

# MARINE SCIENCE AND TECHNOLOGY BULLETIN

## Effect of diet on the fatty acids composition of cultured sea bass (*Dicentrarchus labrax*) liver tissues and histology compared with wild sea bass caught in Eagean Sea.

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### ABSTRACT

In the present study, the effect of diet on the fatty acids composition of cultured European sea bass liver tissues and histology when compared with wild sea bass caught in Eagean Sea was determined. In the study, the fish were fed with commercial feed for 90 days. In the liver tissue samples the amount of total lipid was higher in farmed than in wild fish. Henicosanoic fatty acid was found in commercial feed but not in fish liver. Fatty acid profiles in the livers reflected the fatty acid profiles of the commercial feeds. The dominant fatty acids in livers of cultured and wild sea bass were 16:0, 18:1n9, 18:2n6, C20:5n3 and 22:6n3. The results showed that cultured fish contained a higher level of EPA/DHA ratio and oleic acids, monounsaturated fatty acids, n-6 polyunsaturated fatty acids, whereas wild fish contained a higher level of saturated fatty acids, eicosapentaenoic acids, n-3 polyunsaturated fatty acids and n-3/n-6 ratio ( $P < 0.05$ ). Commercial feed had effect on hepatic lipid droplets accumulation degree and pattern of vacuolization in the observed liver sections. The marked hepatic cell membrane degeneration and haemorrhagy on the liver observed in cultured sea bass was caused by the accumulation of very large lipid droplets. The hepatocyte nucleus size of fish was higher in farmed than in wild fish liver tissue ( $P < 0.05$ ). Hepatocytes enlarged irregular nucleus located at the periphery of the cells. In conclusion, commercial feed had effect on farmed fish liver histomorphology. However, proper choice of dietary lipid would allow the fatty acid composition of cultured fish to be tailored to address the beneficial health aspects and consumer's demands.

### Introduction

Nowadays, aquaculture has become an important industry in Turkey and mainly cultured marine finfish species are sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*). The most important fish species is sea bass with 50796 tons production value (TUIK, 2010). Many fish producers prefer the species because of high market demand and easy culture practices.

In intensive fish culture, the diet is the most important point with its huge expense. Furthermore, the most

important ingredients in fish feed are fish oil and fish meal, in which both of them are very valuable. Increased energy contents in fish feeds and the inclusion of vegetable protein or lipid sources in substitution of fish meal and oil in fish diets (Caballero et al., 1999 and Caballero et al., 2004) lead to an enhanced perivisceral, hepatic and subcutaneous fat deposition which may affect product quality and restrains optimization of fish production concerning fish farmers (Izquierdo et al., 2005). The composition of commercial feed used for cultured fish also influences the total lipid composition of the fish. Different variations have been observed in the reported values of fatty acid concentrations in the same species of fish depending on the diet formulation (Mnari et al. 2007; Seno et al., 2008).

Fish lipids are well known to be rich in long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFA), especially

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Table 1. Average weight and length of cultured and caught wild sea bass (average  $\pm$  SEM)

Time	Weight (gr)		Length (cm)	
	Caught*	Culture**	Caught*	Culture**
Initial	52.3 $\pm$ 0.12	54.4 $\pm$ 0.15	10.3 $\pm$ 0.04	10.6 $\pm$ 0.02
45th Day	102.6 $\pm$ 0.08	87.1 $\pm$ 0.04	13.1 $\pm$ 0.01	14.0 $\pm$ 0.02
Final	165.5 $\pm$ 0.12	151.4 $\pm$ 0.15	18.2 $\pm$ 0.02	17.7 $\pm$ 0.02

\* n = 5x3, \*\*n = 25x3.

eicosapentaenoic acid (EPA or 20:5n-3) and docosahexaenoic acid (DHA or 22:6n-3) (Alasalvar et al., 2002). Fatty acids play a vital role in fish nutrition, disease prevention and health promotion (Horrocks and Yeo, 1999; Ulbricht and Southgate, 1991) and also growth promoting (Mourete and Bell 2006). The marine fish have a high dietary requirement for n-3 highly unsaturated fatty acids (HUFA), reflecting the natural abundance of these nutrients in their cellular and depot lipids and in their natural prey in the marine environment (Sargent and Tacon, 1999). Marine fish are traditionally fed high lipid diets with ingredients of marine origin containing high levels of n-3 fatty acids, particularly n-3 HUFA such as EPA and DHA.

