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INTESTINAL FATTY ACID BINDING PROTEIN GENE VARIATION IN EUROPEAN SEA BASS (*Dicentrarchus Labrax*)

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Abstract: Fatty acid binding proteins (FABPs), which belong to the multigene family, play an important role in homeostasis, lipid uptake and transport in tissues. Intestinal fatty acid binding protein (I-FABP) is a small cytosolic protein and is highly active in intracellular fatty acid metabolism in fish gut. The European Sea bass (*Dicentrarchus labrax*) is an important commercial marine fish species in the Mediterranean region. In the present study, the partial I-FABP gene region of European sea bass was sequenced for detecting single nucleotide polymorphism (SNP) using DNA sequencing. We identified one SNP (g.2450T>C) in the noncoding region of the I-FABP gene in European sea bass. In this study, the relationship between the g.2450T>C locus of the I-FABP (fabp2) gene and body length, post-anal length, body weight and fillet weight was found significant (P<0.05). According to these results, the g.2450T>C locus in I-FABP which could affect growth and muscle fat content, can be used for marker-assisted selection (MAS) studies in European sea bass.

Keywords: SNP, FABP2 gene, teleost, variation

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1. Introduction

Growth and development in animals are affected by the intracellular transport of long-chain fatty acids (FABPs) which are special-formed proteins of the lipid-binding protein (LBP) family (Besnard et al., 2002; Kaitetzidou et al., 2015; Zhang et al., 2019). FAPBs, long-chain fatty acids, consists of 126 to 137 amino acids and their molecular weight is around 150 kDa (Chen and Shi, 2009; Venold et al., 2013). These proteins are involved in fatty-acid metabolism, including absorption, transportation and regulation of the concentration gradient across cellular membranes (Wang et al., 2005). Total of 12 FABP genes have been identified in vertebrate and invertebrate species (Kaitetzidou et al., 2015; Venkatachalam et al., 2017; Zhang et al., 2020). These proteins were named according to the tissue from which they were initially isolated, e.g. liver type fatty acid-binding protein (L-FABP), brain-type fatty acid-binding protein (B-FABP), intestinal-type fatty acid-binding protein (I-FABP or *fabp2*), etc. (Alves-Costa et al., 2008). The intestinal fatty acid binding protein (I-FABP) gene is involved in the synthesis, uptake and intracellular transport of triglyceride-rich lipoproteins in the intestine (Chen and Shi, 2009; Levy et al., 2001). The fatty acids and dietary lipids supply most of the energy needed for vital activities such as the growth, development, swimming and reproduction of fish (Andre et al. 2000). I-FABP is expressed in many tissues such as intestine, brain and muscle in fish (Sharma et al., 2004; Venold et al., 2013; Zhang et al., 2019).

Improving growth and meat quality characteristics is the primary focus of livestock and fish breeding studies. Single nucleotide polymorphisms (SNPs) in candidate genes that regulate yield traits are identified and widely used as markers in breeding studies, for example, gene mapping, association analyses, etc. (Wang et al., 2014). Although a significant correlation between I-FABP gene and fatty acid content in cattle has not been reported before, this gene has been reported as a candidate gene for meat quality as a result of the genome-wide association study (GWAS) (Dawood et al., 2021). Besides, there are significant associations between the single nucleotide polymorphisms (SNPs) of the I-FABP gene and growth characteristics of fish species such as growth, body thickness and etc. (Xia et al., 2013; Zhou et al., 2019).

European sea bass, which belongs to the Moronidae family, lives from the north-eastern Atlantic Ocean to the Mediterranean and the Black Sea (Vandeputte et al., 2019). Türkiye was the largest producer of farmed sea bass and the largest exporter of sea bass products in the worldwide. Türkiye has produced 149.000 tonnes European sea bass in 2020 (FAO, 2022). World aquaculture production of European sea bass was

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276.000 tonnes in 2020 (FAO, 2022). Increasing the growth rate and muscle fat content of *D. labrax* stocks is important for sustainable aquaculture. Molecular markers are very effective tools in breeding programs of aquaculture species.

The aim of this study is to reveal the SNPs in the I-FABP gene region by DNA sequencing method and their associations with the growth traits of 80 European sea bass individuals.

