

Fatty Acid Profiles in Wild Axillary Seabream (*Pagellus acarne*) versus Cage-Aggregated and Cage-Farmed Fish with Reference to Nutritional Contribution for Human Consumers

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Article History

Received 13 July 2018

Accepted 17 October 2018

First Online 31 October 2018

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Keywords

Fillet quality

Fatty acid

Health benefits

Pagellus acarne

Cage aggregation

Abstract

Fatty acid profiles of cage-farmed axillary seabream (*Pagellus acarne*) were compared with their wild representatives aggregated around the cage system and those from a distance area far from the fish farm. Wild fish contained higher levels of polyunsaturated fatty acids (PUFA; 36.47 g/100 g lipid) than the cage-aggregated (30.16 g/100 g lipid) or cage-farmed fish (29.20 g/100 g lipid). However, the most salient difference between wild and farmed-fish was the fat content with two-times higher levels in the latter (7.70% versus 3.05%). This resulted in a higher nutritional contribution of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which totally covered the recommendations of European Food Safety Authority (EFSA) with higher rate in cage-farmed (140%, CFF) and cage-aggregated axillary seabream (130%, CAF) compared to the wild populations of *P. acarne* from distant area (99%, WCF). As a result, all fish either farmed, cage-aggregated or wild-caught individuals met the minimum nutritional contribution for EPA+DHA in the order of CFF > CAF > WCF.

Introduction

Fisheries products are recommended for human consumption for the prevention of cardiovascular diseases in human beings (Leaf, Xiao, Kang, & Billman, 2003). Due to overexploitation of marine resources, fish populations are under threat. The scarcity of wild fish and the increasing demand for fishery products by the growing world population triggered the rapid growth of the aquaculture industry (Tidwell & Allan, 2001). The amount of fish capture from natural resources is no longer capable to meet this demand due to the almost stabilized catch yields since 2011 according to FAO (2016). Fish consumption was reported as 146 million tons in 2014, while only 93.4 million tons were captured in 2014 (FAO, 2016). On the contrary, aquaculture is practiced in a closed-circle from egg to harvest instead of wild fish capture. With

an annual increase of 6%, the aquaculture sector is expected to provide a solution for meeting food demand of the increasing human population, estimated to reach around 9.7 billion in year 2050 (FAO, 2016). Among various aquaculture practices, the cage aquaculture industry with its rapid growth is supplying an important amount of the sea food demand in the world, with a total fish production of nearly 3.4 million tons in 2010 from cage farm operations (FAO, 2012). Therefore, fish production in aquaculture facilities seems to be capable to meet the increasing demand of the growing human population and nowadays consumers are offered farmed fish as alternative to wild fish.

However, there are consumers perceptions about farmed and wild fish, with concerns on the quality of farmed versus wild fish (Claret *et al.*, 2014). Omega-3 long-chain polyunsaturated fatty acids

(PUFA), especially eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3), well known with their pleiotropic effects on health promotion and prevention of cardiovascular diseases (Leaf *et al.*, 2003) are considered among quality indices in fish meat (Pleadin, 2017). The composition of fish muscles is considered as the most important aspect of the quality level, whereas for consumer acceptability, sensory properties and freshness are playing important roles as quality parameters (Grigorakis, 2007). Since marine aquaculture in Southern European seas has been intensified on limited fish species such as gilthead seabream (*Sparus aurata*) and the European seabass (*Dicentrarchus labrax*), problems arising from the overproduction of these finfish species forced farmers to introduce new species to diversify their product range in order to improve their competitive power. Axillary seabream (*Pagellus acarne*), with its high commercial potential, is reported to be a promising alternative species and candidate for the expanding aquaculture industry (Yigit *et al.*, 2016). To our knowledge so far, there are very limited reports published on axillary seabream in cage conditions (Guner, Canyurt, Kizak, & Gulec, 2013; Yigit *et al.*, 2016), however no information is available so far regarding fatty acid composition in axillary seabream, nor on its nutritional aspects in general.

Cage farming facilities attract large numbers of wild fish around the cage system due to loss of uneaten feed particles and serving as a shelter in an exposed marine environment. Axillary seabream is one of the fish species schooling around cage farms in abundance that comprise an important amount of fish catch for the local fishermen and are abundantly available in the local market. Evaluation of dietary fatty acid profiles in fish meat as an aspect to the health benefits on human beings through the consumption of farmed versus wild fish is important for assessing the degree of value of the farmed fish and promoting marketing strategies for potential development of the aquaculture sector. Therefore, the aim of the present study was to evaluate differences in fatty acids of cage-farmed, cage-aggregated and wild axillary seabream and to compare the health benefits for human consuming wild versus farmed fish.