The aim of the study was to determine the effect of diet on the fatty acids composition of cultured European sea bass liver tissues and histology when compared with wild trapped sea bass caught in Aegean Sea.

## Material and methods

### Animals and Experimental Design

In the trial, sea bass was obtained from Gelidonya Su Urunleri Limited Company in Turkey in Bodrum. 2000 fish average weight of 54.4  $\pm$  0.15 g were divided into three circular (250 m<sup>3</sup> for each cage) fish cages for the application of commercial diet. The basal diet obtained from commercial company was fed to the fish by % 2 of the body weight for 90 days. Changes on fatty acid composition of sea bass liver tissue and histology were monitored on every 45 days of the experiment. The wild fish were used in the experiment was caught in the Aegean Sea. The fish used in the study were anaesthetized by 0.1 gr l<sup>-1</sup> MS-222 (Mannerstrom et al., 2001). Liver tissue samples were fixed in immunofix solution and kept in + 4 °C till analysis. Weight and length of cultured and the wild sea bass were given in the Table 1.

### Chemical Analysis

The chemical composition of diet and liver tissues such as dry matter, crude protein, crude ash and total lipid were analyzed in Canakkale Onsekiz Mart University Feed and Nutrient Laboratory according to AOAC (2000). Determination of fatty acid composition of lipids from the diet and sea bass liver tissues were carried out with a mixture of chloroform and methanol (2:1, v/v; (Folch et al., 1957)) containing 0.01% BHT. The lipid extract was dried out overnight under vacuum following the subtraction of solvent by evaporation under a stream of nitrogen.

After being weighed, the lipid extract was redissolved at a known concentration in chloroform/methanol (2:1, v/v) containing 0.1 g kg<sup>-1</sup> BHT and stored at -20 °C prior to analysis. Fatty acid methyl esters (FAME) were prepared according to the procedure of Miyashita et al. (1999) and were purified with the Seppak ® cartridge (Miyashita et al., 1999). Analytical conditions for analysis of FAME by gas liquid chromatography (GC-17A, Shimadzu Corp., Kyoto, Japan) are described in Furuita et al. (2003).

### Liver Collection and Histological Analyses

Seabass were euthanised by using 1 gr l<sup>-1</sup> MS-222. Liver tissues were taken from fish and stored in the burette that contain of immunofix solution. Cutting of the tissue were accomplished in Celal Bayar University Medical Faculty. Liver tissues were fixed in Phosphate-Salt Buffer solution in 3 minutes and this procedure was repeated twice. The tissue was frozen in Leica CM-1100 model frozen microtome and histological sections were cut at 6  $\mu$ , stained with haematoxylin-eosin and mounted permanently for microscopic analysis. All of the slides were examined 'blind' (to eliminate bias in interpretation) for abnormalities, such as lipid infiltration, degeneration and/or necrosis of hepatocytes. Pictures were taken in Olympus BX 51 microscope with Micro Publisher 3.3 RTV photographer. Area and perimeter of hepatocytes and nuclei were made by Capture Pro 5.1.

### Data Processing and Statistical Analysis

The specific growth rate (SGR), and condition factor (CF) were calculated as follows:

Specific Growth Rate (SGR) (% day<sup>-1</sup>) = 100 x [(ln final fish weight) - (ln initial fish weight)] / days fed.

Condition Factor (CF): Fish weight/ (Total fish length)<sup>3</sup> x 100

All variables were tested by one-way ANOVA and differences between means were determined by Tukey's multiple range test, and were reported to be significant if P<0.05. All statistical analyses were performed using Statgraphics Centurion XV program.

## Results

In the study, cultured seabass weight gain was 97 g, respectively. Growth in terms of specific growth rate (SGR)

Table 2. Proximate analysis and fatty acid composition of the commercial diet.

