Hayvansal Üretim (2023) 64 (1): 6-11
Journal of Animal Production (2023) 64 (1): 6-11
https://doi.org/10.29185/hayuretim.1261236

Research Article ***Araştırma Makalesi***

Identification and Characterization of Carnitine Palmitoyltransferase 1A (CPT1A) Gene in European Sea Bass (*Dicentrarchus labrax*)[#]

Avrupa Levreğinde (*Dicentrarchus labrax*) Karnitin Palmitoiltransferaz 1A (CPT1A) Geni Tanımlanması ve Karakterizasyonu

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ABSTRACT

Objective: CPT1A intervenes in the access of the long-chain fatty acyl-CoA to the mitochondrial matrix. In this study, we conducted the genetic polymorphisms of the partial sequence of the CPT1A gene in European sea bass (*Dicentrarchus labrax*) that were cage cultured in the Aegean Sea.

Material and Methods: Genomic DNA was isolated from 100 European sea bass individuals. After amplifying a partial region of the CPT1A gene by thermal cycler, PCR products were sequenced via Sanger method.

Results: We detected two SNPs in partial sequence of the CPT1A gene in European sea bass. TT, TA and AA genotypes were observed for CPT1A g.2080T>A locus with frequencies of 27.04, 49.92 and 23.04%, respectively. The frequencies of the g.2216A>G locus as AA, AG and GG were found to be 1.2, 19.58 and 79.21%, respectively. The CPT1A g.2080T>A locus was in HWE, whereas the g.2216A>G locus was not in HWE.

Conclusion: European sea bass has a high commercial value. These findings suggest that two SNPs in the CPT1A gene could be used for genomic selection programs related to fatty acid composition in European sea bass.

Keywords: Fatty acid, SNP, sea bass, variation

ÖZET

Amaç: CPT1A, uzun zincirli yağ asil-CoA'nın mitokondriyal matrise girişine müdahale eder. Bu çalışmada, Akdeniz'de kafes kültürü yapılan Avrupa levreklerinde (*Dicentrarchus labrax*) CPT1A geninin kısmi dizisinde genetik varyasyonlar araştırılmıştır.

Materyal ve Yöntemler: 100 Avrupa levreği bireyinde genomik DNA izole edilmiştir. CPT1A geninin kısmi bir bölgesi termal döngüleyici ile çoğaltıldıktan sonra, PCR ürünleri Sanger yöntemi ile dizilenmiştir.

Bulgular: Avrupa levreği CPT1A geninde kısmi dizisinde iki SNP belirlenmiştir. g.2080T>A lokusu için sırasıyla %27.04, %49.92 ve %23.04 frekansları ile TT, TA ve AA genotipleri gözlenmiştir. g.2216A>G lokusunun AA, AG ve GG'nin frekansları sırasıyla %1.2, %19.58 ve %79.21 olarak bulunmuştur.

Sonuç: Avrupa levreği yüksek ticari değere sahiptir. Bu bulgular, CPT1A genindeki iki SNP'nin, Avrupa levreğindeki yağ asidi bileşimi ile ilgili genomik seleksiyon programları için kullanılabileceğini düşündürmektedir.

Anahtar Kelimeler: Yağ asidi, SNP, levrek, varyasyon

Citation: Gökçek, E.Ö., Işık, R. 2023. Identification and characterization of carnitine palmitoyltransferase 1A (CPT1A) gene in European Sea Bass (*Dicentrarchus labrax*). Journal of Animal Production 64(1): 6-11. https://doi.org/10.29185/hayuretim.1261236

Atıf: Gökçek, E.Ö., Işık, R. 2023. Avrupa Levreğinde (*Dicentrarchus labrax*) karnitin palmitoiltransferaz 1A (CPT1A) geni tanımlanması ve karakterizasyonu. Hayvansal Üretim 64(1): 6-11. https://doi.org/10.29185/hayuretim.1261236

Geliş tarihi (Received): 07.03.2023 Kabul tarihi (Accepted): 20.05.2023

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[#]Bu çalışma, 31 Ağustos – 3 Eylül tarihleri arasında düzenlenen "International Fish Congress & Fish Expo Brasil 2022" kongresinde sözlü bildiri olarak sunulmuştur.