Materials and methods

Ethical Note

All procedures and applications in the present study were performed in compliance with relevant laws and institutional guidelines and approved by the Ethical Commission of Canakkale Onsekiz Mart University (Ethical Commission Approval Number:

2013/ 10-04) and followed regulations of Animal Behavior Society Guidelines for the Use of Animals in Research.

Experimental Fish and Study Area

Differences in nutritional quality between cage-farmed and wild fish were studied in the Northern Aegean Sea (40°03'42"N-26°20'36"E, 40°03'51"N-26°20'45"E, 40°03'45"N-26°20'55"E, 40°03'36"N-26°20'48"E). A total of 200 axillary seabream (*P. acarne*) individuals were captured with baited trotlines between depths of 0-60 m along 1 km stretch of coastline of Dardanos town in the Starit of Canakkale (formerly, the Dardanelles) and placed into a gravity-type polyethylene fish cage (1.5 m diameter and 4 m net chamber depth) in an offshore cage farm 0.6 nautical miles off the coast of Dardanos town area (Canakkale Province) (40°03'42"N-26°20'36"E).

The axillary seabream (*P. acarne*) is reported to spawn in April and from September to October in the Aegean Sea (Soykan *et al.*, 2015). Further the average length for the mature individuals spawning in April was reported as 13.5 cm (Soykan *et al.*, 2015). Therefore, individuals over a total length of 13.5 cm were assumed as a market-size fish and used in the present study. The wild-caught fish were acclimatized for a period of 1 month to culture conditions in the cage environment and fish behavior was monitored during the course of the adaptation period. Well adapted fish were weighed and the feeding experiment was initiated. Fish in cage environment were fed to satiation once a day for a period of 90 days, using a commercial seabream diet with 47% protein, 17% lipid, 3% cellulose, 13% ash, and 12% moisture with a gross energy and digestible energy level of 20.4, and 17.77 kJ/g diet, respectively. Once the feeding activity was initiated fish started feeding at surface, causing scintillation at water surface while grappling the pellets. After a while the feeding behavior at surface declined and only some of the fish continued feeding approximately one meter below surface, where the greyish coloration of fish belly was still visible during bending of the body and pellet grappling behavior. At this point, fish was assumed to reach satiation and feeding was withheld. Wild individuals were captured from two different locations, namely (a) wild stocks aggregated around the cage system, and (b) wild fish stocks in a distant area of more than 1.0 nautical mile far from the fish farm facility. Special care was taken for the wild fish samples to be similar in weight and length with their cage-farmed representatives. Body weights of fish investigated varied between 39.18-71.87, 44.12-63.79, and 36.87-75.47 g for the cage-farmed fish (CFF), cage-aggregated wild fish (CAF), and wild-

caught from a distant area (WCF), respectively. Random sampling was conducted at the end of the trial in July 2014. During the sampling, fish from the cage environment was taken by a butterfly net, while the wild fish schools around the cage farm and those from a distant area were captured by trotlines.

Biometric Parameters and Bioassay

The comparison of the external characterization of the body shape of the fish in cage with the wild fish stocks of both cage-aggregated and far distance schools were based on biometric parameters of total length, body weight and condition factor. Total length (TL, from snout tip to the posterior point of the caudal fins) and body weight (Wt, body wet weight) was measured to the nearest mm and g, respectively. Additionally, Fulton's condition factor was calculated according to the equation given by Htun-Han (1978) with the following formula:

$$\text{Condition factor (K)} = (\text{Wt} \times 100) / \text{TL}^3$$

where, Wt is the weight of fish in g, and TL for the total length of fish in cm.

Proximate composition analyses were conducted on whole body samples (without liver, viscera and visceral fat) of farmed and wild auxiliary seabream with eight random fish samples for each fish stock. Samples were weighed, lyophilized, homogenized, and dried at 103°C for 24 hr (AOAC, 2000) to a constant weight of ± 0.1 mg difference. Kjeldahl method (AOAC, 2000) was used for determination of crude protein. Whole body ash content was determined after incinerating the sample in a muffle furnace at 550°C for 18 hours according to AOAC (2000) guidelines. Crude lipid was extracted according to Bligh and Dyer (1959) using a 2:1 v/v ratio of chloroform to methanol.