Component	Diet
Crude Protein	46.5 ± 0.03
Total Lipid	12.1 ± 0.01
Moisture	8.5 ± 0.01
Crude Ash	6.7 ± 0.04
<b>Fatty Acids</b>	
C14:0	4.04 ± 0.1
C15:0	0.53 ± 0.1
C16:0	20.48 ± 0.1
C17:0	0.57 ± 0.1
C18:0	5.60 ± 0.1
C20:0	0.63 ± 0.1
C21:0	0.04 ± 0.1
C22:0	0.25 ± 0.1
C23:0	0.01 ± 0.1
C24:0	0.13 ± 0.1
C14:1	0.05 ± 0.1
C15:1	0.08 ± 0.1
C16:1	4.98 ± 0.1
C17:1	0.02 ± 0.1
C18:1n7	2.10 ± 0.2
C18:1n9	20.71 ± 0.1
C20:1n9	0.93 ± 0.1
C22:1n9	0.04 ± 0.1
C20:2	0.21 ± 0.1
C22:2	4.69 ± 0.1
C18:2n6	13.79 ± 0.1
C18:3n6	0.28 ± 0.1
C20:3n6	0.20 ± 0.1
C20:4n6	0.05 ± 0.1
C18:3n3	1.37 ± 0.1
C18:4n3	0.86 ± 0.1
C20:3n3	0.99 ± 0.1
C20:5n3	6.89 ± 0.1
C22:5n3	0.89 ± 0.1
C22:6n3	8.70 ± 0.1
Total SFA	32.24 ± 0.1
Total MUFA	28.88 ± 0.1
Total n-6 PUFA	14.03 ± 0.1
Total n-3 PUFA	19.69 ± 0.2
Total n-3 HUFA	17.46 ± 0.2
Σn-3/Σn-6	1.40 ± 0.1
AA /EPA	0.02 ± 0.1
EPA/DHA	0.79 ± 0.1

Data are expressed as mean±SD (n=3). SFA; Saturated Fatty Acids, MUFA; monounsaturated fatty acids, PUFA; polyunsaturated fatty acids, HUFA; 20-22 Highly Unsaturated Fatty Acids, AA; arachidonic acid (20:4n-6), EPA; eicosapentaenoic acid (20:5n-3), DHA; docosahexaenoic acid (22:6n-3).

and condition factor (CF) of the experimental fish are shown in Table 3. There were no statistically significant differences in CF between the cultured and wild sea bass at any sampling time ( $p > 0.05$ ). However, SGR was significantly higher ( $p > 0.05$ ) in the first 45<sup>th</sup> day of the experiment than the 90<sup>th</sup> day. Crude protein and lipid levels

were higher in the cultured sea bass ( $p < 0.05$ ). Similar results were observed in the last period of the experiment. Proximate analysis is given in Table 4.

Differences were not observed on the fatty acid composition of cultured sea bass in any sampling time of the study ( $p > 0.05$ ). Hencicosanoic acid that identified in the diet was not detected in the liver of the cultured sea bass. C16:0, C18:1n9 and C22:6n3 was higher in the cultured fish than wild caught sea bass ( $p < 0.05$ ), but significant differences were not observed on C18:2n6 level between the fishes ( $p > 0.05$ ). SFA, MUFA, n-6 PUFA, n-3 PUFA, n-3 HUFA, total n-3/n-6 ratio and EPA/DHA ratio were estimated significantly different ( $p < 0.05$ ) between all groups with the exception of AA/EPA ratio's ( $p > 0.05$ ). Fatty acid composition of the cultured sea bass and wild sea bass at 45<sup>th</sup> and 90<sup>th</sup> day of the experiment is given in Table 5.

The aspect of significant accumulation of fat, increasing in the number of vacuoles and deterioration of membrane structures in the cultured fishes were also included in the study (Fig. 1, 2). Total amount of fat in cultured sea bass liver was found higher than the wild sea bass's ( $p < 0.05$ ) and it is pointed to an apparent accumulation of fat. The histology and fatty acid levels of liver tissues were affected by fatty acid composition of the diet. Moreover, the hepatocytes nuclei were migrated to the cell periphery moved to the cap (Fig. 3, 4, 5, 6). Accordingly, the hypertrophy was observed in hepatocytes showed an increase in the diameter of nuclei of hepatocytes ( $p < 0.05$ ) (Table 6)

## Discussion

In this study, the liver fatty acid compositions and some histomorphological parameters of wild sea bass caught in the Aegean Sea in Turkey and cultured sea bass were determined by comparing the effect of commercial feed. Fatty acid composition and fat accumulation in the liver of the fish was affected by fatty acid composition and total fat content of the diet, causing degeneration in the liver. According to Spisni et al. (1998), sea bream fed with diet that had similar content has shown fat deposition on the liver. Mnari et al. (2007) suggested that cultured sea bream have shown high lipid level in liver than wild sea bream. Our study was supported by these results. According to the study, liver enlargement due to diet should be mentioned as well. Many studies have shown the effectiveness of the diet on the liver (Figueiredo et al., 2005; Mourente et al., 2005; Schulz et al., 2005; Skalli et al., 2006; Mourente et al., 2007; Valente et al., 2007).

In the study condition factor was determined very high not only in cultured sea bass but also in wild sea bass.