INTRODUCTION

Dicentrarchus labrax; Moronidae (European sea bass), which has a crucial commercial value, is mainly produced in the Aegean region of Turkey. European sea bass lives in the marine coastal waters along the northeast Atlantic Ocean, throughout the Aegean (Mediterranean) Sea (including Turkey), and the Black Sea (Özcan Gökçek et al., 2020). According to FAO, the aquaculture production of this species was 276,000 tonnes in 2020 and its market value reached 1,360,000 dollars. European sea bass culture started becoming widespread in the 1990s in the Mediterranean region ranking 35th among the world's fish farming industry. Genetic improvement studies for growth traits in European sea bass populations started relatively recently compared with the Atlantic salmon (*Salmo salar*).

The enzyme carnitine palmitoyltransferase 1 (CPT1) is the main regulator of mitochondrial fatty acid oxidation in vertebrates (Lu et al., 2016). CPT1 is also the "rate limiting" enzyme of beta-oxidation in the cell (Eaton, 2002). The CPT system (consisting of CPT1 and CPT2), intervenes in the access of the fatty acyl-CoA to the mitochondrial matrix (Gutiéres et al., 2003). The CPT1 catalyzes the transition of the acyl groups of long-chain fatty acyl-CoAs in carnitine and produces acyl carnitine. CPT1 plays a crucial role in the mitochondrial regulation of β-oxidation (Morash et al., 2008). Decrease in CPT-1A activity reduces the intra-mitochondrial substrate contents for fatty acid beta-oxidation, thus attenuating energy accumulation and it can cause hepatic encephalopathy and hypoketotic hypoglycemia after long periods of fasting in humans (Bennett et al., 2004; Gan et al., 2021). CPT1 is located in the mitochondrial outer membrane and has 3 isoforms in mammals: CPT1A (liver isoform), CPT1B (muscle isoform), and CPT1C (brain isoform) (Morash et al., 2010; Lu et al., 2016). Morash et al. (2010) have reported that taxa-specific variations might be the consequence of genome duplications in finfishes causing an increase to five CPT I isoforms in rainbow trout (*Oncorhynchus mykiss*). PPARA upregulates the expression level of CPT1A, which is contained in lipid catabolism (Guo et al., 2015). In a human study, it was reported that raised expression of the CPT1A gene was related to a decline in plasma triglyceride and affected fish oil intake (Bouchard-Mercier et al., 2014). It has been reported that the balance between the intracellular inflammatory response and lipid accumulation in the yellow-striped gobies (*Mugilogobius chulae*) can be regulated by different expression levels of MGLL and CPT1 (Cai et al., 2021). Liu et al. (2018) observed higher CPT1 activity in the juvenile golden pompano (*Trachinotus ovatus*) group fed with fish oil compared to the soybean oil fed and lard oil fed groups. Relationships between the CPT1 gene expression and lipid metabolism in aquaculture species such as gilthead sea bream (*Sparus aurata*) (Bermejo-Nogales et al., 2014), Nile tilapia (*Oreochromis niloticus*) (Zhang et al., 2019), largemouth bass (*Micropterus salmoides*) (Chen et al., 2020), hybrid grouper (Zou et al., 2022), crab (*Portunus trituberculatus*) (Yuan et al., 2022), common carp (*Cyprinus carpio*) (Xie et al., 2022), Japanese seabass (*Lateolabrax japonicas*) (Zheng et al., 2012), yellow catfish (*Pelteobagrus fulvidraco*) (Zheng et al., 2013), and grass carp (*Ctenopharyngodon idella*) (Shi et al., 2017) have been studied.

