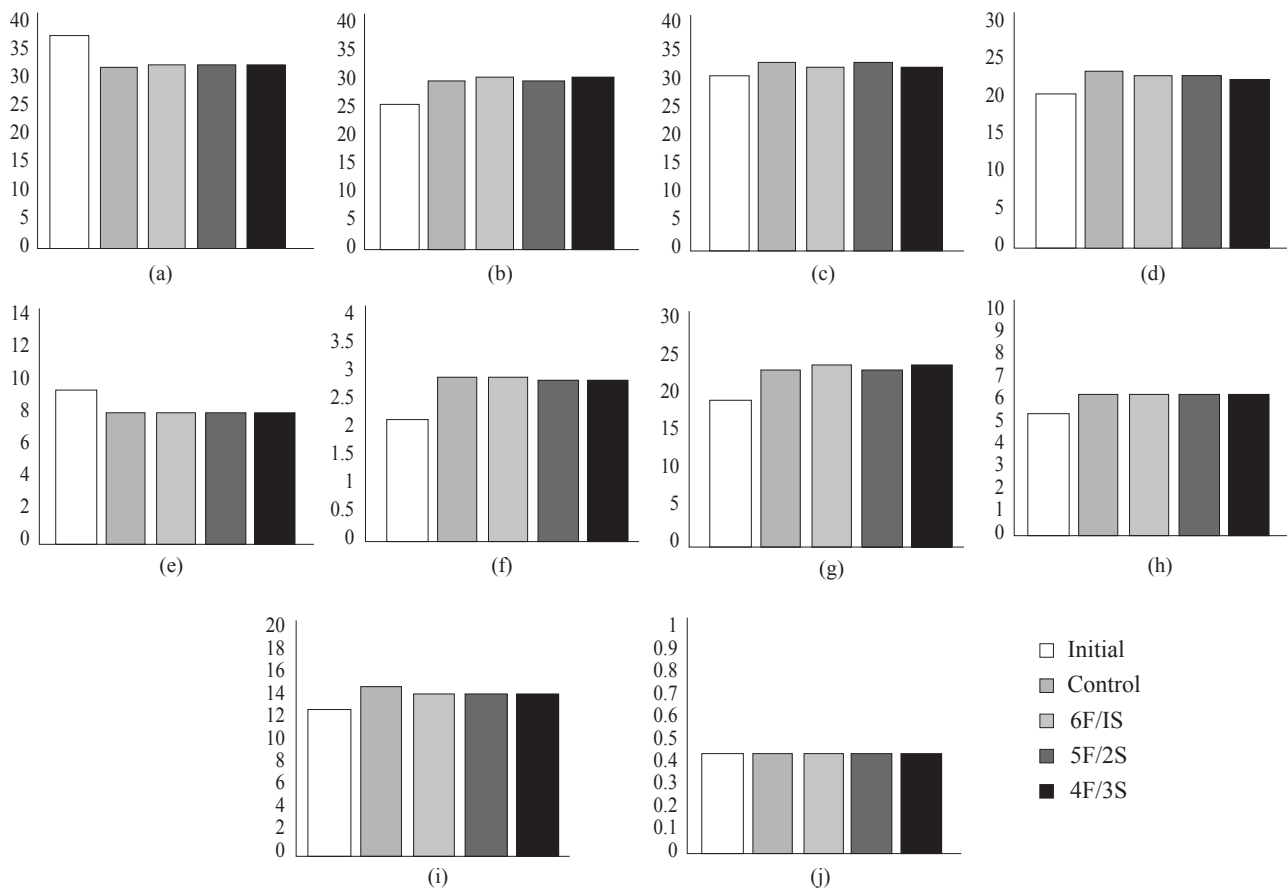


Fig. 3. Fatty acid levels in different experimental groups of European seabass. (a): Σ SFA, (b): Σ MUFA, (c): Σ PUFA, (d): Omega-3, (e): Omega-6, (f): Omega-9, (g): Omega-3/Omega-6, (h): EPA, (i): DHA, (j): EPA/DHA

indicates an important quality parameter of the fish meat was $2.10 \pm 0.01\%$ and it increased in all groups at the end of the experimental feeding. Similar values were obtained for limited feeding groups as well as for control group. Omega-9 value was $18.99 \pm 0.01\%$ at the beginning of the study and it increased in all groups at the end of the study and the differences between the groups were significant ($p < 0.05$).

EPA value was $5.19 \pm 0.01\%$ initially which increased in all groups at the end of the study and the lowest value was recorded in 4F/3S group ($p < 0.05$). DHA value was $12.31 \pm 0.09\%$ at the beginning of the study which increased in all groups on termination of the study. DHA value in the control group was higher compared to those of limited feeding groups ($p < 0.05$).

Results of the correlation analysis performed between starvation periods and SFA, MUFA, PUFA, EPA, DHA, omega-3, omega-6 and omega-3/omega-6 ratio, showed that SFA and MUFA values increased with starvation period whereas DHA, PUFA, EPA, omega-3 and omega-3/omega-6 values decreased.

There was no correlation between starvation period and PUFA and omega-3 values ($r = 0.11$; $r = 0.13$) whereas there was a strong correlation between omega-3/omega-6 and starvation period ($r = 0.94$). De Silva *et al.* (1997) reported that omega-3/omega-6 ratio increased during starvation period with DHA values in direct proportion to starvation period. The cells increased DHA levels in order to protect themselves as DHA is a main component of biological membranes (Tidwell *et al.*, 1992).

The advantages of limited feeding in fish have been reported as the increase in growth and feed conversion ratio (Zhu *et al.*, 2001; 2004). It was reported that fish exhibited a more rapid growth following starvation period and limited feeding protocols can be adopted in commercial aquaculture for achieving best growth values and feed conversion ratios (Zhu *et al.*, 2001; Eroldogan *et al.*, 2006; Gao *et al.*, 2015).

In the present study, the effects of limited feeding on the growth, biochemical and fatty acid compositions in juvenile seabass were investigated. At the end of the

study, it was observed that 1 day starvation per week had a positive effect on the growth parameters in juvenile seabass had no negative effects on the biochemical composition and had a negative effect on fatty acid composition. However, it was detected that two days and longer starvation periods per week, had more negative impact on the growth, biochemical and fatty acid compositions in juvenile seabass. Therefore, it was concluded that limited feeding applications in aquaculture should be carried out during periods when the growth is slower and the protein/energy requirements are lower rather than during juvenile periods when the fish grow rapidly which require high protein/energy.

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