Table 3. Specific growth (SGR) rate and condition factor (CF) of the experimental fishes

	Initial		45th Day		90th Day	
	Culture	Wild	Culture	Wild	Culture	Wild
SGR			1.62 <sup>a</sup> ± 0.2		0.93 <sup>b</sup> ± 0.1	
CF	4.56 <sup>a</sup> ± 0.23	4.78 <sup>a</sup> ± 0.52	3.63 <sup>b</sup> ± 0.31	3.48 <sup>b</sup> ± 0.12	2.73 <sup>c</sup> ± 0.2	2.74 <sup>c</sup> ± 0.14

Values are provided as mean ± standard error (n=15). Values with different letters are significantly different ( $p = 0.05$ ) within the daily groups.

Table 4. Proximate analyses of the culture and wild caught seabass liver.

	Proximate (%)	Cultured	Wild
45 <sup>th</sup> Day	Crude Protein	15.7 ± 0.01 <sup>a</sup>	14.9 ± 0.03 <sup>b</sup>
	Crude Lipid	11.9 ± 0.01 <sup>a</sup>	6.7 ± 0.01 <sup>b</sup>
	Moisture	70.00 ± 0.15 <sup>b</sup>	75.6 ± 0.15 <sup>a</sup>
	Crude Ash	1.5 ± 0.10 <sup>a</sup>	1.5 ± 0.10 <sup>a</sup>
90 <sup>th</sup> Day	Crude Protein	14.1 ± 0.02 <sup>a</sup>	13.5 ± 0.01 <sup>b</sup>
	Crude Lipid	10.3 ± 0.03 <sup>a</sup>	5.5 ± 0.08 <sup>b</sup>
	Moisture	74.00 ± 0.22 <sup>b</sup>	76.00 ± 0.05 <sup>a</sup>
	Crude Ash	1.5 ± 0.10 <sup>a</sup>	1.5 ± 0.10 <sup>a</sup>

Values are provided as mean ± standard error (n=30). Values with different letters are significantly different (p = 0.05) within the daily periods.

Table 5. Fatty acid composition of the cultured sea bass and wild sea bass on 45<sup>th</sup> and 90<sup>th</sup> day of the experiment.