Fatty Acid Composition

For the esterification of the fatty acids, 0.150 g of raw oil sample was weighed in a volumetric flask and 5 ml of methanolic 0.5 N NaOH was added. Then the saponified of oil was ensured with the boiled in the water bath during 15 minutes. Right after addition of the 5 ml BF₃ (Boron trifluoride) reactant on the cooler, oil was boiled for an additional 5 minutes, then 2 ml heptane was added. After boiling for another minute, the coolant was removed and the sample was taken into a 25 ml volumetric flask with precision. The flask was rinsed with saturated NaCl, the rinse was added and 1-2 mL of the top heptane phase was taken with a micropipette and transferred

in to a glass bottle via a test tube. Fatty acid composition was determined by withdrawal of this solution with injector and injection into 1 μ L gas chromatography (GC) (IUPAC, 1987).

Fatty acid contents were determined using a Shimadzu GC-2010 Series gas chromatography. Fatty acid methyl esters were separated on an Omega WAX capillary column and for specification of peak Supelco 37 Component FAMES Mix norm was used. The chromatography operating conditions were identified as follows; 5 minutes at 70 °C, reach 5 °C/min increase up to 250 °C, waiting time of 20 minutes at 250 °C. Helium was used as the carrier gas with a flow and split rates of 1.0 ml/min and 1:10, respectively. All analyses were conducted in triplicate. When the results of triplicates differed by more than 2%, outlier data were excluded and the analysis was repeated.

Nutritional Contribution of LA, ALA and Σ EPA+DHA

Nutritional contribution by consuming auxiliary seabream was estimated using the concentrations for linoleic acid (LA), alpha(α)-linolenic acid (ALA), and sum of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in fish sample of 100 g. The meal size, an important criterion for the determination of fish consumption patterns, was used as 227 g in average as suggested by US-EPA (1995) for an adult consumer body weight of 70 kg. The Australian National Health and Medical Research Council (NHMRC) and the New Zealand Ministry of Health (MoH) reported adequate intake values of 1300 and 800 mg/day for LA and ALA in adult men and women, respectively that are the values found in a population with no apparent essential fatty acid deficiencies (NHMRC, 2006). Thus, in the present study the average of 1050 mg/day for both men and women were used in the estimation of nutritional contribution for LA and ALA in human consumption. According to the European Food Safety Authority (EFSA, 2010), recommended consumption of EPA and DHA for the primary cardiovascular prevention is reported as between 250 and 500 mg/day. The upper level of 500 mg/day was used in the present study for the calculations of the nutritional contribution of EPA+DHA, using the following formula according to Saavedra *et al.* (2017):

$$\text{Nutritional Contribution (NC, \%)} = [(C \times M) / \text{DRI}] \times 100$$

where, C is fatty acid concentration as mg/100 g fish sample, M is the meal portion in g, and DRI is dietary reference intake in mg.

Statistical Analysis

The results are expressed as means±standard deviation (SD). The data were tested for homogeneity of variances at a significance level of $P<0.05$ and probability values less than 0.05 were considered as statistically significant. When normality variances were assumed, one-way ANOVA followed by Duncan multiple comparison test (Duncan, 1955) was performed. Statistical data analysis was performed using SPSS.19 software package (SPSS, Inc., Chicago, IL, USA).

Results

The external characterization of body shapes of the fish from three experimental groups have been demonstrated in Table 1. Proximate composition analyses of farmed and wild axillary seabream both cage-aggregated and far distant stocks resulted in values of 21.21% vs. 19.29% and 19.44% for crude protein, 7.70% vs. 5.10% and 3.05% for crude lipid, 1.62% vs. 1.60% and 1.56% for crude ash, and 70.5% vs.74.1% and 76.1% for moisture levels, respectively (Table 2).

Twenty-eight fatty acids with carbon chain lengths from 14 to 22 were found in whole body fish from three environments, *ie.* CFF, CAF, and WCF, with eleven saturated (SAFA), seven monounsaturated

(MUFA) and ten polyunsaturated fatty acids (PUFA). The most abundant fatty acids in the whole body of CFF (about 65% of the total) were monounsaturated fatty acids (MUFA), especially oleic acid (OA, 18:1n-9; 28.43 g/100 g fat), while in the whole body of CAF and WCF populations, the sum of MUFAs decreased to around 62%, with OA (18:1n-9) of 23.87 and 19.03 g/100 g fat, respectively. Total MUFAs showed a decline from 35.40 g/100 g fat for CFF, to 32.76 and 26.37 g/100 g fat for the CAF and WCF populations. In contraversion, SAFAs increased from 35.41 g/100 g fat for CFF, to 37.08 g/100 g fat for the CAF and 37.16 g/100 g fat for the WCF populations. Similarly, PUFAs also displayed an increase from 29.20 g/100 g fat for CFF, to 30.16 and 36.47 g/100 g fat for the CAF and WCF populations, respectively.