2. Materials and Methods

A total of 80 European sea bass samples were randomly taken from a processing factory in Urla- İzmir. These fish samples were reared in the same cage environment from Çeşme-İzmir. The standard length (SL, cm), head length (HL, cm), body length (BL, cm), pre-anal length (PAL, cm), abdominal length (AL, cm), post-anal length (POSTAL, cm), head width (HW, cm), body width (BW, cm), total weight (TW, g) and fillet weight (FW, g) of fish samples were measured. Muscle tissue samples were collected from each fish and preserved at -20°C until DNA isolation. Genomic DNA was extracted by using the High Pure PCR Template Kit (Roche, Germany) following recommended protocols in the Ege University, Faculty of Fisheries, Laboratory of the Molecular Genetics and Fish Breeding. The concentration and purity of the genomic DNA samples were measured by spectrophotometer (MaestroGenNano).

The primer sequences of partial region of I-FABP gene were designed for European sea bass based on whole genome shotgun sequence (Accession number CBXY010015347) using Primer-BLAST algorithm (https:// www.ncbi.nlm.nih.gov/tools/primer90blast/) from GenBank using the Primer3 program (http://bioinfo.ut.ee) (NCBI, 2022). Primer sequences of I-FABP gene are F: 5'- TCCAGGGTGCGGAATTTACT -3' and R: 5'- CCTTCAACGGCAACTGGAAA -3'. PCR was performed in a 50-µL volume containing 50 ng genomic DNA, 0.5 µM of each primer, 2× MyTaq Mix (Meridian Bioscience, USA), 0,5 U Taq Hot Start DNA (Bioline) polymerase and distilled water. The thermal profile consisted of initial denaturation at 95°C for 4 min; 37 cycles of amplification, including 95°C for 45 s, 56°C for 45 s and 72°C for the 90 s and final extension at 72°C for 10 min. The PCR products were checked on 2% agarose gel using horizontal electrophoresis and the gels were stained using RedSafe (iNtRON) (Figure 1).

The genotyping of the SNPs in the partial region of the I-FABP gene was performed by 3500XL Genetic Analyzer System (Applied Biosystems, USA). The sequence results were aligned and controlled by ChromasPro Version 2.1.10 (Technelysium Pty. Ltd. Australia). Differences of gene sequences between individuals were detected using BioEdit (Hall, 1999). The Hardy–Weinberg equilibrium of the population was estimated using the 'HardyW'einberg' package in R (R Core Team, 2013). The associations between genotypes, haplotypes and growth traits were analysed (via SPSS Inc. V. 18.0, IBM, Chicago, IL, 2009) using the general linear model (GLM; equation 1) and an alpha value of 0.05 was considered significant.

$$\text{Linear Model I} = Yjk = \mu + Gj + ejk \quad (1)$$

Where;

Yijk represents the traits; µ represents the intercept; Gj represents the fixed effect of the I-FABP genotype or each haplotype and eijk is the random error. The significance of differences between genotypes of the locus was determined using Bonferroni multiple range test. The thresholds for significant differences were $P<0.05$.

The sequence data obtained for I-FABP gene region and the reference sequences taken from GenBank were used in the reconstruction of the phylogenetic tree based on Maximum Likelihood (ML) method applying HKY nucleotide substitution model. The nucleotide substitution models were selected based on the results obtained from ModelTest implemented in the software MEGA. In order to test the reliability of the tree topology, bootstrapping (×1000) was performed. Phylogenetic evolutionary analyses of I-FABP gene region of European sea bass were conducted using MEGA version 11 (Tamura et al., 2021).

3. Results and Discussion

European sea bass I-FABP gene contains four exons and three introns, that encode 132 amino acids (KJ130030) (NCBI, 2022). The genetic variation at 754 bp of the partial I-FABP gene was amplified by PCR and it was shown in Fig. 1. The amplified gene region in this study is located between 1845 and 2598 bp in the reference sequence (CBXY010015347) (NCBI GenBank).

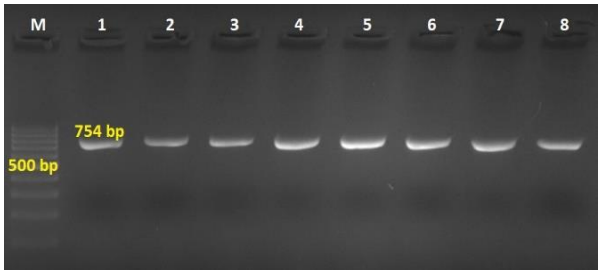


Figure 1. Electrophoresis image of the PCR amplicons of I-FABP gene for the 8 European sea bass samples. M= marker.