Abnormal fat accumulation in finfishes is an important trouble for the economics of the fish industry especially farmed fish, which might be correlated with instability of feed nutrition, over-feeding and the alteration of fish oil to vegetable oil in feed. Hereby, studies on the physiological functions and expression of the CPT genes in fish might be beneficial for inside fat accumulation in fish.

Since the yield differences between animals are caused by genotype and environmental factors, selection programs have been performed on these two factors for years to reach the desired yield levels. The significance of marker-assisted selection (MAS) has increased in animal and fish selection due to the extended generation interval, alongside traditional breeding methods. Genes with major effects are more important than genes with minor effects in the variation observed among individuals, and such genes are considered candidate genes. Polymorphism and association studies on candidate genes in different populations provide basic information for MAS research. Molecular markers were used in breeding programs such as genomic selection, MAS of livestock and fish species (Gutierrez et al., 2015). One of the most widely used markers is SNPs depend on a single variation of a nucleotide (Özcan Gökçek and Işık, 2020). In European sea bass, there is no published article investigating the CPT1A gene polymorphisms with SNP markers. Carnitine palmitoyltransferase 1 controls fatty acid oxidation, and it is important to understand the molecular characterization of lipid metabolism in cultured fish (Lu et al., 2016). This study aimed to investigate the genetic polymorphisms of the partial sequence of the CPT1A gene in European sea bass that is cultured in Aegean conditions.

MATERIALS AND METHODS

Animal Material

The 100 European sea bass individuals were selected from a commercial population. Fish came from two

batches of two commercial hatcheries. The individuals were cage cultured in the same marine environmental conditions on a fish farm in Urla, İzmir.

Method

DNA Isolation and PCR

Muscle tissue samples were taken from the dorsal part of the fish and preserved at -20 °C until DNA isolation. DNA was extracted by using the High Pure PCR Template Kit (Roche, Germany) following recommended protocols at Ege University, Faculty of Fisheries, Laboratory of Molecular Genetics and Fish Breeding. The quantity of extracted DNA samples was estimated with a spectrophotometer.

The primer pairs were designed based on CPT1A nucleotide information of European sea bass deposited in GenBank (accession number KF857302), to amplify a partial region of the CPT1A gene. Primer sequences of the CPT1A gene are F: 5'-CAACCGAGACACACAACCTG-3' and R: 5'-AGAACCTCATGTAACCGGCA-3'.

PCR were carried out in 30 µL reaction volumes containing: 0.5 µM of each primer and 2X MyTaq™ Mix (Meridian Bioscience, USA), 1 U Taq Hot Start DNA (Bioline) polymerase and 100 ng genomic DNA. The amplification was carried out with the following thermal cycle program: an initial denaturing step for 5 min at 95 °C; followed by 37 cycles of 95 °C for 45 s, 58 °C for 30 s, 72 °C for 60 s, and 72 °C for 10 min. The DNA fragments were separated by 1.5% agarose gel electrophoresis.

Data Analyses

The genotyping of the SNPs in the CPT1A gene was performed by Sanger sequencing (3500XL Genetic Analyzer System) of the PCR products. The sequence results were aligned and controlled by ChromasPro Version 2.1.10 (Technelysium Pty. Ltd. Australia). The calculation of observed, allele and genotype frequencies, and the Chi-squared test of the group Hardy-Weinberg equilibrium (HWE) status was performed with the "HardyWeinberg" package in R software (R version R-3.4.3).