The SFAs in whole body of wild fish stocks of both CAF and WCF populations contained large proportion of palmitic acid (PA, C16:0; 21.06 and 21.06 g/100 g fat, respectively), while the CFF in the net cage showed lower level of PA (20.43 g/100 g fat) compared to the CAF and WCF populations. The omega-3 fatty acids, eicosapentaenoic (EPA, 20:5n-3), docosahexaenoic acids (DHA, 22:6n-3) and alpha(α)-linolenic acid (ALA, C18:3n3) were found in whole body of fish from all three environments, however DHA levels were 2.5 times higher in whole body of the WCF from distant areas than the CFF, while the CAF showed almost two times more DHA content

Table 1. Total length (TL, cm), weight (Wt, g) and condition factor (K) parameters of cage-farmed, cage-aggregated, and wild-caught axillary seabream (n=60).

	Cage-farmed fish	Cage-aggregated fish	Wild-caught fish
TL	15.38±0.89 (13.80-16.40)	15.48±0.85 (14.40-16.83)	14.82±1.27 (13.57-17.15)
Wt	58.23±11.9 (39.18-71.87)	51.80±7.1 (44.12-63.79)	50.78±15.8 (36.87-75.47)
K	1.56±0.08 (1.49-1.63)	1.41±0.09 (1.34-1.48)	1.49±0.06 (1.48-1.50)

Values are means ± standard deviation, minimum-maximum range in parenthesis.

Table 2. Whole body bio-chemical composition (% wet basis) of cage-farmed, cage-aggregated and wild-caught axillary seabream (n=60)

	Cage-farmed fish	Cage-aggregated fish	Wild-caught fish
Moisture, %	70.5±2.12 ^a (63.99-74.01)	74.1±1.09 ^b (72.8-75.47)	76.1±1.08 ^b (74.66-77.18)
Crude protein, %	21.2±1.84 ^b (19.15-24.28)	19.3±0.18 ^a (19.01-19.47)	19.4±0.36 ^a (18.88-19.91)
Crude lipid, %	7.70±1.77 ^c (4.94-9.94)	5.10±0.90 ^b (3.82-6.23)	3.05±0.79 ^a (2.22-3.88)
Ash, %	1.62±0.12 (1.45-1.79)	1.60±0.22 (1.42-1.95)	1.56±0.32 (1.09-1.98)

Values are means±standard deviation, minimum-maximum range in parenthesis. Superscript letters represent significant differences at 0.05 levels.

compared to the CFF. The ratio of DHA/EPA for the two wild fish populations were lower in the CAF compared to the WCF, and the DHA/EPA ratio was highest in the WCF and lowest in the CFF populations. Conversely, the whole body alpha(α)-linolenic acid (ALA) value was lowest in the WCF population (0.74 g/100 g fat) and increase to 1.15 g/100 g fat in the CFF and 1.33 g/100 g fat in the CAF fish stock. In contrast

to ALA, the level of Arachidonic acid (ARA, C20:4n6) in the whole body of fish increased from 0.22 and 0.23 in the CAF and CFF to 0.27 g/100 g fat for the WCF population (Table 3).

The content of ω -6 fatty acids in whole body of CFF (15.75 g/100 g fat) was significantly higher ($P < 0.05$) compared to the wild fish populations of both CAF (4.56 g/100 g fat) and WCF populations from

Table 3. Fatty acid composition as percentage of total fatty acids in dry weight basis of cage-farmed fish (CFF) versus cage-aggregated (CAF) and wild axillary seabream (WCF) (n=60).