In this study, PCR products of the I-FABP gene region were investigated with the Sanger sequencing method and a g.2450T>C change was detected in the noncoding region (Figure 2). Generally, in the literature more polymorphisms are found in intronic regions because they are under less selection pressure than exonic regions of genes (Özcan Gökçek and Işık, 2020; Tran et al., 2021). Moreover, SNPs in non-coding regions can affect transcription and translation of mRNA splicing and regulate gene expression (Pagani and Baralle, 2004). Gene expression level should be analyzed, to detect if it is affected from SNP or not. In this study, the g.2450T > C

locus of I-FABP gene was in Hardy–Weinberg equilibrium. The genotype and allele frequencies of the European sea bass I-FABP gene were shown in Table 1.

In the current study, we detected significant associations between the g.2450T>C locus of the I-FABP gene and body length, post-anal length, body weight and fillet weight of European sea bass (P<0.05) (Table 2). According to the results, the CC genotype is superior for all these traits. Similarly, in Asian sea bass (*Lates calcarifer*), Xia et al. (2013) reported that a SNP (SNP1245) in the exon 3 of the IFABP-a gene has a significant relationship with the growth characteristics of 6- and 9-month-old fish by using QTL mapping and association analysis. It has been reported that heterozygosity (CG) is quite high in the large-size fish group for the IFABP-SNP1245 locus (P<0.01). Besides, Wan et al., (2018) performed a genome-wide association study (GWAS) for highly unsaturated fatty acids (HUFA) and eviscerated weight (EW) traits in the large yellow croaker (*Larimichthys crocea*) population. They reported that the FABP gene is one of the candidate genes associated with n-3 HUFA and EW traits in large yellow croacker. Also, Zhou et al. (2019) found that I-FABPa gene on chromosome 24 affects the growth and body thickness (BT) of *L. crocea* with GWAS. The I-FABP gene has the potential to be a candidate gene that affects growth due to its role in the regulation of vertebrate and fish metabolism, such as digestion and intracellular transport of dietary fatty acids (Sharma et al., 2004; Xia et al., 2013).

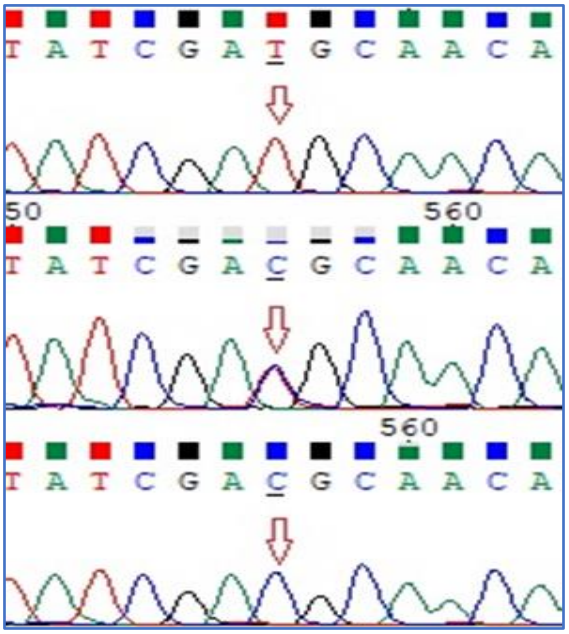


Figure 2. Partial chromatogram for SNPs g.2450T>C in noncoding region of I-FABP gene.

Table 1. Allele and genotype frequencies of I-FABP gene region in European sea bass

Loci		I-FABP Genotypes			Allele Frequency		χ2*
		TT	TC	CC	T	G	
g.2450T>C	O	50	25	5	0.78	0.22	0.58
	E	48.83	27.34	3.83			

*χ2 (0.05; 1), P<0.05

Table 2. Associations between g.2450T>C genotypes of growth traits of *Dicentrarchus labrax* (mean±SE).

	I-FABP Genotypes			
Trait	TT	Y	CC	P*
SL	29.03 ± 0.52	30.87 ± 0.47	32.68 ± 0.78	0.012
HL	7.90 ± 0.17	8.33 ± 0.19	8.59 ± 0.23	0.178
BL	21.16 ± 0.38[a]	22.64 ± 0.37[a]	24.19 ± 0.63[b]	0.005*
PAL	21.11 ± 0.42	22.29 ± 0.38	23.65 ±0.83	0.043
AL	13.49 ± 0.27	14.26 ± 0.26	15.40 ± 0.62	0.038
POSTAL	8.94 ± 0.16[b]	9.68 ± 0.19[a]	10.08 ± 0.28[a]	0.006*
HD	4.96 ± 0.10	5.35 ± 0.12	5.30 ± 0.18	0.063
BD	8.66 ± 0.16	7.85 ± 0.14	7.32 ± 0.35	0.005
BW	434.91 ± 22.38[a,b]	531.37 ± 21.98[a]	676.90 ± 16.46[b]	0.000*
FW	226.05 ± 11.61[a]	284.40 ± 11.27	362.98 ± 7.73[b]	0.000*

*Values with different superscripts (a, b) within the same row differ significantly at P<0.05. SL= standard length, HL= head length, BL= body length, PAL= pre-anal length, AL= abdominal length, POSTAL= post-anal length, HD= head depth, BD= body depth, TW= total weight, FW= fillet weight.