RESULTS AND DISCUSSION

The 621 bp of the CPT1A gene region was investigated by PCR and it is represented in Fig. 1. The partial sequence of the CPT1A gene in the *Dicentrarchus labrax* was studied by DNA sequencing in the current study. The *Dicentrarchus labrax* CPT1A gene includes 18 exons that encode 796 amino acids (FQ310507.3). The amplified CPT1A gene region is located between 4718538-4728712 bp in the NCBI GenBank (FQ310507.3). Two SNPs (g.2080T>A and g.2216A>G) have been defined in the European sea bass CPT1A gene noncoding region (KF857302) (Fig. 2a,b). The noncoding regions of the genome do not encode amino acids but, have important regulatory roles in transcription and translation such as modulation of gene expression, mRNA splicing and can induce changes in biological properties (Pagani and Baralle 2004; Kuhl et al., 2010; Sun et al., 2019). Introns are longer and under less selection pressure (Zhang et al., 2016). The variations of the sequences of the CPT1A gene were reported to the NCBI GenBank database for the first time in this study. Since intronic and noncoding regions are longer and under less pressure from natural selection, mutations are more common in these regions (Hu et al., 2013).

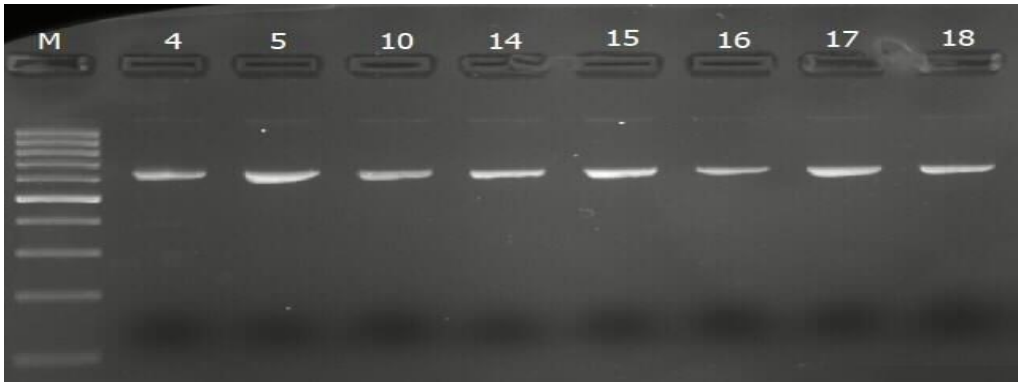

Figure 1. Electrophoresis results of the PCR products of CPT1A gene for the 8 samples. M; Marker
Şekil 1. *8 örneğin CPT1A geni PCR ürünlerine ait elektroforez sonuçları. M; Marker*

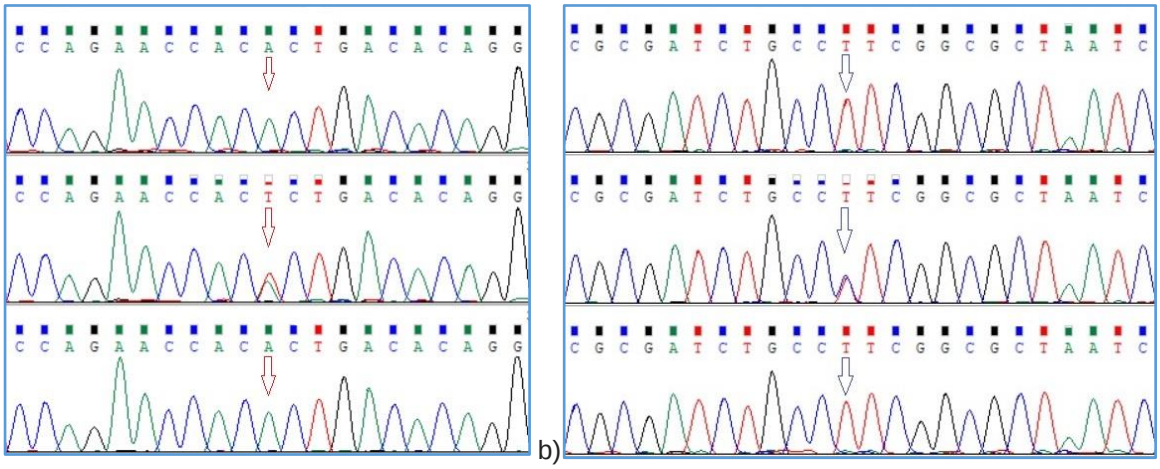

Figure 2. The sequences of CPT1A gene with the SNPs; a) g.2080T>A b) g.2216A>G (showing reverse complement)