	CFF	CAF	WCF
	Percent, dry weight basis		
SAFA (Saturated - fatty acids)			
12:0	0.09±0.05 ^a	0.11±0.06 ^a	0.10±0.06 ^a
13:0	0.01±0.01 ^a	0.02±0.02 ^a	0.02±0.03 ^a
14:0	2.67±0.47 ^a	3.00±0.83 ^a	2.71±0.91 ^a
15:0	0.44±0.26 ^b	0.69±0.22 ^{ab}	0.77±0.21 ^a
16:0	20.43±0.84 ^a	21.06±3.22 ^a	21.06±0.99 ^a
17:0	0.60±0.28 ^a	0.85±0.45 ^a	0.99±0.50 ^a
18:0	8.86±0.86 ^a	8.12±1.33 ^a	8.14±1.50 ^a
20:0	1.10±1.24 ^a	0.76±1.02 ^a	0.67±0.66 ^a
21:0	0.49±0.38 ^a	1.18±1.69 ^a	1.33±1.73 ^a
22:0	0.05±0.04 ^a	0.07±0.09 ^a	0.03±0.03 ^a
23:0	0.38±0.09 ^b	1.10±0.57 ^a	1.18±0.23 ^a
MUFA (Monounsaturated - fatty acids)			
14:1	0.08±0.03 ^a	0.07±0.05 ^a	0.08±0.08 ^a
15:1	0.06±0.04 ^a	0.09±0.08 ^a	0.10±0.08 ^a
16:1	4.37±1.06 ^a	4.94±1.30 ^a	4.66±1.31 ^a
17:1	0.29±0.16 ^b	0.34±0.24 ^{ab}	0.52±0.14 ^a
18:1n9	28.43±2.70 ^a	23.87±7.22 ^{ab}	19.03±3.72 ^b
20:1n9	1.78±1.00 ^a	2.60±1.44 ^a	1.58±1.10 ^a
22:1n9	0.36±0.07 ^a	0.71±0.69 ^a	0.41±0.25 ^a
24:1n9	N.D.	N.D.	N.D.
PUFA (Polyunsaturated - fatty acid)			
18:2n6c	11.26±6.31 ^a	3.85±3.94 ^b	2.01±0.43 ^b
18:2n6t	0.07±0.07 ^a	0.08±0.13 ^a	0.05±0.09 ^a
20:2	0.74±0.37 ^a	0.75±0.53 ^a	0.68±0.42 ^a
22:2	0.15±0.08 ^a	0.34±0.32 ^a	0.25±0.44 ^a
18:3n3	1.15±0.88 ^a	1.33±1.32 ^a	0.74±0.21 ^a
18:3n6	1.00±1.14 ^a	0.41±0.37 ^a	0.38±0.21 ^a
20:3n3+3n6	1.02±0.73 ^a	1.36±1.12 ^a	1.88±1.55 ^a
20:4n6	0.23±0.06 ^a	0.22±0.10 ^a	0.27±0.15 ^a
20:5n3	4.30±2.16 ^b	6.20±2.16 ^{ab}	8.14±1.81 ^b
22:6n3	9.28±3.46 ^b	15.60±9.76 ^{ab}	22.07±8.08 ^a
Total ω -3	15.75±6.31 ^b	24.51±9.63 ^{ab}	32.84±6.47 ^a
Total ω -6	12.56±7.05 ^a	4.56±3.94 ^b	2.71±0.47 ^b
Total ω -9	30.60±2.98 ^a	27.33±8.68 ^{ab}	21.02±4.22 ^b
ω -6 / ω -3 ratio	0.797	0.186	0.083
UNSAT	64.60±1.87 ^a	62.92±5.61 ^a	62.84±1.93 ^a
SAT	35.41±1.87 ^a	37.08±5.61 ^a	37.16±1.93 ^a
UNSAT / SAT	1.83±0.15	1.77±0.54	1.70±0.14
DHA / EPA	2.25±0.41 ^a	2.66±1.42 ^a	3.02±1.97 ^a
Σ SAFA	35.41±1.87 ^a	37.08±5.61 ^a	37.16±1.93 ^a
Σ MUFA	35.40±2.79 ^a	32.76±9.45 ^{ab}	26.37±5.48 ^b
Σ PUFA	29.20±2.89 ^a	30.16±8.11 ^a	36.47±6.48 ^a

distant areas (2.71 g/100 g fat), mostly because of the 3 times higher level of LA (18:2n-6; 11.26 g/100 g fat) over the CAF (3.85 g/100 g fat) and 5 times higher over the WCF populations (2.01 g/100 g fat). Higher ω -6 fatty acid in fish whole body was recorded for the CAF over the WCF population, however the difference was not significant ($P>0.05$). Similar to ω -6, the ω -9 fatty acids in fish whole body showed highest value (30.60 g/100 g fat) for the CFF and lowest (21.02 g/100 g fat) for the WCF from distant population. The level of ω -9 fatty acids in the whole body of CAF around the net cage increased to 27.33 g/100 g fat and was significantly similar ($P>0.05$) to the whole body of CAF fed artificial pellets in the net cage. In contrast to ω -6 and ω -9 fatty acids, the ω -3 fatty acids in fish whole body was highest ($P<0.05$) for the WCF population of distant area, with a value of twice more than the CFF. The ω -3 fatty acid level of the wild fish populations declined from 32.84 g/100 g fat in the fish from distant area (WCF) to 24.51 g/100 g fat in those aggregated around the net cage (CAF) however the difference was not significantly important ($P>0.05$).