	45th Day		90th Day	
	Culture	Wild	Culture	Wild
Total Lipid	11.9 ± 0.01 <sup>a</sup>	6.7 ± 0.01 <sup>b</sup>	10.3 ± 0.03 <sup>a</sup>	5.5 ± 0.08 <sup>b</sup>
Fatty Acids				
C14:0	1.63 ± 0.1 <sup>a</sup>	1.62 ± 0.1 <sup>a</sup>	1.59 ± 0.1 <sup>a</sup>	1.66 ± 0.1 <sup>a</sup>
C15:0	0.24 ± 0.1 <sup>a</sup>	0.20 ± 0.1 <sup>b</sup>	0.21 ± 0.1 <sup>a</sup>	0.25 ± 0.1 <sup>a</sup>
C16:0	16.68 ± 0.1 <sup>b</sup>	24.72 ± 0.1 <sup>a</sup>	18.06 ± 0.1 <sup>b</sup>	22.16 ± 0.2 <sup>a</sup>
C17:0	0.35 ± 0.1 <sup>b</sup>	0.37 ± 0.1 <sup>b</sup>	0.29 ± 0.1 <sup>b</sup>	0.41 ± 0.1 <sup>a</sup>
C18:0	3.80 ± 0.1 <sup>a</sup>	7.44 ± 0.1 <sup>a</sup>	4.32 ± 0.1 <sup>b</sup>	5.82 ± 0.1 <sup>a</sup>
C20:0	0.11 ± 0.1 <sup>a</sup>	0.14 ± 0.1 <sup>a</sup>	0.12 ± 0.1 <sup>a</sup>	0.14 ± 0.1 <sup>a</sup>
C22:0	0.07 ± 0.1 <sup>b</sup>	0.08 ± 0.1 <sup>b</sup>	0.05 ± 0.1 <sup>b</sup>	0.09 ± 0.1 <sup>a</sup>
C23:0	0.21 ± 0.1 <sup>a</sup>	0.29 ± 0.1 <sup>a</sup>	0.12 ± 0.1 <sup>b</sup>	0.29 ± 0.1 <sup>a</sup>
C24:0	0.08 ± 0.1 <sup>a</sup>	0.10 ± 0.1 <sup>a</sup>	0.09 ± 0.1 <sup>a</sup>	0.10 ± 0.1 <sup>a</sup>
C14:1	0.04 ± 0.1 <sup>b</sup>	0.07 ± 0.1 <sup>a</sup>	0.05 ± 0.1 <sup>b</sup>	0.09 ± 0.1 <sup>a</sup>
C15:1	0.07 ± 0.1 <sup>b</sup>	0.07 ± 0.1 <sup>b</sup>	0.05 ± 0.1 <sup>b</sup>	0.09 ± 0.1 <sup>a</sup>
C16:1	4.22 ± 0.1 <sup>b</sup>	4.39 ± 0.1 <sup>b</sup>	4.38 ± 0.1 <sup>b</sup>	6.14 ± 0.1 <sup>a</sup>
C17:1	0.01 ± 0.1 <sup>b</sup>	0.44 ± 0.1 <sup>a</sup>	0.01 ± 0.1 <sup>b</sup>	0.47 ± 0.1 <sup>a</sup>
C18:1n7	3.42 ± 0.1 <sup>a</sup>	3.35 ± 0.1 <sup>a</sup>	3.50 ± 0.1 <sup>a</sup>	3.66 ± 0.1 <sup>a</sup>
C18:1n9	34.73 ± 0.1 <sup>a</sup>	30.25 ± 0.2 <sup>b</sup>	33.35 ± 0.1 <sup>a</sup>	25.45 ± 0.2 <sup>b</sup>
C20:1n9	1.45 ± 0.1 <sup>a</sup>	0.99 ± 0.1 <sup>a</sup>	1.36 ± 0.1 <sup>a</sup>	1.16 ± 0.1 <sup>b</sup>
C22:1n9	0.03 ± 0.1 <sup>a</sup>	0.04 ± 0.1 <sup>a</sup>	0.04 ± 0.1 <sup>a</sup>	0.05 ± 0.1 <sup>a</sup>
C20:2	0.74 ± 0.1 <sup>a</sup>	0.29 ± 0.1 <sup>b</sup>	0.66 ± 0.1 <sup>a</sup>	0.39 ± 0.1 <sup>b</sup>
C22:2	1.59 ± 0.1 <sup>b</sup>	1.97 ± 0.1 <sup>a</sup>	0.87 ± 0.1 <sup>b</sup>	1.70 ± 0.1 <sup>a</sup>
C18:2n6	15.05 ± 0.1 <sup>a</sup>	14.88 ± 0.1 <sup>a</sup>	13.63 ± 0.1 <sup>a</sup>	13.35 ± 0.1 <sup>a</sup>
C18:3n6	0.37 ± 0.1 <sup>a</sup>	0.03 ± 0.1 <sup>b</sup>	0.45 ± 0.1 <sup>a</sup>	0.02 ± 0.1 <sup>b</sup>
C20:3n6	0.11 ± 0.1 <sup>b</sup>	0.07 ± 0.1 <sup>c</sup>	0.11 ± 0.1 <sup>b</sup>	0.12 ± 0.1 <sup>a</sup>
C20:4n6	0.08 ± 0.1 <sup>a</sup>	0.07 ± 0.1 <sup>a</sup>	0.07 ± 0.1 <sup>a</sup>	0.07 ± 0.1 <sup>a</sup>
C18:3n3	1.48 ± 0.1 <sup>a</sup>	0.50 ± 0.1 <sup>b</sup>	1.32 ± 0.1 <sup>a</sup>	0.66 ± 0.1 <sup>b</sup>
C18:4n3	0.42 ± 0.1 <sup>a</sup>	0.43 ± 0.1 <sup>a</sup>	0.39 ± 0.1 <sup>a</sup>	0.47 ± 0.1 <sup>a</sup>
C20:3n3	0.93 ± 0.1 <sup>b</sup>	1.39 ± 0.3 <sup>b</sup>	1.23 ± 0.1 <sup>b</sup>	2.42 ± 0.1 <sup>a</sup>
C20:5n3	3.77 ± 0.1 <sup>b</sup>	4.29 ± 0.1 <sup>a</sup>	3.95 ± 0.1 <sup>b</sup>	5.09 ± 0.1 <sup>a</sup>
C22:5n3	0.80 ± 0.1 <sup>a</sup>	0.70 ± 0.1 <sup>b</sup>	0.76 ± 0.1 <sup>a</sup>	0.82 ± 0.1 <sup>a</sup>
C22:6n3	7.48 ± 0.1 <sup>b</sup>	13.26 ± 0.1 <sup>a</sup>	8.95 ± 0.1 <sup>b</sup>	15.68 ± 0.2 <sup>a</sup>
ΣSFA	23.18 ± 0.1 <sup>b</sup>	34.96 ± 0.1 <sup>a</sup>	24.86 ± 0.1 <sup>b</sup>	30.90 ± 0.2 <sup>a</sup>
ΣMUFA	43.97 ± 0.1 <sup>a</sup>	39.24 ± 0.5 <sup>b</sup>	42.75 ± 0.1 <sup>a</sup>	37.10 ± 0.1 <sup>b</sup>
Σn-6 PUFA	15.61 ± 0.1 <sup>a</sup>	15.02 ± 0.1 <sup>b</sup>	14.26 ± 0.1 <sup>a</sup>	13.56 ± 0.1 <sup>b</sup>
Σn-3 PUFA	14.89 ± 0.1 <sup>b</sup>	20.12 ± 0.4 <sup>a</sup>	16.60 ± 0.1 <sup>b</sup>	25.13 ± 0.1 <sup>a</sup>
Σn-3 HUFA	12.99 ± 0.1 <sup>b</sup>	19.19 ± 0.4 <sup>a</sup>	14.89 ± 0.1 <sup>b</sup>	24.00 ± 0.2 <sup>a</sup>
Σn-3/Σn-6	0.95 ± 0.1 <sup>b</sup>	1.34 ± 0.1 <sup>a</sup>	1.16 ± 0.1 <sup>b</sup>	1.85 ± 0.1 <sup>a</sup>
AA /EPA	0.02 ± 0.1 <sup>a</sup>	0.02 ± 0.1 <sup>a</sup>	0.02 ± 0.1 <sup>a</sup>	0.01 ± 0.1 <sup>a</sup>
EPA/DHA	0.50 ± 0.1 <sup>a</sup>	0.32 ± 0.1 <sup>b</sup>	0.44 ± 0.1 <sup>a</sup>	0.33 ± 0.1 <sup>b</sup>