Şekil 2. *CPT1A genine ait SNP bulunan sekanslar a) g.2080T>A b) g.2216A>G (revers komplementinde gösterilmiştir)*

The three genotypes were seen in the studied population, whereas the AA genotype was seen in very few individuals in g.2216A>G locus. The frequencies of the alleles A and G were 0.11 and 0.89, respectively. The genotype and allele frequencies of the European sea bass CPT1A gene were shown in Table 1. The CPT1A gene g.2080T>A locus is in HWE, while the g.2216A>G locus is not in HWE. The genetic variation in this population is not constant from one generation to the next in the absence of disturbing factors. This shows that the imbalance indicates that the population has been selected. According to the results of the g.2216A>G locus, it may be associated with unknown genes affected by natural selection (Yang et al., 2018).

Table 1. Genotype and allele frequencies of CPT1A gene region

Çizelge 1. *CPT1A gen bölgesi genotip ve allel frekansları*

Loci		CPT1A Genotypes			Allele Frequency		χ2
		TT	TA	AA	T	A	
g.2080T>A	Obs.	27	50	23	0.52	0.48	0.00*
	Exp.	27.04	49.92	23.04			
		AA	AG	GG	A	G	
g.2216A>G	Obs.	6	10	84	0.11	0.89	23.9
	Exp.	1.21	19.58	79.21			

Note: χ2 (0.05; 1), *The population is in HWE

The CPT1A gene was not studied at DNA variation level. Mostly there are gene expression researches about the CPT1A gene. There are no publications investigating the possible relationships between lipid metabolism and the CPT1A gene in fish. Rimoldi et al. (2016) revealed that expression levels of ATGL, HADH and the CPT1A genes were upregulated during the 10 days fasting study of the European sea bass. They reported that the CPT1A is one of the most important markers in monitoring the nutritional status of European sea bass. In another study, CPT1A expression in the rainbow trout liver fed coconut oil (CO) diet was affected by fat storage but not by fat level (Figueiredo-Silva et al., 2012). It was stated that the effect of diets in the experiment on the expression of fatty acid oxidation markers (ACOX and CPT1A) in fish was less regulated in muscle than in liver. Chen et al. (2020) revealed that the addition of soybean oil and L-carnitine to fish food stimulated hepatic CPT1 activity and expression in largemouth bass. Researchers have

reported that L-carnitine supplementation accelerates fat metabolism in fish and reduces fat storage. Horn et al. (2020) reported that the SNPchip they developed for the selection of omega-3 fatty acid content in Atlantic salmon fillet would become more effective with the CPT1A and CPT2, which are marker genes whose effects are known in fish fat metabolism. Thus, Louro et al (2016) reported that CPT1A located in LG6 was found in the QTL confidence interval and is one of the marker genes that affect growth traits of European sea bass. Cai et al. (2021) have found that the alteration of MGLL and CPT1 gene expression at different stages of development in the liver of the yellowstripe goby (*Mugilogobius chulae*) and some innate immune gene family may help to offset high fat accumulation in steatohepatitis and hepatocytes. The researchers indicated that this gene could be related to sex determination and high-fat accumulation in the marine fish liver.

CONCLUSION

European sea bass is a very crucial commercial cultured fish in the Aegean Sea. Fatty acid composition is a significant quality trait in cultured seabass production. This study identified the genetic polymorphisms of the CPT1A gene in *Dicentrarchus labrax* populations in cage cultured European sea bass. Two SNPs were found in the noncoding region of the CPT1A gene in farmed European sea bass. The SNPs in the CPT1A gene could be related to fatty acid composition and this SNP could be exploited further in the genomic selection programs after validation with association studies with a large number of samples.

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