Highest levels of nutritional contribution of LA and ALA were recorded in the CFF group with 55.30 and 5.65%, followed by the CAF and the WCF groups with 11.00 and 3.80%, and 3.18 and 1.17%, respectively. The nutritional contribution obtained for EPA+DHA was highest in the CFF with 140.3%, followed by the CAF and WCF groups with 130.8% and 99.88%, respectively (Table 4).

Discussion

Based on the results of the proximate

composition, it can be noted that protein, lipid and moisture content was influenced by the sampling environment of the fish ($P<0.05$). However, it was found that the origin or sampling environment did not affect on the ash content ($P>0.05$). Protein levels between the cage-farmed fish and the wild populations of axillary seabream differed significantly with higher protein contents in the cage-farmed fish over the wild fish stocks which are in agreement with Grigorakis, Alexis, Taylor, and Hole (2002). The lipid concentrations in the fillets may influence the texture of the fish muscles (Hernández *et al.*, 2009). Johnston *et al.* (2000) reported that a softer texture of fish meat can be obtained with higher levels of fat. In the present study, whole body lipid levels in the cage-farmed fish (7.70%) were significantly higher compared to the wild fish stocks both cage-aggregated (5.10%) or far distant fish populations (3.05%). The axillary seabream in the cage environment fed on artificial pelleted diets showed body lipid content more than twice the lipid level of the wild fish, eventually leading to a possibly softer fillet of the cage-farmed fish over the wild-caught fish fillets. Similar findings regarding differences in the total fat contents between farmed or wild gilthead seabream, seabass, salmon, and meagre were also reported by Johnston *et al.* (2006), Grigorakis (2007), and Saavedra *et al.* (2017). The relatively higher fat contents in the farmed fish over the wild populations might be attributed to the higher fat levels in the pelleted diets as well as feeding frequency (Johnston *et al.*, 2006), or to a variety of factors including availability of diets and type of food, dietary ingredients, and the higher energy consumption of farmed fish compared to the wild individuals (Grigorakis *et al.*, 2002). The findings in the

Table 4. Fatty acid composition as percentage of total fatty acids in mg/100 g wet weight basis and nutritional contribution of cage-farmed fish (CFF) versus cage-aggregated (CAF) and wild-caught axillary seabream (WCF) for human consumption

	CFF	CAF	WCF
Dietary levels (mg/100 g wet weight basis)			
LA	255.77	50.86	14.65
ALA	26.12	17.57	5.39
EPA	97.68	81.90	59.34
DHA	210.8	206.06	160.88
DHA / EPA	2.16	2.52	2.71
∑ EPA+DHA	308.48	287.96	220.22
Dietary reference intake (mg/day)			
LA	1050	1050	1050
ALA	1050	1050	1050
∑ EPA+DHA	500	500	500
Nutritional contribution for human consumption			
LA (%)	55.30	11.00	3.18
ALA (%)	5.65	3.80	1.17
∑ EPA+DHA (%)	140.27	130.75	99.88

LA: linoleic acid, ALA: (α) linolenic acid, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid; Dietary reference intake (DRI, mg/day; average value for adult men+women)

present study are in agreement with the earlier reports in terms of higher fat contents in farmed fish compared to the wild populations. Johnston *et al.* (2000) reported that other factors such as collagen content and muscle cellularity may also influence texture and softness of fish fillets besides higher fat contents in fish body. Eventhough collagen was not determined in the present study, and its potential effect on fish fillet texture could not be evaluated, Periago *et al.* (2005) recorded that the collagen in the tissue causes higher cohesiveness, leading to a firmer texture in fish fillets.

Similar to the differences observed among experimental groups of CFF, CAF, and WCF in terms of whole body fat contents, the whole body fatty acid compositions also showed variations and differences among these groups. Fatty acid profile of fish is dependent on various factors such as the dietary lipid source, water temperature, salinity, season (Yildiz, Şener, & Timur, 2008), in addition to a possibility of a combination of these factors.