Values are provided as mean ± standard error (n=10). Values with different letters are significantly different (α = 0.05) within the daily periods. SFA; Saturated Fatty Acids, MUFA; monounsaturated fatty acids, PUFA; polyunsaturated fatty acids, HUFA; 20:22 Highly Unsaturated Fatty Acids, AA; arachidonic acid (20:4n-6), EPA; eicosapentaenoic acid (20:5n-3), DHA; docosahexaenoic acid (22:6n-3).

Table 6. Diameter of nuclei of hepatocytes (µm).

	Culture	Wild
Initial	4.96 ± 0.1 <sup>a</sup>	4.10 ± 0.2 <sup>b</sup>
45 <sup>th</sup> Day	4.67 ± 0.1 <sup>a</sup>	3.73 ± 0.1 <sup>b</sup>
90 <sup>th</sup> Day	5.00 ± 0.2 <sup>a</sup>	4.65 ± 0.2 <sup>a</sup>

Values are provided as mean ± standard error. Values with different letters are significantly different (p = 0.05) within the daily periods.

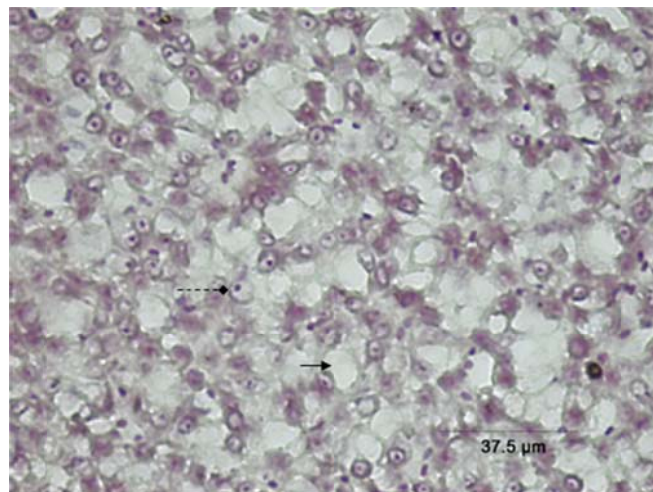


Figure 1. Pathological finding in cultured sea bass at the beginning of the experiment.