According to ML analysis of the evolutionary relationship of the I-FABP sequences obtained from the present study with the other fish species retrieved from GenBank are shown in Figure 3. The ML tree based on HKY nucleotide substitution model revealed *D. labrax, Epinephelus fuscoguttatus, Larimichthys crocea* and partial region (1845-2598) of *D. labrax* whole genome shotgun sequence in the same clade. Kaitetzidou et al. (2015) identified that European sea bass I-FABP gene has 2 paralogs, fabp2a and fabp2b. In this study, the fabp2a gene region was amplified which localized on LG7.

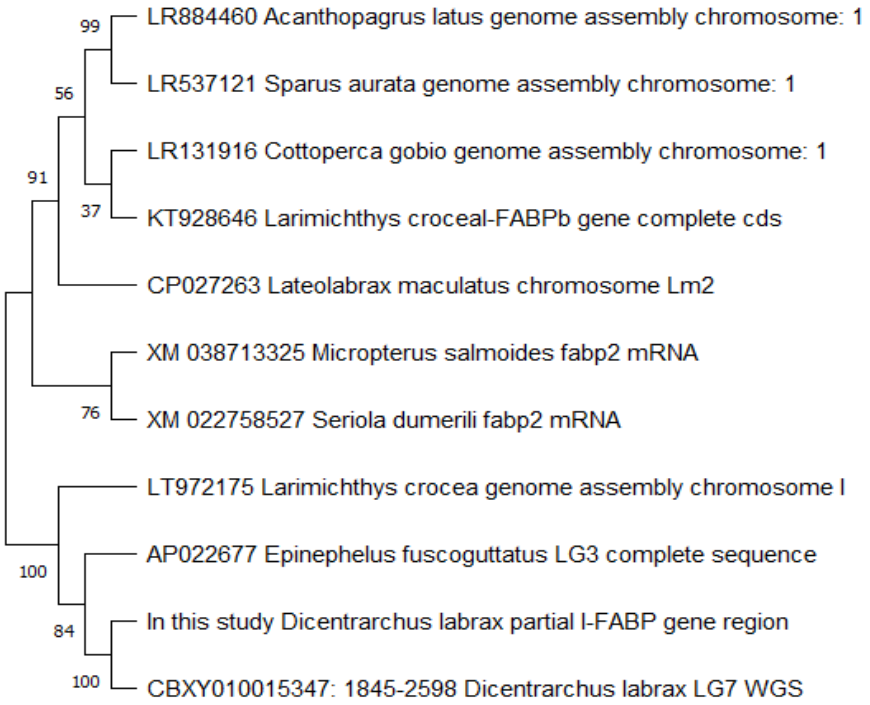


Figure 3. The phylogenic tree of the I-FABP (fabp2) gene sequences retrieved from GenBank database for different species (*Dicentrarchus labrax, Lateolabrax maculatus, Acanthopagrus latus, Epinephelus fuscoguttatus, Sparus aurata, Larimichthys crocea, Cottoperca gobio, Micropterus salmoides, Seriola dumerili, Larimichthys crocea*).

4. Conclusion

The SNP (g.2450T>C) detected in the current study and potential SNPs that can be found in other regions of the I-FABP gene of European sea bass and their relationships with harvest traits such as growth, muscle fat content and body thickness should be investigated with large sample size. The results of this study show that the I-FABP (fabp2) gene has a high potential for MAS studies.

Author Contributions

The percentage of the author(s) contributions is present below. All authors reviewed and approved final version of the manuscript.

	E.Ö.G.	R.I.
C	50	50
D	50	50
S	50	50
DCP	50	50
DAI	50	50
L	50	50
W	50	50
CR	50	50
SR	50	50
PM	50	50
FA	50	50

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on live animals or humans. Ethics committee approval was not obtained because of the dead fish samples were taken from a private facility that breeds and processes fish for commercial purposes. The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to.

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