Among the PUFAs, higher levels of linoleic acid (LA, C18:2n6c (n-6); 11.26 g/100 g fat) were noted in the whole body of CFF compared to the wild fish, both the CAF (LA, C18:2n6c (n-6) 3.85 g/100 g fat) and WCF from far distant populations (LA, C18:2n6c (n-6) 2.01 g/100 g fat). Similar to our finding on elevated LAs, recent studies on fish nutrition demonstrated that the dietary incorporation of soybean oil up to 60% resulted in a 428-647% increased LA in the fish body compared to a diet comprised of fish oil only. Similarly, an incorporation of linseed oil of around 60% is reported to increase LA levels in fish body compared to the fish oil based diets (Hibbeln, Nieminen, Blasbalg, Riggs, & Lands, 2006).

Interestingly, the cage-aggregated wild fish individuals showed reduced EPAs and DHAs, similar to the fish in the cage environment fed on formulated aqua-diets. The reason might explain that the wild fish aggregated around the net cage shared the uneaten and lost pellets scattered to the surrounding water environment from the fish cage, which is in agreement with earlier reports of Skog, Hylland, Torstensen, and Berntssen (2003) and Fernandez-Jover *et al.* (2007). Eventhough, no significance was found, the cage-aggregated fish presented elevated levels of LAs over those of distant areas in the present study. The prepotency of 18:2n-6, 18:3 n-3, linoleic acid (LA, C18:2n6c (n-6)), alpha linolenic acid (α -LA, C18:3n3 (n-3)) and the increased level of n-6/n-3 ratio in the body of cage-farmed fish over the wild fish, both cage-aggregated or far distant populations in the present study was possibly due to the increased levels of dietary incorporation of plant oils as an alternative raw material in diets given to farmed fish as also reported by Dubois, Breton, Linder, Fanni, and

Parmentier (2007). The use of alternative sources of raw materials in fish diets is a common practice in the feed industry, due to the increasing demand and limited production of fish meal or fish oil which is mainly dependent on wild fish resources. Hence, the feed industry tends to incorporate appropriate amounts of plant resources as a substitute for fish meal and fish oil in order to reduce production costs to increase competitiveness of the facilities (Dubois *et al.* 2007).

Our results in terms of increased LAs in farmed fish is in close agreement with the findings of Lenas, Chatziantoniou, Nathanailides, & Triantafillou (2011 in wild and farmed seabass (*Dicentrarchus labrax*), indicating that the farmed fish showed lower nutritional value in terms of FAs, with high levels of LA or α -LA and n-6/n-3 ratio. The transformation capability of LA and α -LA in fish diets to n-6 and n-3 FAs in the body of marine fishes is lower compared to fresh water fish species, possibly due to the lack of necessary enzymes (D-5-6-Desaturase) that elongate the carbonic chain of fatty acids (Kris-Etherton *et al.*, 2000). Sargent, Bell, McEvoy, Tocher, and Estevez (1999) reported that the transformation rates of LAs to AA and of α -LA (18:3 n-3) to EPA or DHA in several marine fishes were insufficient, unequal and negligible or null. Similarly, Fountoulaki, Alexis, Nengas, and Venou (2003) indicated that the replacement of fish oils by soybean oil presented a significant increase in linoleic acid and linolenic acid levels with an increment of the n-6/n-3 ratio in the muscle of farmed fish. The authors also reported a reduction around 72% in the n-3 FAs compared to the fish fed solely fish oil as an oil source in the formulated diet. The disability of transforming LAs to AA and α -LA (18:3 n-3) to EPA or DHA in marine fishes results in the inclusion of LA and α -LA in fillet lipids, that ends in the human body through consumption (Lenas *et al.*, 2011). Overconsumption of LAs may contribute to depression and increased cardiac mortality in human (Fountoulaki *et al.*, 2003). However, if fatty acids were converted into mg/100 g wet weight basis, all fatty acids presented higher levels in farmed axillary seabream, with two fold higher ARAs and EPAs in farmed fish over the wild individuals. Contents of ALAs, EPAs, and DHAs in fish body of the cage-farmed fish were 1.5 to 4 times higher compared to the wild-caught axillary seabream.