Hypertrophy in hepatocyte —◆, Lipid deposition—→

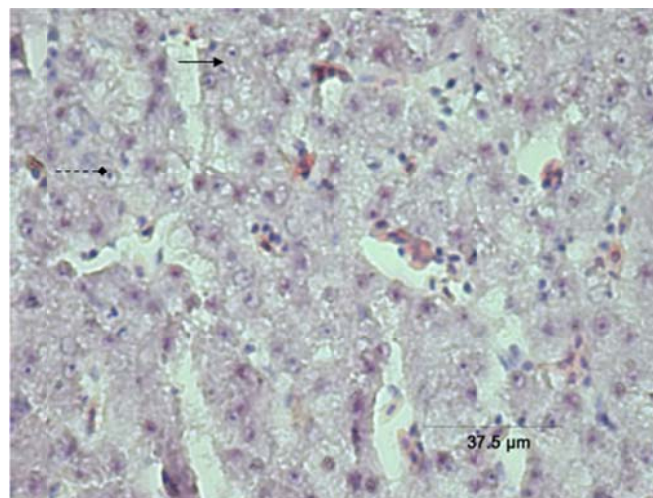


Figure 2. Wild sea bass liver at the beginning.

Nuclei ----◆, Hepatocyte —→

Mourete and Bell (2006), Izquierdo et al. (2003) and Martinez and Vasquez's (2001) findings are drastically lower than this study. The type and amount of fat used in the diet are important factors affecting the condition factor.

Oleic acid was found higher on the cultured sea bass and similar results were observed on seabream (Grigorakis et al., 2002). The high proportion of oleic acid in the liver can be caused by diet (Alasalvar et al., 2002). Linolenic acid (LA, 18:2n-6) and arachidonic acid (AA, 20:4n-6) are important n-6 fatty acids in fishes. Although different results were observed in the amount of LA in the study compared with Grigorakis et al. (2002), results were similar



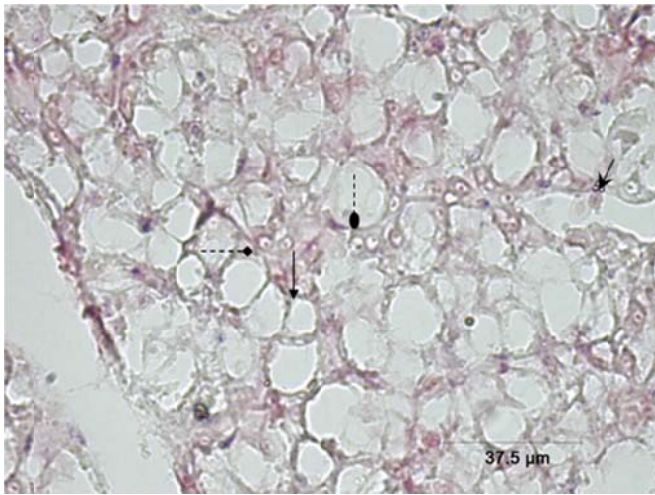


Figure 3. Pathological finding in cultured sea bass at the 45<sup>th</sup> day of the experiment.

Enlarged irregular nuclei located at the periphery of the cell -----♦, Hypertrophy in hepatocyte —→, Macrophage —→●

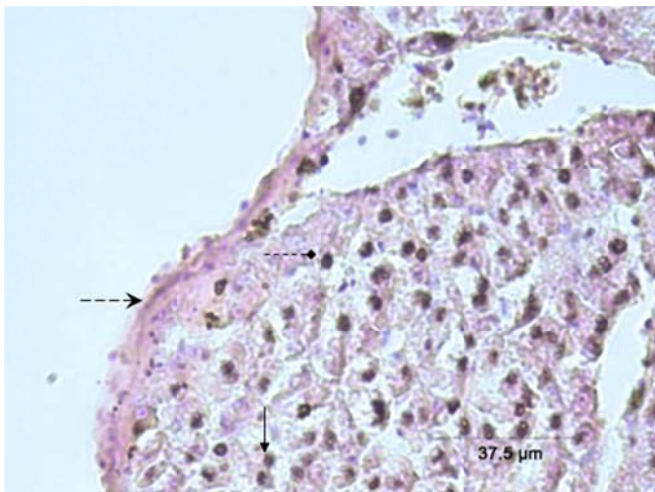


Figure 4. Wild sea bass liver at 45<sup>th</sup> day of the experiment.