Considering the lipid concentration in fish body and converting fatty acid levels into mg/100 g, it was seen that ARAs, ALAs, EPAs and DHAs were higher in the CFF group versus CAF and WCF populations. The estimated nutritional contribution of LA and ALA were highest in the farmed axillary seabream (55.30 and 5.65%), followed by the cage-aggregated fish (11.00

and 3.80%) and wild fish populations (3.18 and 1.17%), respectively (Table 4). Dubois *et al.* (2007) reported that the increase of plant oil contribution in aqua-feed formulations as a raw material alternative to fish oil might lead to an increase of LA levels in fish body, supporting our results in terms of increased nutritional contribution of LA or ALA in the cage-farmed or even the cage-aggregated fish groups compared to the wild populations of *P. acarne*. The nutritional contribution of EPA+DHA was found as highest in the farmed axillary seabream (140.3%), followed by the cage-aggregated fish (130.8%) and wild fish populations (99.88%), respectively (Table 4). Cage-farmed and cage-aggregated fish completely fulfilled the daily intake levels of EPA+DHA recommended by the EFSA (2010) for the prevention of primary cardiovascular disease. Wild fish captured from distant area far from the cage site, almost met the recommended daily amount of EPA+DHA at 99.88%, nearly 100%.

Several recommendations for the consumption amounts of EPA+DHA to prevent cardiovascular diseases are available with different suggestion levels. The American Dietetic Association and Dietitians of Canada suggested an amount of 500 mg/day for EPA+DHA providing by two servings of oily fish per week (one serving is about 112 g) (Kris-Etherton & Innis, 2007). The suggested daily amount for EPA+DHA is given as 450 mg/day by the United Kingdom Scientific Advisory Committee on Nutrition (SACN, 2004), while the World Health Organization (FAO/WHO, 2003) recommended one to two servings per week, each providing an amount of 200-500 mg EPA+DHA. The European Food Safety Authority (EFSA, 2010) suggested a consumption amount of EPA+DHA for the primary cardiovascular prevention as 250 to 500 mg/day. In the estimation of the nutritional contribution of farmed and wild axillary seabream in the present study, suggestions of EFSA (2010) were followed with the upper dietary reference value of 500 mg/day for EPA+DHA. Considering the nutritional contribution of EPA+DHA recorded in the present study, 227 g of farmed or cage-aggregated axillary seabream appears to be more than sufficient (140.3% and 130.8%, respectively) and meets the amount of 500 mg recommended by EFSA (2010) for primary prevention of cardiovascular diseases, whereas the wild fish captured from a distant area not affected from the cage farm was slightly below or just enough (99.9%) to meet the suggested daily amount of EPA+DHA for human consumption and prevention of coronary diseases. Similar to our findings, Saavedra *et al.* (2017) reported that a daily portion of 160 g of farmed meagre (*Argyrosomus regius*) were estimated to meet the amount of 500 mg of EPA+DHA, while the wild meagre were reported to

partially meet the recommended daily amount.

In the present study, the MUFAs showed increased levels, especially in oleic acid (OA, C18:1n9) in the body of cage-farmed fish compared to the wild populations in areas far from the cage farm. However, the cage-aggregated wild fish stocks showed elevated OAs and overall MUFAs similar to the cage-farmed fishes. This gain could be attributed to the consumption of uneaten and lost pellets scattered to the surrounding water environment from the fish cage. In earlier studies, it was reported that farm-aggregated wild fishes consume large amounts of pellets lost from fish cage, which might lead to a change in the feeding behavior with consequences on natural fish populations by changing their body proximate composition or fatty acid levels due to the abundant pellets around the fish cage system (Skog *et al.*, 2003; Fernandez-Jover *et al.*, 2007), that eventually might affect local fishermens activities since the cage-aggregated fishes have commercial value in the local fishery community. It is likely that a certain amount of biomass is transferred from cage farms to natural fish populations, which eventually might reduce environmental effects of cage farming activities with transferring uneaten pellets into natural fish biomass increase, that in long-term could have economic and social benefits to the local fishermens' activities.

As a conclusion, cage-farmed and wild axillary seabream showed differences in proximate composition, and fatty acid profiles. Overall, farmed axillary seabream appears to be promising marine food source with high potential for consumers' health with its meat quality and nutritional contribution of EPA+DHA, not less but even better than its wild representatives. Hence, axillary sea bream reared in a cage environment or wild fish aggregated around the cage farm seems to be beneficial in terms of human health, especially for the nutritional requirements of people suffering from coronary health diseases.

Acknowledgement

This work was supported by the Scientific Research Projects Commission (COMU-BAP) of Canakkale Onsekiz Mart University, Turkey [FBA-2014-237]. Legal and ethical standards for welfare of animals have been followed during the process of farming and handling fish, prior to and during death and during live transportation or storage. All procedures and applications in the present study were performed in compliance with relevant laws and institutional guidelines and approved by the Ethical Commission of Canakkale Onsekiz Mart University (Ethical Commission Approval Number: 2013/ 10-04).

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