Nuclei -----♦, Hepatocyte —→, Endothelial cells ----→

with the studies of Krajnovic et al. (1994) and Pagliarani et al. (1986). Vegetable oils that are used in the diet cause storage of LA (Alasalvar et al., 2002; Mnari et al., 2007). Arachidonic acid (AA) was found higher on wild fishes than cultured ones in some reports (Grigorakis et al., 2002; Alasalvar et al., 2002; Mnari et al., 2007). However, in this study, there were no differences in the amount of AA between the fishes. Deposition of AA in lipids was related to dietary fatty acid levels. The need for AA has been mainly related to stressful reactions of fish (Sargent et al., 1995), and particular needs have been shown for larvae, which are more susceptible to stress (Koven et al., 1993). Dietary eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are considered as essential fatty acids in marine fishes. Low levels of EPA and DHA in the cultured sea bass were observed in the study. Besides, low levels of DHA in cultured sea bass liver indicate highly metabolic activity because of DHA's utilization as an energy substrate and its physiological role in the cells. EPA and DHA ratio is the most important n-3 fatty acids in optimum

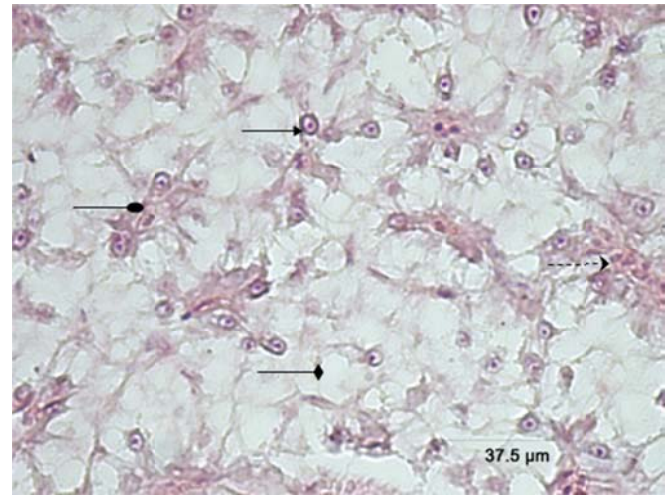


Figure 5. Pathological finding in cultured sea bass at the 90<sup>th</sup> day of the experiment.

Hypertrophy in hepatocyte —→, Nucleus are located at the periphery of the cell and gathered —●, Lipid deposition —♦, Hemorrhagic foci -----→

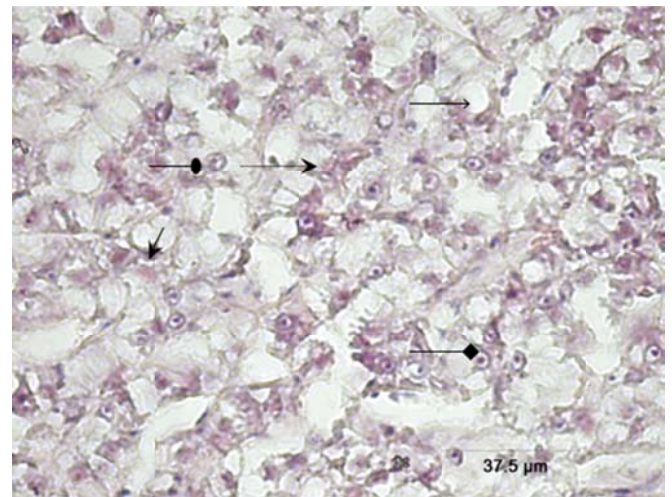


Figure 6. Wild sea bass liver at 90<sup>th</sup> day of the experiment.

Hypertrophy in hepatocyte —●, Nucleus are located at the periphery of the cell and gathered —♦, Lipid deposition —♦, Hemorrhagic foci —→, Macrophage —→

fish development (Rodriguez et al., 1994; Sargent et al., 1995; Skalli and Robin, 2004). The ratio was found higher in cultured sea bass compared with Mnari et al. (2007). However, according to Dias et al. (1998) and Yildiz and Şener (2003), high level of n-3 PUFA in the diet may cause fat deposition on viscera. These results supported our results of fat deposition in liver of cultured sea bass. n-3/n-6 fatty acid composition in cultured sea bass was found lower than wild sea bass and this may be a result of rich monounsaturated fatty acids and poor n-3 polyunsaturated fatty acids in diet (Ackman and Takeuchi, 1986).

We have observed similar results with Coz et al. (2005) as deterioration of membrane structures in hepatocytes, fat infiltration and degeneration in the liver of cultured sea bass pointing out the steatosis which occurs due to extreme dietary intake of lipids (Spisni et al., 1998). Steatosis is a limiting factor in sea bass culture. In order to prevent the disease, fatty acid composition and total lipid level of diet

should be formulated depending on the size of fish, metabolic activity and season.

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